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TOXICOLOGICAL REVIEW OF FORMALDEHYDE INHALATION TOXICITY

(CAS No. 50-00-0)

In Support of Summary Information on the Integrated Risk Information System (IRIS)

VOLUME II of IV

Hazard Characterization

March 17, 2010

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LIST OF ABBREVIATIONS AND ACRONYMS

ACGIH	American Conference of Governmental Industrial Hygienists
ADAF	age-dependent adjustment factors
ADH	alcohol dehydrogenase
ADS	anterior dorsal septum
AIC	Akaike Information Criterion
AIE	average intensity of exposure
AIHA	American Industrial Hygiene Association
ALB	albumin
ALDH	aldehyde dehydrogenase
ALL	acute lymphocytic leukemia
ALM	anterior lateral meatus
ALP	alkaline phosphatase
ALS	amyotrophic lateral sclerosis
ALT	alanine aminotransferase
AML	acute myelogenous leukemia
AMM	anterior medial maxilloturbinate
AMPase	adenosine monophosphatase
AMS	anterior medial septum
ANAE	alpha-naphthylacetate esterase
ANOVA	analysis of variance
APA	American Psychiatric Association
ARB	Air Resources Board
AST	aspartate aminotransferase
ATCM	airborne toxic control measure
ATP	adenosine triphosphate
ATPase	adenosine triphosphatase
ATS	American Thoracic Society
ATSDR	Agency for Toxic Substances and Disease Registry
AUC	area under the curve
BAL	bronchoalveolar lavage
BALT	bronchus associated lymphoid tissue
BBDR	biologically based dose response
BC	bronchial construction
BCME	bis(chloromethyl)ether
BDNF	brain-derived neurotrophic factor
BEIR	biologic effects of ionizing radiation
BfR	German Federal Institute for Risk Assessment
BHR	bronchial hyperresponsiveness
BMC	benchmark concentration
BMCL	95% lower bound on the benchmark concentration
BMCR	binuclated micronucleated cell ratefluoresce
BMD	benchmark dose
BMDL	95% lower bound on the benchmark dose

LIST OF ABBREVIATIONS AND ACRONYMS (continued)

BMR	benchmark response
BN	Brown-Norway
BrdU	bromodeoxyuridine
BUN	blood urea nitrogen
BW	body weight
CA	chromosomal aberrations
CalEPA	California Environmental Protection Agency
CAP	College of American Pathologists
CASRN	Chemical Abstracts Service Registry Number
CAT	catalase
CBMA	cytokinesis-blocked micronucleus assay
CBMN	cytokinesis-blocked micronucleus
CDC	U.S. Centers for Disease Control and Prevention
CDHS	California Department of Health Services
CFD	computational fluid dynamics
CGM	clonal growth model
CHO	Chinese hamster ovary
CI	confidence interval
CIIT	Chemical Industry Institute of Toxicology
CLL	chronic lymphocytic leukemia
CML	chronic myelogenous leukemia
CNS	central nervous system
CO_2	carbon dioxide
COEHHA	California Office of Environmental Health Hazard Assessment
CREB	cyclic AMP responsive element binding proteins
CS	conditioned stimulus
C × t	concentration times time
DA	Daltons
DAF	dosimetric adjustment factor
DDX	DNA-DNA cross-links
DEI	daily exposure index
DEN	diethylnitrosamine
Der f	common dust mite allergen
DMG	dimethylglycine
DMGDH	dimethylglycine dehydrogenase
DNA	deoxyribonucleic acid
DOPAC	3,4-dihydroxyphenylacetic acid
DPC / DPX	DNA-protein cross-links
EBV	Epstein-Barr virus
EC	effective concentration
ED	effective dose
EHC	Environmental Health Committee
ELISA	enzyme-linked immunosorbent assay

LIST OF ABBREVIATIONS AND ACRONYMS (continued)

EPA	U.S. Environmental Protection Agency
ERPG	emergency response planning guideline
ET	ethmoid turbinates
FALDH	formaldehyde dehydrogenase
FDA	U.S. Food and Drug Administration
FDR	fecundability density ratio
FEF	forced expiratory flow
FEMA	Federal Emergency Management Agency
FEV1	forced expiratory volume in 1 second
FISH	fluorescent in situ hybridization
FSH	follicle-stimulating hormone
FVC	forced vital capacity
GALT	gut-associated lymphoid tissue
GC-MS	gas chromatography-mass spectrometry
GD	gestation day
GI	gastrointestinal
GO	gene ontology
G6PDH	glucose-6-phosphate dehydrogenase
GPX	glutathione peroxidase
GR	glutathione reductase
GM-CSF	granulocyte macrophage-colony-stimulating factor
GSH	reduced glutathione
GSNO	S-nitrosoglutathione
GST	glutathione S-transferase
HAP	hazardous air pollutant
Hb	hemoglobin
HCl	hydrochloric acid
HCT	hematocrit
HEC	human equivalent concentration
5-HIAA	5-hydroxyindoleacetic acid
hm	hydroxymethyl
HMGSH	S-hydroxymethylglutathione
HPA	hypothalamic-pituitary adrenal
HPG	hypothalamo-pituitary-gonadal
HPLC	high-performance liquid chromatography
HPRT	hypoxanthine-guanine phosphoribosyl transferase
HR	high responders
HSA	human serum albumin
HSDB	Hazardous Substances Data Bank
Hsp	heat shock protein
HWE	healthy worker effect
I cell	initiated cell
IARC	International Agency for Research on Cancer

LIST OF ABBREVIATIONS AND ACRONYMS (continued)

ICD	International Classification of Diseases
IF	interfacial
IFN	interferon
Ig	immunoglobulin
IL	interleukin
I.P.	intraperitoneal
IPCS	International Programme on Chemical Safety
IRIS	Integrated Risk Information System
K_m	Michaels-Menton constant
KM	Kaplan-Meier
LD_{50}	median lethal dose
LDH	lactate dehydrogenase
LEC	95% lower bound on the effective concentration
LED	95% lower bound on the effective dose
LHP	lymphohematopoietic
LI	labeling index
LM	Listeria monocytogenes
LMS	linearized multistage
LLNA	local lymph node assay
LOAEL	lowest-observed-adverse-effect level
LPS	lipopolysaccharide
LR	low responders
LRT	lower respiratory tract
MA	methylamine
MALT	mucus-associated lymph tissues
MCH	mean corpuscular hemoglobin
MCHC	mean corpuscular hemoglobin concentration
MCS	multiple chemical sensitivity
MCV	mean corpuscular volume
MDA	malondialdehyde
MEF	maximal expiratory flow
ML	myeloid leukemia
MLE	maximum likelihood estimate
MMS	methyl methane sulfonate
MMT	medial maxilloturbinate
MN	micronucleus, micronuclei
MNNG	N-methyl-N-nitro-N-nitrosoguanidine
MOA	mode of action
MoDC	monocyte-derived dendritic cell
MP	macrophage
MPD	multistage polynomial degree
MPS	mononuclear phagocyte system
MRL	minimum risk level

mRNA	messenger ribonucleic acid
MVE-2	Murray Valley encephalitis virus
MVK	Moolgavkar, Venzon, and Knudson
N cell	normal cell
NaCl	sodium choride
NAD+	nicotinamide adenine dinucleotide
NADH	reduced nicotinamide adenine dinucleotide
NALT	nasally associated lymphoid tissue
NATA	National-Scale Air Toxics Assessment
NCEA	National Center for Environmental Assessment
NCHS	National Center for Health Statistics
NCI	National Cancer Institute
NEG	Nordic Expert Group
NER	nucleotide excision repair
NGF	nerve growth factor
NHL	non-Hodgkin's lymphoma
NHMRC/ARMCANZ	National Health and Medical Research Council/Agriculture and Resource Management Council of Australia and New Zealand
NNK	nitrosamine nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-butanone
N^6-hmdA	N^6-hydroxymethyldeoxyadenosine
N^4-hmdC	N^4-hydroxymethylcytidine
N^2-hmdG	N^2-hydroxymethyldeoxyguanosine
NICNAS	National Industrial Chemicals Notification and Assessment Scheme
NIOSH	National Institute for Occupational Safety and Health
NLM	National Library of Medicine
NMDA	N-methyl-D-aspartate
NO	nitric oxide
NOAEL	no-observed-adverse-effect level
NPC	nasopharyngeal cancer
NRBA	neutrophil respiratory burst activity
NRC	National Research Council
NTP	National Toxicology Program
OR	odds ratio
OSHA	Occupational Safety and Health Administration
OTS	Office of Toxic Substances
OVA	ovalbumin
PBPK	physiologically based pharmacokinetic
PC	Philadelphia chromosome
PCA	passive cutaneous anaphylaxis
PCMR	proportionate cancer mortality ratio
PCNA	proliferating cell nuclear antigen
PCR	polymerase chain reaction
PCV	packed cell volume

LIST OF ABBREVIATIONS AND ACRONYMS (continued)

PECAM	platelet endothelial cell adhesion molecule
PEF	peak expiratory flow
PEFR	peak expiratory flow rates
PEL	permissible exposure limit
PFC	plaque-forming cell
PG	periglomerular
PHA	phytohemagglutinin
PLA2	phospholipase A2
PI	phagocytic index
PLM	posterior lateral meatus
PMA	phorbol 12-myristate 13-acetate
PMR	proportionate mortality ratio
PMS	posterior medial septum
PND	postnatal day
POD	point of departure
POE	portal of entry
PTZ	pentilenetetrazole
PUFA	polyunsaturated fatty acids
PWULLI	population weighted unit length labeling index
RA	reflex apnea
RANTES	regulated upon activation, normal T–cell expressed and secreted
RB	reflex bradypnea
RBC	red blood cells
RD_{50}	exposure concentration that results in a 50% reduction in respiratory rate
REL	recommended exposure limit
RfC	reference concentration
RfD	reference dose
RGD	regional gas dose
RGDR	regional gas dose ratio
RR	relative risk
RT	reverse transcriptase
SAB	Science Advisory Board
SCC	squamous cell carcinoma
SCE	sister chromatid exchange
SCG	sodium cromoglycate
SD	standard deviation
SDH	succinate dehydrogenase; sarcosine dehydrogenase
SEER	Surveillance, Epidemiology, and End Results
SEM	standard error of the mean
SEN	sensitizer
SH	sulfhydryl
SHE	Syrian hamster embryo
SLMA	spontaneous locomotor activity

LIST OF ABBREVIATIONS AND ACRONYMS (continued)

SMR	standardized mortality ratio
SNP	single nucleotide polymorphism
SOD	superoxide dismutase
SOMedA	N^6-sulfomethyldeoxyadenosine
Sp1	specificity protein
SPIR	standardized proportionate incidence ratio
SSAO	semicarbozole-sensitive amine oxidase
SSB	single strand breaks
STEL	short-term exposure limit
TBA	tumor bearing animal
TH	T-lymphocyte helper
THF	tetrahydrofolate
TK	toxicokinetics
TL	tail length
TLV	threshold limit value
TNF	tumor necrosis factor
TP	total protein
TRI	Toxic Release Inventory
TRPV	transient receptor potential vanilloid
TWA	time-weighted average
TZCA	thiazolidine-4-carboxylate
UCL	upper confidence limit
UDS	unscheduled DNA synthesis
UF	uncertainty factor
UFFI	urea formaldehyde foam insulation
ULLI	unit length labeling index
URT	upper respiratory tract
USDA	U.S. Department of Agriculture
VC	vital capacity
VOC	volatile organic compound
WBC	white blood cell
WDS	wet dog shake
WHO	World Health Organization
WHOROE	World Health Organization Regional Office for Europe

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4. HAZARD CHARACTERIZATION

4.1. HUMAN STUDIES

This chapter discusses epidemiologic studies of site-specific cancers and other adverse health effects that may be caused by exposure to formaldehyde. The primary focus is on the literature describing inhalation exposure and its potential carcinogenic and noncarcinogenic health risks. In addition, oral, dermal, and ocular exposures to formaldehyde are discussed.

The noncancer health effects section is organized by endpoint, beginning with sensory irritation and followed by pulmonary function, asthma, respiratory tract pathology, immunologic responses, neurological and behavioral responses, and, finally, developmental and reproductive outcomes.

The carcinogenicity section is divided into two parts, respiratory tract and non-respiratory tract cancers. The first part discusses site-specific cancers that are chiefly located in the respiratory tract where direct contact with formaldehyde occurs: nasopharyngeal cancers (NPCs), nasal and paranasal cancers, other respiratory tract cancers, and lung cancers. The second part on non-respiratory tract cancer discusses those cancers at other sites with more distant exposure to formaldehyde than respiratory epithelium—mainly, lymphohematopoietic (LHP) cancer, brain and central nervous system (CNS) cancer, pancreatic cancer, and cancer at other sites.

4.1.1. Noncancer Health Effects

4.1.1.1. *Sensory Irritation (Eye, Nose, Throat Irritation)*

As a reactive gas, formaldehyde is a sensory irritant. Sensory irritation of the eyes and respiratory tract by formaldehyde has been observed consistently in clinical and epidemiologic studies in residential and occupational populations. Binding to sensory nerves at the portal of entry (POE) results in direct sensory responses (e.g., detection of odor and tissue irritation) as well as reflex responses to the sensory irritation and neurogenic sensitization. Reflex responses result from CNS stimulation by the afferent sensory signals and include lacrimation, coughing, sneezing, and bronchial constriction (BC). An additional reflex seen in rodents is reflex bradypnea (RB) (also known as reflex apnea [RA]). Formaldehyde-induced sensory irritation may be evident after acute exposures as well as in chronically exposed individuals. Formaldehyde-induced neurogenic sensitization and atopy may result in lifelong health effects from short-term or transient exposures. For this discussion, sensory irritation will include both direct sensory response to formaldehyde exposure and reflex responses (lacrimation, coughing, sneezing, RB, and BC, and sensitization.

Eye, nose, and throat irritation in response to formaldehyde inhalation exposure is well documented (Doty et al., 2004). Broadly, studies examining these endpoints are either controlled chamber studies with a defined population (e.g., healthy volunteers or sensitive individuals), worker/student studies, or population (e.g., residential) studies. Chamber studies, by design, are acute studies, although some researchers have investigated repeated exposures. Occupational, student, and residential exposures are generally longer duration, although there is variability in exposure and duration among subjects. Endpoints include both local effects and reflex effects of sensory irritation. The endpoints for assessing irritation include self-reporting of adverse symptoms (e.g., pain, burning, itching) as well as objective measures of irritation (e.g., eye-blink counts, lacrimation) (Doty et al., 2004). The following review focuses on eye, nose, and throat irritation but studies have documented other types of irritation, including dermal irritation eczema and dermatitis.

4.1.1.1.1. ***Epidemiologic literature.*** A wide variety of epidemiologic studies have assessed the potential effects of exposure to formaldehyde on endpoints, indicating sensory irritation of the eye, nose, and throat. These studies generally include three different types of exposure populations: (1) Residents and visitors exposed to formaldehyde in homes and mobile buildings, where formaldehyde is present from various sources, including building components, furniture and home furnishings, heating and cooking combustion as well as active and passive smoking; (2) various occupational exposures from industrial processes related to wood products, furniture making, and formaldehyde-based resins; and (3) anatomy students who are exposed under well-defined conditions during academic courses where they are examining formaldehyde-preserved cadavers.

4.1.1.1.1.1. ***Residential epidemiology.*** Among the residential epidemiology studies of formaldehyde effects on sensory irritation, one of the strongest studies based on study design, execution, analysis, and sample size was the observational study undertaken by Ritchie and Lehnen (1987). In this cross-sectional study of nearly 2,000 Minnesota residents living in 392 mobile and 494 conventional homes, personal data and formaldehyde samples were collected from residents that had responded to an offer by the state health department to test homes for formaldehyde. Technicians administered a symptom questionnaire to participating residents at the time of formaldehyde sample collection. Residents were asked to close doors and windows of their homes for 12 hours before testing was conducted, and a standardized collection protocol was used for both sample collection and analysis. Measurements of formaldehyde exposure were taken from two rooms of the home, usually the bedroom and living room, and were kept

refrigerated until analysis. Respondents were not aware of the results of the formaldehyde analyses in their homes at the time they responded to the symptom questionnaire. The results from Ritchie and Lehnen (1987) provide a clear dose-response relationship in the percentage of residential occupants reporting eye, nose, and throat irritation. Specifically, eye irritation responses increase from 1–2% in homes with formaldehyde concentrations lower than 0.1 ppm to 12–32% in homes with formaldehyde concentrations ranging from 0.1 to 0.3 ppm with 86–93% of residents reporting . These effects were found in the same concentration range for people living in either mobile (n = 851) or conventional (n = 1,156) homes. Similar percentages were found for nose/throat irritation. Reports of irritation were reported for smokers, passive smokers, and nonsmokers with higher percentages of irritation among smokers, followed by passive smokers and then nonsmokers. While the participants in this study were self-selected and not a random residential sample, a clear concentration response was observed, and, even if participants sought testing because they suspected that they were being exposed to formaldehyde in their homes, they could not know the measured concentration of formaldehyde when reporting their irritation symptoms, so recall bias cannot explain the concentration response. Neither can confounding be an alternative explanation since the authors reported that formaldehyde was the most important explanatory variable for all the sensory irritation effects of the eye, nose, and throat.

The results of an adverse association of sensory irritation with formaldehyde reported by Ritchie and Lehnen (1987) are corroborated by Hanrahan et al. (1984) who conducted a cross-sectional survey by using a random sample of mobile homes from mobile home parks in Wisconsin. Sixty-one teenage and adult residents participated. Health questionnaires were self-administered by each occupant. Respondents were blinded to the results of their home formaldehyde vapor measurements, which were sampled from two rooms in the homes following instruction to close windows, refrain from smoking, and turn off gas appliances for 30 minutes prior to air sampling. Logistic regression analyses were used to ascertain potential symptom risk ratio dependency on each respondent's age, smoking status, gender, and formaldehyde concentration measures in the home. Formaldehyde concentrations ranged from 0.1 ppm to 0.8 ppm with a geometric mean of 0.16 ppm. Across this concentration range, a clear and statistically significant concentration-response relationship was reported in graphical form, controlling for age, gender, and smoking status. At 0.1 ppm, the regression model showed less than 5% predicted prevalence of burning eyes. At 0.2 ppm, the midpoint of the exposure category in Ritchie and Lehnen (1987) that was reported to be the lowest adverse effect level for eye irritation with 12–32% reporting eye irritation, the regression model of Hanrahan et al. (1984) showed approximately 17.5% predicted prevalence of burning eyes. The prevalence of

burning eyes rose linearly to approximately 65% prevalence at 0.5 ppm, with some diminishment in the rate of rise up to approximately 80% prevalence at 0.8 ppm. While only 65 out of 208 randomly selected homes volunteered to complete the health questionnaires, the investigators were able to complete home formaldehyde vapor measurements on all the homes and reported nearly an identical distribution of formaldehyde concentrations in participating and nonparticipating homes. Demographic characteristics of some of the non-respondents were available and were reported as nearly identical to those of participants. There was no indication of selection bias. Confounding is unlikely to explain such a strong concentration response.

These findings of associations of sensory irritation with residential exposures to formaldehyde are further supported by studies that did not examine concentration response but nonetheless assessed the association of formaldehyde with sensory irritation. Similar findings to those of Ritchie and Lehnen (1987) and Hanrahan et al. (1984) have been reported in other residential studies of increased symptoms in association with formaldehyde exposure (Liu et al., 1991; Thun et al., 1982; Dally et al., 1981). Dally et al. (1981) collected data in 100 "complaint structures" (65% mobile homes, 27% conventional homes). Of these, 60% were from home owners contacting the health department and 30% from physician referrals. Twenty percent of the buildings had concentrations below the limit of detection (0.1 ppm), 20% had levels at or above 0.81 ppm, and overall the concentrations ranged from below detection to above 3 ppm with an overall median of 0.35 ppm. The median levels were 0.47 and 0.10 ppm for mobile and conventional homes, respectively. No other contaminants were measured. Eye, nose, and throat irritation were reported in a high percentage of occupants (eye irritation 68%, burning eyes 60%, runny nose 60%, dry or sore throat 57%, cough 51%), but these were not reported as a function of dose or home type. Thus, there was no control group to which rates of irritation could be compared. However, symptoms reportedly stopped in 89% of occupants when they left the "complaint structure." The most recent residential study was performed on over 1,000 mobile homes with 1,394 participants (Liu et al., 1991). Home formaldehyde concentration ranged from below 0.01 to 0.46 ppm. Analyses used logistic regression to control for potential confounders. Eye irritation was positively associated with formaldehyde with a clear concentration response demonstrated with cumulative exposure. During the summer and winter months, formaldehyde exposure was associated with burning eyes. In the winter months, formaldehyde exposure was associated with sore throat. There was no association of formaldehyde exposure with cough or running nose during either season. Liu et al. (1991) also report a synergistic effect on irritation by formaldehyde exposure and chronic disease prevalence. Thun et al. (1982) reported increased symptoms of itchy skin and "wheezing and difficulty breathing" in residents in 395 homes insulated with urea-formaldehyde foam relative to nearby homes without urea foam

formaldehyde insulation (UFFI); however, there were no measurements of formaldehyde concentration taken in this study.

While not strictly a residential epidemiology study, Olsen and Dossing (1982) studied occupational exposures within mobile and non-mobile daycare centers. They reported the mean concentration in mobile and non-mobile day care centers were 350 (200–450) ppb and 65 (40–90) ppb, respectively. Adverse eye, nose, and throat irritation were significantly elevated in the workers (n = 70) in the mobile units as compared with those in non-mobile units (n = 34). The authors also state that a high percentage of workers in the mobile day cares reported that the symptoms disappeared after working hours; however, the authors did not report any such percentages among those working in non-mobile units.

4.1.1.1.1.2. ***Occupational epidemiology.*** Horvath et al. (1988) compared irritation symptoms between 109 workers at a particleboard manufacturing plant and 264 workers at food plants as a control group. The mean 8-hour time-weighted average (TWA) formaldehyde concentrations between these two groups were 0.69 ppm (range 0.17–2.93) and 0.05 ppm (range 0.03–0.12), respectively. Eye, nose, and throat irritation were more common among the former group (prevalence of symptoms during a work shift: throat sore or burning—test 22.0%, controls 3.9%; cough—test 34.9%, controls 18.9%; burning of nose—test 28.4%, controls 2.0%; stuffy nose—test 33.9%, controls 14.2%; itching of nose—test 21.1%, controls 7.9%; eyes burning or watering—test 39.5%, controls 9.1%; eyes itching—test 19.3%, controls 7.1%).

Similar results were reported for frequency of eye and nasal discomfort in a group of workers involved in the manufacture of formaldehyde resins. These workers were exposed to a mean concentration of 0.40 mg/m^3. Alexandersson and Hedenstierna (1988) reported that the frequency of eye, nose, and throat irritation was significantly greater in 38 workers exposed to formaldehyde and solvents in lacquers (average employment duration 7.8 years) as compared with 18 controls (nonexposed individuals working at the same factory). The frequency of eye irritation was 65.8% among those exposed and 16.7% among controls. No controls reported nose/throat irritation, but about 40% of those exposed did.

A Swedish study conducted at a chemical plant found nasal and eye discomfort were reported by 64 and 24%, respectively, of workers (n = 70) exposed to formaldehyde (range 0.05–0.50 mg/m^3 with a mean of 0.26 mg/m^3) versus 25 and 6%, respectively, of the control group made up of clerks from the local government (n = 36). In addition, the majority of workers exposed to formaldehyde reported that their symptoms were relieved during weekends and vacations (Holmström and Wilhelmsson, 1988). Another study by the same authors (Wilhelmsson and Holmström, 1992) reported similar results. In this study irritation prevalent

among 66 workers from a formaldehyde-producing plant was compared with that seen among 36 community clerks. The workers were exposed to 0.26 mg/m^3 of formaldehyde (range 0.05–0.6 mg/m^3). The clerks were exposed to an average of 0.09 mg/m^3. Nasal and eye discomfort were reported at rates of 53 and 24%, respectively, among the workers. Among the community clerks, 3 and 6%, respectively, reported discomfort.

Holness and Nethercott (1989) reported significant increases in eye irritation (42 versus 21%) and nose irritation (44 versus 16%) among 84 funeral service workers as compared with 38 controls (students and individuals from a service organization). The former group had been actively embalming for approximately 10 years and had nearly twice the pack-years smoked as the controls. The exposure concentration in both groups was 0.36 and 0.02 ppm, respectively.

4.1.1.1.1.3. ***Epidemiology on laboratory students***. Several studies have monitored sensory irritation in medical/physical therapy students exposed to formaldehyde during anatomy courses. These studies have particular advantages: the student population generally has no former occupational exposure, and, oftentimes, pre-class survey data serve as the control, providing a better basis for assessing the effects of formaldehyde exposure.

In a study of 24 formaldehyde-exposed anatomy students (personal breathing zone samples 0.73 ppm, range 0.49–0.93), the prevalence of eye irritation before the start of a cadaver dissection class was 16%, while after the class, the prevalence was 59%. The increase in eye irritation was most pronounced, but increases were also observed in the prevalence of irritation of the nose (21%) and throat (15%) (Kriebel et al., 1993). The authors also reported a tendency for this increase in intensity between the beginning and end of class to diminish over the 10-week course, especially for eye irritation. However, although the intensity of the irritation diminished, eye irritation was still present among the students after 10 weeks of intermittent exposure. The report of increase in post- versus pre-class irritation symptoms in this study was no greater for asthmatic students (n = 5) compared with non-asthmatic students.

Takahashi et al. (2007) showed that 143 medical students reported various symptoms (including eye and throat irritation) and that the percentage of students reporting symptoms increased between the beginning and end of the course 2 months later. After the first day of class, approximately 35% of students reported eye soreness and about 15% reported throat irritation. After the course ended, these rates were close to 70% for eye soreness and slightly above 40% for throat irritation. The reported average room formaldehyde concentration was 2.12 ppm (range 1.7–2.4), while the gas samplers worn on the students' chests averaged 2.4 ppm (range 1.8–3.8). Another study of students in an anatomy laboratory class in Japan (Takigawa et al., 2005) measured formaldehyde concentrations and irritation symptoms before and after the

installation of a ventilation system. This system reduced the median personal formaldehyde exposure concentration from 2.7 to 0.72 ppm. Before installation of the ventilation system, the students complained about exacerbation of all the sensory irritation symptoms on average. The increase in 8 out of 25 symptoms was significantly reduced after installing general ventilation ($p < 0.05$). After installation of the ventilation system, a dose-dependent relationship with formaldehyde was seen for irritated eyes but not for itchy nose.

Akbar-Khanzadeh et al. (1994) detected mean personal area levels of formaldehyde at 1.24 ppm and a range of 0.1–2.94 ppm from personal air sampling devices. Almost 90% of the students in this study reported eye irritation, 74% reported nose irritation, and close to 30% reported throat irritation during or after exposure to formaldehyde during the laboratory period after having completed at least 6 weeks of laboratory sessions with formaldehyde exposure. In addition, Uba et al. (1989) demonstrated that symptoms of eye, nose, and throat irritation were correlated with formaldehyde exposure among medical students by comparing students' responses on a questionnaire completed after a lab with formaldehyde exposure to a questionnaire completed after a lab with no formaldehyde exposure. The authors compared questionnaires completed prior to students' first anatomy lab to a questionnaire completed 7 months later. Reports of cough were more frequent after the 7 months. These students were exposed to a mean level of 1.9 ppm (range 0.1–5.0) while dissecting (measured using portable infrared spectrophotometer), and a TWA from all laboratory activities ranged from below limits of detection to 0.93 (measured using personal sampling devices in the students' breathing zones).

4.1.1.1.2. ***Acute studies: controlled chamber exposures.*** Results from controlled human studies demonstrate eye, nose, and throat irritation in association with formaldehyde exposure (Lang et al., 2008; Yang et al., 2001; Krakowiak et al., 1998; Kulle, 1993; Green et al., 1989, 1987; Kulle et al., 1987; Sauder et al., 1987, 1986; Schachter et al., 1987, 1986; Witek et al., 1987; Day et al., 1984; Bender et al., 1983; Anderson et al., 1983; Weber-Tschopp et al., 1977; Andersen, 1979; Schuck et al., 1966). A key advantage of chamber studies is the ability to monitor and closely control formaldehyde concentrations during exposure. However, chamber studies may also be limited by other aspects of the study design, including small number of participants, use of healthy volunteers, short exposure durations (a few minutes), and often studies were conducted with only one exposure group and at relatively high concentrations (>1 ppm). The lack of multiple exposure levels in many studies limits the understanding of exposure-response relationships. Additionally, numerous reports that demonstrate multiple symptoms of eye, nose, and throat irritation at levels at or above 1 ppm did not explore lower levels of exposure and can

only be used for primary hazard identification (Yang et al., 2001; Green et al., 1989, 1987; Sauder et al., 1987, 1986; Schachter et al., 1987, 1986; Witek et al., 1987; Day et al., 1984).

The National Aeronautical and Space Administration conducted experiments in closed-environment living, including environmental monitoring and air quality. James et al. (2002) quantified air pollutants, including formaldehyde, during 30, 60, and 90-day tests in a closed chamber study of a Lunar-Mars life support chamber. Unfortunately, the detection methods used during the 30-day test were not sensitive enough to detect formaldehyde at levels below 2 mg/m^3. Thus, badge samples were obtained in the 60-day and 90-day tests and provided greater detection sensitivity (to 0.02 mg/m^3). Measured values of formaldehyde increased over time. In the 60-day test, formaldehyde levels were well above accepted limits (data not shown). Health effects data are limited since there were only four crew members. One crew member reported eye and upper airway irritation at formaldehyde concentrations of 0.25 mg/m^3 (308 ppb) on day 15. It should also be noted that astronauts are exceptionally healthy individuals, and these data should be interpreted carefully when determining expected health effects in the general population. The experimenters determined that formaldehyde levels increased as temperature increased. Formaldehyde was also linked to murals lining the chamber and was subsequently removed before executing the 90-day study. Between days 0 and 60, formaldehyde levels remained between 0.02 and 0.04 mg/m^3, with one sharp peak that occurred at day three to 0.07 mg/m^3. Between days 60 and 90, formaldehyde concentrations increased to 0.07 to 0.09 mg/m^3. The increase was attributed to an incomplete oxidation of methanol in a catalytic bed rather than in excessive off-gassing of formaldehyde. No crew members reported any adverse effects in the 90-day study.

A few studies have been conducted that specifically address sensitive populations (asthmatics) and/or individuals during exercise, which can exacerbate asthma (further details of these studies are in Section 4.1.1.3, Effects on Asthmatics). In Sauder et al. (1986), 8-minute bicycle exercise was completed multiple times during the exposure period (3 hours). However, irritation symptoms were only reported after 2 hours of exposure and do not address whether changes occurred during the periods of exercise. Overall, reports of eye, nose, and throat irritation increased with exposure to formaldehyde (3 ppm) compared with reports of irritation with no exposure to formaldehyde. Green et al. (1987) report that eye, nose, and throat irritation symptoms were greater immediately after exercise during exposure to 3 ppm formaldehyde. Additionally, the response levels were similar between asthmatic (n = 16) and non-asthmatic (n = 22) subjects. Similar effects of exercise on certain symptoms, such as throat irritation, were reported in 15 asthmatic subjects exposed to 2 ppm formaldehyde at rest and after exercise (Witek et al., 1987).

Kulle (1993) and Kulle et al. (1987) enrolled 19 healthy volunteers and exposed them to a range of formaldehyde concentrations. At 2 ppm, 53% reported mild or moderate eye irritation (32% mild, 21% moderate). At 3 ppm, 100% of subjects exposed at this level (n = 9) reported irritation. The reported increase in irritation was shown to correspond with increasing formaldehyde concentration in a linear fashion. Mild nose/throat irritation was present among 37% of those exposed to 2 ppm of formaldehyde. Odor detection was very similar to the distribution seen for eye irritation. Nineteen subjects performed light to moderate exercise while exposed to 2 ppm; there was no increase in report of eye irritation, but nose/throat irritation did increase. The data were reanalyzed (Kulle, 1993), and thresholds for irritation were found to be 0.5–1 ppm for eye irritation and 1 ppm for nose/throat irritation.

Yang et al. (2001) reported that eight individuals exposed to varying levels of formaldehyde (1.65, 2.99, and 4.31 ppm) had mild to moderate eye irritation during the 5-minute exposures. The increase in irritation was detected at 30 seconds with exposure to 1.65 ppm of formaldehyde. The highest severity ratings at this concentration occurred between 60 and 90 seconds. Frequency of eye blinking was also measured. The peak in blinking rate occurred after about 1 minute of exposure and then decreased almost back to a normal rate after 5 minutes of exposure. Higher formaldehyde concentrations were associated with increased frequency of blinking compared with the 1.65 ppm exposure.

Other studies have examined responses across multiple exposure levels. For example, Weber-Tschopp et al. (1977) used two different methods of studying irritation resulting from formaldehyde exposure. For one, they exposed subjects (n = 33) to an increasing level of formaldehyde (maximum exposure was 3.2 ppm). This design precluded evaluation of distinct effects at different exposure levels. The researchers addressed this by examining another group of subjects (n = 48) that were exposed to 0, 1, 2, 3, or 4 ppm five times for 90 seconds. Levels of nasal and throat irritation for this discontinuous exposure were slightly higher than the irritation levels reported among those with continuous exposure. However, this was reversed for eye irritation; those with continuous exposure reported higher levels of irritation than those with discrete exposures. An objective measure, eye-blinking rate, was measured for those with continuous exposure and was found to have a statistically significant increase at 1.7 ppm.

Bender et al. (1983) conducted a study that enrolled individuals who “responded” to formaldehyde at 1.3 and 2.2 ppm and did not report irritation to the clean air control. They found that, among these subjects, exposure to 1 ppm of formaldehyde (n = 27) resulted in the reporting of eye irritation with a median response time of 78 seconds. Reports of irritation were given as less than slightly irritating for formaldehyde concentrations of 0.3–0.9 ppm.

Assessment of sensory irritation for pain and discomfort often relies on self-reporting, using symptom questionnaires and severity ratings (e.g., mild, moderate, severe). In the case of formaldehyde, subjective ratings of eye irritation correlate positively with eye-blinking frequency (Lang et al., 2008). Lang et al. (2008) saw an increase in eye blinking after 195 minutes of exposure to formaldehyde at 0.5 ppm with four peak exposures of 1 ppm. After this amount of time and formaldehyde exposure, there was also an increase in moderate eye redness. Weber-Tschopp et al. (1977) reported that, among concentrations ranging from 0.03 to 3.2 ppm, eye-blinking frequency was increased at 1.7 ppm; similarly Yang et al. (2001) reported increased blinking at >1.5 ppm (the lowest concentration examined). There are studies that suggest that psychological factors (e.g., anxiety) can impact the perception of irritation—and perhaps more so at lower concentrations (Lang et al., 2008; Ihrig et al., 2006; Dalton, 2003). However, when Lang et al. (2008) controlled for mood prior to exposure, subjective symptoms of eye, nasal, and olfactory irritation were significantly related to exposure (0.5 ppm)

Schuck et al. (1966) performed a study that also examines self-reported eye irritation as well as blinking rate. Fourteen individuals were exposed to formaldehyde concentrations ranging from 0 to 1 ppm. Increased irritation was reported with increasing formaldehyde concentration. One subject, judged to be the least sensitive, was still able to detect formaldehyde levels as low as 0.01 ppm. In addition, the authors examined the blinking rate of participants, which they found was related to irritation intensity.

Andersen (1979) and Anderson and Molhave (1983) reported on a controlled experiment in which 16 individuals were exposed to varying levels of formaldehyde for five hours and rated their level of discomfort over the exposure period. Discomfort occurred within 1 hour at formaldehyde exposure levels of 1 and 2 mg/m^3 (Andersen and Molhave, 1983) After 2 hours, increasing discomfort was reported among the groups exposed to 0.3 and 0.5 mg/m^3. Subject reported that discomfort was mainly conjunctival irritation and dryness in the nose and throat. Subjects complained at all four concentrations of formaldehyde: 0.3, 0.5, 1.0, and 2.0 mg/m^3 and of 16 subjects, 3, 5, 15, and 15 subjects complained at each respective exposure concentration (Andersen and Molhave, 1983).

Controlled chamber studies have also been conducted on various populations of previously exposed individuals to determine if formaldehyde exposure potentiates an individual's response to acute exposures. Schachter et al. (1987) reported on 15 laboratory workers "frequently exposed to formaldehyde" (no quantification of exposure is given; however, the workers report being exposed for 1 to 7 days per week from a range of 1 to 21 years). Tests performed at the start of the study found that these individuals had pulmonary function similar to that seen in healthy individuals. The workers in this study reported subjective measures of eye,

nose, and throat irritation after 40 minutes of exposure to 2 ppm of formaldehyde. However, the 2 ppm acute exposure in this study may be sufficiently high to induce significant irritation in most individuals. Krakowiak et al. (1998) reported that 10 asthmatics with occupational exposure to formaldehyde (via formaldehyde solutions or pure gaseous formaldehyde) exhibited similar symptom scores to healthy controls (never exposed to formaldehyde in the workplace) exposed to 0.4 ppm formaldehyde for 2 hours. The mean symptom scores and standard deviation (SD), which included information on sneezing, rhinorrhea, mucosal edema, and itching, were 4.6 ± 1.6 (mean ± SD) for asthmatics and 4.3 ± 1.2 for healthy subjects immediately after inhalation. These dropped to 1.8 ± 1.2 and 1.2 ± 1.3, respectively, 4 hours after the exposure. It is unclear if sensitive individuals may not be represented in either of these groups, as the workers were tolerating their exposures during the work shift "healthy worker" effect. However, residents (n = 9) exposed to formaldehyde in their homes, who complained about adverse effects from the material, but with no occupational exposure reported eye, nose, and throat irritation at a similar rate as controls (individuals in homes without formaldehyde or individuals in homes with formaldehyde but not reporting adverse effects [n = 9]) after a 90-minute exposure to 1 ppm (Day et al., 1984). The number of individuals reporting eye irritation, nasal congestion, and throat irritation were seven, three, and two among sensitive individuals and eight, four, and three among controls, respectively. These individuals may be considered a sensitive population since they had "previously complained of various nonrespiratory effects from the UFFI in their homes" (household concentrations unknown).

4.1.1.2. *Pulmonary Function*

Workers chronically exposed to formaldehyde have exhibited signs of reduced lung function consistent with BC, inflammation, or chronic obstructive lung disease. Lung function deficits have been reported both in pre-shift versus post-shift measurements and as a result of chronic exposures (Pourmahabadian et al., 2006; Herbert et al., 1994; Malaka and Kodama, 1990; Alexandersson and Hedenstierna, 1989; Alexandersson et al., 1982). Decreases in spirometric values, including vital capacity (VC), forced expiratory volume (FEV), forced vital capacity (FVC), and FEV/FVC have been reported. Chronic studies also report increased respiratory symptoms, such as cough, increased phlegm, asthma, chest tightness, and chest colds, in exposed workers (Pourmahabadian et al., 2006; Herbert et al., 1994; Malaka and Kodama, 1990; Alexandersson and Hedenstierna, 1989; Alexandersson et al., 1982). Similar findings have been reported for low-level residential formaldehyde exposure, including decreased peak expiratory flow (PEF) rates (Krzyzanowski et al., 1990).

Worker exposures that report cross-shift differences in spirometric values are consistent with formaldehyde-induced sensory irritation. Additionally, concordance has been reported between subjective irritant response and measured changes in pulmonary function, further supporting the possibility that cross-shift and short-term evidence of BC may be a reflexive response to sensory irritation. Absolute values for lung function parameters are likely to vary by gender, age, height, and smoking status and are best compared when normalized to the expected lung function based on these variables (Schoenberg et al., 1978). Individual variation can also be addressed by each subject serving as his/her control with measurements taken before, during, and after exposure. Analysis of the percent change in various parameters in this context may have greater sensitivity to detect exposure-related changes in function.

In addition to individual variation in baseline lung function, there is variation in bronchial responsiveness. Reduced lung function parameters in response to methacholine challenge is a standard test for BC, and this can be used to define responsive, sensitive, or susceptible individuals. Since formaldehyde-induced BC is measured with these lung function tests, variability in bronchial responsiveness may impact interpretation of formaldehyde-induced changes. Experiments with sensitive individuals can help address this question. However, results need to be normalized in some way to account for differences in responsiveness before formaldehyde exposure. Researchers have in some cases excluded hyperresponsive individuals or presented results as a proportion or percent of the unexposed value for each individual. However, excluding sensitive individuals may bias results towards the null.

The American Thoracic Society (ATS) published an official statement on what constitutes an adverse health effect of air pollution (ATS, 2000). According to the ATS statement, exposure that increases the risk of an adverse effect to the entire population can be considered adverse, even though it may not increase the risk of any individual to an unacceptable level. For example, a population of asthmatics could have a distribution of lung function such that no individual has a level associated with significant impairment. Exposure to an air pollutant could shift the distribution to lower levels that still do not bring any individual to a level that is associated with clinically relevant effects. However, this would be considered to be adverse because individuals within the population would have diminished reserve function and therefore would be at increased risk if affected by another agent.

4.1.1.2.1. ***Epidemiologic literature.*** The potential adverse effects of formaldehyde exposure on pulmonary function in humans can be examined on several time scales of interest. The epidemiologic literature supports the assessment of exposures among exposed anatomy medical students where all participants have well-defined and similar duration of exposure (i.e., a

semester-long class) (Kriebel et al., 2001, 1993; Akbar-Khanzadeh and Mlynek, 1997; Akbar-Khanzadeh et al., 1994; Uba et al., 1989; Fleisher, 1987), among individuals living or working in buildings with formaldehyde exposure (Franklin et al., 2000; Krzyzanowski et al., 1990; Main and Hogan, 1983), and among workers (industrial, manufacture, mortuary, hospital staff, etc.) (Ostojic et al., 2006; Herbert et al., 1994; Khamgaonkar and Fulare, 1991; Malaka and Kodama, 1990; Nunn et al., 1990; Alexandersson and Hedenstierna, 1989; Holness and Nethercott, 1989; Holmström and Wilhelmsson, 1988; Horvath et al., 1988; Kilburn et al., 1985; Alexandersson et al., 1982).

The observed effects in the previously unexposed anatomy students provide additional information on acute exposures in two naïve populations (Kriebel et al., 2001, 1993) as well as special insight into the intermediate stages of possible sensitization (Kriebel et al., 1993). Kriebel and colleagues (1993) examined the pre-laboratory and post-laboratory PEF in students attending anatomy classes once per week. They found the strongest pulmonary response when examining the average cross-laboratory decrement in PEF in the first 2 weeks of the study when formaldehyde concentrations collected in the breathing zones had a geometric average concentration of 0.73 ppm. Overall, the students exhibited a 2% decrement in PEF, while the students with any history of asthma showed a 7.3% decrement in PEF. These findings of acute decreases in PEF following students' initial anatomy sessions were corroborated by the Kriebel et al. (2001) study, which used a similar study design applied to another class of anatomy students.

The Kriebel et al. (1993) study also shows how the acute effects of formaldehyde exposure were altered following several weeks of weekly episodic exposure. By the fifth week of class, the pre- and post-laboratory measurements of PEF were no longer reflecting a clearly demonstrated acute effect, but, following the seventh week of episodic exposure, both pre- and post-laboratory PEF continued to drop steadily until the class adjourned after 10 weeks. While the acute effects of formaldehyde exposure appeared to diminish after several weeks of exposure, the intermediate effect across 9 weeks was a 24 L/minute drop in PEF that was statistically significant ($p < 0.01$ after statistical control for random person effects, asthma, interaction between time and asthma, and eye as well as nose symptoms of irritation).

Similar studies among medical students have been performed. In one study, 34 exposed and 12 control students completed pulmonary function tests before and after their work in the laboratory (approximately 3 hours) (Akbar-Khanzadeh et al., 1994). The time-weighted average exposure ranged from 0.07-2.94 ppm. More than 94% of the subjects were exposed to >0.3 ppm and 31.7% were exposed to >0.5 ppm. Comparing pre- and post-exposures among the exposed students, on average FVC decreased by 1.4%, FEV_3 decreased by 1.2%, FEV_1/FVC increased by

1.6%, and $FVC_{25–75\%}$ increased 2.5%. These average percent changes in the control group are –0.3, 1.30, 2.31, and 0.6%, respectively. The researchers also calculated correlation coefficients by examining the relationship between lung function and formaldehyde concentration, but no association was found. Akbar-Khanzadeh and Mlynek (1997) performed another study with 50 exposed students and 36 controls and reported a larger increase in lung function among controls when compared with cases after 1–3 hours of exposure (FVC 3.0 versus 0.9, FEV_1 4.1 versus 1.2, FEV_3 3.3 versus 0.8, forced expiratory flow during the middle of the FVC [$FEF_{25–75\%}$] 6.1 versus 0.7). These differences between cases and controls remained for FEV_1, FEV_3, and $FEF_{25–75\%}$ after 3 hours.

A study of 103 medical students was performed over a period of 7 months in which the students were exposed to formaldehyde at a time weighted average of <1 ppm with peaks of 5 ppm during anatomy laboratory sessions (Uba et al., 1989). Twelve students were asthmatics. Unlike the studies by Kriebel et al. (2001, 1993), these researchers did not find a change in pulmonary function over the course of 7 months. The mean percent change for pulmonary function before and after the exposure did change slightly, with measures showing decreases in function at the end of the laboratory session (measurements taken at the 7-month time point: FVC –0.79%, FEV_1 –0.48%, $FEF_{25–75\%}$ 0.07%, FEV_1/FVC 0.24%).

Finally, Fleisher (1987) gave self-administered questionnaires to medical students after completing an anatomy laboratory session (formaldehyde exposure measures as <1 ppm) and a pathology/microbiology laboratory session (no formaldehyde exposure). Over 8% of students reported experiencing shortness of breath during the laboratory with formaldehyde exposure, but none of the students reported shortness of breath in the laboratory session with no exposure. No objective measurements of formaldehyde exposure were used.

Three studies have been performed that examine formaldehyde exposure from the buildings in which individuals live or work. One study included children 6–13 years of age and measured the levels of formaldehyde in their homes. There was no association between FVC or FEV and the indoor concentrations of formaldehyde, although there were signs of lower airway inflammation as measured by levels of exhaled nitric oxide (NO) in children exposed to average formaldehyde levels ≥0.05 ppm (Franklin et al., 2000). Municipal employees with their children (613 adults and 298 children) were randomly sampled in another study of home exposures (Krzyzanowski et al., 1990). Residential exposures to formaldehyde were based on repeated samples from each individual's kitchen, living area, and bedroom. The average formaldehyde concentration was 26 ppb, with a maximum sample value of 140 ppb. The majority of subjects (83%) lived in homes with 2-week average concentrations below 40 ppb. Subjects' peak expiratory flow rates (PEFRs) were determined four times daily, in the morning, at noon, in the

early evening, and before bed, for 2 weeks. A statistically significant linear relationship between increased formaldehyde exposure and decreased PEFR was reported in children but not adults. All statistical models controlled for socioeconomic status, tobacco smoking (current active or environmental tobacco smoking), and nitrogen dioxide concentrations. Among adults, there was a statistically significant nonlinear relationship with decreased morning PEFR for formaldehyde concentration <40 ppb.

Main and Hogan (1983) reported on a group of individuals (n = 21) working in two mobile trailers for 34 months and exposed to levels of formaldehyde ranging from 0.12 to 1.6 ppm (mean age 38 ± 9 years, 76% male, 19% nonsmokers). The control population was comprised of individuals who did not work in the trailers (n = 18; mean age 30 ± 6, 50% male, 22% nonsmokers). There were no differences between the exposure and control groups' percent predicted FEV_1 or FVC regardless of smoking status.

Several studies allowed for the examination of potential chronic effects of formaldehyde exposure. These included an occupational study by Malaka and Kodama (1990) that reported pre-shift pulmonary function as a percentage of expected among the formaldehyde exposed compared with comparable people not exposed to formaldehyde. This study found that an average 8-hour TWA formaldehyde exposure of 1.13 ppm from area samples was associated with statistically significant decrements in FEV_1, FEV_1/FVC, and $FEF_{25–75\%}$ compared with a referent population. The strongest response was for $FEF_{25–75\%,}$ which showed a 12% drop in observed function compared with expected function in the unexposed, but it is unclear how to interpret the potential chronic adverse effect(s) with just the magnitude of the decrement and the length of the average occupational tenure at this plywood facility (6.5 years), which was not reported by exposure status.

A study comparing oriented strand board workers (exposed to formaldehyde) with oil/gas field plant workers (not exposed to formaldehyde) demonstrated a difference in pulmonary function between the two groups (Herbert et al., 1994). The groups were similar in regard to measured FVC and FEV_1 (controlled for age, height, and smoking), but the workers exposed to formaldehyde had lower FEV_1/FVC. In addition, those exposed to formaldehyde showed a decrease in FVC and FEV_1 after their shift, with an average pre- and post-shift difference of 47 mL ($p = 0.022$) and 39 mL ($p = 0.044$) for FVC and FEV_1, respectively (however change could not be compared with the controls of this study because no post-shift measurements were taken). Two other occupational studies found no association between formaldehyde and lung function (Holness and Nethercott, 1989; Horvath et al., 1988). One of these studies was conducted among funeral workers and an unexposed control group (Holness and Nethercott, 1989). There was no difference in pulmonary function of the two groups at baseline. After

exposure, there was no change in lung function for the exposed or unexposed when comparing lung function tests done immediately before and after an embalming procedure (for controls the repeat measures were taken approximately 2–3 hours after the first measure) (changes in percentage predicted FVC and FEV_1 were 0.88 ± 2.95 and –0.03 ± 2.40 for exposed and 1.13 ± 3.98 and 1.45 ± 4.43 for unexposed). Further analysis showed no association between formaldehyde levels and changes in lung function. Another study (Horvath et al., 1988) found no differences in pre-shift pulmonary function between the exposed (workers at a particleboard and molded products operation, formaldehyde measured using individual monitors ranged from 0.17–2.93 ppm) and controls (workers from nearby food processing facilities, formaldehyde measured using individual monitors ranged from 0.03–0.12 ppm). However, the authors did find a post-shift decline in FVC and FEV_1 among controls and FEV_1 and $FEF_{25–75\%}$ among workers when using paired comparisons for each group. When assigning all controls a formaldehyde exposure value of 0.05 ppm, there was a correlation detected in pre-and post-shift pulmonary function changes and formaldehyde, though no specific details on regression analysis were provided.

A study performed in India (Khamgaonkar and Fulare, 1991) examined individuals working in anatomy and histopathology departments and exposed to formaldehyde (mean 1.00 ppm, range 0.036–2.27). Controls (individuals not working in laboratories with formaldehyde) were exposed to an average of 0.102 ppm formaldehyde (range 0–0.52). Lung function tests were performed on a Monday morning after days of no exposure in order to examine chronic effects. The FVC and FEV_1% of the exposed group, respectively, were 17.12 and 22.94% reduced compared with the control group (Khamgaonkar and Fulare, 1991); however, while the pool of cases and controls were frequency-matched on age and gender, there was no mention by the investigators of normalizing the pulmonary function metrics by gender and height, which would have made for more appropriate comparisons. Kilburn et al. (1985) also demonstrated reduced pulmonary function (lower percent predicted FVC, FEV_1, and $FEF_{25-75\%}$) among workers occupationally exposed to formaldehyde when compared with individuals working at jobs without formaldehyde exposure.

Two occupational studies found no association between formaldehyde exposure and deficits in pulmonary function (Ostojic et al., 2006; Holmström and Wilhelmsson, 1988). Ostojic et al. (2006) examined nonsmoking male health service professionals working in pathoanatomic laboratories with 8 hours of formaldehyde exposure per day at an unspecified concentration for at least 4 years (n = 16). The control group was comprised of sixteen age- and stature-matched nonsmoking male controls. There was no difference in mean FVC or FEV_1 between exposed and controls. The researchers also examined values for diffusing lung capacity

for carbon monoxide and membrane diffusion capacity, which were similar between the exposed and control groups. However, blood volume of pulmonary capillaries was found to be higher in the exposed group. Holmström and Wilhelmsson (1988) recruited individuals from a chemical plant where formaldehyde and formaldehyde products were made (n = 70). Exposure levels varied from 0.05–0.5 mg/m^3. A control group was mostly comprised of clerks for the local government (n = 36). No difference in FEV% was detected between the groups. Mean FVC was lower than expected among the exposed group (expected values were based on age, sex, smoking habits, height, and weight). This study went further and measured changes in pulmonary function for those employed more than 5 years and reported no signs of increasing restrictivity after 5 years. No associations were seen and there was no correlation between pulmonary function and cumulative dose of formaldehyde (Holmström and Wilhelmsson, 1988).

There have been only two studies that have reported on the longitudinal follow-up of workers exposed to formaldehyde (Nunn et al., 1990; Alexandersson and Hedenstierna, 1989). The Alexandersson and Hedenstierna (1989) investigation not only examined the acute effects of exposure across shift but was able to do so among some of the same workers that had been studied 5 years earlier (Alexandersson et al., 1982). Statistically significant decreases in FEV_1/FVC and $FEF_{25–75\%}$ were noted over the intervening 5 years in nonsmokers after correction for normal aging and reference lung function spirometry values. The decrease in $FEF_{25–75\%}$ was 0.212 ± 0.066 L/second (mean ± SD) for each year of exposure and was highly significant ($p <$ 0.01). For comparison with the 12% drop in the same pulmonary metric reported by Malaka and Kodama (1990) over an estimated 6.5 years, the extrapolated percentage decrease in $FEF_{25–75\%}$ was computed for the Alexandersson and Hedenstierna (1989) study by using the reported yearly decrement applied to the pre-shift values at the time of the initial study period. From the predicted value of 4.57 L/second, a decrease of 0.168 L/second for each year of exposure regardless of smoking status was calculated. For 6.5 years of exposure, this would result in a 24% drop in $FEF_{25–75\%}$. Formaldehyde concentrations were estimated at 0.42 ppm in the first Alexandersson et al. (1982) study and at 0.50 ppm in the Alexandersson and Hedenstierna (1989) study. The study by Nunn et al. (1990) assessed the decrease in FEV_1 with age. The researchers calculated the decrease in FEV_1 to be 42 mL/year among workers exposed to formaldehyde and 41 mL/year for workers who were not exposed to formaldehyde. Thus, they showed no association between formaldehyde exposure and decreased FEV_1.

There are a few important limitations to consider in these occupational studies of formaldehyde exposure. First, an often-shared weakness is the absence of data on, and appropriate statistical control of, potential confounding by occupational co-exposures. Also, studies that did not report pre-shift pulmonary function as a percentage of expected function

contribute less to an assessment of potential chronic effects because, post-hoc, it is difficult to calibrate the multiple pulmonary function data for cross-study comparison without knowledge of the age, gender, smoking status, height, year of birth, etc., that are important determinants of the pulmonary function metrics of concern.

4.1.1.2.2. ***Acute studies: controlled chamber exposures.*** Pulmonary effects of acute formaldehyde exposure have been studied in both healthy volunteers and sensitive populations under controlled conditions. Controlled chamber studies have the advantage of measured controlled exposures, but other factors can limit the usefulness of the studies, especially when study populations are small and there is high variability in the measured parameters.

In general, acute formaldehyde exposures (0.5–3 ppm) have not induced significant pulmonary deficits in healthy, non-exercising volunteers (Kulle et al., 1987; Schachter et al., 1986; Witek et al., 1986; Day et al., 1984; Andersen and Molhave, 1983). However, it is unclear whether the data analysis in these reports had the statistical power to substantiate the small deficits reported in occupational and student studies. All four reports had relatively small study groups of healthy individuals (n = 19 [Kulle et al., 1987], n = 16 [Andersen and Molhave, 1983], n = 15 [Schachter et al., 1986], n = 15 [Witek et al., 1986], and n = 9 [Day et al., 1984]), and in some cases the group was further divided by gender. Two studies report the absolute values of the lung function parameters without adjustment to individual expected function or the unexposed baseline for each individual (Kulle et al., 1987; Andersen and Molhave, 1983). As discussed, this decreases the power of the study to detect formaldehyde-induced changes in pulmonary function. In contrast, Witek et al. (1986) and Schachter et al. (1986) report lung function as a percent of baseline (although not normalized for age gender and height). Each study showed an increase in FEV_1 in formaldehyde-exposed individuals at rest and increases in maximal expiratory flow (MEF) at 50% of expired vital capacity (MEF50%) (Witek et al., 1986; Schachter et al., 1986). However, in both reports the SDs of changes in lung function parameters are quite large, nearly equaling the reported value and exceeding it in several cases. The absence of normalized raw data, combined with large individual variation, limit the interpretation of these studies. A small study (Day et al., 1984) that included nine healthy individuals showed no changes in FEV or FVC after 90 minutes of exposure to 1 ppm of formaldehyde. A more recent study (Lang et al., 2008) of 21 healthy volunteers exposed to a range of formaldehyde concentrations (0.0 to 0.15 or 0.3 ppm) reports no formaldehyde-related pulmonary deficits. However, data were not shown, and it is unclear whether the authors compared absolute or relative values of lung function and what variation in lung function was present in the study population. Additionally, the authors did not provide the criteria used to gauge deficit of lung

function. If a clinically significant deficit was defined (e.g., 20%), then more subtle changes in pulmonary function, as supported in other studies, would not have been reported.

Similar to these studies of healthy individuals, acute controlled studies including asthmatics also report no changes in pulmonary function associated with formaldehyde exposure (Ezratty et al., 2007; Harving et al., 1990; Green et al., 1987; Sauder et al., 1987; Witek et al., 1987, 1986), including studies of individuals thought to have formaldehyde-induced bronchial asthma (Krakowiak et al., 1998); however, the number of asthmatic individuals included in each of these studies was small. (The details of these studies have been reported elsewhere in this chapter. Briefly, the number of asthmatics in the study/total number of individuals in the study are as follows: Ezratty et al. [2007]—12/12; Harving et al. [1990]—15/15; Green et al. [1987]—16/38; Sauder et al. [1987]—9/9; Witek et al. [1987]—15/15; Witek et al. [1986]—15/30; Krakowiak et al. [1998]—10/20]. The same is true for individuals who are frequently exposed to formaldehyde either at work (n = 15) (Schachter et al., 1987) or at home (n = 18) (Day et al., 1984).

Small but statistically significant deficits in pulmonary function due to acute formaldehyde exposure (2 or 3 ppm) have been reported in healthy volunteers during exercise (Green et al., 1989, 1987; Sauder et al., 1986; Schachter et al., 1986). Although changes in lung function parameters averaged over experimental groups were generally small, some individuals exhibited clinically significant deficits, even after only 2 hours of exposure. Deficits in FEV_1 and $FEF_{25–75\%}$ in the first 30 minutes of a 2-hour exposure at 3 ppm formaldehyde were 2 and 7%, respectively. Changes in lung function were not statistically significant after 60 and 180 minutes of exposure (Sauder et al., 1986), even when assessed as absolute rather than relative measurements. Thirteen percent (5 of 38 subjects) demonstrated formaldehyde-induced clinically significant deficits when exposed at 3 ppm during exercise (defined by Green et al. (1987) as decrease in FEV_1 > 10% of control).

4.1.1.3. *Asthma*

A large number of studies have investigated the potential association between formaldehyde exposure and a continuum of adverse health effects ranging from decrements in pulmonary function to asthma. In general, epidemiologic studies of adults have reported varied results between null findings and positive findings. However, The National Research Council concluded in its report on Formaldehyde that, “Formaldehyde has been shown to cause bronchial asthma in humans” (NRC, 1981), citing numerous studies demonstrating the induction of asthma following exposure to formaldehyde (Hendrick and Lane, 1975, 1977; Laffont and Noceto, 1961; Nova and Touraine, 1957; Paliard et al., 1949; Popa et al., 1969; Sakula, 1975; Schoenberg and

Mitchell, 1975; Turiar, 1952; Vaughan, 1939). In a subsequent review article on formaldehyde and the health effects that have been associated with it, Stenton and Hendrick (1994) reported on formaldehyde and asthma in occupational settings and starkly describe the "…first detailed case report of formaldehyde asthma confirmed by specific inhalation challenge test occurring in a nursing sister on a renal dialysis unit. Her symptoms were suggestive of late asthmatic reactions occurring 4 to 5 hours after heavy exposures. The occurrence of late reactions was confirmed in a series of challenge tests that involved the painting of formalin onto cardboard pieces within a confined space" (Stenton and Hendrick, 1994; Hendrick 1997). The results of the challenge tests are illustrated in Figure 4-1 .

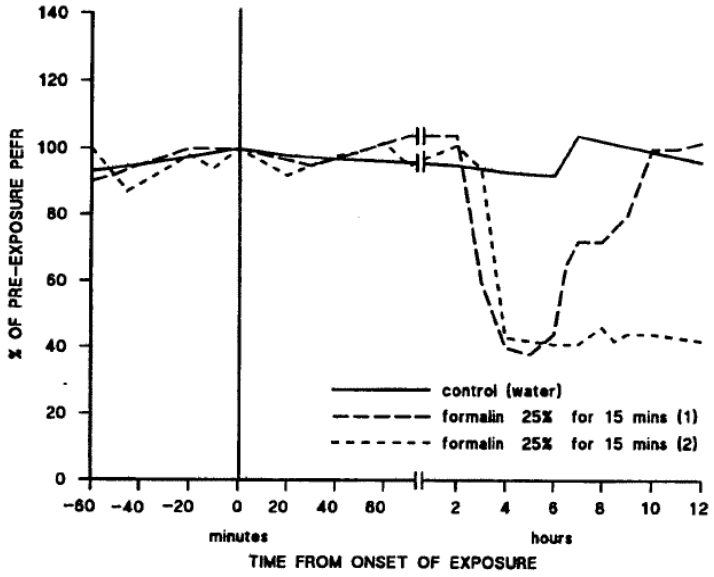

Figure 4-1. Delayed asthmatic reaction following the inhalation of formaldehyde after "painting" 100% formalin for 20 minutes. Challenge 2 was premedicated with inhaled betamethasone 200 µg.
Source: (Stenton and Hendrick, 1994)

Five years later, the two nurses were re-challenged with the nurse who had left the dialysis unit having no response to the subsequent challenge while the nurse who had remained working in the unit developed mild late asthmatic response with peripheral blood eosinophilia (Stenton and Hendrick, 1994; Hendrick et al., 1982). Stenton and Hendrick (1994) concluded that these studies "provide clear evidence of formaldehyde's ability to induce asthma" but no indication of the exposure concentrations to induce it. In a follow-up study of dialysis unit

staffers exposed to formaldehyde as a sterilizing agent, 8/28 people reported respiratory symptoms and a prolonged increase in circadian rhythm of peak expiratory flow rate was seen in one subject (Hendrick and Lane, 1983) implying an increase in airway responsiveness (Stenton and Hendrick, 1994). It should be noted, however, that while there did appear to be a clear response to formalin, it is not known what contribution to the response was attributable to formaldehyde and what contribution might have been attributable to methanol. Further, while the evidence of a causal association between formaldehyde and asthma is clear, the above studies do not offer information on the concentrations at which adverse effects would expected in a population.

There is at least one clinical study in humans that investigated whether exposure to a low level of formaldehyde (500 $\mu g/m^3$) would enhance inhaled allergen responses (Ezratty et al., 2007). Twelve subjects with intermittent asthma were exposed to either formaldehyde or purified air in a double-blind crossover study for 1 hour. Following exposure (8 hours), airway responsiveness to methacholine challenge was measured. No significant effects on methacholine-induced bronchial hyperresponsiveness (BHR) were detected due to formaldehyde exposure.

Numerous epidemiologic studies have investigated adverse effects in populations. Decreased peak expiratory flow rates (PEFR) are an important component in the diagnosis of asthma and there is evidence of formaldehyde-induced decrements in PEFR (see Section 4.1.1.2). However, the diagnosis of asthma is both a more serious health condition and diagnostically more complex than decreased PEFR alone and is evaluated here as a distinct endpoint. A number of epidemiologic studies have investigated the potential association between formaldehyde exposure and a continuum of adverse health effects from pulmonary function to asthma.

The association between formaldehyde and asthma has been studied by examining occupational exposures (Fransman et al., 2003; Malaka and Kodama, 1990), school-related exposures (Zhao et al., 2008; Smedje and Norback, 2001; Norback et al., 2000) and residential exposures (Matsunaga et al., 2008; Tavernier et al., 2006; Gee et al., 2005; Delfino et al., 2003; Rumchev et al., 2002; Garrett et al., 1999; Palczynski et al., 1999; Norback et al., 1995; Krzyzanowski et al., 1990). The two occupational studies examined the respiratory health of plywood workers (Fransman et al., 2003; Malaka and Kodama, 1990). The most recent of these was conducted in New Zealand by Fransman et al. (2003). Personal samples of formaldehyde exposure were taken. The mean level of exposure was 0.08 mg/m^3 (65 ppb) and the majority of samples were below the limit of detection which was reported to be 0.03 mg/m^3 (24 ppb). Compared with those with low levels of formaldehyde exposure, workers with high levels of

exposure were more likely to report having asthma (OR=4.3 [95% CI]: 0.7–27.7]). The association was not seen when examining formaldehyde exposure and use of asthma medication. The second study of plywood workers was completed in Indonesia. Background levels of formaldehyde ranged from 0.003 to 0.07 ppm. The highest concentration of formaldehyde detected in an air sample was in the particleboard unit (range 1.16 to 3.48 ppm). Asthma, which was defined as "have you ever had an attack of wheezing that made you feel short of breath?", was found to be positively associated with formaldehyde exposure (Malaka and Kodama, 1990).

Studies of exposure to formaldehyde at schools have been performed in China (Zhao et al., 2008) and in Sweden (Smedje and Norback, 2001). In the study from China (Zhao et al., 2008), mean levels of formaldehyde were reported to be 2.3 μg/m^3 (range 1.0–5.0 μg/m^3) indoors and 5.8 μg/m^3 (range 5.0–7.0 μg/m^3) outdoors. Cumulative asthma and daytime attacks of breathlessness were found to be associated with outdoor formaldehyde levels. Neither of these outcomes was associated with indoor concentrations of formaldehyde; however, indoor levels were found to be associated with nocturnal attacks of breathlessness. In Sweden (Smedje and Norback, 2001), the levels of formaldehyde measured indoors were higher (mean 4, range <5.0–72 μg/m^3). One difference between this Swedish study and the study performed in China is that the Swedish study examined the incidence of asthma over a 4-year period. This study did not report an association between formaldehyde exposure and the incidence of asthma (OR 1.2 [95% CI: 0.8–1.7]) among the whole study population. However, when the investigators stratified on history of atopy, they reported that among children without a history of atopy, a new diagnosis of asthma was significantly more likely at higher concentrations of formaldehyde (OR 1.7 per 10 μg /m^3 [95% CI: 1.1–2.6]) and at higher total concentrations of mold (OR=4.7 per 10-fold increased in total molds [95% CI: 1.2-18.4] in the classroom air. The finding for adverse effects of formaldehyde and mold did not appear to control for the other exposure and no information on the potential correlation between the two exposures was provided. In order to evaluate the potential for confounding of the reported formaldehyde association by the reported mold association, the magnitude of effects must be compared on an appropriate scale since the magnitude of an odds ratio depends on the magnitude of the change in exposure level that is expected to produce increased risk. Standardizing the units to the reported geometric mean standard deviation, the result for formaldehyde (GSM=2.3 μg /m^3) is OR[1]=1.13 per GSD and the result for mold is OR[2]=1.02 for a comparison of risks at the GSM to 10*GSM and OR[3]=1.06 for

[1] OR per GSD=exp[ln(OR per μg /m^3)/ 10 μg /m^3 * 2.3 μg /m^3]=exp[ln(1.7)/10*2.3]=1.13
[2] OR per GSD=exp[ln(OR per 10-fold increase)/ (9*GSM)*1.6 μg /m^3]=exp[ln(4.7)/162*1.6]=1.02

[3] OR per GSD=exp[ln(OR per 10-fold increase)/ (9*Minimum)*1.6 μg /m^3]=exp[ln(4.7)/45*1.6]=1.06

a comparison of risks at the minimum value of total molds ($5*10^3/m^3$) to 10*minimum. As it appears that the magnitude of the formaldehyde effect is substantially stronger than that of the mold effect (following standardization of exposure increment) it can be concluded that the reported formaldehyde effect could not have been the spurious result of uncontrolled confounding by mold.

The results of studies measuring residential exposure to formaldehyde and asthma are varied, with some demonstrating an association and others finding no relationship. A recent study (Matsunaga et al., 2008) found no association between 24-hour formaldehyde and prevalence of asthma when pregnant women with an exposure ≥47 ppb were compared to those with exposure to <18 ppb. However, they did report at increased risk of atopic eczema. It should be noted that this study did not assess the risk of incident asthma. A study utilizing self-reported asthma prevalence as an outcome also found no association with levels of formaldehyde (mean 25.9 $\mu g/m^3$, range 2.0–66.8 $\mu g/m^3$) (Palczynski et al., 1999) although they did note that the incidence of allergic diseases was highest in the highest formaldehyde exposure group but that the group was too small for statistical evaluation.

A study performed by Tuthill (1984) measured formaldehyde exposure for children grades K through 6 by using a combination of proxy variables. Overall, there was no association, but some individual variables did show an increased risk. For example, the reported risk ratio for having new construction or remodeling performed in the house in the past 4 months was 2.5 (95% CI: 1.7–3.9). The risk ratio for having new or upholstered furniture in the house (brought into the house within the past 4 months) was 2.2 (95% CI: 1.2–3.9).

The study by Delfino et al. (2003) assessed whether ambient formaldehyde concentration measured at a central monitoring site were associated with asthma symptoms. The study examined 22 10–15 year olds with at least 1 year of physician-diagnosed asthma and living in a nonsmoking household. The mean levels of formaldehyde were measured to be 7.21 ppb (range 4.27–14.02 ppb). There was a positive association between asthma symptom scores (comparing children who report symptoms interfering with their daily activities versus those with no symptoms or symptoms not great enough to affect their daily activities) and high current levels of formaldehyde (OR 1.90 [95% CI: 1.13–3.19]).

Three studies (Tavernier et al., 2006; Gee et al., 2005; Garrett et al., 1999) were performed by matching children with and without asthma and comparing the levels of formaldehyde in their homes. Gee et al. (2005) reported median formaldehyde levels of 0.03 ppm in living rooms and 0.04 ppm in bedrooms. Analyses were limited to univariate comparisons of formaldehyde levels for cases of existing asthma and controls without asthma. The concentrations did not differ in a statistically significant manner. The study by Gee et al.

(2005) was followed up with a more sophisticated analysis of the same children in the same homes. Tavernier et al. (2006) reiterated the earlier finding by Gee et al (2005) that formaldehyde was not found to be associated with existing asthma. Tavernier et al. (2006) did not report the measured levels of formaldehyde but gave the OR for the highest tertile of exposure compared with the lowest tertile of exposure as 0.99 (95% CI: 0.39–2.50). The width of this confidence interval suggests that these findings would still be consistent with two-fold increase in risk.

Garrett et al. (1999) reported on the risk of allergy and asthma-like respiratory symptoms due to formaldehyde exposure in a cross-sectional survey of households with children with (n = 53) or without (n = 88) doctor-diagnosed asthma. Formaldehyde exposure was characterized by 4 seasonal in-home sampling events across the year for bedrooms and 4–day passive samples collected in living rooms, kitchens and outdoors. Statistically significant linear trends for increased risk of having asthma were seen with increasing formaldehyde levels ($p < 0.02$); however, the ORs for the association did not remain statistically significant after controlling for parental allergy and asthma (exact ORs and 95% CIs not given). Garrett et al (1999) also evaluated the prevalence and severity of allergic sensitization to 12 common allergens and reported increased prevalence with increasing formaldehyde concentration in the home. The respiratory symptom score was also increased and demonstrated a significant effect for formaldehyde in a multiple regression after adjusting for multiple risk factors and interactions. For the atopy and respiratory symptom endpoints, severity/incidence was increased in the medium (20–50 $\mu g/m^3$) and high (>50 $\mu g/m^3$) exposure groups relative to the low (<20 $\mu g/m^3$) exposure group, based on the highest of four seasonal 4-day formaldehyde measurements in the home. The associations between formaldehyde concentrations and severity of allergic sensitization are clearly shown and further substantiated with multivariate regression controlling for potential confounders. In logistic regressions, both the prevalence and severity of allergic sensitization to 12 common allergens increased with increasing formaldehyde concentration in the home. The crude association for atopy with an increase in formaldehyde concentration per 10 $\mu g/m^3$ was OR=1.34 which increased when adjusted for parental asthma and gender to and odds ratio of 1.42 per 10 $\mu g/m^3$ (95% CI: 0.99-2.04). Passive smoking, the presence of pets, indoor nitrogen dioxide concentrations, airborne fungal spores and house-dust-mite allergens did not influence the effect estimates and were unlikely to be confounders. Additionally, a calculated respiratory symptom score was increased and demonstrated a significant relationship to increased formaldehyde concentration in a multiple linear regression after adjusting for multiple risk factors and interactions. For each of these endpoints, severity/incidence was increased in the medium (20–50 $\mu g/m^3$) and high (>50 $\mu g/m^3$) exposure groups relative to the

low (<20 μg/m^3) exposure group, based on the highest of four seasonal 4-day formaldehyde measurements in the home.

Residential formaldehyde exposure was associated with an increased risk of asthma in a population-based case-control study of 192 children aged 6 months to 3 years (Rumchev et al., 2002). The study, which comprises 88 cases of children discharged from the emergency department of a children's hospital in Perth, Australia, with a primary diagnosis of asthma and 104 controls, provides a positive exposure-response relationship. Seasonal in-home formaldehyde measurements taken in the living room and subject's bedroom were used to assess exposure (8-hour passive sampler). The odds ratios (ORs) for risk of asthma by formaldehyde exposure level category were adjusted for numerous risk factors both familial and environmental including, familial history of asthma, age, sex, smoking, presence of pets, and attributes of the home. Of these, age, allergic sensitization to common allergens, and family history of allergy were independent risk factors for asthma (ORs of 1.09, 2.57, and 2.66, respectively). Categorical analysis of the data indicates the ORs for asthma were increased in the two highest formaldehyde exposure groups, reaching statistical significance for household exposures > 60 μg/m^3 (48 ppb) (OR of 1.39). Analysis of the data with formaldehyde as a continuous variable provides a statistically significant increase in the risk of asthma (3 % increase in risk per every 10 ug/m3 increase in formaldehyde level. All analyses controlled for other indoor air pollutants, allergen levels, relative humidity, and indoor temperature as well as other risk factors.

A study of 202 households (mean formaldehyde level of 26 ppb) found that among children aged 6–15 years old and exposed to environmental tobacco smoke, the prevalence of asthma was 45.5% for those with measured levels of formaldehyde in the kitchen >60 ppb. The prevalence of asthma dropped to 15.1% for levels ≤40 ppb and 0% for 41–60 ppb. No trend in asthma prevalence was seen for children who were not exposed to environmental tobacco smoke (Krzyzanowski et al., 1990).

Finally, a study by Norback et al. (1995) reported mean levels of formaldehyde were 29 μg/m^3 (range <5–110 μg/m^3) in the bedrooms of individuals experiencing nocturnal breathlessness compared with formaldehyde levels of 17 μg/m^3 (<5–60 μg/m^3) among those without nocturnal breathlessness. The OR for this association was 12.5 (95% CI: 2.0–77.9) and the effect was substantially stronger in magnitude than the associations observed for toluene, terpenes and volatile organic compounds which makes confounding by those co-exposures unlikely.

Formaldehyde has clearly been shown to be a cause of bronchial asthma and several epidemiologic studies have identified causal evidence of an adverse effect of exposure on pulmonary function and the incidence of asthma. While there are studies that did not find

associations, many of those were limited by their study design, exposure measurement and the definition of prevalent asthma as the health endpoint.

4.1.1.4. *Respiratory Tract Pathology*

Formaldehyde-induced respiratory tract pathology includes inflammation, rhinitis, goblet cell hyperplasia, metaplastic changes, squamous cell hyperplasia, and impaired mucociliary transport. Formaldehyde may bind to the trigeminal nerve and trigger the release of neurogenic mediators of inflammation that result in tissue edema, lacrimation, mucus production and leukocyte infiltration. How much inflammation, hyperplasia, and metaplastic change are due to sensory irritation-induced inflammatory responses compared with formaldehyde-induced direct cell damage cannot be distinguished. Increased mucus flow and metaplastic changes may progress in relation to the concentration and duration of exposure to protect the underlying tissue. When the exposure exceeds protective and defensive mechanisms, permanent damage results (Swenberg et al., 1983). Nonetheless, these changes serve as a sensitive indicator of formaldehyde exposure, since they occur before gross cellular damage and focal lesions (Monticello et al., 1989), and potentially suggest a point at which the concentration and duration of exposure exceed the protective nature of local responses (increased mucus flow, goblet cell hyperplasia, squamous metaplasia, etc.) (Swenberg et al., 1983). A number of human studies have reported nasal lesions associated with exposure to formaldehyde (Pazdrak et al., 1993; Ballarin et al., 1992; Boysen et al., 1990; Holmström et al., 1989c; Edling et al., 1988), while other studies have documented changes in mucociliary clearance and activity (Holmström and Wilhelmsson, 1988; Andersen and Molhave, 1983). These studies are summarized below.

4.1.1.4.1. ***Nasal lesions.*** Ballarin et al. (1992) did a case-control study of 15 workers from a plywood factory where urea-formaldehyde glue is used. Mean levels of formaldehyde exposure (8-hour average) were estimated to be 0.09, 0.1, and 0.39 mg/m^3 in three regions of the facility (sawmill, shearing press, and warehouse, respectively). Nasal respiratory samples were obtained. Stained cells were scored for histopathology. Cytology examination revealed increased squamous metaplasia cells in 10 of 15 (67%) factory workers (with an average severity score of 2.3) compared with one of 15 (6%) controls (with an average histology severity score of 1.6). In addition, one formaldehyde exposed worker (n = 15) exhibited mild dysplasia and had the highest severity score (3.0). Authors suggest that these results may be due to chronic irritation of the nasal respiratory mucosa. This small study reported only incidence of lesions and did not score based on severity of lesions. The lesion incidence was not reported in relation

to dose, so no dose-response relationship could be determined, precluding the establishment of a point of departure (POD).

Holmström et al. (1989) collected nasal biopsy samples from workers exposed to air or to formaldehyde at a median concentration of 240 ppm. Nasal biopsy samples were scored on a 0–8 range with normal respiratory epithelium as 0 and carcinoma as 8. Observed histologic changes included loss of cilia, goblet cell hyperplasia, and cuboidal and goblet cell metaplasia replacing normal columnar epithelium. The incidence associated with each histologic change was not reported and cannot be compared between formaldehyde-exposed and control individuals. Moreover, these biologically relevant changes were not analyzed independently in the analysis. The mean scores were 1.56 (range, 0–4) for the control group and 2.16 (range, 0–4) for the formaldehyde-exposed group. Although the range of scores in the controls and formaldehyde-exposed groups were the same (0–4), the difference in mean scores (1.56 versus 2.16) was statistically significant ($p < 0.05$); scores were worse in the formaldehyde-exposed group. The authors reported no correlation between the duration of exposure and histologic changes and no correlation between smoking habits and biopsy scores. The loss of cilia, goblet cell hyperplasia, and cuboidal and squamous cell metaplasia replacing the columnar epithelium were increased in the group exposed to formaldehyde and is a biologically relevant change. This study provides a lowest-observed-adverse-effect level (LOAEL) of 0.240 ppm for nasal histopathology.

Edling et al. (1988) collected nasal biopsy samples from workers (n = 75) exposed to formaldehyde at three plants (workers in two of these plants were also exposed to wood dust) compared with a referent group (n = 25). Concentrations ranged from 0.1 to 1.1 mg/m^3 (TWA) with peaks of 5 mg/m^3. Nasal histology was scored from 0 to 8 by increasing severity, from normal respiratory epithelium (0) to carcinoma (8). A normal respiratory epithelium was noted in 3 of 75 workers. A loss of cilia and goblet cell hyperplasia (scores of 2) was reported in eight workers. Mixed cuboid/squamous epithelium (metaplasia), stratified squamous epithelium, and keratosis were reported in 58 of 75 workers (those with scores of 3, 4, and 5 were combined). Dysplasia (score of 6) was reported in 6 of 75 formaldehyde-exposed workers. None of the workers had lesions that warranted a histologic score higher than 6. Histologic scores did not correlate with duration of exposure but could not be confirmed due to poor reporting. Data from the referent group were not included. A POD could not be determined from this study.

Boysen et al. (1990) collected nasal biopsy samples from workers exposed to air (n = 37) or to formaldehyde (n = 37) and sometimes wood dust. The exposed workers were classified into two exposure groups, 0.5–2 ppm and >2 ppm. Nasal biopsy samples were assessed by using a histopathology score range of 0–5, based on the pathology of pseudostratified columnar

epithelium (0) to dysplasia (5). Mean pathology scores for the control were decreased compared with the formaldehyde-exposed group (1.4 and 1.9, respectively) but did not reach statistical significance. Little quantitative pathology data were provided, although qualitative histology revealed a range of observed effects from deciliated epithelial cells to mixed stratified cuboidal, squamous epithelium to dysplasia. None of the control samples received histologic severity scores of 4 or 5, indicating that keratinizing stratified squamous epithelium and dysplasia were not observed in controls. A wider variety of histopathologic lesions were reported in exposed workers compared with controls, and a greater number of exposed workers had histologic changes compared with controls. Incidence data for each type of histopathology were not reported, but the authors wrote that the degree of metaplastic alterations was more pronounced among the exposed workers. An upper range for the high concentration group (>2 ppm) was not reported, and median concentrations were not provided.

Pazdrak et al. (1993) exposed human subjects (six men, three women) to 0.4 ppm formaldehyde in a chamber for 2 hours. Approximately half of the subjects suffered from skin hypersensitivity to formaldehyde, while the other subjects were healthy. An evaluation of nasal lavage pretest and following formaldehyde exposure revealed that the hypersensitive and healthy groups had similarly elevated eosinophil counts at 0 hours after exposure (from 42×10^3 cells/mL to 72×10^3 cells/mL for healthy subjects [$p < 0.05$] and from 39×10^3 cells/mL to 69×10^3 cells/mL for hypersensitive subjects [$p < 0.05$]). Similar eosinophil levels were also seen in both groups at 3 and 18 hours. Both groups had equivalent increases in lavage albumin and total protein levels following exposure, but basophil counts were unchanged. Based on evidence of formaldehyde-induced inflammation, these data provide a LOAEL of 0.4 ppm for nasal histopathology.

4.1.1.4.2. ***Mucociliary clearance.*** In addition to abnormal nasal histopathology, changes in mucociliary clearance were also observed in some of these studies at similar exposure concentrations. The mucociliary apparatus is an important barrier to infection and exogenous agents and, thus, is considered as a potential adverse effect. These effects may be due to direct interaction of formaldehyde with the mucus itself or to SI-induced inflammation in the nasal tissue that affects mucus production and creation of an effective mucosal barrier.

Andersen and Molhave (1983) reviewed five controlled human studies, one of which (Andersen and Lundqvist, 1974) examined mucus flow rate in 16 individuals acutely exposed to 0, 0.3, 0.5, 1, or 2 ppm formaldehyde for 4–5 hours in a chamber. Mucus flow rate was decreased in the anterior and middle third of the ciliated mucosa at 0.3 ppm, but statistical significance was not determined. This study included smokers and nonsmokers. The small

sample size, potential confounder effect from smoking, and lack of dose-response relationship preclude the establishment of a POD.

Holmström and Wilhelmsson (1988) demonstrated reduced mucociliary clearance and nasal mucosal swelling in 70 workers exposed to a median formaldehyde concentration of 0.21 ppm, compared with a referent group of store clerks (n = 36) and was further averaged over years of exposure. Mucosal swelling and mucociliary activity was measured in the nasal turbinates. The authors also reported symptoms not only during the weekdays, but also over weekends and vacation periods. Formaldehyde-exposed subjects self-reported significantly more nasal discomfort, eye discomfort, deeper airway discomfort, and frequent headache than the referent group. Groups exposed to formaldehyde had more pronounced mucosal swelling (10.7 nasal resistance score) compared with the reference group (6.5 nasal resistance score). This difference persisted when data were normalized for differential nasal congestion in the subjects. Decreased mucociliary activity was seen in 3% of controls and 20% of formaldehyde-exposed subjects and reached statistical significance ($p < 0.05$). It is not clear whether impaired mucociliary clearance was a consequence of altered cell morphology or increased mucus viscosity. These data provide a LOAEL of 0.21 ppm based on impaired mucociliary clearance.

Thus, mild nasal epithelial lesions observed in formaldehyde-exposed workers have been observed consistently at levels of about 0.20 ppm to about 2 ppm (Boysen et al., 1990; Holmström et al., 1989; Edling et al., 1988). Of these, Holmström et al. (1989) and Edling et al. (1988) do not appear to be confounded by exposure to wood dust. Nasal biopsy pathology from formaldehyde-exposed workers is consistent with irritant and reactive properties of formaldehyde (Ballarin et al., 1992; Boysen et al., 1990; Holmström et al., 1989; Edling et al., 1988; Berke, 1987). Moreover, these findings are supported by results from animal toxicity and pharmacokinetic and anatomical airflow studies, indicating that, at concentrations less than 1 ppm, inhaled formaldehyde gas does not reach lower regions of the respiratory tract. Of the available human studies that evaluated histopathology, Holmström and Wilhelmsson (1988) appears to be the most robust and sensitive. The study was carefully designed and included a large sample of formaldehyde-exposed subjects who were considered separately from workers exposed to combinations of exposures (formaldehyde and wood dust). Study subjects had been exposed to formaldehyde regularly for many years. The authors reported not only weekday exposures but effects reported on weekends and on vacation. Total exposure was carefully calculated and averaged. The data were controlled for potential confounders, such as smoking. The endpoint of reduced mucociliary clearance has been substantiated by Andersen and Molhave (1983) and Holmström et al. (1989). Animal studies have also reported formaldehyde-induced changes on the nasal mucosa and are highlighted in Section 4.2.1.2.

4.1.1.5. *Immunologic Effects*

Numerous studies have examined the immunologic responses of individuals exposed to formaldehyde. This section will discuss four specific areas related to immunotoxicity after exposure to formaldehyde: increased upper respiratory tract (URT) infections, systemic immune dysfunction, sensitization and atopy, and production of formaldehyde-protein complexes. Some studies report increased incidence of URT infections after exposure to formaldehyde (Lyapina et al., 2004; Krzyzanowski et al., 1990; Holness and Nethercott, 1989). This effect appears to occur independently of systemic immune changes and may be due to damage to the mucosal barrier, thus facilitating pathogen access. A number of studies have investigated the hypothesis that formaldehyde may induce systemic immunomodulation (Ohtani et al., 2004a, b; Erdei et al., 2003; Thrasher et al., 1990, 1987; Pross et al., 1987). Some studies have also evaluated immune system effects by investigating the role of reactive oxygen species (ROS) from respiratory burst associated with immune cells (Lyapina et al., 2004; Gorski et al., 1992) and by assessing chromosomal damage in immune cells (Orsière et al., 2006; Yu et al., 2005). In addition to the effects of formaldehyde on asthmatics and the potential for formaldehyde exposure to exacerbate asthmatic responses, reviewed in Section 4.1.1.2, numerous studies have investigated whether formaldehyde may directly induce sensitization and atopic responses by measuring immunoglobulin E (IgE) levels associated with formaldehyde exposure (Ohmichi et al., 2006; Vandenplas et al., 2004; Doi et al., 2003; Baba et al., 2000; Palczynski et al., 1999; Krakowiak et al., 1998; Wantke et al., 1996a, b; Liden et al., 1993; Salkie, 1991; Grammer et al., 1990; Kramps et al., 1989). Findings are largely negative and suggest that formaldehyde-induced IgE production is not likely. Lastly, studies have investigated the production of formaldehyde-specific antibodies, formaldehyde-albumin complexes, and formaldehyde-heme complexes (Kim et al., 2001; Carraro et al., 1997; Grammer et al., 1993, 1990; Dykewicz et al., 1991; Thrasher et al., 1990). Heme complex formation is not a strict immunologic endpoint but may trigger antibody formation and thus it will be discussed in this section. This section will thus summarize the human studies that have specifically addressed the increased incidence of URT infections, immunotoxic endpoints, atopy and sensitization, and formation of formaldehyde-heme and formaldehyde-albumin complexes.

4.1.1.5.1. *Increased URT* ***infections.*** Diverse studies have investigated the possibility that formaldehyde exposure leads to increased URT infections (Lyapina et al., 2004; Krzyzanowski et al., 1990; Holness and Nethercott, 1989). Lyapina et al. (2004) studied 29 workers who were occupationally exposed occupationally to formaldehyde for an average of 12.7 years through contact with carbamide-formaldehyde glue. The mean values of the average shift concentrations

of formaldehyde in the application of carbamide-formaldehyde glue to be 0.71 ppm TWA with a range of 0.32 to 1.57 ppm. The workers were divided into two subgroups, one (n = 12) that suffered from either a long history (with clinical findings) of chronic mucous inflammation of the URT with multiple relapses and a second group (n = 17) whose URT inflammations were short, acute, and predominantly viral. Twenty-one healthy subjects served as controls. A statistically significant association of self-reported chronic bronchitis and decreased resistance to URT infection was reported in all the exposed workers compared with controls ($p = 0.02$). Of the workers, 41% had a history of chronic respiratory infection and frequent long-lasting infectious inflammatory relapses (group 1a). Another group (group 1b) consisted of 17 exposed workers, 12 of whom had no history of recurrent viral infections of the URT. There was a statistically significant association of frequency and duration of inflammatory relapses between groups 1a and 1b. No dates were provided regarding when these measurements were made or over what period of time they were calculated.

Krzyzanowski et al. (1990) measured formaldehyde levels in homes and recorded, by way of a questionnaire, health histories from adult and child residents. Formaldehyde levels were reported from samples taken for two 1-week periods in various rooms of the home (kitchen, living room, subject's bedroom). The average formaldehyde level was 26 ppb in 202 homes, and levels were stratified into homes with exposure levels below 40 ppb, between 40 and 60 ppb, and above 60 ppb. Incidences of doctor-diagnosed chronic bronchitis were more prevalent in children (under age 15) living in homes with higher formaldehyde (>60 ppb) readings in the kitchen ($p < 0.001$). This effect was more pronounced ($p < 0.001$) in children simultaneously exposed to environmental tobacco smoke. The prevalence of chronic cough was also increased in adults living in homes with measurable levels of formaldehyde, but data were not shown. Holness and Nethercott (1989) assessed chronic bronchitis in 87 funeral workers, where the average formaldehyde exposure was reported at 0.38 ± 0.19 ppm. Chronic bronchitis was observed in 20 funeral workers (n = 87) exposed to formaldehyde compared with 3 cases of chronic bronchitis in nonexposed referent controls (n = 38).

These studies suggest that exposure to formaldehyde may be associated with increased incidence of chronic bronchitis. The mechanism for this association has not been elucidated. Pathogens may gain access to the URT via a compromised mucosal barrier, as has been shown in histopathology studies (Section 4.1.1.4).

4.1.1.5.2. ***Immune function.*** A number of studies have evaluated the ability of formaldehyde to induce systemic immunotoxic effects (Ohtani et al., 2004a, b; Erdei et al., 2003; Thrasher et al., 1990, 1987; Pross et al., 1987). Some studies have reported altered innate immune responses

associated with formaldehyde exposure (Erdei et al., 2003), while others have noted adaptive immune response suppression associated with formaldehyde exposure (Thrasher et al., 1990, 1987) and changes associated with alterations to a predominant T—lymphocyte helper 2 (Th2) pattern (Ohtani et al., 2004a, b). In contrast, Pross et al. (1987) did not observe formaldehyde-associated changes in systemic immune function.

Erdei et al. (2003) found that *Haemophilus influenzae* humoral biomarker (H.in.IgG), *Klebsiella pneumoniae* biomarker (K.pn.IgG), and elevated monocyte concentrations were significantly associated with high formaldehyde concentrations in asthmatic children, compared with nonsensitive children. Briefly, Erdei et al. (2003) compared the immune system responses in 9- to 11-year-old Hungarian school children whose respiratory systems were immunologically compromised (chronic respiratory disease, asthma) and normal children who were exposed to indoor air pollutants, including formaldehyde. In the homes of the children with the highest levels of pollutants, 49.3% of formaldehyde measurements exceeded the Hungarian indoor standard of 0.01 ppm, while 20% exceeded the World Health Organization's (WHO's) suggested indoor level of 0.09 ppm. The authors excluded from consideration all measurements that exceeded WHO's air quality guidelines in one unidentified city to prevent a "city-related bias," since these measurements occurred entirely in that city. The average formaldehyde concentration in the 123 homes tested was 14 ppm with a range of 0.5 to 46 ppm. H.in.IgG and K.pn.IgG were significantly associated with high formaldehyde concentrations ($p < 0.013$ and $p < 0.049$, respectively) in sensitive children compared with nonsensitive children. These markers were also correlated with high levels of nitrogen dioxide, the number of cigarettes smoked, and exposure to paint, volatile organic compounds, and solvents. Additionally, indoor formaldehyde exposure was significantly associated with increased monocyte concentrations ($p < 0.017$) that are important to the innate immune response (inflammation) in diseased tissue. The authors concluded that the elevation of immune biomarkers in sensitive children with respiratory disease is likely the result of high concentrations of toxic indoor air pollutants, including formaldehyde.

Thrasher et al. (1987) assessed the effects of formaldehyde exposure on cellular immunity and antibody formation in eight exposed and eight unexposed individuals. The exposed group consisted of three males and five females. Seven of the exposed individuals had resided in mobile homes for periods ranging from 2 to 7 years; the eighth was a laboratory worker who resided in a newly decorated, energy-efficient apartment. Air monitoring in four of the homes revealed formaldehyde vapor concentrations ranging from 0.07 to 0.55 ppm. Venous blood samples were collected from all subjects and T- and B-cells were counted and monitored for blastogenesis. When IgG and IgE antibodies to formaldehyde were monitored in serum, no

IgE antibodies to formaldehyde were detected in exposed or control subjects. IgG antibody titers in exposed subjects ranged from 1:8 to 1:256 but essentially were undetected (1:4) in seven of the controls. T- and B-cell numbers were significantly lower ($p < 0.05$) in mobile home residents (48 and 12.6%, respectively) compared with those of control subjects (65.9 and 14.75%, respectively). As determined by incorporation of ^{3}H-thymidine into 48-hour unaltered lymphocytes, phytohemagglutinin-stimulated T- and B-cell blastogenesis was significantly depressed ($p < 0.01$) in cells of mobile home residents compared with those of control subjects (17,882 and 28,576 cpm, respectively). Thrasher et al. (1987) concluded that exposure to formaldehyde decreases the proportion of peripheral T cells.

In a later study, Thrasher et al. (1990) evaluated five groups of subjects with varying levels and durations of formaldehyde exposure. The groups consisted of (1) asymptomatic chiropractic students exposed during anatomy classes (controls with only intermittent exposure to formaldehyde), (2) mobile home residents, (3) office workers, (4) patients with multiple symptoms who had been removed from the source of formaldehyde for at least a year, and (5) occupationally exposed patients. All groups were assessed for immunologic function via white cell, lymphocyte, and T-cell counts, T-helper/suppressor ratios and B-cell counts. When compared with controls (chiropractic students), the patient groups had significant elevations in formaldehyde antibody titers and B-cell titers.

Ohtani et al. (2004a, b) reported effects of exposure to formaldehyde and diesel exhaust particles on cytokine production by human monocyte-derived dendritic cells (MoDCs) and T cells in vitro. Dendritic cells were stimulated with CD40 ligand and interferon (IFN)-γ, T cells with anti-CD3/CD28 antibodies. Cytokine proteins and mRNA levels were measured in supernatants by enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR), respectively. Formaldehyde and diesel exhaust particles significantly increased tumor necrosis factor (TNF)-α levels and suppressed interleukin (IL)-12p40 protein and mRNA levels in MoDCs. The same treatment suppressed protein synthesis and mRNA expression of IFN-γ and IL-10 in T cells. The authors concluded that their findings support a role of formaldehyde and diesel exhaust particles in altering the immune response to a Th2-dominant pattern that furthers allergic inflammation. Further details, such as exposure concentrations and experimental protocols, are not available.

In contrast, Pross et al. (1987) concluded that formaldehyde does not induce altered immune activity. The authors evaluated the immunologic response of asthmatic subjects exposed to UFFI off-gas products. Subjects consisted of 23 individuals with a history of asthmatic symptoms attributed to UFFI and 4 individuals with asthma unrelated to UFFI off-gas products. All subjects were exposed in an environmental chamber according to the following

sequence: (1) room air (placebo) for 30 minutes; (2) 1 ppm formaldehyde gas for 3 hours; (3) UFFI particles (4 μm diameter, 0.5 particles/mL) for 3 hours, commencing 48 hours after formaldehyde gas exposure; and (4) UFFI off-gas products for 3 hours, commencing 48 hours after UFFI particle exposure. There was a significant increase in the percentage and absolute number of eosinophils and basophils in the subjects who lived in UFFI homes but no differences between exposure groups with respect to lymphocyte subpopulations either before or after UFFI exposure. However, when T8 suppressor cells were counted, values in the UFFI-exposed group pre-exposure and postexposure, a small but statistically significant ($p < 0.01$) increase in T8 cell count was observed. The biological significance of this increase in T8 cell count in exposed asthmatics is not known. Pross et al. (1987) concluded that short-term exposure to formaldehyde was not immunosuppressive and did not result in systemic immune reactivity.

Respiratory burst from immune cells creates ROS that can incur further cellular damage. Several studies have evaluated, either directly or indirectly, the potential role of ROS as potential mediators of formaldehyde-associated effects, particularly those caused by immune cells. Gorski et al. (1992) measured chemiluminescence resulting from the release of free radicals from granulocytes of healthy and formaldehyde-sensitive subjects. Thirteen subjects with contact dermatitis who were occupationally exposed to formaldehyde and five healthy volunteers participated in the study. All underwent skin-prick tests for common allergens as well as a histamine inhalation provocation test. Subjects were exposed to 0.5 mg/m^3 (0.41 ppm) formaldehyde for 2 hours, and the PEFR was measured immediately before exposure, after 60 and 120 minutes of exposure, and 6 and 21 hours after completion of exposure. Peripheral blood granulocyte chemiluminescence was measured in the presence of luminol. Free radical production was increased significantly within 30 minutes of beginning the exposure in subjects with allergic dermatitis and remained elevated for 24 hours compared with baseline values. Gorski et al. (1992) concluded that granulocyte chemiluminescence did not increase in healthy, formaldehyde-exposed patients but was diagnostic for formaldehyde-sensitive patients. These results also suggest a putative role for oxidative damage associated with formaldehyde exposure, particularly in sensitized individuals.

Lyapina et al. (2004) also reported effects of formaldehyde exposure on neutrophil respiratory burst activity (NRBA), the capacity of polymorphonuclear leukocytes to produce reactive oxygen radicals in response to chemical or microbial stimuli using flow cytometry. Briefly, Lyapina et al. (2004) studied 29 workers who were occupationally exposed to formaldehyde for an average of 12.7 years through contact with carbamide-formaldehyde glue with a mean value of the average shift concentration of formaldehyde reported as 0.71 ppm TWA with a range of 0.32 to 1.57 ppm. The workers were divided into two subgroups, one (n =

12) that suffered from either a long history (with clinical findings) of chronic mucous inflammation of the URT with multiple relapses, and a second group (n = 17) whose URT inflammations were short, acute, and predominantly viral. Twenty-one healthy subjects served as controls. A suite of hematological tests and flow cytometric analysis for respiratory burst activity were performed. Although no significant difference was observed in the spontaneous and stimulated NRBA (median percentage of oxidizing cells) between the 29 exposed workers with URT inflammation and the healthy controls (0.83 versus 1.35, respectively), a separate comparison of the NRBA of 12 workers with chronic, repeating URT infections and 17 workers with short, infrequent episodes of URT inflammations was significant (0.45 versus 1.00, p = 0.037). When the NRBA of the group with chronic URT infections (n = 12) was separately compared with that of the healthy controls (n = 21), the results were also significant (0.45 versus 1.35, p = 0.012). Individuals with chronic URT infections have reduced NRBA that could be due to formaldehyde exposure. Neutrophils respond to tissue damage or local invasion of microorganisms and act to phagocytize foreign cells. If neutrophilic activity is hampered or altered by formaldehyde exposure, then the ability to fight infection will be diminished, leading to prolonged infection. However, no dose-response pattern of formaldehyde exposure could be determined from this study.

Other investigators have reported chromosomal damage in immune cells due to formaldehyde (Orsière et al., 2006; Yu et al., 2005). Yu et al. (2005) evaluated chromosomal damage in lymphocytes from 151 exposed and nonexposed workers from a plywood factory detected by comet assay. The authors reported that chromosomal damage was statistically elevated in lymphocytes from formaldehyde-exposed workers compared with controls. However, no information on exposure duration or levels was provided. Orsière et al. (2006) studied DNA damage in lymphocytes from 59 hospital employees with formaldehyde exposures from pathology and anatomy laboratories in five hospitals. Controls were 37 workers from the same hospitals, matched on gender, age, and smoking habits, with no known exposure to genotoxic agents. Study participations were excluded if workers had a history of radio- or chemotherapy or had used therapeutic medications that were known to be mutagenic. Occupational exposure was determined through 15-minute and 8-hour personal air sampling during a typical workday. Mean formaldehyde concentrations were 2 ppm (range: <0.1–20.4 ppm) for 15-minute sampling and 0.1 ppm (range: <0.1–0.7 ppm) for 8-hour sampling. No change in DNA damage was found between the beginning and end of the workday among exposed workers (3.9 ± 0.6 versus 3.6 ± 0.5 relative light units/ng DNA). However, exposed workers had significant elevations in the binucleated micronucleated cell rate (BMCR) per 1,000 cells compared with controls (16.9 ± 9.3 versus 11.1 ± 6.0%; p < 0.001), but BMCR did

not appear to be correlated with formaldehyde concentration. Linear regression analysis showed that the effect for exposure remained after adjusting for gender, age, smoking, and drinking habits. For 18 exposed and 18 control workers who underwent cytokinesis-blocked micronucleus assay (CBMA) combined with fluorescent in situ hybridization (FISH) with pan-centromeric DNA probe, results showed that the frequency of micronuclei (MN) containing only one centromere (C1+MN) was elevated among the exposed compared with unexposed workers ($11.0 \pm 6.2\%$ versus $3.1 \pm 2.4\%$; $p < 0.001$). The effect of exposure remained significant after controlling for gender, age, smoking, and drinking habits. Results from Yu et al. (2005) and Orsière et al. (2006) suggest that formaldehyde exposure may promote chromosomal damage leading to micronucleated lymphocytes.

Compromised lymphocyte function may significantly contribute to altered immune status. The mechanism underlying this effect has not been elucidated.

4.1.1.5.3. ***Sensitization and atopy.*** Numerous studies have documented formaldehyde-induced exacerbation of asthmatic responses (see Section 4.1.1.2). The mechanism of this effect has not been clarified and has led investigators to assess the potential for formaldehyde to directly induce formation of formaldehyde-specific antibodies, leading to allergic responsiveness. One case report showed systemic allergic reactions (e.g., anaphylaxis) to formaldehyde in a patient undergoing hemodialysis (Maurice et al. [1986] referenced in Thrasher et al. [1990]). Some studies have evaluated the potential association of formaldehyde-specific IgE in already-sensitized individuals (Baba et al., 2000; Palczynski et al., 1999). Other studies have investigated whether formaldehyde can directly induce IgE in nonsensitized individuals. Most of the studies have not identified presence of formaldehyde-specific IgE (Ohmichi et al., 2006; Krakowiak et al., 1998; Grammer et al., 1993, 1990; Kramps et al., 1989; Thrasher et al., 1987) and are summarized below. A few studies (Vandenplas et al., 2004; Doi et al., 2003; Liden et al., 1993) reported positive IgE against formaldehyde, associated with exposure, but the IgE titers were either transient (Vandenplas et al., 2004) or were positive in a small subset of previously sensitized subjects (2 of 15) (Liden et al., 1993). Doi et al. (2003) detected IgE against formaldehyde in two asthmatic children (out of 122 asthmatic children), but the response severity did not correlate with exposure level.

Palczynski et al. (1999) evaluated whether exposure to formaldehyde might facilitate specific sensitization to common allergens. The study population was comprised of residents of apartments built in 1989–1990. Only households with children from 5–15 years were eligible for the study. A random sample of 120 apartments was selected in which lived a total of 465 persons aged 5–65 years. Individual demographic characteristics and medical histories were

determined by questionnaire. Residents were tested, using the skin-prick method, for allergen response to a variety of materials, such as household dust, pollens, and feathers. Total serum IgE levels were measured, and the presence of formaldehyde-specific IgE antibodies was determined. Measured mean levels of formaldehyde were 21.05 ± 8.94 ppb. No significant relationship between respiratory allergy prevalence and indoor exposure to formaldehyde was detected. Significant increases in serum IgE levels were found in children exposed to both environmental tobacco smoke and formaldehyde.

Baba et al. (2000) investigated whether production of formaldehyde-specific IgE could be detected in adult asthmatics. Formaldehyde exposure levels were not documented. Formaldehyde-IgE was detected in two asthmatic patients (n = 80), one male and one female, but the titer of IgE did not parallel the severity of the asthmatic responses and could not be linked to formaldehyde exposure. Thus, formaldehyde-specific IgE-mediated allergy was rare in adult chronic asthmatics.

Several studies have examined serum for formaldehyde-specific IgE antibodies in groups of formaldehyde-exposed humans (Ohmichi et al., 2006; Krakowiak et al., 1998; Wantke et al., 1996a, b; Salkie, 1991; Grammer et al., 1990; Kramps et al., 1989). While formaldehyde-specific IgE was reported in one study (Wantke et al., 1996a), results from most other studies failed to find a consistently strong association between formaldehyde-specific IgE or IgG antibodies in groups of formaldehyde-exposed humans.

Wantke et al. (1996a) detected elevated levels of formaldehyde-specific IgE in 24 of 62 8-year-old children who were students in three particleboard-paneled classrooms in which the estimated formaldehyde air concentrations were 0.075, 0.069, and 0.043 ppm. In a health survey, the children reported headaches (29/62), fatigue (21/62), dry nasal mucosa (9/62), rhinitis (23/62), cough (15/62), and nosebleeds (14/62). The number of children with symptoms in each classroom decreased with decreasing formaldehyde concentration (49, 47, and 24, respectively, for the 0.075, 0.069, and 0.043 ppm classrooms). However, the investigators reported that elevated levels of specific IgE did not correlate with the number and severity of symptoms. When the children were evaluated after 3 months in a new school that did not have particleboard paneling and had lower ambient formaldehyde concentrations (0.029, 0.023, and 0.026 ppm), the number of children reporting symptoms decreased significantly from earlier figures, and, when measured in 20 of the children, the mean serum levels of formaldehyde-specific IgE declined significantly compared with pre-moving mean levels.

In contrast, a study by Krakowiak et al. (1998) measured serum IgE levels in asthmatic and healthy subjects as part of a larger study to characterize the mechanism of formaldehyde-induced nasal and bronchial response in asthmatic subjects with suspected

formaldehyde allergy. Ten subjects reported to have formaldehyde rhinitis and asthma and 10 healthy subjects underwent a 2-hour inhalation challenge in an exposure chamber with formaldehyde at a concentration of 0.5 mg/m^3 (0.41 ppm). Formaldehyde-specific serum IgE antibodies were measured, and cellular, biochemical, and mediator changes were assessed in nasal lavage before, immediately after, and at 4 and 24 hours after challenge. Challenges with formaldehyde caused only transient symptoms of rhinitis in both groups. Furthermore, none of the subjects thought to have occupational asthma developed clinical symptoms of bronchial irritation. No specific IgE antibodies to formaldehyde were detected in persons with occupational exposure to formaldehyde. No differences in the nasal response to formaldehyde were found between subjects reported to have occupational allergic respiratory diseases and healthy subjects ($p > 0.05$). The study showed that inhaled formaldehyde at a level as low as 0.5 mg/m^3 did not induce a specific allergic response either in the upper or in the lower part of the respiratory tract. In addition, it demonstrated that there was no difference in nasal response to formaldehyde between asthmatic subjects occupationally exposed to formaldehyde and healthy subjects.

Similarly, formaldehyde-specific IgE antibodies were detected in only 1 serum sample (out of 86) from four groups of formaldehyde-exposed subjects (Kramps et al., 1989). The subject with detected formaldehyde-specific IgE displayed allergic symptoms. The groups included (1) 28 subjects living or working in places with formaldehyde-containing construction materials (e.g., chipboard) and estimated formaldehyde concentrations ranging from 0.08 to 0.37 ppm, (2) 18 occupationally exposed subjects from an anatomy laboratory and in other unspecified industries where air concentrations were not measured, (3) 12 hospital attendants who worked with formaldehyde-sterilized hemodialysis equipment, and (4) 28 hemodialysis patients coming into contact with equipment sterilized with formaldehyde. Other subjective symptoms, such as headache, eye irritation, and respiratory complaints, were reported by 24/28 subjects in the construction material group and confirm that formaldehyde is an irritant (reviewed in Section 4.1.1.1). Durations of exposure or length of employment were not reported for the subjects in this study.

Grammer et al. (1990) studied the immunologic nature of formaldehyde sensitivity in 37 workers who were examined by a group of physicians in response to complaints of formaldehyde-related illness. Air sampling of formaldehyde ranged from 0.003 to 0.078 ppm, but specific levels were not tied to specific workplace areas. Blood samples were collected and assayed for IgE and IgG activity against formaldehyde. None of the workers had IgG activity against formaldehyde. No IgE antibodies were detected in the other 32 workers. The authors

concluded that there was no evidence of an immunologically mediated response to formaldehyde in this group of workers.

Formaldehyde-specific IgE was not detected in any of a group of 45 medical students before or after the students attended a 4-week anatomy dissecting course (Wantke et al., 1996b). Estimates of ambient air concentrations of formaldehyde ranged from 0.059 to 0.219 ppm (0.124 ± 0.05 ppm; mean ± SD). However, the survey revealed frequencies of irritation symptoms that were consistent with other studies (e.g., itching of the skin in 33/45 students, headache in 15/45, and burning eyes in 13/45).

Similarly, Ohmichi et al. (2006) were unable to correlate formaldehyde exposure with specific IgE production among eight students attending a gross anatomy laboratory. Formaldehyde exposure was estimated to range from 0.33 to 1.47 ppm during the laboratory sessions. The sample size was small, and IgE levels varied substantially (ranging from <19 to >5,000 international units/mL). Compared with IgE levels taken 90 minutes prior to the start of the first session, IgE levels measured shortly after the last session and up to 23 days following the last session showed no association with exposure.

Salkie (1991) investigated the prevalence of formaldehyde-specific IgE in practicing pathologists who complained of formaldehyde sensitivity. Exposure levels were not reported. Serum samples were assayed for total IgE and formaldehyde-specific IgE. Of the 46 subjects, 29 self-reported atopy that was confirmed in 12 subjects by positive IgE. Moreover, 29 subjects complained of formaldehyde-specific sensitivity. However, zero subjects had formaldehyde-specific IgE, and there was no evidence that atopic individuals were more sensitive to formaldehyde than non-atopic individuals. The authors noted that atopic individuals may have selectively reduced their exposure to formaldehyde.

Vandenplas et al. (2004) evaluated a case study of a 31-year-old male who was accidentally exposed to formaldehyde for 2 hours. The exposure level was not provided. The subject had smoked a pack of cigarettes a day for 13 years and was admitted to the emergency room for asthmatic symptoms. Eight days following exposure, increased levels of formaldehyde-specific IgE antibodies were detected but could not be detected in subsequent assessments.

A clinical study by Liden et al. (1993) evaluated IgE-specific antibodies against formaldehyde in 23 subjects who had previously tested positive for skin sensitization by skin prick test. Subjects were exposed to formaldehyde by skin patch (1% formaldehyde in water). Ten of the subjects were classified as atopic Though 15 of 23 of the sensitized subjects were also sensitive to formaldehyde applied by skin patch, formaldehyde-IgE was positive in 2 of 15

individuals who were not classified as atopic. No dose-response relationship could be determined from the study design of this study.

Doi et al. (2003) conducted a clinical study in 155 children of which 122 were asthmatics. No specific exposure to formaldehyde was documented. IgE against formaldehyde was determined in blood. Formaldehyde-specific IgE was found in two asthmatic children. Thus, while several studies have documented formaldehyde-specific IgE, the occurrence is rare and may be transient. Asthmatic children may be more predisposed to form formaldehyde-specific IgE than non-atopic individuals or adults. The formation of formaldehyde-specific IgE is quite rare.

4.1.1.5.4. ***Formaldehyde-albumin and formaldehyde-heme complexes***. Numerous studies have shown that formaldehyde can bind to blood proteins as formaldehyde-heme and formaldehyde-human serum albumin (formaldehyde-HSA) complexes (Carraro et al., 1997; Grammer et al., 1993, 1990; Dykewicz et al., 1991; Thrasher et al., 1990). Kim et al. (2001) failed to identify IgE against formaldehyde-HSA complexes in one case-control subject following industrial occupational exposure to formaldehyde. These complexes may serve to traffic formaldehyde throughout the bloodstream and throughout the body. While formaldehyde may be too small to engender an immune response, these complexes may be able to trigger formaldehyde-protein-specific antibodies, leading to an immune response, including sensitization.

Thrasher et al. (1990) evaluated five groups of subjects as follows with varying levels and durations of formaldehyde exposure: asymptomatic chiropractic students exposed during anatomy classes (controls with only intermittent exposure to formaldehyde), mobile home residents, office workers, patients with multiple symptoms who had been removed from the source of formaldehyde for at least a year, and occupationally exposed patients. All groups were assessed for production of antibodies against formaldehyde-HSA. The level of autoantibodies was significantly elevated in patients exposed long-term to formaldehyde. From the data, Thrasher et al. (1990) concluded that exposure to formaldehyde stimulates IgG antibody production to formaldehyde-HSA.

Grammer et al. (1990) studied the immunologic nature of formaldehyde sensitivity in 37 workers who were examined by a group of physicians in response to complaints of formaldehyde-related illness. Air sampling of formaldehyde ranged from 0.003 to 0.078 ppm, but specific levels were not tied to specific workplace areas. Blood samples were collected and assayed for IgE and IgG activity against formaldehyde and formaldehyde-HSA. None of the workers had IgG activity against formaldehyde. Five workers had IgE against both HSA alone and against formaldehyde-HSA complexes. No IgE antibodies were detected in the other 32

workers. The authors concluded that there was no evidence of an immunologically mediated response to formaldehyde in this group of workers.

Grammer et al. (1993) described the evaluation of a worker with bronchospasm symptoms caused by formaldehyde exposure. The worker was evaluated by means of ELISA, cutaneous tests, and methacholine and formaldehyde inhalation challenges. The ELISA showed that the worker had positive IgE and IgG titers to formaldehyde-HSA. The worker also had a positive cutaneous test for formaldehyde-HSA but a negative methacholine challenge at 25 mg/mL and negative formaldehyde inhalation challenges at exposure concentrations of 0.3, 1, 3, and 5 ppm for 20 minutes. The worker might have developed a positive response if a higher concentration of formaldehyde had been used for the challenge, but it is more probable that the worker's symptoms were not caused by immunologically mediated asthma.

Dykewicz et al. (1991) evaluated whether IgE or IgG antibodies to formaldehyde were related to formaldehyde exposure or to respiratory symptoms arising from such an exposure. The authors studied 55 potentially exposed subjects (hospital histology technicians, internal medicine residents, pathology residents, current smokers, and persons with known workplace exposure to formaldehyde) and compared them to controls with no history of formaldehyde exposure. Reported workplace formaldehyde concentrations were 0.2–0.64 ppm for pathology residents, 0.64 ppm for histology technicians, and 0.6–11 ppm for miscellaneous formaldehyde exposure scenarios. Workplace air concentrations were not measured for the other occupations. Occupational exposure to formaldehyde averaged 12.45 years for histology technicians, 0.38 years for medical residents, 3.21 years for pathology residents, and 18.34 years for five subjects exposed to formaldehyde in miscellaneous workplaces. Blood samples were analyzed for IgE and IgG reactivity with formaldehyde-HSA complexes. Three subjects had IgE against -HSA; these three and two others had low levels of anti-formaldehyde-HSA IgG. The presence of IgG and IgE antibodies to formaldehyde was not clearly related to formaldehyde exposure or pack-years of smoking. One subject had both IgE and IgG antibodies and also suffered from eye and respiratory symptoms when exposed to formaldehyde at his workplace. However, the authors concluded that they could not establish a relationship between IgE and IgG levels and formaldehyde exposure. This study has several limitations. First, the volunteers (hospital workers) may not be representative of exposed workers in the general population. One of the exposure groups comprised cigarette smokers. Although the study focused on formaldehyde antibodies, which would be unaffected by the other chemicals, respiratory symptoms among smokers would reflect exposures to the constituents of smoke. Dykewicz et al. (1991) concluded that immunologically mediated asthma caused by formaldehyde is extremely rare and may not exist at all.

Carraro et al. (1997) reported development of a reliable assay to effectively measure formaldehyde-HSA complexes in smokers, ex-smokers, and nonsmokers. A correlation between formaldehyde-HSA antibodies and smoking status was detected. This study did not correlate formaldehyde exposure and formaldehyde-HSA antibodies.

Given that formaldehyde is a sensory irritant that is particularly bothersome to individuals with compromised lung function or asthma, numerous studies have assessed the ability of formaldehyde to induce immunotoxic effects. Some studies have documented increased rates of chronic bronchitis and URT infections associated with exposure to formaldehyde, which suggests a possible immunomodulatory effect. However, of the numerous articles that have investigated systemic immunomodulatory effects due to formaldehyde (Lyapina et al., 2004; Gorski et al., 1992; Thrasher et al., 1990, 1988, 1987; Pross et al., 1987), few have reported significant immune modulation related to formaldehyde exposure. Significant decreases in specific adaptive immune cell populations do not appear correlated to formaldehyde exposure (Erdei et al., 2003; Gorski et al., 1992; Thrasher et al., 1990, 1987; Pross et al., 1987). Thus, the tendency for increased infection rates associated with formaldehyde may not be related to altered immune function. Perhaps altered mucociliary clearance and disturbed mucosal barrier may provide greater access for pathogens and result in greater infection rates. Moreover, formaldehyde has been associated with exacerbation of asthmatic or atopic responses, particularly in sensitized individuals. However, this effect does not appear to occur by increased IgE or formaldehyde-specific IgE levels (Ohmichi et al., 2006; Palczynski et al., 1999; Krakowiak et al., 1998; Wantke et al., 1996b; Salkie, 1991; Grammer et al., 1990; Kramps et al., 1989). Thus, formaldehyde-associated enhanced allergic responses does not appear to be due to direct induction of sensitization and may not occur via an immunologic mechanism. Lastly, the formation of formaldehyde-heme and formaldehyde-HSA complexes has been well documented (Grammer et al., 1993, 1990; Dykewicz et al., 1991; Thrasher et al., 1990) and may serve as a biomarker of exposure (Carraro et al., 1997). Moreover, these complexes may provide a means by which formaldehyde travels throughout the bloodstream and may drive antibody formation that may lead to immune activation.

4.1.1.6. *Neurological/Behavioral*

There is some suggestion of neurological impairment in humans following occupational exposure to formaldehyde; the data are limited and the results from several studies are potentially confounded by exposure to other solvents. Two studies of histology technicians with occupational exposure to formaldehyde and other solvents found neurological deficits and poorer performance on neurocognitive tests associated with formaldehyde exposure (Kilburn et al.,

1987, 1985). In another study, Kilburn and Warshaw (1992) found no change from initial performance, for as long as 4 years, in follow-up evaluations of histology technicians with continuing exposure to formaldehyde. In a preliminary report from a prospective study, Weiskopf et al. (2009) found a strong association between duration of formaldehyde exposure and death from amyotrophic lateral sclerosis (ALS). In a controlled exposure study, Bach et al. (1990) found that, when workers with chronic formaldehyde exposure were challenged with an acute formaldehyde exposure, they exhibited poorer performance on some neurocognitive tests compared with workers without chronic exposure undergoing the same acute challenge conditions. In another controlled exposure study, Lang et al. (2008) found equivocal changes in reaction time following an acute exposure.

4.1.1.6.1. ***Epidemiological studies.*** Kilburn et al. (1985) reported that a group of 76 female histology technicians displayed statistically significantly greater frequencies of neurobehavioral deficits (lack of concentration and loss of memory, disturbed sleep, impaired balance, variations in mood, and irritability), than did a referent group of 56 unexposed female clerical workers. The technicians had been employed from 2 to 37 years (mean 12.8 years). Analysis of workplace air samples indicated the presence of several solvents, ranging from 0.2 to 1.9 ppm for formaldehyde, 3.2 to 102 ppm for xylene, 2 to 19.1 ppm for chloroform, and 8.9 to 12.6 ppm for toluene. Subsequently, Kilburn et al. (1987) administered a battery of 10 tests to 305 female histology technicians to assess various aspects of cognitive and motor function. The researchers analyzed the results by regression analysis with age, years of smoking, and hours per day of exposure to formaldehyde and other solvents as explanatory variables. Increased daily hours of exposure to formaldehyde were significantly correlated with decreased performance in several tests (including several types of memory, dexterity, and balance), whereas hours of daily exposure to other solvents were only correlated with decreased performance in a single memory test. In a later prospective study of performance, 318–494 histology technicians were tested in a battery of neurobehavioral tests, and testing for a subset of subjects was repeated yearly for up to 4 years. No statistically significant decrement in performance was found when initial test results were compared with retest results to evaluate effects of continuing occupational exposure to formaldehyde (or other solvents) or possible effects of aging (Kilburn and Warshaw, 1992). Kilburn (1994) later reported that three anatomists and one railroad worker, occupationally exposed to airborne formaldehyde for 14–30 years, each showed impaired performance on several neurobehavioral tests (e.g., choice reaction time, abnormal balance, digit symbol, and perceptual motor speed).

Weisskopf et al. (2009) evaluated the association between chemical exposure and death from ALS, using the cohort of 987,229 people from the prospective Cancer Prevention Study II of the American Cancer Society. From 1989–2004, 1,156 deaths from ALS were identified from mortality records from the National Death Index. Exposure assessment occurred prior to follow-up and was based on a questionnaire; participants were asked about current exposure to 12 types of chemicals and whether they had been regularly exposed in the past. After controlling for a number of potentially confounding factors (including age, sex, smoking status, military service, education, alcohol intake, occupation, vitamin use, and exposure to other chemicals), it was found that exposure to formaldehyde for a known duration was statistically significantly associated with increased risk of death from ALS ($p < 0.0001$) with a relative risk (RR) of 2.47 (95% CI: 1.58–3.86) based on 22 deaths. Weisskopf et al. (2009) reported that the association had a strongly significant dose-response relationship, with increased duration of exposure associated with increased RR of ALS mortality with a reported *p* value for continuous trend of 0.0004. Multivariate adjusted rate ratios were 1.5 for known formaldehyde exposures less than 4 years, 2.1 for 4–10 years, and 4.1 for >10 years. Although the authors indicated that these results need independent verification, the results of this study of the nearly one million people followed for 15 years is unlikely to be biased due to its longitudinal design.

4.1.1.6.2. ***Controlled exposure studies.*** Bach et al. (1990) examined whether cognitive and motor performance of humans responded acutely to formaldehyde exposure and whether previous chronic exposure to formaldehyde affected the responses observed following acute exposure. Thirty-two men with at least 5 years of occupational exposure to formaldehyde and 29 matched controls were exposed to formaldehyde at concentrations of 0.04, 0.21, 0.48, or 1.10 ppm for 5.5 hours. During the exposure period, symptoms were assessed by using a standardized questionnaire, and subjects were evaluated in four tests designed to estimate several aspects of cognitive function. Testing was performed once prior to exposure and twice during the exposure period. The authors noted that the typical dose-related symptoms of respiratory irritation were not seen in this study. Previously unexposed subjects reported more headaches, "heavy head," and physical tiredness than the exposed workers. In both occupationally exposed and unexposed subjects, decreased performance in an addition test was significantly correlated with increasing concentration of formaldehyde. Compared with previously unexposed subjects, occupationally exposed subjects showed significantly decreased performance in three other tests, although the effect was not dose related. The study did not adjust for several potential confounders, including prior exposure to other chemical agents, and the age and health status of the cases and controls. Authors concluded that their data indicated that acute exposure to

formaldehyde might cause acute effects on CNS functions but that more investigation was needed to verify their results.

In a study evaluating chemosensory irritation, Lang et al. (2008) assessed possible changes in reaction time during an acute (4-hour) exposure to formaldehyde concentrations between 0–0.5 ppm (some exposure sessions also included short concentration peaks of up to 1 ppm) with or without a masking agent (ethyl acetate). Twenty-one healthy volunteers were exposed once per day to each of 10 different exposure combinations in random order (for a total of 10 sessions per subject). Reaction time was tested before and after each exposure session. Significant increases in reaction time were seen at 0.3 ppm formaldehyde, with or without masking agent, but not at 0.5 ppm. The significance of these findings is unclear.

Performance of 16 healthy volunteers on addition, multiplication, and card punching tasks was measured by Andersen and Molhave (1983) before and during a 5-hour exposure to formaldehyde at concentrations up to 2 mg/m^3. The authors reported that formaldehyde exposure had no effect on performance, but results were not presented.

4.1.1.6.3. ***Summary.*** The limited information currently available from human studies does not permit a definitive conclusion regarding an association between formaldehyde exposure and human neurotoxicity. There is, however, sufficient information to raise a serious concern for this type of effect, and additional studies are needed.

4.1.1.7. ***Developmental and Reproductive Toxicity***

Epidemiologic studies suggest a convincing relationship between occupational exposure to formaldehyde and adverse reproductive outcomes in women. Several of these studies deal with spontaneous abortion following maternal occupational formaldehyde exposure (Taskinen et al., 1999, 1994; John et al., 1994; Seitz and Baron, 1990; Hemminki et al., 1985, 1982; Axelsson et al., 1984), but not all reported a significant association between exposure and spontaneous abortion. A study of fecundability found an increase in time to pregnancy among female workers exposed to formaldehyde (Taskinen et al., 1999). Three studies that examined the effect of occupational exposures on the incidence of congenital malformation produced mixed results (Dulskiene and Gražulevičiene, 2005; Taskinen et al., 1994; Hemminki et al., 1985). A population-based, semi-ecologic study found an association between atmospheric formaldehyde exposure and low birth weight (Gražulevičiene et al., 1998).

4.1.1.7.1. ***Spontaneous abortion.*** Several epidemiologic studies report a relationship between occupational exposure to formaldehyde and increases in risk of spontaneous abortion following maternal occupational formaldehyde exposure (Taskinen et al., 1999, 1994; John et al., 1994;

Seitz and Baron, 1990; Axelsson et al., 1984). Increased RRs were in the range of 1.7 to more than 3.0. However, other studies (Hemminki et al., 1985, 1982) found no association between occupational formaldehyde exposure and spontaneous abortion. Paternal occupational exposure to formaldehyde was not related to spontaneous abortion (Lindbohm et al., 1991).

The earliest report of an association between spontaneous abortion and formaldehyde exposure comes from a Swedish cohort study of female laboratory workers (Axelsson et al., 1984). Subjects were women born in 1935 or later and worked in a university laboratory during 1968–1979. There were 745 women who responded to a mailed questionnaire (response rate = 95%), 556 of whom reported on 1,180 pregnancies that resulted in 997 births. Exposure to formaldehyde was estimated based on answers to the questionnaires. Formaldehyde exposure was reported only in connection with 10 pregnancies, of which 5 went to term, 3 were reported as miscarriages, and 2 were terminated by induced abortion. Excluding the latter, the spontaneous abortion rate among women exposed to formaldehyde in the first trimester was 3/8 (37.5%) compared with 14/148 (9.5%) in the population of laboratory workers not exposed to any solvent in the first trimester.

While not computed by the authors, the OR can be calculated as 5.7 (95% CI: 1.2–26.6). The exposure assessment on which this result is based was methodologically weak but unlikely to be a source of bias. Given the exploratory nature of this study, potential confounders were not controlled for, but no other co-exposure was more strongly related to the increased risk of miscarriage, so this result is not likely to be explained by confounding. Selection bias is also an unlikely explanation given the high participation rate. However, although this association of an increased risk of pregnancy loss with formaldehyde exposure is statistically significant, the CI is wide and chance may be a possible explanation for this finding.

In a 1988 Health Hazard Evaluation, the National Institute for Occupational Safety and Health (NIOSH) investigated complaints of adverse reproductive outcomes at a plant where work pants were cut and sewn with a fabric that was treated with a resin that releases formaldehyde (Seitz and Baron, 1990). In a NIOSH laboratory, the fabric released 163 to 1,430 µg of formaldehyde/gram of cloth. TWA personal breathing space formaldehyde levels ranged from trace to 0.46 ppm, while workstation values ranged from 0.32 to 0.70 ppm. The investigators studied the outcomes by using a mailed questionnaire. The response rate for current employees was 98%. There were 296 pregnancies among a cohort of 188 women. The investigators found increased rates of spontaneous abortion, premature birth, and congenital malformations. The crude rate of spontaneous abortion was 21% among women working at the plant while pregnant (4 of 19 pregnancies), 15% among women employed elsewhere while pregnant (11 of 71 pregnancies), and 5% among women at home while pregnant (10 of 206

pregnancies). The investigators did not explain how workers employed elsewhere or at home during pregnancy were categorized compared with current workers, nor did they calculate RRs. As calculated from data presented in Table 5 of the monograph, the crude OR (not corrected for multiple observations per woman) for those pregnant while currently working at the plant compared with all others was 3.2 (95% CI: 0.8–12). There were also excess congenital malformations (13 versus 2%) and premature births (13 versus 4%) among the live births (both based on two births each in the exposed group) from the women who were pregnant while employed at the textile plant compared with women who stayed at home. After the NIOSH investigation, changes were made in the plant to improve ventilation.

Because the report provides insufficient details of the methodology and the fact that there was no personal exposure classification in this study, it is difficult to validate the findings in this report. The results did not take into account other potential risk factors for spontaneous abortion or correct for multiple pregnancies per woman. Furthermore, the marked differences between the "home" and "work" pregnancies were difficult to interpret.

A cohort study of effects of paternal occupational exposures in Finland by Lindbohm et al. (1991) found that exposure to formaldehyde had little effect on the rate of spontaneous abortions among 99,186 pregnancies listed in the national hospital discharge register. The analysis was limited to births/spontaneous abortions in 1976 and from May 1980 to April 1982. Spontaneous abortion incidence came from the hospital discharge register and data from outpatient clinics. There were 808 pregnant wives among potentially formaldehyde-exposed fathers. Exposure to formaldehyde was based on employment information listed in the Finnish 1980 census. Compared with pregnancies among wives of unexposed spouses, the age and socioeconomic level-adjusted ORs were 1.1 for low paternal exposure to formaldehyde and 1.0 for moderate to high paternal exposure. Paternal occupational exposures to ethylene oxide, gasoline/benzene, and rubber industry chemicals were associated with spontaneous abortion. The authors hypothesized that the mode of action (MOA) for spontaneous abortion following male exposure to chemicals is genetic damage to germ cells.

The indirect exposure assessment was a substantial limitation of this study. Some confounders in a study of this type could not be controlled for (smoking history, previous spontaneous abortions, alcohol use), and census data could not provide completely accurate information, potentially masking associations between paternal formaldehyde exposure and spontaneous abortion.

A case-control study by Taskinen et al. (1994) of effects of maternal occupational exposure to chemicals used in laboratories in Finland indicated that exposure to formalin, which is a 37% aqueous solution of formaldehyde, was related to an increased risk of spontaneous

abortion. The investigators studied subjects from payrolls of Finnish state-employed laboratory workers, the laboratory workers' union, and a register of workers occupationally exposed to carcinogens. These records were cross-referenced with the hospital discharge register. The investigators selected women who had a single spontaneous abortion during the period 1973–1986 and two controls who had delivered a baby without malformations. The final sample size was 208 cases and 329 controls after refusals and other exclusions. The response rate was 82.4%.

Information on occupational exposure, health status, medication, contraception, and pregnancy history came from mailed questionnaires. Industrial hygienists' construction of an exposure index was based on the subjects' descriptions of their work assignments, use of solvents including estimates of quantity used, and use of a fume hood. ORs were adjusted for employment, smoking, alcohol consumption, parity, previous miscarriage, birth control failure, febrile disease during pregnancy, and other organic solvents found in laboratory work. Spontaneous abortion was associated with 3–5 days per week of formalin exposure (OR 3.5 [95% CI: 1.1–11.2]). A contemporaneous study of formaldehyde concentrations in similar Finnish workplaces (pathology and/or histology laboratories) reported workroom air to range from 0.01 to 7 ppm with a mean of 0.45 ppm formaldehyde (Heikkilä et al., 1991 [as cited in Taskinen et al., 1994]) and that the highest exposures occurred during emptying of sample containers, dish washing, and preparation of formaldehyde solution.

Although the results of this study indicate an increased risk between spontaneous abortion and exposure to formaldehyde/formalin, the women were also exposed to several chemicals concurrently, of which toluene (OR 4.7 [95% CI: 1.4–15.9]) and xylene (OR 3.1 [95% CI: 1.3–7.5]) were also significantly associated with the incidence of spontaneous abortion. However, the investigators reported that the women were more likely to be co-exposed to formalin and xylene, which would make confounding by toluene less likely, and, since xylene was not as strongly associated with the outcome as was formaldehyde, it too is unlikely to fully explain the reported relationship between formaldehyde and increased risk of spontaneous abortion. While it is possible that exposure misclassification may have occurred because of the indirect assessment of workplace chemical exposure, an overall conclusion is that, since the exposure assessment was conducted by industrial hygienists, it is unlikely that this form of bias will have impacted the results of the study to any great extent.

In a U.S. study (John et al., 1994), the results of a case-control study of cosmetologists also supported an association between spontaneous abortion and the use of formaldehyde-based disinfectants. The study population came from the 1988 North Carolina cosmetology license registry. Women on this list who were 22–36 years of age were screened to find those who were

recently pregnant. The cases were full-time cosmetologists who experienced a spontaneous abortion before gestational week 20 during 1983–1988. The most recent spontaneous abortion was used as the reference case. Controls were full-time cosmetologists who delivered a live infant during the same time period.

Information was based on mailed questionnaires. Women were not told the purpose of the study in order to avoid selection and recall bias. Of 8,356 women who received the screening questionnaires, 72.5% responded. Of those, 1,696 qualified for the study and 73.6% completed a more detailed questionnaire. Among them, 96 women were "absolutely sure" they had a spontaneous abortion and qualified as cases. There were 1,058 live births that qualified as controls. Exposure assessment included identification of disinfectants used as well as types of chemicals used on hair, use of gloves, hours worked, number of procedures involving chemicals, and use of manicure products. Presence of formaldehyde in the cosmetology profession in general was confirmed in two NIOSH hazard reports (Almaguer and Klein, 1991; Almaguer and Blade, 1990). ORs were adjusted for age, smoking, pregnancy characteristics, other jobs, hours worked, education (cosmetology school), hours standing per week, number of chemical procedures per week, hair dyes per week, bleachings per week, permanents per week, use of gloves, beauty salon characteristics, and use of alcohol or formaldehyde disinfectants.

An elevated OR of 2.1 (95% CI: 1.0–4.3) was reported with the use of formaldehyde-based disinfectants adjusted for maternal characteristics and other workplace exposures. Other chemical exposures were also associated with spontaneous abortion, including number of chemical services per week, hair dyes, bleaches, and alcohol-based disinfectants. Strengths of this study include adjustment for important confounding risk factors for spontaneous abortion, detailed collection of interview-based information, a favorable response rate, and the fact that the index population had a high likelihood of formaldehyde exposure. These data provide overall support for an association between formaldehyde exposure and spontaneous abortion.

In a retrospective cohort study by Taskinen et al. (1999) of female woodworkers in Finland, exposure to formaldehyde was associated with delayed conception and spontaneous abortion. The subjects, recruited from a woodworkers' union and other businesses involving wood processing, were linked to a national register of births. Women were included if they were born between 1946 and 1975, had a live birth at age 20–40 years during 1985–1995, had worked in the wood processing industry for at least 1 month, and had first employment in the wood processing industry beginning at least 6 months before the index pregnancy. The first pregnancy that fulfilled the above criteria was the index pregnancy. There were 1,094 women with these criteria. Information about personal characteristics, pregnancies, and exposures were collected from mailed questionnaires for which the response rate was 64%. After other exclusions

(primarily infertility history, unknown time to pregnancy, and contraceptive failure), the final sample included 602 women.

Estimates of mean daily exposure to formaldehyde were based on measurements taken at the women's factories of employment during the early 1990s. Where measurements were unavailable, measurements from equivalent industries were used. An exposure index representing a TWA exposure was established for every person in the study based on the concentration of workplace formaldehyde multiplied by the proportion of the workday exposed to formaldehyde. The investigators categorized TWA formaldehyde exposure into categories of low (mean of 18 ppb), medium (mean of 76 ppb), and high (mean of 219 ppb) exposure.

Time-to-pregnancy data were analyzed by a discrete proportional hazards regression procedure with, as the outcome, a fecundability density ratio (FDR), in which a ratio of average incidence densities of pregnancies for exposed women was compared with that of the employed, unexposed women. As explained by Taskinen et al. (1999), an FDR significantly below unity suggests that conception was delayed. The age-, employment-, smoking-, alcohol consumption-, parity-, and menstrual irregularity-adjusted FDR was 0.64 (95% CI: 0.43–0.92) for women exposed to high formaldehyde levels compared with the unexposed controls, indicating that there was a substantial delay in time to conception in this group of women. Among a subset of women with high exposure who did not use gloves, the FDR was even lower (0.51 [95% CI: 0.28–0.92]), suggesting that these results might be explained in part through dermal contact with formaldehyde or might indicate an individual's failure to follow appropriate precautions, which might have increased inhalation exposures in other ways. Exposure to solvents, wood dust and other dusts, and phenols was not associated with decreased fecundability.

The investigators further conducted an analysis of the risk of spontaneous abortion after carefully including only women who had the same workplace during the year of the spontaneous abortion as they had during the beginning of the time-to-pregnancy period. Spontaneous abortion was associated with formaldehyde exposure in the low exposure group (OR = 2.4 [95% CI: 1.2–4.8]), in the medium exposure group (OR = 1.8 [95% CI: 0.8–4.0]), and in the high exposure group (OR = 3.2 [95% CI: 1.2–8.3]). Endometriosis was also associated with the highest formaldehyde level (OR = 4.5 [95% CI: 1.0–20.0]).

This study by Taskinen et al. (1999) was a well-designed population-based case-control study that appears to have been well executed and appropriately analyzed. The study population of Finnish women was well defined and adequately selected so as to allow for meaningful comparisons of health effects between individuals with different levels of exposure to formaldehyde. The participation rate was 64%, which is low enough to raise a concern about the potential for selection bias. However, the authors noted that selection bias has not influenced the

results of other reproductive epidemiology studies reporting results on smoking, irregular menstruation, and earlier miscarriages, which are known to lengthen the time to pregnancy (Bolumar et al., 1996; Sallmén et al., 1995; Baird and Wilcox, 1985). Furthermore, there is no evidence to support conjecture that an individual's decision to participate in this study would be differential with respect to their workplace formaldehyde exposures while being non-differential with respect to the other exposures of interest, including organic solvents, wood dust, and phenols. Since the women who chose to participate in this study were not likely to be aware of the specific hypotheses under investigation, nor could they have known the formaldehyde exposures that were independently estimated by an industrial hygienist, selection bias is not a likely explanation for the findings of adversity.

Data on pregnancy history, including spontaneous abortions, were collected by questionnaire. Spontaneous abortion is the most common adverse outcome of pregnancy (Klein et al., 1989), and retrospective self-report of spontaneous abortion has been found to match well with prospectively collected reproductive histories (Wilcox and Horney, 1984). Many spontaneous abortions, however, are missed with self-reporting with the magnitude likely exceeding 25%, but only rarely do women self-report false positive events (Wilcox and Horney, 1984). The effect of such an undercount is to cause a bias towards the null when the likelihood of undercounting is unrelated to formaldehyde exposure. The implication is that the observed association of increased risk of spontaneous abortion associated with occupational exposure to formaldehyde may be an underestimation of the true risk.

Two studies (Hemminki et al., 1985, 1982) specifically assessed the effects of formaldehyde exposure and reported no significant increase in the risk of spontaneous abortion. Hemminki and colleagues (1982) conducted a retrospective cohort study of nurses who were potentially exposed to chemical sterilizing agents, including formaldehyde, ethylene oxide, and glutaraldehyde. The risk of having a spontaneous abortion among the women on the sterilizing staff was compared with that among the control population of nursing auxiliaries whom the supervisory nurses thought to be unexposed to the chemical sterilizing agents during the previous three decades. However, no measurements of the chemical sterilizing agents were taken. Information about exposure to chemical sterilizing agents was obtained from the supervising nurses. When the women were conducting sterilizing procedures during their pregnancies, the frequency of spontaneous abortion was 15.1% compared with 4.6% for the nonexposed pregnancies among the sterilizing staff. The increased frequency of spontaneous abortion correlated with exposure to ethylene oxide but not with exposure to glutaraldehyde or formaldehyde. The investigators reported that ethylene oxide concentrations have been measured in many sterilizing units in Finnish hospitals; 8-hour weighted mean concentrations

have ranged from 0.1 to 0.5 ppm with peak concentrations up to 250 ppm (measurements by the Finnish Institute of Occupational Health) (Hemminki et al, 1982). No measurements of glutaraldehyde concentrations were available. Hemminki et al. (1982) reported that exposure to formaldehyde in the sterilization units may be minimal, particularly when gas chambers are used. The range of formaldehyde concentrations measured in sterilizing units has been reported as 0.03–3.5 ppm.

It is not clear that the unexposed women who served as controls were an appropriate comparison group to the sterilizing staff. The investigators reported that, among the sterilizing staff, those women who were unexposed during pregnancy experienced a rate of spontaneous abortion of 4.6% but that, among the comparison population of nursing auxiliaries who were presumed to be unexposed, the rate of spontaneous abortion was 10.5%. Had the nursing auxiliaries been an appropriate comparison group, it would be expected that their rate of spontaneous abortion would be similar to the unexposed sterilizing staff. Given this anomaly in study design and the unknown concentrations of formaldehyde exposure that were assessed as positive or negative by supervisory nurses regarding occupational exposures in the previous 30 years, it is concluded that this report of no association between formaldehyde exposure and the risk of spontaneous abortion does not temper the conclusion that formaldehyde exposure has been shown to increase the risk of spontaneous abortion.

A second study by the same lead author (Hemminki et al., 1985) used a different study design to reassess the hypothesis that chemical exposures common in the field of nursing could be risk factors for spontaneous abortion. This case-control study found no increase in the risk of spontaneous abortion associated with exposure to formaldehyde. The head nurses at each hospital were asked by the investigators whether each case or control had been exposed to formaldehyde during a given 3-month period corresponding to the first trimester of a study participant's pregnancy during 1973–1979. Formaldehyde exposure was assessed as positive or negative for either use as a sterilizing agent or use of sterilized instruments. The reported crude OR for formaldehyde exposure was 0.6; no CIs were provided. From the data reported in Table 2 in Hemminki et al. (1985), the unadjusted OR and its CI can be computed post hoc as OR (0.70 [95% CI: 0.28–1.73]). The authors acknowledged that the study failed to distinguish between sterilizing work and the use of sterilized instruments, where only very small exposures could be expected. Given the likelihood of extreme exposure misclassification and the presentation of only crude results without control of potential confounding for formaldehyde, these results do not appear to be exculpatory of a true causal association between formaldehyde exposure and the risk of spontaneous abortion.

A meta-analysis of formaldehyde exposure and spontaneous abortion was conducted by Collins et al. (2001b). However, the published results should be interpreted with caution. This meta-analysis included one very large null study of paternal formaldehyde exposure along with seven studies of maternal exposure. The two null studies by Hemminki et al. (1985, 1982) were also included without consideration of the potentially extreme exposure misclassification that may have attenuated any true adverse effect. Nevertheless, the overall reported meta-analytic RR for parental formaldehyde exposure based on eight maternal and paternal exposure studies was 1.6 (95% CI: 0.9–2.7). For case-control studies the RR was 1.8 (95% CI: 0.7–4.8), and for cohort studies the RR was 1.7 (95% CI: 1.2–2.3). Collins et al. (2001b) argued that the method of exposure evaluation may have influenced the observed results; they stated that several of the studies whose exposures were based on the investigator's judgment were likely misclassified, which may have obscured the true relationship, while other studies that assessed exposure based on self-reporting could have suffered from recall bias. They report that RRs were higher for studies based on self-reported exposures (RR = 1.9 [95% CI: 1.3–2.6]) than those based on objective exposure assessments (RR = 1.5 [95% CI: 0.6–3.7]) and suggested that this difference might reflect recall bias in the exposure assessment. However, for recall bias to have been operable in these studies, the women who provided self-reported data on pregnancy history and occupational exposure would have had to appreciate that the hypothesis of interest was the specific effect of formaldehyde on the risk of spontaneous abortion. In the specific case of the study by Taskinen and colleagues (1999), the investigator also looked at the effects of other exposures, such as organic solvents, dust, and phenols, and did not report adverse effects. It is therefore unlikely that the women providing exposure data were doing so in a manner indicative of recall bias. If the supposition of non-differential misclassification error in exposure is indeed correct, the observed results of the meta-analysis would likely have been biased towards the null. Therefore, the true RR for maternal formaldehyde could be higher than Collins et al. (2001b) reported and would likely be statistically significant. Had the study of paternal exposure been set aside, the meta-analysis almost surely would have shown a statistically significant increase in the risk of spontaneous abortion associated with maternal formaldehyde exposure. This single study reported a null finding based on exposure assessment from census records of employment, and, as the largest of the studies in the meta-analysis, it contributed the greatest weight.

Lastly, Collins and coworkers (2001b) suggested that there were potential confounding factors in each of the workplaces that might have produced the observed findings of increased risk of spontaneous abortion associated with formaldehyde. While each of these occupational studies focused on women who were co-exposed to formaldehyde and other chemicals, the occupational groups were quite different and had different sets of co-exposures. The

woodworkers in the Taskinen et al. (1999) study were potentially co-exposed to organic solvents related to painting and lacquering, dusts, and phenols, none of which was shown to be an independent predictor of adverse risk. The cosmetologists studied by John et al. (1994) were co-exposed to hair dyes, bleach, alcohol-based disinfectants, and chemicals specific to services, such as fingernail sculpturing, but, in analyses that were specifically adjusted for other work exposures and their potentially confounding effects, the investigators reported an OR of 2.1 (95% CI: 1.0–4.3) for the use of formaldehyde-based disinfectants. The laboratory workers studied by Axelsson et al. (1984) were potentially co-exposed to a wide range of solvents, but the miscarriage rate was highest among those exposed to formaldehyde, and, for a potential confounder to entirely explain an observed effect of another exposure, it must be more strongly associated with the adverse outcome.

It does not appear that the collective results of formaldehyde exposures associated with increased risk of spontaneous abortion—often in spite of exposures being crudely measured—can be explained by information bias or confounding.

The findings by Taskinen et al. (1999) of reduced fertility and increased risk of spontaneous abortion are internally consistent and coherent with other reports of increased risk of pregnancy loss associated with exposure to formaldehyde (John et al., 1994; Taskinen et al., 1994; Seitz and Baron, 1990; Axelsson et al., 1984). Absent evidence of alternative explanation for these findings, it is concluded that exposure to formaldehyde is associated with pregnancy loss and diminished fertility.

4.1.1.7.2. ***Congenital malformations.*** Only three studies have reported on the epidemiologic evidence of an association between formaldehyde exposure and the risks of births having congenital malformations. In the earliest study by Hemminki et al. (1985), the investigators presented an analysis of 34 congenital malformations from the Finnish Register of Congenital Malformations and compared them with a group of 95 controls from those used in the larger study. An association was found between formaldehyde exposure and malformations based on three exposed cases (OR = 1.8).

The case-control study by Taskinen et al. (1994) of effects of occupational exposure to chemicals used in laboratories in Finland examined the potential effects of exposure to formalin on both spontaneous abortions and congenital malformation. The investigators reported on a study of 36 laboratory workers with a child registered in the Finnish Register of Congenital Malformations and 105 controls. There was no association between formalin and congenital malformations.

A Lithuanian study (Dulskiene and Gražulevičiene, 2005) for which only a brief summary is available in English investigated the risk of congenital heart malformations as a result of exposure to 43 different agents. The number of births included in the study was not given in the English abstract. Exposure to residential ambient formaldehyde concentrations of >2.42 μg/m^3 (0.002 ppm) was associated with a 24% increase in the risk of congenital heart malformations (OR = 1.24 [95% CI: 0.81–2.07]). The details of this study are unavailable in English translation, making it impossible to critically analyze details, such as co-exposure and other possible confounders.

4.1.1.7.3. ***Low birth weight.*** A case-control study by Gražulevičiene et al. (1998) examined the association of low birth weight (<2,500 grams) and air pollutants, including formaldehyde, particulates, sulfur dioxide, lead, ozone, and nitrogen dioxide, measured in 12 areas in the city of Kaunas, Lithuania. This city has conducted environmental pollutant measurements since 1993, and the investigators classified formaldehyde exposure based on the area of residence of the study subjects. Formaldehyde levels in the 12 districts of Kaunas in 1994 ranged from 1.36 to 5.28 μg/m^3 (0.0011–0.0043 ppm), with a citywide average of 3.14 μg/m^3 (0.0026 ppm). Information on infants came from a birth registry. There were 244 cases of low birth weight and 4,089 normal controls born in 1994. Personal data came from record-based prenatal interviews, and pregnancy data came from hospital records.

The crude RR of low birth weight among women exposed to the highest airborne formaldehyde level was 1.68 (95% CI: 1.24–2.27). After adjustment for age, occupation, hazardous work, education, marital status, smoking, hypertension, and other air pollutants, the OR was still elevated but no longer statistically significant (OR 1.37 [95% CI: 0.90–2.09]). Although formaldehyde exposure was the only single air pollutant associated with low birth weight, factors such as smoking, marital status, and pregnancy-related factors had more of an impact on birth weight. Total suspended particulates (OR 2.58 [95% CI: 1.34–4.99]) and hazardous work (OR 2.62 [95% CI: 1.12–6.10]), which was not defined by the authors, were also related to low birth weight.

Aside from studies of birth weight deficits from tobacco smoke and occupational exposure, the literature on exposure to ambient air pollutants to support the investigators' hypothesis is limited. The strength of the association between total suspended particulates and low birth weight supports the idea that incidence of birth weight <2,500 grams may be related to atmospheric pollution, although this finding may not be specific to formaldehyde. Because of the large number of variables evaluated in the analysis, large fluctuations in the atmospheric

formaldehyde measurements, co-exposure to other pollutants, and geographic variability of low birth weight, it is difficult to estimate the impact of formaldehyde alone on low birth weight.

4.1.1.7.4. ***Summary.*** Although all studies on potential developmental toxicity of formaldehyde have limitations and do not uniformly report positive results, the associations between spontaneous abortion, delayed conception, or reproductive outcomes and formaldehyde exposure in multiple studies cannot be dismissed, because several studies report concordant findings across several populations and study methodologies. The results of most of the studies with positive findings were adjusted for many potentially confounding factors that may be related to spontaneous abortion and infertility, including smoking and alcohol use, pregnancy and reproductive history, and other chemical exposures.

The association between fertility and formaldehyde (Taskinen et al., 1999) stands out because of its strong quantitative statistical analysis, adequate sample size, and rigorous exposure assessment. This study was designed to specifically assess the effect of formaldehyde on reproductive outcomes. Furthermore, it was the only study with an exposure assessment based on quantitative measurements from the subject's workplace. Moreover, the investigators conducted a multivariable survival analysis that approximates a longitudinal life table or person-year analysis while simultaneously adjusting for important confounders. The findings were strengthened by statistically significant associations between formaldehyde and spontaneous abortion and endometriosis. The fact that the use of gloves may reduce the reproductive effect of formaldehyde supports the dose-response relationship in this study, and the lack of an association between time to pregnancy and any other workplace exposures strengthens the specificity of formaldehyde effects. The results also support associations reported between formaldehyde and increased risk of spontaneous abortion because subfertility and spontaneous abortion are biologically linked (subclinical pregnancy losses are increased among women with fertility problems) (Gray and Wu, 2000; Hakim et al., 1995), and both subfertility and spontaneous abortion may be related to sensitivity to environmental agents (Correa et al., 1996).

4.1.1.8. *Oral Exposure Effects on the Gastrointestinal Tract*

No human epidemiology studies exist to determine an association between oral exposure of formaldehyde and adverse health effects in the gastrointestinal (GI) tract.

4.1.1.9. *Summary: Noncarcinogenic Hazard in Humans*

Formaldehyde has clearly and consistently been shown to be a potent sensory irritant with a variety of adverse health effects. Eye, nose, and throat irritation as a result of

formaldehyde exposure has been documented in a wide range of epidemiologic studies. Workers chronically exposed to formaldehyde have exhibited signs of reduced lung function consistent with BC, inflammation, or chronic obstructive lung disease. A well-conducted residential epidemiology study has convincingly shown a concentration response for decreased pulmonary function among children with increased formaldehyde exposures. Several cross-sectional studies have described associations between increased concentrations of formaldehyde and increased prevalence of asthma. However, two case-control studies that focused on risk factors for the initial physician diagnosis of asthma, which is indicative of atopic switching, have also shown concentration-dependent adverse effects associated with formaldehyde exposure.

Results of research on the effects of formaldehyde on tissue histology suggest that formaldehyde is also responsible for reduced mucociliary clearance and the induction of histopathologic lesions in the nose. In addition, there is evidence of neurological impairment in several studies of formaldehyde-exposed histology technicians, but confounding exposures to other neurotoxic solvents and inconsistent results prevent drawing definitive conclusions concerning the neurotoxicity of formaldehyde from these studies.

Finally, there is epidemiologic evidence that formaldehyde is associated with adverse reproductive outcomes. Four of six occupational studies found an increased risk of spontaneous abortion among formaldehyde-exposed women. Results of other studies suggested associations among formaldehyde and congenital malformations, low birth weight, and endometriosis. The strongest evidence of an association between formaldehyde and an adverse reproductive outcome came from a well-conducted study of infertility in women employed in the wood processing industry. This study found a greater than threefold increased risk of spontaneous abortion, a nearly 50% decrease in a measure of delayed conception indicating reduced fertility, and increased time to pregnancy associated with average daily formaldehyde exposures of 0.15–1 ppm.

4.1.2. Cancer Health Effects

4.1.2.1. *Respiratory Tract Cancer*

4.1.2.1.1. *NPC.* NPC is a very rare form of cancer. The incidence is less than 1 per 100,000 persons throughout most parts of the world. The most common form of NPC arises from the epithelial cells lining the nasopharynx. This presentation constitutes between 75 and 100% of all NPCs. There are two types, squamous cell carcinoma (SCC) and nonkeratinizing carcinoma. In the U.S., the 5-year survival rate for NPC is about 25% (Burt et al., 1992). Certain risk factors have been implicated in its etiology, including Epstein-Barr virus (EBV), wood dust and

particles applied to wood in its treatment, exhaust fumes, occupational smoke, and nitrosamines. The epidemiologic studies of NPC are summarized in Table 4-1.

4.1.2.1.1.1. *Cohort studies.* The International Agency for Research on Cancer (IARC) reported on eleven cohort studies of formaldehyde-exposed industry workers (Marsh et al., 2002, 1996, 1994; Andjelkovich et al., 1995; Gardner et al., 1993; Bertazzi et al., 1989, 1986; Stayner et al., 1988; Blair et al., 1987, 1986; Edling et al., 1987) and results from eight cohort studies of professional workers (Hall et al., 1991; Hayes et al., 1990; Stroup et al., 1986; Harrington and Oakes, 1984; Levine et al., 1984; Walrath and Fraumeni, 1984, 1983; Friedman and Ury, 1983) (IARC, 2006).

Several of these studies measured exposure to formaldehyde at 10 production facilities that contributed to a cohort that has been studied by Blair et al. (1987, 1986) and Hauptmann et al. (2004). The National Cancer Institute (NCI) conducted a mortality study of solid tumors among a cohort of 25,619 workers who were employed in these 10 plants that produced or used formaldehyde in the U.S. before 1966 (Blair et al., 1987, 1986). Subjects were followed to January 1, 1980, accruing approximately 600,000 person-years of follow-up. Hauptmann et al. (2004) updated the cohort to December 31, 1994 and reported a significant excess risk of NPC in exposed workers based on U.S. population death rates (standardized mortality ratio [SMR] = 2.1 [95% CI: 1.05–4.21]). In addition to the SMR based on an external comparison population, RRs were presented based on internal comparisons of similar workers in order to minimize potential selection bias due to the well-known healthy worker effect (HWE). For NPC, RRs increased with several different exposure metrics, including average exposure intensity, cumulative exposure, highest peak exposure, and duration of exposure to formaldehyde (*p* values for tests for trends were 0.066, 0.025, <0.001, and 0.147, respectively). These results were based on primary data analyses of the health and exposure data collected by the NCI, according to their research protocol and analyzed accordingly. As such, the reported statistical *p* values may be appropriately interpreted as showing that these workers were at increased risk of NPC associated with exposure to formaldehyde. These NCI investigations controlled for potential selection bias due to the HWE and for several potential confounders, including calendar year, age, sex, race, and pay category. There was no evidence of any differential measurement error that could have produced the observation of a spurious association. Any non-differential measurement error would likely have led to an observed effect of formaldehyde that was less than that which would otherwise have been observed in the absence of measurement error.

Table 4-1. Cohort and case-control studies of formaldehyde cancer and NPC

Reference	Study design	Exposure assessment	Results; statistical significance (number observed deaths)				
Hauptmann et al. (2004)	Retrospective cohort mortality study of 25,619 workers employed at 10 formaldehyde plants in the U.S. followed from either plant start-up or first employment through 1994. The 10 plants produced formaldehyde (3 plants), molding compounds (3 plants), photographic film (2 plants), plywood (1 plant), and formaldehyde resins (6 plants).	Exposure estimates[a] based on job titles, tasks, visits to plants by study industrial hygienists, and monitoring data measurements. Peak exposure = short-term excursions >8-hour TWA formaldehyde intensity and knowledge of job tasks. Workers contributed pre-exposure person time to nonexposed category. RRs were from Poisson regression models, using a 15-year lag to account for tumor latency.	Overall				
			Nonexposed	SMR	1.56	(95% CI: 0.39–23)	(2)
			Exposed	SMR	2.10	(95% CI: 1.05–21)	(8)
			Peak exposure (ppm)				
			0	RR[b]	1.00	(95% CI: NS)	(2)
			>0 to <2.0		N/A	(95% CI: NS)	(0)
			2.0 to <4.0		N/A	(95% CI: NS)	(0)
			4.0 or greater		1.83	(95% CI: NS)	(7)
						Trend p < 0.001	
			Average intensity of exposure (ppm)				
			0	RR[b]	1.00	(95% CI: NS)	(2)
			≤0.5		N/A	(95% CI: NS)	(0)
			0.5 to <1.0		0.38	(95% CI: NS)	(1)
			1.0 or greater		1.67	(95% CI: NS)	(6)
						Trend p = 0.066	
			Cumulative exposure (ppm-years)				
			0	RR[b]	2.40	(95% CI: NS)	(2)
			>0 to <1.5		1.00	(95% CI: NS)	(3)
			1.5 to <5.5		1.19	(95% CI: NS)	(1)
			5.5 or more		4.14	(95% CI: NS)	(3)
						Trend p = 0.025	
			Duration (years)				
			0	RR[b]	1.77	(95% CI: NS)	(2)
			>0 to <5		1.00	(95% CI: NS)	(4)
			5 to <15		0.83	(95% CI: NS)	(1)
			15 or more		4.18	(95% CI: NS)	(2)
						Trend p = 0.147	
Marsh et al. (2002)	Retrospective cohort mortality study of 7,328 workers hired up to 1984	Worker-specific exposure[a] from job exposure matrix based on available	<u>Cohort study</u>				
			Overall				
			U.S.	SMR	4.94	(95% CI: 1.99–10)	(7)

Table 4-1. Cohort and case-control studies of formaldehyde cancer and NPC

Reference	Study design	Exposure assessment	Results; statistical significance (number observed deaths)					
	and followed until 1998 in one plant from Hauptmann et al. (2004). Mortality was compared with death rates in two Connecticut counties and the U.S.	sporadic sampling data from 1965−1987, job descriptions, and verbal job descriptions by plant personnel and industrial hygienists. Exposures ranked on a 7-point scale with exposure range assigned to each rank. 17% of jobs validated with company monitoring data; remaining 83% based on professional judgment. Assumed pre-1965 exposure levels same as post-1965 levels.					
				County	SMR	5.00	(95% CI: 2.01–10)	(7)
			Short-term worker (<1 year)					
					SMR	5.35	(95% CI: 1.46–14)	(4)
			Long-term worker (1 or more years)					
					SMR	4.59	(95% CI: 0.95–13)	(3)
			Year of hire					
				1941−1946	SMR			(0)
				1947−1956	SMR	8.13	(95% CI: 2.98–18)	(6)
				1957 or later	SMR	2.63	(95% CI: 0.07–15)	
			Cumulative exposure (ppm-years) county					
				Unexposed	SMR			(0)
				0 to <0.004	SMR	3.97	(95% CI: 0.10–22)	(1)
				0.004–0.219	SMR	5.89	(95% CI: 1.22–17)	(3)
				0.22+	SMR	7.51	(95% CI: 1.55–22)	(3)
			Average exposure (ppm) county					
				Unexposed	SMR			(0)
				0 to <0.03	SMR	2.41	(95% CI: 0.06–13)	(1)
				0.03–0.159	SMR	15.30	(95% CI: 4.16–39)	(4)
				0.16+	SMR	4.13	(95% CI: 0.50–15)	(2)
			Duration of exposure to >0.2 ppm (years)					
				Unexposed	SMR	3.01	(95% CI: 0.36–11)	(2)
				0 to <1	SMR	4.81	(95% CI: 0.58–17.4)	(2)
				1–9	SMR	4.04	(95% CI: 0.10–22.51)	(1)
				10+	SMR	27.60	(95% CI: 3.34–100)	(2)
			Duration of exposure to ≥0.7 ppm (years)					
				Unexposed	SMR	3.64	(95% CI: 0.99–9.31)	(4)
				<1	SMR	9.51	(95% CI: 1.15–34.4)	(2)
				1+	SMR	11.07	(95% CI: 0.28–61.67)	(1)

Table 4-1. Cohort and case-control studies of formaldehyde cancer and NPC

Reference	Study design	Exposure assessment	Results; statistical significance (number observed deaths)				
Marsh et al. (2002)	A nested case-control analysis of all pharyngeal cancer cases also conducted with four controls randomly selected from cohort and matched on age, year of birth, race, and sex. Conditional logistic model used for nested case-control analysis.		Nested case-control analysis[c]				
			Duration of exposure to >0.2 ppm (years)				
			Unexposed	OR	1.00		(8)
			0 to <1	OR	1.13	(95% CI: 0.24–5.29)	(6)
			1–9	OR	1.38	(95% CI: 0.18–9.03)	(3)
			10+	OR	9.49	(95% CI: 0.55–701)	(5)
			Duration of exposure to ≥0.7 ppm (years)				
			Unexposed	OR	1.00		(16)
			<1	OR	0.52	(95% CI: 0.08–2.45)	(4)
			1+	OR	1.11	(95% CI: 0.06–11.3)	(2)
Hayes et al. (1990)	Proportionate mortality cohort study of 4,046 U.S. male embalmers and funeral directors who died between 1975 and 1985.	Exposure presumed.	Overall	PMR	2.16		(4)
Hansen and Olsen (1995)	Proportionate incidence study of 2,041 men with cancer who died between 1970 and 1984, identified from the Danish Cancer Registry and matched with the Danish Supplementary Pension Fund, whose longest work experience occurred at least 10 years before the cancer diagnosis. The SPIR measured the proportion of cases of NPC in formaldehyde-associated companies relative to the proportion of cases of NPC among all employees in Denmark.	Linked companies through tax records to the national Danish Product Register.	Overall	SPIR	1.3	(95% CI: 0.03–3.2)	(4)

Table 4-1. Cohort and case-control studies of formaldehyde cancer and NPC

Reference	Study design	Exposure assessment	Results; statistical significance (number observed deaths)
Olsen et al. (1984)	Case-control study of 314 cases of NPC from Danish Cancer Registry linked to the Registry during 1970–1982. Three controls/case sampled with cancer of the colon, rectum, breast, and prostate by age, sex, and year of diagnosis of cases.	Employment histories after 1964 from files maintained by Danish Cancer Registry evaluated by industrial hygienists.	Men OR 0.7 (95% CI: 0.3–1.7) Women OR 2.6 (95% CI: 0.3–22)
West et al. (1993)	Case-control study of 104 non-Chinese incident NPC cases from the Philippine General Hospital matched with 104 hospital and 101 community controls.	Personal interview, including job history. Industrial hygienists blinded to case-control status reviewed and rated jobs as likely or unlikely to be exposed. Analysis by length of exposure, length of exposure lagged 10 years, time since first exposure, and age at first exposure, based on date of interview or death.	Length of exposure (years) <15 RR[d] 2.7 (95% CI: 1.1–6.6) 15 or more 1.2 (95% CI: 0.5–3.2) Length of exposure lagged 10 years (years) <15 RR[d] 1.6 (95% CI: 0.7–3.8) 15 or more 2.1 (95% CI: 0.7–6.2) Years since first exposure <25 RR[d] 1.3 (95% CI: 0.6–3.2) 25 or more 2.9 (95% CI: 1.1–7.6) Age at first exposure (years) <25 RR[d] 2.7 (95% CI: 1.1–6.6) 25 or older 1.2 (95% CI: 0.5–3.3)

Table 4-1. Cohort and case-control studies of formaldehyde cancer and NPC

Reference	Study design	Exposure assessment	Results; statistical significance (number observed deaths)
Roush et al. (1987)	Population-based case-control study of 173 male cases from the Connecticut Tumor Registry who died of any cause from 1935–1975. 605 male controls randomly selected from state death certificates during same time period.	Four categories: I, probably exposed most of working life; II, probably exposed most of working life and probably exposed 20+ years before death; III, probably exposed most of working life and probably to high level in some year; IV, probably exposed most of working life and probably exposed to high level 20+ years before death.	Exposure levels I — OR[e] 1.0 (95% CI: 0.6–1.7) II 1.3 (95% CI: 0.7–2.4) III 1.4 (95% CI: 0.6–3.1) IV 2.3 (95% CI: 0.9–6.0)
Vaughan et al. (1986a)	Population-based case-control study of 27 incidence cases of NPC (during 1980–1983) from a 13-county area (Washington State Cancer Surveillance System) and 552 matched controls from random digit dialing in same area, for occupational exposures.	Interview-based information on lifetime occupational exposure to formaldehyde with cases, next of kin, and controls. Exposure from available hygiene data, NIOSH and other data, and NCI job exposure linkage system. Exposure levels based on investigator's judgment. Exposure score A: weighted sum of no. years spent per job (weight = estimated formaldehyde level). B: weighted sum of no. years spent per job with 15-year lag (latency).	Intensity Low — OR[f] 1.2 (95% CI: 0.5–3.3) Medium/high 1.4 (95% CI: 0.4–4.7) No. years exposed 1–9 — OR[f] 1.2 (95% CI: 0.5–3.1) 10 or more 1.6 (95% CI: 0.4–5.8) Exposure score A: no lag 5–19 — OR[f] 0.9 (95% CI: 0.2–3.2) 20 or more 2.1 (95% CI: 0.6–7.8) Exposure score B: 15-year lag 5–19 — OR[f] 1.7 (95% CI: 0.5–5.7) 20 or more 2.1 (95% CI: 0.4–10)

Table 4-1. Cohort and case-control studies of formaldehyde cancer and NPC

Reference	Study design	Exposure assessment	Results; statistical significance (number observed deaths)				
Vaughan et al. (1986b)	Population-based case-control study of 27 incidence cases on NPC (during 1980–1983) from a 13-county area (Washington State Cancer Surveillance System) and 552 matched controls from random digit dialing in same area, for residential exposures.	No direct measurements. Interview information from cases/controls or next of kin: residence in past 50 years, use of particleboard or plywood, and lifetime occupational and chemical exposure history.	Years of residence in mobile home				
				1–9	OR[g]	2.1	(95% CI: 0.7–6.6)
				10 or more		5.5	(95% CI: 1.6–19)
			Years of exposure to particleboard				
				1–9	OR[g]	1.4	(95% CI: 0.5–3.4)
				10 or more		0.6	(95% CI: 0.2–2.3)
			Exposure source				
				Occupation only	OR[g]	1.7	(95% CI: 0.5–5.7)
				Mobile home only		2.8	(95% CI: 1.0–7.9)
				Both		6.7	(95% CI: 1.2–39)
Vaughan et al. (2000)	Population-based case-control study of 196 incident epithelial NPC patients identified from 5 U.S. cancer registries from 1987–1993 matched with 244 controls from random digit dialing in the same geographic regions.	Interviewed for lifetime occupational and chemical exposure. Exposure estimates by industrial hygienist without knowledge of case-controls status. Probability of exposure: definitely not or unlikely (<10%); possible (≥10% and <50%); probable (>50% and <90%); and definite ≥90%). Jobs with potential exposure assigned estimated concentration levels based on TWA: low (<10 ppm), moderate (≥10 and <50 ppm), and high (≥50 ppm).	Possible, probable, or definite exposure (61 cases, 76 controls)				
				Ever	OR[h]	1.6	(95% CI: 1.0–2.8)
				Duration (years)			
				1–5	OR[h]	0.9	(95% CI: 0.4–2.1)
				6–17		1.9	(95% CI: 0.9–4.4)
				18 or more		2.7	(95% CI: 1.2–6.0)
							Trend p = 0.014
			Cumulative exposure (ppm-years)				
				0.05–0.40	OR[h]	0.9	(95% CI: 0.4–2.0)
				0.41–1.10		1.8	(95% CI: 0.8–4.1)
				≥1.10		3.0	(95% CI: 1.3–6.6)
							Trend p = 0.033
			Probable or definite exposure (27 cases, 30 controls)				
				Ever	OR[h]	2.1	(95% CI: 1.1–4.2)
				Duration (years)			
				1–5	OR[h]	2.0	(95% CI: 0.8–5.0)
				6–17		3.3	(95% CI: 0.9–12)
				18 or more		1.6	(95% CI: 0.5–5.6)
							Trend p = 0.069

Table 4-1. Cohort and case-control studies of formaldehyde cancer and NPC

Reference	Study design	Exposure assessment	Results; statistical significance (number observed deaths)			
			Cumulative exposure (ppm-years)			
			0.05–0.40	OR[h]	1.9	(95% CI: 0.7–4.9)
			0.41–1.10		2.6	(95% CI: 0.7–9.5)
			≥1.10		2.2	(95% CI: 0.7–7.0)
						Trend p = 0.13
			Definite exposure (10 cases, 2 controls)			
			Ever	OR[h]	13.3	(95% CI: 2.5–70)
Hildesheim et al. (2001)	Population-based case-control study of 375 incident cases from two Taiwanese hospitals between 7/15/91 and 12/31/94. 325 controls came from a random sample of households from a national household registration system and were age, sex, and area-of-residence matched. Tumors were histologically confirmed. All subjects were tested for the EBV. Exposure metrics were stratified by seropositivity.	In-person interviews collected information on risk factors and job history for jobs held >1 year, including length of time job held, type of industry, and tasks, tools, and materials used on the job. Industrial hygienist assigned Standard Industry Classification/ Standard Occupational Classification codes to jobs, assigning each a probability and intensity of exposure on a 0–9 scale. Exposure metrics were duration, average intensity (intensity scale), average probability (probability scale), cumulative (average intensity), years since 1st exposure, and age at 1st exposure. Analysis of	Ever exposed	RR[i]	1.4	(95% CI: 0.9–2.2)
			Duration (years)			
			≤10	RR[i]	1.3	(95% CI: 0.69–2.3)
			>10	RR[i]	1.6	(95% CI: 0.91–2.9)
						Trend p = 0.08
			EBV pos[j]			
			Ever exposed	RR[i]	2.7	(95% CI: 1.2–6.2)
			≤10	RR[i]	2.8	(95% CI: 0.8–9.7)
			>10		2.6	(95% CI: 0.9–7.7)
			Cumulative exposure (average intensity-years)			
			<25	RR[i]	1.3	(95% CI: 0.7–2.4)
			≥25	RR[i]	1.5	(95% CI: 0.9–2.7)
						Trend p = 0.10
			EBV pos[j]			
			<25	RR[i]	4.0	(95% CI: 0.9–17)
			≥25	RR[i]	2.2	(95% CI: 0.8–5.8)
			Years since 1st exposure			
			<20	RR[i]	2.3	(95% CI: 1.0–5.8)
			≥20	RR[i]	1.2	(95% CI: 0.8–2.0)

Table 4-1. Cohort and case-control studies of formaldehyde cancer and NPC

Reference	Study design	Exposure assessment	Results; statistical significance (number observed deaths)				
		nonkeratinizing or undifferentiated tumors yielded similar results as overall analysis.	EBV pos[h]				
				<25	RR[i]	2.3	(95% CI: 0.5–10)
				≥25	RR[i]	2.8	
			Age at 1st exposure				
				<20	RR[i]	1.3	(95% CI: 0.8–2.0)
				≥20	RR[i]	3.4	(95% CI: 0.9–12)
			EBV pos[j]				
				<25	RR[i]	2.6	(95% CI: 1.1–6.5)
				≥25	RR[i]	3.1	(95% CI: 0.4–24)

[a]Exposure estimates by Hauptmann et al. (2004) were 10 times higher than those of Marsh et al. (2002).
[b]Adjusted for calendar year, age, sex, race, and pay category (salaried versus wage).
[c]Results for cumulative and average intensity of exposure are not included here because condition logistic regression produces unstable estimates for this small number of cases.
[d]Adjusted for years since first exposure to dust and exhaust fumes.
[e]Adjusted for age at death, year at death, and availability of occupational information (Roush et al., 1987).
[f]Adjusted for cigarette smoking, alcohol consumption, gender, and age.
[g]Adjusted for ethnic origin and cigarette smoking.
[h]Adjusted for age, sex, race, SEER site, cigarette usage, proxy status, and education.
[i]Adjusted for age, sex, education, and ethnicity.
[j]EBV seropositives defined as positive for one of the following anti-EBV antibodies known to be associated with NPC: viral capsid antigen IgA, EBV nuclear antigen 1 IgA, early antigen IgA, DNA binding protein IgG, and anti-DNase IgG.
N/A = not applicable, NS = not significant, PMR = proportionate mortality ratio.

Following these reports of increased risk of NPC associated with formaldehyde exposure, a series of post hoc analyses of similar data were undertaken by Marsh and coworkers (Marsh et al., 2007a, b, 2002, 1996; Marsh and Youk, 2005). Briefly, these studies focused on the specific findings from a single plant in the NCI cohort (Wallingford, Connecticut) that generated the majority of the NPC cases.

In the most recent subsequent report, Marsh et al. (2007a) continues to argue against a formaldehyde-NPC association. Although earlier reports speculated on anecdotal evidence that the statistically significant excess risk of NPC observed at the Wallingford, Connecticut, plant reflected the influence of unmeasured nonoccupational risk factors associated with employment outside the plant, the new report (Marsh et al., 2007a) suggests that occupational or hobby-related work in silversmithing may have confounded the observed effect of formaldehyde on the increased risk of NPC. In this report, Marsh et al. (2007a) show that their subjectively assessed work in silversmithing is strongly associated with NPC. While the reported ORs are indeed quite high, the estimates are extremely unstable and it is not clear how many a priori hypotheses were tested for statistical significance. There are no citations of an association between silversmithing exposures and NPC in the medical literature. Marsh and coworkers mention that there was concordance of silver manufacturing history in the Wallingford, Connecticut, area. If silversmithing exposures are indeed independent risk factors for NPC, it would be expected that the rates of NPC in the surrounding counties with historical silver-related exposures would be elevated. However they are not increased, as evidenced by the comparability of the increased rates of NPC among the plant workers compared with both the national and local county rates that were very similar (Marsh et al., 2007a). The comparable rates indicate the counties' rates of NPC were very similar to the national rates and weaken an association between silversmithing and NPC. Given the many post hoc reexaminations of alternative hypotheses to explain the original NCI findings, it is more likely that silversmithing is an artifactual confounder.

4.1.2.1.1.2. ***Professional cohort studies.*** Two cohort studies of professional groups, such as anatomists, pathologists, embalmers, and funeral directors, examined the risk of NPC and formaldehyde exposure. In general, measurements of formaldehyde concentrations were not available in studies of professionals but are generally below 1 ppm (IARC, 1995; Korczynski, 1994; Stewart et al., 1992; Moore and Ogrodnik, 1986). Hayes et al. (1990) reported an excess risk of NPC among male professional embalmers and funeral directors, based on 4 deaths with 1.9 expected based on age, gender, and calendar-year-specific proportions of deaths in the U.S. population. Hansen and Olsen (1995) studied male Danish cancer patients employed in companies in which formaldehyde was used or produced. Only a slight excess risk of NPC was

found, based on 4 cases with 3.2 expected (standardized proportionate incidence ratio [SPIR] 1.3). Hansen and Olsen (1995) also reported on a significantly elevated risk of sinonasal cancer.

4.1.2.1.1.3. ***Case-control studies.*** Five case-control studies (West et al., 1993; Roush et al., 1987; Vaughan et al., 1986a, b; Olsen et al., 1984) reviewed by IARC in 1995 provided evidence of excess risks of NPC due to formaldehyde. Most of these studies report significant and nonsignificant elevations in risk of NPC in the range of 1.5–3.0, with some higher than 5.0. In its report, IARC (1995) concluded that, taking the data as a whole, formaldehyde appears to have a causal role in the induction of NPC, recognizing that the conclusion is based on small numbers of cancer cases.

Three case-control studies have been conducted since the 1995 IARC report. Armstrong et al. (2000) found no association between formaldehyde exposure and NPC (adjusted OR = 0.71 [95% CI: 0.34–1.43]), controlling for wood dust and industrial heat. Using data from the Surveillance Epidemiology and End Results (SEER) program, Vaughan et al. (2000) found an OR for ever-exposed persons of 3.1 (95% CI: 1.0–9.6) among cases of epithelial NPC, suggesting differences in the etiology of cancers at this site. There was a trend of increasing risk of NPC with increasing duration of exposure and cumulative exposure, controlling for wood dust exposure. Finally, Hildesheim et al. (2001) found that exposure to formaldehyde produced modest risk elevations for duration of exposure (OR = 1.6 for 10 years or less and 1.2 for over 10 years of exposure), for cumulative exposure (ORs were 1.3 for <25 years of exposure and 1.5 for 25+ years of exposure), and for years since first exposure. Among those with EBV, the OR was 2.7 (95% CI: 1.2–6.2) for ever-exposed persons. The risk was significantly higher among exposed persons whose work history was within the last 10 years (OR = 4.7 [95% CI: 1.1–20.0]) and for those followed 20+ years after exposure (OR = 2.8 [95% CI: 1.1–7.6]).

4.1.2.1.1.4. ***Summary of NPC studies.*** Findings from the large NCI cohort studies of NPC risk due to formaldehyde exposure clearly show a consistent pattern of increased risk with increased exposures. Post hoc reanalyses have challenged the interpretation of these findings but have not been able to dispute the reported excess in NPC mortality (Marsh et al., 2007a, b, 2002, 1996; Marsh and Youk, 2005). The major questions that have been raised by Marsh and coworkers highlight the observation that the NPC findings appear to depend on the results of 1 of the 10 plants that made up the NCI cohort. While it is theoretically possible for coexposures at that plant or among those workers to act as potential confounders or modifiers of the observed effect of formaldehyde on increased risk of NPC, there is no solid evidence of such a relationship that would outweigh or supersede the reported adverse effects of formaldehyde exposure. While all

of the cohort members at the Wallingford, Connecticut, plant were also exposed to particulates, the NCI investigators did observe a dose-response relationship with formaldehyde among individuals with high particulate exposures, thereby strengthening the causal interpretation of the formaldehyde relationship with an increased risk of NPC. The described association of a potential occupational relationship with silversmithing and NPC has no basis in the medical literature and is inconsistent with the supposition that this activity is common in the locality of Wallingford, Connecticut, but has not been associated with increased rates of NPC in surrounding New Haven County (Connecticut). Marsh and coworkers did report significantly increased rates of pharyngeal cancer (including NPCs) among workers from the Wallingford plant compared with both the county and national rates. It is more plausible that the observed association at the Wallingford plant reflects higher formaldehyde exposures than at other plants. The exposure levels at Plant 2 were even higher than at the Wallingford plant and were associated with a fivefold increase in risk associated with NPC, even though this was based on a single observed case and was not significant.

In addition to the evidence from the NCI cohort studies, modest additional evidence is found in the professional cohort studies of Hayes et al. (1990) and Hansen and Olsen (1995). The rarity of the disease and difficulties in obtaining valid and reliable historical exposure estimates are substantial limitations of these cohort studies. Further evidentiary support comes from the results of several case-control studies that support an increased risk of NPC from exposure to formaldehyde. The studies of Vaughan et al. (2000) and Hildesheim et al. (2001) provide evidence of an association of NPC with exposure to formaldehyde. Vaughan et al. (2000) found a dose-response relationship of NPC with increasing exposure to formaldehyde, as did Hildesheim et al. (2001). These studies, in general, are easier to conduct and may provide more statistical power for a specific level of risk estimate than do cohort studies.

4.1.2.1.2. ***Nasal and paranasal cancer***

4.1.2.1.2.1. *Case-control studies.* Eight case-control studies were evaluated in the 1995 IARC monograph regarding the risk of nasal cavity and accessory sinuses from exposure to formaldehyde (Luce et al., 1993; Roush et al., 1987; Hayes et al., 1986; Olsen and Asnaes, 1986; Vaughan et al., 1986a, b; Brinton et al., 1984; Olsen et al., 1984). Of three studies that identified a cell type, two reported a positive finding of sinonasal cancer (Hayes et al., 1986; Olsen and Asnaes, 1986). One of the positive studies did not report any exposure to the potentially confounding influence of wood dust, while the other two did report an adjustment for exposure to wood dust. Of the remaining five studies where a cell type was not identified, only Roush et al. (1987) and Olsen et al. (1984) found positive results. The remaining studies (Vaughan et al.,

1986a, b; Brinton et al., 1984) from the 1995 IARC monograph did not find associations between exposure and sinonasal cancer. Study details of the epidemiologic studies of nasal and paranasal cancer are summarized in Table 4-2. Vaughan et al. (1986b) matched 53 sinonasal cancer patients to 552 controls. Potential residential exposure to formaldehyde was estimated by utilizing residence in a mobile home with or without the presence of UFFI or particleboard or plywood as a surrogate for exposure. The authors found an OR of 1.5 for sinonasal cancer in subjects reporting residence of 10 or more years in a mobile home with UFFI before diagnosis. A higher OR (1.8) was reported for less than 10 years of mobile home residence. However, because actual formaldehyde levels in the subjects' mobile homes are unknown, the exposure estimates are, at best, imprecise surrogates that typically have the effect of attenuating any true risk. In Vaughan et al. (1986a), the same cases and controls were examined for occupational exposures to formaldehyde, but no increase in risk of nasal or paranasal cancer was reported.

More recently, Luce et al. (2002) pooled data from 12 case-control studies. Combined, these studies had 195 adenocarcinomas and 432 SCCs of the sinonasal passages compared with 3,136 controls. The authors reported a significant increase in the risk of sinonasal adenocarcinoma in men (adjusted OR = 3.0 [95% CI: 1.5–5.7]; 91 cases) and in women (adjusted OR = 6.2 [95% CI: 2.0–19.7]; 5 cases) with a high probability of exposure to formaldehyde. For SCCs, the ORs were more modest: OR = 1.2 in men and OR = 1.5 in women for a high probability of exposure to formaldehyde. In an analysis of 11 formaldehyde-exposed cases of sinonasal adenocarcinomas who were not exposed to wood dust, there was an elevated risk in men (OR = 1.9; 3 cases) and a significantly increased risk in women (OR = 11.1 [95% CI: 3.2–38.0]; 5 cases) with a high probability of exposure to formaldehyde. Limitations of these studies were the lack of information about the actual levels or intensity of exposure to formaldehyde, exposure to multiple occupational carcinogens, and the small number of cases in some subgroups. In spite of those limitations, which generally obfuscate the observation of a true underlying effect, these studies identified effects of formaldehyde that were statistically significant predictors of sinonasal cancers.

Table 4-2. Case-control studies of formaldehyde and nasal and paranasal cancer

Reference	Study design	Exposure assessment	Results, statistical significance (number of cases)				
Olsen and Asnaes (1986)	Case-control study of histologically confirmed cases of squamous cell carcinoma/lymphoepithelioma of the sinonasal cavities and paranasal cancers in 215 men and adenocarcinomas of the sinonasal cavities and paranasal cancers in 39 men matched with 2,465 controls with other cancers from the Danish Cancer Registry, 1970–1982.	Employment histories after 1964 from files maintained by Danish Cancer Registry estimated by industrial hygienists.	Squamous cell carcinoma/lymphoepithelioma				
			Ever vs. never				
				Formaldehyde only	RR	2.0	(95% CI: 0.7–5.9)
				Formaldehyde + wood dust	RR	1.6	(95% CI: 0.8–3.3)
			10 or more years since first exposure				
				Formaldehyde only	RR	1.4	(95% CI: 0.3–6.4)
				Formaldehyde + wood dust	RR	1.8	(95% CI: 0.7–4.4)
			Adenocarcinoma				
			Ever vs. Never				
				Formaldehyde only	RR	7.0	(95% CI: 1.1–44)
				Formaldehyde + wood dust	RR	40.0	(95% CI: 22–71)
			10 or more years since first exposure				
				Formaldehyde only	RR	9.5	(95% CI: 1.6–58)
				Formaldehyde + wood dust	RR	44.0	(95% CI: 22–88)
Hayes et al. (1986)	Case-control study of 91 men with SCC of the nasal cavity and paranasal sinuses, from clinical records of six medical institutions in the Netherlands. 195 controls from living and deceased males from municipal residence registries, from 1978–1981.	Cases selected from clinical records of six institutions in the Netherlands. 91 male patients and 195 controls from living and deceased males from municipal residence registries with little or no exposure to wood dust. Industrial hygienists evaluated job histories according to probability of exposure based on job records.	Industrial hygienist A				
				Any exposure	RR	3.0	(90% CI: 1.3–6.4)
				Moderate exposure		2.7	(90% CI: 1.0–7.2)
				High exposure		3.1	(90% CI: 0.9–10.0)
			Industrial hygienist B				
				Any exposure	RR	1.9	(90% CI: 1.0–3.6)
				Moderate exposure		1.4	(90% CI: 0.5–3.4)
				High exposure		2.4	(90% CI: 1.1–5.1)

Table 4-2. Case-control studies of formaldehyde and nasal and paranasal cancer

Reference	Study design	Exposure assessment	Results, statistical significance (number of cases)				
Roush et al. (1987)	Population-based case-control study of 198 male cases of sinonasal cancer from the Connecticut Tumor Registry who died of any cause in 1935–1975. Controls were 605 males dying in Connecticut during the same time period, randomly selected from state death certificates.	Occupations from city directories and evaluation of job by industrial hygienist who classed exposure into I, probably exposed to some level most of working life; II, probably exposed to some level most of working life and probably exposed to some level 20+ years before death; III, probably exposed to some level most of working life and probably exposed to a high level in some years; IV, probably exposed to some level most of working life and probably exposed to a high level 20+ years before death.	Exposure levels	Sinonasal cancer			
			I	OR[a]	0.8	(95% CI: 0.5–1.3)	
			II		1.0	(95% CI: 0.5–1.8)	
			III		1.0	(95% CI: 0.5–2.2)	
			IV		1.5	(95% CI: 0.6–3.9)	
Luce et al. (1993)	Case-control study of men with sinonasal cancer (histologically confirmed), 77 with adenocarcinoma, 59 with squamous cell carcinomas, and 25 tumors of other types, matched with 409 controls from 27 French hospitals and	Industrial hygienist estimation based on job histories from personal interviews. Subjects were broken out into no exposure, possible exposure, or probable/definite	Adenocarcinoma[b]				
			Possible exposure	OR[c]	1.28	(95% CI: 0.16–10)	
			Probable/definite exposure				
			Average level				
			≤2	OR[c]	4.15	(95% CI: 0.96–18)	
			>2		5.33	(95% CI: 1.28–22)	
			Duration (years)				
			≤20	OR[c]	1.03	(95% CI: 0.18–5.77)	
			>20		6.86	(95% CI: 1.69–28)	

Table 4-2. Case-control studies of formaldehyde and nasal and paranasal cancer

Reference	Study design	Exposure assessment	Results, statistical significance (number of cases)				
	from lists of names supplied by patients.	exposure. Those classed as probable/definite exposure further categorized into three levels of frequency of exposure during a normal workweek: 1 = <5% of the time; 2 = 5–30% of the time; and 3 = >30% of the time. Concentration was categorized into 3 levels: low (<0.0 ppm); medium (0.1–1 ppm); and high (>1 ppm). The exposure index = concentration × frequency. Cumulative level = sum of exposure indices. Average level = cumulative level/duration and ranged from 1 to 9. Nearly all cases had had wood dust exposure.	Cumulative level (years)				
			≤30	OR[c]	1.13	(95% CI: 0.19–6.95)	
			30–60		2.66	(95% CI: 0.38–19)	
			>60		6.91	(95% CI: 1.69–28)	
			Age 1st exposed (years)				
			≤15	OR[c]	9.99	(95% CI: 1.85–54)	
			16–20		4.12	(95% CI: 0.95–18)	
			>20		2.74	(95% CI: 0.58–13)	
			Date 1st exposed (years)				
			After 1954	OR[c]	6.02	(95% CI: 1.18–31)	
			Before 1954		4.26	(95% CI: 1.06–17)	
			Other cell type carcinoma[b]				
			Possible exposure	OR[c]	0.81	(95% CI: 0.15–4.36)	
			Probable/definite exposure				
			Average level				
			≤2	OR[c]	1.67	(95% CI: 0.51–5.42)	
			>2		3.04	(95% CI: 0.95–9.7)	
			Duration (years)				
			≤20	OR[c]	2.82	(95% CI: 0.94–8.4)	
			>20		1.62	(95% CI: 0.48–5.51)	
			Cumulative level (years)				
			≤30	OR[c]	2.18	(95% CI: 0.65–7.31)	
			>30		2.21	(95% CI: 0.73–6.73)	
			Age 1st exposed (years)				
			≤20	OR[c]	2.03	(95% CI: 0.63–6.54)	
			>20		2.36	(95% CI: 0.76–7.33)	
			Date 1st exposed (years)				
			After 1954	OR[c]	0.48	(95% CI: 0.05–4.35)	
			Before 1954		3.27	(95% CI: 1.15–9.33)	
Luce et al. (2002)	Pooled analysis of 195 adenocarcinomas and 432 squamous cell carcinomas of	Job exposure matrix based on interview data developed for pooled	Adenocarcinoma				
			High probability of exposure				
			Men	OR[d]	3.0	(95% CI: 1.5–5.7)	

Table 4-2. Case-control studies of formaldehyde and nasal and paranasal cancer

Reference	Study design	Exposure assessment	Results, statistical significance (number of cases)				
	the sinus/nasal cavity matched with 3,136 controls from 12 case-control studies.	analysis. Industrial hygiene data used to develop indices of exposure. 11 formaldehyde cases reported no exposure to wood dust.	Women	OR[e]	6.2	(95% CI: 2.0–20)	
			Squamous cell carcinoma				
			High probability of exposure				
			Men	OR[e]	1.2	(95% CI: 0.8–1.8)	
			Women	OR[e]	1.5	(95% CI: 0.6–3.8)	
Brinton et al. (1984)	Case-control study of 160 patients with cancer of the nasal cavity and paranasal sinuses from four North Carolina and Virginia hospitals matched with 290 hospital controls with other conditions, based on occupational exposures.	Interview data on job history. Estimation of exposure based on industry type. Only two cases employed in industry associated with formaldehyde. There were no deaths in the high exposure category.	Overall male and female	RR	0.35	(95% CI: 0.1–1.8)	
			Residence in mobile home	OR[f]	0.6	(95% CI: 0.2–1.7)	
			Years of exposure to particleboard				
			1 to 9	OR[f]	1.8	(95% CI: 09–3.8)	
			10 or more		1.5	(95% CI: 0.7–3.2)	
Olsen et al. (1984)	Case-control study of 488 cases of nasal cancer linked to the Danish Cancer Registry during 1970–1982. Controls were individuals with cancer of the colon, rectum, breast, and prostate. Three controls per case were selected for the same distributions of age, sex, and year of diagnosis as cases.	Employment histories after 1964 from files maintained by Danish Cancer Registry estimated by industrial hygienists.	Men				
			Formaldehyde only				
			Ever exposed	RR	2.8	(95% CI: 1.8–4.3)	(33)
			Exposure to wood dust and formaldehyde	RR	3.1	(95% CI: 1.8–5.3)	(23)
			Ever exposed	RR	3.5	(95% CI: 2.2–5.6)	(28)
			1st exposure >10 years or more before diagnosis	RR	4.1	(95% CI: 0.2.3–7.3)	(20)
Hansen and Olsen (1995)	Proportionate incidence study of 2,041 men with sinonasal cancer who died between 1970 and 1984 identified from the Danish Cancer Registry matched with the Danish	Linked companies through tax records to national Danish Product Register, where companies must report amount of	Overall	SPIR	2.3	(95% CI: 1.3–4.0)	(13)
			Low formaldehyde	SPIR	0.8	(95% CI: 0.02–4.4)	(1)
			Formaldehyde, no wood dust	SPIR	3.0	(95% CI: 1.4–5.7)	(9)
				SPIR	5.0	(95% CI: 0.5–13)	(2)
			Unknown	SPIR	1.0	(95% CI: 0.03–6.1)	(1)

Table 4-2. Case-control studies of formaldehyde and nasal and paranasal cancer

Reference	Study design	Exposure assessment	Results, statistical significance (number of cases)				
	Supplementary Pension Fund whose longest work experience occurred at least 10 years before the cancer diagnosis. The measure of risk was the SPIR, which measured the proportion of cases of sinonasal cancer in formaldehyde-associated companies relative to the proportion of cases of sinonasal cancer among all employees in Denmark.	formaldehyde used per year.					
Coggon et al. (2003)	Cohort mortality study of 14,014 men employed in 6 factories of the chemical industry in Great Britain from periods during which formaldehyde was produced. Cohort followed through 2000. Sinonasal cancer mortality SMRs based on English and Welsh age and calendar-year-specific mortality rates.	Exposures assessment based on data abstracted from company records. Each job categorized as background, low, moderate, high, or unknown levels. For analysis of sinonasal cancer, no gradient used because of small number of observed cases.	Overall	SMR	0.87	(95% CI: 0.11–3.14)	(2)

[a]Adjusted for age at death, year at death, and availability of occupational information.
[b]All had medium to high exposure to wood dust.
[c]Adjusted for age and exposure to glues and adhesives.
[d]Adjusted for age and study.
[e]Adjusted for age, study, and cumulative exposure to dust.
[f]Adjusted for cigarette smoking, alcohol consumption, gender, and age.

4.1.2.1.2.2. ***Cohort studies of nasal and paranasal cancer.*** IARC (1995) also reported the results of several cohort studies of professional and industrial workers for nasal and paranasal cancer (Andjelkovich et al., 1995; Gardner et al., 1993; Hall et al., 1991; Hayes et al., 1990; Bertazzi et al., 1989; Edling et al., 1987; Blair et al., 1986; Stroup et al., 1986; Harrington and Oakes, 1984; Levine et al., 1984; Walrath and Fraumeni, 1984, 1983; Friedman and Ury, 1983). Only a few studies reported any cases of sinonasal cancer. No cases of this type of cancer were reported in any of the studies of professional workers examined by the IARC. Only 2 cases (2.2 expected) were reported by Blair et al. (1986), and only 1 case (1.7 expected) was reported by Gardner et al. (1993). The likelihood of finding this rare tumor type in a long-term cohort study is low.

Three subsequent cohort studies reported on nasal and paranasal cancer. Hansen and Olsen (1995), in a proportional incidence study, found a significantly increased risk of sinonasal cavity cancer (SPIR = 2.3 [95% CI: 1.3–4.0]; 13 observed) in 265 Danish industries, where 2,041 of 91,182 cancer patients had at least 10 years of continuous formaldehyde-related work experience before diagnosis. Coggon et al. (2003), in a cohort study of 14,014 employees in six chemical factories in Great Britain, found only 2 deaths from sinonasal cancer (2.3 expected based on national death rates in Great Britain). Finally, Hauptmann et al. (2004) evaluated the sinonasal cancer risk in the NCI cohort and found three cases (SMR = 1.19 [95% CI: 0.38–3.68]) among those with a 15-year lag period.

4.1.2.1.2.3. ***Summary of nasal and paranasal cancers.*** The pooled case-control study of Luce et al. (2002) provides strong evidence of an association between formaldehyde exposure and increased risk of sinonasal adenocarcinoma. The cohort studies may not have had sufficient statistical power to show an association, and studies that did not distinguish cancer type may have aggregated a truly causal relationship with a noncausal relationship with SCC. In summary, there appears to be increased risk of sinonasal cancer associated with formaldehyde exposure with or without exposure to wood dust. The effect appears to be stronger when the risk is stratified by cancer type with higher risks of adenocarcinoma compared with SCC. Taken together with the NPC findings in the neighboring tissue, it is concluded that there is evidence of higher risks of sinonasal cancer associated with exposure to formaldehyde.

4.1.2.1.3. ***Other respiratory tract cancers***. Of six cohort studies of buccal/pharynx cancer in studies of professionals reviewed by IARC (Hayes et al., 1990; Logue et al., 1986; Stroup et al., 1986; Levine et al., 1984; Walrath and Fraumeni, 1984, 1983), no evidence of a risk associated with exposure to formaldehyde was reported (see Table 4-3). In studies of industrial worker cohorts where buccal/pharynx cancer was examined (Andjelkovich et al., 1995; Stayner et al.,

1988; Blair et al., 1986), only one (Stayner et al., 1988) reported an excess risk of death from this tumor (SMR = 3.4, based on four deaths in a cohort of 6,741 white women). Three case-control studies (Merletti et al., 1991; Vaughan et al., 1986a, b) did not find an association between oral cavity, oropharyngeal, and hypopharyngeal cancers and formaldehyde exposure. However, Merletti et al. (1991) found an elevated OR of 1.8 associated with probable or definite exposure to formaldehyde in a study of 86 patients matched with 373 controls. There was no risk of laryngeal cancer associated with formaldehyde in a case-control study (Wortley et al., 1992) of 235 patients with laryngeal cancer and 547 controls. The OR in that study was 1.0 (adjusted for age, smoking, drinking, and level of education). IARC (1995) concluded that there was little evidence of an increased risk of laryngeal cancer.

4.1.2.1.3.1. ***Cohort studies of other respiratory tract cancers.*** Hansen and Olsen (1995) reported a 10% increase in the risk of cancer of the buccal cavity and pharynx (SPIR = 1.1) in their proportional incidence study of Danish workers. Marsh et al. (1996) reported no excess risk of buccal cavity cancer cases (SMR = 1.31) based on U.S. rates and no excess based on state mortality rates (SMR = 1.0). For oropharyngeal cancer, the SMR was 1.84 (based on two cases), the SMR for hypopharyngeal cancer was 1.41 (based on one case), and the SMR for laryngeal cancer was 1.47 (based on six cases). The latter risks were elevated even when SMRs were derived from Connecticut mortality rates.

The Marsh et al. (2002) update also derived elevated risk estimates for oropharyngeal, hypopharyngeal, and pharyngeal-unspecified cancers. The SMRs ranged from 2.11 to 2.25 based on U.S. rates and 1.52 to 1.89 based on county rates. When combined with NPC International Classification of Death (ICD) codes 146–149 to increase statistical power, the total pharyngeal cancer SMRs were significant based on U.S. death rates (SMR = 2.63, n = 22, $p < 0.01$) and county death rates (SMR = 2.23, n = 22, $p < 0.01$) and remained significant for both short-term (less than 1 year) and long-term exposures. Furthermore, using the exposure estimates of Marsh et al. (1996), both cumulative and average exposure to formaldehyde resulted in elevated SMRs, some of which were significant for pharyngeal cancer. Coggon et al. (2003) identified 14 cases of cancer of the larynx (13.1 expected) in their cohort of formaldehyde-exposed factory workers. Pinkerton et al. (2004) found an excess risk of mortality from buccal cavity cancer (SMR = 1.33, four deaths observed) and a deficit of the risk of pharyngeal cancer (SMR = 0.64, three observed). Since the number of observed deaths was small and the risk estimates were subject to much variation for both studies, no conclusions about cancer risk can be drawn.

Table 4-3. Epidemiologic studies of formaldehyde and pharyngeal cancer (includes nasopharyngeal cancer)

Reference	Study design	Exposure assessment	Results, statistical significance (number observed deaths for cohort study)				
Marsh et al. (2002)	Retrospective cohort mortality study of 7,328 workers hired up to 1984 and followed until 1998 in one plant from Blair et al. (1986, 1987) and Hauptmann et al. (2004). Mortality was compared with death rates in two Connecticut counties and U.S. A nested case-control analysis was also conducted with 4 controls matched on age, year of birth, race, and sex randomly selected from cohort. Conditional logistic model was used for nested case-control analysis.	Worker-specific exposures from job exposure matrix were based on available sporadic sampling data from 1965–1987, job descriptions, and verbal job descriptions by plant personnel and industrial hygienists. Exposures were then ranked on a 7-point scale. An exposure range was assigned to each rank. 17% of jobs validated with company monitoring data, remaining 83% based on professional judgment. Pre-1965 levels of formaldehyde were assumed to be the same as post-1965 levels.	<u>Cohort study</u>				
			Overall				
			U.S.	SMR	2.63	(95% CI: 1.65–3.98)	(22)
			County	SMR	2.23	(95% CI: 1.40–3.38)	(22)
			Short-term worker (<1 year)				
				SMR	2.35	(95% CI: 1.22–4.11)	(12)
			Long-term worker (1 or more years)				
				SMR	2.10	(95% CI: 1.01–3.86)	(10)
			Cumulative exp. (ppm-years) county				
			Unexposed	SMR	1.24	(95% CI: 0.15–4.49)	(2)
			>0 to <0.004	SMR	3.31	(95% CI: 1.22–7.21)	(6)
			0.004–0.219	SMR	2.06	(95% CI: 0.83–4.24)	(7)
			0.22+	SMR	2.30	(95% CI: 0.92–4.73)	(7)
			Average Exposure (ppm) county				
			Unexposed	SMR	1.24	(95% CI: 0.15–4.49)	(2)
			>0 to <0.03	SMR	2.02	(95% CI: 0.74–4.40)	(6)
			0.03–0.159	SMR	3.82	(95% CI: 1.54–7.88)	(7)
			0.16+	SMR	2.03	(95% CI: 0.82–4.19)	(7)
			Exposure to ≤0.2 ppm	SMR	1.72	(95% CI: 0.74–3.39)	(8)
			Exposure to >0.2 ppm	SMR	2.68	(95% CI: 1.46–4.49)	(14)
			Exposure to ≤0.7 ppm	SMR	2.12	(95% CI: 1.21–3.45)	(16)
			<u>Nested case-control analysis</u>				
			Cumulative exp. (ppm-years)				
			<0.004	OR	1.00		(8)
			0.004–0.219	OR	0.71	(95% CI: 0.20–2.43)	(7)
			0.22+	OR	0.79	(95% CI: 0.18–3.20)	(7)
			Average exposure (ppm)				
			<0.03	OR	1.00		(8)
			0.03–0.159	OR	1.71	(95% CI: 0.47–6.10)	(7)
			0.16+	OR	0.99	(95% CI: 0.27–3.55)	(7)
			Exposure to >0.2 ppm	OR	1.35	(95% CI: 0.45–4.25)	(14)
			Exposure to >0.7 ppm	OR	1.60	(95% CI: 0.15–9.77)	(6)

Table 4-3. Epidemiologic studies of formaldehyde and pharyngeal cancer (includes nasopharyngeal cancer)

Reference	Study design	Exposure assessment	Results, statistical significance (number observed deaths for cohort study)				
Coggon et al. (2003)	Cohort mortality study of 14,014 chemical workers employed in 6 British factories.	Based on data abstracted from company records. Each job was categorized as having background, low, moderate, high, or unknown levels of formaldehyde.	Overall High exposure	SMR SMR	1.55 1.91	(95% CI: 0.87–2.56) (95% CI: 0.70–4.17)	(15) (6)
Shangina et al. (2006)	Multicentered, hospital-based case-control study in four European countries; men only. Cancer cases: 34 hypopharyngeal; 316 laryngeal. Controls: 728 hospital patients with various conditions.	Exposures determined by local industrial hygienists, chemists, and physicians. Coding was established and standardized. Categories were developed for 73 agents; frequency was estimated as the proportion of time a worker was exposed. Linear trends were examined for duration in years, weighted duration in hours, and cumulative exposure.	<u>Laryngeal cancer:</u> Formaldehyde Ever vs. never Highest cumulative (>22,700 mg/m^3-hours) vs. lowest Tests of trends: Years exposed Cumulative exposure	 OR OR p = 0.06 p = 0.07	 1.68 3.12	 (95% CI: 0.85–3.31) (95% CI: 1.23–7.91)	

Hauptmann et al. (2004) combined URT cancers (cancers of the salivary gland, mouth, nasopharynx, nasal cavity, and larynx). For average intensity of exposure (AIE), the RR was 1.69 for the medium exposure category (0.5 to <1.0 ppm) and 2.21 ($p < 0.05$) for the high exposure category (>1.0 ppm). For peak exposure, the RR was 1.24 for the medium exposure category (2.0 to <4.0 ppm) and 1.65 for the high exposure category (>4.0 ppm). For cumulative exposure, the RR was 1.92 for the medium exposure category (1.5 to <5.5 ppm) but 0.86 in the high exposure category (>5.5 ppm-years). The dose trends for these analyses, while suggestive for average and peak exposures, were not statistically significant.

4.1.2.1.3.2. ***Case-control studies of other respiratory cancers.*** Gustavsson et al. (1998) conducted a case-control study of 545 cases of SCC of the oral cavity, oropharynx, hypopharynx, larynx, and esophagus, frequency-matched by age and region with 641 controls. Regression analyses among 545 male cases showed elevated but nonsignificant risks of SCC of the oral cavity (OR = 1.28), esophagus (OR = 1.90), and larynx (RR = 1.45) associated with formaldehyde exposure. However, several of the carcinoma types were statistically significantly associated with exposure to welding fumes, polyaromatic hydrocarbons, asbestos, and metal dust.

In a case-control study, Laforest et al. (2000) examined 201 patients with squamous cell hypopharyngeal cancer and 296 patients with squamous cell laryngeal cancer, who were matched to 296 controls with cancers of other sites in 15 French hospitals. Adjusting for potential confounders, the OR of hypopharyngeal cancer in patients with a high probability of exposure to formaldehyde was 3.78 (95% CI: 1.50–9.49). The ORs were significantly increased with both exposure durations and high cumulative level of exposure.

Marsh et al. (2002) conducted a nested case-control study of the 22 pharyngeal cancer deaths in the Wallingford, Connecticut, plant cohort. Each of the pharyngeal cancer deaths was matched on race, sex, age, and year of birth to four controls from the cohort. Twenty of the 22 cases were exposed to formaldehyde, yielding an OR of 3.04 after adjustment for smoking and year of hire. There was little or no association of pharyngeal cancer incidence in these workers with either average or cumulative exposure, based on the exposure estimates in this study. There was a suggested trend of increasing OR with increasing duration of exposure for any formaldehyde exposure as well as for formaldehyde exposure >0.2 ppm. The results of this nested case-control analysis are inconclusive because of its low statistical power and questionable exposure estimates, which differed substantially from those estimated by the NCI (see Section 4.1.1.1). In addition, the relatively flat dose-response curve in the nested case-

control study contradicts the positive dose-response curves reported (particularly for NPCs) in the same study, based on SMRs derived from county and U.S. death rates in the cohort analysis.

Shangina et al. (2006) conducted a multicentered case-control study in Europe and Russia of 34 cases of hypopharyngeal cancer, 316 cases of laryngeal cancer, and 728 controls. With regard to formaldehyde exposure, a nonsignificant positive association was found for laryngeal cancer (OR = 1.68, 95% CI: 0.85–3.31). Trends over increasing exposure were found for duration of exposure in years ($p = 0.06$) and for cumulative exposures ($p = 0.07$). The investigators reported an OR of 3.12 (95% CI: 1.23–7.91) for the highest cumulative exposure group (>22,700 mg/m^3-hours) compared with the unexposed group.

4.1.2.1.3.3. ***Summary of other respiratory cancers.*** The evidence for a compound-specific effect on the risk of buccal/pharynx, oral cavity, oropharynx, hypopharynx, and laryngeal cancers as a result of exposure to formaldehyde is minimal. Only the study by Laforest et al. (2000) and, to a lesser extent, that by Shangina et al. (2006) provided evidence of an association between formaldehyde and these tumors. However, even the study of Laforest et al. (2000) had major limitations that made the evidence of an association suggestive at best.

4.1.2.1.4. ***Lung Cancer.*** None of the cohort studies of workers in specific professions indicated excess risks of lung cancer. Of the professional studies reviewed, the RRs range from an extremely low value (SMR) of 0.2 in Hall et al. (1991), based on nine deaths, to an RR (proportional mortality ratio) of 1.1, based on 70 lung cancer deaths in Walrath and Fraumeni (1983).

4.1.2.1.4.1. ***Industrial worker cohort studies of lung cancer.*** Evidence of a relationship between formaldehyde exposure and lung cancer is conflicting, with some studies showing modest increases while others show significant deficits in risk. There is, at best, only weak evidence from several studies to suggest that exposure to formaldehyde is associated with lung cancer.

Several industrial cohort studies (Andjelkovich et al., 1995; Bertazzi et al., 1989, 1986; Stayner et al., 1988; Edling et al., 1987) reported no significant excess risks of lung cancer from exposure to formaldehyde. No consistent association between formaldehyde exposure and lung cancer was found in several reports of the NCI 10-plant cohort originally investigated by Blair et al. (1987, 1986). Hauptmann et al. (2004) gives the most recent report on this cohort, which has been studied in part or in its entirety by several others (Marsh et al., 1994, 1992a, b; Sterling and Weinkam, 1994, 1989a, b, 1988; Robins et al., 1988; Liebling et al., 1984; Fayerweather et al.,

1983; Wong, 1983; Marsh, 1982). Marsh et al. (1996) reported small but significantly increased risks of respiratory cancer in males at one plant from the cohort. The SMRs were 1.22 based on U.S. rates, 1.34 based on Connecticut rates, and 1.28 based on county rates.

Similarly, Gardner et al. (1993) and Acheson et al. (1984) found a significant but modest association between lung cancer and formaldehyde exposure (SMR = 1.2 [95% CI: 1.1–1.4]). In workers hired after 1964, the SMR was 1.1. No trends by level or duration of exposure were found. Pinkerton et al. (2004) and Stayner et al. (1988) studied a cohort of 11,030 workers in three garment plants and found an SMR of 1.1 for lung cancer. Some studies have found modest elevations in risk of lung cancer with formaldehyde exposure, some of which were significant. Coggon et al. (2003) updated the Gardner et al. (1993) study of industrial workers. By taking data from all six factories together, results showed a statistically significant excess risk of lung cancer in the high-exposure category when compared with British national mortality rates (SMR = 1.58 [95% CI: 1.40–1.78]) and to local mortality rates (SMR = 1.28 [95% CI: 1.13–1.44]). Callas et al. (1996) reanalyzed the cumulative exposure of 279 lung cancer cases among white male workers from the NCI study, which comprised 80% of the NCI cohort (Blair et al., 1986). The analysis revealed modest RRs of 1.46, 1.27, and 1.38 for lung cancer in the cumulative exposure categories 0.05 to 0.5, 0.51 to 5.5, and greater than 5.5 ppm-years, respectively. None of these RRs were significant. Finally, Matanoski (1991) reported a significant deficit in the risk of respiratory cancer (SMR = 0.56 [95% CI: 0.44–0.70]; 77 observed) in pathologists presumably exposed to formaldehyde based on U.S. mortality rates.

4.1.2.1.4.2. ***Case-control studies.*** Several case-control studies of lung cancer (Partanen et al., 1990; Gerin et al., 1989; Bond et al., 1986; Coggon et al., 1984; Fayerweather et al., 1983; Anderson et al., 1982) showed no excess lung cancer risk associated with potential exposure to formaldehyde when analyzed by length of exposure, intensity, and potential exposure 5, 10, or 15 years before death or by combinations of these factors. By contrast, Coggon et al. (1984) reported a statistically significant increase in risk of lung cancer among male patients with any potential exposure to formaldehyde based on occupations listed on death certificates (SMR = 1.5 [95% CI: 1.2–1.8]).

De Stefani et al. (2005) conducted a case-control study of 338 adenocarcinomas of the lung in male patients admitted to four Montevideo hospitals from 1994 to 2000. The highest ORs were for smoking (6.0 [95% CI: 3.3–11]) and for former smokers (4.0 [95% CI: 2.1–7.3]). In addition, three agents (i.e., asbestos, silica dust, and formaldehyde) indicated significant excess risks of lung adenocarcinoma after adjusting for smoking history. A significant exposure-

duration relationship was found for formaldehyde for 21+ years of exposure (OR = 3.0 [95% CI: 1.6–5.8]; p = 0.004 for trend).

4.1.2.1.4.3. ***Summary of lung cancer.*** The evidence for an association between formaldehyde and lung cancer is limited. Only one study has found a statistically significant effect (Coggon et al., 2003). However, there may be other explanations rather than exposure to formaldehyde for this association. Except for the findings of De Stefani et al. (2005), other studies of lung cancer and exposure to formaldehyde have not supported this finding, including several well-done cohort studies that were specifically designed to evaluate lung cancer. Until the Coggon et al. (2003) study of British formaldehyde workers is replicated or reevaluated to determine the cause of the excessive lung cancer risk, evidence from that study alone is insufficient at this time to support an association between lung cancer and formaldehyde exposure.

4.1.2.1.5. ***Summary of respiratory tract cancers.*** Recent studies of NPC continue to support an association with exposure to formaldehyde even at low levels. In some studies, the association between formaldehyde and NPC persisted even when adjusted for the effect of potential confounders (Hauptmann et al., 2004). Data from some reports have suggested a dose-response relationship (Hauptmann et al., 2004; Marsh et al., 2002; Vaughan et al., 2000).

The risk of NPC was significantly elevated among industrial workers with cumulative exposure, average exposure, and peak exposure to formaldehyde (Hauptmann et al., 2004). The studies of the single Wallingford plant by Marsh et al. (2002, 1996, 1994) and Marsh and Youk (2005) also revealed a dose-response trend, although the absolute exposure level estimates were much lower. The relatively flat dose-response curve seen in the nested case-control study by Marsh et al. (2002) of all pharyngeal cancers was inconsistent with the positive dose-response curves reported in the same paper based on county and U.S. death rates, particularly for NPC. Also of interest was the finding of a statistically significant increase in the risk of NPC in formaldehyde-exposed workers who were seropositive for EBV in the Hildesheim et al. (2001) study.

The pooled analysis by Luce et al. (2002) provides evidence of a relationship of sinonasal cancer, particularly adenocarcinoma, with formaldehyde. However, as with some of the studies of NPC, the findings are potentially confounded by concurrent exposure to wood dust. When wood dust exposure was adjusted for in the analysis, the resulting risks were still positive but based on small numbers and, as a result, subject to much variability. The more recent studies continued to reveal small significant and nonsignificant associations among cancers of

buccal/pharynx, oral cavity, hypopharynx, and larynx and exposure to formaldehyde. However, the estimates were always based on small numbers.

A recent study that reported statistically significant lung cancer in association with a high level of exposure to formaldehyde was conducted by Coggon et al. (2003). The investigators suggested that unknown lifestyle factors, including smoking, could be responsible for the finding. Despite the results of their analysis, the authors were unconvinced that formaldehyde was the agent responsible for the elevation in lung cancer risk. However, De Stefani et al. (2005) also reported a statistically significant risk of lung adenocarcinoma in formaldehyde-exposed hospital patients even when smoking was controlled for in their analyses.

In all studies of formaldehyde and lung cancer, smoking remains an important confounder and possibly an effect modifier. Residual confounding of smoking or other respiratory exposures (e.g., wood dust or chemical or particular exposures) must always be considered.

4.1.2.2. *Non-Respiratory Tract Cancer*

4.1.2.2.1. *LHP cancers*. Cancers of the hematopoietic system include lymphosarcoma, reticulosarcoma, Hodgkin's disease, non-Hodgkin's disease, multiple myeloma, and all types of leukemia, including lymphoid and myeloid. Virtually all of the studies of LHP cancers and formaldehyde are cohort studies and are divided into two groups: professional and industrial. Several of the studies of professional groups were reviewed in an IARC (1995) monograph and are briefly discussed in the next section regarding their findings on cancer of the LHP system. One case-control study of non-Hodgkin's lymphoma is discussed at the end of this section.

4.1.2.2.1.1. *Professional cohort studies.* Several cohort studies have been undertaken by professional groups (i.e., anatomists, pathologists, embalmers, and funeral directors) because their careers are likely to bring them into contact with formaldehyde. Some studies have reported an increase in the risk of myelogenous leukemia and other LHP cancers (see Table 4-4). A few of the increased risks were statistically significant. None of the studies of professionals have used personal exposure measurements of formaldehyde or other chemicals, making specificity for any single exposure difficult to determine.

Harrington and Shannon (1975) conducted a cohort mortality study of 2,079 British pathologists (1955–1973) and 12,944 British medical laboratory technicians (1963–1973). When compared with death rates for England and Wales, the all-cause SMR for the pathologists was 0.60 versus 0.67 for the laboratory technicians. There was a significant increase in the risk of lymphatic and hematopoietic neoplasia (SMR 2.0; 8 observed with 3.3 expected; $p < 0.01$)

among male pathologists. However, the SMR for technicians was only 0.6 (3 observed). The low SMRs suggest that these professionals have a healthier profile compared with the British population. No actual exposure estimates are available.

Harrington and Oakes (1984) expanded the above study to include 2,307 male and 413 female pathologists and laboratory technicians. Mortality was only examined from 1973 until 1980; deaths that occurred before 1974 were not included in the update. The SMR for leukemia was 0.91 in men and 9.26 (based on one case) in women. Although the earlier LHP cancer deaths were not included in this analysis, the investigators say in their conclusion that their previous suggestion of an increase in certain lymphatic neoplasia was not confirmed in the present study because of small numbers. The exceptionally low SMRs suggest that this group of professionals enjoyed a healthier lifestyle compared with the British population as a whole. Just as in the earlier studies of these professionals, no exposure estimates are available.

Hall et al. (1991) expanded the above study by including the newest members of the Royal College of Pathologists. The cohort totaled 4,512 individuals, although only 3,069 males and 803 females were included in the analysis. The reasons for this discrepancy were not specified, although the authors mentioned that an unknown number of expected deaths for Northern Irish and female Scottish pathologists were not calculated, 32 pathologists were lost in follow-up, and cause of death was unknown for 9 individuals. Follow-up was extended from 1980 to 1986. Mortality was enumerated from 1974 to 1987, a period of time that differed from both of the earlier studies described above. There were statistically not significant excess risks for lymphatic and hematopoietic cancer (SMR 1.44; 10 observed) and leukemia (SMR 1.52; 4 observed) for both sexes combined, based on mortality rates in England and Wales. Separately, there was 1 female death in the lymphatic and hematopoietic cancer category (0.57 expected). The most striking observation in this study is that, despite the low cancer mortality (SMR 0.45 for all cancer; 53 observed but 118.19 expected), there was still an excess (but not statistically significant) risk of hematopoietic cancers. This finding of an extremely low risk for all cancers suggests that population death rates may not be appropriate as a referent group—for example, the SMRs for lung cancer (0.19) and nonneoplastic respiratory diseases (0.23) were significantly decreased, suggesting a lower prevalence of smoking among the pathologists compared with the general population of England and Wales. However, the finding of a possibly increased risk of LHP cancers should be analyzed further by selecting a more appropriate reference population (another professional group without exposure to formaldehyde) or by utilizing internal comparisons.

Walrath and Fraumeni (1983) conducted a proportionate mortality study of all embalmers and funeral directors licensed in the state of New York between 1902 and 1980 who were known

to have died between 1925 and 1980. The investigators requested death certificates for 1,678 persons but received only 1,263 (75%). The investigators restricted their analysis to 1,132 males. The distribution of the causes of death was compared with the age-, race-, and calendar-year-specific proportions of deaths for each cause among the male U.S. population. Duration of exposure was approximated by time since first license. While the methodology could not be applied in all calculations because of data gaps, excess risks were found for lymphatic and hematopoietic cancers, with a proportionate mortality ratio (PMR) of 1.2 (observed 25), and for leukemia, with a PMR of 1.32 and proportionate cancer mortality ratio (PCMR) of 1.19 (12 observed). The PMRs were not affected when the estimates were stratified by latency (<35 years or 35 years since first license) or by age at first license. Because the cause of death could not be determined for nearly 25% of the study group, the risk estimates could be underestimated. The metrics, PMR, and PCMR are not stochastic processes. An increase in one cause would produce decreases in all the other causes.

Using the proportionate mortality method, Walrath and Fraumeni (1984) studied 1,007 deceased white male embalmers, members of the California Bureau of Funeral Directing and Embalming, whose deaths occurred between 1925 and 1980. The decedents had to have been licensed to practice between 1916 and 1978. For lymphatic and hematopoietic cancer, the PMR was 1.22 (19 deaths observed). For leukemia alone, the PMR was 1.75 and significant (12 deaths observed, $p < 0.05$). Among embalmers licensed for 20 years or longer, the risk of leukemia increased and was also significant (PMR 2.21; 8 observed; $p < 0.05$). But this study, like the study of New York embalmers, had the same limitations discussed above. The investigators did not provide information on the number of embalmers for whom no cause of death could be found.

Levine et al. (1984) conducted a cohort mortality study of 1,477 male Ontario undertakers first licensed between 1928 and 1957 and followed until the end of 1977. Out of 359 subjects who had died, there were 8 deaths from lymphatic and hematopoietic cancers compared with 6.5 expected. Additionally, there were 4 deaths from leukemia versus 2.5 expected. Because death rates were not available for Ontario before 1950, person-years and deaths before 1950 could not be counted. No actual exposure estimates are available for these undertakers.

Stroup et al. (1986) conducted an historic cohort mortality study of 2,317 men who were members of the American Association of Anatomists between 1888 and 1969. The investigators derived SMRs from the U.S. white male population and used members of the American Psychiatric Association (APA) as a comparison group. Vital status was ascertained between 1925 and 1979. Women were excluded from analysis because of the small numbers. Only

738 deaths were observed versus 1,133.9 expected, based on U.S. death rates (SMR 0.65), possibly indicating a sizable HWE. However, a slight increase in the risk of lymphatic and hematopoietic cancers (SMR 1.2; 18 observed) and the risk of leukemia (SMR 1.5; 10 observed) was evident. A significant increase in the risk of brain cancer (SMR 2.7; 10 observed; $p < 0.05$) was also reported. When the leukemia analysis was restricted to the myeloid type, the SMR increased to 8.8, based on five deaths ($p < 0.05$). The analysis using the APA group was restricted to deaths that occurred between 1900 and 1969. This restriction removed five leukemia deaths and person-years from the analysis because they likely died after 1969. Because of this, there were only 3 leukemia deaths versus 3.6 expected, based on APA death rates. The investigators concluded that the etiological agent had not been definitively identified, mentioning that a wide range of solvents, stains, and preservatives, including formaldehyde, are used to prepare biological specimens.

Logue et al. (1986) conducted a cohort study of male radiologists and pathologists registered with the Radiation Registry of Physicians and the College of American Pathologists (CAP) between 1962 and 1977. Although the main focus was on determining mortality in radiologists from exposure to ionizing radiation, mortality was also ascertained for pathologists alone. To derive SMRs, expected deaths were the sum of the products of person-years times death rates for both cohorts during the follow-up period in white males only. However, there were no exposure measurements, and the SMRs were not adjusted for calendar time. Of 5,585 members of the CAP, 496 had died by December 31, 1977. Although the SMR was 0.48 for pathologists for cancer of the lymphatic and hematopoietic system, for the more specific category of leukemia and aleukemia the SMR was 1.06 (neither was significant). For radiologists, the SMRs were 0.78 and 1.55, respectively, also not significant. Cause of death could not be determined for 8% of the deaths. Although age-adjusted rates for leukemia were also calculated for each cohort, they were only used for comparison between the two separate professional groups.

Hayes et al. (1990) conducted a proportionate mortality study of 3,649 deceased white and 397 deceased nonwhite U.S. male embalmers and funeral directors who had died between 1975 and 1985, using records from local licensing boards, state funeral directors' associations in 32 states and the District of Columbia, the National Funeral Directors' Association, and state offices of vital statistics (n = 894). Expected deaths by cause were derived from 5-year age- and calendar-year-specific proportions of deaths among appropriate race groups from the U.S. population. No measured exposure data were available. A PCMR would be derived by excluding noncancer causes of death. Statistically significant excesses in hematopoietic and lymphatic cancers were found in white (PMR 1.31 [95% CI: 1.06–1.59]; 100 observed) and

nonwhite (PMR 2.41 [95% CI: 1.35–3.97]; 15 observed) embalmers and funeral directors. The combined PMR was 1.39 (95% CI: 1.15–1.63). The excess risk was higher for myeloid leukemia (ML) (PMR 1.61 [95% CI: 1.02–2.41]; 23 observed) and for other unspecified leukemias (PMR 2.08 [95% CI: 1.21–3.34]; 17 observed) in white males. The risks were elevated in nonwhite males based on only a few cases (PMR 1.33 [95% CI: 1.10–1.60]; 4 observed).

Matanoski (1991) conducted a study of 6,111 male pathologists for NIOSH. Members of the cohorts were part of an earlier unpublished study. Twenty-nine thousand psychiatrists were used as a comparison group. Both samples were selected from the membership rolls of professional associations. A total of 3,787 pathologists died between 1940 and 1978. Women were excluded from the analysis. Of the population of psychiatrists, 4,788 died by 1980. U.S. age- and calendar-time-specific death rates from 1925 were used to develop SMRs. Separate SMRs were based on psychiatrists' death rates. The risk of hematopoietic cancer (excluding Hodgkin's disease) was elevated (SMR 1.25; 57 observed) based on U.S. white males. For leukemia, the SMR was 1.35 (31 observed). The SMR for leukemia among psychiatrists was 0.83 (35 observed). Compared with leukemia in psychiatrists, the SMR for pathologists was 1.68 (95% CI: 1.14–2.38). The SMR for other lymphatic cancers was 1.53 (16 observed) and for LHP cancer 1.22 (64 observed). Comparing the pathologists' death rates to those of psychiatrists could be thought to have greater validity than if death rates for the U.S. population as a whole had been used, because of shared socioeconomic circumstances and access to medical care between the two professional groups. Differences in access to health care might have been greater for subjects in the earlier part of the study, because improved diagnosis and medical care for LHP cancers became more broadly available later in the study period. By using SMRs based on U.S. death rates, which include those who do not have adequate access to medical care, the difference between expected and observed deaths would be reduced. This is less likely to occur when one professional group is compared with another professional group, assuming psychiatrists and pathologists have equal access to care.

4.1.2.2.1.2. ***Industry worker cohort studies.*** This section discusses updated industrial worker studies that show associations between LHP cancer and formaldehyde. The studies by Marsh et al. (1994), Blair et al. (1986), and Acheson et al. (1984) and the later update by Gardner et al. (1993) provide estimates of exposure to formaldehyde. The remaining studies generally rely either on duration of exposure (number of years in the job) as a surrogate (Pinkerton et al., 2004) or provide no exposure assessment.

Marsh et al. (1994), in an early study of the Wallingford plant, which is also part of the Hauptmann et al. (2004, 2003) and Blair et al. (1986) studies, found SMRs of 0.89 and 0.91, based on U.S. and county death rates, respectively (25 observed deaths). The authors did not further discuss this cancer site until after Hauptmann et al. (2003) was published. Blair et al. (1986) reported on 4,396 deaths from all causes in the 10 formaldehyde-associated factories that made up the NCI cohort of 26,561 workers employed before January 1, 1966. There was little evidence of an association with LHP system cancer (SMR 0.91; 56 observed) in exposed white men, who dominated the cohort.

Hauptmann et al. (2003) updated the cohort mortality study of Blair et al. (1986) that consisted of predominantly the same (25,619) workers from 10 plants. The primary focus of this analysis was cancer of the LHP system, including leukemia. The description and demographics of the current study are the same as those reported by Blair et al. (1986) and Stewart et al. (1986). In the current update, follow-up was extended through December 31, 1994. The additional 15 years of follow-up increased the number of deaths from 4,349 to 8,486. Exposures were not updated for the 4% of workers still in exposed jobs in 1980, but eliminating exposure estimates for these workers did not change the results since exposures received after this date were considered so low as to contribute little to the analysis by the authors.

Peak exposure categories were defined as nonexposed, low (0.1–1.9 ppm), medium (2.0–3.9 ppm), and high (4.0 ppm or greater). Average intensity categories of exposure were defined as nonexposed, low (0.1–0.4 ppm), medium (0.5 to <0.9 ppm), and high (≥1.0 ppm). Cumulative exposure was defined as nonexposed, low (0.1–1.4 ppm-years), medium (1.5–5.4 ppm-years), and high (≥5.5 ppm-years). Duration of exposure was defined as 0, 0.1–4.9 years, 5.0–14.9 years, and ≥15 years. The median TWA exposure level was 0.45 ppm, range 0.01–4.25 ppm. Only 2.6% of the workers had average exposure intensities of 2 ppm or higher, and 14.3% had peak exposures of 4 ppm or higher. A total of 3,201 workers had no exposure. The median duration in formaldehyde-exposed jobs was 2 years. The median TWA intensity for formaldehyde exposure was 0.5 ppm among exposed workers.

A Poisson regression model was stratified for calendar year, age, sex, race, and pay category (salary/wage). A minimum latency period of 2 years between exposure and death from a potentially exposure-related LHP cancer was assumed by the investigators to prevent the inclusion of exposures not likely to contribute to the development of LHP cancer because of their timing. Other lag times were evaluated that did not improve the regression models.

There were 2,099 cancer deaths. Hauptmann et al. (2003) reported that mortality from all causes, all cancers, and LHP malignancies were significantly lower among the unexposed (SMRs 0.77, 0.65, and 0.62, respectively). Among the exposed, the SMRs were 0.95, 0.90, and 0.80,

respectively, for mortality from all causes, all cancers, and LHP malignancies. These SMRs in part reflect the HWE caused by using external U.S. population death rates as a referent. Unexposed workers also may have differed from the exposed workers in other ways. The remaining analyses used internal comparisons, avoiding the HWE. The referent group in this analysis was the low exposure group rather than the unexposed group, because nonexposed workers, who are primarily managers, secretaries, and other non-production personnel, were considered likely to have different socioeconomic characteristics than workers in the production areas.

Statistically significant positive associations were found for LHP malignancies and leukemia, particularly myeloid, in certain higher exposure categories in comparison with employees in the lowest exposure categories (Table 4-4). In the highest peak exposure level, the RR for LHP malignancies was 1.87 (95% CI: 1.27–2.75; 64 observed) and for ML was 3.46 (95% CI: 1.27–9.43; 14 observed) compared with employees in the low exposure peak level. For workers with high peak exposure levels, the RR for LHP malignancies was 1.71 (95% CI: 1.14-2.58; 49 observed) and 2.43 (95% CI: 0.81–7.25) for ML. The trend tests for slope were highly statistically significant for both LHP malignancies ($p < 0.002$) and ML ($p < 0.009$).

Significant results for LHP cancers were also seen with the average intensity exposure metric. RRs were 1.63 ($p < 0.05$) and 1.50 ($p < 0.05$) for the medium and high categories, respectively. The risk of ML was also significantly increased (RR = 2.49) in the highest exposure category. In contrast, Hauptmann et al. (2003) did not find statistically significant associations of formaldehyde with LHP cancer, either by cumulative exposure or years of duration. However, there were positive associations for leukemia (RR = 1.39) and ML (RR = 1.35) when exposure was 15 years or longer.

The authors concluded that formaldehyde may cause leukemia, particularly ML, in humans. However, because results from other studies were inconsistent, they suggested caution in drawing definite conclusions. A biological basis for the significant excess risk of LHP cancer remains unclear. The authors pointed out several studies that indicate changes that are consistent with chromosomal changes in formaldehyde-exposed persons, such as increased frequencies of (MN (He et al., 1998; Kitaeva et al., 1996; Suruda et al., 1993), sister chromatid exchanges (SCEs) (Shaham et al., 2002, 1997; Yager et al., 1986), chromosomal aberrations (CAs) (He et al., 1998; Bauchinger and Schmid, 1985), and DNA-protein cross-links (DPXs) (Shaham et al., 1997, 1996a) in peripheral lymphocytes of humans exposed to formaldehyde.

Hauptmann et al. (2003) identified 11 suspected carcinogens used at the plants: antioxidants (unspecified), asbestos, carbon black, dyes and pigments, hexamethylenetetramine, melamine, phenol, plasticizers, urea, wood dust, and benzene. Some workers were employed as

chemists and laboratory technicians. The investigators did not find substantial changes in the risk estimates after adjusting for exposure to these substances or for working as a chemist or laboratory technician. They also eliminated the 586 benzene-exposed persons from their analysis and found similar results (benzene is a known human leukemogen). Smoking was not likely to explain an increased risk of leukemia in this cohort, because no increase was seen for smoking-related diseases, including lung cancer. The cohort consisted predominantly of males (88%). Strengths include the fact that the cohort was large and there was a long period of follow-up that was 96.6% successful. Internal analyses eliminated the HWE. One potential limitation that could lead to an underestimate of risks is the 3.4% or 866 lost to follow-up.

The study by Hauptmann et al. (2003) has been criticized extensively by several experts representing the formaldehyde industry (Tarone and McLaughlin, 2005; Casanova et al., 2004; Cole and Axten, 2004; Collins, 2004; Collins and Lineker, 2004). Most of the same criticisms have been repeated in other critiques by the above-mentioned authors and have been addressed in the discussions concerning the details of the methodology. However, a few new issues have arisen from these critiques, as follows. One issue pertains to a concern that person-years at risk of death may have been assigned wrongly to the highest "peak" category of exposure for the duration of the study period. For example, there is inconsistency in the fact that only 4% of the original cohort (Blair et al., 1986) had average exposures equaling or exceeding 2 ppm yet 45% of the person-years were assigned to the peak exposure category. Average exposures are time-weighted exposures that can have brief excursions over 4 ppm and still average 2 ppm or less. Only for the peak exposure surrogate were person-year values assigned to the peak category following the exposure, because it is a test for the possibility that biological changes could have been initiated from that brief high exposure that might increase the risk of cancer. If these genetic changes are irrevocable, then the risk of cancer could be increased and subsequent person × years should be assigned to that higher risk category.

According to Casanova et al. (2004), the assignment of peak exposures in the Hauptmann et al. (2003) study was questionable because they were based on professional judgement. However, there are adequate grounds for hypothesizing that the assignment of peak exposure was completed before determination of vital status and cause of death. It is always possible that some subjects may be subject to misclassification. Hauptmann et al. (2003) chose this metric partly because it more closely resembled the exposure that embalmers and pathologists may have received from formaldehyde. This same criticism could be said about the Coggon et al. (2003) study as well.

Hauptmann et al. (2003) have also been criticized because the metric "cumulative exposure" was not significant and did not show a trend. No adequate explanation has been given

by Hauptmann et al. (2003) except that it is possible that this metric is not as sensitive for this agent. Duration of exposure was only weakly associated with a trend of increasing risk. After 15 years' duration, there appeared to be a slight increase in ML based on 10 cases (RR = 1.35 [95% CI: 0.56–3.24]). For all leukemia the risk in workers who were exposed 15 or more years was somewhat higher (RR = 1.39 [95% CI: 0.78–2.49]) based on 22 cases.

Another of the criticisms from these authors discussed the lack of a biologically plausible explanation for how leukemia could result from exposure to formaldehyde when there appears to be no recognizable indication of the presence of formaldehyde in excessive quantities in the blood of animals or any associated metabolites in experimental research animals. Hauptmann et al. (2004b) responded that there is evidence that genotoxic effects can be detected in vivo in the bone marrow of rats and in human peripheral lymphocytes.

Stayner et al. (1988) conducted a cohort study of 11,030 workers (82% female) followed from 1955 or the beginning date of exposure through 1982 in three garment factories. Personnel records from three garment manufacturing facilities, one in Pennsylvania and two in Georgia, were used to assemble a cohort of workers who attained a minimum of 3 months of exposure after the introduction of formaldehyde into these facilities. Formaldehyde resins were used to treat fabrics, beginning in 1955 and 1959. Although formaldehyde levels were available on a subset of the employees from monitoring data available from surveys completed in 1981 and 1984, they were not used in this analysis. Instead, the results were stratified by duration and latency. SMRs were based on U.S. population mortality rates. Based on six cases, the SMRs for leukemia were 2.43 and 3.81 among workers with 20 or more years since first exposure or at least 10 years of exposure, respectively. In their conclusions, the authors suggested that, although the numbers of deaths from LHP cancers were small, the risks were related to duration and latency.

Pinkerton et al. (2004) updated the Stayner et al. (1988) study by adding 16 years of follow-up. No new exposure information was added. The mean TWA exposure in 1981–1984 for the three plants was 0.15 ppm. No additional information regarding earlier industrial hygiene data was available, although the authors stated that the levels of exposure to formaldehyde were greater in the years before 1980. Stayner et al. (1988) cited independent studies of exposure levels in similar garment factories in the 1960s that seemed to indicate that the formaldehyde levels during that period ranged from 0.9 to 2.7 ppm (Blejer and Miller, 1966) in one garment manufacturing area. Another report (Shipkovitz, 1966) of 10-minute personal exposure samples indicated a range from 0.3 to 2.7 ppm in eight garment plants. In another study (Ahmad and Whitson, 1973), the levels ranged from 2 to 10 ppm. Goldstein (1973) calculated that concentrations in the cutting rooms of garment plants dropped from 10 ppm in 1968 to less than

2 ppm in 1973 because of an improvement in the resin treating process. The authors assumed that exposure ceased in 1981 and 1983. This produced an underestimate of exposure based on duration of employment for about 11% of the cohort who were still actively employed after those dates. Stayner et al. (1988) speculated that the risks of cancer of the buccal cavity, leukemia, and other LHP neoplasia may have been due to exposure to the highest potential formaldehyde levels in the industry between 1955 and 1962, because the resin used to treat permanent press fabrics still contained a relatively large amount of formaldehyde.

The SMRs were derived from age-, race-, and calendar-time-adjusted U.S. mortality rates. The analysis was repeated using Georgia or Pennsylvania mortality rates. In addition to the primary analysis of the underlying cause of death, the analysis used all causes listed on the death certificates to evaluate multiple cause mortality. As a referent for this, the analysis relied on multiple cause death rates available since 1960 from the National Death Index maintained by the U.S. Centers for Disease Control and Prevention (CDC).

Altogether, 608 cancer deaths were observed. The SMR for all cancer was 0.89 (95% CI: 0.82–0.97). The overall SMR for leukemia was 1.09 (24 deaths) and 1.44 (15 deaths) for ML. After 10 years of exposure, the risk for ML was 2.19. Exposure prior to 1963 was associated with a risk of 1.61. Among garment workers followed for 20 or more years from initial exposure, the SMR was significantly elevated for ML (1.91; $p < 0.05$; 13 deaths), as was the SMR for multiple cause leukemia (1.92 [95% CI: 1.08–3.17]; 15 deaths) in the subgroup with 10 or more years of exposure to formaldehyde and who were followed for 20 or more years after first exposure. The multiple cause mortality for ML for this subgroup of workers was also significant (SMR 2.55 [95% CI: 1.10–5.03]; 8 deaths).

The study by Stayner et al. (1988) has only limited power to detect excess risks of rare cancers, such as NPC and nasal cancer (13 and 16%, respectively). Limitations to the interpretations of the findings include a lack of any monitoring data before 1981, particularly during the critical time period 1955 to 1962, and lack of personal exposure estimates for any members of the cohort. The possibility exists that misclassification may still be present because the intensity of exposure to formaldehyde decreased as improvements were made in the resin systems used to treat fabrics (e.g., a person who worked 5 years beginning in 1955 might have been subject to greater exposure than a person who worked 5 years beginning in 1993). However, workers from the 1950s and 1990s were both placed in the same category of having worked fewer than 10 years. The median duration of exposure was 3.3 years. Work histories were not updated in the follow-up study; however, the low or background exposure levels that probably existed after 1981 were not likely to contribute substantially to the risk of cancer. The use of mortality data to estimate risk, when the case fatality rate was less than 100% for most

cancer sites evaluated, could potentially produce an underestimate of the actual risk. Despite these limitations, this study provides additional evidence of an association between leukemia, especially ML, and formaldehyde in comparison with the general population.

Gardner et al. (1993) reported that the risk of leukemia was not statistically significant (SMR 0.9) based on 15 deaths among workers employed before 1965. Only four leukemia deaths were observed after 1964 through 1989, producing an SMR of 0.9.

When Coggon et al. (2003) updated the above cohort study of 14,014 men first employed before 1965 in six factories by adding 11 additional years of follow-up (ending December 31, 2000), no increase in the risk of leukemia or related cancers of the hematopoietic system was reported, either in the entire cohort (SMR 0.91; 31 observed) or in the group with the highest formaldehyde exposure (>2 ppm) (SMR 0.71; 8 observed). Similar results were obtained for Hodgkin's disease, non-Hodgkin's lymphoma, and multiple myeloma. No other cancers of the hematopoietic system were evaluated, and no additional analyses were performed to assess a possible leukemia risk. However, the main finding from this study was a marked association of lung cancer with formaldehyde (discussed in the lung cancer section). This study's main focus was respiratory disease, lung cancer, and stomach cancer, not LHP cancers. For cancers of the LHP system, there was neither latency evaluation nor internal comparisons. The HWE is also potentially a problem.

Andjelkovich et al. (1995) studied a cohort of 3,929 male iron foundry workers potentially exposed to formaldehyde between January 1, 1960, and December 31, 1989, in which 127 cancer deaths had occurred during the observation period. An industrial hygienist, after reviewing work histories, categorized formaldehyde exposure into four levels corresponding to the approximate midpoint of the ranges: none, low (0.05 ppm), medium (0.55 ppm), and high (1.5 ppm) for exposure to formaldehyde. Boundaries of these exposure categories were not given. The authors warned that the assignment of exposure levels was not perfect because "subjective judgment had to be applied in many instances." SMRs were based on U.S. male mortality rates, but actual ranges were not specified. The authors also compared the exposed to 2,032 nonexposed workers from the same company. The population-based SMR for hematopoietic cancer in the exposed population was 0.59 (based on seven observed deaths). For leukemia the SMR was 0.43, based on two deaths. There were no other analyses for leukemia or LHP cancers in this study. Because of the uncertainty about workers' true formaldehyde exposure, there was no analysis by level of exposure, duration, or latency. There were also very few LHP cancers in the cohort. Thus, these results neither support nor refute an association of formaldehyde exposure with LHP cancers. The main focus of this paper was on lung cancer risk.

Bertazzi et al. (1989, 1986), in a cohort mortality study, followed 1,330 male workers from 1959 through 1986 at a formaldehyde resin plant in Italy. The workers had to have been employed for at least 30 days at the plant sometime between 1959 and 1980 to be included in the study. Their mortality was compared with national and local rates adjusted for age and calendar time period. No individual exposure estimates were available, but mean levels were estimated to be between 0.2 and 3.8 mg/m^3 (0.16 and 3.1 ppm) during the period 1974–1979. The authors found an SMR of 2.01 (five deaths observed) for cancer of the lymphatic and hematopoietic system. The study's limitations included incomplete work histories, small numbers of deaths, and a follow-up period that may not have been sufficient to allow for a latency period for the development of LHP cancers. As before, the results neither support nor refute an association of formaldehyde exposure with LHP cancers.

Edling et al. (1987) reported on the incidence of disease in a cohort of 521 blue collar Swedish workers in plants where abrasives bound with formaldehyde resins were manufactured. Formaldehyde levels ranged from 0.1 to 1.0 mg/m^3 (0.08–0.8 ppm). The workers in the cohort were employed between 1955 and 1983, and incidence rates were calculated from 1958 through 1981. There were only 24 total cancer cases (28.5 expected) of which 2 (1.0 expected) were lymphomas and 2 (0.5 expected) were multiple myelomas. Expected cases were determined through the Swedish National Cancer Register. No other LHP cancers were observed. This study lacked the power to detect any significant associations between LHP cancer and exposure to formaldehyde.

Dell and Teta (1995) conducted a cohort mortality study of 5,932 male employees of a New Jersey plastics manufacturing, research, and development facility. The workers, who had been employed during the period 1946–1967, were followed-up for an average of 32 years. SMRs were based on U.S. and New Jersey mortality rates. Hourly workers (n = 3,853) were analyzed separately from the 2,079 salaried employees. Although no excess risk was evident for hematopoietic cancer in hourly workers (SMR 0.93; 28 observed), there was an SMR of 1.69 (95% CI: 1.07–2.53; 23 observed) among salaried workers. This association was further narrowed to mainly research and development workers (eight leukemia deaths observed with three expected, for an SMR of 2.67). No common exposure was found when work history records were examined. The decedents were mostly associated with process development in two research pilot plants, where chemical engineers, lab technicians, and plant operators executed small-scale product development. Although notebooks referred to benzene and toluene solvents, no definite connection was made with formaldehyde or any of the solvents. No ambient air measurements of formaldehyde were available. The findings cannot be assumed to be due to formaldehyde exposure because of the presence of other potential leukemogens.

Blair et al. (1993) conducted a study that evaluated the risk of non-Hodgkin's lymphoma from exposure to formaldehyde. This was a population-based, case-control, interview-based study of 1,867 white males of whom 622 cases had the disease and 1,245 were controls. Subjects had lived in Iowa and Minnesota between 1980 and 1983. This study was exploratory and designed to find associations with any environmental exposures and non-Hodgkin's lymphoma. Subjects or next of kin were interviewed to determine what exposures the cases and controls may have received based on agricultural exposures, work histories, medical conditions, and family history. Extra effort was made to collect information about occupation, industrial exposures, and other selected exposures. The analysis revealed an OR of 1.2 for exposure to formaldehyde. Similar associations were found for metals and other substances in the study. This study, because it did not select cases and controls from a population with possible formaldehyde exposure, could not detect specific relationships between formaldehyde and non-Hodgkin's lymphoma.

4.1.2.2.1.3. *Summary of non-respiratory tract cancers.* The Hauptmann et al. (2003) study appears to provide the strongest evidence of an association for ML in particular. Statistically significant positive associations were found for LHP malignancies and leukemia, particularly ML, in certain higher exposure categories in comparison with employees in the lowest exposure categories. In the highest peak exposure level, the RR for LHP malignancies was 1.87 (95% CI: 1.27–2.75; 64 observed) and for ML was 3.46 (95% CI: 1.27–9.43; 14 observed) compared with employees in the low-exposure peak level. For workers with high-peak exposure levels, the RR for LHP malignancies was 1.71 (95% CI: 1.14–2.58; 49 observed) and 2.43 (95% CI: 0.81–7.25) for ML. The trend tests for slope were highly statistically significant for both LHP malignancies ($p < 0.002$) and ML ($p < 0.009$). Significant results for LHP cancers were also seen with the average intensity exposure metric. RRs were 1.63 ($p < 0.05$) and 1.50 ($p < 0.05$) for the medium and high categories, respectively. The risk of ML was also significantly increased (RR = 2.49) in the highest exposure category. In contrast, results showed no associations of formaldehyde with LHP cancer, either by cumulative exposure or years of duration.

Additional support linking LHP cancer and formaldehyde comes from a study of garment workers (Pinkerton et al., 2004) and studies of pathologists and other medical workers exposed to formaldehyde (Matanoski, 1991; Blair et al., 1990; Hayes et al., 1990; Stroup et al., 1986; Walrath and Fraumeni, 1984, 1983; Harrington and Shannon, 1975). Hayes et al. (1990) and Stroup et al. (1986) also reported significant excess risks of ML.

Several reports have challenged the association between LHP cancer and formaldehyde (Casanova et al., 2004; Cole and Axten, 2004; Collins, 2004; Collins and Lineker, 2004; Coggon

et al., 2003; Casanova and Heck, 1987; Heck et al., 1985). These papers argue that a biological explanation for the excess risk of LHP cancer or leukemia remains unclear. Absent a plausible MOA by which formaldehyde could cause these cancers, many investigators have been unable to accept the reported increased risks identified in the epidemiologic literature. Some researchers have argued against the biological plausibility of formaldehyde-induced lymphoreticular cancers based solely on the assumption that formaldehyde as a reactive gas does not penetrate past the POE. This argument is relevant to diseases for which transformation of stem cells in the bone marrow is essential. However, cancers that arise from more mature cells present outside of the bone marrow compartment cannot be dismissed with this argument. Although often grouped for analysis, the lymphohemoreticular system cancers represent many distinct malignancies that may arise from discrete cell types in different tissues throughout the body. For example, acute lymphocytic leukemia (ALL) is believed to arise from the transformation of a lymphoid stem cell in the bone marrow, resulting in a blood-borne leukemia of immature cells of the lymphoid cell line. However, if transformation occurs in a mature lymphocyte (e.g., post-germinal center B cell), a chronic lymphocytic leukemia (CLL) results. Although etiologically different, these cases would both be lymphocytic leukemia. When considering biological plausibility of an exogenous agent increasing the incidence of ALL, bone marrow toxicity would be expected. However, when considering the biological plausibility of CLL, bone marrow toxicity would not be essential. Mutation or epigenetic changes attained in the mature cell may be passed on to daughter cells during response to antigen and eventually lead to transformation. So the etiologies of these two leukemias need not be similar. In contrast, a non-Hodgkin's lymphoma results from transformation of a mature B or T cell resulting in a solid tumor. The etiology of this cancer is actually similar to CLL. A recent reclassification of lymphoid malignancies by the WHO designates adult B-cell leukemias and lymphomas as the same disease, with ALL as a separate disease. Therefore, mortality analysis by ICD code and the standard groupings of those codes does not reflect the biology of the cancers.

Considering the whole class of LHP cancers, there is a range of biological plausibility for an agent whose primary action is at the POE. Acute leukemias (ALL and acute myelogenous leukemia [AML]), believed to arise from transformation of stem cells in the bone marrow, are less plausible, although trafficking of stem cells to different tissues would be an alternative etiology for exogenous compounds acting at the POE. In contrast CLL, lymphomas, multiple myelomas (from plasma B cells), and unspecified cancers may involve an etiology in peripheral tissues to include cells, cell aggregates, germinal centers, and lymph nodes. An association of these cancers to an exogenous agent acting at the POE is biologically plausible.

Table 4-4. Epidemiologic studies of formaldehyde and lymphohematopoietic cancers

Reference	Study design	Exposure assessment	Results, statistical significance (number of observed deaths for cohort study)				
Harrington and Shannon (1975)	Cohort mortality study of 2,079 pathologists and laboratory technicians from the Royal College of Pathologists and the Pathological Society of Great Britain from 1955–1973. The comparison population came from national mortality data.	Presumed exposure to formaldehyde tissue fixative.	Pathologists				
			All cause mortality	SMR	0.60		
			LHP cancers	SMR	2.0	$p < 0.01$	(8)
			Hodgkin's disease	SMR	1.4		(1)
			Leukemia	SMR	0.6		(1)
			Technicians				
			All cause mortality	SMR	0.67		
			LHP cancers	SMR	0.5		(3)
			Hodgkin's disease	SMR	–		(0)
			Leukemia	SMR	0.5		(1)
Harrington and Oakes (1984)	Cohort mortality study of 2,720 pathologists from the Royal College of Pathologists and the Pathological Society of Great Britain from 1974–1980. Vital status obtained from the census, a national health registry, and other sources. SMRs developed from the English, Scottish, Irish, and Welsh populations.	Presumed exposure to formaldehyde tissue fixative.	All causes				
			Men	SMR	0.56		
			Women	SMR	0.99		
			Leukemia				
			Men	SMR	0.91	(90% CI: 0.05–4.29)	(1)
			Women	SMR	9.26	(90% CI: 0.47–43.9)	(1)
			Other LHP cancers				
			Men	SMR	0.53	(90% CI: 0.03–2.54)	(1)
			Women	SMR	–	–	(0)
Hall et al. (1991)	Cohort mortality study of 4,512 pathologists from the Royal College of Pathologists and the Pathological Society of Great Britain from 1974–1987. Vital status obtained from the census, a national health registry, and other sources. SMRs developed from the English and Welsh populations.	Presumed exposure to formaldehyde tissue fixative.	All cause mortality				
			Men	SMR	0.43		(176)
			Women	SMR	0.65		(18)
			Hodgkin's disease	SMR	1.21	(95% CI: 0.03–6.71)	(1)
			All cancers	SMR	1.44	(95% CI: 0.69–2.63)	(10)
			Leukemia	SMR	1.52	(95% CI: 0.41–3.89)	(4)
Levine et al. (1984)	Cohort mortality study of 1,477	Presumed exposure to	All LHP cancers	SMR	1.24		(8)

Table 4-4. Epidemiologic studies of formaldehyde and lymphohematopoietic cancers

Reference	Study design	Exposure assessment	Results, statistical significance (number of observed deaths for cohort study)				
	male Ontario undertakers first licensed 1928–1957, followed from 1950 to 1977. SMRs developed from Ontario mortality rates.	formaldehyde tissue fixative.	Leukemia	SMR	1.60		(4)
Stroup et al. (1986)	Cohort mortality study of 2,317 white male members of the American Association of Anatomists from 1888 to 1969 who died 1925–1979. SMRs developed using U.S. population mortality rates.	Presumed exposure to formaldehyde tissue fixative.	All cause mortality	SMR	0.65	(95% CI: 0.60–0.70)	(738)
			All LHP cancers	SMR	1.2	(95% CI: 0.7–2.0)	(18)
			Lymphosarcoma and reticulosarcoma	SMR	0.7	(95% CI: 0.1–2.5)	(2)
			Hodgkin's disease	SMR	–	–	(0)
			Leukemia	SMR	1.5	(95% CI: 0.7–2.7)	(10)
			Other lymphatic	SMR	2.0	(95% CI: 0.7–4.4)	(6)
Logue et al. (1986)	Cohort mortality study of 4,485 pathologists who were members of the College of American Pathologists, 1962–1972, followed for mortality through 1977. SMRs developed from U.S. population mortality rates.	Presumed exposure to formaldehyde tissue fixative.	LHP cancer other than leukemia	SMR	0.48		(NR)
			Leukemia	SMR	1.06		(NR)
Matanoski (1991)	Cohort mortality study of 6,111 male pathologists from membership rolls of the American Medical Association 1912–1950. Mortality was followed through 1978. SMRs developed from U.S. population white male mortality rates.	Presumed exposure to formaldehyde tissue fixative.	All cancer	SMR	0.78	(95% CI: 0.71–0.85)	(508)
			All LHP cancers	SMR	1.25	(95% CI: 0.95–1.62)	(57)
			Lymphosarcoma and reticulosarcoma	SMR	1.31	(95% CI: 0.66–2.35)	(11)
			Hodgkin's disease	SMR	0.36	(95% CI: 0.04–1.31)	(2)
			Leukemia	SMR	1.35	(95% CI: 0.92–1.92)	(31)
			Other lymphatic	SMR	1.54	(95% CI: 0.82–2.63)	(13)
Hauptmann et al. (2003)	Retrospective cohort mortality study of 25,619 workers employed at 10 formaldehyde plants in the U.S. followed from either the plant start-up or first employment through 1994.	Exposure estimates based on job titles, tasks, visits to plants by study industrial hygienists, and monitoring data through	All LHP cancers				
			Exposed	SMR	0.80	(95% CI: 0.69–0.94)	(161)
			Unexposed	SMR	0.62	(95% CI: 0.39–1.00)	(17)
			Peak exposure (ppm)				
			0	RR	1.08	(95% CI: 0.60–1.94)	(17)

Table 4-4. Epidemiologic studies of formaldehyde and lymphohematopoietic cancers

Reference	Study design	Exposure assessment	Results, statistical significance (number of observed deaths for cohort study)				
	SMRs calculated using sex-, age-, race-, and calendar-year-specific U.S. mortality rates. RRs estimated using Poisson regression stratified by calendar year, age, sex, and race; adjusted for pay category.	1980. Peak exposure defined as short-term excursions exceeding the 8-hour TWA formaldehyde intensity and knowledge of job tasks. Exposures to 11 other compounds were identified. Workers contributed pre-exposure person-time to nonexposed category. Poisson regression models used a 2-year lag to account for tumor latency.	0.1–1.9	RR	1.00	*Reference value*	(48)
			2.0 to <4.0	RR	1.71	(95% CI: 1.14–2.58)	(49)
			4.0 or greater	RR	1.87	(95% CI: 1.27–2.75)	(64)
			Trend p = 0.002				
			Average exposure (ppm)				
			0	RR	0.91	(95% CI: 0.52–1.59)	(17)
			0.1–0.4	RR	1.00	*Reference value*	(81)
			0.5 to <1.0	RR	1.63	(95% CI: 1.11–2.37)	(42)
			1.0 or greater	RR	1.50	(95% CI: 1.01–2.24)	(38)
			Trend p = 0.050				
			Cumulative exposure(ppm-years)				
			0	RR	0.74	(95% CI: 0.42–1.30)	(17)
			0.1–1.4	RR	1.00	*Reference value*	(94)
			1.5 to 5.4	RR	0.79	(95% CI: 0.52–1.21)	(29)
			5.5 or greater	RR	1.03	(95% CI: 0.70–1.52)	(38)
			Trend p = 0.157				
			Leukemia				
			Peak exposure (ppm)				
			0	RR	0.78	(95% CI: 0.25–2.43)	(4)
			0.1–1.9	RR	1.00	*Reference value*	(16)
			2.0 to <4.0	RR	2.04	(95% CI: 1.04–4.01)	(20)
			4.0 or greater	RR	2.46	(95% CI: 1.31–4.62)	(29)
			Trend p = 0.001				
			Average exposure (ppm)				
			0 ppm	RR	0.56	(95% CI: 0.19–1.66)	(4)
			0.1–0.4	RR	1.00	*Reference value*	(32)
			0.5 to <1.0	RR	1.52	(95% CI: 083–2.79)	(16)
			1.0 or greater	RR	1.68	(95% CI: 0.91–3.08)	(17)

Table 4-4. Epidemiologic studies of formaldehyde and lymphohematopoietic cancers

Reference	Study design	Exposure assessment	Results, statistical significance (number of observed deaths for cohort study)				
			Trend p = 0.193				
			Cumulative exposure (ppm-years)				
			0	RR	0.48	(95% CI: 0.16–1.42)	(4)
			0.1–1.4	RR	1.00	*Reference value*	(35)
			1.5–5.4	RR	0.90	(95% CI: 0.47–1.73)	(13)
			5.5 or greater	RR	1.14	(95% CI: 0.63–2.07)	(17)
			Trend p = 0.183				
			Hodgkin's disease				
			Peak exposure (ppm)				
			0	RR	0.51	(95% CI: 0.06–4.52)	(1)
			0.1–1.9	RR	1.00	*Reference value*	(5)
			2.0 to <4.0	RR	3.45	(95% CI: 0.98–12.2)	(7)
			4.0 or greater	RR	3.35	(95% CI: 0.97–11.6)	(8)
			Trend p = 0.014				
			Average exposure (ppm)				
			0	RR	0.46	(95% CI: 0.05–3.93)	(1)
			0.1–0.4	RR	1.00	*Reference value*	(7)
			0.5 to <1.0	RR	4.70	(95% CI: 1.61–13.8)	(8)
			1.0 or greater	RR	3.12	(95% CI: 0.91–10.7)	(5)
			Trend p = 0.022				
			Cumulative (ppm-years)				
			0	RR	0.29	(95% CI: 0.04–2.34)	(1)
			0.1–1.4	RR	1.00	*Reference value*	(12)
			1.5–5.4	RR	1.35	(95% CI: 0.45–3.99)	(5)
			5.5 or greater	RR	1.17	(95% CI: 0.31–4.46)	(3)
			Trend p = 0.037				
			ML				
			Peak exposure (ppm)				
			0	RR	0.67	(95% CI: 0.12–3.61)	(2)
			0.1 to 1.9	RR	1.00	*Reference value*	(6)
			2.0 to <4.0	RR	2.43	(95% CI: 0.81–7.25)	(8)
			4.0 or greater	RR	3.46	(95% CI: 1.27–9.43)	(14)

Table 4-4. Epidemiologic studies of formaldehyde and lymphohematopoietic cancers

Reference	Study design	Exposure assessment	Results, statistical significance (number of observed deaths for cohort study)				
			Trend p = 0.003				
			Average exposure (ppm)				
			0	RR	0.41	(95% CI: 0.08–1.95)	(2)
			0.1 to 0.4	RR	1.00	*Reference value*	(14)
			0.5 to <1.0	RR	1.15	(95% CI: 0.41–3.23)	(5)
			1.0 or greater	RR	2.49	(95% CI: 1.03–6.03)	(9)
			p = 0.086				
			Cumulative (ppm-years)				
			0	RR	0.32	(95% CI: 0.07–1.51)	(2)
			0.1-1.4	RR	1.00	*Reference value*	(17)
			1.5-5.4	RR	0.57	(95% CI: 0.19–1.73)	(4)
			5.5 or greater	RR	1.02	(95% CI: 0.40–2.55)	(7)
			Trend p = 0.123				
Pinkerton et al. (2004)	Cohort mortality study of 11,098 workers in 3 garment plants exposed ≥3 months after formaldehyde was introduced. Women comprised 81.7% of the cohort. Vital status was followed through 1998. SMRs were calculated by using sex-, age-, race-, and calendar-year-specific U.S. mortality rates. Multiple cause SMRs were derived from all contributing causes from death certificates.	Data for 549 randomly selected employees in 5 departments in 1981 and 1984 used to estimate overall exposure levels. Levels presumed to be 0.09–0.20 ppm.	All LHP cancers	SMR	0.97	(95% CI: 0.74–1.26)	(59)
			Lymphosarcoma and reticulosarcoma	SMR	0.85	(95% CI: 0.28–1.99)	(5)
			Hodgkin's disease	SMR	0.55	(95% CI: 0.07–1.98)	(2)
			Other lymphatic	SMR	0.97	(95% CI: 0.64–1.40)	(28)
			Leukemia	SMR	1.09	(95% CI: 0.70–1.62)	(24)
			Mortality since 1960				
			Lymphocytic leukemia	SMR	0.60	(95% CI: 0.12–1.75)	(3)
			ML	SMR	1.44	(95% CI: 0.80–2.37)	(15)
			10+ years of exposure	SMR	2.19	NS	(8)
			20+ years since 1st exposure	SMR	1.91	*p* > 0.05	(13)
			Multiple cause leukemia				
			10+ years of exposure and 20+ years since 1st exposure	SMR	1.92	(95% CI: 1.08–3.17)	(15)

Table 4-4. Epidemiologic studies of formaldehyde and lymphohematopoietic cancers

Reference	Study design	Exposure assessment	Results, statistical significance (number of observed deaths for cohort study)				
			Multiple cause ML				
			20+ years since 1st exposure	SMR	2.02	(95% CI: 1.13–3.34)	(15)
			10+ years of exposure and 20+ years since 1st exposure	SMR	2.55	(95% CI: 1.10–5.03)	(8)
Coggon et al. (2003)	Cohort mortality study of 14,014 men employed in 6 factories of the chemical industry in Great Britain from periods during which formaldehyde was produced. Cohort mortality followed from 1941 through 2000. SMRs based on English and Welsh age- and calendar-year-specific mortality rates.	Exposure assessment based on data abstracted from company records. Jobs categorized as background, low, moderate, high, or unknown levels.	Non-Hodgkin's lymphoma				
			Overall	SMR	0.98	(95% CI: 0.67–1.39)	(31)
			High exposure	SMR	0.89	(95% CI: 0.41–1.70)	(9)
			Leukemia				
			Overall	SMR	0.91	(95% CI: 0.62–1.29)	(31)
			High exposure	SMR	0.71	(95% CI: 0.31–1.39)	(8)
			Multiple myeloma				
			Overall	SMR	0.86	(95% CI: 0.48–1.41)	(15)
			High exposure	SMR	1.18	(95% CI: 0.48–2.44)	(7)
Andjelkovich et al. (1995)	Cohort mortality study of 3,929 automotive industry iron foundry workers exposed from 1960–1987 and followed through 1989. SMRs calculated using sex-, age-, race-, and calendar-year-specific U.S. mortality rates.	Exposure assessment based on review of work histories by an industrial hygienist.	All LHP cancers	SMR	0.59	(95% CI: 0.23–1.21)	(7)
			Leukemia	SMR	0.43	(95% CI: 0.05–1.57)	(2)
Bertazzi et al. (1986)	Cohort mortality study of 1,330 male workers in an Italian resin plant. Subjects were employed any time between 1959 and 1980 for at least 30 days. Vital status followed through 1986. SMRs calculated using sex-, age-, race-, and calendar-year-specific national and local mortality rates.	Exposure assessment based on reconstruction of work history.	All LHP cancers	SMR	2.01		(5)

Table 4-4. Epidemiologic studies of formaldehyde and lymphohematopoietic cancers

Reference	Study design	Exposure assessment	Results, statistical significance (number of observed deaths for cohort study)				
Edling et al. (1987)	Cohort mortality and incidence study of 521 Swedish workers in an abrasive production plant with at least 5 years of employment between 1955 and 1983, followed through 1983.	Exposure level of 1–5 µg/m^3.	Lymphoma	SMR	2.0	(95% CI: 0.2–7.2)	(2)
			Multiple myeloma	SMR	4.0	(95% CI: 0.5–14)	(2)
Dell and Teta (1995)	Cohort mortality study of 5,932 male employees of a New Jersey plastics manufacturing, research and development facility.	Examination of work histories to identify jobs where formaldehyde was involved.	All LHP cancers				
			Hourly workers	SMR	0.93		(28)
			Salaried workers	SMR	1.69		(23)
			Leukemia				
			Hourly workers	SMR	0.98		(12)
			Salaried workers	SMR	1.98		(11)
Walrath and Fraumeni (1983)	Proportionate mortality cohort study of 1,132 white male embalmers licensed to practice between 1902 and 1980 in New York who died between 1925 and 1980 identified from registration files. Deaths were compared with age-, race-, and calendar-year-expected numbers of deaths from the U.S. population.	No direct measurements. Presumed exposure to formaldehyde tissue fixative.	All LHP cancers	PMR	1.15		(21)
			Lymphosarcoma and reticulosarcoma	PMR	1.08		(4)
			Hodgkin's disease	PMR	1.0		(2)
			Other lymphatic lymphoma	PMR	1.18		(5)
			Leukemia	PMR	1.32		(10)
Walrath and Fraumeni (1984)	Proportionate mortality cohort study of 1,007 white male embalmers from the California Bureau of Funeral Directing and Embalming who died between 1925 and 1980. Deaths were compared with age- and calendar-year-expected numbers	No direct measurements. Presumed exposure to formaldehyde tissue fixative.	All LHP cancers	PMR	1.22		(19)
			Lymphosarcoma and reticulosarcoma	PMR	0.97		(3)
			Hodgkin's disease	PMR	–		(0)
			Other lymphatic lymphoma	PMR	1.33		(4)

Table 4-4. Epidemiologic studies of formaldehyde and lymphohematopoietic cancers

Reference	Study design	Exposure assessment	Results, statistical significance (number of observed deaths for cohort study)				
	of deaths from the U.S. population.		Leukemia				
				PMR	1.75	$p < 0.05$	(12)
			Licensed <20 years	PMR	1.24		(4)
			Licensed ≥20 years	PMR	2.21	$p < 0.05$	(8)
Hayes et al. (1990)	Proportionate mortality cohort study of 3,649 deceased white and 397 deceased nonwhite U.S. male embalmers and funeral directors, derived from licensing boards and funeral director associations in the 32 states and the District of Columbia. Occupation was confirmed on death certificate. Deaths were compared with age- and calendar-year-expected numbers of deaths from the U.S. population.	No direct measurements. Presumed exposure to formaldehyde tissue fixative.	All LHP cancers				
				PMR	1.39	(95% CI: 1.15–1.63)	(115)
			Race				
			White	PMR	1.31	(95% CI: 1.06–1.59)	(100)
			Nonwhite	PMR	2.41	(95% CI: 1.35–3.97)	(15)
			Occupation on death certificate				
			Embalmer	PMR	1.23	(95% CI: 0.78–1.85)	(23)
			Funeral director	PMR	1.56	(95% CI: 1.23–1.94)	(78)
			Other	PMR	1.30	(95% CI: 0.67–2.28)	(12)
			Age at death				
			<60	PMR	1.35	(95% CI: 0.88–1.98)	(26)
			60–74	PMR	1.72	(95% CI: 1.33–2.19)	(66)
			≥75	PMR	1.16	(95% CI: 0.74–1.74)	(23)
			Hodgkin's disease				
				PMR	0.72	(95% CI: 0.15–2.10)	(3)
			Non-Hodgkin's lymphoma				
				PMR	1.26	(95% CI: 0.87–1.76)	(34)
			Lymphosarcoma and reticulosarcoma				
				PMR	1.12	(95% CI: 0.58–1.96)	(12)
			Multiple myeloma				
				PMR	1.37	(95% CI: 0.84–2.12)	(20)
			Other lymphatic lymphoma				
				PMR	1.35	(95% CI: 0.85–2.01)	(22)
			Lymphatic leukemia				
				PMR	0.74	(95% CI: 0.29–1.53)	(7)
			ML				
				PMR	1.57	(95% CI: 1.01–2.34)	(24)
			Other leukemia				

Table 4-4. Epidemiologic studies of formaldehyde and lymphohematopoietic cancers

Reference	Study design	Exposure assessment	Results, statistical significance (number of observed deaths for cohort study)				
				PMR	2.28	(95% CI: 1.39–3.52)	(20)
Blair et al. (1993)	Population-based case-control study of 622 white men with LHP cancers. Cancers selected from Iowa and Minnesota cancer surveillance networks diagnosed between 10/80 and 9/82. 1,245 matched controls for living cases selected by random digit dialing if younger than age 65 and from Medicare records if 65 or older. Study focused on agricultural exposures.	Personal interviews of subjects or next of kin included job histories, agricultural exposures, and chemical exposures. Job titles used to create job exposure matrix. Industrial hygienist estimated probability and intensity of exposures to large numbers of substances.	Non-Hodgkin's lymphoma (formaldehyde exposure)	OR[a]	1.2	(95% CI: 0.9–1.7)	
			Funeral service worker	OR[a]	2.1	(95% CI: 0.5–7.9)	(6)

[a]Adjusted for age, state, smoking, family history of malignant proliferative disease, agricultural exposure to pesticides, use of dye, and direct/surrogate response to interview.

4.1.2.2.2. *Brain and CNS cancer*. Several studies of professional groups discussed earlier investigated brain and other CNS cancers among those exposed to formaldehyde on the job. Several of these studies found that exposure increased risk two to three times among exposed professionals (Hall et al., 1991; Stroup et al., 1986; Walrath and Fraumeni, 1984), while others found modest or no increase in risk (Hayes et al., 1990; Levine et al., 1984; Walrath and Fraumeni, 1983).

None of the industrial cohort worker mortality studies of exposure to formaldehyde found a clear relationship between formaldehyde exposure and risk of brain or CNS cancer (Pinkerton et al., 2004; Coggon et al., 2003; Andjelkovich et al., 1995; Gardner et al., 1993; Stayner et al., 1988; Blair et al., 1987, 1986). To date, no case-control studies of brain and CNS cancer have been completed. In the Hauptmann et al. (2004) study, the authors reported that no clear association was seen for cancer of the brain and CNS and exposure to formaldehyde.

4.1.2.2.3. *Pancreatic and other cancers.* Two studies (Kernan et al., 1999; Dell and Teta, 1995) have found increases in the risk of pancreatic cancer in association with possible exposure to formaldehyde. Collins et al. (2001a) conducted a meta-analysis of fourteen studies (Kernan et al., 1999; Andjelkovich et al., 1995; Hansen and Olsen, 1995; Gardner et al., 1993; Hall et al., 1991; Matanoski, 1991; Hayes et al., 1990; Gerin et al., 1989; Stayner et al., 1988; Blair et al., 1986; Stroup et al., 1986; Levine et al., 1984; Walrath and Fraumeni, 1984, 1983) and found a small increase in risk (RR = 1.1 [95% CI: 1.0–1.3]).

Other sites that have been examined are stomach cancer (Coggon et al., 2003) (SMR = 1.47; $p < 0.05$), intraoccular melanoma (Holly et al., 1996) (OR = 2.9 [95% CI: 1.2–7.0]), and thyroid cancer among women (Wong et al., 2006) (OR = 8.33 [95% CI: 1.16–60.0]; 2 cases). However, without further substantiation, it is difficult to infer causation based on these isolated results alone.

4.1.2.3. *Summary: Carcinogenic Hazard in Humans*

The weight of the epidemiologic evidence at this time supports a link between formaldehyde exposure and NPC in humans. This conclusion is based on the longitudinal cohort study of Hauptmann et al. (2004) as well as the case-control studies of NPC and formaldehyde exposure completed by Hildesheim et al. (2001), Vaughan et al. (2000), and several additional case-control studies described in the text. With the exception of Hauptmann et al. (2004), most of the other cohort studies found little or no increased risk of NPC from exposure to formaldehyde. However, Hauptmann et al. (2004) employed different exposure metrics and based their analyses on conservative internal comparisons that limited the potential for the HWE

to obscure true effects. The case-control studies that provide additional evidence of an association between NPC and formaldehyde have more power and generally rely on better diagnoses of NPC. Better ascertainment of histologic types of tumors can sometimes also be obtained if the cases are taken from cancer registries. The NPC risk is also supported by experimental evidence in animals in which formaldehyde induces nasal cancers (Section 4.2.2). Since the physiology of the rat nasal passage is somewhat different from that of humans, it is not possible to obtain a direct site-specific correspondence between the species. However, in both species, the tumors are found within the same area of the URT where maximum exposure can be expected to occur.

Several researchers have challenged the conclusion of a relationship between formaldehyde and NPC. Those critical of the link argue that, given the wide variability in results across studies and competing explanations, conclusions about any link from the existing studies are premature. The difficulty in attaining consensus on whether formaldehyde influences the risk of NPC in humans arises from several limitations inherent in epidemiologic methods and exposure assessment, as well as from the characteristics of the disease. The most prominent of these limitations are the rarity of the cancer and imprecise estimates of exposure. Because NPC is a very rare cancer with an incidence of less than 1 per 100,000, it is difficult to obtain precise estimates of risk from cohort studies. Although case-control studies are better suited for studying rare conditions, they are limited in obtaining valid and precise exposure assessments. A further problem with exposure assessment is isolating formaldehyde exposure from other potential chemical or particulate exposures that may influence risk of NPC. Imprecise exposure assessment and the inability to isolate formaldehyde exposure from other exposures are largely the bases on which Marsh and coworkers have challenged the NCI cohort study (Marsh et al., 2007a, b, 2002, 1996; Marsh and Youk, 2005). Marsh and coworkers (Marsh et al., 2007a) show that subjectively assessed exposure to silversmithing is tentatively associated with NPC. Given that there were no prior citations of an association between silversmithing exposures and NPC in the medical literature and given the many post hoc reexaminations of alternative hypotheses to explain the original NCI findings, it is more likely that silversmithing is an artifactual potential confounder.

It may be expected that, without new approaches for obtaining more accurate and precise estimates of exposure, further follow-up of current cohorts and future epidemiologic studies of formaldehyde and NPC will face the same limitations and criticisms found with existing studies. These limitations notwithstanding, the epidemiologic studies reviewed here represent what may be currently discernable about a formaldehyde-NPC link in humans by using rigorous observational methods. As such, concluding any influence of formaldehyde must be made on the

weight of all human and animal evidence in the face of known and expected limitations in study design and exposure assessment.

The results of two well-designed cohort studies found a positive association between formaldehyde-exposed professionals, such as pathologists, embalmers, and funeral directors, and LHP cancer, particularly ML. The largest cohort study of formaldehyde has the most extensive exposure assessment (Blair et al., 1986; Stewart et al., 1986), and the cohort was followed for a median duration of 35 years (Hauptmann et al., 2003). By using cumulative exposure measures not previously used and by using internal comparison groups, significant increases in the risk of cancer of the LHP system, particularly ML, were reported. This study demonstrated that formaldehyde was a risk factor for LHP cancers, independent of other risk factors, such as benzene and smoking. Hauptmann et al. (2003) found statistically significant dose trends for peak exposure and AIE. Pinkerton et al. (2004) also found a significant increase in the risk of ML in garment workers 20 years after their initial exposure and in workers with 10 or more years of exposure. Additionally, several studies of pathologists, embalmers, and other medical workers reported greater numbers of observed deaths from leukemia than expected although many studies of these groups suffer from a substantial HWE based on comparisons with external death rates. Two of these studies, Hayes et al. (1990) and Stroup et al. (1986), also report a significantly excess risk of ML in embalmers, funeral directors, and anatomists.

There is a range of biological plausibility for an agent whose primary action is at the POE. Acute leukemias (ALL and AML), believed to arise from transformation of stem cells in the bone marrow, are less plausible. In contrast chronic lymphatic leukemia, lymphomas, multiple myelomas (from plasma B cells), and unspecified cancers may involve an etiology in peripheral tissues to include cells, cell aggregates, germinal centers, and lymph nodes. An association of these cancers to an exogenous agent acting at the POE is biologically plausible.

It is the conclusion of this assessment that the weight of the epidemiologic evidence at this time supports a link between formaldehyde exposure and carcinogenicity in humans.

4.2. ANIMAL STUDIES

This section discusses the available laboratory animal data on the toxicity of inhalation, oral, and dermal exposures to formaldehyde. A comprehensive database of laboratory animal studies is available for formaldehyde, including numerous 2-year bioassays by both the inhalation and oral exposure routes and dermal application studies. Although a large portion of the literature reports studies focused on toxic effects at the site of contact or POE, general systemic effects as well as neurobehavioral effects, reproductive and developmental effects, immunologic changes, and sensitization are well represented in the literature as well.

RB is a reduction in ventilation rate, minute volume, and other physiological parameters experienced by rodents exposed to an irritant/reactive gas. Although humans and nonhuman primates do not exhibit the same change in respiratory rate, these studies are included in Section 4.2.1.1 in order to better understand the effects on RB in interpreting rodent studies presented in the balance of the chapter. Additionally, although binding to the trigeminal nerve and subsequent downstream events do not result in pulmonary changes in humans, the mechanism itself plays a role in understanding other adverse health effects observed in humans.

The available data for the three exposure routes confirm direct formaldehyde-induced toxicity in tissues present at the POE. These observations are consistent with the physicochemical characteristics, reactivity, and metabolic pathways of formaldehyde as discussed in Chapter 3. Indications of cell damage, cell proliferation, and inflammatory responses are similar for each route of exposure, therefore effects at the POE for inhalation and oral exposures are described first (Sections 4.2.1.2 and 4.2.1.3, respectively). Given the well-established nature of these health effects and the wealth of literature for inhalation exposures, complete study summaries for respiratory tract effects are provided. Studies are organized by study duration—acute, subchronic and chronic—where some of the chronic bioassays were designed to address carcinogenic potential. Section 4.2.2 pulls together the findings of chronic studies across the routes of exposure to evaluate the carcinogenic potential of formaldehyde exposure.

Although a majority of the oral and inhalation studies focus on health effects at the POE—respiratory tract and GI tract—the general systemic toxicity of formaldehyde is addressed where it was integral to the study. Therefore, body weight and organ weight changes, gross pathology, organ histopathology outside of the POE, blood and urine chemistry, and other biochemical measures may be included in these study summaries. An overview of general systemic findings is provided in Section 4.4 for all routes of exposure.

Studies addressing immune function, neurobehavioral effects, sensitization, and reproductive and developmental effects are addressed across routes of exposure. The specialized nature of these studies requires discrete treatment, and inclusion of data across routes of exposure allows for a synthesis of the available information.

4.2.1. Noncancer Health Effects

4.2.1.1. *Reflex Bradypnea*

Reflex bradypnea (RB), which is believed to be a protective response, is often observed in rodents exposed to reactive gases. It is primarily characterized by marked decreases in activity, respiratory rate, body temperature, and metabolic rate. RB is not seen in humans and

nonhuman primates. An understanding of the RB is important to the interpretation of many of the animal bioassays examining formaldehyde-induced health effects. Of chief concern is that the physiological effects of RB, described below, may interfere with appropriate interpretation of adverse effects noted with formaldehyde exposure. It is important to distinguish between an effect directly related to RB versus formaldehyde exposure. Additionally the effects of RB may mask or alter formaldehyde-induced health effects. Secondly, differential respiratory effects of RB due to species and strain will result in differential inhaled doses at the same exposure level. This needs to be considered both when comparing the results of animal studies and in extrapolation to humans. Finally, although humans do not experience RB, the mechanism of RB as a reflex response to trigeminal nerve stimulation assists in understanding human health related to localized and reflex responses due to trigeminal nerve stimulation.

Irritant gases have been shown to decrease body temperature, heart rate, and blood pressure as well as alter blood chemistry in rodents (Pauluhn, 2003, 1996; Jaeger and Gearhart, 1982). Because of their small size, mice can rapidly lower their body temperatures and thus their metabolic rate and ventilation rate. The hypothermia that results from RB can directly affect nearly all biological processes (Gordon et al., 2008). Formaldehyde exposure can dramatically lower ventilation rate and reduce body temperature in mice by as much as 4°C, and it has been posited that decreased oxygen supply is likely to have profound effects on organisms with substantial oxygen demands (Jaeger and Gearhart, 1982). The effects of RB are reversible, though it may take several minutes to several hours to return to pre-exposure conditions (Pauluhn, 1996; Jaeger and Gearhart, 1982).

The literature on sensory irritation is broad; many studies have investigated species differences, dose response relationships, tolerance, and cross-tolerance to other sensory irritants (see Tables 4-5 and 4-6). This discussion focuses on the changes in respiratory rate and minute volume during formaldehyde exposure. Sensory irritation is often quantified as the statistically derived exposure concentration that results in a 50% reduction in respiratory rate (RD_{50}) in rodents (ASTM, 2000; Kane et al., 1979). Kane and Alarie (1977) evaluated various aspects of sensory irritation, including establishing the RD_{50}, exploring the reproducibility of response, investigating the effect of tracheal cannulation, and determining the potential for tolerance with repeated exposure or pre-exposure in male Swiss-Webster mice, caused by formaldehyde and acrolein. The RD_{50} was established by exposing four mice for 10 minutes at each concentration across a range representing approximately 10 to 80% reduction in respiration and calculated by using least squares regression. The RD_{50} and its 95% CI for formaldehyde were calculated to be 3.1 (2.1–4.7) ppm (3.8 [2.58–5.77] mg/m^3). The tracheal cannulation experiments demonstrated that the effect on respiratory rate was caused by URT sensory irritation.

Table 4-5. Respiratory effects of formaldehyde-induced reflex bradypnea in various strains of mice

Species/strain	No./ group	Treatment[a]	Respiratory effects	Reference
Male Swiss-Webster mice	4	Duration: 10 minutes. Exposure: up to 100 ppm.	RD_{50}[a] = 3.1 ppm (95% CI: 2.1–4.7).	Kane and Alarie (1977)
Male Swiss-Webster mice	8	Duration: 3 hours/day for 3 days. Exposure: 0.52, 0.44, 1.16, 1.83, 3.10, 5.35, 5.60, and 11.2 ppm.	RD_{50} = 3.4 ppm (95% CI: 2.4–4.7).	Kane and Alarie (1977)
Male Swiss-Webster mice	4	Duration: 10 minutes (head only). Exposure: up to 10 ppm.	RD_{50} = 3.2 ppm (95% CI: 2.1–4.7).	Steinhagen and Barrow (1984)
Male Swiss OF_1 mice	6	Single 5-minute exposure to four unspecified concentrations.	RD_{50} = 5.3 ppm.	De Ceaurriz et al. (1981)
Male B6C3F1 mice	4	Duration: 10 minutes (head only). Exposure: Range up to 10 ppm.	RD_{50} = 4.9 ppm (95% CI: 3.9–6.4).	Steinhagen and Barrow (1984)
Male B6C3F1 mice	4	Duration: 10 minutes (head only). Exposure: up to 15 ppm Pretreatment: 2, 6, or 15 ppm 6 hours/day for 4 days.	Naïve mice: RD_{50} = 4.4 ppm (95% CI: 0.9–5.0) Pretreated mice: RD_{50} = 4.3 ppm (95% CI: 3.4–5.5).	Chang et al. (1981); Barrow et al. (1983)
Male C57BL6/F1 mice	3	Whole-body exposure for up to 2 hours.	After 1.25 hours: Tidal volume reduced by 33%; 68% reduction in respiratory frequency; CO_2 production reduced by 50%; %; body temperature dropped from 37.8 to 34.7°C.	Jaeger and Gearhart (1982)

[a]Exposure concentration that results in a 50% reduction in respiratory rate.

Table 4-6. Respiratory effects of formaldehyde-induced reflex bradypnea in various strains of rats

Species/strain	No./ group	Treatment	Respiratory effects	Reference
Male Crl-CD rats	4		RD_{50} = 13.8 ppm.	
Male Wistar rats	4	30-minute nose-only exposure to a range of formaldehyde concentrations.	RD_{50} = 10.0 ppm.	Cassee et al. (1996)
Male F344 rats	4	Duration: 10 minutes (head only). Exposure: up to 56 ppm. Pretreatment:2, 6, or 15 ppm 6 hours/day for 4 days	Naïve rats: RD_{50} = 13.1 ppm (95% CI: 10.6–17.5) Pretreated rats: RD_{50} = 10.8 ppm (95% CI: 7.6–16.9)	Chang et al. (1981); Barrow et al. (1983)
Male F344 rats	4	Single 10-minute head-only exposure to a range of concentrations. Pretreatment: 15 or 28 ppm formaldehyde or 10 ppm chlorine.	Baseline RD_{50} = 31.7 ppm. Pre-exposure to formaldehyde-induced tolerance at 28 ppm (RD_{50} = 20.2 ppm) but not 15 ppm. Pre-exposure to chlorine-induced tolerance to formaldehyde (RD_{50} ranged from 64.5 to 115 ppm, depending on exposure duration).	Chang and Barrow (1984)
Male F344 rats	ND	10 minute exposure to acrolein or acetaldehyde (head only). Pre-exposed to formaldehyde at 15 ppm for 6 hours/day for 9 days.	Pre-exposure to formaldehyde-induced tolerance: Acetaldehyde (RD_{50} = 2,991 ppm in naive versus 10,601 ppm in preconditioned animals) Acrolein (RD_{50} = 6 ppm in naïve versus 29.6 ppm in preconditioned animals).	Babiuk et al. (1985)
Male Charles Rivers CD rats	3	Whole-body exposure for up to 2 hours.	After 0.7 hours: Tidal volume reduced by 22%; 20% reduction in respiratory frequency; CO_2 production unaffected.	Jaeger and Gearhart (1982)

Across the literature there is fairly good agreement on RD_{50} values for various strains of mice (Table 4-5), ranging from 3.1 ppm in male Swiss-Webster mice to 4.9 ppm in male B6C3F1 mice. Rats are less sensitive, with RD_{50} values ranging from 10 ppm in male Wistar rats to 31.7 ppm in male F344 rats. No reported RD_{50} for female rodents exposed to formaldehyde exists.

Jaeger and Gearhart (1982) evaluated the effect of formaldehyde on respiratory rate, tidal volume, minute volume, carbon dioxide (CO_2) production (exhaled to air) as a reflection of total metabolism, and core body temperature in male Charles River CD rats and male C57BL6/F1

mice. Animals (three/concentration) were exposed to 15 ppm (18.4 mg/m^3) formaldehyde for up to 2 hours. Mice exhibited a greater decrease in respiratory frequency and minute volume compared with the rats. CO_2 production and body temperature were also affected to a greater extent in the mice (Table 4-5). The authors postulated that the decreased body temperature in mice would likely lead to decreased biologic action of formaldehyde in the tissue.

4.2.1.1.1. ***Tolerance.*** Tolerance is defined as an increase in the concentration required to elicit the same degree of RB response and was evaluated by Kane and Alarie (1977). In the first set of experiments, mice (four/concentration) were exposed 3 hours/day for 4 days at the concentration associated with either a 30 or 50% decrease in respiratory frequency (specific concentrations not given) (Kane and Alarie, 1977). Naïve animals served as controls for each day. The maximum response increased with each additional day of exposure, and the diminution of response that was typically exhibited after 60 minutes of exposure in naïve animals was markedly delayed. In the second set of experiments, mice were exposed to a formaldehyde concentration at one-tenth the RD_{50} (i.e., 0.3 ppm) 3 hours/day for 3 days. On the fourth day the animals underwent a similar exposure protocol to identify the concentration that resulted in an RD_{50}, following the above protocol. No change in the RD_{50} was demonstrated. Both of these experiments indicate no change in tolerance with either type of pretreatment in Swiss-Webster mice.

Chang and Barrow (1984) tested whether tolerance would develop in male F344 (CDF[F344]Crl/Br) rats exposed to formaldehyde. Exposure to formaldehyde at 15 ppm (18.4 mg/m^3) for 6 hours/day, 5 days/week failed to induce tolerance. However, tolerance was observed following exposure to 28 ppm (34.4 mg/m^3) formaldehyde for 4 days. The concentration-response curve in these animals was significantly different than that of naïve animals, with an increase in the RD_{50} estimate for this exposure duration from 31.7 to 70.2 ppm.

4.2.1.1.2. ***Cross-species differences in inhaled dose.*** Formaldehyde-induced RB lowers both respiratory rate and tidal volume and thus reduces the inhaled dose of formaldehyde at a given exposure concentration. Chang et al. (1983) and Barrow et al. (1983) evaluated the species differences and the effective inhaled dose between rats and mice, since mice seem to be more sensitive to formaldehyde-induced RB and do not exhibit tolerance as shown in F344 rats. Groups (four/concentration) of male F344 rats and male B6C3F1 mice were exposed to formaldehyde concentration ranges of 6.2–48 ppm (7.6–59 mg/m^3) or 0.78–14.0 ppm (0.96–17.2 mg/m^3), respectively, for 10 minutes. Pretreated animals used in the tolerance experiments were exposed to formaldehyde at 2, 6, or 15 ppm (2.45, 7.36, or 18.4 mg/m^3) 6 hours/day for 4 days prior to determination of the RD_{50} and concentration response across the same ranges.

A concentration-dependent decrease in respiratory rate was seen in both naïve and pretreated rats during formaldehyde exposure. Tolerance (defined as a decrease in respiratory rate followed by a subsequent return to control values) occurred after 4 minutes of exposure and was more pronounced at concentrations above 4 ppm. Concentration-response relationships were very similar for naïve and pretreated rats, and the RD_{50}s were similar for both groups (naïve = 13.1 ppm [95% CI: 10.6–17.5]; pretreated = 10.8 ppm [95% CI: 7.6–16.9]). In contrast, naïve or pretreated mice did not develop tolerance during exposures. An examination of concentration-response relationships for mice showed similar RD_{50} values (naïve = 4.4 ppm [95% CI: 0.9–5.0] and pretreated = 4.3 ppm [95% CI: 3.4–5.5]) compared with rats, although the slopes of the concentration-response regressions were statistically different (Figure 4-2).

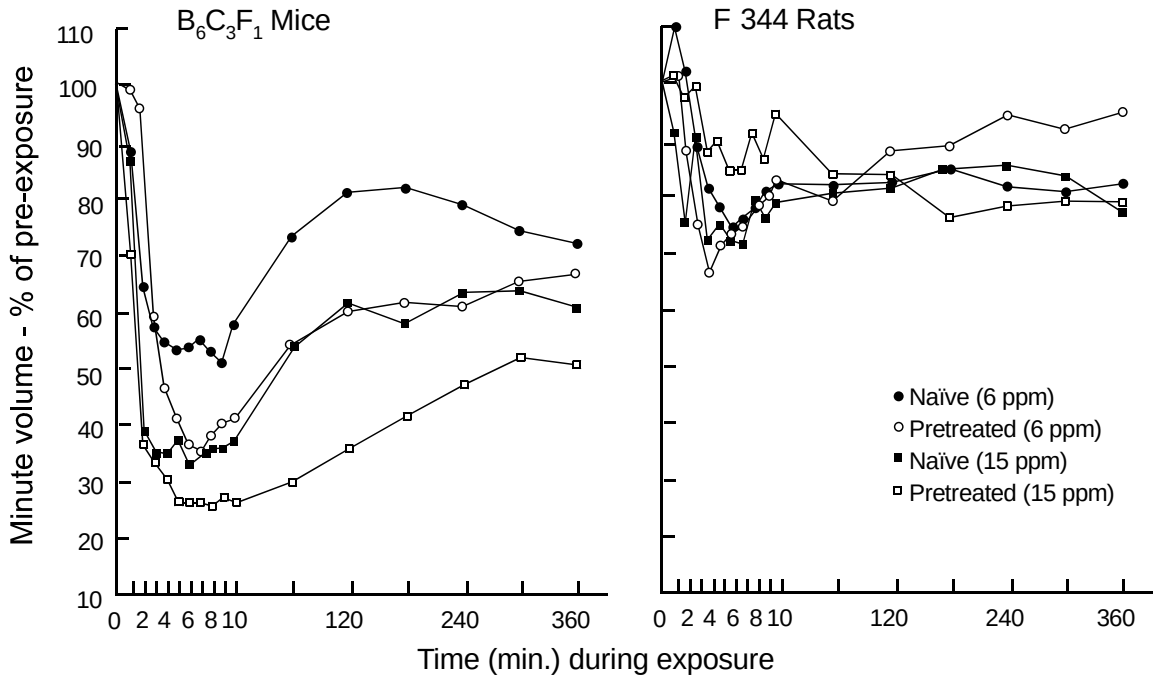

Figure 4-2. Formaldehyde effects on minute volume in naïve and formaldehyde-pretreated male B6C3F1 mice and F344 rats.

Source: Redrawn from Chang et al. (1983).

Exposure of naïve or pretreated rats resulted in an increased (compensatory) tidal volume. However, the increase in tidal volume did not compensate entirely for the decrease in ventilation rate and was only concentration dependent in pretreated rats. Comparison of tidal volume from naïve and pretreated mice exposed to formaldehyde showed a slight increase in naïve animals but a decrease in pretreated ones. The effect of formaldehyde exposure on tidal

volume was concentration dependent in both groups of mice. These results indicate that tidal volume does not compensate entirely for the decrease in respiratory rate and that the compensation is slightly greater in rats than in mice.

These studies (Barrow et al., 1983; Chang et al., 1983) showed that B6C3F1 mice sustain RB, whereas F344 rats develop tolerance more readily both during exposure and with pretreatment. Thus, these results suggest that the rat may be the more sensitive species for the effects of inhaled formaldehyde due in part to the difference in sensitivity between mice and rats as evidenced by an RD_{50} of 4.9 versus 31.7 ppm and the ability of rats to develop tolerance while mice appear to sustain RB. Barrow et al. (1983) used the results of these experiments to estimate an inhaled dose equivalent to the exposure concentration of 15 ppm for the strains of mice and rats used in the chronic formaldehyde bioassays by Kerns et al. (1983) and Monticello and Morgan (1994) described in Section 4.1.2 as follows:

Inhaled dose ($\mu g/min\text{-}cm^2$) =

$$\frac{\textit{HCHO concentration } (\mu g/L) \times \textit{minute volume } (L/min)}{\textit{Nasal cavity surface area } (cm^2)} \qquad (5\text{-}1)$$

As shown in Table 4-7, because mice were observed to be able to decrease their minute volume by approximately 75% as compared with 45% in rats, a twofold higher inhaled dose would be expected in rats versus mice. This difference may be relevant to the increased incidence of SCC in the nasal cavity seen in F344 rats when compared with B6C3F1 mice.

Table 4-7. Inhaled dose of formaldehyde to nasal mucosa of F344 rats and B6C3F1 mice exposed to 15 ppm

Parameter	F344 rats	B6C3F1 mice
HCHO concentration (μg/L)	18.4	18.4
Minute volume (L/min)	0.114	0.012
URT surface area (cm^2)	13.44	2.89
Inhaled dose ($\mu g/min/cm^2$)	0.156	0.076

Source: Barrow et al. (1983).

4.2.1.1.3. ***Cross-tolerance.*** Cross-tolerance of chemically-induced reflex responses has been examined in several systems in order to better understand the specificity and nature of the interaction of reactive chemicals (such as formaldehyde with chlorine) with the trigeminal nerve involved in the RB. Development of cross-tolerance to formaldehyde following preexposure to

chlorine or to chlorine following preexposure to formaldehyde was shown to be a function of the duration of the pretreatment in male F344 rats (Chang and Barrow, 1984) (Table 4-8). A 7-day recovery period resulted in only a slight loss of cross-tolerance from a 4-day pre-exposure to either chlorine or formaldehyde (data not shown). The cross-tolerance between formaldehyde and chlorine demonstrated in the Chang and Barrow (1984) study suggests that these chemicals may act via a common mechanism and may involve the trigeminal nerve. In rats, cross-tolerance was induced after chlorine exposure but not after formaldehyde exposure, which suggests that the trigeminal nerve may have different reactive sites that are differentially activated, depending on the stimulus.

Table 4-8. Exposure regimen for cross-tolerance study

Pre-exposure			Chlorine RD_{50}	
			FA-pretreated	**Naïve**
Formaldehyde	15 ppm 6 hours/day	1 day	22.6 ppm	10.9 ppm
		4 days	16.8 ppm	
		10 days	64.5 ppm	
Pre-exposure			**Formaldehyde RD_{50}**	
			Cl-pretreated	**Naive**
Chlorine	10 ppm 6 hours/day	1 day	64.5 ppm	31.7
		4 days	66 ppm	
		10 days	115 ppm	

Source: Chang and Barrow (1984).

Babiuk et al. (1985) evaluated the potential for formaldehyde pretreatment to cause cross-tolerance with various other inhaled aldehydes, including acetaldehyde and acrolein. Male F344 rats were pretreated with 15 ppm (18.4 mg/m^3) formaldehyde 6 hours/day for 9 days and challenged on the 10^{th} day with the second aldehyde for 10 minutes at various concentrations (four rats/concentration) to establish an RD_{50}. Exposure to acetaldehyde and acrolein, the two smallest molecules in the series of aldehydes tested, resulted in cross-tolerance. The RD_{50} and its 95% CI for acetaldehyde were estimated at 2,991 (95% CI: 2,411–3,825) ppm in the naïve rats, and this was increased by approximately 3.5-fold to 10,601 (95% CI: 7,902–15,442) ppm in the rats pretreated with formaldehyde. With acrolein, the RD_{50} increased approximately fivefold, from 6.0 (95% CI: 3.5–18.1) ppm to 29.6 (95% CI: 15.6–93.0) ppm. Cross-tolerance with formaldehyde has only been demonstrated with acetaldehyde, acrolein, and chlorine (Babiuk et al., 1985; Chang and Barrow, 1984), suggesting that it is not a generalized phenomenon.

Whether the phenomenon of tolerance involves modulation of specific trigeminal nerve receptors or whether it results from less specific chemical injury of the nasal mucosa has not

been determined. For example, different mechanisms lead to stimulation of the trigeminal nerve and are likely to control the decrease in respiratory rate. In particular, acetaldehyde might interact with sensory nerves via an amino group (Steinhagen and Barrow, 1984; Schauenstein et al., 1977), whereas the receptor-binding site for formaldehyde and acrolein is believed to be a thiol group. Furthermore, different binding sites exist on the trigeminal nerve for different irritants (Nielsen, 1991). Thus, Bos et al. (1992) concluded that the data on tolerance or "desensitization" versus "sensitization" (as defined strictly on the basis of the respiratory apneic response) may be the result of adaptation or reversible/irreversible adverse changes. The mechanisms underlying sensitization or desensitization are not well characterized.

4.2.1.1.4. ***Formaldehyde binding and activation of trigeminal nerve afferent activity.*** Kane and Alarie (1978) evaluated the effect of 11 combinations of acrolein and formaldehyde on respiratory rate in outbred specific-pathogen-free male Swiss-Webster mice. Exposure concentrations ranged from 0.12–8.97 ppm (0.28–21 mg/m^3) for acrolein and 0.37–9.73 ppm (0.45–11.9 mg/m^3) for formaldehyde. The data were evaluated using a simple model of competitive antagonism. Comparing the observed and predicted responses indicated no apparent differences, and paired t-tests showed no statistical significance. The authors concluded that acrolein and formaldehyde acted at the same receptor site and acted as competitive antagonists when exposure occurred simultaneously.

Kulle and Cooper (1975) investigated the effects of formaldehyde on trigeminal nerve afferent activity in adult male Sprague-Dawley rats. The authors isolated both the ethmoid and nasopalatine branches of the trigeminal nerve and recorded afferent signaling as electrical activity while reactive gases (formaldehyde, ozone, and amyl alcohol) were passed through the nasal passages of the anesthetized animals. The authors reported that both branches of the trigeminal nerve responded similarly to all three chemicals, and they therefore conducted the balance of their experiments on the nasopalatine branch of the nerve. Nerve response was calculated as the difference between exposed and control activity, and the threshold for a positive response was arbitrarily defined as an increase of 0.1 spikes per second. The sensory threshold was determined by extrapolation from the measured nerve response to a range of formaldehyde concentrations (0.5–2.5 ppm) or ozone (5.0–29 ppm) for an exposure duration of 2 minutes. Amyl alcohol exposure (0.3–10.0 ppm) lasted for 25 seconds. Threshold was arbitrarily defined as an increase of 0.1 spikes per second. The mean thresholds were 0.25 ppm for formaldehyde, 5.0 ppm for ozone, and 0.30 ppm for amyl alcohol, suggesting that the trigeminal nerve is highly sensitive to formaldehyde and amyl alcohol compared with ozone exposure.

In a second set of experiments, Kulle and Cooper (1975) investigated the effects of prolonged formaldehyde-exposure on the odor response to amyl alcohol. Rats were pre-exposed to a series of amyl alcohol concentrations (0.3, 0.7, 1.0, 3.3, 6.7, or 10.0 ppm [1.08, 2.52, 3.6, 11.9, 24, or 36 mg/m^3]) then a 1-hour continuous formaldehyde exposure (0, 0.5, 1.0, 1.5, or 2.0 ppm [0, 0.61, 1.23, 1.84, or 2.45 mg/m^3]). There was a progressive decrease in odor response to amyl alcohol with increasing stimulus of formaldehyde concentration ($p < 0.01$, analysis of variance [ANOVA]). The response to formaldehyde concentration was described by a power function $Y = 0.741 \times X^{1.47}$, where X is the formaldehyde concentration. The effects of exposure to 2.0 ppm were similar, regardless of whether it was presented immediately as a separate exposure or as the final concentration of a progressively increasing series. The response to amyl alcohol did not fully recover within the 1-hour extended recovery period. Thus, it appeared that the afferent function depression was not due to receptor adaptation or insufficient time for formaldehyde diffusion away from receptor sites.

In an attempt to elucidate the basis of the differential effects of various types of aldehydes on sensory irritation, Tsubone and Kawata (1991) recorded the afferent activity of the surgically isolated ethmoidal nerve (a branch of the trigeminal nerve) during delivery of 0.32–4.7 ppm (0.39–5.77 mg/m^3) formaldehyde, 0.18–7.2 ppm (0.41–16.5 mg/m^3) acrolein, and 134–2,232 ppm (241–4,021 mg/m^3) acetaldehyde into the cannulated URT of male Wistar rats (six/aldehyde) at a flow rate of 200 mL/minutes for 22 seconds. Only one aldehyde was used in each animal and each exposure was repeated two to four times at different concentrations. The activity of the nerve was recorded as the number of electrical discharges for a total period of 100 seconds, including pre-inhalation (30 second), inhalation (22 second), and post-inhalation (48 second) periods. Nitrogen was used as the control gas and as the vehicle to dilute the aldehyde gases in order to not interfere with the gas chromatography used to analyze the exposures. The vapor concentrations associated with a 50% increase in nerve activity over the level of control gas were calculated as approximately 1.8, 1.2, and 908 ppm for formaldehyde, acrolein, and acetaldehyde, respectively. These results are consistent with the findings of Steinhagen and Barrow (1984) and the hypothesis that the differences in RD_{50} are due to differences in chemical reactivity in the tissue.

In summary, RB is a phenomenon observed in rodents exposed to reactive gases, believed to be a protective response to the irritant properties of the gas. In comparative studies, rats appear more sensitive to irritant gases since they have a more pronounced RB response compared with mice at a given concentration of formaldehyde and because the dose required to elicit a bradypneic response is higher in rats than in mice. Interestingly, only rats appear to develop tolerance to irritant gases, while mice sustain an RB response. When formaldehyde

exposure is studied in concert with other reactive gases like chlorine and other aldehydes like acetaldehyde and acrolein, cross-tolerance developed. However, the mechanism underlying this response is unknown. It is thought that RB may occur as a result of stimulation of the trigeminal nerve. Thus, although RB appears to be a phenomenon specific to rodents, the mechanism by which it occurs, trigeminal nerve stimulation, may be applicable to understanding MOAs in other species, such as primates and humans, particularly in regard to sensitization.

4.2.1.2. *Respiratory Tract Pathology*

The database for evaluating the POE toxicity in the respiratory tract of inhaled formaldehyde is robust, with well-designed studies that span a duration range of a few hours to chronic 2-year bioassays. Toxicity testing has been performed in various species, including mice, rats, hamsters, guinea pigs, dogs, and nonhuman primates. Although a few studies include examination of tissues outside of the URT, the majority of studies focus on changes in cell proliferation and cell pathology in the nasal mucosa. Both mice and rats are well-defined animal models with standard histologic sections established to evaluate various regions of the nasal passages, divided into Levels 1 to 5 and illustrated in Figure 4-3. Pathology of the nasal mucosa will be discussed with reference to these sections, and the region examined will be stipulated (e.g., nasoturbinates, maxilloturbinates, or ethmoid turbinates [ETs]). Additionally, pathology of the respiratory epithelium will be distinguished from effects on the olfactory epithelium, although the nature of the lesions is similar.

4.2.1.2.1. *Mucociliary clearance*. The mucociliary apparatus of the URT is the first line of defense against airborne toxicants. Comprising a thick mucus layer (epiphase), hydrophase, and ciliated epithelium, the mucociliary apparatus may entrain, neutralize, and remove particulates and airborne chemicals from inspired air (Figure 4-4). The mucus serves to entrain or neutralize and remove exogenous agents from the nasal epithelium (e.g., particles, reactive chemicals). As reviewed by Kim et al. (2003), the nasal mucus contains proteins, glycoprotein, and lipids but is primarily water (95%) and is propelled along by movement of the underlying cilia. Degradation in the continuity or function of the mucociliary apparatus, which provides protection to the nasal epithelium, would result in higher levels of gases and particles reaching the nasal epithelium itself and greater penetration of chemicals into the respiratory tract. Therefore, breakdown and disruption of mucociliary function are adverse effects, since a key bodily defense to exogenous agents (including infectious agents) is damaged.

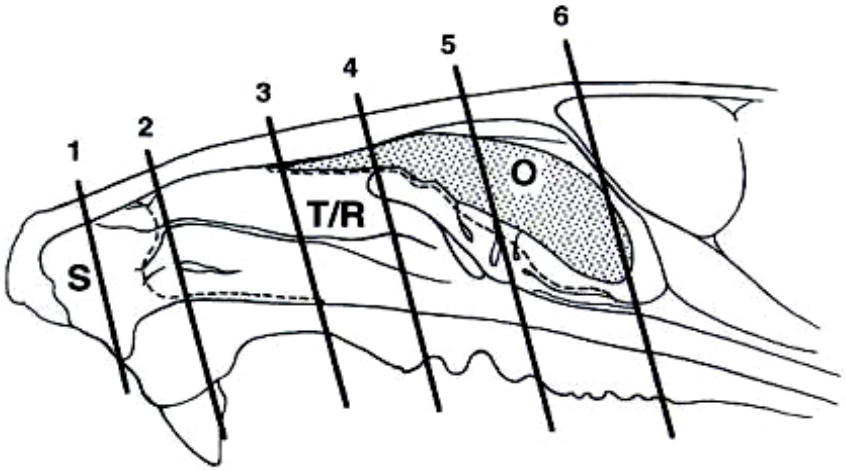

Figure 4-3. Sagittal view of the rat nose (nares oriented to the left).

Note: The figure shows the normal distribution of nasal mucosae and the section levels used in contemporary histopathology (Brenneman et al., 2000; Mery et al., 1994). Sections 1, 2, 4, and 5 correspond to Levels I, II, III, and IV as proposed by Young (1981). S = squamous, T/R = transitional/respiratory, O = olfactory mucosa.

Source: Brenneman et al. (2000).

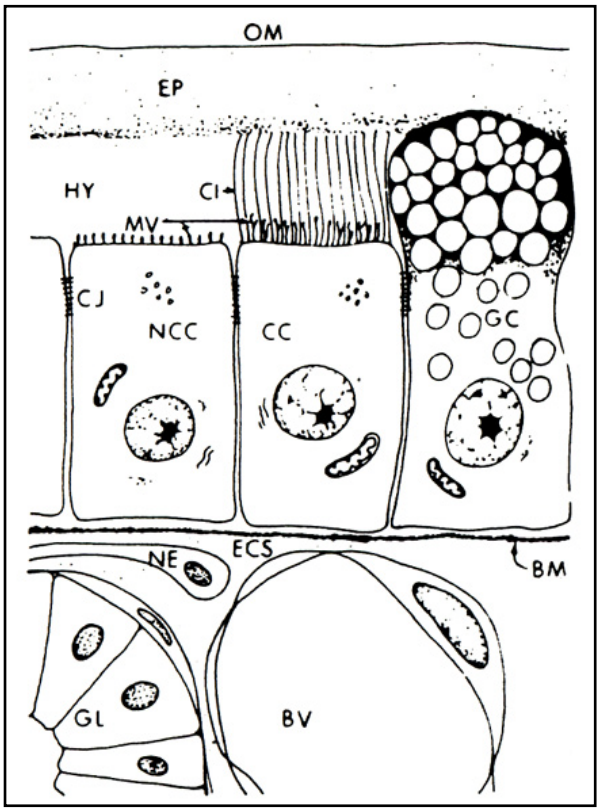

Figure 4-4. Main components of the nasal respiratory epithelium.

Note: OM = osmiophilic membrane; EP = epiphase; HY = hypophase; Cl = cilia; MV = microvilli; CJ = cell junction; CC = ciliated cell; NCC = non-ciliated cell; GC = goblet cell; NE = nerve; GL = gland; BV = blood vessel; ECS = extracellular space; BM = basement membrane.

Source: Morgan et al. (1986d).

Mucus flow slows upon formaldehyde exposure, despite an increase in the ciliary beat of the underlying epithelial cells, which propel the mucus across the nasal epithelium (Morgan et al., 1986a, c, d; 1983). These findings are consistent with other studies since airborne pollutants and reactive gases have been shown to decrease mucus flow rates in several animal models (Mannix et al., 1983; Iravani, 1974; Carson et al., 1966; Dalhamn, 1956; Cralley, 1942). In addition to slowing flow, the mucus layer has been observed breaking up as it floats on the epiphase, creating gaps in the epiphase and revealing the hydrophase below (Morgan et al., 1986c, d). Formaldehyde reacts with glycoproteins in the mucus of the epiphase, creating cross-links between these large molecules; this is believed to increase the viscosity of the mucus.

In their first experiments, Morgan et al. (1983) describe progressive mucostasis (slowing of mucous flow) and ciliastasis (disruption of ciliary beat) with increasing days of exposure to formaldehyde in male F344 rats (15 ppm; 6 hours/day for 1, 2, 4, or 9 days). Ciliastasis occurred with greater frequency and across more regions of the nasoturbinate with subsequent days of exposure. After 9 days, mucostasis was recorded in all but two regions evaluated. Although the severity and time course of these changes varied across regions of the nose, the process followed a similar pattern: decreased flow, increased ciliary action, mucostasis, and ciliastasis. Since the formaldehyde-induced deficits in mucociliary function increased with days of exposure, activity did not fully recover between exposures (18 hours) (Morgan et al., 1983). Therefore, the severity and extent of adverse effects are dependent on both the concentration of exposure and duration (in this case, days of repeated exposures).

In subsequent studies, Morgan et al. (1986c) examined the exposure-response relationship of formaldehyde effects on mucociliary function and functional recovery 18 hours after exposure ceased. Exposure regimens similar to the above experiment included additional exposure concentrations (0.5, 2, and 6 ppm) and an additional time point of 15 days duration. Exposure at 2 and 6 ppm resulted in the same progression of effects on mucus flow and ciliary beat. Considering both severity and extent of effects a clear exposure-response relationship was demonstrated. Additionally, within each exposure group, effects progressed both in severity and extent by duration of exposure to formaldehyde (from 1 to 4, 9, and 15 days of exposure) (Morgan et al., 1986c).

Flow and ciliary beat were not reduced, but rather increased, in epithelium from rats exposed to 0.5 ppm formaldehyde. Mucus flow in 2 of 10 areas assessed was clearly increased (275 and 200% of controls) after 4 days of exposure to 0.5 ppm formaldehyde. Two other epithelial regions showed a similar trend (150% of controls), but this change was not statistically significant. Interestingly, measurements made in corresponding areas after 9 days of exposure did not show an increase, and measurements in one region were reduced to 37% of control. Although it is not known whether the observed increase in mucus flow rate is a subtle indication of an adaptive response to a low level irritant, the increase appears to be transient. It is not known if flow rate would continue to decrease below control levels for repeated exposures at 0.5 ppm for longer than 9 days.

The regions affected at 15 ppm generally included the lateral aspects of the nasoturbinate and both the dorsal and medial aspects of the maxilloturbinate. In general there was an anterior to posterior effect with increasing concentration and time. Additionally, impaired mucociliary function was more extensive with greater concentration and length of exposure. Nasal lesions were seen on the nasal epithelium and correlated with those areas where some inhibition of

ciliary function was measured. Areas without mucus flow but that still retained ciliary function did not develop epithelial lesions. Morgan et al. (1986c) reported "coagulated mucus," viewed as a "continuous membrane" over the epithelium after 6 hours of exposure to 15 ppm formaldehyde. Minor cell damage and infiltrating neutrophils and monocytes were also seen in these areas. The coagulated mucus was not seen in similarly exposed rats that were allowed 18 hours of recovery before sacrifice. However, ciliated cells were damaged, and there was a greater presence of neutrophils and macrophages (MPs) after this recovery period. The authors noted that, as the exposure continued, these areas exhibited increased signs of inflammation and epithelial damage, eventually resulting in "severe degenerative changes."

Morgan et al. (1986a) refined their study design to implement a nose-only exposure to formaldehyde in order to better examine the progression of changes in mucociliary function during short-term exposure, allowing examination of mucus flow immediately following exposure. Three F344 rats/group were exposed to 15 ppm (18.4 mg/m^3) formaldehyde for 10, 20, 45, or 90 minutes or 6 hours. Two groups of rats were exposed to 2 ppm to determine a no effect level for 90 minutes or 6 hours. The extent and severity of mucostasis and ciliastasis seen after a 6-hour 15 ppm (18.4 mg/m^3) formaldehyde exposure and a 1-hour recovery period were similar to the earlier study (Morgan et al., 1986a), indicating that similar exposure conditions were reached with this nose-only apparatus. Ciliastasis and mucostasis were both less severe and less extensive in a time-dependent manner and at the earlier time points of 10, 20, 45, and 90 minutes. Significant recovery was seen in mucociliary function by allowing a 1-hour recovery between exposure and sacrifice. Regions of both the nasal septum and lateral wall, which exhibited no mucus flow when examined immediately after a 6-hour exposure, had measurable flow after the 1-hour recovery period. Similar recovery was seen at all durations of exposure. No decreases in mucociliary function were seen after exposure for either 90 minutes or 6 hours at 2 ppm formaldehyde. However, given evidence of recovery (Morgan et al., 1986a) and the time taken to dissect and view the tissues ex vivo may have obscured more subtle effects.

To assess more immediate effects on mucociliary apparatus, Morgan et al. (1984a) have examined formaldehyde effects on the mucociliary apparatus of isolated frog palates. This system allowed observation of mucociliary function during exposure. Unexposed frog palates were covered by a continuous sheet of mucus of variable thickness, which was observed to flow in streams across the palette, exhibiting a wave-like form in some areas of the epiphase. The authors reported particle movement in a lower, less viscous layer that was consistent with a less viscous underlying hydrophase, similar to that described in rat mucosa. The basal mucus flow rate was 0–4 mm/minute, with localized ciliary activity. Short periods of increased mucus flow were associated with seemingly spontaneous increases in ciliary beat.

Formaldehyde exposure resulted in an initial increase in ciliary beat and mucus flow rate in all palates exposed at 1.37, 4.36, and 9.58 ppm formaldehyde (but not 0.23 ppm). With increasing formaldehyde concentration and time of exposure, mucostasis was evident as mucus became stiff and eventually rigid. Ciliary beat continued after mucostasis was reached until palates were exposed to 4.36 and 9.48 ppm formaldehyde, when ciliastasis was reached. The time course to peak mucus flow rate, mucostasis, and ciliastasis was concentration dependent, with mucostasis reached in less than 3 minutes at 9.48 ppm. In contrast, increased mucus flow peaked at 8 minutes in palettes exposed at 1.52 ppm formaldehyde, which, though declining, remained above basal levels after 25 minutes with no mucostasis or ciliastasis noted at this level.

Fló-Neyret et al. (2001) demonstrated reduced mucociliary clearance and decreased frequency of ciliary beats by using a similar isolated frog palette mucociliary apparatus. However the palates were exposed by formaldehyde in the Ringer's solution in which the palates were placed (0, 1.25, 2.5, or 5 ppm). Also, mucus was removed from the palettes and did not come into direct contact with the formaldehyde. Despite these differences, formaldehyde caused mucociliary clearance to decrease in a time- and concentration-dependent manner; mucostasis occurred after 60 minutes of exposure to 5 ppm formaldehyde (Figure 4-5). Ciliary beat was decreased in a time-dependent manner at 2.5 and 5 ppm exposure but increased at 1.25 ppm formaldehyde (Figure 4-5). Reduced mucociliary clearance at 2.5 and 5 ppm was consistent with the reduced ciliary beat. However, clearance decreased at 1.25 ppm formaldehyde, where there was an apparent increase in ciliary beat. The authors suggest this may be a result of disrupting the harmonic movement of the cilia, impairing effective mucociliary clearance. Based on study results, the authors hypothesize that changes in ciliary beat, including excitation at lower exposures, are likely to be a direct effect of formaldehyde on epithelial cells or other cellular components of the mucosa.

In summary, numerous studies have identified impaired mucociliary clearance activity associated with formaldehyde exposure (Table 4-9). Although low-dose and short-term exposures first increase ciliary beat, impaired mucus flow, slowed ciliary beat, and eventual mucostasis and ciliastasis have been demonstrated in both in vivo and in vitro exposure systems. These effects are both concentration and duration dependent and can be seen in as few as 15 minutes from exposure. Repeated inhalation exposures in rats indicate the effect does not fully recovery in an 18-hour period between exposures, contributing to greater impairment over extended periods of exposure.

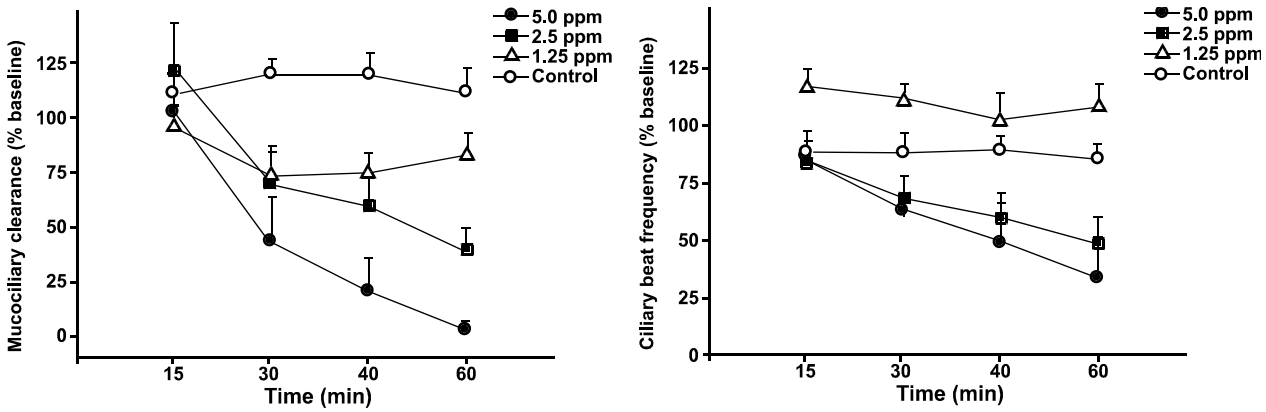

Figure 4-5. Decreased mucus clearance and ciliary beat in isolated frog palates exposed to formaldehyde after 3 days in culture.

Source: Fló-Neyret et al. (2001).

Morgan et al. (1983) suggested that the initial stimulation of ciliary activity may be a defensive response to the irritant gas, possibly indicating some penetration of formaldehyde to the underlying epithelial cells. Later effects of mucostasis may be a result of cross-linking of mucus glycoproteins by formaldehyde, creating a rigid mucus that is not able to flow even with a rigorous ciliary beat. It is unknown if the eventual cessation of ciliary beat is a result of compound-related effects on ciliated epithelium as formaldehyde diffuses through the mucus or an indirect effect associated with mucostasis. However, in vitro experiments by Fló-Neyret et al. (2001) indicate that formaldehyde in solution, supporting isolated frog palates without mucus, resulted in the same sequence of effects, including increased ciliary beat at the lowest exposure. These data suggest a role of formaldehyde beyond its ability to form protein cross-links in mucociliary proteins.

4.2.1.2.2. ***Cellular pathology*** **.** This section summarizes studies that have investigated cellular pathology in the URT and in the lung. Below, full study descriptions are provided for both short-term and subchronic duration studies (including, where appropriate, how cell proliferation relates to the observed formaldehyde-induced pathology).

Table 4-9. Summary of formaldehyde effects on mucociliary function in the upper respiratory tract

Species	N[a]	Treatment	Measure of mucociliary function	Summary of results by location	Reference
Male F344 rats	10	15 ppm formaldehyde 6 hours/day for 1, 2, 4, or 9 days	Mucus flow and ciliary beat	Mucostasis in regions 2, 3, 4, 5, and 8 for all rats after a single dose.Mucostasis in all but two regions evaluated by day 9. Ciliastasis followed mucostasis.	Morgan et al. (1983)
Male F344 rats	6	0, 0.5, 2, 6, or 15 ppm formaldehyde 6 ourshours/day for 1, 4, 9, or 15 days	Mucus flow and ciliary beat and histopathologic analysis	Flow or ciliary beat were increased at 0.5 ppm. After1After 1 day, slowed or halted mucociliary flow at 15 ppm after 6 hours. After 9 days, slowed or halted mucociliary flow decreased or completely stopped in all nasal regions evaluated. Regions affected included lateral aspect of the nasoturbinate and dorsal and medial aspects of maxilloturbinate.	Morgan et al. (1986c)
Male F344 rats	3 per group	15 ppm formaldehyde for 10, 20, 45, or 90 minutes or 6 hours	Mucus flow and ciliary beat	Ciliastasis and mucostasis increased in a time- and concentration-dependent manner, with maximal response at 6 hours. Significant recovery was observed when a 1-hour recovery period occurred between exposure and sacrifice.	Morgan et al. (1986a)
Isolated frog palates	Not stated	0.23, 1.37, 4.36, or 9.58 ppm formaldehyde	Mucus flow rates and histopathology	Ciliary beat and mucus flow increased from baseline at 1.37, 4.36, and 9.58 ppm. Over time, mucus became rigid, and ciliastasis occurred	Morgan et al. (1984a)
Isolated frog palates	4	0, 1.25, 2.5, and 5 ppm formaldehyde every 15 minutes for 60 minutes	Mucociliary clearance and ciliary beat	Ciliary beat decreased in a time-dependent manner at 2.5 and 5,0 ppm but was increased at 1.25 ppm. Mucostasis occurred after 60 minutes at 5 ppm.	Fló-Neyret et al. (2001)

N = number of animals in study.

4.2.1.2.2.1. ***Nasal pathology short-term studies.*** Inhalation of formaldehyde for a few hours has been shown to result in damage of the nasal mucosa, depending on the exposure concentration. Bhalla et al. (1991) observed changes in cell morphology in male Sprague-Dawley rat nasal epithelia after a single 4-hour exposure to 10 ppm (12.3 mg/m^3) formaldehyde. Three exposed rats were sacrificed 1 hour and 24 hours after exposure, with two control rats at each time point. Noses were fixed, decalcified, and sliced along the midsagittal plane through the nasal septum. The exposed turbinates were examined by scanning electron microscopy. Transverse sections through the hard palate, at the level of the incisive papillae, were prepared for light microscopy from similarly exposed rats (n = 10). The authors provided detailed descriptions of cell epithelial organization in untreated rat turbinates and changes observed in formaldehyde-treated rats, as set forth below. No statistical analysis was provided.

Scanning electron microscope examination of nasoturbinates showed increased mucus, erythrocyte infiltration, swelling of microvillus cells, and some cell separation in formaldehyde-treated rats. Nasoturbinates examined 1 day after exposure showed greater effects, including cell damage, matted cilia, and blebbing of cell membranes. Damage to microvillus cells of the maxilloturbinate included deformed cilia, cell swelling and rupture, and lack of typical microvilli on the cell margins. As in the nasoturbinates, damage was more marked 24 hours after exposure. The epithelium of the ETs exhibited less cell damage than in the nasal and maxillary regions, with the slight lesions noted in the upper (ET1) portion and little to no damage noted on the mid and lower (ET2 and ET3) regions. Examination of transverse tissue sections revealed swollen goblet cells and stretched epithelial cells that formed an epithelial lining approximately 40% taller than the lining seen in control rats. There was also a patchy loss of ciliated cells in the respiratory epithelium, where columnar cells were present.

Buckley et al. (1984) investigated the respiratory tract lesions associated with several sensory irritants. As part of this investigation, male Swiss-Webster mice were exposed to 3.13 ppm (3.85 mg/m^3) formaldehyde 6 hours/day for 5 days. A total of nine chemicals were tested in parallel. The report indicates there were 24–34 mice in each group, although not detailed for each chemical. One-half of the treatment group and unexposed controls were sacrificed immediately after the last exposure. The remaining exposed mice were sacrificed 72 hours later. The head, trachea, and lungs were fixed and heads decalcified. Five sections were taken of each nose at levels equivalent to standard levels 2–6 (Figure 4-3) and were examined by light microscopy. Details on lung and trachea sections were not given. Formaldehyde induced lesions in the respiratory epithelium of exposed mice, including inflammation, exfoliation, erosion, ulceration, necrosis, and squamous metaplasia. The section

level for these effects was not given. No effects were reported in the squamous epithelium, olfactory epithelium, trachea, or lungs of formaldehyde-exposed mice.

Monteiro-Riviere and Popp (1986) evaluated damage to the respiratory epithelium due to acute formaldehyde exposures. Male F344 (CDF [F344]/CrlBr) rats (three to five per group) were exposed at 0.5, 2.0, 6.0, or 15 ppm (0.62, 2.5, 7.4, or 18.5 mg/m^3) formaldehyde 6 hours/day for either 1, 2, or 4 days. Rats were sacrificed either immediately after exposure or 18 hours later (Table 4-10). After fixation and decalcification, blocks of tissue were collected from transverse sections of the skull. The first block of tissue, 1 µm thick, was taken just posterior of the incisor teeth. The second block was taken halfway between the first block and the incisive papillae. The dorsal nasal conchae, lateral wall, and ventral nasal conchae were microdissected, postfixed, and viewed by transmission electron microscopy (Monteiro-Riviere and Popp, 1986).

Table 4-10. Concentration regimens for ultrastructural evaluation of male CDF rat nasoturbinates

Formaldehyde[a,b]	Duration	Time of sacrifice	Observations
0.5 ppm (3)	6 hours for 1 day 6 hours for 4 days	18 hours later	No lesions. Altered ciliary configuration.
2.0 ppm (3)	6 hours for 1 day 6 hours for 4 days	18 hours later	No lesions. Altered ciliary configuration.
6 ppm (5 each group)	6 hours for 1 day 6 hours for 1 day 6 hours for 2 days 6 hours for 4 days	Immediately 18 hours later	Focal lesions on dorsal and nasal conchae and lateral wall. Severity of lesions increased with exposure duration.
15 ppm (5 each group)	6 hours for 1 day 6 hours for 2 days	18 hours later	Focal lesions on dorsal and nasal conchae and lateral wall. Severity of lesions increased with exposure duration. Severity of lesions increased with concentration.

[a]Number of exposed rats is shown in parentheses.
[b]Five control rats were examined for each experiment.
Source: Monteiro-Riviere and Popp (1986).

No lesions were observed at either 0.5 or 2.0 ppm formaldehyde for either 1 day or 4 days, evaluated 18 hours after exposure. However, an unusual altered ciliary configuration, including blebbing of the cell membrane, was observed in almost all formaldehyde-treated rats, whereas it was only "occasionally noted" in control rats. Focal lesions in the dorsal and ventral conchae and lateral wall were seen in rats exposed at 6 and 15 ppm for 1 day and sacrificed immediately after exposure. These lesions included cytoplasmic and autophagic vacuoles, loss of microvilli, and hypertrophy. Lesions increased in severity with both exposure concentration

and duration. Neutrophil infiltration and intercellular edema were seen after 1 day at 6 and 15 ppm. Nonkeratinized squamous metaplasia was noted after 4 days at 6 and 15 ppm in treated rats. Cell death and sloughing were noted after only 2 days of exposure at 6 ppm formaldehyde.

As described above, Cassee and Feron (1994) examined the effects of intermittent exposure to formaldehyde (3.5 ppm [4.3 mg/m^3]), ozone (0.44 ppm [0.86 mg/m^3]), or a combination of the two on changes to the rat nasal epithelium. Exposure occurred through six consecutive 12-hour cycles in which rats were exposed for 8 hours and then not exposed for a further 4 hours. Rats were weighed before the first and after the last exposure periods and sacrificed immediately after the last exposure. To collect tissue for biochemical analysis, skulls were split sagittally and the respiratory epithelium collected. Tissues from six rats were pooled and homogenized to enable the measurement of glutathione (GSH) and the activities of the following enzymes: glutathione S-transferase (GST), glutathione peroxidase (GPX), glucose-6-phosphate dehydrogenase (G6PDH), glutathione reductase (GR), alcohol dehydrogenase (ADH), and formaldehyde dehydrogenase (FALDH). The remaining heads were fixed, decalcified, and sectioned (standard cross sections [Figure 4-3]).

All groups, including controls, lost weight during the course of treatment. Rats exposed to formaldehyde, ozone, or both lost more weight than controls ($p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively). Formaldehyde treatment alone increased GPX from 48.6 to 64.0 µmole/minute-mg protein ($p < 0.05$) (Table 4-11). Formaldehyde exposure, in conjunction with ozone, decreased GST from 490 to 389 µmole/minute-mg protein ($p < 0.05$). No other enzyme activities or tissue GSH levels were affected by formaldehyde exposure.

Table 4-11. Enzymatic activities in nasal respiratory epithelium of male Wistar rats exposed to formaldehyde, ozone, or both

Enzyme	Controls[a]	Formaldehyde (3.5 ppm)	Ozone (0.4 ppm)	Both[b]
ADH	2.66 (0.99)	3.53 (0.13)	3.40 (0.33)	2.42 (0.61)
GST	490 (32)	494 (24)	514 (4)	389 (28)[c]
GPX	48.6 (4.3)	64.0 (7.9)[c]	55.6 (2.0)	54.5 (0.3)
G6PDH	58.9 (7)	60.8 (4.7)	65.8 (1.0)	45.5 (6.8)
GR	275 (16)	288.2 (16)	279 (17)	236 (14)
FALDH	0.77 (0.03)	0.68 (0.04)	0.68 (0.07)	0.80 (0.08)

[a]Values shown are the means and SDs of three measurements of a pooled sample. Units are µmole/minute/mg of cytosolic protein.
[b]Rats were exposed intermittently, 12-hour cycles of 8 hours exposed and 4 hours unexposed, for 3 days.
[c]Different from control, $p < 0.05$.

Source: Cassee and Feron (1994).

Formaldehyde-exposed rats exhibited lesions in the nasal epithelium at levels 2 and 3 of the nose, with effects slightly more severe in level 2. Lesions observed include necrosis, hyperplasia accompanied by squamous metaplasia, and rhinitis. Exposure to formaldehyde in the presence of ozone resulted in more severe squamous metaplasia (statistics not given). These findings are similar to those of Monteiro-Riviere and Popp (1986), indicating that single or repeated exposures can result in cell damage and death. Cell death and increased cell proliferation were seen here after 3 days of repeated exposures to 3.5 ppm formaldehyde. While no increases were seen in olfactory epithelium, frank necrosis, squamous metaplasia, and hyperplasia of both ciliated and nonciliated epithelium were noted at level 2 and 3.

Javdan and Taher (2000) exposed male and female albino Wistar rats (five/group) at 0, 2, or 5 ppm (0, 2.5, or 6.2 mg/m^3) formaldehyde 8 hours/day for either 3 or 30 days. Transverse tissue sections at the base of the incisive teeth and the first palatine folds were examined by light microscopy. Lesions reported after 3 days of exposure to 2 ppm formaldehyde included chorion congestion, cell disarrangement, squamous hyperplasia, atypical mitosis, and epithelial hyperplasia. Similar lesions were seen after 30 days but were more severe. Effects at 5 ppm formaldehyde included goblet cell proliferation, olfactory epithelial hyperplasia, calcified regions, and an abscess on the chorion. These lesions were more severe after 30 days of exposure.

Kamata et al. (1996a, b) conducted several high-dose studies by inhalation in rats. Specifically they exposed male F344 rats to 0, 128.4, or 294.5 ppm (0, 158, or 362 mg/m^3) formaldehyde for 6 hours (Kamata et al., 1996a). In a subsequent study in the same laboratory, male F344 rats were exposed to either 0, 15, or 145 ppm (0, 18.5, or 178 mg/m^3) formaldehyde nose only for 6 hours (Kamata et al., 1996b). Congestion was noted in the nasal cavities of formaldehyde-exposed rats and was more severe at 145.6 ppm (Kamata et al., 1996b). Rats exposed to 15 ppm formaldehyde had lesions in the nasal turbinate and trachea (not detailed) (Kamata et al., 1996b). A slight hypersecretion of mucus was noted in the tracheal epithelium in the absence of histopathologic changes. Rats exposed to 145.6 ppm had more dramatic lesions that penetrated more deeply into the respiratory tract. Hyperkeratosis of the squamous epithelium was found at level 1 of the nasal cavity. Hypersecretion, desquamation, and irregular mucosal epithelium were seen in levels 2, 3, 4, and 5 of the nasal cavity, with more severe changes noted in the nasal septum. Increased secretion and desquamation of mucosal cells occurred in the trachea, and a slight hyperplasia of the alveolar wall was noted in rats exposed to 145.6 ppm formaldehyde (Kamata et al., 1996b)

Hester et al. (2003) carried out a transcriptional analysis of the nasal epithelium of male F344 rats 24 hours after nasal instillation of 40 µL of 400 mM formaldehyde. Immediately after

sacrifice, cells were harvested from the nasal cavity for RNA extraction. The authors found several phase I and II enzymes, indicative of oxidative stress, to be elevated. They also reported the greatest increase in inflammatory genes, such as iNOS and neuropeptides. In an effort to phenotypically link any gene changes to pathology, Hester et al. (2003) noted that this exposure scenario has been demonstrated to induce regenerative hyperplasia with minimal cytotoxicity. In this regard, they observed no significant change in nine genes involved in three apoptotic pathways.

In an expansion of their earlier study, Hester et al. (2005) carried out a transcriptional analysis of the nasal epithelium of male F344 rats that had been exposed to formaldehyde by nasal instillation for a single exposure, 5 days of exposures, or 28 days of exposure. In addition, this study also attempted to characterize the comparative toxicity of glutaraldehyde with structurally similar formaldehyde (van Birgelen et al., 2000). Thus, four animals per group were instilled with 40 µL of deionized water (control group), 40 µL of 400 mM formaldehyde, or 40 µL of 20 mM glutaraldehyde. Phenotypically, both aldehydes induced similar histopathologic changes.

Both aldehydes induced similar changes in DNA repair and apoptotic pathways initially, but the patterns of gene changes were different after about 5 days of exposure. Eight genes were differentially expressed between formaldehyde and glutaraldehyde that indicated different pathways for DNA repair, including recombination, base excision repair, and nucleotide excision repair. Within this group, replication protein 70 and DNA excision repair ERCC1 showed a twofold induction by formaldehyde compared with glutaraldehyde. Since both of these genes and their products function by recognizing and removing damaged DNA bases, Hester et al. (2005) hypothesized that formaldehyde-exposed cells may remove damaged bases more efficiently than glutaraldehyde-exposed cells

4.2.1.2.2.2. ***Lung pathology: short-term studies.*** In addition to nasal pathology, several researchers specifically investigated formaldehyde-induced effects in the trachea, bronchi, and pulmonary tissues of the deep respiratory tract in a variety of species (Lino dos Santos Franco et al., 2006; Kamata et al., 1996a, b; Schreibner et al., 1979; Ionescu et al., 1978).

Ionescu et al. (1978) described progressive damage in pulmonary tissue of adult male rabbits exposed to an aerosol of 3% formaldehyde solution 3 hours/day for up to 50 days (method of aerosol generation or particle size were not provided). An equivalent air concentration was not reported and cannot be derived from the information given. Animals were sacrificed at several time points (3, 7, 15, 20, 30, and 50 days), and fragments of the caudal lobes of both lungs were taken to examine bronchi (intrapulmonary and distal) and lung parenchyma.

Enzymatic activity was characterized in frozen sections for β-galactosidase, adenosine triphosphatase (ATPase), adenosine monophosphatase (AMPase), lactate dehydrogenase (LDH), malate dehydrogenase, succinate dehydrogenase (SDH), acid phosphatase, Tween-60 esterase, naphthol-AS-D-acetate esterase, proline oxidase, hydroxyproline epimerase, leucyl aminopeptidase, and β-glucuronidase. A portion of the lung was fixed and sectioned and viewed by light microscopy to determine changes in cell populations and tissue pathology.

In addition, biochemical analysis revealed that enzymatic activity of β-galactosidase, ATPase, AMPase, LDH, malate dehydrogenase, and SDH were all unchanged by formaldehyde exposure across the course of treatment (Ionescu et al., 1978). The activities of several enzymes were increased through the course of exposure, including acid phosphatase, Tween-60 esterase, naphthol-AS-D-acetate esterase, proline oxidase, and hydroxyproline epimerase. Although no details were reported, the authors described the changes as progressive, with the increase in proline oxidase and hydroxyproline epimerase seen only in the second half of the treatment course. The activities of two enzymes, leucyl aminopeptidase and β-glucuronidase, were observed to decrease rapidly (time frame not provided) (Ionescu et al., 1978)

Histologic changes in the lung tissue were noted after only 3 days of exposure and were generally progressive throughout the course of treatment. Early changes in the bronchial epithelium included increased mucus secretion, hyperplasia, and hypertrophy of epithelial cells. Lymphocyte infiltration was noted in many areas, and a limited thickening of the alveolar walls was reported after 3 days of exposure. Epithelial cell lesions, thickening of the alveolar, and infiltration of lymphocytes increased as exposure continued. Mucus cells increased as much as 40% after 40 days of treatment. After 40 days of treatment, Ionescu et al. (1978) observed "destructive and fibrotic lesions" and provided a detailed description of progressive lesions.

Schreiber et al. (1979) also examined histologic changes in lung tissue after high formaldehyde exposures. Syrian golden hamsters (34, sex not stated) were exposed to 250 ppm (308 mg/m^3) formaldehyde 1 hour/day for 1, 2, 5, or 15 days. Five hamsters in each treatment group were sacrificed 2 days after exposure was ended. Three hamsters in each group were sacrificed 1, 2, or 6 weeks after exposure ended to determine if formaldehyde-induced changes regressed over time. Tracheal washing was carried out to collect cytologic samples in each animal prior to sacrifice. Samples were fixed, stained, and examined by light microscopy. Lungs and tracheae were removed en bloc and fixed, and 20, 1 μm thick cross sections were taken (location not detailed). The remaining respiratory tissue was sectioned at 200 μm intervals. Sections were stained and viewed by light microscopy.

Abnormal epithelial cells were found in tracheal washings from formaldehyde-exposed hamsters. Schreibner et al. (1979) described cells with lobulated nuclei and a coarse chromatin

pattern, especially in cells showing signs of degeneration (e.g., vacuolization of nuclei and cytoplasm) (Schreiber et al., 1979). Cell number and damage were not quantified, and there was no discussion of the effects of exposure duration on treatment, if any, on these observations. Tracheal washing was normal 2 and 6 weeks after the end of exposure, indicating that the cytological changes were reversible (Schreiber et al., 1979).

Formaldehyde exposure caused multifocal lesions in the mucociliary epithelium in the trachea and larger bronchi. Dysplastic and poorly differentiated squamous metaplastic foci replaced ciliated epithelium (Schreiber et al., 1979). Abnormal nuclear membranes, tonofibrils around the nuclei, the appearance of nucleoli, and heterochromatin condensation were distinct in the formaldehyde-treated hamsters. These changes, observed 2 days after formaldehyde exposure, were reversible over time and not seen 2 and 6 weeks later.

Because of the similarity of form and physiology of rhesus monkey URTs to the human respiratory tract, the effects of short-term formaldehyde exposure were evaluated in both nasal and lung tissue in these monkeys by Monticello et al. (1989). Male rhesus monkeys (nine/group) were exposed to 6 ppm formaldehyde (7.4 mg/m^3) 6 hours/day for 5 days/week for either 1 or 6 weeks. Control animals were exposed to the same regimen of filtered air for 1 week. Monkeys were weighed during the course of exposure and observed for clinical signs of irritation or sickness. Monkeys were intravenously injected with [^{3}H]-thymidine 18 hours after the last formaldehyde treatment to evaluate induced cell proliferation. Sections of the nasal passages, trachea, larynx, lung carina, and duodenum were processed for histoautoradiography. Tissues fixed and sectioned for examination by light microscopy included nose, adrenal, sternum (bone marrow), duodenum, esophagus, eyes, gallbladder, heart, kidney, liver, lymph nodes, pancreas, stomach, spleen, and tongue. The nose was cut into a series of transverse sections, 3 µm thick, and sections from five levels were examined (Figure 4-6). Lung lobes were trimmed midsagittally and sectioned with care to include airway bifurcations. Sections of the nasal passages, trachea (cross section), larynx (cross section), lung carina (frontal section), and duodenum were also processed for histoautoradiography.

There were no significant changes in body weight over the course of the experiment. Oronasal breathing was noted in the first 15 minutes of formaldehyde exposure (Monticello et al., 1989). Monkeys did experience eye irritation (mild lacrimation and conjunctival hyperemia) during exposure.

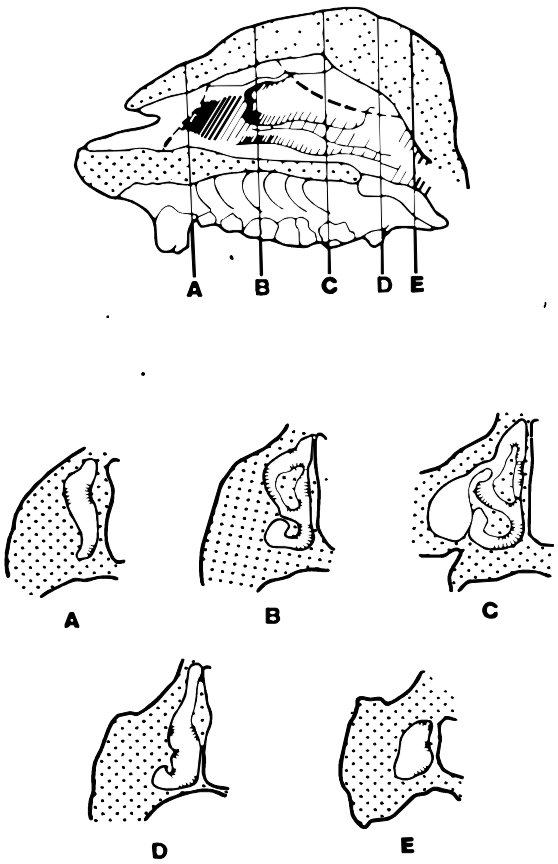

Figure 4-6. Diagram of nasal passages, showing section levels chosen for morphometry and autoradiography in male rhesus monkeys exposed to formaldehyde.

Source: Redrawn from Monticello et al. (1989).

Formaldehyde-related lesions were reported in the nasal passages, tracheas, and in the larynx of treated animals (Figure 4-7) (Monticello et al., 1989). Nasal epithelium from treated animals exhibited many of the histologic lesions described in rodent studies, including loss of goblet cells, loss of cilia, epithelial hyperplasia, squamous metaplasia, and neutrophilic inflammatory response in the respiratory epithelium. The lesions were more severe after 6 weeks of exposure and were present over a greater percentage of the epithelium compared with the 1-week exposure group ($p < 0.05$) (Figure 4-7).

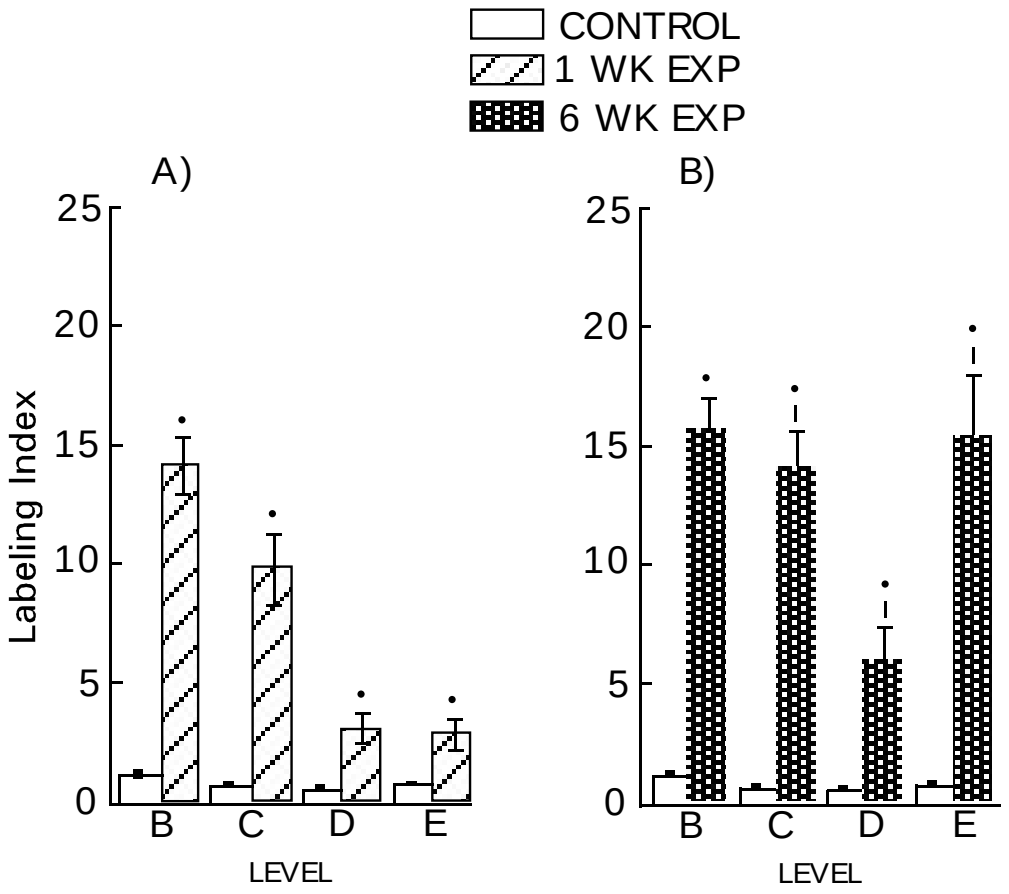

Figure 4-7. Formaldehyde-induced cell proliferation in male rhesus monkeys exposed to formaldehyde

Note: Animals were exposed to 6 ppm formaldehyde 6 hours/day, 5 days/week for 1 or 6 weeks. Bar graph depicting mean labeling indices for the respiratory epithelium at levels B−E. A: One-week exposure group. B: Six-week exposure group. *Statistically different from controls ($p \leq 0.05$). Statistically different from 1-week exposure group ($p \leq 0.05$).

Source: Redrawn from Monticello et al. (1989).

There was a distinct anterior to posterior gradient in both 1-week and 6-week treatment groups in which the anterior regions had a higher percentage of impacted epithelium (Monticello et al., 1989). However, the longer duration exposure produced significantly more lesions in the larynx and trachea compared with those observed after only 1 week of exposure ($p < 0.05$). No formaldehyde-related lesions were reported for the epithelium of the maxillary sinus, a structure not present in rodents. Labeling indices (LIs) from the histoautoradiograms indicated increased cell proliferation in transitory, respiratory, and olfactory epithelial cells after the 6-week

formaldehyde exposure (Figure 4-8) (Monticello et al., 1989). Similar trends were seen after only 1 week but were statistically significant only in the respiratory epithelium. Although increased proliferation in the trachea and carina was statistically significant after 1 week of exposure, the greater increases seen after 6 weeks of exposure, compared with controls, were not statistically significant. A small sample size (n = 3) and high variability may have contributed to the lack of statistical significance. Monticello et al. (1989) noted that increased cell proliferation was seen in locations with minimal histologic changes, indicating proliferation may be a more sensitive predictor of adverse health effects of formaldehyde exposure.

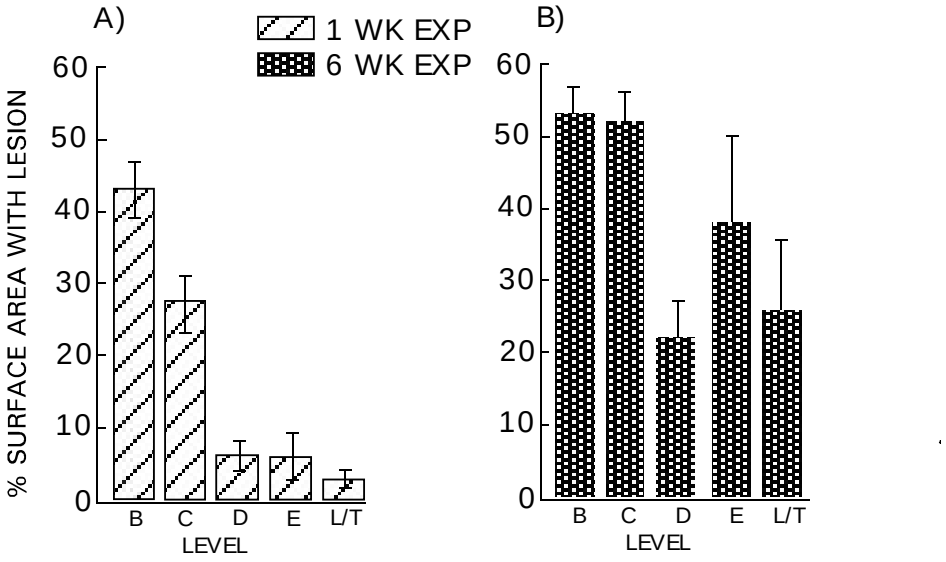

Figure 4-8. Formaldehyde-induced lesions in male rhesus monkeys exposed to formaldehyde.

Note: Animals were exposed to 6 ppm formaldehyde 6 hours/day, 5 days/week for 1 or 6 weeks. Bar graph showing levels B–E of the nasal passages and the larynx/trachea (L/T), depicting percent surface area with formaldehyde-induced lesions. Morphometry of level A was excluded due to the similarity of normal features of transitional epithelium to formaldehyde-induced lesions in the respiratory epithelium. A: One-week exposure group. B: Six-week exposure group.

*Statistically different from controls ($p \leq 0.05$).

| Statistically different from 1-week exposure group ($p \leq 0.05$).

Source: Redrawn from Monticello et al. (1989).

There are two reports in the literature assessing changes in pulmonary tissues after acute formaldehyde exposures (Kamata et al., 1996a, b). Kamata et al. (1996a) exposed male F344

rats to 0, 128.4, or 294.5 ppm (0, 158, or 362 mg/m^3) formaldehyde for 6 hours. Lung lavage samples were collected and the fluid analyzed for the lipids, free cholesterol, phosphatidyl ethanolamine, phosphatidyl choline, sphingomyelin, and triglyceride.

The bronchoalveolar lavage (BAL) was analyzed for triglycerides, cholesterol, and phosphatidyl choline. As in the first experiment (Kamata et al., 1996a), triglyceride concentration was reduced in the lavage of treated animals, in this case, to 16% of controls in lavage in those rats exposed to 145.6 ppm formaldehyde (Table 4-12). Cholesterol concentration was unchanged and phosphatidyl choline was increased to 220% of that of control rats as a result of exposure to 145.6 ppm formaldehyde. However, BAL lipids were unchanged in 15 ppm exposed rats. Triglycerides were reduced in unwashed lung tissue from formaldehyde-treated rats in a concentration-dependent manner and free fatty acids were reduced in rats exposed to 145.6 ppm formaldehyde. Neither triglyceride nor sphingomyelin was detected in lung lavage fluid from the high treatment group.

Table 4-12. Lipid analysis of lung tissue and lung lavage from male F344 rats exposed to 0, 15, or 145.6 ppm formaldehyde for 6 hours

	Control[a]	15 ppm[a]	145 ppm[a]
Lung tissue			
Free fatty acids (mg/g lung)	3.30 (0.7)	3.11 (1.23)	1.41 (0.63)[b]
Triglyceride (mg/g lung)	1.55 (0.23)	0.74 (0.14)[c]	0.62 (0.17)[c]
Cholesterol (mg/g lung)	1.72 (0.10)	1.41 (0.25)	1.16 (0.55)
Phosphatidyl ethanolamine (mg/g lung)	7.41 (1.81)	7.46 (2.28)	5.49 (1.78)
Phosphatidyl choline (mg/g lung)	11.0 (1.49)	9.65 (3.21)	7.53 (3.52)
Sphingomyelin (mg/g lung)	3.44 (0.75)	3.13 (1.28)	2.51 (0.95)
Lung lavage			
Triglyceride (mg/lung)	0.31 (0.10)	0.24 (0.09)	0.05 (0.02)[c]
Cholesterol (mg/lung)	0.04 (0.01)	0.04 (0.01)	0.04 (0.01)
Phosphatidyl choline (mg/lung)	0.66 (0.23)	0.84 (0.35)	1.45 (0.31)[c]

[a]SD given in parentheses.
[b]Significant difference from controls ($p < 0.05$).
[c]Significant difference from controls ($p < 0.01$).

Source: Kamata et al. (1996b).

Concentration-dependent decreases were seen in nonprotein sulfhydryl (SH) groups and lipooxygenase in nasal mucosa homogenate and nonprotein SH groups in lung tissue homogenate (Table 4-13). Increases in both lipooxygenase and LDH activities were found in lung tissue homogenate from formaldehyde-exposed rats.

Table 4-13. Formaldehyde effects on biochemical parameters in nasal mucosa and lung tissue homogenates from male F344 rats exposed to 0, 15, or 145.6 ppm formaldehyde for 6 hours

	Control[a]	15 ppm[a]	145 ppm[a]
Nasal mucosa[b]			
Nonprotein SH groups (μM/g tissue)[c]	1.64 (0.50)	1.29 (0.28)	0.73 (0.21)[f]
Lipid peroxides (μM/g tissue)	118 (23)	71 (16)[f]	59 (18)[f]
Glucose-6-dehydrogenase (U/g tissue)[d]	1.96 (0.10)	1.87 (0.07)	2.07 (0.13)
Lung[e]			
Nonprotein SH groups (μM/g tissue)[c]	1.83 (0.18)	1.70 (0.11)	1.29 (0.28)[f]
Lipid peroxides (μM/g tissue)	72 (8)	95 (15)[f]	93 (8)[g]
Glutathione reductase (U/g tissue)[d]	0.42 (0.25)	0.25 (0.05)	0.22 (0.05)
Lactate dehydrogenase (U/g tissue)[d]	77.37 (9.28)	88.69 (7.66)	93.62 (4.99)[f]

[a]SD given in parentheses.
[b]5 or 10% nasal mucosa homogenates.
[c]nmol malonaldehyde/g tissue.
[d]Units per gram tissue.
[e]20% lung homogenates.
[f]Significant difference from controls ($p < 0.01$).
[g]Significant difference from controls ($p < 0.05$).

Source: Kamata et al. (1996b).

Lino dos Santos Franco et al. (2006) studied the effects of inhaled formaldehyde on lung injury and changes in airway reactivity in rats. The extent of local and systemic inflammation was assessed by changes in leukocyte counts in BAL fluid, blood, bone marrow, and spleen. Changes of reactivity of isolated tracheae and intrapulmonary bronchi in response to methacholine were monitored in response to formaldehyde exposure. The authors exposed male Wistar rats to formaldehyde generated from a 1% solution of formalin. However, they provided insufficient information for the exposure concentration to be determined. Groups of six animals were exposed to formaldehyde for either 0, 30, 60, or 90 minutes on 4 consecutive days. All experiments were carried out 24 hours after the final exposure.

The authors reported a significantly increased number of leukocytes in the BAL fluid of animals exposed to formaldehyde via inhalation. The effect reached a maximum for the longer exposure duration (90 minutes). Compared with controls, rats exposed to formaldehyde 90 minutes/day for 4 days also displayed an increase in the number of total blood leucocytes ($1.4 \pm 0.06 \times 10^4$ versus $0.8 \pm 0.01 \times 10^4$ cells/mm^3). These values are means ± standard error of the mean (SEM) for six animals/group. The effect appeared to reflect changes in the mononuclear cell population ($1.1 \pm 0.02 \times 10^4$ versus $0.6 \pm 0.003 \times 10^4$ cells/mm^3) rather than peripheral blood neutrophils ($0.2 \pm 0.003 \times 10^4$ cells/mm^3 in test animals and controls). There was also an apparently compound-related increase in the total cell count in the spleen

($112.7 \pm 4.4 \times 10^6$ versus $94.2 \pm 5.5 \times 10^6$ cells). However, a change in the number of cells eluted from bone marrow did not reach statistical significance ($54.6 \pm 1.3 \times 10^6$ versus $45.0 \pm 4.3 \times 10^6$ cells). Lino dos Santos Franco et al. (2006) provided data on dose-dependent changes in methacholine-induced contractions in isolated tracheae and bronchi obtained from formaldehyde-exposed and control rats. Although the maximal contractile response induced by methacholine in tracheae of formaldehyde-treated rats was unchanged compared with controls, contractions in isolated bronchi were significantly weaker than those observed in controls.

The authors examined the effect of formaldehyde inhalation on rat lung mast cells. Degranulation and significant neutrophil infiltration were features of the response to formaldehyde (Table 4-14).

Table 4-14. Mast cell degranulation and neutrophil infiltration in the lung of rats exposed to formaldehyde via inhalation

Treatment group	Mast cell degranulation (cells/mm^2)[a]	Neutrophil infiltration (cells/mm^2)[a]
Controls	0[b]	0.3 ± 0.2
Formaldehyde-exposed	2.0 ± 0.4[c]	5.2 ± 1.7[c]

[a]Values are means ± SEM; n = 6.
[b]4.2 ± 0.6 cells/mm^2 intact mast cells were found in the lungs of controls.
[c]No statistical analysis was provided by the authors for these changes.

Source: Lino dos Santos Franco et al. (2006).

Selected pharmacological agents were used to explore the mechanism by which exposure to formaldehyde might have brought about the observed lung infiltration and bronchial hyporesponsiveness. Lino dos Santos Franco et al. (2006) provided data showing that separate pretreatment of the animals with compound 48/80, sodium cromoglycate (SCG), and indomethacin reduced the formaldehyde effect on neutrophil release into BAL but had no effect on mononuclear cell counts. Compound 48/80 and SCG also reversed the formaldehyde-induced reduction in bronchial response to methacholine, but indomethacin had the opposite effect (causing an additional decrease in bronchial responsiveness). In broad terms, these findings were thought to implicate mast cells as a possible mediator of the toxicological effects of formaldehyde. Histologically, a significantly increased number of degranulated mast cells were evident in the pulmonary tissue of rats that were exposed to formaldehyde.

Lino dos Santos Franco et al. (2006) also examined the regulatory role of NO on formaldehyde-induced bronchial activity. Nitrites generated by cultured cells of BAL from formaldehyde-treated rats increased about threefold compared with those from controls.

However, pretreatment with the NO synthase inhibitor, N-nitro-L-arginine methyl ester, prevented the formaldehyde-induced bronchial hyporesponsiveness to methacholine but had no effect on pulmonary leukocyte recruitment. These data implicate the existence of distinct mechanisms for the induction of lung inflammation versus bronchial hyporeactivity. Further support for this concept came from an experiment in which rats were pretreated with capsaicin to examine the involvement of sensory fibers in lung inflammation and the bronchial hyporesponsiveness induced by formaldehyde inhalation. Although the treatment did not influence formaldehyde-induced bronchial hyporesponsiveness to methacholine, the number of leukocytes recovered in the BAL fluid were reduced compared with those of rats exposed to formaldehyde alone.

4.2.1.2.2.3. ***Extrapulmonary effects: short-term studies.*** Kamata et al. (1996a) exposed male F344 rats to 0, 128.4, or 294.5 ppm (0, 158, or 362 mg/m^3) formaldehyde for 6 hours. In addition, blood samples were monitored for hematology and clinical chemistry parameters, including red blood cell (RBC) count, hemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), white blood cell (WBC) count, and plasma levels of total protein (TP), albumin (ALB), blood urea nitrogen (BUN), glucose, phospholipids, triglycerides, total cholesterol, cholinesterase, and LDH. Male rats exposed to 294.5 ppm formaldehyde had increased RBC count, Hb, hematocrit (HCT), MCV, and serum glucose ($p < 0.05$) compared with controls (Kamata et al., 1996a). There were concentration-related decreases in serum measures of TP, ALB, and phospholipids ($p < 0.05$). BUN was decreased in rats exposed to 128.4 ppm but increased in the higher treatment group ($p < 0.05$). Phospholipid analysis of the lung surfactant indicated a decrease in the production in formaldehyde-treated animals ($p < 0.05$). Total free cholesterol, phosphatidyl ethanolamine, and phosphatidyl choline were reduced to 60, 55, and 38% of controls for rats treated with 294.5 ppm formaldehyde ($p < 0.05$). Sphingomyelin was reduced to 32% of controls in the low treatment group ($p < 0.05$).

In a subsequent study in the same laboratory (Kamata et al., 1996b), male F344 rats were exposed to either 0, 15, or 145 ppm (0, 18.5, or 178 mg/m^3) formaldehyde nose only for 6 hours (Kamata et al., 1996b). Fifteen animals were treated at each level and separated into subgroups of five animals each for tissue collection and the determination of other endpoints. Blood samples were collected from one subgroup to determine such hematological and clinical chemistry parameters as RBC count, Hb, PCV, MCV, MCHC, WBC count, and plasma levels of TP, ALB, BUN, glucose, phospholipids, triglycerides, total cholesterol, LDH, alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and

G6PDH. BAL was collected from five animals of each group and analyzed for phospholipids. Lung homogenate from five animals in each treatment group was analyzed for nonprotein SH groups, lipid peroxides, and total lipids. The 20,000 × g supernatant of the lung homogenate was assayed for the activities of GR, G6PDH, and LDH. Similarly, nonprotein SH groups and lipid peroxidase were measured in homogenates of excised nasal mucosa. At autopsy, organs (brain, heart, lung, liver, kidney, spleen, and testis) were weighed and tracheae and nasal turbinates examined. After fixation and decalcification, five sections across the nose were taken, corresponding to standard sections 1–5 (Figure 4-3).

Several blood parameters were affected after these acute exposures. The WBC count was slightly increased, from 4.7×10^3 cells/mm^3 in control rats to 5.1×10^3 cells/mm^3 and 6.1×10^3 cells/mm^3 at 15 and 145.6 ppm formaldehyde, respectively (Kamata et al., 1996b). Serum levels of AST and LDH decreased in an apparent concentration-dependent manner (AST 68 and 54% of controls and LDH 48 and 28% of controls, respectively). Serum levels of G6PDH and ALT were decreased similarly across exposure groups at 45 and 78% of controls, respectively.

A synopsis of respiratory pathology findings following short-term exposure to formaldehyde is presented in Table 4-15.

4.2.1.2.2.4. ***Nasal pathology: subchronic studies.*** In a study by Maronpot et al. (1986), female and male B6C3F1 mice (10/group) were exposed at 0, 2, 4, 10, 20, or 40 ppm (0, 2.46, 4.92, 12.3, 24.6, or 49.2 mg/m^3) formaldehyde 6 hours/day, 5 days/week for 13 weeks. Clinical observations were made daily, and mice were weighed weekly. At autopsy, tissue sections from each organ system (approximately 50 tissues per mouse) were fixed, stained, and examined by light microscopy. Noses were fixed, decalcified, and transversely trimmed at three levels: the incisor teeth, midway between the incisor teeth and first molar teeth, and the second molar teeth (corresponding to sections 2, 3, and 4 in Figure 4-3).

Although control mice gained weight, mice exposed to 40 ppm formaldehyde lost weight during the 13-week exposures. Expressed by the authors as a percent of weight gain in controls, the weight losses were −235% in males and −168.6% in females. Early mortality for both male and female mice exposed to 40 ppm was 80%. Although gross and histochemical effects in excised pieces from each organ system were evaluated, endometrial hypoplasia in mice treated with 40 ppm was the only effect noted outside the respiratory system. The authors considered this effect secondary to the observed respiratory tract lesions and frank toxicity at 40 ppm formaldehyde.

Table 4-15. Summary of respiratory tract pathology from inhalation exposures to formaldehyde—short-term studies

Species/strain	No./ group	Treatment	Respiratory effects	LOAEL/NOAEL	Reference
			Nasal pathology		
Male Sprague-Dawley rats	3	Single 4-hour exposure to 10 ppm formaldehyde.	Marked histopathologic changes to the nasoturbinates, maxilloturbinates, ethmoidal turbinates, and goblet and microvillus cells.	LOAEL = 10 ppm.	Bhalla et al. (1991)
Male Swiss-Webster mice	24–34	0 or 3.13 ppm formaldehyde 6 hours/day for 5 days.	Histopathologic lesions to the respiratory epithelium, including inflammation, exfoliation, erosion, ulceration, necrosis, and squamous metaplasia.	LOAEL = 3.13 ppm.	Buckley et al. (1984)
Male F344 rats	3–5	0, 0.5, 2, 6, or 15 ppm 6 hours/day for 1, 2, or 4 days.	Histopathologic lesions to the nasal conchae, lateral wall, and ventral nasal conchae.	NOAEL = 2 ppm for focal lesions. Some changes in ciliary configuration were evident at all exposures.	Monteiro-Riviere and Popp (1986)
Male Wistar rats	20	0 or 3.5 ppm formaldehyde through six consecutive 12-hour cycles in which rats were exposed for 8 hours; 10 were unexposed for 4 hours.	The activity of GPX was increased in respiratory epithelium homogenates. The nasal respiratory epithelium showed frank necrosis.	LOAEL = 3.5 ppm.	Cassee and Feron (1994)
Male F344 rats	5	0, 6, or 15 ppm [^{14}C]-formaldehyde 6 hours/day for a single day (naïve group). A pretreated group was exposed to 6 or 15 ppm formaldehyde 6 hours/day for 4 days prior to [^{14}C]-formaldehyde exposure.	Cellular necrosis to the nasal epithelium. 10.05% cellular proliferation.	LOAEL = 6 ppm.	Chang et al. (1983)
Male and female Wistar rats	5/sex	0, 2, or 5 ppm formaldehyde 8 hours/day for 3 or 30 days.	Cell disarrangement, squamous hyperplasia, atypical mitosis, and epithelial hyperplasia.	NOAEL = 2 ppm.	Javdan and Taher (2000)
Male F344 rats	15	0, 15, or 145.6 ppm formaldehyde for a single 6-hour exposure.	Histopathologic lesions in the nasal turbinates and trachea	LOAEL = 15 ppm.	Kamata et al. (1996b)
Male rhesus monkeys	9	0 or 6 ppm formaldehyde 6 hours/day for 1 or 6 weeks. [^{3}H]-thymidine was injected prior to sacrifice.	Histopathologic lesions, including loss of goblet cells, loss of cilia, epithelial hyperplasia, squamous metaplasia, and neutrophilic inflammation.	LOAEL = 6 ppm.	Monticello et al. (1989)

Table 4-15. Summary of respiratory tract pathology from inhalation exposures to formaldehyde—short-term studies

Species/strain	No./ group	Treatment	Respiratory effects	LOAEL/NOAEL	Reference
Tracheal and lung pathology					
Syrian golden hamsters (sex unstated)	5	0 or 250 ppm 1 hour/day for 1, 2, 5, or 15 days.	Abnormal cells in tracheal lavage, an effect that was reversed on cessation of treatment.	LOAEL = 250 ppm.	Schreiber (1979)
Male rabbits (strain unstated)	ND	Aerosol generated from a 3% formaldehyde solution 3 hours/day for up to 50 days (air concentration unknown).	Necrosis of the bronchi and lung parenchyma. Increased activities of acid phosphatase, Tween-60 esterase, naphthol-AS-D-acetate esterase, proline oxidase, and hydroxyproline epimerase. Reduced activities of leucyl aminopeptidase and β-glucuronidase. Adverse histopathologic changes.	ND.	Ionescu et al. (1978)
Male F344 rats	6	0, 128.4, or 294.5 ppm for a single 6-hour exposure.	Phospholipid content was reduced in lung surfactant, for example, sphingomyelin to 43% of controls in the low-concentration group.	LOAEL = 128.4 ppm.	Kamata et al. (1996a)
Male F344 rats	15	0, 15, or 145.6 ppm formaldehyde for a single 6-hour exposure.	Biochemical changes in lung homogenates. Altered lipid content of BAL in high concentration rats.	LOAEL = 15 ppm.	Kamata et al. (1996b)
Male Wistar rats	6	Aerosol generated from a 1% formalin solution 0, 30, 60, or 90 minutes/day on 4 consecutive days (air concentration unknown).	Increased leukocyte count in bronchoalveolar fluid. Degranulation of mast cells and increased neutrophil infiltration.	ND.	Lino dos Santos Franco et al. (2006)
Extrapulmonary effects					
Male F344 rats	6	0, 128.4, or 294.5 ppm for a single 6-hour exposure.	.	LOAEL = 128.4 ppm.	Kamata et al. (1996a)
Male F344 rats	15	0, 15, or 145.6 ppm formaldehyde for a single 6-hour exposure.	.	LOAEL = 15 ppm.	Kamata et al. (1996b)

ND = not determined; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level.

While no statistical comparison was provided, respiratory tract lesions showed an increased incidence with concentration, as well as an increased distribution throughout the respiratory tract (Table 4-16). No lesions were seen in the nasal cavity, larynx, trachea, or lung of control mice or mice treated with 2 ppm formaldehyde. Minimal squamous metaplasia in the nasal cavity was noted in 1 of 10 male mice treated with 4 ppm formaldehyde, but none were observed in the female mice. However, squamous metaplasia was observed in all mice in the higher treatment groups (10, 20, and 40 ppm). Lesions became more severe and penetrated more deeply into the respiratory tract as exposure concentration increased. Where lesions were present in the nasal cavities of all mice exposed to 10 ppm, similar lesions were reported in the larynx and trachea of some animals exposed to 20 ppm and all animals exposed to 40 ppm formaldehyde. Mice exposed to 40 ppm formaldehyde exhibited lesions as deep as the lung, including squamous metaplasia, submucosal fibrosis inflammation, and epithelial hyperplasia.

The findings of Maronpot et al. (1986) indicated a no-observed-adverse-effect level (NOAEL) of 4 ppm and a LOAEL of 10 ppm in mice, based on squamous metaplasia in the nasal epithelium. Although a LOAEL of 10 ppm was observed, there was 80% mortality for both sexes at 40 ppm, indicating a very narrow range between the first observed adverse health effects and frank effect concentrations in mice for this 13-week treatment.

In a study by Woutersen et al. (1987), male and female albino SPF Wistar rats (10/group) were exposed to 0, 1, 10, or 20 ppm (0, 1.23, 12.3, or 24.6 mg/m^3) formaldehyde 6 hours/day, 5 days/week for 13 weeks. Rats were checked daily and weighed weekly. Three longitudinal sections of lungs, trachea, and larynx and six standard cross sections of the nose were taken for microscopic examination. Two rats per exposure group were similarly treated for 3 days and sacrificed 18 hours later, and nasoturbinates were dissected to measure cell proliferation.

Woutersen et al. (1987) noted that the majority of the dose-dependent increases in cell proliferation seen at section level 3 after 3 days of repeated 6-hour exposures to 10 and 20 ppm (12.3 and 24.6 mg/m^3) formaldehyde occurred in areas of the epithelium showing "clear squamous metaplasia and hyperplasia." Cell proliferation rates in metaplastic epithelium of 29.5 and 33.2% were much higher than the 1.4 to 2.8% proliferation in the visibly unaffected respiratory epithelium from rats exposed at 10 ppm formaldehyde. Although there was a slight trend towards increased cell proliferation in the visibly unaffected epithelium of exposed animals compared with unexposed controls, the majority of increased cell proliferation resulting from exposure to 10 and 20 ppm formaldehyde was attributed to the metaplastic epithelium (Woutersen et al., 1987).

Table 4-16. Location and incidence of respiratory tract lesions in B6C3F1 mice exposed to formaldehyde

Location of respiratory tract lesions	Control		2 ppm		4 ppm		10 ppm		20 ppm		40 ppm	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Nasal cavity												
Squamous metaplasia	–[a]	–	–	–	1/10	–	10/10	10/10	10/10	10/10	10/10	10/10
Seropurulent inflammation	–	–	–	–	–	–	4/10	–	10/10	8/10	10/10	10/10
Larynx												
Squamous metaplasia	–	–	–	–	–	–	–	–	6/9	3/9	10/10	7/8
Trachea												
Squamous metaplasia	–	–	–	–	–	–	–	1/10	3/10	5/10	10/10	10/10
Epithelial hyperplasia	–	–	–	–	–	–	–	–	4/10	2/10	2/10	---
Seropurulent inflammation	–	–	–	–	–	–	–	–	–	–	8/10	5/10
Submucosal fibrosis	–	–	–	–	–	–	–	–	–	–	9/10	5/10
Lung (Bronchus)												
Squamous metaplasia	–	–	ND[b]	ND	ND	ND	–	–	–	–	4/10	3/10
Inflammation	–	–	ND	ND	ND	ND	–	–	–	–	3/10	2/10
Submucosal fibrosis	–	–	ND	ND	ND	ND	–	–	–	–	2/10	–

[a]Dash indicates no lesions recorded in that treatment group.
[b]ND = no data.

Source: Maronpot et al. (1986).

Statistically significant increases were seen in focal respiratory epithelial hyperplasia and keratinization in both male and female rats at the highest treatment level (20 ppm) (Table 4-17). Male rats also had statistically significant increases in observed respiratory epithelial squamous metaplasia, focal olfactory epithelial thinning, and rhinitis. Both male and female rats treated with 10 ppm formaldehyde showed statistically significant increases in squamous metaplasia, hyperplasia, and keratinization of the respiratory epithelium (Woutersen et al., 1987). Disarrangement of the respiratory epithelium was only significantly increased in female rats, but this change was observed at both the 10 and 20 ppm treatment levels. Although some lesions were observed in animals treated with 1 ppm formaldehyde, their incidences were not statistically significant and the findings were equivocal.

Feron et al. (1988) examined recovery of formaldehyde-induced nasal lesions after subchronic exposures. Male albino SPF Wistar rats (50–55/group) were exposed to 0, 10, or 20 ppm (0, 12.3, or 24.6 mg/m^3) formaldehyde 6 hours/day, 5 days/week for either 4, 8, or 13 weeks. All groups were observed for a total of 130 weeks, including treatment and recovery. Rats were weighed weekly for the first 13 weeks and monthly thereafter. Rats (five/group) were sacrificed immediately after the end of exposure (4, 8, or 13 weeks). The balance of the rats were sacrificed after 130 weeks, inclusive of exposure time. At sacrifice, noses were fixed and sectioned by using standard section levels.

Formaldehyde exposure (20 ppm) was associated with reduced body weight throughout the exposure period (4, 8, or 13 weeks). However, body weight in these groups matched that of controls after 8, 40, and 100 weeks, respectively. Rats exposed to 10 ppm for 8 or 12 weeks had slightly decreased body weight (further details not given).

Nonneoplastic lesions were reported in the nasal mucosa of rats exposed to either 10 or 20 ppm formaldehyde and examined immediately after exposure was discontinued (4, 8, or 13 weeks). Lesions increased in severity with both exposure duration and concentration (details of severity and incidence were not provided). Rhinitis, hyperplasia, and squamous metaplasia of the respiratory epithelium were seen in rats from both dose groups, but changes in olfactory epithelia were only seen in rats exposed to 20 ppm, where cell disruption, thinning of the epithelium, and simple cuboidal or squamous metaplasia were also reported. Changes in the dorsomedial region, at the junction of the respiratory and olfactory epithelium, were similar to those seen in the olfactory epithelium of rats exposed to 20 ppm formaldehyde. A similar concentration- and duration-dependent increase in histopathologic changes in nasal epithelium was observed after the full 130 weeks, which included 126, 122, or 117 weeks of recovery for the three duration groups, 4, 8, and 13 weeks, respectively (Table 4-17).

Table 4-17. Formaldehyde effects (incidence and severity) on histopathologic changes in the noses and larynxes of male and female albino SPF Wistar rats exposed to formaldehyde 6 hours/day for 13 weeks

		Concentration of formaldehyde (ppm)							
		0	1	10	20	0	1	10	20
Respiratory epithelium	***Severity***	***Males***				***Females***			
Diffuse squamous metaplasia	Slight	–[a]	–	–	–	–	–	–	3
	Moderate	–	–	–	5[b]	–	–	–	4
	Severe	–	–	–	5[b]	–	–	–	3
Focal squamous metaplasia	Very slight	–	1	–	–	–	–	1	–
	Slight	–	1	6[b]	–	–	–	7[c]	–
	Moderate	–	–	4	–	–	–	2	–
Focal hyperplasia	Very slight	–	–	1	1	–	–	2	1
	Slight	–	–	6[b]	7[c]	–	1	6[b]	6[b]
	Moderate	–	–	1	–	–	–	–	–
Focal disarrangement	Very slight	–	–	1	–	–	–	2	1
	Slight	–	–	3	–	–	1	6[b]	6[b]
	Moderate	–	–	1	–	–	–	–	–
Focal keratinization	Very slight	–	2	6[b]	1	–	–	6[b]	6[b]
	Slight	–	–	3	6[b]	–	–	2	4
	Moderate	–	–	–	1	–	–	–	–
Olfactory epithelium									
Focal thinning	Slight	–	–	–	2	–	–	–	2
	Moderate	–	–	–	1	–	–	–	2
	Severe	–	–	–	5[b]	–	–	–	2
Focal squamous metaplasia	Slight	–	–	–	4	–	–	–	3
	Moderate	–	–	–	4	–	–	–	1
Focal keratinization	Very slight	–	–	–	1	–	–	–	–
	Slight	–	–	–	2	–	–	–	–
Rhinitis		–	2	5[b]	10[c]	–	–	3	2
Larynx									
Squamous metaplasia	Very slight	–	–	–	3	–	NE[d]	NE	–
	Slight	–	–	–	1	–	NE	NE	–
	Moderate	–	–	–	1	–	NE	NE	–
Keratinization	Slight	–	–	–	2	–	NE	NE	–

[a] Dash indicates no lesions reported.
[b] Different from control, $p < 0.05$.
[c] Different from control, $p < 0.01$.
[d] NE = not evaluated.

Source: Woutersen et al. (1987).

Feron et al. (1988) did not provide a direct comparison among lesions reported at the interim sacrifice and terminal sacrifice after the extended recovery period. However, similar lesions were reported after the recovery period, including focal hyperplasia and stratified squamous metaplasia of the respiratory epithelium, stratified cuboidal or squamous metaplasia in the dorsomedial area, and replacement of olfactory epithelium. The incidence and severity of

these lesions in rats exposed to 20 ppm formaldehyde were statistically different from control animals, regardless of exposure duration (Table 4-18).

Table 4-18. Formaldehyde-induced nonneoplastic histopathologic changes in male albino SPF Wistar rats exposed to 0, 10, or 20 ppm formaldehyde (6 hours/day, 5 days/week) and examined at the end of 130 weeks inclusive of exposure

	4 Weeks			8 Weeks			13 Weeks		
Formaldehyde, ppm	**0**	**10**	**20**	**0**	**10**	**20**	**0**	**10**	**20**
Total noses examined	**44**	**44**	**45**	**45**	**44**	**43**	**45**	**44**	**44**
Respiratory epithelium focal hyperplasia									
Very slight	0	0	0	0	1	3	0	5[a]	2
Slight	0	3	8[b]	2	2	12[b]	1	6	14[b]
Moderate	0	0	1	0	1	0	0	0	4
Respiratory epithelium focal stratified squamous metaplasia									
Very slight	3	6	14[b]	8	16	17[a]	2	10[a]	2
Slight	4	2	19[b]	2	1	20[b]	3	18[b]	26[b]
Moderate	0	2	3	0	0	2	1	5	14[b]
Severe	0	0	0	0	0	0	0	0	1
Respiratory/olfactory epithelium stratified cuboidal or squamous metaplasia	0	0	4	0	0	17[b]	0	2	23[b]
Rhinitis	7	7	18[a]	4	6	22[a]	8	11	23[b]
Olfactory epithelium replacement by respiratory epithelium and regeneration									
Very slight	0	0	0	0	0	2	0	0	1
Slight	1	0	6	0	0	14[b]	0	0	12[b]
Moderate	0	0	1	0	0	3	0	0	12[b]
Severe	0	0	0	0	0	1	0	0	1

[a]Significantly different from control, $p < 0.05$.
[b]Significantly different from control, $p < 0.01$.

Source: Feron et al. (1988).

Although a slight increase in changes to the olfactory epithelium and dorsomedial area was seen in rats treated with 20 ppm formaldehyde for only 4 weeks, these differences were significant and more severe in the 8- and 13-week treatment groups. Replacement of olfactory epithelium by respiratory epithelium was described as slight after 8 weeks of exposure and slight to moderate after 13 weeks of exposure in the 20 ppm treatment groups. Therefore, formaldehyde-induced lesions were not resolved after a considerable nonexposure recovery period of up to 126 weeks (Feron et al., 1988).

Feron et al., (1988) derived a correlation between the development of nonneoplastic changes in nasal epithelium and the development of nasal tumors as a result of these subchronic formaldehyde exposures. Two SCCs were reported in rats exposed to 10 ppm formaldehyde but

were not considered to be formaldehyde related because of their locations (nasolacrimal duct, incisor tooth). Six tumors were observed in the 20 ppm, 13-week exposure group (Table 4-19) of which three of the tumors were SCCs similar to those observed as a result of chronic formaldehyde exposure. Two polypoid adenomas also were reported in rats exposed to 20 ppm formaldehyde. Feron et al. (1988) concluded that subchronic exposures to 20 ppm formaldehyde could result in an increase in nasal tumors, an effect that followed observation of cellular proliferation.

Table 4-19. Formaldehyde-induced nasal tumors in male albino SPF Wistar rats exposed to formaldehyde (6 hours/day, 5 days/week for 13 weeks) and examined at the end of 130 weeks inclusive of exposure

Tumor type	0 ppm	10 ppm	20 ppm
No. of rats exposed for 4 weeks	**44**	**44**	**45**
Polypoid adenoma	0	0	1[a]
SCC	0	0	1
No. of rats exposed for 8 weeks	**45**	**44**	**43**
Polypoid adenoma	0	0	1[a]
SCC	2	1	1
No. of rats exposed for 13 weeks	**45**	**44**	**44**
SCC	0	1	3[a]
Cystic squamous cell carcinoma	0	0	1
Carcinoma in situ	0	0	1[a]
Ameloblastoma	0	0	1

[a]Tumor considered to be associated with formaldehyde exposure.

Source: Feron et al. (1988).

A companion study from the same laboratory examined the effects of lower concentration formaldehyde exposures (Zwart et al., 1988). Male and female albino Wistar rats (50/group) were exposed to 0, 0.3, 1, or 3.0 ppm (0, 0.37, 1.2, or 3.7 mg/m^3) formaldehyde 6 hours/day, 5 days/week for 13 weeks. Body weight, general condition, and behavior were recorded weekly. No effects of formaldehyde exposure on body weight changes were noted, and growth was considered comparable among different exposure groups and controls. Rats were sacrificed during week 14, and noses were fixed and sectioned (exact time after exposure ended not given). Six standard cross sections were examined for each animal by light microscopy, anterior to posterior. Noses were fixed and decalcified, and six standard cross sections were taken and developed.

No formaldehyde-related lesions were reported in the respiratory epithelium at section level 3 after 13 weeks of formaldehyde exposure (0.1 ppm, 1 ppm, or 3 ppm). Signs of

inflammation (rhinitis, sinusitis, mononuclear cell infiltrates) were observed in formaldehyde-treated rats, but there was no concentration-response relationship (data not provided). Formaldehyde-related pathology in the anterior part of level 2 epithelium was reported in 37/50 males and 21/50 female rats exposed to 3.0 ppm for 13 weeks. Both keratinized and unkeratinized squamous metaplasia were present, and disarranged cells and hyperplastic respiratory epithelium were found in the transitional zone between squamous and pseudostratified epithelium at level 2. Foci of keratinized squamous epithelium, glandularization of goblet cells, and deciliated epithelium were observed by electron microscopy in anterior sections of level 2 of rats exposed to 3 ppm formaldehyde. Epithelial cells with irregularly shaped and strongly indented nuclei were described at level 2 in animals exposed to 0.3 and 1 ppm formaldehyde and were considered to be disarranged as well at 3 ppm formaldehyde exposures.

Although early cell proliferation at level 3 corresponded to basal cell hyperplasia at 3 days, neither effect persisted for the course of the exposure. The authors speculate that this is an indication of an adaptive response, perhaps through increased function of the mucociliary apparatus present at level 3. In contrast, the early changes at section level 2 were less dramatic but persisted through 13 weeks, including clear formaldehyde-related pathology.

Concentration times time (C × t) issues have been investigated for histopathology as well as for cellular proliferation, outlined above. Specifically, Wilmer et al. (1989, 1987) compared the effects of 8-hour continuous and 8-hour intermittent formaldehyde exposure in two studies. Fifty male albino Wistar rats (10/group) were exposed to different exposure regimens to achieve similar compound-related C × t products. A C × t product of 40 ppm-hours (49.2 mg/m^3-hours) was attained by an 8-hour exposure to 5 ppm (6.2 mg/m^3) or a 4-hour exposure to 10 ppm (12.3 mg/m^3) (Wilmer et al., 1987). Similarly, an 80 ppm-hours (98.4 mg/m^3-hours) C × t product was attained from continuous 10 ppm exposure or intermittent 20 ppm (24.6 mg/m^3) exposure. Rats were exposed to one of these regimens 8 hours/day for either 3 days (two/group) or 4 weeks (eight/group). Eighteen hours after exposure ended, rats were injected with [^{3}H]-thymidine and sacrificed 2 hours later. Noses were fixed and decalcified, and six standard cross sections were taken and developed.

Thinning and disarrangement of the respiratory epithelium, squamous metaplasia, basal cell hyperplasia, and rhinitis were seen in formaldehyde-treated rats. Lesions were most severe in group 4 (20 ppm intermittent). Groups 2 and 3 had similar lesions (10 ppm intermittent and continuous). Rats in group 1 had mild lesions. Formaldehyde concentration was the major determinate in severity of nasal lesions. Formaldehyde effects were less severe in group 1 than in group 3, even though the C × t product was the same, indicating concentration rather than

duration or cumulative exposure correlates to severity. Epithelial lesions in group 3 rats were similar among rats exposed to 10 ppm, regardless of duration (groups 2 and 3).

In a follow-up study, Wilmer et al. (1989) assessed both cellular proliferation and histologic lesions in Wistar rats exposed to formaldehyde in groups that differed by concentration and time. Group A served as a control group (0 ppm). Group B was exposed to 1 ppm for 8 hours, group C to 2 ppm for 8 hours, group D to 2 ppm for 4 hours (30 minutes for 8 hours), and group E to 4 ppm for 4 hours (30 minutes for 8 hours). The experimental design and cellular proliferation results are illustrated in Table 4-20. Intermittent exposures at 2 and 4 ppm resulted in formaldehyde-related histopathologic lesions similar to those reported by Zwart et al. (1988). Disarrangement and squamous metaplasia in respiratory epithelium were observed at 4 ppm (Table 4-20). Disarrangement, nest-like infolds, goblet cell hyperplasia, and rhinitis were observed at 2 ppm. Rats exposed continuously for 8 hours at 2 ppm formaldehyde had fewer lesions than rats intermittently exposed to 2 ppm and were not statistically different from controls. Although lesions were noted in rats given the continuous 1 ppm, 8-hour treatment, their incidence was not significantly different from the controls (Table 4-20). It should be noted that the control rats in this study were reported to have a higher frequency of lesions than controls in two previous studies from this laboratory employing the same techniques (Zwart et al., 1988; Woutersen et al., 1987). For example, lesions noted in the respiratory epithelium of 25 control rats included 13 disarrangements, 13 basal cell hyperplasia, and 5 each of goblet cell hyperplasia, nest-like infolds, and squamous metaplasia. This is in contrast to the data of Woutersen et al. (1987), who reported no lesions in the respiratory epithelium of 20 control rats (male and female). Although Zwart et al. (1988) discussed inflammatory lesions in control rats, no mention was made of the other scored lesions in control animals. Overall, Wilmer et al. (1989) reported clear adverse effects at 2 ppm formaldehyde, resulting from intermittent exposure for 8 hours/day, 5 days/week for 13 weeks. The indication of no effects at 1 ppm and 2 ppm continuous exposure should be considered with some caution, given the unusual incidence of lesions in the control animals.

The results reported by Wilmer et al. (1989, 1987) indicate a greater influence of concentration, rather than exposure regimen (continuous versus intermittent) on formaldehyde toxicity. However, these studies were conducted as repeated 8-hour exposure regimens over a course of days or weeks. Therefore both regimens allowed for a 16-hour recovery time before the next reexposure and do not represent a true continuous exposure. This research group has speculated that defensive adaptation of the nasal mucosa may include the function of the mucociliary apparatus (Feron et al., 1989). Morgan et al. (1986a) have shown formaldehyde effects on mucus flow and ciliary beat in F344 rats to result from hourly exposures to 15 ppm

formaldehyde. However, effects seen in repeated 8-hour exposures may not correspond to those effects resulting from shorter duration exposures to higher formaldehyde concentrations.

Table 4-20. Formaldehyde effects on nasal epithelium for various concentration-by-time products in male albino Wistar rats

	Exposure regimen (number of animals)				
	A (25)	**B (22)**	**C (24)**	**D (23)**	**E (25)**
	0 ppm	**1 ppm**	**2 ppm**	**2 ppm**	**4 ppm**
Respiratory epithelium at crosssection level 2		**8-Hour continuous**	**8-Hour continuous**	**8-Hour intermittent**	**8-Hour intermittent**
Disarrangement					
Focal	12	4	8	3[a]	8
Diffuse	1	1	0	15[b]	11[c]
Necrosis					
Focal	4	3	0	2	3
Diffuse	0	0	0	2	2
Basal cell hyperplasia					
Focal	9	4	6	11	10
Diffuse	4	0	0	4	11
Squamous metaplasia					
Focal	5	0	1	7	16[c]
Keratinization	0	0	1	0	3
Nest-like infolds					
Focal	5	4	11	14[c]	7
Diffuse	0	3	1	0	1
Goblet cell hyperplasia					
Focal	0	1	1	2	1
Diffuse	5	2	8	13[b]	10
Rhinitis	3	2	3	16[c]	8

[a] $p < 0.05$, compared with group A.
[b] $p < 0.001$, compared with group A.
[c] $p < 0.01$, compared with group A.

Source: Wilmer et al. (1989).

Rusch et al. (1983a, b) performed a comparative study of formaldehyde effects on the nasal epithelium in F344 rats, Syrian golden hamsters, and cynomolgus monkeys. Groups of animals were exposed at 0, 0.2, 1, or 3 ppm (0, 0.25, 1.2, or 3.7 mg/m^3) formaldehyde 22 hours/day, 7 days/week for 26 weeks. Six male monkeys, 10 male and 10 female hamsters, and 20 male and 20 female rats were exposed at each exposure level. The experiment was run in two trials, each with its own control group: trial 1 at 0.2 or 1 ppm and trial 2 at 3 ppm. Animals were weighed weekly and physically assessed (details not given). At sacrifice, organ weights were recorded for the kidney, adrenals, heart, and liver. Tissue sections of the lung (4), trachea, and nasal turbinates (4) of each animal were examined by light microscopy (section locations not

given). Additionally, sections were examined by electron microscopy for rats in the control and 1 ppm treatment groups (five rats per group).

Body weights of both male and female rats in the 3 ppm treatment group were depressed by 20% between week 2 and the end of the 26-week exposure. Absolute liver weights were decreased in these animals as well (26% lower in males and 12% lower in females, $p < 0.05$). This decrease in liver weight remained significant for male rats when normalized for body weight (a ratio of 2.9 in treated versus 3.16 in controls) but not for female rats. No significant body weight or organ weight changes were seen in hamsters or monkeys. Increased incidences of congestion (36/156), hoarseness (32/156), and nasal discharge (62/156) were observed in monkeys in the 3.0 ppm treatment group versus no hoarseness or congestion and only five observations of nasal discharge in 156 observations for control monkeys. Increased nasal congestion was noted in the two lower treatment groups of monkeys: 30/156 and 45/156 observations, respectively, versus 9/156 observations in nasal discharge in the controls. The authors reported an increase in nasal discharge and lacrimation in treated hamsters but no increases in symptoms in rats. However, observations of adverse symptoms in the control rats were greater than 10% on some measures.

Rhinitis increased in rats in the 3 ppm treatment group, and the incidence in controls was notable (Table 4-21). All groups of monkeys showed some rhinitis, and no treatment effects were observed in either monkeys or hamsters. Monkeys and rats in the high treatment group (3 ppm) had a greater incidence of lesions in the nasoturbinate epithelium (Table 4-22). Rusch et al. (1983a, b) noted that most lesions were mild to moderate but were "somewhat more severe" in the high treatment group. Hamsters did not exhibit a similar increase, with few lesions noted in the nasal epithelium. Overall, these studies show a clear increase in adverse health effects at 3 ppm for rats and monkeys, with no adverse effects seen in hamsters at this treatment level or rats and monkeys at the lower concentrations (0.2 ppm and 1 ppm).

Table 4-21. Rhinitis observed in formaldehyde-treated animals; data pooled for male and female animals

	F344 rats	Cynomolgus monkeys	Syrian golden hamsters
Trial 1:			
I, Control	17/38	4/6	0/14
II, 0.2 ppm	14/39	4/6	0/4
III, 1 ppm	14/38	5/6	0/11
Trial 2:			
IV, Control	12/40	2/6	0/9
V, 3 ppm	25/39	4/6	2/16

Source: Rusch et al. (1983a, b).

Table 4-22. Epithelial lesions found in the middle region of nasoturbinates of formaldehyde-treated and control animals; data pooled for males and females

	F344 rats		Cynomolgus monkeys	Syrian golden hamsters
	Basal cell hyperplasia	Squamous metaplasia/ hyperplasia	Squamous metaplasia/ hyperplasia	Nasal epithelium
Trial 1:				
I, Control	0/38	2/38	0/6	No lesions noted
II, 0.2 ppm	0/38	1/38	0/6	
III, 1 ppm	0/36	3/36	1/6	
Trial 2:				
IV, Control	4/39	3/39	0/6	No lesions noted
V, 3 ppm	25/37	23/37	6/6	

Source: Rusch et al. (1983a, b).

Andersen et al. (2008) examined the effect of formaldehyde exposure at several concentrations and durations. This study comprised histopathology and cell proliferation data, as well as genomic analyses at Level II of the nasal cavity. Toxicogenomics analysis was performed only at Level II because this was the region where the most severe lesions have been reported in chronic bioassays (Andersen et al., 2008; Monticello et al., 1991; Kerns et al., 1983). More specifically, Andersen et al. (2008) stated that the histopathologic and cell proliferation effects at Levels II and III (with similar tissue structure) (Monticello et al., 1991) provided phenotypic anchoring for the genetic analysis. Table 4-23 summarizes many of the broad phenotypic findings.

The primary conclusions of this study with regard to the histopathology and cell proliferation are as follows:

- The presence of inflammatory cell infiltrates in the nasal epithelial tissue of F344 rats is highly variable and provides no coherent pattern with dose or duration at levels below 6 ppm.
- Hyperplasia was observed following exposure to ≥2 ppm.
- Metaplasia was observed at 6 ppm on day 5, but not before or after.
- Cell proliferation (as measured by labeling indices) was significantly elevated in Levels I–III at 6 ppm on day 5 and Level I on day 15, leading to the conclusion that significant changes in cell proliferation may not occur at exposures to ≤2 ppm.
- A significant decrease in cell density was observed at Level I in animals exposed to 6 ppm formaldehyde for 15 days, which was posited to be related to tissue remodeling in response to this concentration.

Table 4-23. Cellular and molecular changes in nasal tissues of F344 rats exposed to formaldehyde

ppm / Response	D1					D1R					D5					D6					D6R					D15				
	0	**0.7**	**2**	**6**	**15**	**0**	**0.7**	**2**	**6**	**15**	**0**	**0.7**	**2**	**6**	**15**	**0**	**0.7**	**2**	**6**	**15**	**0**	**0.7**	**2**	**6**	**15**	**0**	**0.7**	**2**	**6**	**15**
I	0	1	6	8	–	4	2	1	7	–	1	1	5	8	–	5	2	4	7	–	6	1	3	7	–	3	1	0	5	–
H	0	0	0	0	–	0	1	3	8	–	0	0	3	8	–	0	0	1	8	–	0	0	2	8	–	0	0	2	7	–
M	0	0	0	0	–	0	0	0	0	–	0	0	0	7	–	0	0	0	0	–	0	0	0	0	–	0	0	0	0	–
P1											39±9	37±15	65±40	155±89[a]												79±55	56±37	51±44	119±38[a]	
P2											–	–	–	[a]												–	–	–	–	
P3											–	–	–	[a]												–	–	–	–	
CD											321±30	336±64	377±141	400±61												362±61	340±57	321±37	293±53[b]	
G	–	0	1	42	745	–	0	0	0	–	–	0	15	28	–	–	0	0	9	–	–	–	–	–	–	–	0	0	54	

D = day; R = recovery.
I = infiltrations (number out of 8 total animals); H = hyperplasia (number/8); M = metaplasia (number/8).
P1–P3 = proliferation at levels I–III (ULLI).
CD = cell density (cells/mm) at Level I.
G = genes significantly altered at Level II of nasal epithelial tissue.
[a]Significantly elevated ULLI and LI at Level I on day 5 or significantly elevated lLI lat Level I on day 15; index [a] without numerical value indicates significant increases in ULLI in all subregions of Levels II and III at day 5.
[b]Statistically significant difference from control ($p < 0.05$).

Source: Andersen et al. (2008).

Based on their analysis of the microarray data, Andersen et al. (2008) concluded that no genes were significantly altered by exposure to 0.7 ppm from 1 to 15 days. Exposure to 2 ppm primarily resulted in gene changes at 5 days of exposure, but not thereafter. One gene was significantly increased on day 1, but the authors did not identify that gene. At 6 and 15 ppm, 42 and 745 genes were altered at day 1, respectively. After 5 days, gene changes were only observed at 6 ppm (15 ppm was not examined after day 1). These findings support conclusions reached by their laboratory in an earlier analysis. Thus, the primary conclusion in the Andersen et al. (2008) study is that genomic changes, including those suggestive of mutagenic effects, did not temporally precede or occur at lower doses than phenotypic changes in the tissue. The implications of this finding will be examined later in Section 4.5.

4.2.1.2.2.5. ***Lung pathology: subchronic studies.*** Studies have also investigated the ability for formaldehyde to induce pathology in the trachea, bronchi, and lung tissue. These studies have reported tracheal tissue changes, lung inflammation, necrosis, changes to the biochemistry of BAL fluid and lung surfactant in a variety of species. Özen et al. (2003a) noted changes in zinc concentration in the lung tissue following exposure for formaldehyde. Dallas et al. (1989) and Dinsdale et al. (1993) observed changes in P450 enzyme activity in the lung associated with formaldehyde exposure.

Özen et al. (2003a) measured zinc, copper, and iron content in lung tissue from formaldehyde-exposed Wistar rats. Adult male rats were exposed to 0, 5, or 15 ppm (0, 6.2, or 18.5 mg/m^3) formaldehyde 8 hours/day, 5 days/week for either 4 or 13 weeks. Rats were checked daily and weighed weekly. At sacrifice, rats were autopsied and examined for gross pathological changes. Lung tissue was homogenized and analyzed for zinc, copper, and iron.

Body weight gain was depressed in all treatment groups in a concentration-dependent manner ($p < 0.001$) (Table 4-24). Formaldehyde-exposed rats consumed less food and water than controls and showed unsteady breathing, increased nose cleaning, excessive licking, frequent sneezing, and nasal mucosa hemorrhages. Significant decreases were seen in the zinc content of lungs after either 5 or 10 ppm formaldehyde exposure (Table 4-25). Copper content was unchanged from controls in all treatment regimens, whereas iron content was increased after 4 weeks of 5 ppm exposure and after 13 weeks of either 5 or 10 ppm formaldehyde exposure (Özen et al., 2003a).

Table 4-24. Percent body weight gain and concentrations of iron, zinc, and copper in cerebral cortex of male Wistar rats exposed to formaldehyde via inhalation for 4 and 13 weeks

Exposure (mg/m^3)	Weight gain (%)[a]	Zinc (mg/kg)[a]	Copper (mg/kg)[a]	Iron (mg/kg)[a]
4-week data				
0	20.11 ± 2.87	120 ± 6.03	4.60 ± 0.42	25.07 ± 2.83
6.1	7.27 ± 1.49[e]	130 ± 7.26[c]	5.60 ± 0.50[b]	23.00 ± 2.32
12.2	5.24 ± 1.52[e]	185 ± 10.36[e]	5.80 ± 0.60[d]	22.14 ± 1.95[b]
13-week data				
0	60.53 ± 7.84	123 ± 6.22	4.67 ± 0.38	24.92 ± 2.84
6.1	38.41 ± 2.53[e]	155 ± 7.94[e]	5.41 ± 0.56[c]	22.00 ± 2.41
12.2	25.87 ± 1.32[e]	163 ± 6.03[e]	6.10 ± 0.73[e]	21.00 ± 1.96[b]

[a]Values are means ± SDs (n = 7).

Statistical significance of differences versus controls, as calculated by the authors:
[b]$p < 0.05$. [c]$p < 0.02$. [d]$p < 0.002$. [e]$p < 0.001$.

Source: Özen et al. (2003b).

Table 4-25. Zinc, copper, and iron content of lung tissue from formaldehyde-treated male Wistar rats

Concentration	Duration[a]	Zinc[b,c]	Copper[b,c]	Iron[b,c]
0 ppm	Control	20.7 (1.6)	0.39 (0.05)	12.5 (0.8)
5 ppm	4 weeks	16.1 (1.3)[d]	0.32 (0.07)	12.9 (1.0)
10 ppm	4 weeks	13.8 (1.2)[e]	0.36 (0.04)	17.5 (1.3)[e]
0 ppm	Control	20.0 (1.6)	0.39 (0.05)	12.7 (0.4)
5 ppm	13 weeks	15.3 (1.4)[e]	0.37 (0.04)	17.9 (1.1)[e]
10 ppm	13 weeks	13.0 (1.1)[e]	0.39 (0.05)	22.4 (1.4)[e]

[a]Rats were exposed 8 hours/day, 5 days/week for the number of weeks indicated.
[b]Concentrations are expressed as moles/mg of tissue, wet basis.
[c]Values are means (n = 7); SDs shown in parentheses.
[d]$p < 0.005$, compared with controls, as calculated by authors.
[e]$p < 0.001$, compared with controls, as calculated by the authors.

Source: Özen et al. (2003a).

There are two reports of lung cytochrome P450 levels after formaldehyde exposure. The first report by Dallas et al. (1989) describes concentration- and duration-dependent changes in P450 levels. Male Sprague-Dawley rats were exposed at 0, 0.5, 3.0, or 15 ppm (0, 0.62, 3.7, or 18.5 mg/m^3) formaldehyde 6 hours/day, 5 days/week for 1 day, 4 days, 12 weeks, or 24 weeks. There were six rats in each exposure group, but the experiment was run in two parts, with three rats in each subgroup. Rats were sacrificed after 1 day, 4 days, 12 weeks, or 24 weeks of exposure, and liver microsomes were prepared. TP and P450 content were determined on each sample.

Average P450 levels in control groups ranged from 17–76 pmol P450/mg protein. However, no P450 was detected in lung from formaldehyde-treated animals after 1 day of exposure, with a method detection limit of approximately 10 pmol P450/mg protein. In contrast, P450 levels were elevated significantly above controls in a concentration-dependent manner after 4 days of formaldehyde exposure (Table 4-26). Although P450 levels remained elevated in some experimental groups after 12 and 24 weeks of exposure, results were variable and less dramatic.

Table 4-26. Total lung cytochrome P450 measurements of control and formaldehyde-treated male Sprague-Dawley rats

	1 Day[a,b]		4 Days		12 Weeks		24 Weeks	
Formaldehyde	**Expt. 1**	**Expt. 2**	**Expt. 1**	**Expt. 2**	**Expt. 1**	**Expt. 2**	**Expt. 1**	**Expt. 2**
0 ppm	17 (6)	44 (13)	39 (11)	23 (3)	29 (10)	19 (23)	76 (49)	18 (11)
0.5 ppm	ND	ND	103 (52)	137 (14)[e]	87 (11)[d]	35 (7)	172 (12)[c]	38 (9)
3.0 ppm	ND	ND	357 (10)[e]	278 (100)[e]	91 (10)[d]	67 (34)	92 (103)	30 (15)
15 ppm	ND	ND	362 (38)[e]	334 (4)[e]	130 (2)[e]	56 (6)	151 (9)	48 (7)[c]

[a]Rats were exposed 6 hours/day, 5 days/week for the duration shown.
[b]Cytochrome P450 expressed as pmol P450/mg of protein. Values are means (SDs) (n = 3).
[c]Different from control, $p < 0.05$.
[d]Different from control, $p < 0.01$.
[e]Different from control, $p < 0.001$, as calculated by the authors.
ND = not detected above the limit of detection, approximately 10 pmol/mg protein.

Source: Dallas et al. (1989).

A later study by Dinsdale et al. (1993) attempted to confirm the increase in P450 levels reported by Dallas et al. (1989). In their first experiment, Dinsdale et al. (1993) treated male Sprague-Dawley rats at approximately 10 ppm (12.3 mg/m^3) formaldehyde 6 hours/day for 4 days. The formaldehyde vapor was generated from formalin by a concentric jet atomizer. For the second experiment, Dinsdale et al. (1993) similarly exposed rats to formaldehyde, but the gas was generated by the thermal depolymerization of paraformaldehyde as was done by Dallas et al. (1989). The concentration of P450 and activity of several P450 isozymes were measured in lung microsomes (pentoxyresorufin O-dealkylase, benzyloxyresorufin O-dealkylase, ethoxyresorufin O-dealkylase, and 2-aminofluorene N-hydroxylation). ALP and γ-glutamyl transpeptidase activity were measured in BAL fluid collected from each animal. No changes were seen in BAL enzyme activity or the activity of lung microsomes for the P450 substrates tested. Cytochrome P450 levels were unchanged in experiment 1, where formaldehyde was generated from formalin. Cytochrome P450 levels were increased in experiment 2 with formaldehyde generated from paraformaldehyde (Table 4-27).

Table 4-27. Cytochrome P450 levels in formaldehyde-treated rats

Group	Experiment 1 (formalin)[a] (nmol/mg protein)	Experiment 2 (paraformaldehyde)[a] (nmol/mg protein)
Control	82 ± 30	85 ± 5
10 ppm formaldehyde	73 ± 27	125 ± 23[b]

[a]Values are means ± SDs (n = 3–5).
[b]Different from controls, $p < 0.05$.

Source: Dinsdale et al. (1993).

4.2.1.2.2.6. ***Extrapulmonary toxicity: subchronic studies.*** Several studies have investigated toxicity in organs other than those associated with the respiratory tract. An earlier cross-species study examined changes in lung tissue resulting from continuous exposure (Coon et al., 1970). Animals were exposed to 3.7 ppm (4.6 mg/m^3) formaldehyde for 90 days. Five species of animals were studied: male and female Sprague-Dawley and Long-Evans derived rats (15), male and female Princeton-derived guinea pigs (15), male New Zealand albino rabbits (3), male squirrel monkeys (*Saimiri sciureus*) (3), and purebred male beagle dogs (2). Blood samples were taken for Hb concentration, HCT, leukocyte counts, and serum levels of BUN, AST, ALT, ALP, and LDH. Sections of heart, lung, liver, kidney, and spleen were fixed and examined from each species (details of method not provided). Brain, spinal cord, and adrenal tissue also were examined in monkeys and dogs as well as thyroid from dogs. Liver and kidney sections were stained for reduced nicotinamide adenine dinucleotide, lactate, isocitrate, and β-hydroxybutyrate. Tissue sections of the nasal mucosa were not examined in this study.

Hematological parameters were unaffected by formaldehyde treatment. The lung tissue of all species exhibited interstitial inflammation after 90 days of formaldehyde exposure (detailed description not provided). Formaldehyde-treated rats and guinea pigs also had focal chronic inflammation in heart and kidney tissue sections. However, the authors were uncertain whether the observed changes to heart and kidney were due to formaldehyde exposure.

As mentioned above, Woutersen et al. (1987) exposed male and female albino SPF Wistar rats (10/group) to 0, 1, 10, or 20 ppm (0, 1.23, 12.3, or 24.6 mg/m^3) formaldehyde 6 hours/day, 5 days/week for 13 weeks. Rats were checked daily and weighed weekly. During week 13, blood samples were taken for Hb, PCV, RBC count, and a differential count of leukocytes. Urine samples were also analyzed. At sacrifice, blood samples were analyzed for ALB, creatinine, glucose, TP, BUN, and the enzyme activities (AST, ALT, and ALP). GSH and protein content were determined in liver homogenates. Organs were examined and weighed: adrenals, brain, heart, kidneys, liver, lungs, ovaries, pituitary, spleen, testes, thymus, and thyroid.

No gross pathological changes were seen upon autopsy, but body weights decreased in both male and female rats at the 20 ppm treatment level. Of the organs weighed, 6 of 11 had significantly increased relative rates in male rats exposed to 20 ppm formaldehyde. Relative brain weight was increased in female rats at the same treatment level (Woutersen et al., 1987).

Clinical chemistry parameters of liver and kidney function and hematological parameters were also measured after the 13-week treatment by Woutersen et al. (1987). Compared with those of controls, activities of AST, ALT, and ALP were significantly elevated in plasma from the 20 ppm treated male rats (by 124, 132, and 126%, respectively; $p < 0.05$). Total plasma protein was reduced to 95% of controls in the same animals. Although there was an observed increase in BUN in male rats treated with 1 ppm, this was not considered a treatment effect. Furthermore, no statistically significant differences were seen for these parameters in female rats at any concentration level (Woutersen et al., 1987).

Sul et al. (2007) exposed Sprague-Dawley rats to 0, 5, and 10 ppm formaldehyde for 6 hours/day (5 days/week) for 2 weeks and collected lung samples for tissue damage and genomic analysis. According to their results, 21 genes were altered in a dose-dependent manner by microarray analysis; 2 were up regulated and 19 were down regulated in the lung tissue of animals exposed to formaldehyde. However, six of the nine genes further analyzed by PCR did not show dose dependency (authors did not comment). Although the authors briefly describe the functions and potential implications for changes in the expression of some of the altered genes, there is no discussion of the relationship between these altered genes (i.e., there is no pathway analysis).

In 2006, Im et al. (2006) published a proteomic analysis using the same exposure protocols (possibly using the same animals as in the Sul et al. [2007] study, although neither study makes reference to the other). Im et al. (2006) examined DNA damage in lymphocytes and liver tissues, as well as protein and lipid oxidation in plasma and liver samples. Similar to changes reported in the lung (discussed elsewhere), using two-dimensional electrophoresis and matrix-assisted laser desorption ionization time-of-flight mass spectrometry, the authors also reported dose-dependent changes in the levels of 32 proteins in plasma (19 up, 13 down). None of the changes in plasma proteins correspond to the changes in lung reported by Sul et al. (2007). Again, no pathway analysis was provided. Interestingly, Im and colleagues (2006) also demonstrated a dose-dependent increase in plasma IL-4 and dose-dependent decrease in IFNγ, perhaps indicative of Th-2-mediated inflammatory response. An overview of formaldehyde exposure-related pathology in the respiratory system of laboratory animals is presented in Table 4-28.

Table 4-28. Summary of respiratory tract pathology from inhalation exposures to formaldehyde, subchronic studies

Species/strain	No./ group	Treatment[a]	Respiratory effects	LOAEL/NOAEL	Reference
Nasal pathology					
B6C3F1 mice (male and female)	10	0, 2, 4, 10, 20, or 40 ppm formaldehyde 6 hours/day, 5 days/week for 13 weeks	Minimal squamous metaplasia in 1 of 10 mice (4 ppm). Squamous metaplasia observed in all mice at 10 and 20 ppm.	NOAEL = 4 ppm	Maronpot et al. (1986)
SPF Wistar Rats (male and female)	10	0, 1, 10, or 20 ppm formaldehyde 6 hours/day, 5 days/week for 13 weeks	Increased respiratory epithelial hyperplasia and keratinization at 20 ppm; squamous metaplasia at 10 ppm in males and females.	NOAEL = 1 ppm	Woutersen et al. (1987)
SPR Wister rats (male)	50–55	0, 10, or 20 ppm formaldehyde for 6 hours/day, 5 days/week for 4, 8, or 13 weeks	Rhinitis, hyperplasia, and squamous metaplasia in respiratory epithelium at all doses (number of weeks not specified). Squamous metaplasia of olfactory epithelium at 20 ppm (number of weeks not specified)	NOAEL = 1 ppm	Feron et al. (1988)
Wistar rats (male and female)	50	0, 0.3, 1, or 3.0 ppm formaldehyde 6 hours/day, 5 days/week for 13 weeks	Keratinized and non-keratinized squamous metaplasia in level 2 epithelium in 37/50 male and 21/50 female rats at 3 ppm for 13 weeks.	NOAEL = 1 ppm	Zwart et al. (1988)
Wistar rats (male)	10	40 ppm-hours (8 hours at 5 ppm, 4 hours at 10 ppm) or 80 ppm hours (10 ppm continuous or 20 ppm intermittently)	Thinning and disarrangement of respiratory epithelium, squamous metaplasia, most severe in 20 hours intermittent exposure	NOAEL = 10 ppm	Wilmer et al. (1987)
Wistar rats (male)	10	0, 8, or 16 ppm, given either continuously or intermittently	Disarrangement and squamous metaplasia at 4 ppm. Continuous exposure yielded less severe lesions than intermittent exposure	LOAEL = 8 ppm	Wilmer et al. (1989)
F344 rats (male and female), Syrian golden hamsters (male and female), cynomolgus monkeys	20 rats, 10 hamsters, 6 monkeys	0.0.2, 1, or 3 ppm 22 hours/day, 7 days/week, 26 weeks	Rats: rhinitis at 3 ppm, increased incidence of nasal lesions at 3 ppm. Monkeys: rhinitis at all doses, increased incidence of nasal lesions at 3 ppm. Hamsters: no significant nasal lesions.	NOAEL = 1 ppm	Rusch et al. (1983a, b)
Tracheal and lung pathology					
Wistar rats (male)	6	0, 5, 15 ppm for 8 hours/day, 5 days/week, 4 or 13 weeks	Significant decreases in zinc content in lung, copper unchanged, iron increased in lung.	LOAEL = 5 ppm	Özen et al. (2003)

Table 4-28. Summary of respiratory tract pathology from inhalation exposures to formaldehyde, subchronic studies

Species/strain	No./ group	Treatment[a]	Respiratory effects	LOAEL/NOAEL	Reference
Sprague-Dawley rats (male)	6 but n = 5 in some trials	0, 0.5, 3.0, 15 ppm 6 hours/day, 5 days/week, for 1 day, 4 days, 12 weeks, 24 weeks	Increased P450 levels after 4 days at 3 ppm.	NOAEL = 0.5 ppm	Dallas et al. (1989)
Sprague-Dawley rats (male)	5	0 or 10 ppm 6 hours/day, 4 days using both formalin and paraformaldehyde	P450 levels increased at 10 ppm only in groups treated with paraformaldehyde.	LOAEL = 10 ppm	Dinsdale et al. (1993)
Extrapulmonary effects					
Rats and guinea pigs	15	3.7 ppm for 90 days	Focal chronic inflammation in heart and kidney tissue.	LOAEL = 3.7 ppm	Coon et al. (1970)
SPF Wistar rats (male and female)	10	0, 1, 10, or 20 ppm 6 hours/day, 5 days/week for 13 weeks	Relative brain weight increased in female rats at 20 ppm; increased AST, ALT, ALP in plasma at 20 ppm.	NOAEL = 10 ppm	Woutersen et al. (1987)

4.2.1.2.3. ***Chronic inhalation bioassays.*** The respiratory pathology observed in chronic bioassays is consistent with the subchronic studies. As exposure concentration and duration of exposure are increased, the pathology becomes more severe and penetrates more deeply into the respiratory tract. These effects are progressive over time. Tumors are reported in several bioassays, primarily SCCs. Experimental results regarding both the severity of respiratory tract pathology as well as the tumor incidence vary by species strain and experimental design. As discussed above rodents experience RB, and species differences in respiratory and physiological depression would result in differences in absorbed dose in the respiratory tract, given the same exposure concentration (Chang and Barrow, 1983). Additionally, differences in the nasal architecture result in species-dependent variation of formaldehyde absorption (flux) within the respiratory tract (see Section 3.4). Therefore, chronic studies are discussed by species for greater clarity.

4.2.1.2.3.1. ***Mice.*** Early experiments by Horton et al. (1963) subjected mice (C3H, sex unspecified) to extreme formaldehyde concentrations (0, 0.05, 0.1, and 0.2 mg/L or 41–163 ppm) in an attempt to simulate lung pathology reported in humans exposed to cigarette smoke. The mice were exposed 1 hour/day, 3 days a week for up to 35 weeks. The authors did not note the effects of RB or provide any information on pathology of the URT. There was a clear increase in histologic changes in the tracheobronchial epithelium by exposure, including basal-cell hyperplasia, stratification squamous cell metaplasia and atypical metaplasia. Subsequent exposures to various combinations of formaldehyde and coal tar did result in squamous cell tumors. The findings of Horton et al. (1963) suggest a role for formaldehyde in lung cancer under some conditions. However, the exposure design and early deaths in the treatment groups severely limit the usefulness of these data in human health risk assessment.

In a comprehensive study conducted by Swenberg et al. (1980) (also reported in Kerns et al. [1983]) in conjunction with Chemical Industry Institute of Toxicology (CIIT) and Battelle Columbus Laboratories, male and female C57BL/6 × C3H F_1 (B6C3F_1) mice (approximately 120/sex/concentration) were exposed to 0, 2.0, 5.6, or 14.3 ppm (0, 2.45, 6.87, or 17.5 mg/m^3) formaldehyde 6 hours/day, 5 days/week for 24 months. This exposure period was followed by up to 6 months of nonexposure to evaluate recovery. Interim sacrifices were conducted at 6, 12, 18, 24, 27, and 30 months (due to unscheduled deaths, no male mice were sacrificed at 18 or 27 months). Exposure generation was accomplished by sublimation of paraformaldehyde, and exposures were conducted in whole-body chambers. Detailed sectioning and examination of the nasal passages were conducted at each interim sacrifice, beginning at 12 months, and for all unscheduled deaths. Gross organ pathology was noted for all animals and complete

histopathologic examination was conducted on all animals in the control and high-exposure groups. There were no differences in survival in any exposure group compared with controls. Generally, poor survival in all groups of male mice was attributed to fighting and infections of the urogenital tract associated with group housing; 78, 77, 81, and 82 unscheduled deaths were recorded before 24 months in the 0, 2.0, 5.6, and 14.3 ppm treatment groups, respectively (n = 119, 120, 120, and 119 males, respectively). After the interim sacrifices (6 and 12 months) only 17–22 male mice survived to the 24-month scheduled sacrifice. Female mice had much greater survival with only 30, 34, 19, and 34 unscheduled deaths prior to the 24-month sacrifice. The authors did not note the effects of RB in mice, although the RD_{50} for a 10-minute exposure for male B6C3F1 mice has been reported at 4.9 ppm and 4.4 ppm (Steinhagen and Barrow, 1984; Chang et al., 1981).

The first examination of the nasal cavities was conducted at the 12-month interim sacrifice. Inflammation in the nasal turbinates was evident in mice in the 2 and 6 ppm treatment groups (14/20 and 18/20, respectively), including adenitis of the nasal lacrimal duct, lacrimal duct, and vomeronasal gland. Inflammation was not present in mice exposed at 15 ppm, although serous rhinitis was seen in 4 of 20 animals. At 18 months, mice exposed at 2 and 6 ppm no longer exhibited adenitis in the nasoturbinates. Epithelial dysplasia was evident in 4 of 20 mice at 6 ppm exposure. Mice in the high-exposure group had significantly greater nasal pathology; epithelial dysplasia and squamous metaplasia were reported in 18/19 and 17/19 female mice, respectively, exposed to 15 ppm. After 24 months, squamous epithelial hyperplasia of the nasolacrimal duct (29/45) and atrophy of the olfactory epithelium (18/45) were also noted in animals from the high-exposure group (male and female) (Battelle Columbus Laboratories, 1981). Similar pathology was reported in only a small fraction of mice exposed at 2 and 6 ppm (5/48 and 11/60, respectively).

Three months after cessation of exposure, only nine female mice were available for sacrifice, but within this small sample the data suggested recovery of nasal lesions: epithelial dysplasia (4/9), squamous metaplasia (2/9), atrophy of the olfactory epithelium (1/9), and squamous epithelial hyperplasia of the nasolacrimal duct (1/9) (Battelle Columbus Laboratories, 1981).

Of the 17 male mice that survived to 24 months in the 14.3 ppm exposure group, 2 had SCC in the nasal cavity ($p < 0.05$). Of the two tumor-bearing mice, one exhibited significant epithelial pathology, including rhinitis, dysplasia, squamous metaplasia, and hyperplasia. Squamous metaplasia of the nasolacrimal duct was the only related pathology reported for the second mouse. No SCCs were found in female mice, although 48 mice survived to 24 months. The authors reported no other formaldehyde-related tumors. However, comparisons were based

on summary tables by organ. Although lymphomas were analyzed by organ and site (e.g., increase in salivary gland lymphoma considered separately from mandibular lymphoma), later reanalysis of lymphoma in female mice, based on tumor-bearing animals (TBAs), does indicate an association with formaldehyde exposure.

4.2.1.2.3.2. ***Rats.*** Holmström et al. (1989a) evaluated co-exposure of inhaled formaldehyde with wood dust in 16 female Sprague-Dawley rats/group. Rats were exposed in whole-body chambers for 6 hours/day, 5 days/week for 104 weeks to formaldehyde alone at 12.4 ± 1.1 ppm (15.21 ± 1.35 mg/m^3), wood dust alone (25 mg/m^3), or both wood dust (25 mg/m^3) and formaldehyde (12.7 ± 1.0 ppm) or to room air as the control. The wood dust was generated from grinding of beech. Microscopic measurements of the wood particles indicated that approximately 70% had a geometric diameter of about 10 μm, while 10–20% were about 5 μm or less. Animals were sacrificed at 104 weeks and histopathology was performed on five transverse sections of the nasal cavity (Figure 4-3) and the lungs (not otherwise specified).

There were no differences in mortality among the groups at any time during the study period. Rats exposed to formaldehyde were reported to have exhibited yellow discoloration of the fur, and many displayed eye irritation. Formaldehyde exposure, with and without wood dust, induced squamous metaplasia, keratinization, and dysplasia of the nasal epithelium (Table 4-29).

Table 4-29. Histopathologic findings and severity scores in the naso- and maxilloturbinates of female Sprague-Dawley rats exposed to inhaled formaldehyde and wood dust for 104 weeks

Treatment	Pronounced squamous metaplasia	Pronounced squamous metaplasia with keratinization	Pronounced squamous metaplasia with presence of dysplasia	Sum of rats with pronounced metaplasia and/or dysplasia	CCSCC	Histologic scores at the level of naso- and maxilloturbinates (mean ± SD)
Formaldehyde group (n = 16)	7	2	1	10	1	2.25 ± 1.73[a]
Formaldehyde-wood dust group (n = 15)	7	1	4	12	0	2.6 ± 1.88[a]
Wood dust group (n = 15)	0	0	0	0	0	1.86 ± 0.83[b]
Control group (n = 15)	0	0	0	0	0	1.07 ± 0.70

[a]$p < 0.01$.
[b]$p < 0.05$.

Source: Holmström et al. (1989a).

Among the five levels of the nasal cavity that were examined, Holmström et al. (1989a) presented findings for the naso- and maxilloturbinates since formaldehyde-induced tumors had been associated with this level (Morgan et al., 1986a, b). The data also suggested an effect of wood dust on formaldehyde-induced nasal pathology, with a slightly higher histologic score and greater incidence of dysplasia than formaldehyde exposure alone. One SCC (1/16) occurred in the group exposed to formaldehyde only but not in the group exposed to formaldehyde and wood dust. Microscopic examination of the lungs revealed that emphysema (diagnostic criteria not specified) was more prevalent in both groups exposed to wood dust compared with the control group ($p < 0.05$). There was no significant difference in pulmonary epithelial histopathology among the groups.

Tobe et al. (1985) also evaluated F344 rats (32/group) exposed to inhaled formaldehyde for 28 months. Exposures were for 6 hours/day, 5 days/week to formaldehyde concentrations of 0, 0.3, 2, and 14 ppm (0, 0.37, 2.45, and 17.2 mg/m^3). Fourteen of 32 rats (44%) in the high concentration group developed nasal SCCs, compared with none in the other exposed groups and the control group. Tobe et al. (1985) reported increased rhinitis, hyperplasia, and squamous metaplasia of the nasal respiratory epithelium, including in the low-exposure group (0.3 ppm.) However, some level of rhinitis, hyperplasia, and metaplasia were also present in controls. Without a more complete report, it is unknown whether or not the pathology reported at 0.3 ppm was a formaldehyde-related effect.

Kamata et al. (1997) evaluated the effects of inhaled formaldehyde in male F344 (F344/DuCrj) rats (32/group) exposed for 28 months. Formaldehyde exposure was generated by metering 37% formalin (containing 10% methanol) into a sprayer in a glass bottle and diluting with room air. Concentration in the chamber was monitored twice daily by the acetyl acetone method. Exposures were for 6 hours/day, 5 days/week at nominal formaldehyde concentrations of 0, 0.3, 2.0, and 15 ppm (0, 0.37, 2.45, and 18.4 mg/m^3). Actual levels were 0, 0.3 ± 0.07, 2.17 ± 0.32, and 14.85 ± 2.22 ppm (mean ± SD). Rats in the 0 ppm group were given methanol to inhale at the same concentration (4.2 ppm) as the 15 ppm group. A room control no-exposure group was also included in the study. All animals were observed for clinical signs once a day during the study. Body weights and food consumption were recorded weekly. Five animals per group, randomly selected at the end of 12, 18, and 24 months, and all surviving animals at 28 months were sacrificed for hematological measurements (Hb, RBCs, PCV, MCV, mean corpuscular hemoglobin [MCH], MCHC, and WBCs), biochemical determinations (TP, ALB, BUN, ALP, AST, ALT, glucose, albumin/globulin ratio, phospholipids, triglycerides, and total cholesterol), and pathological examinations. Wet weights were taken on brain, heart, lungs, liver, kidneys, spleen, testes, and adrenal gland of each rat. Histopathology was performed on all

moribund or dead animals and those at specified sacrifices on all gross lesions and the following tissues: pituitary, thyroid, nasal cavity, trachea, esophagus, stomach, small and large intestines, prostate gland, urinary bladder, muscle, femur, sciatic nerve, spinal cord, and mesenteric lymph nodes. Histopathologic sections of the nose were obtained from five anatomical levels, but these did not correspond to the typical levels taken in other bioassays. Most notably, section level B was anterior and not posterior to the incisor teeth. The incidence data for nasal histopathology were not reported with respect to section level location, with the exception that the nonproliferative lesions and tumors reported were described to occur predominantly at levels B and C.

Yellow discoloration of the coats occurred in animals exposed at the 2 and 15 ppm levels. Significant decreases in body weight and food consumption were observed in the high concentration (15 ppm) group throughout the exposure period, and elevated mortality was noted at 28 months (88.3 versus 31.8% in controls). The first death occurred after 6 versus 18 months in the control group. Other effects noted in the 15 ppm exposure group include decreased triglycerides, reduced liver weight (both relative and absolute), and increased relative adrenal weights.

Treatment-related macroscopic and histopathologic findings were limited to the nasal cavity. Squamous cell metaplasia was reported in all treatment groups: 16% (0.3 ppm), 37.5% (2 ppm), and 91% (15 ppm) of exposed rats. Epithelial hyperplasia was similarly present in 12.5, 22, and 91% of the animals, respectively. Since a no-effect level could not be determined, the authors reported benchmark doses (BMDs) of 0.25 and 0.24 ppm for squamous cell metaplasia and epithelial hyperplasia (10% response.). Additional lesions only occurring in the 15 ppm dose group were papillary hyperplasia (2/32), SCC (13/32), squamous cell papilloma (3/32), and sarcoma (1/32). The majority of the tumors were located at levels B and C of the nasal cavity.

Albert et al. (1982) and Sellakumar et al. (1985) reported on a set of lifetime studies performed in male Sprague-Dawley rats to evaluate the effects of inhaled formaldehyde alone and in combination with hydrochloric acid (HCl). Rats were exposed 6 hours/day, 5 days/week for life. In the first experiment (Albert et al., 1982), 8-week-old male inbred Sprague-Dawley rats (n = 99) were exposed to a mixture of 10 ppm (12.3 mg/m^3) HCl and 14 ppm (17.2 mg/m^3) formaldehyde, and there were two control groups: air-sham and untreated (n = 50). Bis(chloromethyl)ether (BCME), a known animal carcinogen (Albert et al., 1975; Kuschner et al., 1975; Figueroa et al., 1973; Laskin et al., 1971), is formed when formaldehyde and HCl are mixed. BCME concentrations were estimated at about 1 ppb in the formaldehyde-HCl mixed exposures, based on levels in the mixing chamber. Complete necropsies were conducted when animals died naturally or were killed when moribund. Histologic sections were taken from the

nasal cavity, larynx, trachea, pulmonary lobes, liver, bladder, kidney, spleen, and other organs with gross pathologic alterations.

Exposure to the mixed gases (formaldehyde-HCl-BCME) induced nasal lesions, including epithelial hyperplasia (71%), squamous metaplasia (64%), squamous papilloma (3%), and SCC (25%) (Albert et al., 1982). Although a few squamous metaplasia were noted in the larynx, trachea, and bronchi, these lesions were also noted in controls. Mortality in exposed rats was significantly increased over controls and was approximately 30% when the first carcinoma was reported (233 days). Mortality in exposed rats rose quickly to approximately 60% after the first year of exposure. Therefore, the authors used a life-table method to calculate a mortality-corrected cumulative incidence, reporting a corrected cumulative incidence of 77% at 720 days after first exposure.

In the second experiment performed in this laboratory (Sellakumar et al., 1985; Albert et al., 1982), Sprague-Dawley rats were similarly exposed to HCl (10 ppm) alone, formaldehyde alone (15 ppm), or a combination of both. The combination exposure was generated in two different ways to better understand the influence of BCME formation on study results: premixed at high concentrations and gases fed separately into the inlet air supply at the target concentrations. BCME concentration measured by a gas chromatography/mass spectrometry method in the premixed chamber varied between 0.1 and 0.4 ppb. Cage-side observations and necropsy procedures were as described in Albert et al. (1982) with the exception of the histologic preparation of the head. The head was cut transversely into four tissue blocks, and sections were taken from the face of each.

Animals exposed to formaldehyde alone and formaldehyde-HCl (premixed or non-premixed) showed a marked decrease in body weight after 16 weeks. After 32 weeks rats exposed to the premixed formaldehyde-HCl (with BCME) had higher mortality compared with the other mixed gas exposures ($p < 0.05$). Nasal pathology was similar among rats exposed to formaldehyde alone or the mixed gases (Table 4-30). Desquamation of respiratory epithelial cells was reported in the respiratory epithelium that covers the nasomaxillary turbinates and the nasal septum (approximately section levels 2 and 3). Olfactory epithelium in the ET frequently showed an inflammatory reaction with seropurulent exudate filling the lumen. Squamous metaplasia and hyperplasia were reported in the larynx and trachea in all treatment groups.

Tumors arose primarily from the nasomaxillary turbinates and nasal septum. The SCCs were predominantly moderate to well differentiated, with excessive amounts of keratin occluding the lumen, killing the animals by asphyxiation. Statistical comparisons by the log rank test (Peto test) showed that tumor incidence was increased in the premixed formaldehyde-HCl combined exposure group over formaldehyde alone or the combined formaldehyde-HCl (not premixed).

There were no significant differences in the latency among groups, with the average latency varying from 603 to 645 days. Rats exposed to HCl exposure alone did not develop tumors.

The esthesioneuroepithelioma is a unique tumor type observed with a high incidence in an earlier inhalation study of rats exposed to BCME (Kuschner et al., 1975), suggesting that the higher incidence of nasal tumors observed in the premixed-combined formaldehyde-HCl-exposure group may have been due to BCME (Krimsky, 1986) since this premixed protocol was the one most likely to generate BCME. Sellakumar et al. (1985) refuted this assertion, stating that this singular tumor occurred in the absence of other changes in the ethmoid region or in the lungs where BCME was also demonstrated to cause tumors. Furthermore, exposure was approximately one-tenth the cumulative dose in the Kuschner et al. (1975) study that was associated with a single similar tumor. Sellakumar et al. (1985) attributed the higher incidence in the premixed-combination group to traces of other alkylating agents (not BCME) that could have been formed. The results demonstrate that animals exposed to either a combination of formaldehyde-HCl or to formaldehyde alone develop nasal tumors, principally SCCs, at about the same frequency, indicating that HCl plays little or no role in the carcinogenicity of inhaled formaldehyde.

Table 4-30. Histopathologic changes (including tumors) in nasal cavities of male Sprague-Dawley rats exposed to inhaled formaldehyde or HCl alone and in combination for a lifetime

Observation	Premixed HCl-HCHO	Non-premixed HCl-HCHO	HCHO	HCl	Air	Colony
Number of animals examined	100	100	100	99	99	99
Rhinitis	74	75	74	81	72	70
Epithelial or squamous hyperplasia	54	53	57	62	51	45
Squamous metaplasia	64	68	60	9	5	6
Polyp or papilloma	13	11	10	0	0	0
SCC	45	27	38	0	0	0
Adenocarcinoma	1	2	0	0	0	0
Mixed carcinoma	0	0	1	0	0	0
Fibrosarcoma	1	0	1	0	0	0
Esthesioneuroepithelioma	1	0	0	0	0	0
Larynx						
Hyperplasia	11	22	21	22	2	2
Squamous metaplasia	10	15	4	0	0	0
Trachea						
Hyperplasia	18	32	21	26	6	2
Squamous metaplasia	9	8	7	0	0	0

Source: Sellakumar et al. (1985).

In a companion study to the chronic mouse study described above (Kerns et al., 1983; Swenberg et al., 1980), groups of F344 rats (approximately 120/sex/concentration) were exposed to 0, 2.0, 5.6, or 14.3 ppm (0, 2.45, 6.87, or 17.5 mg/m^3) formaldehyde 6 hours/day, 5 days/week for 24 months. This exposure period was followed by up to 6 months of nonexposure to evaluate recovery. Interim sacrifices were conducted at 6, 12, 18, 24, 27, and 30 months. Study parameters and methods were as described above.

Formaldehyde exposure increased mortality of both male and female rats in all treatment groups ($p < 0.05$ for 6 and 15 ppm groups). Severe treatment-related mortality was seen at the highest exposure group beginning at 12 months with only 30% surviving to the 24 months. There were no alterations in clinical chemistry, neurofunctional, or ophthalmological measurements considered to be related to formaldehyde exposure. A concentration-dependent increase in yellow discoloration of the hair coat was observed. This discoloration dissipated over the 3-month postexposure period. Rats in the highest-concentration group were dyspneic ($p < 0.01$) and emaciated ($p < 0.05$) and had many facial swellings that on closer examination were revealed to be carcinomas protruding through the nasal cavity. Neoplastic lesions in the URT were first observed clinically at day 358 in females and day 432 in males. Macroscopically, these lesions originated in the anterior portion of the nasal cavity and, in a few instances, extended into the ETs.

Figure 4-9 shows the frequency of squamous metaplasia by location in the noses of rats sacrificed at various time points along the 2-year exposure period. Histopathologic lesions were confined to the nasal cavity and proximal trachea in concentration-dependent fashion. The morphologic diagnosis of squamous metaplasia was used to designate zones of altered epithelium that were characterized by the presence of a well-differentiated germinal layer (stratum germinativum) and superficial layers of epithelium (stratum spinosum and stratum corneum). Keratin was produced only in areas of squamous metaplasia. Epithelial dysplasia was detected earlier than squamous metaplasia and was characterized by a mucosa that had undergone a transition from nonciliated simple cuboidal to one that was several cells thick and squamoid with an organization and polarity of the individual cells that had changed from vertical to horizontal with respect to the basement membrane. Similar histomorphologic changes have also been called basal cell hyperplasia and epidermoid metaplasia (e.g., Albert et al. [1982]). Figure 4-9 clearly illustrates that concentration is a dominant determinant of lesion distribution. At low concentrations the lesions occur only in the most anterior region (cross-section level 1). At 5.6 ppm, the squamous metaplasia in levels 1, 2, and 3 was also associated with purulent rhinitis and epithelial dysplasia. At the highest concentration, the lesions progress to the more distal URT, with lesions evident in level 5 and no difference in the incidence at level 1 or 2

across the various sacrifice times. Statistically significant ($p < 0.05$) regression of the lesion was evident at most locations at the 27-month sacrifice (3 months postexposure) (e.g., level 1 in the 2 ppm group, all levels of the 5.6 ppm group, and levels 4 and 5 of the 14.3 ppm group).

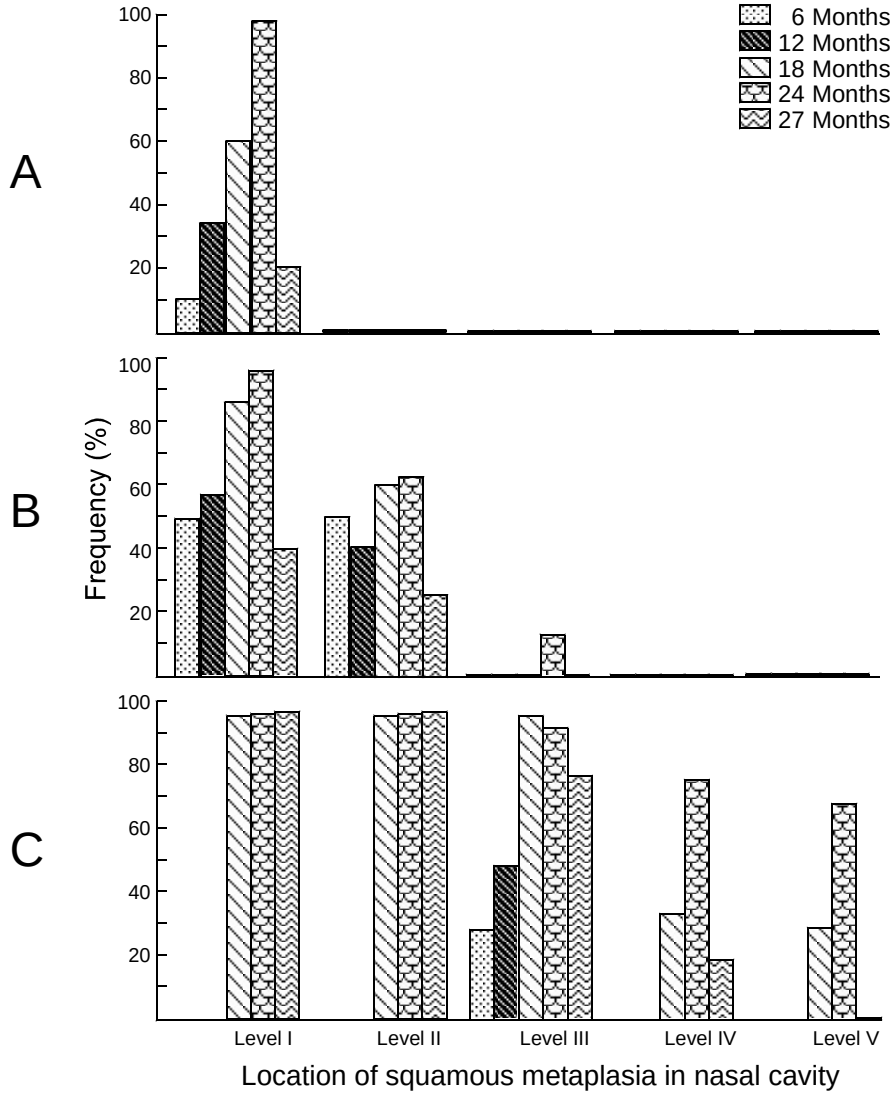

Figure 4-9. Frequency and location by cross-section level of squamous metaplasia in the nasal cavity of F344 rats exposed to formaldehyde via inhalation.

Note: Exposure concentrations were 2.0 ppm (A), 5.6 ppm (B), or 14.3 ppm (C). Nasal cavity levels 2, 3, 4, and 5 were not evaluated at the 6- and 12-month interim sacrifices in the 14.3 ppm exposure group.

Source: Redrawn from Kerns et al. (1983).

Furthermore, progression of lesions distally to the lower respiratory tract (LRT) occurred only in the high concentration group. Tracheal pathology observed at 18 months included multifocal areas of minimal to mild epithelial hyperplasia, epithelial dysplasia, or squamous metaplasia. There were no significant tracheal lesions present in the 0, 2.0, or 5.6 ppm exposure groups, and tracheal lesions were not observed during the postexposure period in the 14.3 ppm exposure group.

Table 4-31 provides the summary data of all neoplastic lesions in the nasal cavity of exposed rats. The adjusted cumulative incidence rates of SCC in male and female rats from the 14.3 ppm exposure group at 24 months were 67 and 87%, respectively. In this group, the formation of zones of squamous metaplasia with zones of squamous epithelial hyperplasia and increased keratin production appeared to precede areas of squamous papillary hyperplasia with foci of cellular atypia. More advanced lesions included carcinoma in situ and invasive SCC of the nasal turbinates. The neoplasia were extremely osteolytic and were associated with excessive keratin production and mild to severe purulent rhinitis. In many animals from the high-exposure group (with or without carcinoma), the excessive accumulation of keratin and inflammatory exudates within the lumen of the URT caused severe dyspnea and death. Polypoid adenomas were also observed in eight rats (four/sex) from the low-exposure group, six male rats from the intermediate-exposure group, and six rats (five males, one female) from the high-exposure group in level 1, 2, or 3. One control male rat had a similar lesion. When adjusted and unadjusted data were analyzed, no significant differences were observed in pair-wise analyses; however, a significant adjusted trend ($p < 0.05$) was reported for male rats. There was no evidence of progression from polypoid adenoma to SCC.

Table 4-31. Summary of neoplastic lesions in the nasal cavity of F344 rats exposed to inhaled formaldehyde for 2 years

Formaldehyde (ppm)	Sex	No. of nasal cavities evaluated	SCC	Nasal carcinoma	Undifferentiated carcinoma or sarcoma	Carcino-sarcoma	Polypoid adenoma	Osteo-chondroma
0	M	118	0	0	0	0	1	1
	F	114	0	0	0	0	0	0
2.0	M	118	0	0	0	0	4	0
	F	118	0	0	0	0	4	0
5.6	M	119	1	0	0	0	6	0
	F	116	1	0	0	0	0	0
14.3	M	117	51	1[a]	2[a]	1	4	0
	F	115	52	1	0	0	1	0

[a]One rat in this group also had an SCC.

Source: Kerns et al. (1983).

Morgan et al. (1986b) performed an additional analysis of the slides and tissues from the Kerns et al. (1983) study to more precisely determine the location of each tumor recorded. Additional sections were cut from the existing tissue blocks if a full slide set (i.e., five sections) was unavailable for each animal. For each animal, the location of each tumor was recorded on diagrams of the cross section of the nose, and an attempt to determine the site of origin was made based on the center of the tumor mass. The results for each case were assigned an accuracy rating that was based on the degree of confidence that the pathologist had in the designated site of origin. Results for SCCs are shown in Table 4-32.

Table 4-32. Apparent sites of origin for the SCCs in the nasal cavity of F344 rats exposed to 14.3 ppm of formaldehyde gas in the Kerns et al. (1983) bioassay

Sex	Accuracy rating	Number of animals	Total SCC (%)[a]				
			Area 1[b]	Area 2[b]	Area 3[b]	Area 4[b]	Unable to determine
Male	High	36	56	28	14	3	NA
	Low	25	56	20	8	0	16
Female	High	45	62	27	7	4	NA
	Low	15	47	33	13	0	7
Totals		121	57	26	10	3	4

[a]Rounded to nearest whole number.
[b]Area 1 = lateral aspect of the nasoturbinate and adjacent lateral wall; Area 2 = midventral septum; Area 3 = dorsal septum and roof of dorsal meatus; Area 4 = dorsal and lateral aspect of the maxilloturbinate.
NA = not applicable.

Source: Morgan et al. (1986b).

In the 14.3 ppm exposure group, 98/103 rat noses had adequate numbers and quality of slides for mapping the SCC distribution. Single neoplasia were present in 80 (40/sex), while multiple neoplasia were present in 9 males (21 neoplasia) and 9 females (20 neoplasia). The results were similar for cases with high or low accuracy. For example, more than half (57%) of the SCCs occurred on the lateral side of the nasoturbinate and adjacent lateral wall at the front of the nose (levels 1 and 2); approximately 25% were located on the midventral nasal septum (levels 2 and 3); and about 10% were on the dorsal septum and roof of the dorsal meatus (levels 1, 2, and 3). A small number (3%) were found on the maxilloturbinate (levels 2 and 3), which only involved the medial aspect. All other regions of the nose where SCC was found were considered to be involved as a result of invasion from one or more of the above sites. There

were two tumors in the 5.6 ppm group: one male had a single neoplasm on the ventral nasal septum (level 3) while a female had an SCC from the lateral aspect of the maxilloturbinate to the adjacent lateral wall (level 2).

On the basis of the morphology of 19 small neoplasia in this study and in additional work described below (Morgan, 1997; Monticello et al., 1996), it was further concluded that the SCCs arose from the epithelium lining the airway and not from the underlying glandular epithelium. This mapping procedure and that of Monticello et al. (1996) described below were in good concordance and showed a clear site specificity; most of the SCC arose in the anterior lateral meatus (ALM) (57%), which is lined by transitional epithelium, and the midventral nasal septum (26%), which is lined by respiratory epithelium (Morgan, 1997).

The CIIT performed a second bioassay on inhaled formaldehyde in 9-week-old male F344 (CDF[F344]/CrlBr) rats (Monticello et al., 1996). The rats were exposed 6 hours/day, 5 days/week for 24 months to 0, 0.7, 2, 6, 10, and 15 ppm (0, 0.86, 2.45, 7.36, 12.3, and 18.4 mg/m^3) formaldehyde. Study objectives were to repeat the Kerns et al. (1983) bioassay, better defining the concentration response relationship and to seek a correlation between localized data on tumor sites and concomitant cell proliferation assays. Histopathology was performed on six cross-section levels of the nasal cavity on every animal of an unscheduled death and all those of the terminal sacrifice after 24 months. The distribution of lesions for each individual animal was recorded onto epithelial maps of the nasal cavity at 30 selected levels designed to permit accurate localization (Mery et al., 1994). Cell proliferation was measured in a subset of animals (five per treatment group) at 3, 6, 18, and 24 months of exposure in each of the nasal regions to which tumors were mapped (Table 4-33).

Table 4-33. Incidence and location of nasal squamous cell carcinoma in male F344 rats exposed to inhaled formaldehyde for 2 years

Formaldehyde concentration (ppm)	No. of nasal cavities examined	Nasal location							No. of animals with SCC[a]
		Anterior lateral meatus	Posterior lateral meatus	Anterior mid-septum	Posterior mid-septum	Anterior dorsal septum	Anterior medial maxillo-turbinate	Maxillary sinus	
0	90	0	0	0	0	0	0	0	0
0.7	90	0	0	0	0	0	0	0	0
2	96	0	0	0	0	0	0	0	0
6	90	1	0	0	0	0	0	0	1
10	90	12	2	0	0	0	0	0	20
15	147	17	9	8	1	3	4	0	69

[a]Total number of animals with SCCs, including those too large to allocate and those located in a site not listed in this table.

Source: Monticello et al. (1996).

Yellow discoloration of the fur, a consistent response to formaldehyde in rats, was observed in the rats exposed to 10 and 15 ppm formaldehyde. There were numerous premature deaths in the 15 ppm exposure group, resulting in significantly decreased survival relative to controls (18.8 versus 35.7%; $p < 0.001$). Survival was higher in the three lowest exposure groups and statistically comparable to controls in the 10 ppm exposure group (35.7 versus 31.3%, respectively).

Control animals showed no histopathologic evidence of disease in the nasal passages. Buccal cavity SCC, not associated with the nasal cavity, was present in 2 of 90 control animals. This was considered an incidental finding and within the spontaneous incidence range reported for this strain of rat. Buccal SCCs were observed in three animals at 15 ppm and in one animal at 2 ppm. All other neoplastic responses in the respiratory tract were confined to the nose and considered to have originated from the epithelium lining the nasal airways. The nasal neoplasia included SCCs and polypoid (transitional) adenomas and were similar in morphologic characteristics to those described in the Kerns et al. (1983) chronic bioassay. The incidence of nasal SCCs by location is summarized in Table 4-33, which demonstrates a clear concentration-response relationship. No SCCs occurred in the two lowest exposure groups or in the controls. One nasal rhabdomyosarcoma and two nasal adenocarcinomas were reported in animals in the highest treatment groups.

Regional analysis indicated that the SCCs arose in nasal regions lined with transitional or respiratory epithelium and were most common in the lateral meatus and the midseptum (Table 4-33). Within the lateral meatus and mid-septum, there was clear evidence of a higher tumor incidence rate in the anterior sample site ($p = 0.001$ and 0.02, respectively). Smaller numbers of SCCs were observed on the medial aspect of the maxilloturbinate and the dorsal septum and on the posterior lateral wall and lining of the nasopharyngeal meatus (data not shown). No SCCs were observed in the maxillary sinus, with the exception of one animal exposed to 15 ppm that had a small tumor in the wall of the ostium of this sinus. Tumor rates across the seven nasal epithelial sites are presented in Table 4-33. There was an increasing tumor response between the 10 and 15 ppm exposure groups in all sites, except in the ALM. The SCC rates at 10 and 15 ppm were virtually identical (13.3 and 11.6%, respectively), which is probably attributable to the occurrence of many large neoplasia in the lateral meatus site that were not suitable and not counted in the analysis.

The nonlinear tumor response is mirrored by a highly nonlinear response in cell proliferation measured after 3, 6, 12, and 18 months of exposure. Significant treatment-induced responses in cell proliferation indices at these time points were only observed at the two highest exposure concentrations (10 and 15 ppm). Other treatment-induced lesions, predominantly

epithelial hypertrophy, hyperplasia, squamous metaplasia, and mixed inflammatory cell infiltrate, were also most severe at these two exposure concentrations. Significant distortion and destruction of the nasoturbinate architecture occurred in many animals exposed to 15 ppm. Nasal turbinate adhesions and olfactory degeneration (usually confined to the walls of the anterior dorsal medial meatus) also occurred in animals exposed to 10 and 15 ppm. Lesions in the 6 ppm exposure group were limited to focal squamous metaplasia in the anterior regions.

As discussed briefly above, small numbers of polypoid adenomas were also induced by formaldehyde exposure and were similar in acinar-like structure and location to those in the Kerns et al. (1983) bioassay. No polypoid adenomas occurred in the control animals or in the 0.7, 2, or 6 ppm exposure groups. A clear concentration response was observed in the 10 and 15 ppm exposure groups. Five of 90 animals (5.6%) in the 10 ppm exposure group and 14 of 147 animals (9.5%) in the 15 ppm exposure group had a polypoid adenoma. Most of these polypoid adenomas (79%) were located in or adjacent to the lateral meatus. The significance of these tumors for risk assessment remains to be determined (Morgan, 1997).

Appelman et al. (1988) studied the effects of bilateral intranasal electrocoagulation damage on susceptibility to inhaled formaldehyde in male SPF Wistar (Cpb: WU) rats. Rats were exposed 6 hours/day, 5 days/week for 13 or 52 weeks to 0, 0.1, 1.0, or 10 ppm (0, 0.12, 1.23, or 12.3 mg/m^3) formaldehyde. These concentrations were chosen because the various short-term studies performed in the same laboratory (described in Section 4.2.1.2) showed that formaldehyde was noncytotoxic to the nasal mucosa at levels of 0.3, 1.0, and 2.0 ppm, slightly cytotoxic at 3 and 4 ppm, and strongly cytotoxic at 10 and 20 ppm (Zwart et al., 1988; Wilmer et al., 1987; Woutersen et al., 1987). Furthermore, because nasal tumors have only been found at exposure concentrations that also induced severe degenerative, hyperplastic, and metaplastic changes in the nasal epithelium (Griesemer et al., 1985; Squire and Cameron, 1984), Feron et al. (1984) and the investigators at the TNO-CIVO Toxicology and Nutrition Institute postulated that formaldehyde at a subcytotoxic concentration was only a very weak initiator without promoting activity. Appelman et al. (1988) used an electrocoagulation method in this study to evaluate if damage to the mucosa followed by compensatory cell proliferation might render the epithelium vulnerable to subcytotoxic levels of formaldehyde. One-half of the rats used in the study (10/group) were damaged bilaterally and then subjected to the first 6-hour exposure to formaldehyde approximately 20–26 hours after the electrocoagulation procedure. Ten undamaged rats/group were also exposed at each concentration for either 13 or 52 weeks. Histopathologic examination included six standard cross-section levels in the nose; livers of all rats killed at 14 weeks and of all control and 10 ppm exposed rats killed in week 53; larynges, tracheas, and lungs of all rats of the control and 10 ppm exposed rats killed in week 53; and

organs and tissues of control and 10 ppm exposed rats with an undamaged nasal mucosa killed in week 53.

Yellow discoloration of the fur occurred in all animals of the two highest exposure groups. Growth retardation was observed in the animals killed with or without damaged noses after 2 weeks of exposure to 10 ppm formaldehyde. No toxicologically significant findings in the body weights or organ weights of any animals in the other exposure groups were observed. No relevant differences between groups were found in any of the hematological or urinary parameters with the exception of frequent oliguria ($p < 0.05$) in the top exposure group without nasal coagulation and killed in week 53. Three-way ANOVA revealed a significant increase in TP content of the liver in rats with damaged noses as compared with rats with undamaged noses, and there was a significant negative correlation between the formaldehyde exposure level and TP in these same rats. Hepatic GSH was positively correlated with both nasal damage and age of the animals. No treatment-related gross findings were observed in animals sacrificed at either 14 or 53 weeks except for yellow discoloration of the fur in rats exposed at the two highest concentrations. No changes observed in the larynx, trachea, lungs, liver, or other tissues evaluated were regarded as related to formaldehyde.

Few nasal lesions were noted in intact rats exposed at 0.1 or 1 ppm for either 13 or 52 weeks (n = 10/group). Focal squamous metaplasia was noted in a single animal exposed at 1 ppm for 13 weeks. Rats exposed at 10 ppm formaldehyde demonstrated clear pathology in the respiratory epithelium progressing from 13 to 52 weeks, including squamous metaplasia, basal cell hyperplasia, and focal rhinitis. Additionally, focal nest-like infolds of the epithelium were present in 4 of 10 rats at 52 weeks, and minor changes to the olfactory epithelium were noted (thinning/disarrangement and focal basal cell hyperplasia.)

All rats with damaged nasal passages exhibited similar minor pathology of the respiratory epithelium at 13 and 52 weeks (squamous metaplasia, focal basal cell hyperplasia, and focal rhinitis). Formaldehyde-related effects were note at 52 weeks, where the squamous metaplasia of the respiratory epithelium was no longer noted in controls (versus 13 weeks) but was clearly present in all formaldehyde-treatment groups, including progression from focal to diffuse lesions (at 1 and 10 ppm) and keratinization (3/10 and 4/10 at 0.1 ppm and 10 ppm, respectively). The formaldehyde effects on the respiratory epithelium were much more severe in rats with damaged nasal passages, with all animals demonstrating thinning and disarrangement of the olfactory epithelium and 8 of 10 rats exhibiting “loosely arranged submucosal tissue.” Squamous metaplasia and focal rhinitis of the olfactory epithelium were seen in less than half of the formaldehyde-treated rats with damage. No changes in the olfactory epithelium due only to electrocoagulation were encountered.

The most notable effects of nasal damage from electrocoagulation were the ones at the highest formaldehyde exposure (10 ppm) on the olfactory epithelium. Damage to the respiratory epithelium also occurred more posteriorly in rats with damaged noses. Since electrocoagulation often induced damage that included partial or complete loss of turbinates and septal perforation, a likely explanation for the posterior distribution of the damage is an abnormal airflow pattern. This gross damage to the nasal structure may have also disrupted normal mucous production and flow. Therefore, formaldehyde-induced pathology appearing deeper in the nasal passages, including the respiratory epithelium, may be due to formaldehyde penetrating more deeply into the nasal passages and resulting in greater tissue doses in these areas.

Woutersen et al. (1989) conducted a lifetime study in parallel to the 1-year study described above for Appelman et al. (1988). Male Wistar rats with nasal damage induced by electrocoagulation (60/group) or without nasal damage (30/group) were exposed 6 hours/day, 5 days/week to the same concentrations as in the previous study (0, 0.1, 1.0, and 10 ppm) for 28 months or for 3 months followed by a 25-month observation period. The general condition and behavior of the animals were checked daily. Body weight, organ weight, and gross pathology were evaluated as described for Appelman et al. (1988). Histopathologic examination was conducted on all animals at the standard six cross sections (see Figure 4-3).

No remarkable findings on behavior were observed except for yellowing of the fur in animals at the two highest concentrations. There were no relevant differences in mortality (data not shown). Growth retardation was observed relative to controls in animals with or without damaged noses exposed to 10 ppm from day 14 onward. Body weights were generally slightly lower in formaldehyde-exposed animals with an intact nasal mucosa and slightly higher in exposed animals with damaged noses than in the corresponding controls.

The effects of formaldehyde exposure on the respiratory and olfactory epithelium after 28 months of exposure were similar to those reported for 52 weeks exposure (Appelman et al., 1988): rhinitis, squamous metaplasia with some keratinization of the respiratory epithelium, and thinning/disarrangement and slight squamous metaplasia of the olfactory epithelium at the 10 ppm exposure. Effects attenuated from the anterior to posterior sections (I–II, III, IV, and V–VI). A low incidence of olfactory epithelium replaced by respiratory epithelium (<10%) and vacuolation and atrophy of olfactory cells (<10%) was reported, this in part may be due to the larger study size (30 rats per group versus 10). Squamous metaplasia in levels I–II of the respiratory epithelium at 10 ppm was the only treatment-related pathology remaining in rats exposed for 3 months followed by a 25-month recovery period.

Similarly, as reported by Appelman et al. (1988), rats with noses damaged by electrocoagulation did demonstrate increased pathology of the respiratory epithelium.

Formaldehyde exposure at 10 ppm exacerbated these changes, and effects were noted in more posterior sections than in rats without nasal damage (levels III, IV, and V). Olfactory pathology was also greater in formaldehyde-treated rats: basal cell hyperplasia, replacement of olfactory epithelium by respiratory epithelium (10–20% at level III and <10% at level IV). Although the incidences are low, there is some evidence that effects on the olfactory epithelium may be increased at the lower formaldehyde exposures (0.1 and 1 ppm.) Analysis of the number of animals with olfactory pathology would be helpful to better understand the potential of low-level formaldehyde effects on these less frequent lesions. Interestingly, the recovery of the olfactory and respiratory epithelium seen in rats with undamaged nasal cavities after a 25-month recovery period was not evident in rats with damaged noses. Formaldehyde-exposure effects are only present at the 10 ppm exposure for the respiratory epithelium (squamous metaplasia, basal cell hyperplasia), and the formaldehyde-related effects on the olfactory epithelium (thinning/disarrangement, basal cell hyperplasia, and replacement by respiratory epithelium) are seen at 0.1 and 1.0 ppm as well.

A single SCC, 1 out of 30 rats, was found in each 28-month formaldehyde-treatment group (1/26, 1/28, and 1/26, respectively) but not in any control animals (n = 52). SCCs were also noted in rats with noses damaged by electrocoagulation (1/54, 1/58, 0/56, and 15/58 for control rats and the formaldehyde-treatment groups, respectively). These data clearly indicate a synergistic effect of high formaldehyde exposure and nasal damage on the formation of SCCs in rats. One adenosquamous carcinoma and one adenocarcinoma were also reported as increasing the frequency to 17/58 for all tumors. Additionally SCC was present in two rats in the 0.1 and 1 ppm 3-month exposure groups with damaged noses only, although only one SCC was reported in the 10 ppm 3-month groups with and without damaged noses. Rats not surviving to 28 months are included in these results, as well as the histopathology reported above. Since no mortality data are reported, it should be noted that the incidence of both nasal lesions and tumors are not controlled for early deaths.

In total, 30 tumors were examined from this study. In general, the tumors (26/30 or 87%) were SCCs, and 69% (18/26) of these clearly originated from the respiratory epithelium lining the septum or nasal turbinates. The eight other SCCs, derived from the epithelium lining the nasolacrimal duct, were seen in connection with severe odontodystrophy and periodontitis or might have originated from the skin or salivary glands. Four remaining rats bearing a nasal tumor developed a small polypoid adenoma located on the nasoturbinate, an adenocarcinoma originating from the olfactory epithelium, an adenosquamous carcinoma of the respiratory epithelium lining the septum or turbinates, or a carcinoma in situ of epithelium in the nasolacrimal duct.

4.2.1.2.3.3. ***Hamsters***. Dalbey (1982) examined the effects of inhaled formaldehyde alone for a lifetime or combined with diethylnitrosamine (DEN) in an initiation-promotion study design using male Syrian golden hamsters. For the first experiment, hamsters were exposed at either 0 or 10 ppm (0 or 12.3 mg/m^3) formaldehyde in whole body chambers 5 hours/day, 5 days/week for a lifetime (132 controls, 88 exposed). Histopathologic evaluations were carried out on two transverse sections of the nasal turbinates (otherwise not specified), longitudinal sections of larynx and trachea, and all lung lobes cut along the bronchus prior to embedding. In the formaldehyde-only (10 ppm) experiment, mortality was increased relative to unexposed controls ($p < 0.05$). No tumors and little evidence of toxicity to the nasal epithelium were observed. There was no increase in rhinitis. Epithelial hyperplasia and metaplasia were increased in formaldehyde-treated animals (5% incidence) versus none observed in controls.

The second set of experiments by Dalbey (1982) examined interaction of formaldehyde exposure on tumor formation from DEN administered subcutaneously. The five treatment groups included: (1) controls (n = 50); (2) formaldehyde only (n = 50); (3) DEN 0.5 mg, once per week for 10 weeks (n = 100); (4) formaldehyde exposure for life with DEN injection for the first 10 weeks given 48 hours after formaldehyde exposure (n = 27); and (5) DEN injection for 10 weeks, followed by formaldehyde exposure for life (n = 23). In all groups hamsters were exposed at 30 ppm formaldehyde 5 hours/day, once a week. Histopathologic examinations were conducted as above.

Although weekly exposures to formaldehyde alone (30 ppm once a week) did not influence mortality, treatment with DEN alone significantly ($p < 0.05$) increased mortality above that of untreated controls, and mortality was further elevated ($p < 0.05$) in the two groups exposed to both DEN and formaldehyde compared with DEN alone. No respiratory tract tumors were observed in untreated animals or those receiving only formaldehyde. DEN treatment alone resulted in a high incidence (77%) of tumors (nasal, larynx, trachea, and lung). Formaldehyde pre- or posttreatment did not further increase the number of TBAs-. All tumors observed were classified as adenomas. Formaldehyde pretreatment nearly doubled the number of tumors per animal in the trachea (but not lung or larynx) ($p < 0.05$). This increase in tumors initiated by DEN given 48 hours after formaldehyde exposure suggests a role of formaldehyde- induced changes in the respiratory tract in tumor promotion (e.g., cell proliferation and inflammation).

4.2.1.2.3.4. ***Summary.*** Chronic rodent studies of inhalation exposure to formaldehyde provide a consistent picture of the agent's toxicity—especially on the URT—on which most studies focus. All three species tested—hamsters, mice, and rats—had some degree of hyperplastic and metaplastic change in the nasal passages. The pathology defined in acute and subchronic

exposures is similarly described in chronic studies, where progression, severity, and presence in more posterior sections of the nose increase with both the concentration and duration of exposure.

Pathology of the respiratory epithelium includes rhinitis, goblet cell hyperplasia, pseudoepithelial cell hyperplasia, squamous metaplasia, and dysplasia (see Table 4-34). At higher exposures and longer durations of exposure, similar effects are seen on the olfactory epithelium, present further into the nasal passages. In addition to hyperplasia and squamous metaplasia, thinning and disarrangement of the olfactory epithelium noted and, in a few cases, cell damage and replacement of olfactory epithelium with respiratory epithelium appear, including loss of sensory cells (Woutersen et al., 1989; Kerns et al., 1983; Battelle Columbus Laboratories, 1981).

Clear species differences in the severity of lesions are present. Although the bioassays in mice, hamsters, and rats do represent similar exposure concentrations and duration of exposure, hamsters exhibit little pathology and rats (three strains tested) exhibit gross toxicity and even increased mortality. Mice similarly exposed exhibit a range of effects on the respiratory epithelium but not near the severity seen in rats. Many factors may contribute to these observed species differences. As Chang and Barrow (1983) reported, the increased RB of mice seems to be protective of POE damage in comparison to that of rats. The reduced ventilation rate and minute volume of rodents in the presence of a reactive gas can reduce the effective delivered dose at the same exposure concentration (Chang and Barrow, 1983). Additionally, as illustrated in the computational fluid dynamic (CFD) modeling (see Section 3.5), there are species differences in nasal architecture that influence areas of formaldehyde absorption or flux into the tissue. Localized differences in mucus flow and production as well as metabolic enzymes have also been posited as having roles in differential toxicity of formaldehyde on the URT (see Chapter 3).

Formaldehyde-induced tumors were present in exposed rats and mice and primarily involved SCCs later in life (Kamata et al., 1997; Tobe et al., 1985; Kerns et al., 1983; Swenberg et al., 1980). Although exposure of male Syrian hamsters to either 10 or 30 ppm did not result in formaldehyde-induced nasal tumors, a classic initiation-promotion assay with DEN-induced tumor formation did indicate that formaldehyde increased the tumor burden per animal, where DEN induced tumors in 77% of the animals (Dalbey, 1982). This study suggests a role for promotion in the observed carcinogenicity of formaldehyde. Less clear are the implications of the synergistic effect of formaldehyde exposures and gross damage to the respiratory epithelium by electrocoagulation on tumor formation (Woutersen et al., 1989).

Table 4-34. Summary of respiratory tract pathology from chronic inhalation exposures to formaldehyde

Species/strain	No./ group	Treatment[a]	Respiratory effects	Noncancer LOAEL/NOAEL	Reference
Chronic bioassays					
Mice					
C3H mice (sex unstated)	60	0, 41, 82, or 163 ppm 1 hour/day, 3 days/week for up to 35 weeks. Low- and mid-group mice then exposed at either 122 or 244 ppm during weeks 35–70.	**Pathology:** Histologic changes in the tracheobronchial epithelium by exposure, including basal-cell hyperplasia, stratification squamous cell metaplasia, and atypical metaplasia. **Carcinogenicity:** No SCC formation was evident in mice exposed to formaldehyde alone.	LOAEL = 41 ppm No evidence of carcinogenicity	Horton et al. (1963)
Male and female B6C3F1 mice	120/sex	0, 2, 5.6, or 14.3 ppm 6 hours/day, 5 days/week for 24 months. The protocol featured a 6-month recovery period. Interim sacrifices occurred at 6, 12, 18, 24, and 30 months.	**Pathology:** Rhinitis; hyperplasia, dysplasia, and squamous metaplasia of the nasal epithelium; atrophy of the olfactory epithelium; glandular adenitis and nasolacrimal duct hyperplasia and metaplasia. **Carcinogenicity:** Nasal SCC in male mice at 24 months (2/17). No SCC in female mice.	LOAEL = 2 ppm Evidence of carcinogenicity	Swenberg et al. (1980); Kerns et al. (1983); CIIT (1982) ; Battelle Columbus Laboratories (1981)
Rats					
Female Sprague-Dawley rats	16	0 or 12.4 ppm formaldehyde ± wood dust 6 hours/day, 5 days/week for 104 weeks.	**Pathology:** Squamous metaplasia and dysplasia. **Carcinogenicity:** One of 16 rats exposed to formaldehyde alone developed SCCs.	LOAEL = 12.4 ppm Support for carcinogenicity	Holmström et al. (1989a)
Male and female F344 rats	32/sex	0, 0.3, 2, or 14 ppm 6 hours/day, 5 days/week for 28 months.	**Pathology:** Increased rhinitis, hyperplasia, and squamous metaplasia of the nasal respiratory epithelium **Carcinogenicity:** Nasal SCCs in high concentration rats (44%).	LOAEL = 0.3 ppm Support for carcinogenicity	Tobe et al. (1985)
Male F344 rats	32	0, 0.3, 2, or 15 ppm 6 hours/day, 5 days/week for 28 months.	**Pathology:** Squamous cell metaplasia and epithelial hyperplasia. **Carcinogenicity:** SCC (13/32), squamous cell papilloma (3/32), and sarcoma (1/32).	LOAEL = 0.3 ppm BMD_{10} = 0.24 ppm Evidence of carcinogenicity	Kamata et al. (1997)

Table 4-34. Summary of respiratory tract pathology from chronic inhalation exposures to formaldehyde

Species/strain	No./ group	Treatment[a]	Respiratory effects	Noncancer LOAEL/NOAEL	Reference
Male Sprague-Dawley rats	100	0 or 15 ppm 6 hours/day, 5 days/week for life.	**Pathology:** Squamous metaplasia, epithelial hyperplasia, and polyps/papillomas. **Carcinogenicity:** SCCs formed in the nasomaxillary turbinates and nasal septum (25%).	LOAEL = 15 ppm Evidence of carcinogenicity	Albert et al. (1982); Sellakumar et al. (1985)
Male and female F344 rats	120/sex	0, 2, 5.6, or 14.3 ppm 6 hours/day, 5 days/week for 24 months. The protocol featured a 6-month recovery period. Interim sacrifices occurred at 6, 12, 18, 24, and 30 months.	**Pathology:** Lesions of the nasal cavity were the primary effects, including squamous metaplasia and epithelial dysplasia, hyperkeratosis, goblet cell hyperplasia, and rhinitis. Salivary gland: atrophy, squamous metaplasia, and sialadenitis. **Carcinogenicity:** SCCs were evident in the nasal cavity of high concentration rats, plus some polypoid adenomas.	LOAEL = 2 ppm Evidence of carcinogenicity	Swenberg et al. (1980); Kerns et al. (1983); CIIT (1982); Battelle Columbus Laboratories (1981); Morgan et al. (1986b)
Male F344 rats	90 and 150 controls	0, 0.7, 2, 6, 10, or 15 ppm 6 hours/day, 5 days/week for 24 months.	**Pathology:** Olfactory degeneration, squamous metaplasia, epithelial hypertrophy and hyperplasia, and mixed inflammatory cell infiltrate. **Carcinogenicity:** SCCs and polypoid adenomas in the nasal cavity	LOAEL = 2 ppm Evidence of carcinogenicity	Monticello et al. (1996)
Male SPF Wistar rats	10	0, 0.1, 1, or 10 ppm 6 hours/day, 5 days/week for 13 or 52 weeks. An electrocoagulation method was applied to damage the noses of ½ of each study group.	**Pathology:** Formaldehyde-induced focal changes to the respiratory and olfactory epithelium, including rhinitis, hyperplasia, and metaplasia (10 ppm). In rats with damaged noses: squamous metaplasia of the respiratory epithelium increased at all formaldehyde exposures. Pathology of the olfactory epithelium increased at the 10 ppm exposure. **Carcinogenicity:** No tumors noted; 1-year study	LOAEL = 0.1 ppm in rats with damaged nasal passages NOAEL = 1 ppm for rats with intact noses	Appelman et al. (1988)

Table 4-34. Summary of respiratory tract pathology from chronic inhalation exposures to formaldehyde

Species/strain	No./ group	Treatment[a]	Respiratory effects	Noncancer LOAEL/NOAEL	Reference
Male Wistar rats	30 (without nasal damage), 60 (with nasal damage)	0, 0.1, 1, and 10 ppm 6 hours/day, 5 days/week for 28 months or for 3 months with a 25-month observation period. An electrocoagulation method was applied to damage the nasal cavity.	**Pathology:** Intact noses: squamous metaplasia in the high concentration group exposed for 28 months and degeneration of the olfactory epithelium. Changes were more severe in animals with damaged noses . **Carcinogenicity:** SCCs developed in 15/60 rats with damaged noses exposed at 10 ppm. In other groups, the incidence of nasal tumors was low irrespective of the state of nasal damage.	NOAEL = 1 ppm Evidence of carcinogenicity	Woutersen et al. (1989)
			Hamsters		
Male Syrian golden hamsters	88 treated 132 controls.	0 or 10 ppm formaldehyde 5 hours/day, 5 days/week for life.	**Pathology:** Increased mortality. Epithelial hyperplasia and metaplasia increased in formaldehyde-treated animals (5% incidence) **Carcinogenicity:** No tumors reported.	LOAEL = 10 ppm No evidence of carcinogenicity	Dalbey (1982)
Male Syrian golden hamsters	50	0 or 30 ppm 5 hours/day, 1 day/week for life ± injections with 0.5 mg DEN.	**Pathology:** Increased mortality in conjunction with DEN—above DEN-only treated animals. Respiratory pathology not reported. **Carcinogenicity:** Only hamsters receiving DEN developed tumors (77%, adenomas). There was an increase in the number of tumors per TBAs in the trachea of animals exposed to formaldehyde 48 hours prior to DEN (but no increase in TBAs).	LOAEL = 30 ppm. Evidence for formaldehyde as a promoter	Dalbey (1982)

4.2.1.2.4. *Summary of respiratory pathology.* The progressive pathology of the nasal passages from inhalation exposure to formaldehyde is well documented, especially in rodents (rats and mice) (see Tables 4-9, 4-15, 4-28, 4-34). Although there are species differences in tissue dose (Section 3.4) due to variations in nasal architecture and breathing patterns, the nature and progression of the pathology is fairly well conserved across species, including nonhuman primates. The observed formaldehyde-induced pathology includes disruption of the mucociliary apparatus, rhinitis (**s**erous and purolent), hyperplasia (cell proliferation), metaplasia (transition of cell type), dysplasia (disarrangement of cells), nest-like infolds and invaginations of the epithelium, thinning of the epithelial layer and focal to diffuse lesions, atrophy of the olfactory epithelium, thickening and keratinization (usually of squamous metaplasia), tumors (adenoma, sarcoma, carcinoma) (Section 4.2.2).

Progression of lesions can be viewed as progression from the anterior to posterior sections of the nasal cavity or as a progression in severity of lesions at a particular location (e.g., level or region) of the nasal passages. In both cases, progression is evident with increasing exposure concentration and with increasing duration of exposure (Kamata et al., 1997; Monticello et al., 1996; Morgan et al., 1986b; Takahashi et al., 1986; Sellakumar et al., 1985; Kerns et al., 1983; Albert et al., 1982). The data suggest that concentration and duration of exposure do not act in a simply cumulative manner (e.g., $C \times t$). Additionally the influence of concentration, duration, and repeated exposure may be different for various effects. For example, some lesions may be transient (e.g., low-exposure cell proliferation), others may have a threshold and vary little after that (e.g., rhinitis). Additionally, as the nasal epithelium responds with both adaptive and adverse epithelial changes, the absorption of formaldehyde into the tissue at that location may be reduced. As respiratory epithelium transitions to squamous metaplasia, the effective tissue dose of formaldehyde increases posterior to these lesions. As barriers to formaldehyde flux into the tissue develop (e.g., squamous metaplasia, keratinization), formaldehyde penetrates more deeply into the nasal passages (Kimbell et al., 2006). Therefore, although both concentration and duration of exposure do effect the adverse effect, the relationship is difficult to define and in fact may be different for various adverse effects.

Respiratory histopathology has been commonly reported in response to exposure to formaldehyde in rats and mice (Lino dos Santos Franco et al., 2006; Javden and Taher, 2000; Kamata et al., 1996a, b; Cassee and Feron, 1994; Bhalla et al., 1991; Monteiro-Riviere and Popp, 1986; Buckley et al., 1984; Chang et al., 1983), rabbits (Ionescu et al., 1978), hamsters (Schreibner et al., 1979), and rhesus monkeys (Monticello et al., 1989). The histopathologic lesions ranged from inflammation to ulceration, necrosis, and metaplasia that occurred in nasal turbinates, maxilloturbinates, and goblet and microvillus cells (Bhalla et al., 1991). These effects

were observed at a variety of doses (e.g., 10 ppm for 4 hours, 3.13 ppm for 6 hours for 1, 2, or 4 days, 6 or 15 ppm). Wilmer et al. (1989, 1987) assessed whether a dose and time-dependent interaction (C × t) is associated with histopathologic lesions. Results indicated that concentration, rather than duration or cumulative exposure, correlates best with severity of lesions (Wilmer et al., 1989, 1987).

Histopathologic lesions and changes to biochemistry have been reported in the lung as well, though these effects were observed following a high dose of formaldehyde. In addition, changes in clinical chemistry, P450 expression and activity in lung tissue, and gene expression that is phenotypically anchored to the observed respiratory pathology have been reported. Extrapulmonary effects have also been noted, including changes in liver chemistry, relative brain weight, and focal, chronic inflammation in the heart and kidney. Most of these changes occurred at exposures of 20 ppm, and those that occurred at lower formaldehyde exposures (3.7 ppm) could not be strictly correlated with formaldehyde exposure.

Some researchers have reported formaldehyde-induced effects in the pulmonary region in rats, mice, and rabbits. Kamata et al. (1996a) observed reduced lipid content of pulmonary surfactant in rats exposed to 128.4 or 294.5 ppm formaldehyde. Kamata et al. (1996b) reported biochemical changes in lung homogenates and altered lipid content of BAL at 145.6 ppm formaldehyde. Lino dos Santos Franco et al. (2006) observed increased leukocytes (and neutrophils) and degranulated mast cells recovered in BAL fluid (concentration of 1% formalin not provided). In rabbits, Ionescu et al. (1978) observed frank necrosis of lung parenchyma after aerosol inhalation of 3% formalin for 3 hours/day for 50 days (concentration of formaldehyde not provided). These pulmonary effects may be due to frank toxicity resulting from the high dose of formaldehyde used in these studies.

Several recent toxicogenomics studies have assessed gene expression changes in nasal and lung tissue in animals and in humans by using in vivo and in vitro approaches. Hester et al. (2005, 2003) documented changes in gene expression associated with DNA repair and apoptosis in nasal tissue from male rats after a single instillation of formaldehyde. Other gene expression changes were observed in those genes related to xenobiotic metabolism and in cell cycle and repair. These preliminary results provide an initial basis for forming a phenotypically anchored set of gene expression changes associated with exposure to formaldehyde and may assist in determining the underlying MOA, as will be discussed in Section 4.5. Sul et al. (2007) investigated gene expression genes in lung tissue from formaldehyde-exposed rats. Yang et al. (2005) performed a proteomics analysis by using lung tissue extracted from formaldehyde-exposed rats. Two studies used human tracheal cell lines to investigate formaldehyde-induced gene expression changes in vitro (Lee et al., 2008, 2007). However, the relevance of these

findings to actual exposures remains unknown. In total, toxicogenomics studies hold promise, but they must be interpreted with caution until results can be replicated and phenotypically linked to observable changes.

Thus, formaldehyde-induced respiratory pathology has been commonly described in the nasal passages and includes cellular proliferation, mucociliary function, and histopathologic lesions. Pulmonary effects have been documented as well but at high doses. The nasal pathology may occur as a result of both concentration and duration components of exposure.

4.2.1.2.5. ***Cell proliferation.*** Formaldehyde-induced cell proliferation has been demonstrated under range of exposure conditions in vivo and in vitro as well (Chapter 3). Formaldehyde-induced mitogenesis may be a primary effect (as demonstrated in the in vitro work) or secondary to adaptive responses and tissue remodeling (Swenberg et al., 1983). This section provides a comprehensive discussion of formaldehyde effects on cell proliferation in the epithelial tissues in the respiratory tract. The majority of the work discussed investigates cell proliferation with in vivo labeling of proliferating cells, although additional methods, such as flow-cytometry, have been employed in some instances.

Swenberg et al. (1986) conducted a series of experiments in rodents to assess cell proliferation in the nasal mucosa after formaldehyde inhalation. Radiolabeled thymidine [^{3}H]-thymidine was injected intraperitoneally (I.P.) into male F344 rats and B6C3F1 mice after formaldehyde exposure to assess the extent of in vivo incorporation into proliferating cells. Two hours later, animals were sacrificed and the nasal passages were fixed, embedded, and sectioned to examine the nasal mucosa. Slides were exposed for 12 weeks and developed to identify cells that incorporated the radiolabeled thymidine. The percentage of labeled cells, as indicated by the presence of five or more grains over the nucleus, was determined by visual count. A total of 4,000 or 1,500 cells were counted per section for rats and mice, respectively.

The first set of studies reported by Swenberg et al. (1986) compared the dose response of rats and mice. Animals were exposed to 0, 0.5, 2, 6, or 15 ppm (0, 0.61, 2.45, 7.36, or 18.4 mg/m^3) formaldehyde 6 hours/day for 3 days. Tritiated thymidine for cell labeling was injected 2 hours after the end of exposure. No change in the percentage of labeled cells was seen after 0.5 or 2 ppm formaldehyde exposure. However, the nasal passages of rats exposed at 6 and 15 ppm showed 10- to 20-fold increases over controls in LI at level 2. A similar cell proliferation response was seen in mice treated with 15 ppm formaldehyde, although no increase over control was seen in mice exposed to 6 ppm formaldehyde. These findings are consistent with other data that indicate rats are more sensitive to formaldehyde exposure than mice. This may be due to differences in the reflex apneic response between the two species. As discussed in

Section 4.2.1.1, mice maintain decreases in minute volume in response to formaldehyde, which results in a lower overall effective internal dose to the mice.

Comparing cell proliferation rates after 2 versus 18 hours of exposure, Swenberg et al. (1986) found that the longer exposure duration gave twice the cell proliferation rates after repeated exposures. Therefore, these researchers conducted a second dose-response study to examine cell proliferation 18 hours after exposure instead of the shorter exposure duration. The dose-response study varied dose as well as duration of treatment. Rats were exposed 6 hours/day to either 0.5, 2, or 6 ppm (0.61, 2.45, or 7.36 mg/m^3) formaldehyde over periods of 1, 3, or 9 days. Formaldehyde exposure at 0.5, 2, or 6 ppm for 1 day increased cell proliferation in the nasal epithelium. However, these increases were transient, and cell proliferation was not increased after 3 or 9 days of exposure to 0.5 ppm or 2 ppm formaldehyde. Although still elevated after a 3-day exposure to 6 ppm formaldehyde, cell proliferation returned to control values after 9 days of exposure to 6 ppm formaldehyde (Swenberg et al., 1986). Therefore, although concentration is a major determinant of cell proliferation, duration of exposure also influenced formaldehyde-induced cell proliferation in the nasal epithelium.

Swenberg et al. (1986) directly tested the effects of cumulative exposure versus concentration for both mice and rats. Animals were treated with one of three regimens, resulting in the same C × t product: 3 ppm × 12 hours, 6 ppm × 6 hours, or 12 ppm × 3 hours, each exposure resulting in 36 ppm-hours. The animals were exposed once a day for either 3 or 9 days. Tritiated thymidine was injected 18 hours after exposure to label of proliferating cells. Tissue sections from levels 1 and 2 of the nasal passages were examined in each case, and the percentage of cells labeled was reported as the percentage of proliferating cells (Figure 4-10).

Cell proliferation at level 1 in the nasal cavity was much greater than at level 2 for all C × t combinations of formaldehyde exposure in both mice and rats (Figure 4-10). The authors noted that level 1 is more anterior and lacks significant defense from the mucociliary apparatus, which may account for the observed greater sensitivity to formaldehyde. At all C × t exposure products, 3 days of exposure resulted in greater cell proliferation than 9 days of exposure. This was true for both species and for both examined levels of the nasal cavity. The decrease in cell proliferation by day 9 is consistent with data on rats labeled 18 hours postexposure (Swenberg et al., 1986).

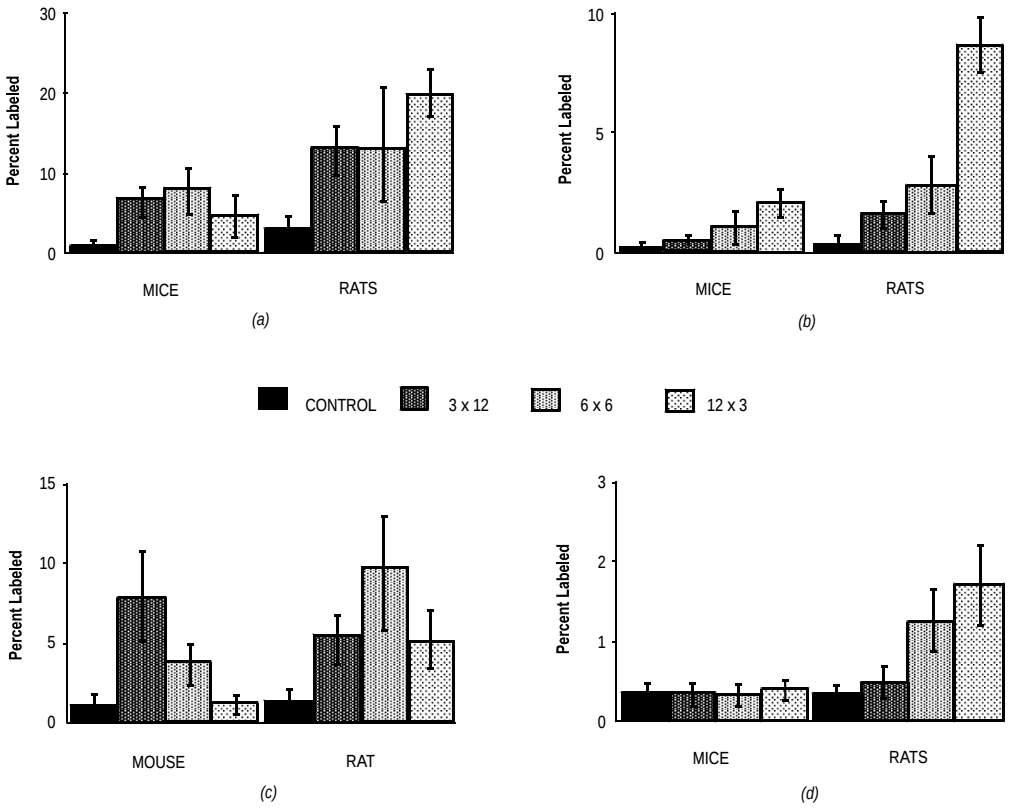

Figure 4-10. Effect of formaldehyde exposure on cell proliferation of the respiratory mucosa of rats and mice.

Note: *a* and *b* are data following 3 days of exposure; *c* and *d* are for 9 days of exposure. *a* and *c* are from level 1 (most anterior); *b* and *d* are from level 2. [^{3}H]-thymidine was administered 18 hours after the last exposure.

Source: Swenberg et al. (1986).

When comparing C × t exposures for a single species and location, the findings are more complex. Cell proliferation in level 2 of the nasal passages appeared to be more dependent on concentration than on duration or cumulative exposure, with the strongest response seen for 12 ppm formaldehyde in combination with the shortest exposure period, 3 hours (Figure 4-10). This pattern was observed in both rats and mice after 3 days of exposure and in rats after 9 days of exposure. No increases in cell proliferation at level 2 were seen for any C × t combination in mice after 9 days. In contrast, increases in cell proliferation at level 1 of the nasal passages were not strictly concentration dependent. After a 3-day exposure, no clear differences were seen among different C × t treatments for either mice or rats, suggesting cumulative exposure may be

the important metric. Therefore, it may be concluded that cell proliferation for level 1 of the nasal passages, where there is less protection of the epithelium, is influenced by concentration, time, and duration of exposure. Cell proliferation at level 2 appeared to be more dependent on concentration than time of exposure (Swenberg et al., 1986).

Cassee and Feron (1994) reported a qualitative increase in histochemical staining for proliferating cell nuclear antigen (PCNA) in the respiratory epithelium of the nasoturbinates, maxilloturbinates, septum, and lateral wall at levels 2 and 3 of rat nasal passages after repeated exposures to 3.5 ppm (4.29 mg/m^3) formaldehyde 22 hours/day for 3 days. While no increases were seen in olfactory epithelium, frank necrosis, squamous metaplasia, and hyperplasia of both ciliated and nonciliated epithelium were noted at these section levels.

Quantitative cell proliferation studies have been conducted by several researchers in the same laboratory (Reuzel et al., 1990; Wilmer et al., 1989; Zwart et al., 1988; Wilmer et al., 1987; Woutersen et al., 1987) (Summary Table 4-39). These studies build off of those of Swenberg et al. (1986), who labeled proliferating cells with [^{3}H]-thymidine in assessing cell proliferation within the nasal mucosa. The studies, all performed in male albino Wistar rats and using a similar experimental design, provide the basis for comparing different exposure levels and dose regimens across studies. Wilmer et al. (1987) demonstrate a concentration-dependent increase in cell proliferation after 3 days of repeated 8-hour exposures at 5, 10, or 20 ppm (6.13, 12.3, or 24.6 mg/m^3) formaldehyde, regardless of continuous versus interrupted exposure conditions (2.83, 8.87, and 19.8 versus 0.86% proliferation in controls). Similar trends were seen when the repeated continuous exposures were extended for 4 weeks, but cell proliferation was not maintained at the same levels. As observed by Swenberg et al. (1986), these results suggest that duration of repeated exposures may be an important determinant of cell proliferation rates.

Woutersen et al. (1987) reported that the majority of the dose-dependent increases in cell proliferation seen at section level 3 after 3 days of repeated 6-hour exposures to 10 and 20 ppm (12.3 and 24.6 mg/m^3) formaldehyde occurred in areas of the epithelium showing "clear squamous metaplasia and hyperplasia." Cell proliferation rates in metaplastic epithelium of 29.5 and 33.2% were much higher than the 1.4 to 2.8% proliferation in the visibly unaffected respiratory epithelium from rats exposed at 10 ppm formaldehyde. Although there was a slight trend towards increased cell proliferation in the visibly unaffected epithelium of exposed animals compared with unexposed controls, the majority of increased cell proliferation resulting from exposure to 10 and 20 ppm formaldehyde was attributed to the metaplastic epithelium.

Similarly, dose-dependent increases in cell proliferation seen at level 3 after 3 days of repeated 6-hour exposures at 0.3, 1, and 3 ppm (0.37, 1.23, and 3.68 mg/m^3) formaldehyde ($p < 0.001$) corresponded to focal basal cell hyperplasia and loss of cilia (Woutersen et al., 1987).

No necrosis or focal erosion was noted at these levels of formaldehyde exposure. Cell proliferation was not sustained at this location, and no lesions were noted after 13 weeks of repeated 6-hour exposures. The authors hypothesized that defensive mechanisms, such as the mucociliary apparatus, may have provided greater protection of the mucosa at level 3. Swenberg et al. (1986) drew a similar conclusion when evaluating extended exposures, suggesting that more posterior sections had a greater adaptive ability than those anterior sections with little mucociliary function. Both Woutersen et al. (1987) and Swenberg et al. (1986) reported sustained cell proliferation and development of lesions in the more anterior cross section. Repeated exposures to 3 ppm formaldehyde (6 hours/day) resulted in significant increases in cell proliferation in the epithelial cells at level 2, with accompanying disarrangement, focal hyperplasia, and squamous metaplasia (Woutersen et al., 1987). Although no cell death was observed at level 2 when viewed by light microscopy, "strongly indented and disarranged nuclei" were seen by electron microscopy, which may be consistent with apoptosis (Woutersen et al., 1987). However, later work in the same laboratory indicated no increased cell proliferation at levels 2 or 3 in male Wistar rats exposed to formaldehyde at 1 or 2 ppm (1.23 and 2.45 mg/m^3) (8-hour repeated exposures for 3 days or 13 weeks) and only minimal response in rats exposed at 4 ppm formaldehyde (interrupted 8-hour exposures for 3 days or 13 weeks) (Wilmer et al., 1989).

Reuzel et al. (1990) published the only report in which formaldehyde effects on cell proliferation were studied for longer daily exposure durations: 22 hours/day versus 6–8 hours/day. Male Wistar rats were exposed to formaldehyde, ozone, or the combination of the two 22 hours/day for 3 consecutive days. The concentrations of formaldehyde were 0.3, 1.0, or 3.0 ppm (0.37, 1.23, or 3.68 mg/m^3). Rats were injected with [^{3}H]-thymidine 2 hours rather than 18 hours after the last exposure. Cell proliferation was quantified by enumerating the percentage of labeled cells in fixed and stained tissue sections. Cell proliferation on the nasoturbinates, maxilloturbinates, lateral wall, and septum at levels 2 and 3 were quantified and reported separately. Cell proliferation was increased at all locations in level 2 at 3 ppm formaldehyde exposure ($p < 0.05$) but not at 0.3 or 1 ppm exposures (Summary Table 4-39). Whereas proliferation of cells in the nasoturbinate, maxilloturbinate, and septum was nearly undetectable in control animals, 4, 5, and 3% proliferation was reported after repeated 22-hour exposures to 3 ppm formaldehyde. Basal proliferation in the lateral wall was greater than in other areas, approximately 1% increasing to 6% after exposure to 3 ppm formaldehyde. Although basal levels of cell proliferation were slightly higher in all areas of level 3, formaldehyde had no significant effects on cell proliferation in the level 3 areas evaluated. There was a slight trend for increases at 3 ppm, but all proliferation rates were below 1%. Exposure to 3 ppm formaldehyde

also damaged the respiratory epithelium at levels 2 and 3, where cell disarrangement and hyperplastic and metaplastic lesions were reported.

Roemer et al. (1993) investigated the effects of formaldehyde exposure on cell proliferation in the trachea and lung in addition to nasal mucosa. Male Sprague-Dawley rats were exposed head only to 2, 6, or 20 ppm (2.45, 7.36, or 24.5 mg/m^3) formaldehyde 6 hours/day for either 1 or 3 days. Proliferating cells were labeled with 5-bromodeoxyuridine (BrdU), the label injected 16–22 hours after formaldehyde exposure ended. Free lung cells were harvested by tracheal lavage, and the majority of isolated cells were MPs (>97%). Epithelial cells were isolated from the nasal and tracheal mucosa by dissection, physical disaggregation, and enzyme treatment to release epithelial cells. All cells were fixed and stained with fluorescent dyes to detect BrdU and total DNA. Flow cytometry was used to determine the percentage of BrdU-labeled cells as a measure of cell proliferation. Cells undergoing unscheduled DNA synthesis (e.g., DNA repair) were excluded by cell cycle analysis.

The proportion of BrdU-labeled cells from the nose and trachea increased two- to threefold above control values after a single 6-hour exposure to formaldehyde (Table 4-35). The lowest effective dose for increased cell proliferation was 2 ppm for nose and tracheal cell proliferation ($p < 0.05$). However, increased proliferation in the nasal mucosa at the lowest dose was transient, returning to control levels after a 3-day exposure. Cell proliferation remained increased in the nasal mucosa after exposure to 6 or 10 ppm (7.36 or 12.3 mg/m^3) formaldehyde for 3 days. In contrast, proliferation of tracheal cells appeared to be reduced as a result of a 3-day exposure to 2 or 6 ppm formaldehyde. A similar trend was seen in free lung cells, but the differences were not statistically significant.

The flow cytometry employed by Roemer et al. (1993) allowed for subtle changes in proliferation rates to be measured with good discrimination. However, the method of cell isolation did not allow examination of proliferation rates in discrete regions of the mucosa, which may have attenuated the magnitude of the response. Additionally, proliferation rates represent a mix of cell types that were not separated in this analysis, making the findings difficult to interpret. This may be especially noteworthy in the free lung cells that were reportedly primarily MPs.

Table 4-35. Cell proliferation in nasal mucosa, trachea, and free lung cells isolated from male Wistar rats after inhalation exposures to formaldehyde

1 Day[a]	Control	2 ppm	6 ppm	20 ppm
Nose	1.3[b]	2.4[c]	3.7[c]	2.7
Trachea	1.2	3.1[c]	2.1[c]	2.8
Lung[d]	1.8	2.6	3.3	3.1
3 Days	**Control**	**2 ppm**	**6 ppm**	**20 ppm**
Nose	1.3	1.4	2.5[c]	2.3[c]
Trachea	1.2	0.3[c]	0.6[c]	2.5[c]
Lung	1.8	2.2	2.4	5.1

[a]Exposures were 6 hours/day.
[b]Proliferation is measured as the percent of BrdU-labeled cells.
[c]Statistically different from controls ($p < 0.05$).
[d]The majority of free lung cells were MPs (97%).

Source: Roemer et al. (1993).

Monticello et al. (1990) investigated whether changes in cell proliferation rate correlated with areas of cell injury or with areas that developed tumors due to formaldehyde exposures by using a unique metric of cell proliferation. They hypothesized that treatment-related effects on cell populations could influence the apparent cell proliferation measured as LI, even though no proliferative effect had occurred. For example, cell death could give an apparent increased proliferation as a LI (% cells proliferating) by reducing the total number of cells present. This would be especially true for a stratified epithelium, where the number of basal cells in active proliferation may not change but cells above the basal layer might die or slough off, thereby reducing the overall number of population of cells counted. The unit length labeling index (ULLI) metric was developed to normalize proliferation rates against length of basal membrane rather than cell population. However, application of a ULLI to the pseudostratified epithelium of the nasal mucosa introduced additional complexities. First, undamaged mucosa has a single layer of epithelial cells that have the capability for cell proliferation. Second, cells only become layered in response to cell damage as a protective measure. Therefore, the total cells present and the linear cell density should be considered, as well as the number and density of proliferating cells, in developing an understanding of the proliferative response of these tissues to toxic insult.

Monticello et al. (1990) directly compared the apparent effects of formaldehyde exposure on cell proliferation when quantified as an LI or as a ULLI. Male F344 rats were divided into groups (n = 6) and exposed to 0, 2, 6, or 15 ppm (0, 2.45, 7.36, or 18.4 mg/m^3) formaldehyde 6 hours/day, 5 days/week for 12 weeks. Rats were administered [^{3}H]-thymidine continuously for the last 5 days of exposure by surgically implanted osmotic pumps. After sacrifice, nasal

passages were fixed, and sections from standard level 3 were prepared for examination. Cell proliferation was quantified at the midseptum and the lateral meatus at this level. Basement membrane length, total number of cells present, and number of labeled proliferating cells were recorded for each location. Each of these areas also was scored for the presence of nasal lesions.

The formaldehyde-related lesions included epithelial hyperplasia, squamous metaplasia, and acute inflammation. These lesions were most severe in animals exposed to 15 ppm, mild at 6 ppm, but absent at 2 ppm. Cell proliferation, measured either as LI or ULLI, was increased in the level 3 septum and lateral meatus after 13 weeks of exposure to 15 ppm formaldehyde but not to 6 or 2 ppm (Table 4-36). There was a slight increase in both cell number and labeled cells in the lateral meatus of rats exposed to 6 ppm formaldehyde, but both measures of proliferation were unchanged from controls. The increased proliferation in the lateral meatus at 15 ppm was entirely due to an increased number of labeled cells. Total cells were unchanged at 15 ppm; therefore, both LIs demonstrated a similar increase over control. In addition to increased labeled cells in the septum at 15 ppm, total cells were increased from 470 to 640 ($p < 0.05$). Where the total cells and linear cell density were increased, the ULLI was proportionally increased over the LI. These observations are consistent with the development of squamous metaplasia and hyperplasia seen at 15 ppm. However, while both LI and ULLI showed an eightfold increase in cell proliferation in the lateral meatus, they gave different results in the septum where cell number was increased by formaldehyde treatment. LI increased 19-fold and ULLI 25-fold with repeated exposures to 15 ppm formaldehyde. Although these data are based on only 5–6 animals/group, and only in an extended study, the results suggest that the ULLI and LI may not be proportional under all conditions studied. In similar experiments the LI and ULLI provided different indices of proliferation in the olfactory epithelium after methyl bromide exposure (Monticello et al., 1990). Methyl bromide exposure decreased cell number/mm of basement membrane in a time-dependent manner, and the LI and ULLI were not proportional across these changes. At day 3 there was an increase in labeled cells but a decrease in total cells; therefore, the LI was increased greater than 20-fold, where the ULLI was only increased eightfold. The authors endeavored to explain why the ULLI and LI yielded different findings. Where ULLI is a more time-efficient method of assessing cell proliferation, the authors suggested that representative areas should be quantified by LI to better understand the nature of increased ULLI.

Table 4-36. The effect of repeated formaldehyde inhalation exposures for 3 months on cell count, basal membrane length, proliferation cells, and two measures of cell proliferation, LI and ULLI, in male F344 rats

	Formaldehyde exposure level (6 hours/day, 5 days/week for 3 months)			
	0 ppm	**2 ppm**	**6 ppm**	**15 ppm**
Lateral meatus				
Total cells	1,800 ± 100	1,800 ± 150	2,300[a] ± 1700	1,900 ± 160
BM length (mm)[b]	12.7 ± 0.6	11.9 ± 0.5	13.4 ± 0.3	11.6 ± 0.7
Cells/mm BM	150 ± 5	150 ± 10	170 ± 10	150 ± 5
Labeled cells	130 ± 10	130 ± 20	210 ± 30	1,400 ± 130
LI	7.2%[c]	7.2%	9.1%	73.7%
ULLI	10.2 cells/mm[d]	10.9 cells/mm	15.7 cells/mm	120.7 cells/mm
Septum				
Total cells	470 ± 20	460 ± 30	470 ± 20	640[a] ± 20
BM length (mm)	2.9 ± 0.1	2.7 ± 0.1	2.9 ± 0.1	2.9 ± 0.1
Cells/mm BM	160 ± 10	170 ± 10	160 ± 3	220[a] ± 10
Labeled cells	20 ± 1	40 ± 10	10 ± 2	250 ± 50
LI	4.3%	8.7%	2.1%	39%
ULLI	6.9 cells/mm	14.8 cells/mm	3.45 cells/mm	86.2 cells/mm

[a]Different from control, $p < 0.05$.
[b]BM is basal membrane length in mm.
[c]Calculated from group averages: LI = (labeled cells)/total cells
[d]Calculated from group averages: ULLI = (labeled cells)/BM length

Source: Monticello et al. (1990).

Monticello et al. (1990) reported similar results in a contemporary abstract; although treatment groups were slightly different than in the above experiments, the findings were similar. Rats were exposed to 0, 0.7, 2, 6, 10, or 15 ppm (0, 0.86, 2.45, 7.36, 12.3, or 18.4 mg/m^3) formaldehyde 6 hours/day for 4 days, 6 weeks, or 3 months. ULLIs were determined in the septum and lateral meatus (methods not detailed). It is not stated whether [^{3}H]-thymidine labeling was carried out by injection or continuous infusion. Significant increases in cell proliferation were reported after repeated exposures to 6, 10, and 15 ppm for 4 days and 6 weeks. After 3 months of exposure, cell proliferation was still increased in rats exposed to 10 and 15 ppm formaldehyde. The authors noted that, although increased cell proliferation was seen at earlier time points, sustained increased cell proliferation was only seen at 10 and 15 ppm, which they considered the clearly carcinogenic doses.

Monticello et al. (1991) applied the ULLI measurements in evaluating formaldehyde effects on cell proliferation after short-term and subchronic repeated exposures. Six male F344 rats/group were exposed to 0, 0.7, 2, 6, 10, or 15 ppm (0, 0.86, 2.45, 7.36, 12.3, or 18.4 mg/m^3)

formaldehyde 6 hours/day for 1, 4, or 9 days and for 6 weeks, using a 5 days/week regimen. Rats were injected with [^{3}H]-thymidine 18 hours postexposure to label proliferating cells. All animals were sacrificed 2 hours later. Nasal passages were fixed, and sections from levels 2 and 3 were prepared for examination. Cell proliferation was quantified for three locations in level 2 (specifically, the lateral meatus, midseptum, and medial aspect of the maxilloturbinate) and for two regions of level 3 (the lateral wall and midventral septum). Each of these areas also was scored for the presence of nasal lesions.

As discussed above, proliferating cells were visually identified by the number of grains over the nucleus, 10 grains indicating a proliferating cell. Cell proliferation was quantified as the number of proliferating cells per length of basement membrane (cells/mm) and reported as a ULLI. The report does not indicate the length of membrane viewed for each section as an indication of how representative the counts are for each region. Lesions associated with formaldehyde exposure may change the density of cells/mm of basement membrane (Monticello et al., 1990). Areas of disarranged cells, erosion, metaplasia, or layering of epithelial cells may exhibit different cell profiles. These processes would alter cell density, and therefore the ULLI, independent of differential proliferation rates. As such, it is not expected to be proportional to cell proliferation rates across conditions that have the potential to change cell density (Monticello et al., 1990).

No formaldehyde-induced epithelial lesions or increases in the ULLI were seen in rats exposed to 0.7 or 2.0 ppm formaldehyde, regardless of duration (Table 4-37). Formaldehyde-induced lesions were present in all regions of the nasal epithelium after exposures to 10 and 15 ppm formaldehyde, regardless of duration (Monticello et al., 1991). Incidence and severity of the lesions increased with concentration and duration of treatment and were correlated to areas with increased cell proliferation. Rats exposed to 6 ppm formaldehyde developed lesions in the level 2 nasal passages, where the ULLI was clearly elevated, but not in the deeper level 3 passages. For example, no formaldehyde-related lesions were seen at the lateral meatus and septum of level 3 at 1, 4, and 9 days of repeated exposure at 6 ppm, although cell proliferation was increased. This transient increase in ULLI returned to near-control levels after 6 weeks of repeated exposure (Table 4-37). Monticello et al. (1991) suggested that cell proliferation is a more sensitive indicator of cellular response and not necessarily dependent on cellular necrosis.

Table 4-37. Formaldehyde-induced changes in cell proliferation (ULLI) in the nasal passages of male F344 rats exposed 6 hours/day

Location[a]	Exposure concentration					
	0 ppm	0.7 ppm	2 ppm	6 ppm[b]	10 ppm[b]	15 ppm[b]
Level 2: lateral meatus						
1 day	2.16	1.31	2.36	16.9[b]	11.2[b]	12.7[b]
4 days	1.46	1.37	1.72	30.5[b]	20.9[b]	25.8[b]
9 days	1.44	1.20	1.73	23.5[b]	28.6[b]	24.6[b]
6 weeks	0.91	0.88	1.36	14.4[b]	23.9[b]	28.7[b]
Level 2: midseptum						
1 day	1.08	1.01	1.69	3.85	17.9[b]	16.7[b]
4 days	1.03	0.97	0.67	10.0[b]	26.1[b]	29.1[b]
9 days	1.09	0.80	0.97	10.9[b]	19.6[b]	29.1[b]
6 weeks	0.41	0.24	0.68	2.10	21.4[b]	25.9[b]
Level 2: medial maxilloturbinate						
1 day	2.49	1.75	2.81	18.15[b]	5.9	5.3
4 days	1.36	1.54	1.09	25.03[b]	20.3[b]	19.4[b]
9 days	1.38	0.80	1.48	22.54[b]	21.0[b]	28.7[b]
6 weeks	1.02	1.21	1.11	16.32[b]	26.1[b]	25.1[b]
Level 3: lateral meatus						
1 day	1.83	1.72	2.46	7.53[b,,c]	14.5[b]	16.4[b]
4 days	1.10	1.27	1.09	8.77[b,,c]	20.0[b]	30.8[b]
9 days	1.36	1.40	1.74	7.35[b,,c]	30.6[b]	40.4[b]
6 weeks	0.98	0.91	0.86	2.08	24.2[b]	34.8[b]
Level 3: midseptum						
1 day	3.02	1.74	2.39	4.20	24.4[b]	19.3[b]
4 days	2.81	3.09	1.43	9.22[b,,c]	18.7[b]	34.4[b]
9 days	1.68	1.06	1.43	9.50[b,,c]	28.6[b]	32.5[b]
6 weeks	2.18	1.54	2.57	2.58	14.0[b]	27.5[b]

[a]ULLI is expressed as the number of labeled cells/mm of basement membrane.
[b]Indicates significantly different from control, $p < 0.05$.
[c]Indicates a location where epithelial lesions were not seen by light microscopy.

Source: Monticello et al. (1991).

The sustained cell proliferation at the lateral meatus and midseptum in rats exposed to 10 and 15 ppm formaldehyde, locations where SCCs are known to arise, supports a role for compensatory cell proliferation in tumor development. However, Monticello et al. (1991) noted that regional differences in sustained cell proliferation do not always correspond to the occurrence of nasal tumors, primarily SCCs, in formaldehyde-exposed rats. Where sustained cell proliferation has been demonstrated in the medial maxilloturbinate (MMT) at level 2 (Monticello et al., 1991), SCCs have not been found to originate in this area at similar exposures (Monticello et al., 1996; Woutersen et al., 1989). Monticello et al. (1991) suggested that the findings of Bermudez and Allen (1984), indicating that the epithelial cells of the maxilloturbinate

are more resistant to the genotoxic effects of DEN, support the possibility that differences in regional tissue susceptibility may contribute to site specificity of formaldehyde-related SCCs.

Monticello et al. (1996) further explored the correlation between measures of cell proliferation and tumor site by modifying the ULLI to take into consideration the total number of cells in a region that may be subject to increased cell proliferation. The population weighted ULLI (PWULLI) is the product of the expected number of cells on a three-dimensional surface in the nasal mucosa and the ULLI of a cross section of that surface. For this series of experiments, six male F344 rats/group were exposed to 0, 0.7, 2, 6, 10, or 15 ppm (0, 0.86, 2.45, 7.36, 12.3, or 18.4 mg/m^3) formaldehyde for up to 24 months with interim sacrifices at 3, 6, 12, and 18 months. Before each interim sacrifice [^{3}H]-thymidine was continuously injected for the last 5 days of exposure through a surgically implanted pump. Nasal passages were prepared, and six standard sections were taken and developed as above for [^{3}H]-thymidine-labeled cells. Stained tissue sections were viewed in order to map all nasal tumors. A ULLI was determined for each region (details not provided). The total cell population of each nasal region was estimated from control animals sacrificed at 3 months (Table 4-38). Cell profiles were counted across 0.5 mm of basement membrane length at two locations for each region (site not specified). Total cells per region were estimated from these counts and the modeled surface area expected in each region (Fluid Dynamics Analysis Package version 7.0). It is unclear if one or more rats were used to quantify cell population. Cell counts and variability were not reported.

Table 4-38. Cell population and surface area estimates in untreated male F344 rats and regional site location of squamous cell carcinomas in formaldehyde-exposed rats for correlation to cell proliferation rates

Nasal region	Total cells (number)[a]	Area (mm^2)[b]	Cell density (cell/mm^2)	SCC incidence[c]	
				10 ppm	15 ppm
Anterior lateral meatus	976,000	59.5	16,400	12	17
Anterior midseptum	184,000	10.5	17,500	0	1
Anterior dorsal septum	128,000	3.84	33,300	0	3
Anterior medial maxilloturbinate	104,000	7.63	13,600	0	4
Posterior lateral meatus	508,000	38.1	13,300	2	9
Posterior midseptum	190,000	10.8	17,600	0	1
Maxillary sinus	884,000	38	23,300	0	0
Region not specified[c]	--	--	--	6	25

[a]Total cell number determined in unexposed rats as a product of representative cell counts and expected surface area of the region.
[b]Modeled surface area of the defined region by FDIP version 7.0.
[c]The number of animals bearing a tumor located in the region. Animals were exposed 6 hours/day for 24 months prior to sacrifice.
Source: Monticello et al. (1996).

ULLIs were quantified by region of the nasal passages in order to correlate with regional localization of tumors. For example, the anterior midseptum included cells from the midseptum from approximately standard section levels 2 to 3. An anterior to posterior pattern of formaldehyde effects, especially differences in cell proliferation rates, has been well established. As such, cell proliferation rates would be expected to vary across the nasal regions used in this analysis. Areas considered to possibly be preneoplastic were not quantified for this work. Monticello et al. (1996) reported increased ULLIs in the ALM and the MMT of rats exposed to 10 or 15 ppm at all time points (3, 6, 12, and 18 months) but provided no indication of variability or a statistical analysis, making it difficult to determine where true differences may exist. Some caution should be used in interpreting the ULLI counts assigned for each region.

The PWULLI was calculated by multiplying the reported ULLIs by the calculated cell populations by region. SCC incidence by region had a greater correlation to the calculated PWULLI than the ULLI, $R^2 = 0.88$ versus $R^2 = 0.46$, respectively. The authors noted that the relative lack of correlation with the ULLI was influenced by findings at the maxilloturbinate where cell proliferation was high but SCC incidence was low. Other tumor types were not included in the analysis (polypoid adenomas, adenocarcinomas, and rhabdomyosarcomas). Additionally, 54 of the SCC tumors could not be accurately localized and were excluded from the analysis, resulting in exclusion of 30 and 39% of animals with SCCs in the 10 and 15 ppm treatment groups, respectively. The authors cautioned that the absence of these data might have skewed the regional analysis of tumor location. Although the purpose of weighting the ULLIs by total population of cells available in each region is to better represent the chance of a tumor arising in each region, the cancer incidence was represented by the number of animals, not the number of tumors, per region. Based on the exclusion of location data (up to 40% of the animals), lack of variability and significance reported for the ULLI for cell counts, and SCC incidence considered by animal rather than by tumor, the significance of a greater correlation by PWULLI versus ULLI is of questionable value.

Monticello et al. (1989) also assessed formaldehyde-induced cell proliferation and regional site location of lesions in the respiratory tract of rhesus monkeys (see Section 4.2.1.2.2.2 for a full study description). LIs from the histoautoradiograms indicated increased cell proliferation in transitory, respiratory, and olfactory epithelial cells after the 6-week formaldehyde exposure. Similar trends were seen after only 1 week but were statistically significant only in the respiratory epithelium. Although increased proliferation in the trachea and carina was statistically significant after 1 week of exposure, the greater increases seen after 6 weeks of exposure were not statistically significant. A small sample size (n = 3) and high variability may have contributed to the lack of statistical significance. The authors noted that

increased cell proliferation was seen in locations with minimal histologic changes, indicating proliferation may be a more sensitive predictor of adverse health effects of formaldehyde exposure. Table 4-39 provides a summary of formaldehyde-induced cell proliferation data.

4.2.1.3. *Gastrointestinal Tract and Systemic Toxicity*

As with inhalation, the POE is thought to be the principal target tissue in response to oral exposure. A concentration-dependent pattern of toxicity longitudinally down the GI tract has been observed upon oral exposure. Some evidence (Til et al., 1989, 1988) suggests that, with regard to oral exposure, duration in addition to concentration is important in the development of toxicity.

Formalin and paraformaldehyde were used to dose animals in oral toxicity studies. Formalin contains 12–15% methanol as a preservative to inhibit the polymerization of formaldehyde and subsequent precipitation as paraformaldehyde (Kiernan, 2000). The presence of methanol in formalin may confound the results of a formaldehyde study. Methanol has been shown to be a developmental and neurologic toxin (e.g., Degitz et al. [2004a, b]; Rogers et al. [2004, 2002]; Weiss et al. [1996]; Sharpe et al. [1982]). Oral dosing with paraformaldehyde is preferred because it allows for the preparation of methanol-free formaldehyde in the laboratory by dissolving paraformaldehyde in slightly basic water.

4.2.1.3.1. ***Short-term and subchronic studies.*** Til et al. (1988) evaluated the oral toxicity of formaldehyde and acetaldehyde in a subacute study in Wistar (Cpb:WU; Wistar random) rats. Groups of rats (10/sex/dose) were exposed to paraformaldehyde dissolved in drinking water at 0, 5, 25, and 125 mg/kg-day for 4 weeks. The control group was comprised of 20 rats of each sex. To account for potential effects of decreased water consumption in treated animals, an additional control group of 10 male and 10 female rats was given drinking water in an amount equal to the amount of liquid consumed by the group given the highest dose. Examination of the GI tract was performed in all dose groups and included the tongue, esophagus, and stomach. Histopathology for the other tissues was performed on high-dose and control animals.

Table 4-39. Summary of formaldehyde effects on cell proliferation in the upper respiratory tract

Species	N[a]	Treatment[b]	Measure of cell proliferation	Summary of results by location[c]	Reference
Male F344 rats; male B6C3F1 mice	NR[d]	0.5, 2, 6, or 15 ppm 6 hours/day for 3 days	LI: percent labeled cells on tissue sections (^{3}H-thymidine I.P.[d] 2 hours postexposure)	Level 2: Rats exhibited greater increased cell proliferation than mice. No increase seen in rats or mice at 0.5 or 2.0 ppm. No increase seen in mice at 6 ppm, but rats had 20-fold increase in proliferation. 10- to 20-fold increase seen in both rats and mice at 15 ppm.	Swenberg et al. (1986)
Male F344 rats	NR	0.5, 2, or 6 ppm 6 hours/day 1, 3, or 9 days	LI: percent labeled cells on tissue sections (^{3}H-thymidine I.P. 18 hours postexposure)	Level 2: Transient increase in cell proliferation on day 1 at 0.5 and 2.0 ppm. Increase in cell proliferation on days 1, 3, and 9 by 6 ppm.	Swenberg et al. (1986)
Male F344 rats; male B6C3F1 mice	NR	3 ppm for 12 hours, 6 ppm for 6 hours, or 12 ppm for 3 hours 3 or 9 days	LI: percent labeled cells on tissue sections (^{3}H-thymidine I.P. 18 hours postexposure for 2 hours)	Level 1: 3 days: Greater increased proliferation in rats than mice. Increases similar for various concentrations yielding the same C × t product. 9 days: Mice exhibited duration-dependent increases in proliferation, inverse to concentration for constant C × t. Level 2 3 days: Concentration-dependent increase in cell proliferation. 9 days: Concentration-dependent increase in cell proliferation in rats; no increase in mice.	Swenberg et al. (1986)
Male F344 rats	4–5	15 ppm 6 hours/day 1 or 5 days	LI: percent labeled cells on tissue sections (^{3}H-thymidine I.P. 18 hours postexposure for 2 hours)	Level 2: Increase in cell proliferation in respiratory epithelium, nasoturbinates, maxilloturbinates, and lateral wall. 1 day: 5.51[f] versus 0.43% in controls 5 days: 10.1%[f]	Chang et al. (1983)[e]
Male BC3F1 mice	4–5	15 ppm 6 hours/day 1 or 5 days	LI: percent labeled cells on tissue sections (^{3}H-thymidine I.P. 18 hours postexposure for 2 hours)	Level 2: Increase in cell proliferation in respiratory epithelium, nasoturbinates, maxilloturbinates, and lateral wall. 1 day: 2.14[f] versus 0.27% in controls 5 day: 3.42%[f]	Chang et al. (1983)[e]
Male albino Wistar rats	5[d]	3.5 ppm 8 hours, twice a day for 3 days	Qualitative staining for PCNA on tissue sections	Levels 2 and 3: Increase in cell proliferation in respiratory epithelium, nasoturbinates, maxilloturbinates, septum, and lateral wall.	Cassee and Feron (1994)[e]

Table 4-39. Summary of formaldehyde effects on cell proliferation in the upper respiratory tract

Species	N[a]	Treatment[b]	Measure of cell proliferation	Summary of results by location[c]	Reference
Male albino Wistar rats	3	0, 5, or 10 ppm 8 hours/day continuously for 3 days or 4 weeks, or 0, 10, or 20 ppm 8 hours/day intermittent[g] for 3 days or 4 weeks	LI: percent labeled cells on tissue sections (^{3}H-thymidine I.P. 18 hours postexposure for 2 hours)	Section level not stipulated in report. 3 days: 0.86% in controls 2.83%[f] at 5 ppm continuous 8.87%[f] at 10 ppm continuous 9.80%[f] at 10 ppm interrupted 19.8%[f] at 20 ppm interrupted 4 weeks: 0.68% in controls 1.33% at 5 ppm continuous 8.85%[h] at 10 ppm continuous 3.41%[f] at 10 ppm interrupted 13.9%[f] at 20 ppm interrupted	Wilmer et al. (1987)[e]
Male albino Wistar rats	2	0, 1, 10, or 20 ppm 6 hours/day for 3 days	LI: percent labeled cells (18 hour postexposure ex vivo ^{3}H-thymidine labeled excised mucosa)	Level 3 Metaplastic epithelium: increased proliferation 31.4% at 10 ppm, 37.6% at 20 ppm Visibly unaffected respiratory epithelium 1.6% in controls 2.6% at 10 ppm, 2.8% at 20 ppm	Woutersen et al. (1987)[e]
Male albino Wistar rats	5	0, 1, or 2 ppm 8 hours/day continuously for 3 days or 4 weeks, or 0, 2, or 4 ppm 8 hours/day intermittent[g] for 3 days or 4 weeks	LI: percent labeled cells on tissue sections (^{3}H-thymidine I.P. 18 hours postexposure for 2 hours)	Level 2 3 days: No change from controls 0.60% in controls 0.34% at 1 ppm continuous 0.61% at 2 ppm continuous 0.29% at 2 ppm interrupted 0.58% at 4 ppm interrupted 4 weeks: no change from controls 1.03% in controls 0.81% at 1 ppm continuous 0.91% at 2 ppm continuous 1.16% at 2 ppm interrupted 2.86% at 4 ppm interrupted	Wilmer et al. (1989)[e]
Male and female albino Wistar rats	5	0, 0.3, 1, 3 ppm 6 hours/day, 5 days/week for 3 days or 13 weeks.	LI: percent labeled cells on tissue sections (^{3}H-thymidine I.P. 18 hours postexposure for 2 hours)	Level 2: Increased cell proliferation at days 3 and 13 weeks ($p < 0.001$). Level 3: Transient dose-dependent increase at 1 and 3 ppm; only seen at day 3 ($p < 0.001$). Note: Results pooled by sex. Data shown graphically on log-normal scale.	Zwart et al. (1988)[e]
Male Wistar rats	5	0, 0.3, 1, or 3 ppm 22 hours/day for 3 days	LI: percent labeled cells on tissue sections (2 hours postexposure ex vivo ^{3}H-thymidine-labeled excised mucosa)	Level 2: 3 ppm increased cell proliferation in nasoturbinates, maxilloturbinates, septum, and lateral wall ($p < 0.05$). Level 3: No significant increases in cell proliferation.	Reuzel et al. (1990)

Table 4-39. Summary of formaldehyde effects on cell proliferation in the upper respiratory tract

Species	N[a]	Treatment[b]	Measure of cell proliferation	Summary of results by location[c]	Reference
Male Sprague-Dawley rats	3–5	0, 2, 6, or 20 ppm 6 hours/day for 1 or 3 days	LI: percent labeled cells by flow cytometry (5-bromodeoxyuridine I.P. 18 hours postexposure for 2 hours)	Respiratory and olfactory epithelial cells. 1 day: 1.3% in controls 2.4% at 2 ppm[f] 3.7% at 6 ppm[f] 2.7% at 20 ppm[f] Tracheal epithelial cells 1 day: 1.2% in controls 3.1% at 2 ppm[f] 2.1% at 6 ppm 2.8% at 20 ppm[f] Free lung cells (>97% MPs): no significant change. 3 days: 1.4% at 2 ppm 2.5% at 6 ppm[f] 2.3% at 20 ppm[f] 3 days: 0.3% at 2 ppm[f] 0.6% at 6 ppm[f] 2.5% at 20 ppm[f]	Roemer et al. (1993)
Male F344 rats	6	0.7, 2, 6, 10, or 15 ppm 6 hours/day, 5 days/week for 1, 4, or 9 days or 6 weeks	ULLI (unit length LI) (^{3}H-thymidine I.P. 18 hours postexposure for 2 hours)	Level 3 No increases in cell proliferation at 0.7 or 2 ppm. Level 4 ULLI increases in locations without lesions at 6 ppm. Increases in ULLI at all locations at 10 and 15 ppm.	Monticello et al. (1991)
Male rhesus monkeys	3	6 ppm 6 hours/day for 1 or 6 weeks	LI: percent labeled cells on tissue sections (^{3}H-thymidine I.P. 18 hours postexposure for 2 hours)	Nasal passages: Duration-dependent increase in cell proliferation at all levels (B–E) in transitional, respiratory, and olfactory epithelium. Increased cell proliferation in areas with minimal lesions. Larynx: trend for increased proliferation Trachea: increased cell proliferation 1 week : 1.14 versus 0.55% in controls 6 weeks: 3.73% Carina of trachea: increased cell proliferation. 1 week: 1.34 versus 0.43% in controls 6 weeks: 3.60% Respiratory bronchioles: no increase in proliferation.	Monticello et al. (1989)

[a]N = number of animals per treatment group.
[b]Treatment is given as the concentration of formaldehyde, duration of exposure each day, and length of the experiment in days and weeks.
[c]Standard section levels of the nasal passages as shown in Figure 4-3 are given for experiments in rats or mice.
[d]NR = not reported; I.P. = intraperitoneally.
[e]Study is described in full in section 4.2.1.2.2.4. .
[f]Different from control, $p < 0.05$.
[g]Intermittent exposures were 30 minutes per hour for 8 hours.
[h]Data from one animal only.

The rats appeared to be healthy throughout the study, and no effects on growth occurred despite significant decreases in food and water intake that occurred at the high dose (125 mg/kg-day). Yellow discoloration of the fur occurred in the rats on the high dosage from week 3 onward. There were no significant changes in hematology among the exposed groups except for slight (not statistically different) increases in PCVs in the water-restricted group and in high-dose males. The high-dose groups of the formaldehyde exposed and in the water-restricted controls had slightly increased urine density, but again this was not statistically significant. Plasma TP and ALB levels were decreased in the males of the highest dose group. No changes in organ weights occurred except for relative kidney weights that were slightly increased in the females of the high-dose group. Gross pathological findings were restricted to the GI tract and revealed a thickening of the limiting ridge of the forestomach in all animals exposed at the highest dose that was accompanied by a yellowish discoloration of the mucosa. These latter changes were not observed in the acetaldehyde-exposed animals. Treatment-related histopathologic changes were seen in the GI tract only. Slight (8/20) or moderate (12/20) focal hyperkeratosis of the forestomach and slight focal atrophic gastritis occurred in animals of the high-dose groups only (Table 4-40). One female had moderate focal papillomatous hyperplasia. No histopathologic changes were observed in any animals of the lower-dose groups. The study established a LOAEL and NOAEL for epithelial changes in the GI tract of male and female Wistar rats exposed to formaldehyde in drinking water at 125 mg/kg-day and 25 mg/kg-day, respectively.

Johannsen et al. (1986) performed a subchronic study by using rats and dogs exposed to paraformaldehyde dissolved in drinking water. Groups of albino Sprague-Dawley rats (15/sex) were administered the equivalent of 0, 50, 100, or 150 mg/kg-day in their drinking water for 91 consecutive days. Pure-bred beagle dogs (four/sex/group) were fed a diet with added aqueous formaldehyde to approximate 0, 50, 75, or 100 mg/kg-day. Dogs were observed daily and rats at frequent intervals for behavioral reactions. Body weights and food and water intake were recorded on a weekly basis in both species. Hematology (HCT, Hb, total and differential leukocyte counts), clinical chemistry (blood sugar, BUN, ALP, AST and ALT in dogs only), and urine analyses (color, appearance, pH, specific gravity, sugar, protein, and microscopic elements) were evaluated in 10 male and 10 female rats selected from each test group and in all dogs. Organ weights were recorded for the adrenals, gonads, hearts, kidneys, livers, lungs, and thyroids in each species. Histopathology was performed on a set of over 20 or 30 tissues and organs from rats or dogs, respectively, in the high-dose and control groups only.

Table 4-40. Summary of lesions observed in the gastrointestinal tracts of Wistar rats after drinking-water exposure to formaldehyde for 4 weeks

	Formaldehyde (mg/kg-day)			
	0	5	25	125
	Number of male rats examined			
Type of lesion	20	10	10	10
Focal hyperkeratosis of forestomach				
Very slight	3	0	0	0
Slight	1	0	0	4
Moderate	0	0	0	6
Focal gastritis				
Slight	0	0	0	2
Moderate	0	0	0	1
Dilated fundic glands (single or a few)	0	0	0	0
Submucosal mononuclear cell infiltrate	0	0	0	1
	Number of female rats examined			
Type of lesion	20	10	10	10
Focal hyperkeratosis of forestomach				
Very slight	6	0	0	2
Slight	0	0	0	2
Moderate	0	0	0	6
Focal gastritis				
Very slight	0	0	0	1
Slight	0	0	0	1
Moderate	0	0	0	1
Focal papillomatous hyperplasia	0	0	0	1
Polymorphonuclear leukocytic infiltration	0	0	0	1

Source: Til et al. (1988).

No deaths or abnormal reactions were observed in either species. Significant reductions in weight gain were observed in dogs of both sexes at 100 mg/kg-day, in rats of both sexes at 150 mg/kg-day, and in male rats at 100 mg/kg-day of formaldehyde. There was a dose-related decrease in liquid consumption of both sexes in rats given formaldehyde, but there was no overall difference in mean food intake or feed efficiency, so the reductions in body weight gain were considered to be systemic effects. Dogs administered formaldehyde had reduced food consumption and feed efficiency at all doses tested. No significant effects on hematology, clinical chemistry, or urine analyses were observed in either species. No effects in either species were reported on organ weights. The GI mucosa in both species was reported to appear normal with no indication of irritation. This study suggests a NOAEL of 150 mg/kg-day in Sprague-Dawley rats and of 100 mg/kg-day in beagle dogs for formaldehyde in drinking water. Differences in the results for the rats with those reported in other studies (Til et al., 1989, 1988; Tobe et al., 1989) may be due to strain differences or duration of the exposure. The dog may be a more sensitive species than the rat based on these results and on those of 2-week pilot studies.

4.2.1.3.2. ***Chronic bioassays: oral exposure to formaldehyde***. The same laboratory that tested formaldehyde and acetaldehyde in a 4-week study (Til et al., 1988) performed a chronic bioassay with formaldehyde in drinking water. Til et al. (1989) administered paraformaldehyde dissolved in drinking water to Wistar rats (Cpb: WU; Wistar random) (70/sex/dose). Interim sacrifices (10/sex/dose) were performed at 12 and 18 months. Formaldehyde was administered in drinking water to provide target doses of 0, 5, 25, and 125 mg/kg-day. The mean formaldehyde doses administered were 0, 1.2, 15, or 82 mg/kg-day for males and 0, 1.8, 21, or 109 mg/kg-day for females. Concentrations were adjusted weekly for the first 12 weeks based on dose estimates derived from body weight and liquid consumption data. Such adjustments were made every 4 weeks from weeks 12 to 52 and kept constant. Fresh solutions of the test concentrations were prepared weekly and stored at 15ºC.

Endpoints examined included daily observations for condition and behavior, body weight at weekly intervals for the first 12 weeks and then every 4 weeks thereafter, liquid intake weekly, and food intake weekly for the first 12 weeks and then every 2 weeks for the remainder of the study. Samples of blood were taken for hematological and clinical chemistry analyses on weeks 26 and 103. Analysis of blood glucose and urine pH, density, and volume was performed on samples at weeks 27, 52, 78, and 104. Pooled urine samples were also evaluated for glucose, occult blood, ketones, urobilinogen, and bilirubin in samples at weeks 27 and 104. Weights of all major organs were recorded at interim sacrifices and at term. Gross and histopathologic examinations were carried out on all major tissues of the rats in the high-dose and control groups. The livers, lungs, stomach, and noses were examined in all rats. Additionally, the adrenals, kidneys, spleens, testes, thyroids, ovaries, pituitaries, and mammary glands (for females) were examined in all sacrificed animals at weeks 53 and 79 and at term.

The general health and behavior of the rats were not affected in any of the formaldehyde-exposed groups. Slight yellowing of the fur did occur in the animals exposed at the mid and high doses from week 3 onward. The mean body weights were decreased in the males from week 1 and in the females from week 24 onward. At the high dose, liquid consumption was significantly decreased in both sexes, and food intake was significantly decreased in the males. There were no toxicologically significant effects on hematological, urinary, or clinical chemistry parameters. Decreases in absolute heart, liver, and testis (males) weights were attributed to lower body weights. Relative kidney weights were increased in females of the high-dose group, and relative brain weights were increased in both sexes of the high-dose group. Relative testis weight was increased in males. Treatment-related changes in gross pathology were restricted to the forestomach. Histopathologic examinations at the two interim sacrifices and final sacrifice

revealed GI tract changes. Renal changes were observed in the high-dose group at final sacrifice. There was no indication of treatment-related effects in other tissues.

As shown in Table 4-41, significant histopathology in the GI tract was limited to the forestomach and stomach of rats in the high-dose groups. Some progression with duration of exposure may have occurred by week 105 because GI lesions were observed in the lower dose groups at this time point, whereas none were observed in these groups at interim sacrifices. The histopathologic changes included papillary epithelial hyperplasia in the forestomach that was frequently accompanied by hyperkeratosis on the limiting ridge or its vicinity. The mucosa showed an irregular layer of hyperplastic basal cells, but no atypical nuclei or other subcellular structures were observed. Chronic atrophic gastritis occurred to varying degrees in the stomachs of all high-dose rats. In some cases the inflammatory process involved the entire mucosa and was seen to extend to the whole muscularis mucosae and met the criteria for ulceration.

Histologic examination also showed that the incidence and degree of renal papillary necrosis was increased in animals of the high-dose groups at the terminal sacrifice. This change was located at the tip of the papilla and was characterized by patchy necrosis of interstitial cells, capillaries, and loops of Henle. There was no evidence of a dose-related response in chronic nephropathy. The incidence of chronic nephropathy was lower in the males of the high-dose group than in controls. In females, the incidence was slightly higher in the test groups than in controls but only achieved statistical significance at the lowest dose. It is likely that the decrease in liquid intake incurred in the high-dose groups contributed to the increased incidence and degree of renal papillary necrosis observed in the high-dose animals because dehydration has been shown to enhance its production by various analgesics.

The results of this chronic bioassay indicated that formaldehyde is cytotoxic to the epithelial mucosa of the nonglandular (forestomach) and glandular stomach with a LOAEL of 82 and 109 mg/kg-day and a NOAEL of 15 and 21 mg/kg-day in males and females, respectively. The findings provided no evidence of carcinogenicity in either the GI tract or systemic sites for formaldehyde administered in drinking water to Wistar rats at doses as high as 82 mg/kg-day.

Table 4-41. Incidence of lesions observed in the gastrointestinal tracts of Wistar rats after drinking-water exposure to formaldehyde for 2 years

	Incidence of lesions with formaldehyde dose (mg/kg-day)[a]							
	Males				Females			
	0	1.2	15	82	0	1.8	21	109
Week 53								
Number of rats examined[b]	**9**	**10**	**10**	**10**	**10**	**10**	**10**	**9**
Forestomach								
Focal papillary epithelial hyperplasia	0	0	0	7	0	0	0	5
Glandular stomach								
Chronic atrophic gastritis	0	0	0	10[c]	0	0	0	9[c]
Focal ulceration	0	0	0	3	0	0	0	1
Focal mononuclear cell infiltrate	1	0	3	0	2	0	0	0
Atypical glandular hyperplasia	0	0	0	0	0	0	0	1
Week 79								
Number of rats examined	**10**	**10**	**10**	**10**	**10**	**9**	**10**	**10**
Forestomach								
Focal papillary epithelial hyperplasia	2	1	1	8	1	0	1	9
Glandular stomach								
Chronic atrophic gastritis	0	0	0	10[c]	0	0	0	10[c]
Focal ulceration	0	0	0	2	0	0	0	0
Focal squamous metaplasia	0	0	0	1	0	0	0	0
Submucosal inflammatory cell infiltrate	1	2	0	0	0	0	0	0
Focal mononuclear cell infiltrate	0	1	0	0	1	1	0	0
Glandular dilation	2	4	4	1	2	2	4	0
Week 105								
Number of rats examined[b]	**47**	**45**	**44**	**47**	**48**	**49**	**47**	**48**
Forestomach								
Focal papillary epithelial hyperplasia	1	2	1	45[c]	1	0	2	45[c]
Focal hyperkeratosis	2	6	4	24[c]	3	5	3	33[c]
Focal ulceration	1	1	1	8	0	0	2	5
Focal acanthosis	1	0	2	1	0	0	0	1
Focal basic cell hyperplasia	0	1	1	0	1	0	0	0
Diverticulum	0	0	1	0	0	0	0	0
Exophytic papilloma	0	1	0	0	1	0	0	0
Glandular stomach								
Chronic atrophic gastritis	0	0	0	46[c]	0	0	0	48[c]
Focal ulceration	0	0	0	11[c]	0	0	0	10[c]
Glandular hyperplasia	0	1	0	20[c]	0	0	0	13[c]
Mineralization	3	2	1	0	0	0	0	0
Focal inflammatory cell infiltrate	5	3	2	0	2	3	1	0

[a]Incidence in rats that died or were killed when moribund during the experiment or were killed at week 53, 79, or 105.
[b]A few rats were lost because of advanced autolysis.
[c]The values differ significantly (Fisher's exact test) from the control value ($p < 0.001$).

Source: Til et al. (1989).

Tobe et al. (1989) performed a chronic toxicity study of Wistar rats (Slc:Wistar) exposed to paraformaldehyde dissolved in drinking water. Groups of 20 male and 20 female rats were given formaldehyde solution in their drinking water at concentrations of 0, 0.02, 0.10, and 0.50% for 24 months. Interim sacrifices of six randomly chosen rats from each group were performed after 12 and 18 months. Based on the estimated average amount of water intake and body weight, the actual doses of formaldehyde in either sex were reported to be 0, 10, 50, and 300 mg/kg-day. Fresh test solutions were prepared twice each week. The rats were observed daily for the entire study. Body weights and water and diet intake were measured once weekly or biweekly. Hematology (RBC, WBC, and Hb) and serum clinical chemistry (TP, ALB, BUN, uric acid, total cholesterol, inorganic phosphorous, ALP, AST, and ALT) were made at each necropsy. Organ weights were measured for the brain, heart, lung, liver, kidney, spleen, adrenal, testis or ovary, pituitary, and thyroid. These organs and the stomach, small and large intestine, pancreas, uterus, lymph nodes, and all tumors were examined histopathologically.

The general condition of animals in the high-dose group was poor with significantly reduced body weight gain as well as intake of water and diet. An increase in mortality was also observed in this group. Some clinical chemistry parameters were altered in this group. No significant changes in absolute or relative organ weights were observed. Mortality was 100% in the high-dose group by 24 months. At the 12-month sacrifice, hyperplasia of the squamous epithelium with or without hyperkeratosis was observed in the forestomach of all high-dose animals (12/12). Basal cell hyperplasia with growth into the submucosa was also observed in most cases (10/12). Erosions and/or ulcers with submucosal inflammatory cell infiltrates were observed in the glandular stomach of most rats (10/12). Regenerative changes of the glandular epithelium (glandular hyperplasia) were noticed in most cases (10/12) along the limiting ridge of the fundic mucosa. No lesions were observed in the glandular stomach at the 50 mg/kg-day dose, and forestomach hyperplasia was observed in only one of six males and in one of eight females at 18 and 24 months. No lesions in either the forestomach or glandular stomach were observed in rats treated at 10 mg/kg-day.

This study corroborates the Til et al. (1989) study and shows that the main targets for formaldehyde toxicity administered by drinking water to rats are the forestomach and glandular stomach. Although the lesions observed at the 50 mg/kg-day were minimal in this study, Tobe et al. (1989) designated the NOAEL at 10 mg/kg-day, further supporting the NOAEL of 15 mg/kg-day from the Til et al. (1989) study.

Takahashi et al. (1986) studied the effects of formaldehyde in an initiation-promotion model of stomach carcinogenesis in male outbred Wistar rats (Shizuoka Laboratory Center, Shizuoka). Rats (n = 17) were given 100 mg/L of N-methyl-N^1-nitro-N-nitrosoguanidine

(MNNG) in drinking water and a diet supplemented with 10% sodium chloride (NaCl) for the first 8 weeks as an initiation phase. This was followed by 0.5% formalin (which contains 12–15% methanol) in drinking water for 32 weeks as the promotion phase of the protocol. A comparison group (n = 10) was given stock water and diet without any supplementation for the first 8 weeks followed by 0.5% formalin in drinking water for 32 weeks. Animals were observed daily and weighed once every 4 weeks. Small pieces of the stomach and other tissues in the peritoneal cavity were fixed for histopathologic examination.

Body weight gain was reduced by exposure to MNNG with sodium chloride, and formaldehyde exposure during the promotion phase exacerbated this effect. Histopathologic investigations were restricted to the GI tract. Formaldehyde was shown to statistically increase the incidence of lesions in the forestomach and stomach in the animals initiated with MNNG with NaCl as compared with controls receiving no initiation (Table 4-42). Increases in papilloma in the forestomach, adenomatous hyperplasia in the fundus, and adenocarcinoma in the pylorus were observed. Histopathology in the animals receiving formaldehyde alone during weeks 9 through 32 showed an increase in forestomach papillomas but with no lesions in the glandular stomach (Table 4-42). The adenomatous hyperplasia were defined as proliferative, noninvasive mucosal lesions, and the adenocarcinomas were defined as well differentiated and composed of typical glandular structures, demonstrating a tubular pattern and cellular or structural atypism without metastasis. No definition of criteria for papilloma diagnosis was provided. The findings in this study are inconsistent with those of Til et al. (1989), who found no evidence of carcinogenicity in a 2-year bioassay at comparable concentrations (assuming 37% formaldehyde in formalin results in 0.19% formaldehyde in this study). As discussed above, the differences may be due to differences in the strains of rat or in the diagnostic criteria. The lack of more than one test concentration precludes dose-response analysis of this study and provides only a stand-alone LOAEL of 0.2% formaldehyde in drinking water. The lack of consumption data precludes an estimation of dose in mg/kg-day.

Table 4-42. Effect of formaldehyde on gastroduodenal carcinogenesis initiated by MNNG and NaCl in male Wistar rats exposed to formaldehyde (0.5% formalin) in drinking water for 8 weeks

No MNNG initiation prior to 8-week oral exposure to formaldehyde (0.5% formalin in drinking water)							
			Glandular stomach tumors				
			Fundus		Pylorus		Duodenum
	Gastroduodenal carcinoma	Forestomach papillomas	Adenocarcinoma	Adenomatous hyperplasia	Adenocarcinoma	Preneoplastic hyperplasia	Adenocarcinoma
Control	0%	0%	0%	0%	0%	0%	0%
Formaldehyde	0%	80%[a]	0%	0%	0%	0%	0%
MNNG initiation (100 mg/L in drinking water for 8 weeks) prior to 8-week oral exposure to formaldehyde (0.5% formalin in drinking water)							
			Glandular stomach tumors				
			Fundus		Pylorus		Duodenum
	Gastroduodenal carcinoma	Forestomach papillomas	Adenocarcinoma	Adenomatous hyperplasia	Adenocarcinoma	Preneoplastic hyperplasia	Adenocarcinoma
Control	13.3%	0%	0%	0%	3.3%	23.3%	10.0%
Formaldehyde	29.4%	88.2%[a]	0%	88.2%[a]	23.5%[b]	41.2%	5.9%

[a]Significantly different from control animals with MNNG initiation, $p < 0.01$.
[b]Significantly different from control animals with MNNG initiation, $p < 0.05$.

Source: Takahashi et al. (1986).

Soffritti et al. (1989) administered formaldehyde in drinking water to Sprague-Dawley rats at different ages. Formaldehyde was produced via the Formox process, which yields carbon monoxide, dimethyl ether, and small quantities of CO_2 and formic acid. In one experiment denoted as BT 7001, rats (50/sex/dose) that were 7 weeks old were administered 0, 10, 50, 100, 500, 1,000, or 1,500 mg/L for 104 weeks. As is usual for experiments carried out at the Ramazzini Foundation, all animals were maintained until natural death at which point they were necropsied and examined histopathologically. In experiment BT 7005, 25-week-old breeder rats or offspring (beginning postnatal day [PND] 12) were provided drinking water with 0 or 2,500 mg/L formaldehyde for 104 weeks. Fluid and food consumption were measured weekly for the first 13 weeks and then every 2 weeks thereafter. Individual body weights were recorded for the first 13 weeks and then every 2 weeks thereafter. Histopathology was routinely performed on all major tissues, including oral and nasal cavities, the GI tract (esophagus, stomach, and intestines [4 levels]), primary and secondary lymph organs (thymus, spleen, subcutaneous lymph nodes, mesenteric lymph nodes, mediastinal lymph nodes, and femur [bone marrow]), head and face bones, and other organ systems (liver, kidney, bladder, reproductive, and various glands). Noncancer health effects were not reported.

No GI neoplasia were observed in any of the control rats (experiments BT 7001 and BT 7005). Historical controls for the BT experimental colony (n = 5,259) indicate an incidence of approximately 1% for benign neoplasia (papillomas and acanthomas) and an incidence of 0.19% for malignant stomach neoplasia (adenomas, SCCs, and adenocarcinomas, fibrosarcomas, and leiomyosarcomas taken together). Therefore, the size of the control groups (18–50 rats/sex) makes detection of background neoplasia unlikely. Similarly, one or two tumors noted in a treatment group (n = 18–50) would represent an apparent increase in these relatively rare tumors. Although stomach and intestinal tumors were found in rats exposed to formaldehyde in drinking water, the low incidence makes it difficult to discern any dose-response effect for individual neoplasia. The authors report formaldehyde-induced GI tract neoplasia to include benign tumors (papillomas and acanthomas of the forestomach and adenomas) and malignant tumors (adenocarcinomas and leiomyosarcomas). The majority of malignant tumors were present in the duodenum, jejunum, and ileum. Benign and malignant neoplasia were consistently noted in the two highest exposure groups: 1500 mg/mL formaldehyde in experiment BT 7001 and 2,500 mg/mL in experiment BT 7005 (Table 4-43). Comparison of overall GI neoplasia in breeder and offspring rats of experiment BT 7005 suggests that rats exposed beginning on PND 12 had a greater incidence of malignant tumors. However, it should be noted that, although there may be an apparent increase in overall tumors, summing across sites and locations is needed before a response can be seen. Even then, several data points are based on a single

observed tumor. However, nonspecific MOAs, such as mutagenicity and regenerative proliferation, would be expected to act on all cell types at the POE, and summing tumor types may have some utility. These results constitute a weak positive result for cancer due to oral exposure to formaldehyde.

Table 4-43. Summary of benign and malignant gastrointestinal tract neoplasia reported in male and female Sprague-Dawley rats exposed to formaldehyde in drinking water at different ages

Experiment	Dose	Sex	Total benign tumors	Total malignant tumors	All tumors
Historical controls	Not applicable	M	1.08%	0.31%	1.4%
		F	0.97%	0.41%	1.4%
BT 7001 (7 weeks old)	Control	M	–[a]	–	–
		F	–	–	–
	1,000 mg/mL	M	–	2%[b]	2%[b]
		F	2%[b]	–	2%[b]
	1,500 mg/mL	M	4%	6%	10%
		F	6%	–	6%
BT 7005 breeders (25 weeks old)	Control	M	–	–	–
		F	–	–	–
	2,500 mg/mL	M	–	5.6%[b]	5.6%[b]
		F	5.6%[b]	–	5.6%[b]
BT 7005 offspring (PND 12)	Control	M	–	–	–
		F	–	–	–
	2,500 mg/mL	M	5.6%	8.3%	13.9%
		F	–	21.6%	21.6%

[a]Dash indicates no tumors reported. An incidence was not reported.
[b]Percentage is based on the observation of a single neoplasm.

Source: Soffritti et al. (1989).

Oral exposure to formaldehyde resulted in a dose-dependent increase in all hemolymphoreticular neoplasia in both male and female rats in experiment BT 7001 (Table 4-44) (Soffritti et al., 1989). The most frequent neoplasia noted were lymphoblastic leukemias and lymphomas. The authors combined lymphoblastic leukemias and lymphosarcomas for analysis and summing across sites. This analysis is appropriate since there is broad consensus that "neoplasms presenting as solid tumors and those presenting with blood and marrow involvement are biologically the same disease but with different clinical presentations," as stated in the recent WHO reclassification of hematological malignancies (Harris et al., 2000b). Inspection of the data tables suggests that the only treatment-related effects occurred at 1,000

and 1,500 mg/L. The reported incidence of lymphoid neoplasia and of all hemolymphoreticular neoplasia in rats exposed to the vehicle (methyl alcohol, 15 mg/L) was similar to results reported in rats exposed to 50 or 100 mg/L formaldehyde in drinking water. Soffritti et al. (1989) provided no statistical analysis of the data. Although the authors report a similar increase in experiment BT 7005, the apparent 5% increase is representative of a single animal in a treatment group of 18 and may not represent a true increase, the study's usefulness for hemolymphoreticular neoplasia being somewhat limited by study size.

Table 4-44. Incidence of hemolymphoreticular neoplasia reported in male and female Sprague-Dawley rats exposed to formaldehyde in drinking water from 7 weeks old for up to 2 years (experiment BT 7001)

Treatment	Sex	Lymphoid neoplasia (%)	Other leukemias (%)	All leukemias and lymphomas (%)
Control	M	4	–[a]	4
	F	1	2	3
Vehicle control	M	10	–	10
	F	2	4	6
10 mg/mL	M	2	–	2
	F	4	–	4
50 mg/mL	M	10	–	10
	F	8	–	8
100 mg/mL	M	8	2	10
	F	4	4	8
500 mg/mL	M	12	4	16
	F	4	4	8
1,000 mg/mL	M	12	–	12
	F	10	4	14
1,500 mg/mL	M	22	–	22
	F	10	4	14

[a]Dash indicates no neoplasm was reported.

Source: Adapted from Soffritti et al. (1989).

The study of Soffritti et al. (1989) does provide qualitative corroboration of evidence from other studies that observed formaldehyde toxicity in the forestomach and stomach. However, the dosages required to induce such lesions in this study were higher than in other studies. Soffritti et al. (1989) is the only animal study of oral exposure to formaldehyde that reports an increase in lymphoblastic leukemia or lymphosarcoma. These occurred at the two highest doses of 1,000 and 1,500 mg/L used in the study of animals exposed from 7 weeks of age (experiment BT 7001).

4.2.1.3.3. ***Summary of toxicity in the GI tract.*** Short-term and subchronic exposures to formaldehyde via drinking water for 4 weeks yielded slight to moderate histopathologic lesions (focal hyperkeratosis) at 125 mg/kg-day in male and female Wistar rats, as well as slight focal gastritis and submucosal infiltrate in one to two animals of both sexes (Til et al., 1988). No histopathologic lesions were noted in albino Sprague-Dawley rats or beagle dogs that received oral doses of formaldehyde in drinking water for 91 days (Johannsen et al., 1986). In both studies, decreases in weight gain were noted in exposed animals compared with controls.

In a chronic drinking water study, Til et al. (1989) reported that formaldehyde is cytotoxic to the epithelial mucosa of the nonglandular (forestomach) and glandular stomach with a LOAEL of 82 and 109 mg/kg-day and a NOAEL of 15 and 21 mg/kg-day in males and female Wistar rats, respectively. The findings provided no evidence of carcinogenicity in either the GI tract or systemic sites for formaldehyde administered in drinking water to Wistar rats at doses as high as 82 mg/kg-day. The incidence and degree of renal papillary necrosis was increased in animals of the high-dose groups at the terminal sacrifice (Til et al., 1989). Findings by Tobe et al. (1989) corroborate the Til et al. (1989) study and show that the main targets for formaldehyde toxicity administered by drinking water to rats are the forestomach and glandular stomach. Takahashi et al. (1986) studied the effects of formaldehyde in an initiation-promotion model of stomach carcinogenesis in male outbred Wistar rats (Shizuoka Laboratory Center, Shizuoka, Japan). In contrast to Til et al. (1989), Takahashi et al. (1986) found increases in incidence of papilloma in the forestomach, adenomatous hyperplasia in the fundus, and adenocarcinoma in the pylorus in a 2-year bioassay at comparable concentrations (assuming 37% formaldehyde in formalin results in 0.19% formaldehyde in this study). Soffritti et al. (1989) administered formaldehyde in drinking water to Sprague-Dawley rats at different ages. Rats (50/sex/dose, age 7 weeks) were administered 0, 10, 50, 100, 500, 1,000, or 1,500 mg/L formaldehyde in drinking water for 104 weeks. The authors reported formaldehyde-induced GI tract neoplasia that included benign tumors (papillomas and acanthomas of the forestomach and adenomas) and malignant tumors (adenocarcinomas and leiomyosarcomas), albeit at a relatively low incidence after summing across sites and locations. Interestingly, oral exposure to formaldehyde resulted in a dose-dependent increase in all hemolymphoreticular neoplasia in both male and female rats (Soffritti et al., 1989). The most frequent neoplasia noted were lymphoblastic leukemias and lymphomas.

4.2.1.4. ***Immune Function***

Leach et al. (1983) documented potential immunomodulatory effects of formaldehyde inhalation exposure. F344 rats were exposed nose only to formaldehyde 6 hours/day,

5 days/week for up to 30 days. The target concentrations for exposure were 0, 3, 16, 61, and 99 ppm formaldehyde (0, 3.7, 19.7, 75.0, and 122 mg/m^3). Body weight and food consumption were recorded, and blood samples for standard hematology and immune assays were collected (details not given). Immune measures referenced include in vitro lymphocyte transformation, hemagglutination assays, and enumeration of B cells, WBCs, and RBCs. No effects were seen at 3 ppm formaldehyde. However, dose-dependent responses were reported for weight loss, decreased food consumption, increased WBCs, increased segmented neutrophils and nucleated RBCs, and decreased ability to produce antibodies to sheep RBCs. The results of the lymphocyte transformation assay were inconsistent, with a 25–30% reduction in stimulation after exposure to 99 ppm but an initial stimulation seen after 16 and 61 ppm exposures. Further details were not available, making it difficult to determine if these reported immunomodulatory effects may have been, in part or in full, secondary to effects on the URT. Subchronic exposures at 61 and 99 ppm formaldehyde would be expected to result in frank toxic effects in mice (see Section 4.2.1). However, these findings suggest possible immunomodulatory effects due to formaldehyde exposure and require further exploration.

Dean et al. (1984) investigated the effects of formaldehyde exposure on a range of indicators of immune function. Female B6C3F1 mice were exposed to 15 ppm formaldehyde (18.4 mg/m^3) 6 hours/day, 5 days/week for 3 weeks. Three trials were run with a total of 255 formaldehyde-treated mice. Body and organ weights were recorded at sacrifice for control and formaldehyde-exposed mice (10 per group). Measures of host susceptibility, cell-mediated immunity MP function, and antibody reactions were conducted 2 to 6 days after the end of exposure (Table 4-45). Lymphocyte subsets, spleen cellularity, bone marrow cellularity, and progenitor cell subsets were enumerated. Host susceptibility and delayed type hypersensitivity were measured in vivo. Lymphocyte proliferation, natural killer cell activity, phagocytosis, hydrogen peroxide production, and IgM plaque-forming cells (PFCs) were measured ex vivo after in vivo stimulation in some cases (Table 4-45).

Body weight, organ weights and cellularity, progenitor cell populations, blood cell counts, and differentials were unchanged in formaldehyde-treated mice (Dean et al., 1984). Circulating blood monocytes were decreased in treated mice, which may be a reflection of the local inflammatory response expected in the nasal epithelium (Dean et al., 1984). However, there was no corresponding decrease in peritoneal MPs. There was a trend, but no statistical significance, for decreased spleen weight, cellularity, and B cell precursors (87, 83, and 78% of controls, respectively). The mean body weight of formaldehyde-treated mice was 21.1 versus 20.9 g in control mice, and thymus and spleen weights were not normalized by body weight.

Table 4-45. Battery of immune parameters and functional tests assessed in female B6C3F1 mice after a 3 week, 15-ppm formaldehyde exposure (6 hours/day, 5 days/week)

Immune function	Model	Challenge	Metric
Host susceptibility	Tumor resistance	PYB6 sarcoma cells	Subcutaneous injection, followed by skin palpation to track tumor development
	Tumor resistance	16F10 melanoma cells	Lung tumor burden determined by [^{125}I]UdR incorporation
	Bacterial resistance	*Listeria monocytogenes*	Survival after challenge
Cell-mediated immunity	Delayed type hypersensitivity	Keyhole limpet hemocyanin	Radiometric index of delayed hypersensitivity responses
	Lymphocyte proliferation	T-cell mitogen, PHA[a] B-cell mitogen, LPS[b] (ex vivo)	Ex vivo proliferation, 3 days, measured by [^{3}H]-thymidine incorporation
	Lymphocyte subsets	None	Percentage of cells positive for cell surface markers (Thy-1, Mac-1, Lyt-1)
	Natural killer cell activity	Yac-1 target cells (^{51}Cr labeled) (ex vivo)	% cytotoxicity by ^{51}Cr release
MP function (both resident and MVE-1 elicited MP)	Phagocytosis	Sheep RBCs (^{51}Cr labeled) (ex vivo)	^{51}Cr incorporation as a measure of RBCs phagocytized
	Hydrogen peroxide production	Pharmacologic stimulation (ex vivo)	H_2O_2 release in culture
Humoral cell immunity	Antibody PFC responses, IgM PFCs	Sheep RBCs, TVF-LPS, or TNF-Ficoll	Plaques formed
Progenitor cells	Bone marrow cellularity (femur)	None	Cell enumeration by a Coulter counter
	Granulocyte-MP progenitors	None	Cell enumeration by a Coulter counter
	B-cell precursors	None	Clonogenic assay

[a]T-cell mitogen, phytohemagglutinin (PHA-P).
[b]B-cell mitogen, lipopolysaccharide (*Escherichia coli*).

Source: Dean et al. (1984).

All indicators of natural killer cell function, cell-mediated immunity, and humoral immunity in formaldehyde-treated mice were unchanged from controls (Dean et al., 1984). Phagocytic capacity of both resident and elicited peritoneal MPs was unchanged by formaldehyde treatment. However, hydrogen peroxide production in elicited peritoneal MPs was significantly increased in formaldehyde-treated mice, 78 versus 42 nmol/mg protein ($p < 0.05$) (Dean et al., 1984).

As shown in Table 4-46, several indicators of host resistance in the female B6C3F1 mice were increased after formaldehyde exposure (Dean et al., 1984). Tumor mass and pulmonary

foci after B16F10 melanoma cell challenge were significantly reduced in formaldehyde-treated mice, indicating improved tumor immunity ($p < 0.05$). However, following PYB6 sarcoma cell challenge, formaldehyde-treated mice had a 7.1% tumor incidence versus 11.1% in controls, which was not statistically different. Mortality due to *Listeria monocytogenes* (LM) was decreased from 70 to 30% ($p < 0.05$). Because resistance to LM is primarily MP dependent, the authors speculated that this enhanced resistance might be due in part to increased bactericidal activity as was also suggested by increased hydrogen peroxide production ex vivo in elicited peritoneal MPs from female mice (Dean et al., 1984).

Table 4-46. Summary of the effects of formaldehyde inhalation on the mononuclear phagocyte system (MPS) in female B6C3F1 mice after a 3-week, 15 ppm formaldehyde exposure (6 hours/day, 5 days/week)

In vivo indicators of MPS	Metric	Formaldehyde effect
Cellularity	Circulating monocytes	Decreased[a]
	CMF progenitor cells	No change[a]
	Resident peritoneal MP	No change[a,b]
	Elicited peritoneal MP	No change[a,b]
In vivo test of host resistance	LM	Increased resistance[a]
	B16F10 tumor challenge	Increased resistance[a]
	PYB6 tumor challenge	No significant increase[a]
Ex vivo indicators of MPS	**Cell type/activation**	**Formaldehyde effect**
H_2O_2 production	Resident, no PMA[c]	None detected [a,b]
	Resident, with PMA	None detected [a,b]
	Elicited, no PMA[d]	None detected [a,b]
	Elicited, with PMA	Increased [a,b]
Phagocytosis	Resident	No change[a]
	Elicited	No change[a]
Assessment of MP maturation	Resident	Decreased[b]
	Elicited	No change[b]
Leucine aminopeptidase content	Resident	No change[b]
	Elicited	No change[b]
Acid phosphatase content	Resident	No change[b]
Binding of tumor cells	Elicited	No change[b]
	Resident	No change[b]
Lysing of tumor cells	Elicited	Increased at mid-range target-to-effector cell ratio[b]

[a]Dean et al. (1984).
[b]Adams et al. (1987).
[c]Phorbol 12-myristate 13-acetate (PMA).
[d]Peritoneal MPs were elicited with the pyran copolymer Murray Valley encephalitis virus (MVE-2).

Sources: Adams et al. (1987); Dean et al. (1984).

Overall, the observations of increased hydrogen peroxide production and increased host resistance in peritoneal MPs distant from the POE suggest that formaldehyde has an effect on the mononuclear phagocyte system (MPS). The authors postulated that this effect may be indirect, due in part to the tissue inflammatory response in the URT or a direct systemic effect on the MPS by formaldehyde exposure (Dean et al., 1984). Subsequent studies by the same researchers explored the possibility of systemic effects of formaldehyde exposure on MPS function and maturation stage (Adams et al., 1987). Female B6C3F1 mice were exposed to 15 ppm (18.4 mg/m^3) formaldehyde 6 hours/day, 5 days/week for 3 weeks, as before (Adams et al., 1987). Both resident and Murray Valley encephalitis virus (MVE-2)-elicited peritoneal MPs were examined for hydrogen peroxide production, enzymatic activity, phagocytic ability, binding, and lysis of tumor cells (Adams et al., 1987).

Similar to the findings of Dean et al. (1984), formaldehyde treatment increased hydrogen peroxide production almost twofold in MVE-2 elicited peritoneal MPs (Adams et al., 1987). As summarized in Table 4-46, no treatment differences were seen in phagocytic ability in either resident or elicited MPs (Adams et al., 1987). Resident peritoneal MPs from formaldehyde-treated mice were not different in their ability to bind or lyse tumor cells. Although formaldehyde treatment did not increase the ability of elicited MPs to bind tumor cells, lysis of the target cells (P815 tumor cells) was increased from 28 to 37% by formaldehyde treatment but only at the midrange target-to-effector-cell ratio tested in the assay ($p < 0.05$) (Adams et al., 1987). Although this is statistically significant, the authors questioned the biological significance of this result since it was not observed at all three target cell ratios tested. However, an increase in tumor cell lysis in vitro would be consistent with the in vivo increased tumor resistance previously reported (Dean et al., 1984). The in vitro lysis response curve suggests that assay conditions may result in a maximum cytolysis near 40%. If so, any treatment effects on lysis would be difficult to discern at higher effector cell ratios.

Jakab (1992) investigated the effect of formaldehyde exposure on the alveolar MPs and resistance to respiratory infections. The first set of experiments assessed bactericidal activity by directly quantifying the pulmonary bacterial loading after exposure to *Staphylococcus aureus*. White female Swiss mice were exposed to formaldehyde after bacterial infection (regimens A and C), before bacterial infection (regimen B), or before and after infection (regimen D) (Table 4-47).

Table 4-47. Formaldehyde exposure regimens for determining the effects of formaldehyde exposure on pulmonary *S. aureus* infection

	Pre-infection treatment	Postinfection treatment	Results
Regimen A	None	4 hours 0, 1, 5, 10, or 15 ppm[a]	15 ppm, increased bacterial loading
Regimen B	18 hours 0, 0.5, or 1 ppm[b]	None	No effect
Regimen C	None	4 hours 0, 0.5, or 1 ppm	No effect
Regimen D	18 hours 0, 0.5, or 1 ppm	4 hours 0, 0.5, or 1 ppm	1 ppm, increased bacterial loading

[a]0, 1.2, 6.2, 12.3, or 18.5 mg/m^3 formaldehyde.
[b]0, 0.62, or 1.2 mg/m^3 formaldehyde.

Source: Jakab (1992).

For regimen A, mice were exposed to 0, 1, 5, 10, or 15 ppm (0, 1.2, 6.2, 12.3, or 18.5 mg/m^3) formaldehyde. For regimens B–D, mice were exposed to 0, 0.5, or 1 ppm (0, 6.2, or 1.2 mg/m^3) formaldehyde. A 30-minute exposure to an infectious aerosol of *S. aureus* deposited 2×10^5 staphylococci in the lungs. Bacterial loading was determined in homogenized lung tissue by culturing diluted aliquots for an estimate of bacteria present immediately after loading and 4 hours later. Bacterial loading was expressed as a percentage change between control and formaldehyde-exposed animals. Mice exposed to 15 ppm formaldehyde for the 4 hours following bacterial infection (regimen A) had approximately an 8% increase in bacteria, indicating decreased host resistance ($p = 0.006$) (Jakab, 1992) (Table 4-47). Mice receiving lower concentrations of formaldehyde following bacterial infection did not have increased pulmonary bacterial loading. Pre-infection exposure to 0.5 or 1.0 ppm did not change bacterial loading 4 hours after infection (regimen B). However, combining an 18-hour pre-infection formaldehyde exposure with a 4-hour postinfection 1 ppm formaldehyde exposure increased pulmonary bacterial loading by approximately 6.5% ($p < 0.05$). This effect was not seen with only a 0.5 ppm pre- and posttreatment regimen. Increased bacterial loading indicates that formaldehyde exposure (regimens A and D) reduced pulmonary bacterial resistance. This is in apparent contradiction to the findings of increased host resistance by Dean et al. (1984). However, there are important differences between the studies. The studies by Jakab (1992) are acute studies examining effects at the respiratory tract where direct effects are possible. Additionally, in some cases, the exposures were concurrent with bacterial infection, and it is difficult to distinguish the potential for formaldehyde effects directly on the mucociliary apparatus as a barrier to infection.

A second set of experiments in the same report (Jakab, 1992) examined the effects of co-exposure to formaldehyde and carbon black on pulmonary infection with *S. aureus.* The particle size distribution of the carbon black aerosol was less than a 5 μm aerodynamic diameter and, therefore, 98% respirable. Female Swiss mice were exposed nose only to formaldehyde and carbon black. Experiments were run at two target concentrations: (1) 2.5 ppm (3.1 mg/m^3) formaldehyde and 3.5 mg/m^3 carbon black or (2) 5 ppm (6.2 mg/m^3) formaldehyde and 10 mg/m^3 carbon black. Co-exposure was given either for 4 hours after a 30-minute *S. aureus* infection or 4 hours/day for 4 days as a pretreatment prior to *S. aureus* infection. Bacterial loading was determined 0 and 4 hours after the *S. aureus* infection to assess bacterial survival. Formaldehyde-carbon black co-exposure did not alter bacterial survival either as a pretreatment or posttreatment to bacterial exposure. However, this exposure regimen was not run for formaldehyde or carbon black separately, and the 4 hours/day for 4 days pretreatment was not included in the formaldehyde alone experiments (Table 4-47).

Jakab (1992) also assessed the phagocytic activity of alveolar MPs collected by lavage at various time points after formaldehyde, carbon black, or co-exposure. Female Swiss mice were co-exposed to 5 ppm (6.2 mg/m^3) formaldehyde and 10 mg/m^3 carbon black 4 hours/day for 4 days. Mice were sacrificed and alveolar MPs harvested 1, 3, 5, 25, and 40 days after exposure. Mice exposed only to formaldehyde or carbon black were sacrificed 3, 10, 25, and 40 days after exposure. Fc-receptor-mediated phagocytosis was assessed ex vivo by using sensitized sheep RBCs. The phagocytic index (PI) was reported as the total number of RBCs in 100 MPs. Neither formaldehyde nor carbon black exposure alone significantly changed the PI (Jakab, 1992). These findings are consistent with the first co-exposure experiment, since no changes in PI were seen immediately after exposure. However, co-exposure did decrease the PI of alveolar MPs in a time-dependent manner, with maximal decrease to less than 70% of controls by 25 days after exposure (Figure 4-11). Decreases in the PI reflect changes in both the percentage of phagocytic MPs and the number of RBCs phagocytized (Jakab, 1992). The PI recovered to control levels by 40 days postexposure.

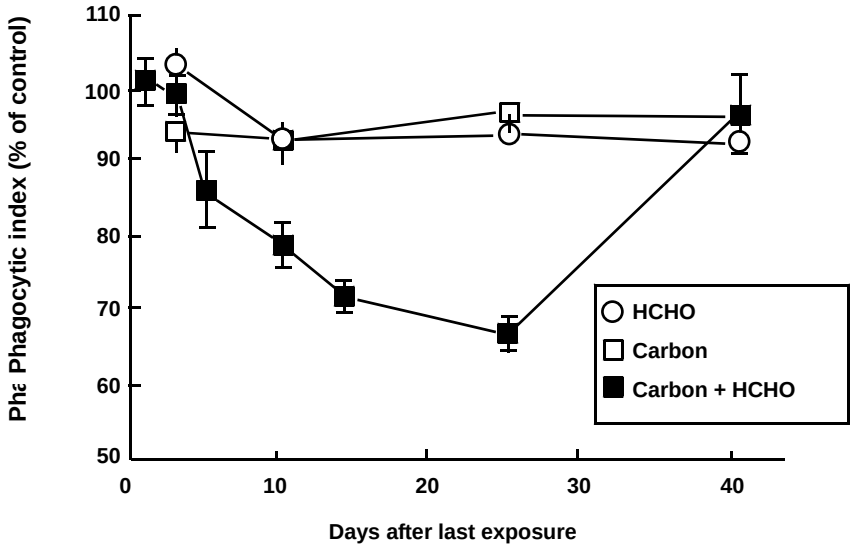

Figure 4-11. Alveolar MP Fc-mediated phagocytosis from mice exposed to 5 ppm formaldehyde, 10 mg/m^3 carbon black, or both.

Note: Exposure was 4 hours/day for 4 days. Each value represents the mean ± SEM of five determinations.

Source: Redrawn from Jakab (1992).

Holmström et al. (1989b) evaluated the effects of long-term formaldehyde exposure on antibody production. Female Sprague-Dawley rats were exposed to 12.6 ppm formaldehyde (15.5 mg/m^3) 6 hours/day, 5 days/week for 22 months. Body weight, tumor incidence, and pathology were reported elsewhere (Holmström et al., 1989b). Rats were given a subcutaneous injection of pneumococcal polysaccharide antigens or tetanus toxoid 21 to 25 days prior to sacrifice. The two vaccines chosen represent T-cell-dependent and T-cell-independent antigens, respectively. Antibody titers (IgG and IgM) were determined prior to vaccination and at sacrifice. Formaldehyde treatment had no effect on antibody titers either before or after vaccination (Holmström et al., 1989b).

4.2.1.4.1. ***Summary of formaldehyde effects on immune function.*** Although there were initial reports of systemic immunomodulation attributed to formaldehyde exposure (Leach et al., 1983), formaldehyde effects on measures of humoral and cell-mediated immunity were not confirmed by Dean et al. (1984). The authors did report increased host resistance to both tumor and bacterial tumor challenges after a 3-week exposure to 15 ppm formaldehyde. An increased

resistance to these challenges, presented distal to the site of formaldehyde exposure (administered subcutaneously or intravenously), suggests a systemic effect of formaldehyde exposure. In addition, increased host resistance and hydrogen peroxide release from peritoneal MPs were reported and confirmed (Adams et al., 1987; Dean et al., 1984). Chronic inflammation and tissue damage to the respiratory mucosa expected with formaldehyde exposure may result in an up regulation of the MPS and therefore increase host immunity. It is unclear if this response would be specific to formaldehyde or similar to enhancement of immune function seen with chronic inflammation.

Jakab (1992) demonstrated decreased pulmonary resistance to bacterial infection where animals were exposed to 15 ppm formaldehyde immediately after bacterial loading or when they were given an 18-hour pre-exposure to formaldehyde followed by 1 ppm formaldehyde exposure after bacterial loading. The authors speculated that formaldehyde may directly act on pulmonary MPs, reducing their effectiveness. However, Jakab (1992) showed that there was no change in Fc-mediated phagocytosis of alveolar MPs immediately after formaldehyde exposures. Degradation of the protective mucus layer and possible epithelial cell damage may contribute to more effective bacterial infection in the presence of formaldehyde without a direct action on MP function. As mentioned above, degradation of the mucus layer may result in a more potent inoculation and therefore higher bacterial loading.

Although neither formaldehyde nor carbon black alone impacted Fc-mediated phagocytosis of alveolar MPs, Jakab (1992) demonstrated that there was decreased Fc-mediated phagocytosis after formaldehyde and carbon black co-exposure. Carbon black may have acted as a carrier for formaldehyde, allowing higher levels of formaldehyde to be delivered more deeply into the lungs than would be seen with formaldehyde alone.

Formaldehyde is known to break down the mucus layer protecting the respiratory tract, allowing exposure of the underlying epithelium (Morgan et al., 1986a, c, d). Additionally, formaldehyde can directly induce tissue inflammation through sensory irritation via substance P from the trigeminal nerve (Fujimaki et al., 2004a). These actions together could contribute to some of the observed effects on immune response attributed to formaldehyde exposures. Degradation of the protective mucus layer would make antigens more available to the immune system. It has been shown that direct application of an antigen to the nasal associated lymph tissue, bypassing the mucus layer, is a more effective delivery of antigen (Hou et al., 2002). Therefore, increased availability of these antigens to the immune system may in part explain observed increased antibody production seen against ovalbumin (OVA) or common dust mite allergen (Der f) during formaldehyde exposure (Sadakane et al., 2002; Riedel et al., 1996; Tarkowski and Gorski, 1995). Neurogenic inflammation may also contribute to more efficient

antigen processing and presentation by activation of resident MPs. These factors are consistent with the observation that formaldehyde exposures do not affect antibody production to antigens administered outside of the respiratory tract, even after chronic exposures (Holmström et al., 1989b).

This effect was initially observed several days after exposure was ended with maximal suppression seen 25 days after a 4-day formaldehyde exposure. The delayed onset of this response, however, suggests an effect beyond the POE effects observed at the time of exposure. Table 4-48 presents a summary overview of the effects of formaldehyde on immune function in laboratory animals.

4.2.1.5. *Hypersensitivity and Atopic Reactions*

Adverse reactions in humans exposed to formaldehyde in the workplace and homes have been reported, which are consistent with an allergic response or a chemical sensitivity (see Section 4.1.1 for details). Rashes and skin reactions are reported in some individuals after dermal exposures, and in some cases exacerbation of asthma is reported after inhalation of formaldehyde. However, the reports of human reactions do not allow a clear determination of whether this sensitization is immunogenic or neurogenic in origin. Formaldehyde-induced sensitization may have both neurogenic and immunologic components. Numerous animal studies have been conducted in order to understand the potential for sensitization to formaldehyde. Although hypersensitivity and allergic sensitization are often considered solely immunologic in origin, neurogenic mechanisms may result in bronchial hypersensitivity and increased immunologic sensitization. Therefore, the animal studies regarding formaldehyde-induced sensitization are evaluated discretely in order to examine these etiologic possibilities.

Classically, hypersensitivity is characterized as an immune response to an antigen, resulting in an inflammatory reaction that itself damages the tissues or is otherwise harmful (Kuby, 1991). These reactions may be localized, as in topical dermatitis, or systematic, as in anaphylactic shock from an allergen. Hypersensitivity can be mediated by a humoral immune response or by a cell-mediated immune response. Four classes of hypersensitivity are generally recognized that differ in their immune system components and functions. Although a single agent (e.g., penicillin) may induce all four types of hypersensitivity, it is more usual for an agent to primarily induce one form of hypersensitivity.

Table 4-48. Summary of immune function changes due to inhaled formaldehyde exposure in experimental animals

Species	No./ group	Treatment[a]	Observations	LOAEL/ NOAEL	Reference
F344 rats	8	0, 3, 16, 61, 99 ppm 6 hours/day, 5 days/week for 4 weeks	No effects at 3 ppm. Mixed results at higher doses that were not consistent.	NA[b]	Leach et al. (1983)
B6C3F1 mice (female)	10	15 ppm 6 hours/day, 5 days/week for 3 weeks	Increased H_2O_2 production, and increased host resistance to tumor formation, but other immune parameters unchanged.	LOAEL 15 ppm	Dean et al. (1984)
B6C3F1 mice (female)	Pooled MPs from a number of mice	15 ppm 6 hours/day, 5 days/week for 3 weeks	Increased H_2O_2 production in MVE-2-elicited peritoneal MPs.	LOAEL 15 ppm	Adams et al. (1987)
White Swiss mice (female)	18	0, 1, 5, 10, or 50 ppm for 18 hours before and/or 4 hours after a 30-minute exposure to bacterial infection (*S. aureus*)	Combining an 18-hour pre-exposure to formaldehyde with 4-hour postexposure to formaldehyde increased bacterial loading at 1 ppm by 6.5%.	LOAEL 1 ppm	Jakab (1992)_
White Swiss mice (female)	18	5 ppm (2.6 mg/m^3) formaldehyde and 10 mg/m^3 carbon black 4 hours/day for 4 days	Phagocytic index was decreased by co-exposure to formaldehyde and carbon black but not by either insult alone.	NA	Jakab (1992)
Sprague-Dawley rats (female)	5	12.6 ppm 6 hours/day, 5 days/week, 22 months	Formaldehyde treatment had no effect on antibody titers either before or after vaccination with pneumococcal polysaccharide antigen or tetanus toxoid.	NA	Holmström et al. (1989b)

NA = not applicable.

Chemical sensitivity generally implies a neurogenically induced sensitization (Meggs, 1995). A chemical may directly interact with sensory nerves, releasing mediators that trigger inflammation, such as substance P (a tachykinin). Repeated exposure to the same chemical is hypothesized to potentiate neurogenic inflammation (Meggs, 1995). The resulting signs of tissue inflammation may be similar to immunogenic inflammation, but there would be no requirement that the immune system recognize the chemical as an antigen for this type of response. Therefore, a chemical may induce one or more clinical signs of atopic asthma without a type 1, IgE-mediated hypersensitivity response. One form of sensitivity, , directly affects sensory nerve endings, resulting in neurogenic inflammation and is a well-known health effect attributed to formaldehyde. Neurogenic responses may result from the direct and acute interaction of the chemical with sensory nerve ending receptors of the trigeminal nerve that may lead to persistent rhinitis and an asthma-like reactive airway dysfunction syndrome that may develop after short-term human exposures (Brooks et al., 1985). Thus, there is evidence to suggest that neurogenic inflammation may contribute to observed increases in formaldehyde-induced airway hyperresponsiveness and atopic responses. The available animal studies that have investigated formaldehyde-induced airway hyperresponsiveness and atopic responses are summarized below.

4.2.1.5.1. ***Inhalation studies in experimental animals.*** This section summarizes animal studies informing the role of formaldehyde-induced chemical sensitization. The symptoms of sensitization (atopy, airway hyperresponsiveness) are frequently associated with immunologic markers (cytokine production, leukocyte infiltration histamine release, and antibody production) but may be mediated by neurogenic sensory irritation, principally by activation of the trigeminal nerve (see Section 4.1.1.1 for a discussion of sensory irritation). The animal studies that illuminate these neurogenic and immunologic responses are discussed outside of the classic neurotoxicology and immunotoxicology study summary sections to allow synthesis of these data.

Sensitization to chemical exposure by inhalation often manifests as an allergic or asthmatic response as characterized by BC or BHR. This sensitization may be a result of immune involvement, as in the case of hypersensitivity, or a neurogenic sensitization, where a chemical may directly stimulate inflammation. Asthma is a specific manifestation of IgE-mediated hypersensitivity, characterized by BHR and airway inflammation, resulting in lower airway obstruction (Fireman, 2003; Kuby, 1991). In asthma, an allergen capable of cross-linking membrane-bound IgE on mast cells initiates immunogenic inflammation resulting in an influx of eosinophils, neutrophils, and lymphocytes. Mediators of BC, including histamine, eicosanoids, and bradykinin (Kuby, 1991), are released during this process. Prior exposure to the allergen can

increase allergen-specific IgE, potentiating the allergic reaction; this is immunogenic sensitization.

Biagini et al. (1989) evaluated the effect of a single pulmonary exposure of formaldehyde on pulmonary mechanics, including BC. The researchers chose cynomolgus monkeys known to be hyperreactive to methacholine (acetyl-β-methacholine chloride), which is a direct-acting stimulant of BC (Cain, 2001). Measures of pulmonary mechanics included pulmonary flow resistance; dynamic compliance; PEFR; FVC; FEV; $FEF_{25–75\%}$, and 50% of VC; and FEFs normalized for VC. Nine cynomolgus monkeys were exposed to increasing levels of methacholine for 1 minute at 10-minute intervals (0, 0.125, 0.5, 2, and 8 mg/mL) as an aerosol (0.065 mL/minute with a mean aerodynamic diameter of 1.0–1.5 μm). Pulmonary mechanics were measured to establish each monkey's response to methacholine. Methacholine challenge, as the positive control, increased pulmonary flow resistance at increasing levels of methacholine (0.125, 0.5, 2, and 8 mg/mL) to 196 ± 16, 285 ± 57, 317 ± 64, and 461 ± 120 % of baseline levels, respectively. After a 2-week recovery period, each methacholine-sensitized monkey was exposed to 2.5 ppm formaldehyde (generated from formalin, 15% methanol) for 10 minutes. Measures of pulmonary function were performed at 2, 5, and 10 minutes after exposure.

Formaldehyde exposure increased pulmonary flow resistance from 11.3 ± 1.4 cm H_2O prior to formaldehyde exposure to 16.1 ± 2.1, 16.9 ± 2.8, and 20.0 ± 3.4 cm H_2O at 2, 5, and 10 minutes after 2.5 ppm formaldehyde exposure (with 142, 150, and 177 % change, respectively). All other measures of formaldehyde-induced pulmonary mechanics were not significantly different from controls. Increased pulmonary flow resistance, a measure of increased BC, was induced by formaldehyde challenge in previously sensitized mice. However, the differences between methacholine challenge and formaldehyde challenge were not statistically significant. Although both formaldehyde challenge and methacholine challenge increased pulmonary flow resistance, there was no correlation between individual methacholine responsiveness and the magnitude of effect after formaldehyde exposure ($p > 0.1$). Therefore, although formaldehyde exposure stimulated BC similarly to a known direct stimulating agent, formaldehyde may not work through the same site of action as methacholine.

Swiecichowski et al. (1993) assessed pulmonary resistance and airway reactivity due to formaldehyde exposure alone and in response to increasing doses of acetylcholine chloride (a direct-acting BC agent) after formaldehyde exposure in vivo. Male Hartley guinea pigs (eight per group) were exposed at 0.86, 3.4, 9.4, or 31.1 ppm (1.1, 4.2, 11.6, or 38.3 mg/m^3) formaldehyde for 2 hours or at 0.11, 0.31, 0.59, or 1.05 ppm (0.14, 0.38, 0.73, or 1.29 mg/m^3) formaldehyde for 8 hours. Total pulmonary resistance increased after 2 hours formaldehyde exposure at 9.4 and 31.1 ppm and reached similar peak resistance at the end of the exposure

period. This effect was rapidly reversible, with values returning to baseline within 30 minutes after exposure. Although 2-hour exposures at 3 and 1 ppm did not increase pulmonary resistance, 8-hour exposures at 0.3 and 1 ppm did increase pulmonary resistance to similar levels as the 2-hour exposure at 30 ppm. The results indicate that both concentration and exposure time impacted the measured increase in pulmonary resistance. However, a simple multiplicative model (e.g., C × t) does not adequately represent the effects observed. It is noted that an 8-hour exposure at 1 ppm (8 ppm-hours), reached approximately the same pulmonary resistance as 2 hours at 9.4 ppm (19 ppm-hours). This may in part be due to a maximum practical increase in pulmonary resistance in the animals. Conversely, there was no effect at 3 ppm for 2 hours (6 ppm-hours), although significant increase in pulmonary resistance was recorded after an 8-hour exposure at 0.3 ppm (2.4 ppm-hours). Formaldehyde does not appear to exert its effects via a classic C × t paradigm. Exposure concentration, however, did seem to impact recovery time.

In addition, specific pulmonary resistance and airway reactivity to increasing doses of intravenous acetylcholine chloride, a direct respiratory stimulant, were measured immediately after formaldehyde exposure for up to 60 minutes. Formaldehyde-induced airway hyperreactivity was defined as a decrease in the level of acetylcholine chloride needed to produce twice the basal specific resistance (effective dose $[ED]_{200}$). The dose of acetylcholine chloride required to double the specific pulmonary resistance (ED_{200}) and airway reactivity was decreased in animals exposed for 2 hours to formaldehyde. When the duration was extended to 8 hours of formaldehyde exposure, the effective dose of formaldehyde required to elicit a doubled pulmonary resistance (ED_{200}) in the presence of acetylcholine chloride was decreased to 1.07 ppm. Lower ED_{200}s were recorded in formaldehyde-treated animals. This indicates that less acetylcholine was needed to produce BC when formaldehyde was present. Thus, formaldehyde can exacerbate BHR. Additionally the formaldehyde-induced effect increased with duration of exposure, indicating that time as well as exposure concentration are factors in the magnitude of the response. Directly induced increases in airway hyperreactivity peaked 1 hour after exposure and persisted 6 hours after exposure.

In a second set of experiments, male Hartley guinea pigs were treated for 8 hours at 3.4 ppm (4.2 mg/m^3) in order to measure airway hyperreactivity ex vivo (Swiecichowski et al., 1993). After formaldehyde exposure, tracheae were excised and mounted in tissue baths, where tracheal contraction was measured in response to direct application of acetylcholine and then carbachol. Tracheae from similarly exposed guinea pigs were fixed and sectioned for histologic examination and were assessed for signs of inflammation. Formaldehyde exposure did not increase ex vivo tracheal constriction and suggests that changes in airway reactivity were

produced due to both local humoral changes and neural reflexes. However, no changes in epithelial cell morphology or influx of inflammatory cells were recorded even up to 4 days after formaldehyde exposure ended. The authors speculated on possible MOAs for BHR, such as the role of an irritant receptor or altered epithelial cell biochemistry. It may be that the window of acute inflammation occurred early in the exposure protocol and was resolved by the time of first measurement, after 8 hours of exposure. The absence of inflammatory markers may argue against a classic type 1 sensitivity.

The binding of an allergen to receptor bound IgE triggers degranulation of mast cells and basophils, releasing mediators of type 1 hypersensitivity, including the histamine responsible for BC. Brown Norway (BN) rats are known for their high capacity for IgE production and airway hyperresponsiveness in response to allergens or other chemicals; they have often been used as a model of allergic respiratory disease. Ohtsuka et al. (1997) compared the effects of formaldehyde exposure on the nasal epithelium of F344 and BN rats. If the formaldehyde-induced inflammatory response in the nasal epithelium is IgE mediated, BN rats would be expected to display more severe effects of formaldehyde exposure than F344 rats. Both strains of age- and sex-matched rats were exposed to formaldehyde aerosol for 3 hours/day, 5 days/week for 2 weeks. The aerosol was generated from a 1% formaldehyde solution by a two-fluid atomizer, and formaldehyde level was maintained at 2 mg (1% sol.)/L (approximately 16 ppm or 20 mg/m^3), by adjusting the flow rate for formaldehyde solution to the atomizer. During the course of exposure, the following clinical signs were monitored: abnormal respiration, stridor wheezing, nasal discharge, and sneezing. Rats were weighed weekly. Two days postexposure, rats were sacrificed and tissues from the head, trachea, and lungs were fixed and sectioned. Transverse sections were taken at the following palatal landmarks from three animals: level 1 (lateral edge of incisor teeth), level 2 (between incisive papilla and the first palatal ridge), and level 3 (on the second upper molar). The nasal septa of the remaining two animals were revealed for examination by electron microscopy.

Formaldehyde-treated F344 rats showed less body weight gain over the 2-week treatment, resulting in lower body weight at week 1 and week 2 than F344 controls ($p < 0.05$ and 0.01). Body weights of formaldehyde-treated BN rats were unchanged from BN controls. The authors observed fewer clinical signs of respiratory irritation in the formaldehyde-exposed BN rats compared with formaldehyde-exposed F344 rats, such as abnormal respiration (three versus five) and nasal discharge (three versus five). Histologic analysis of lung and trachea tissues revealed no distinct signs of inflammation in either strain. Formaldehyde exposure induced cell damage in URT tissues. Epithelial cell damage was milder and impacted a smaller portion of the URT in BN rats compared with F344 rats. Squamous metaplasia were present in the respiratory

epithelium (levels 1 and 2) in both strains in formaldehyde-treated rats. However, a distinct keratinized layer was noted in level 1 epithelium of F344 rats, and the extent of lesions in level 2 respiratory epithelium was much greater than that seen in BN rats. Additionally, the olfactory epithelium (level 2) in formaldehyde-exposed F344 rats exhibited degeneration, necrosis, and desquamation not seen in BN rats. Mild squamous metaplasia was noted in level 3 of the respiratory epithelium in the treated F344 rats but not the treated BN rats. No pulmonary function measurements were taken, and, thus, no direct comparison in BHR or BC between BN and F344 rats in response to formaldehyde can be made. It appears that BN rats are more resistant to formaldehyde-induced cell damage than are F344 rats, despite the fact that BN rats are known to be IgE responders. These results suggest that IgE responsiveness may be protective of formaldehyde-induced cell damage, or IgE may not play a role at all. The authors note that their earlier research indicated the BN rats have well-developed submucosal glands and speculate that greater mucus flow may be partly responsible for the greater resistance of BN rats to the histologic signs of formaldehyde toxicity.

In a subsequent study in the same laboratory, Ohtsuka et al. (2003) compared histology and cytokine profiles in the nasal mucosa of formaldehyde-treated F344 and BN rats. Formaldehyde aerosol was generated as above and rats (nine per group) were exposed 3 hours/day for 5 days to approximately 16 ppm of formaldehyde (20 mg/m^3). Clinical signs were recorded daily, and monitored respiratory parameters included abnormal respiration, stridor wheezing, nasal discharge, and sneezing. Tissue sections of the nose (five rats per group) were prepared for light microscopy as above: transverse sections at levels 1, 2, and 3. Th-1 cytokines (IFN-γ, IL-2) and Th2 cytokines (IL-4 and IL-5) were determined from the whole nasal mucosa in four rats of each treatment group.

As expected, lesions and neutrophilic infiltration were more severe in F344 formaldehyde-exposed rats compared with treated BN rats. In addition, lesions were observed in all three levels of epithelium examined in F344 rats and impacted both respiratory and olfactory epithelium. Mucosal lesions in formaldehyde-treated BN rats impacted the respiratory epithelium of levels 1 and 2 only. Changes in formaldehyde-induced cytokine mRNA expression were modest in both strains. Th-1-related cytokines (IFN-γ, Il-2) in formaldehyde-treated BN rats were significantly decreased compared with control BN rats. A similar, although not statistically significant, decrease in Th-2 cytokines (IL-4, IL-5) was observed in formaldehyde-treated BN rats compared with unexposed BN rats. There were no treatment differences in either Th-1 or Th-2 cytokine expression in formaldehyde-treated F344 rats compared with unexposed F344 rats. The modest changes in cytokine profile reported in formaldehyde-treated BN rats were not consistent with type 1 hypersensitivity since type 1

hypersensitivity reactions generally result in increased Th-2 cytokines. The mRNA expression results were not corroborated with protein levels and may not have been captured at their peak expression levels.

Lee et al. (1984) evaluated the potential for formaldehyde to act as a sensitizing agent through different routes of exposure in guinea pigs. The inhalation studies will be highlighted here. Dermal exposure and associated contact sensitivity results will be discussed in the dermal exposure section (4.2.1.5.2). Three groups of male English smooth-haired guinea pigs (four/group) were exposed via inhalation to either 6 or 10 ppm (7.4 or 12.3 mg/m^3) formaldehyde 6 hours/day for 5 consecutive days. Depending on the group, animals were then subjected to bronchial provocation challenge with 2 or 4 ppm formaldehyde on day 7 or days 7, 22, and 29 after exposure (see Table 4-49 for clarification).

Table 4-49. Study design for guinea pigs exposed to formaldehyde through different routes of exposure: inhalation, dermal, and injection

	Formaldehyde exposure	Bronchial provocation challenge	Skin test	Blood drawn for antibody titer
Group I—Inhalation	6 ppm formaldehyde[a], days 1–5	Day 7 2 ppm formaldehyde[a] for 1 hour	Day 9	Day 14
Group II—Inhalation	10 ppm formaldehyde, days 1–5	Day 7 2 ppm formaldehyde for 1 hour	Day 9	Day 14
Group III—Inhalation	10 ppm formaldehyde, days 1–5	Days 7, 22, and 29 4 ppm formaldehyde for 4 hours	Day 31	Day 14
Group IV—Dermal	100 μL formalin, days 1 and 3	Day 22 2 ppm formaldehyde for 1 hour 4 ppm formaldehyde for 4 hours	Day 7	Day 14
Group V—Injection	37 mg formaldehyde with Freund's adjuvant	Day 19 2 ppm formaldehyde	Day 7	Day 14

Source: Lee et al. (1984).

Dermal and injection groups are shown for comparison. Pulmonary hypersensitivity was assessed by measuring respiratory rate and tidal volume in response to exposure to 2 ppm formaldehyde challenge for 1 hour, 2 days postexposure for all three groups, and additional measurements were taken 22 and 29 days postexposure for group III. Blood was drawn to characterize IgE antibodies to formaldehyde in a passive cutaneous anaphylaxis (PCA) assay. Respiratory rate was measured following initial formaldehyde exposure and again after bronchial challenge with formaldehyde.

Respiratory rate (exhibited as a pause during expiration) was depressed by 45% following exposure to 10 ppm formaldehyde during the first hour of exposure. During the first hour of exposure, decreased respiratory rate was accompanied by a pause during expiration that has been categorized as RB and indicated sensory irritation. The decreased respiratory rate is consistent with URT sensory irritation and induction of the trigeminal (neurogenic) reflex (Lee et al., 1984). After the first hour of exposure, decreased respiratory rate was characterized by a pause between breaths, which is similar to the breathing pattern seen in mice exposed to formaldehyde via tracheal cannula (Alarie, 1981). This suggests a separate effect of formaldehyde on the LRT after deep penetration of formaldehyde and suggests pulmonary irritation (Lee et al., 1984).

However, subsequent bronchial provocation challenge with either 2 or 4 ppm formaldehyde for either 1 or 4 hours failed to elicit immediate or delayed-onset respiratory sensitization (Table 4-50). Respiratory rates were reported as being within ±20% of pre-challenge levels (data not shown) and did not reflect statistical significance (Lee et al., 1984). Moreover, increased respiratory sensitivity was not observed in animals that had received an emulsification of formaldehyde and Freund's complete adjuvant by injection. Only two to four animals given formaldehyde injections in the presence of Freund's complete adjuvant developed a low titer of antibodies to formaldehyde (Lee et al., 1984).

Table 4-50. Sensitization response of guinea pigs exposed to formaldehyde through inhalation, topical application, or footpad injection

Exposure route	Pulmonary sensitization	Dermal sensitization	Antibody production
Inhalation			
6 ppm (Group I)	0/4	0/4	0/4
10 ppm (Group II)	0/4	0/4	0/4
10 ppm (Group III)	0/4	2/4	0/4
Topical	0/8	8/8	0/8
Injection	0/4	4/4	2/4

Source: Lee et al. (1984).

Thus, inhalation exposure to 6 or 10 ppm formaldehyde (8 hours/day for 5 days) followed by bronchial challenge with 2 or 4 ppm formaldehyde failed to result in respiratory sensitivity defined as greater than 20% change in respiratory rate. Second, for animals that received an injection of formaldehyde with Freud's adjuvant, it was not effective in inducing pulmonary sensitivity. While neither inhaled formaldehyde challenge nor injected formaldehyde and Freud's adjuvant emulsion were effective in producing pulmonary sensitivity, this study relied on increased respiratory rate as an indication of hyperresponsiveness and may not be an accurate

measure of hyperresponsiveness. Thus, overall, conclusions are uninformative due to study design flaws.

Riedel et al. (1996) tested the effects of formaldehyde inhalation on the development of sensitization to a known allergen. Female Perlbright-white Dunkin-Hartley guinea pigs (12 per group) were exposed to 0, 0.13, or 0.25 ppm formaldehyde 8 hours/day for 5 consecutive days. On day 5, the animals were sensitized to the common model allergen, OVA, in a 3-minute, head-only exposure to an aerosol of a 5% OVA solution. A booster sensitization with OVA occurred on day 19. A compressor nebulizer with an output rate of 0.75 mL/minute generated the aerosol. Particle size ranged from 0.5 to 5.0 μm. On day 26, bronchial provocation testing was conducted with 1% OVA challenge (aerosol). Blood samples were taken and anti-OVA IgG antibodies were quantified by ELISA. Significant airway obstruction was defined as an increase in compressed air in the lung that cannot be expired. Three guinea pigs were exposed to formaldehyde (0.20 ppm) or clean air for 5 days. Immediately after exposure, lung and tracheal tissues were fixed for histologic and morphometric evaluation. Wall thickness of bronchial and alveolar septa was measured systematically with a microscope-digitizing-table set.

Significant airway obstruction as measured by compressed air was seen in 3 of 12 controls, 8 of 12 0.13 ppm-exposed, and 10 of 12 0.25 ppm-treated animals after OVA challenge. The average airway obstruction was increased after 0.25 ppm (mean = 0.35 mL, $p < 0.01$) but not after 0.13 ppm formaldehyde exposure. However, individual response to OVA sensitization was highly varied, and animals exhibiting a 10-fold increase in obstruction (measured as compressed air) were seen in both treatment groups (0.13 and 0.25 ppm). Even at the lower exposure (0.13 ppm), biologically significant responses were seen in individuals (Figure 4-12).

Specific anti-OVA antibodies (IgG1 class) were not detected in animals prior to sensitization or in control-treated animals after sensitization . Measurable anti-OVA antibodies were elevated in 3 of 12 (at 0.13 ppm) and 6 of 12 (at 0.25 ppm) formaldehyde-treated guinea pigs after sensitization (Figure 4-13). The average anti-OVA titer for the high-dose group was significantly higher than for controls ($p < 0.05$). The individual responses at the 0.13 ppm exposure level indicate that, although the average group OVA titer may not have reached statistical significance, there was a measureable biological response in three individuals. These results indicate that formaldehyde exposure can sensitize previously naïve (non-sensitized) animals to OVA.

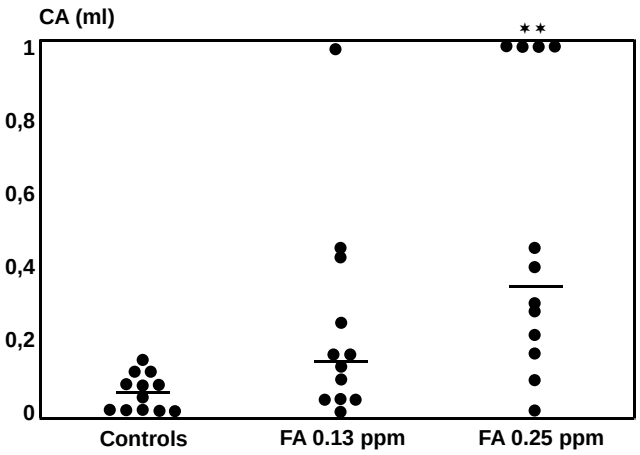

Figure 4-12. Compressed air in milliliters as parameter for airway obstruction following formaldehyde exposure in guinea pigs after OVA sensitization and OVA challenge.

Note: CA = compressed air; FA = formaldehyde; **—** = median; ** = $p < 0.01$.

Source: Redrawn from Riedel et al. (1996).

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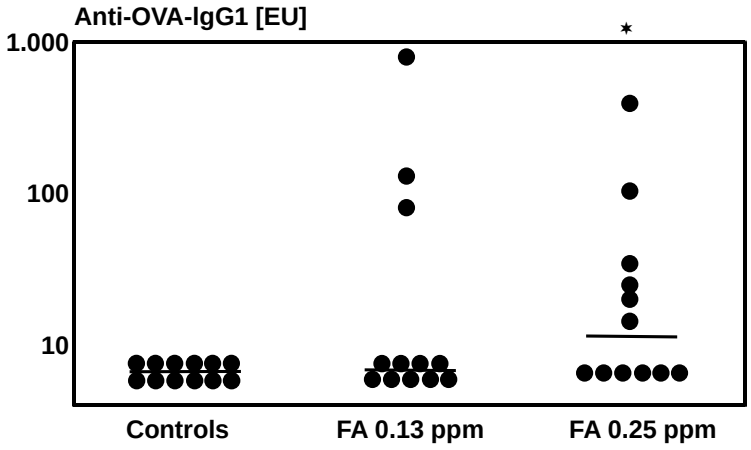


Figure 4-13. OVA-specific IgG1 (1B) in formaldehyde-treated sensitized guinea pigs prior to OVA challenge.

Note: EU = experimental units; FA = formaldehyde; **—** = median; ** = $p < 0.01$.

Source: Redrawn from Riedel et al. (1996).

The only significant, treatment-related histologic change was bronchial edema, with thickening of the bronchial wall in formaldehyde-exposed animals compared with non-treated animals subjected to OVA sensitization and subsequent OVA challenge. Bronchial walls were measured as 40.9 ± 2.5 versus 28.2 ± 1.2 μm. No signs of inflammation in the bronchial mucosa were seen with this edema.

Tarkowski and Gorski (1995) exposed female Balb/C mice to 0 or 6.63 ppm (0 or 2 mg/m^3) formaldehyde for either 6 hours/day for 10 days or 6 hours/day once a week for 7 weeks. All mice were sensitized intranasally to OVA for 10 days or once a week for 7 weeks. IgE anti-OVA titers were determined from sera collected from four mice every 8 days (1 day after OVA booster) by PCA. A parallel experiment to compare the role of the route of administration was conducted with I.P. rather than intranasal sensitization (1 μg OVA once every 7 days).

OVA titers increased similarly in control mice and mice exposed to formaldehyde once a week (Figure 4-14). In contrast, mice exposed to formaldehyde 6 hours/day for 10 consecutive days at the beginning of the experiment had increased anti-OVA beginning after the fourth OVA sensitization, which continued to increase through seven doses of OVA to a peak of 70 PCA units ($p < 0.01$) (Figure 4-14). Anti-OVA IgE titers were significantly different between formaldehyde-treated and nonexposed mice.

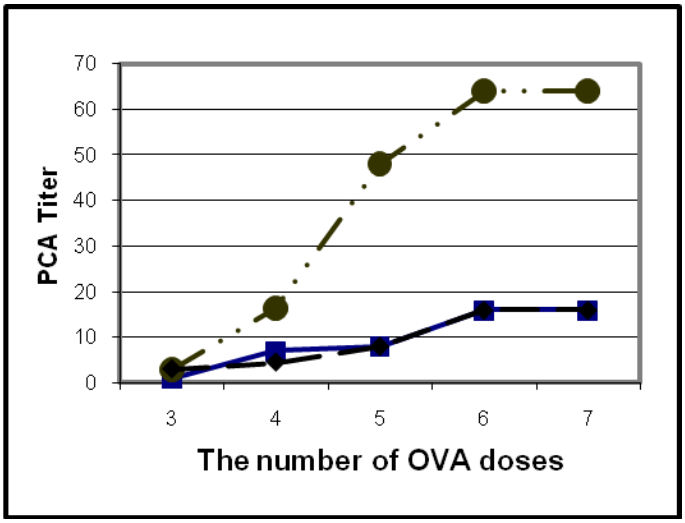


Figure 4-14. Anti-OVA titers in female Balb/C mice exposed to 6.63 ppm formaldehyde for 10 consecutive days or once a week for 7 weeks.

Note: ■ = control mice; ◆ = formaldehyde once a week × 7; ● = formaldehyde 10 days.
Source: Redrawn from Tarkowski and Gorski (1995).

Intraperitoneal sensitization to OVA was much more effective than intranasal sensitization, resulting in titers as high as 1,000 after 4 weeks. However, there were no differences between controls and animals treated with formaldehyde via the I.P. route of exposure. Thus, formaldehyde administered intranasally 6 hours/day for 10 days may facilitate the sensitization to allergens. These changes were not observed when formaldehyde was administered intranasally once a week for 10 weeks or via I.P. injection (Tarkowski and Gorski, 1995). The authors speculate that formaldehyde may increase permeability of respiratory epithelium and destruction of immunologic barriers. Thus the respiratory tract may become vulnerable to inhaled allergens after formaldehyde exposure (Tarkowski and Gorski, 1995).

Ito et al. (1996) conducted three experiments to examine the effects of acute formaldehyde exposure on bronchoconstriction and the mediators of vascular permeability. Male Wistar rats (five to eight per group) were exposed to 0, 2, 5, 15, or 45 ppm (0, 2.5, 6.2, 18.5, or 55.4 mg/m^3) formaldehyde for 10 minutes. Baseline pulmonary insufflation and blood pressure were determined prior to formaldehyde exposure and monitored throughout the experiment. Vascular leakage was measured by injection of Evans blue dye prior to the experiment and determining extravasation 5 minutes postexposure. Briefly, lungs were perfused with 0.9% saline through an aortic cannula. The lower portion of the trachea and main bronchi were removed, and the Evans blue dye remaining was determined and expressed as ng dye/g tissue. A second experiment was conducted to determine if dye leakage continued to increase after exposure. Seven rats were exposed to 15 ppm formaldehyde for 10 minutes, as above. Evans blue dye was injected 5 minutes postexposure, and tissues were perfused and excised 15 minutes later. The final experiment was conducted to determine the effect of certain receptor agonists on the formaldehyde-induced microvascular leakage. Ten groups of Wistar rats (four to seven per group) were exposed to 15 ppm formaldehyde and injected with Evans blue dye, as before. However, each receptor agonist under test or saline sham was injected 4–5 minutes prior to the 10-minute formaldehyde exposure. Agonists tested included tachykinin NK_1 receptor antagonist (CP-99,994) at 1, 3, or 6 mg/kg; a bradykinin B_2 receptor antagonist (HOE 140) at 0.65 mg/kg; and a histamine H_1 receptor antagonist (ketotifen) at 1 mg/kg.

Formaldehyde exposure did not change pulmonary insufflation pressure or blood pressure. Formaldehyde increased vascular permeability in a concentration-dependent manner in both the trachea and main bronchi for the first 5 minutes after exposure, as measured by Evans blue dye extravasation (Ito et al., 1996) (Figure 4-15). Vascular permeability was not increased by formaldehyde exposure from 5 to 15 minutes postexposure (experiment 2). Administration of a selective NK_1 receptor antagonist (CP-99,994) inhibited the formaldehyde-induced vascular

permeability, reducing Evans dye extravasation to control levels at the 3 and 6 mg/kg doses (Figure 4-16).

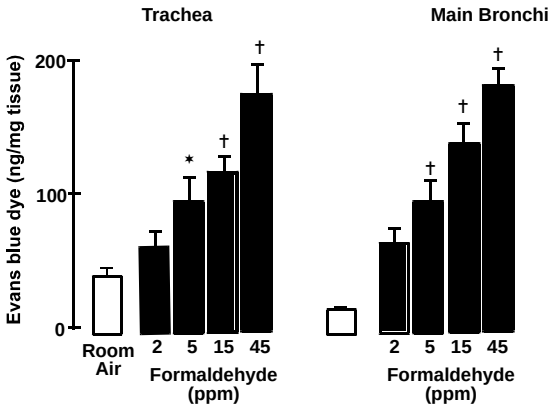


Figure 4-15. Vascular permeability in the trachea and bronchi of male Wistar rats after 10 minutes of formaldehyde inhalation.

Note: Vascular permeability was tested by an increase in Evans blue dye extravasation in the tissue. Solid bars: formaldehyde; open bars: room air, n = 7. Values are the means ± SEM of five to seven animals. $^{*}p < 0.05$ and $^{\dagger}p < 0.01$ versus room-air-exposed group (Williams' test).

Source: Redrawn from Ito et al. (1996).

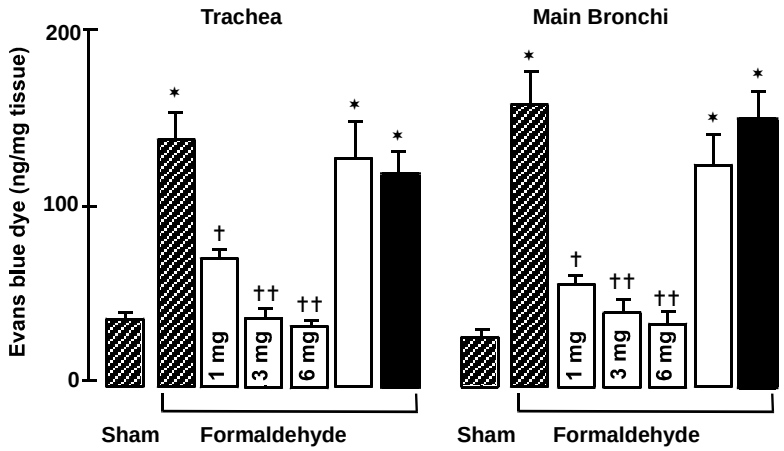


Figure 4-16. Effect of select receptor antagonists on formaldehyde-induced vascular permeability in the trachea and bronchi male of Wistar rats.

Note: Vascular permeability was tested by an increase in Evans blue dye extravasation. Rats were treated i.v. with 1, 3, or 6 mg/kg CP-99,996 (open bars), 0.65 mg/kg HOE 140 (hatched bars), 1 mg/kg ketotifen (solid bars), or vehicle (shaded bars) before formaldehyde challenge. Sham: animals were exposed to the sham gas for 15 ppm formaldehyde (10 minutes) after pretreatment with 0.9% saline (0.5 mL/kg i.v.). Data are the means ± SEM of six to seven rats/group. *$p < 0.05$ versus sham-stimulated group (unpaired Student's *t* test or Welch's test). †$p < 0.05$. ††$p < 0.01$ versus 0.9% saline-pretreated, formaldehyde-exposed control group (Williams' test).

Source: Redrawn from Ito et al. (1996).

Neither the bradykinin B_2 nor histamine H_1 receptor agonists affected formaldehyde-induced vascular permeability (Ito et al., 1996). Therefore, the immediate effect of formaldehyde exposure on vascular permeability is mediated, at least in part, through the NK_1 receptor but does not seem to require the B_2 or H_1 receptors. This implies a role for tachykinins in formaldehyde-induced vascular permeability. These findings suggest a neurogenic inflammatory response because the tachykinins are released from sensory nerve endings in the trachea and bronchi, whereas bradykinin is released from mast cells.

Sadakane et al. (2002) investigated the effects of formaldehyde exposure on airway inflammation caused by Der f. Two groups of male outbred ICR mice (18/group) were exposed to an aerosol of 0.5% formaldehyde solution produced by an ultrasonic nebulizer for 15 minutes, once a week for 4 weeks. Two groups were similarly treated but exposed to saline aerosol only. Details of the aerosol generation and resulting magnitude of exposure were not given. One group each of control and formaldehyde-exposed mice was sensitized to Der f by an injection 1 day prior to formaldehyde exposure (1.5 mg/animal). The same groups were challenged with

intratracheal instillation of Der f (10 μg/animal) after 4 weeks. Three days after allergen challenge, mice were sacrificed and blood plasma and lung tissue were collected. Blood plasma was analyzed for Der f-specific immunoglobulins (IgG1 and IgE). Lungs from nine mice in each treatment group were homogenized, and Th1 cytokine IL-2, Th2 cytokines IL-4 and IL-5, granulocyte macrophage-colony-stimulating factor (GM-CSF), and the "chemokine regulated upon activation, normal T-cell expressed and secreted" (RANTES) protein levels were quantified in the supernatant via ELISA. Lungs from nine mice in each group were fixed, sectioned, and stained to evaluate eosinophil infiltration, lymphocyte infiltration, goblet cell proliferation, and localization of RANTES in the airway epithelium.

Der f-specific IgG1 was present in blood plasma of sensitized mice but was unchanged by formaldehyde exposure (Sadakane et al., 2002). IgE was too low to titer. IL-2 and GM-CSF were undetected in lung homogenate supernatant, and Il-4 was unchanged by sensitization or formaldehyde exposure. However, RANTES was increased by both formaldehyde exposure and allergen sensitization and challenge (Table 4-51). These increases were more pronounced but less than additive for formaldehyde-exposed, allergen-sensitized mice. IL-5 was increased by allergen but unaffected by formaldehyde exposure only. However, formaldehyde exposure potentiated the IL-5 increase seen with allergen challenge.

Table 4-51. Cytokine and chemokine levels in lung tissue homogenate supernatants in formaldehyde-exposed male ICR mice with and without Der f sensitization

Group	Formaldehyde	Der f	GM-CSF	IL-2	IL-4	IL-5	RANTES
1	-	-	ND[a]	ND	68.1 ± .9	4.4 ± 0.3	200.1 ± 19.7
2	+	-	ND	ND	59.5 ± 4.3	4.1 ± 0.2	390.6 ± 37.4[b]
3	-	+	ND	ND	70.7 ± 4.9	13.6 ± 1.6 [c,e]	479.6 ± 80.0[c]
4	+	+	ND	ND	62.3 ± 5.8	21.6 ± 2.7 [c,e,f]	593.3 ± 58.2[c,d]

[a]None detected.
[b]$p < 0.05$ from control.
[c]$p < 0.001$ from control.
[d]$p < 0.05$ from Group 2.
[e]$p < 0.001$ from Group 2.
[f]$p < 0.001$ from Group 3.

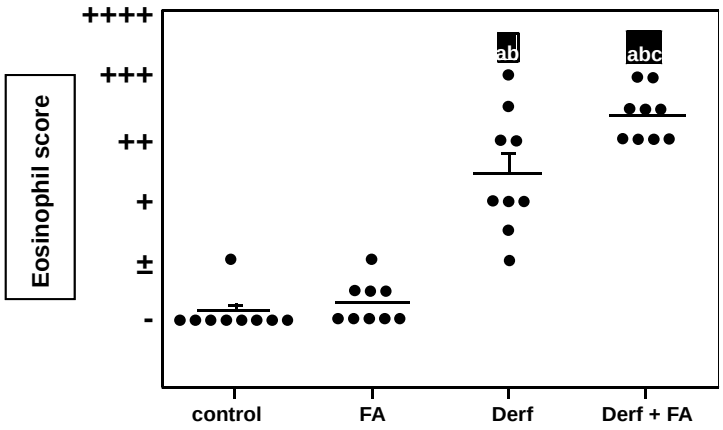
Source: Sadakane et al. (2002).

Der f sensitization and challenge increased eosinophil infiltration into the interstitium around the bronchi and bronchioles as well as goblet cell proliferation in the bronchial epithelium (Figure 4-17). Formaldehyde exposure exacerbated the eosinophilic and goblet cell responses to a challenge dose of Der f ($p < 0.05$) (Sadakane et al., 2002). Formaldehyde-induced

eosinophilic infiltration in the absence of sensitization and challenge was not different from non-treated, non-sensitized mice.

These results suggest that formaldehyde exposure may aggravate eosinophilic infiltration and goblet cell proliferation that accompanies allergic responses. This response is associated with an increase in IL-5, an eosinophilic attractant, and an increase in RANTES, which recruits eosinophils by chemotaxis in formaldehyde-exposed and Der f challenged animals, although the effect was not statistically significantly elevated compared with Der f challenge-induced levels of IL-5 and RANTES alone.

Panel A



Panel B

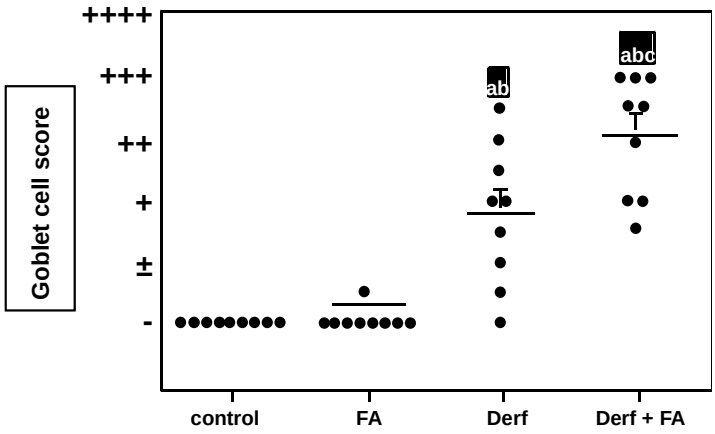


Figure 4-17. The effects of formaldehyde inhalation exposures on eosinophil infiltration (Panel A) and goblet cell proliferation (Panel B) after Der f challenge in the nasal mucosa of male ICR mice after sensitization and challenge.

Note: [a]$p < 0.001$ compared with control group; [b]$p < 0.001$ compared with formaldehyde group; [c]$p < 0.05$ compared with Der f group.
Source: Redrawn from Sadakane et al. (2002).

Fujimaki et al. (2004a) investigated the long-term effects of low-dose formaldehyde exposure on immunologic and neurological inflammation. Female C3H/He mice were exposed to 0, 0.082, 0.393, or 1.87 ppm (0, 0.1, 0.48, or 2.3 mg/m^3) formaldehyde 16 hours/day, 5 days/week for 12 weeks. Six mice at each exposure level were given injections of OVA plus adjuvant before the initial exposure and in weeks 3, 6, 9, and 11 of the experiment. Five mice at each formaldehyde-exposure level did not receive OVA injections. One day after the last exposure, mice were weighed and blood, BAL, spleen, and thymus were collected from each animal. After weighing, spleens were disaggregated and spleen cells harvested for cell culture. Immunophenotype of the spleen cells was determined by flow cytometry (CD4, CD8, CD3, and CD19 positive cells). Lymphocyte proliferation in response to lipopolysaccharide (LPS), phytohemagglutinin A (PHA), or OVA was determined after 72 hours in culture. Splenocytes were cultured for 48 hours in the presence of LPS, PHA, and OVA (immunized mice only), and supernatants were collected for cytokine analysis (IL-4, IL-5, and IFN-γ). Splenocytes were cultured for 24 hours in the presence or absence of OVA to assess chemokine production (MCP-1 and MIP1-α). Anti-OVA IgE, IgG_1, IgG_2, and IgG_3 were quantified in blood plasma.

Body and thymus weights were unchanged by formaldehyde exposure or OVA injection (Fujimaki et al., 2004a), while, in non-immunized mice, spleen weights were reduced by formaldehyde exposure from 152 mg in controls to 128, 118, and 121 mg in mice exposed to 0.08, 0.4, and 1.8 ppm formaldehyde, respectively. Spleen weights tended to increase in groups exposed to 400 and 2,000 ppb formaldehyde compared with controls in OVA-immunized mice (control: 117.8 mg compared with 400 ppb: 168.6 mg and control: 121.0 mg compared with 2,000 ppb:153.2 mg, respectively) but were not statistically significant.

To gain insight on the overall pulmonary inflammatory response of mice exposed to formaldehyde in both immunized and non-immunized mice, the total number and differential count of MPs, neutrophils, lymphocytes, and eosinophils in BAL were counted and were found to be unchanged by formaldehyde in non-immunized mice. By contrast, in immunized mice exposed to 1.8 ppm formaldehyde, the total number of BAL cells, MPs, and eosinophils were significantly increased compared with non-immunized controls (9.65 versus 2.84, 7.22 versus 2.74, and 2.0 versus 0.02 $\times 10^4$ cells, respectively).

To further assess the pulmonary inflammatory response, protein levels of inflammatory cytokines were determined by ELISA in BAL fluid. Levels of IL-1β in BAL of immunized mice were decreased by formaldehyde exposure ($p < 0.05$ at 1.8 ppm formaldehyde), but IL-1β levels after formaldehyde exposure were not different from controls in non-immunized mice (Fujimaki et al., 2004a). All other cytokines or chemokines were either unchanged (TNF-α, IL-6, and GM-CSF) or not detected (eotaxin, MIP-1α, and MCP-1).

Various neuropeptides, such as brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), and substance P are released from vagal nerve endings and mediate a neurogenic inflammatory response. Levels of BDNF, NGF, and substance P were assessed in BAL fluid and/or in plasma. BDNF was not detected in BAL or in plasma. NGF levels in immunized mice were significantly higher than in non-immunized mice in both BAL fluid and in plasma. NGF levels in immunized mice were significantly attenuated by 0.08 and 0.4 ppm formaldehyde exposure (Figure 4-18) in both BAL fluid and in plasma. Plasma level of substance P (a mediator of neurogenic inflammation) was increased by formaldehyde exposures in non-immunized mice (Figure 4-19) in both BAL fluid and plasma. This increase appears to be dose-dependent and reaches statistical significance at 2,000 ppb formaldehyde exposure in non-immunized mice compared with non-immunized controls. Similar to NGF, levels of substance P increased in OVA-immunized mice compared with non-immunized mice in both BAL fluid and plasma. Similar to NGF, levels of substance P in OVA-immunized mice were attenuated by formaldehyde exposure at 80 ppb.

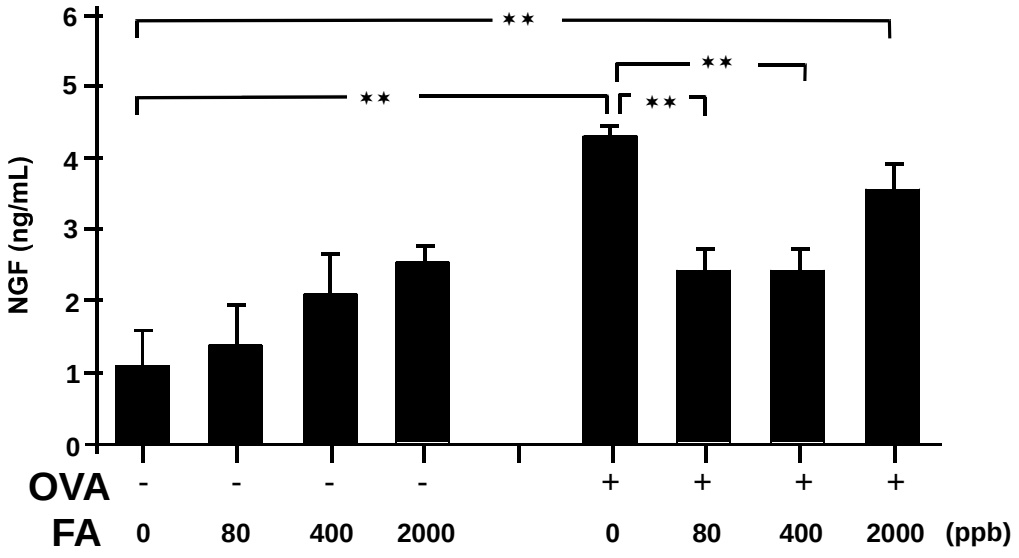


Figure 4-18. NGF in BAL fluid from formaldehyde-exposed female C3H/He mice with and without OVA sensitization.

Note: The day after the final formaldehyde inhalation, BAL fluid was collected from formaldehyde-exposed, non-immunized and formaldehyde-exposed, OVA-immunized mice, and the production of NGF was determined by ELISA. Data are mean ± SEM from five to six animals. $^{**}p < 0.01$.

Source: Redrawn from Fujimaki et al. (2004a).

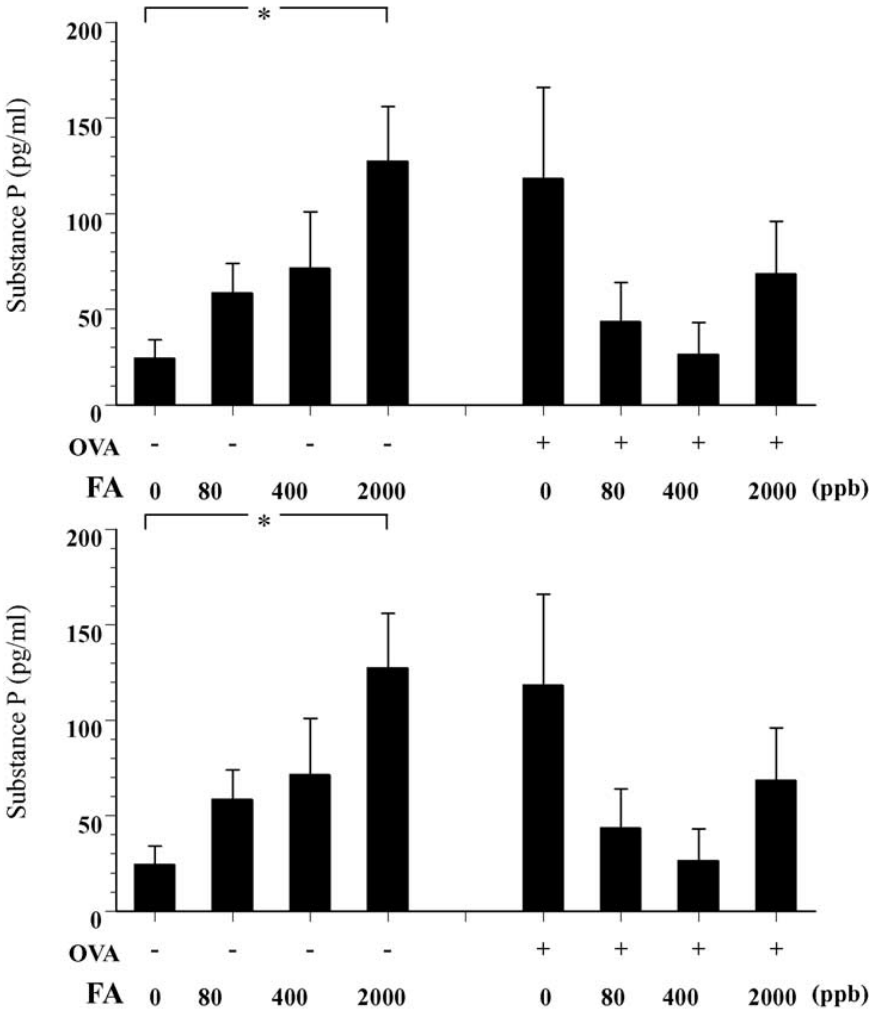


Figure 4-19. Plasma substance P levels in formaldehyde-exposed female C3H/He mice with and without OVA sensitization.

Note: The day after the final formaldehyde inhalation, plasma samples were collected from formaldehyde-exposed, non-immunized, and formaldehyde-exposed OVA-immunized mice, and the levels of substance P were determined by ELISA. Data are mean ± SEM from five to six animals. *$p < 0.05$. FA = formaldehyde.

Source: Redrawn from Fujimaki et al. (2004a).

Fujimaki et al. (2004a) further investigated the effect of low-level formaldehyde exposure from both immunized and non-immunized mice on the systemic immune response. Spleens were removed from formaldehyde-exposed mice and were cultured in the presence of LPS or PHA (for non-immunized samples) or OVA (for immunized samples). The secretory ability of immunized and non-immunized spleen cells was assessed by measuring IFN-γ release by ELISA.

Formaldehyde exposure (1.8 ppm) increased IFN-γ fourfold in LPS-stimulated cultured spleen cells from non-immunized mice. No other cytokine or chemokine was changed by formaldehyde exposure in cultured spleen cells from non-immunized mice. In OVA-immunized mice, formaldehyde had no significant effect on cytokines from stimulated spleen cells. OVA in vitro stimulation significantly increased the chemokines MIP-1 and MCP-1 for control and formaldehyde-treated OVA-immunized mice. The OVA-stimulated release of MCP-1 in vitro was enhanced by formaldehyde exposure in a concentration-dependent manner, increasing threefold and fourfold at 0.40 and 1.8 ppm, respectively. Increases in MCP-1 correlate with reported increases in the associated cytokine, RANTES, which recruits eosinophils by chemotaxis (Sadakane et al., 2002). These formaldehyde-induced increases in cytokine levels contribute to pulmonary inflammation. The inflammatory response is not mediated by lymphocytes, since lymphocyte subsets and in vitro cell proliferation were unchanged by OVA immunization or formaldehyde treatment (Fujimaki et al., 2004a).

Anti-OVA (IgE and IgG_{2a}) levels in plasma were unchanged by formaldehyde exposure. Anti-OVA IgG_1 was reduced in immunized mice exposed to 400 ppb formaldehyde compared with nonexposed animals. However, this effect did not persist as dose increased. Anti-OVA IgG_3 was depressed in immunized mice exposed to 0.08 and 0.4 ppm formaldehyde (Fujimaki et al., 2004a). Formaldehyde exposure did not induce an inflammatory response in lung or tracheal epithelium in sections viewed by light microscopy (Fujimaki et al., 2004a). Although there was a mild infiltration of mast cells into the epithelium of OVA-immunized mice, there were no effects of formaldehyde treatment on mast cell infiltration.

A recent study by Lino dos Santos Franco et al. (2009) exposed male Wistar rats for 3 days, 90-minutes/day, to 1% formaldehyde (by weight; exact doses not reported) by inhalation. Of these, one group was sensitized I.P. to OVA (10 μg), a common allergen, immediately following formaldehyde exposure, and subsequently challenged with OVA 2 weeks later. Other rats were sensitized and challenged but were not exposed to formaldehyde. PCA reaction as well as BAL analysis and whole blood analysis were conducted. Immunohistochemical analysis of platelet endothelial cell adhesion molecule-1 (PECAM-1) expression, an inflammatory mediator, in lung tissue was also measured. When formaldehyde exposure was followed by OVA sensitization and challenge, decreased lung inflammation was reported compared with the group that was OVA-sensitized but had not been exposed to formaldehyde. Reduced lung mast cell degranulation was also reported in the formaldehyde/OVA group compared with the nonexposed OVA group. Total circulating leukocytes, total bone marrow cells, and lung protein expression levels of PECAM-1 were also significantly decreased in formaldehyde/OVA rats compared with non-formaldehyde exposed OVA rats. The reduction in inflammatory parameters in response to

formaldehyde may be attributed to different study designs, since in this study animals were sensitized after exposure rather than prior to exposure. The results suggest that formaldehyde may functionally alter the activity of certain cells, like mast cells, that may downgrade an appropriate immune response to antigen and might serve to threaten lung homeostasis. Due to the unique experimental design of this study, it cannot be directly compared with Sadakane et al. (2002) or Fujimaki et al. (2004a). In addition, this study did not intend to measure whether formaldehyde can exacerbate an asthmatic response but rather set out to identify whether formaldehyde could affect immune homeostasis.

In summary, studies suggest that formaldehyde exposure may induce a predominantly neurogenic inflammatory response via release of neuropeptide, such as NGF and substance P from vagal nerve endings. Formaldehyde does not appear to potentiate a systemic immune response. However, localized pulmonary inflammation can be potentiated by formaldehyde exposure, as indicated by the increased presence of eosinophils and certain proinflammatory cytokines (IFN-γ). This response does not appear to be mediated by classic immunogenic mechanisms since studies have failed to report elevated levels of anti-formaldehyde-specific IgE. Several studies have shown that exposure to formaldehyde can facilitate allergic sensitization in previously naïve animals, and it is thought that this effect may occur due to formaldehyde's ability to increase microvascular leakage in the nasal epithelium and by causing damage to the nasal barrier (Ito et al., 1996). Sadakane et al. (2002) demonstrated that formaldehyde exposure can also exacerbate allergic responses by enhancing the response to challenge allergen. Thus, formaldehyde may exacerbate allergic responsiveness by aggravating the sensitization response in previously naïve animals by altering the permeability of the mucosal barrier in nasal compartments. Neurogenically derived inflammation, including stimulation of the trigeminal nerve and release of braykinin, suggests that the MOA for sensitization may ultimately have its roots in neurogenic inflammation rather than an immunogenic response. In addition, using a different protocol, Lino dos Santos Franco et al. (2009) suggest that formaldehyde exposure can adversely affect lung homeostasis by reducing the activity of important inflammatory mediators (mast cells, circulating leukocytes, PECAM-1 expression) when it occurs prior to sensitization, thus downgrading an appropriate immune response.

4.2.1.5.2. ***Dermal sensitization.*** Wahlberg (1993) used Hartley strain guinea pigs as test animals to determine the skin irritancy of a suite of industrial chemicals, including formaldehyde. Aqueous solutions of the compound in a 0.1 mL volume were applied to the shaved flanks of guinea pigs and gently rubbed into the skin with a cotton-tipped applicator. Sites were left open and the treatments repeated once daily for 10 days. A number of indices of

acute skin irritation were monitored, including erythema via visual scoring and edema and skin-fold thickness using Harpenden calipers. Varying concentrations of formaldehyde (up to a 10% solution) induced a dose-dependent increase in skin-fold thickness. Responses also showed shorter latencies at the higher concentrations. For example, erythema was first observed on day 2 when 10% formaldehyde was applied, day 5 (for 3%), and day 6 (for 1%).

Lee et al (1984) investigated the role of different routes of exposure in formaldehyde-induced allergic sensitization. Two sets of four male English smooth-haired guinea pigs received topical applications of 100 μL 37% w/v formalin distributed over two shaved, depilated dorsal sites two times over the course of 2 days at different sites. The total dose was calculated as 74 μg/animal. In addition, eight animals received a single topical application onto a 15 mm area of the dorsal surface. The applied dose of 25 μL formaldehyde was dissolved in saline. Two other groups of guinea pigs were exposed to either 6 ppm (6 hours/day for 5 days) or 10 ppm (6 hours/day for 5 days) formaldehyde by inhalation. A third group of guinea pigs was exposed to 10 ppm formaldehyde for 8 hours/day for 5 consecutive days by inhalation. All animals were evaluated for contact sensitivity by topical application of 20 mL formaldehyde diluted with saline and distributed in a 15 mm area on the backs of the shaved guinea pigs (Lee et al., 1984). Sites were visually inspected for erythema at 1, 6, 24, and 48 hours following the topical application, and reactions were scored. No erythema was observed in control animals. None of the guinea pigs in the 6 hours/day inhalation groups (6 and 10 ppm formaldehyde) developed skin sensitivity tested on day 9 (4 days after the initial exposure regimen ended). Two of four guinea pigs exposed to 10 ppm formaldehyde for 8 hours/day for 5 consecutive days developed mild skin sensitization tested on day 31. Contact sensitivity increased in a dose-dependent fashion in groups of animals that had been sensitized via the dermal route. Thus, dermal exposure resulted in contact sensitivity. Inhalation exposure did not consistently produce contact sensitivity.

Arts et al. (1997) used a local lymph node assay (LLNA) and the induction of IgE to monitor the sensitization of female Wistar rats (low IgE-responders) and BN rats (high IgE responders). For the LLNA assay, animals were sensitized by the application of varying concentrations of formaldehyde in raffinated olive oil on the dorsum of both ears on days 0, 1, and 2. Control animals were treated with raffinated olive oil alone. Animals received an I.P. injection of BrdU on day 5 and were subsequently sacrificed. Ear-draining lymph nodes were collected, fixed, and sectioned, and the mitotic activity was monitored following successive incubation of the sections in anti-BrdU, biotin-labeled rabbit anti-mouse antibody, peroxidase-conjugated streptavidin, and 3,3-diaminobenzidine tetrahydrochloride. For serum IgE responses, 150 μL of different concentrations of formaldehyde were applied to the shaved flanks of rats on

day 1, then 75 µL of the same chemical at 50% of the initial concentration were applied to the dorsum of each ear on day 7. The amount of IgE in the blood was measured using ELISA but appeared to be little affected by formaldehyde treatment in either species of rat. However, the ear-draining lymph nodes of both strains of rat showed a comparative increase in weight in response to formaldehyde, and proliferation (BrdU positive) of paracortical cells was observed in response to increasing doses of the compound. This response was most notable in BN rats treated with 10% formaldehyde. Arts et al. (1997) concluded that the irritant and sensitizing properties of formaldehyde may act through non-IgE-immune mechanisms.

Hilton et al. (1998) used the LLNA assay in female CBA/Ca (H-2^k haplotype) mice to compare the skin sensitizing potencies of formaldehyde and glutaraldehyde. The comparison was set on a quantitative basis by determining the concentration of each compound necessary to induce a threefold increase in lymph node cell proliferative activity (effective concentration [EC_3]). While both aldehydes induced a dose-dependent proliferative response, the incorporation of [^{3}H]-methylthymidine was far greater in animals exposed to glutaraldehyde versus formaldehyde (with EC_3 values of 0.002–0.006 mol/L for glutaraldehyde versus 0.11–0.18 mol/L for formaldehyde). These data indicate the potential of both chemicals to induce skin sensitization, although the potency of glutaraldehyde was far greater than that of formaldehyde.

Xu et al. (2002) evaluated the extent to which the expression of some cytokines may change as a result of cutaneous exposure to formaldehyde in mice. Female Balb/C mice were skin painted with three topical applications of 100 µL of 17.5% formaldehyde or distilled water with a 1-day interval between each application. Spleen and draining lymph nodes were harvested on days 3, 5, 7, 9, or 12 after the last skin painting. In some animals, contact hypersensitivity was induced by applying 2% formaldehyde to both sides of mouse ears on day 3 following the last skin painting. For this endpoint, the percent increase in thickness of the ears was monitored. For the cytokines, mRNA expression levels of IL-2, IL-4, IL-5, IL-10, IL-12, IL-13, IL-15, IL-18, and INF-γ were determined semiquantitatively by measuring the amount of individual mRNAs following amplification with the reverse transcriptase (RT)-PCR. The relative amounts of cytokine mRNAs were calculated as the ratio of cytokine mRNA to that of glyceraldehyde-3-phosphate dehydrogenase, as revealed in specific bands on an agarose gel.

Cutaneous formaldehyde treatment was associated with the long-lasting expression of IL-4 and IFN-γ mRNAs in mouse spleen and draining lymph nodes and with IL-15 mRNA only in mouse spleen. Only IL-13 mRNAs displayed a transient increase in expression in both spleen and draining lymph nodes. Levels of IL-2, IL-12, and IL-15 were increased in the mouse spleen but not the lymph nodes. The mouse ear swelling test gave positive correlations with enhanced expression of mRNA for IL-4 and IFN-γ (Table 4-52).

Table 4-52. Correlation coefficients among ear swelling responses and skin mRNA levels in contact hypersensitivity to formaldehyde in mice

Variables	Correlation coefficients		
	IL-2	IL-4	IFN-γ
Ear swelling	0.50	0.74[a]	0.67[a]
IL-2	–	0.39	0.60
IL-4	–	–	0.79[a]

[a]Statistically significant ($p < 0.05$).

Source: Xu et al. (2002).

4.2.1.5.3. ***Summary of sensitization studies.*** Several animal studies report increased airway resistance and BC due to inhalation exposures to formaldehyde (Nielsen et al., 1999; Swiecichowski et al., 1993; Biagini et al., 1989; Amdur, 1960). Changes in pulmonary resistance were observed as early as 10 minutes after exposure (Biagini et al., 1989), and reported effect levels ranged from 0.3–13 ppm. Other pulmonary effects were reported in conjunction with BHR, such as increased tracheal reactivity and decreased pulmonary elasticity (Swiecichowski et al., 1993; Amdur, 1960). Although BHR is a common result of Type I hypersensitivity reaction to an allergen, the observation of BHR alone is not sufficient to demonstrate that an agent induces Type 1 hypersensitivity.

BHR may be directly induced both pharmacologically and neurogenically (Joos, 2003; Cain, 2001; Meggs, 1995). There is little evidence that formaldehyde itself is an allergen recognized by the immune system, especially via inhalation (Lee et al., 1984). Although formaldehyde exposure has been reported to alter cytokine levels and immunoglobulins in some experimental systems, these immunomodulatory effects do not support a type 1 hypersensitivity. IgE was unchanged (Fujimaki et al., 2004a; Lee et al., 1984), and cytokine profiles were not consistent with the Th-2 cytokines expected in IgE mediated hypersensitivity (Fujimaki et al., 2004a; Ohtsuka et al., 2003).

Formaldehyde-induced dermal sensitization show parallel results. The physical signs of irritation and sensitization are consistently shown (e.g., rashes, edema). Some involvement of the immune response has been demonstrated with positive LLNA assays, indicating proliferation of lymphocytes in lymph nodes draining the affected area (Hilton et al., 1998; Arts et al., 1997). Increased expression of Th-2 cytokines in the lymph nodes of mice given dermal applications of formaldehyde does indicate an immune component to the observed sensitization. However, the response does not seem to be mediated by IgE (Arts et al., 1997; Lee et al., 1984).

Ito et al. (1996) reported that a tachykinin NK_1 receptor, but not the histamine H_1 or bradykinin B_2 receptors, is involved in formaldehyde-induced vascular permeability. Neuropeptides NGF and substance P were affected in BAL and stimulated splenocytes from formaldehyde-exposed mice, with greater effects seen in OVA-immunized mice. Tachykinins (e.g., substance P and neurokinin A) are produced by nerve cells and can directly stimulate bronchoconstriction (Van Schoor et al., 2000). Substance P is also a mediator of neurogenic inflammation. Therefore, although formaldehyde may induce some of the symptoms of type 1 hypersensitivity, these symptoms are more likely neurogenic than immunogenic in origin.

In contrast, formaldehyde enhances immunogenic hypersensitivity of known allergens (Sadakane et al., 2002; Riedel et al., 1996; Tarkowski and Gorski, 1995). This potentiation varied based on sensitization protocols (respiratory tract versus systemic, frequency and timing of immunization, allergen, etc.) and formaldehyde exposure regimens (concentration, continuous versus intermittent exposures). Taken as a whole, the results support the finding that formaldehyde exposure can aggravate a type 1 hypersensitivity response (Table 4-53).

4.2.1.6. *Neurological and Neurobehavioral Function*

4.2.1.6.1. ***Inhalation exposure.*** There are a number of published reports examining the effects of formaldehyde exposure on nervous system structure and function. The reports evaluating behavioral effects fall into three main categories: (1) behavioral responses evaluated during or immediately following formaldehyde exposures, which may include effects due to the potential irritant properties of the chemical, (2) acute or short-term exposures followed by behavioral assessments conducted 2–24 hours after termination of formaldehyde exposure, which reflect sustained effects of chemical exposure independent of its irritant properties, and (3) repeated exposures to formaldehyde followed by neurological assessments performed throughout the treatment period or several days to weeks after termination of treatment. In addition to reports evaluating changes in behavior, there are several reports evaluating neuropathological effects or changes in brain chemistry.

Table 4-53: Summary of sensitization and atopy studies by inhalation or dermal sensitization due to formaldehyde in experimental animals

Species	No./ group	Treatment[a]	Observations	LOAEL/ NOAEL	Reference
Inhalation studies					
Cynomolgus monkeys	9	Methacholine-sensitive monkeys exposed to 2.5 ppm formaldehyde for 10 minutes	Formaldehyde increased pulmonary resistance after 2, 5, and 10 minutes.	LOAEL 2.5 ppm	Biagini et al. (1989)
Hartley guinea pigs (male)	8	0.86, 3.4, 9.4, 31.1 ppm formaldehyde for 2 hours or 0.11, 0.31, 0.59, 1.05 ppm for 8 hours	Total pulmonary resistance increased after 2 hours exposure at 9.4 and 31.1 ppm. Effect was reversible and returned to baseline within 30 minutes. Total pulmonary resistance was increased after 8 hours exposure at 0.3 and 1 ppm. Amount of acetylcholine needed to achieve doubled pulmonary resistance was decreased in animals after 2 hours exposure.	NA	Swiecichowski et al. (1993)
Hartley guinea pigs (male)	5–7	3.4 ppm for 8 hours	No changes in ex vivo tracheal constriction or inflammation.	NA	Swiecichowski et al. (1993)
F344 rats and BN rats	5	16 ppm 3 hours/day, 5 days	Modest changes in inflammatory cytokine expression, but respiratory and olfactory epithelial lesions were more severe in F344 rats than in BN rats.	NA	Ohtsuka et al. (2003)
English smooth-haired guinea pigs	4	6, 10 ppm, 6 hours/day, 5 days, combined with provocation challenge (2 or 4 ppm on day 7, or days 7, 22, and 29)	Inhalation challenge with 6 or 10 ppm followed by bronchial challenge failed to increase respiratory sensitivity	NA	Lee et al. (1984)
Perlbright-white, Duncan-Hartley guinea pigs (female)	12	0, 0.13, 0.25 ppm 8 hours/day, 5 days. The animals were sensitized to OVA (3 minutes exposure to 5% OVA aerosol)	Anti-OVA titer was significantly elevated over controls in animals exposed to 0.25 ppm formaldehyde and showed that formaldehyde may sensitize previously naïve animals to OVA.	NA	Riedel et al. (1996)
Balb/C mice (female)	4	0, 6.63 ppm 6 hours/day for 10 days or 6 hours/day once/week for 7 weeks. All mice were sensitized to OVA	Formaldehyde administered intranasally for 6 hours/day for 10 days may facilitate sensitization to allergens since anti-OVA titers were elevated over control animals. However, the length and duration of exposure appears to affect development of sensitization.	NA	Tarkowski and Gorski (1995)

Table 4-53: Summary of sensitization and atopy studies by inhalation or dermal sensitization due to formaldehyde in experimental animals

Species	No./ group	Treatment[a]	Observations	LOAEL/ NOAEL	Reference
Wistar rats (male)	5–8	0, 2, 5, 15, 45 ppm for 10 minutes	Pulmonary insufflation or blood pressure were not altered. Vascular permeability increased in concentration-dependent manner and could be reduced by adding a NK1 selective antagonist.	NA	Ito et al. (1996)
Outbred ICR mice (male)	18	0.5% formaldehyde for 15 minutes, once/week for 4 weeks. Both control and exposed groups were exposed to Der f by I.P. injection 1 day before formaldehyde and then challenged with Der f after 4 weeks.	More pronounced RANTES production in formaldehyde-treated and sensitized rats than in sensitized rats that had not been exposed to formaldehyde. Formaldehyde also potentiated IL-5 production associated with sensitization.	NA	Sadakane et al. (2002)
C3H/HeJ mice (female)	6	0, 0.082, 0.393, 1.87 ppm 16 hours/day, 5 day/week, 12 weeks. Mice also given OVA plus adjuvant before exposure, and again 3, 6, 9, 11 weeks after exposure. Some formaldehyde mice did not receive any OVA	Substance P and NGF were increased dose dependently in formaldehyde-treated, non-immunized mice but were attenuated in formaldehyde-treated immunized mice compared with nonexposed, immunized controls.	LOAEL 0.082 ppm	Fujimaki et al. (2004a)
Wistar rats (male)	NA	1% Formaldehyde by weight for 90 minutes for 3 days. One group was sensitized to OVA after to formaldehyde exposure and then challenged with OVA afterwards. Others were sensitized and challenged but not exposed to formaldehyde.	Total circulating leukocytes, bone marrow cells, and lung protein PECAM expression were significantly decreased in formaldehyde/OVA rats compared with OVA rats.	NA	Lino dos Santos Franco et al. (2009)
Dermal sensitization					
Hartley guinea pigs	5	Skin painted once/day for 10 days with 0.1 mL of 1, 3, or 10% formaldehyde	Varying concentrations (up to 10%) induced dose-dependent increase in skin-fold thickness. Erythema seen earlier at higher doses (2 days at 10% formaldehyde vs 5 days at 3% or 6 days at 1%).		Wahlberg et al. (1993)

Table 4-53: Summary of sensitization and atopy studies by inhalation or dermal sensitization due to formaldehyde in experimental animals

Species	No./ group	Treatment[a]	Observations	LOAEL/ NOAEL	Reference
English smooth-haired guinea pigs	4	Group 1: skin painted, 100 µL 37% formalin twice over 2 days, Group 2: single topical application of 25 µL formaldehyde Group 3: 10 ppm 6 hours/day for 5 days by inhalation	Two of four guinea pigs from group 3 had mild skin sensitization after day 31. Contact sensitivity developed in a dose-dependent manner in the dermal groups (group 1 and 2).		Lee et al. (1984)
Wistar and BN rats (female)	4	Application of formaldehyde to ears on days 0, 1, 2, followed by an I.P. injection of BrdU.	Ear-draining lymph nodes increased in weight in response to formaldehyde, reflected in increased number of BrdU-stained cells, most notably in BN rats (high IgE responders) treated with 10% formaldehyde		Arts et al. (1997)
CBA/Ca mice	NA	Compared glutaraldehyde to formaldehyde to induce a local lymph node assay	Glutaraldehyde and formaldehyde induced a dose-dependent proliferative response that was greater in glutaraldehyde-treated animals		Hilton et al. (1998)
Balb/c mice (female)	3–5	Skin painted with 100 µL of 17.5% formaldehyde every other day for days 3, 5, 7, 9, 12	Cutaneous treatment associated with long-lasting expression of various cytokines from draining lymph nodes and spleen.	NA	Xu et al. (2002)

NA = not applicable.

4.2.1.6.1.1. ***Behavioral response***

Clinical signs

Several studies that were focused on general toxicity or carcinogenicity of formaldehyde also assessed clinical signs in exposed animals, which may be related to adverse effects on the nervous system. Procedural details for the assessments, or specific data regarding findings, were not provided. Signs recorded included uncoordinated locomotion and climbing of cage walls at 20 ppm formaldehyde in rats (Woutersen et al., 1987); restlessness at 15 ppm formaldehyde in rats (Morgan et al., 1986a); dyspnea, listlessness, and hunched posture at 20 ppm and ataxia at 40 ppm in mice (Maronpot et al., 1986); and dyspnea in rats at 14.3 ppm formaldehyde (Kerns et al., 1983). Given the lack of information regarding procedures used for these evaluations and the limited reporting of results, the utility of these data is limited.

Irritant threshold detection

Wood and Coleman (1995) evaluated the irritant properties of acute formaldehyde exposure in mice. Adult male Swiss mice (eight/group) were initially trained to terminate a 60-second exposure to an irritant gas (ammonia, 1,000 ppm) by poking their noses into a conical sensor five times to produce a 60-second facial shower of clean air. Each test session consisted of 25 exposure trials. Following training, response to formaldehyde was evaluated, using the same testing scenario. Each day mice had a morning exposure session to ammonia and an afternoon session to formaldehyde. Formaldehyde concentrations tested were different each day, in sequence from 0, 1, 1.8, 3, 5.6, and 10 ppm (0, 1.23, 2.21, 3.68, 6.87, and 12.3 mg/m^3) and then stepping back again from 10 to 0 ppm. Half of the animals were tested in an ascending order of formaldehyde concentrations, the other half in a descending order. The frequency of terminating exposure, error rate, and the time lapse to termination were recorded. The concentration at which 50% of the formaldehyde deliveries would be expected to be terminated was estimated (AC_{50}) by simple linear regression or by analysis of covariance on the logit transform of percentage terminated as a function of log concentration.

All mice were trained successfully to terminate 100% of ammonia exposures, but varied responses were observed with formaldehyde exposure. In general, time taken to terminate formaldehyde exposure decreased significantly with increasing formaldehyde concentration. Mice terminated more exposures to 1 ppm formaldehyde than to air alone ($p < 0.0005$), and the error rate, generally below 40%, did not significantly differ with formaldehyde concentration tested. Each animal had two test sessions with each formaldehyde concentration (once during the ascending sequence and again during the descending sequence); both the time to termination ($p < 0.0012$) and AC_{50} were decreased in the second series of tests. One method of estimation by

the authors yielded an AC_{50} of 3.63 ppm for the first series of tests versus 1.88 ppm for the second series. A two-way repeated measures ANOVA with replication and concentration as within variables was highly significant ($p < 0.00005$). These studies indicate mice are sensitive to the irritant properties of formaldehyde at exposure concentrations as low as 1 ppm, and animals reacted more swiftly and with greater accuracy to terminate formaldehyde exposure as the concentration increased. However, a wide variety of responses was noted on an individual animal basis. Two of the eight mice terminated 90% of the trials during 1 ppm exposures and 80–100% of trials at all other tested formaldehyde concentrations. One mouse terminated fewer than 10% of the formaldehyde exposure trials (1–10 ppm) during the testing regimen but had a 92% response rate to 20 ppm formaldehyde. The remaining five mice responded with increasing termination frequency as formaldehyde concentration increased from 1 to 10 ppm, with an AC_{50} of 2.72 ppm (3.34 mg/m^3).

4.2.1.6.1.2. ***Motor activity and habituation.*** Malek et al. (2003a) examined open field behavior of rats after acute formaldehyde exposures. Male and female LEW.1K rats (15/sex/group) were exposed to 0, 1, 2.5, or 5 ppm (0, 1.23, 3.08, or 6.15 mg/m^3) formaldehyde for 2 hours. Formaldehyde was vaporized from aqueous solutions directly below the exposure chamber. Formaldehyde levels were checked 16 times throughout the 2-hour exposure periods. Mean formaldehyde levels of 1.01 ± 0.29 ppm, 2.51 ppm (SD is missing), and 5.0 ± 0.27 ppm were achieved. Locomotor activity was assessed for 3 minutes in an open field 2 hours after termination of formaldehyde exposure and again 24 hours later, using an automated device to count the number of squares crossed. Other behaviors were noted, including grooming (face cleaning, fur licking, and scratching), rearing, sniffing (air and floor), wall climbing, and defecation.

The authors reported no signs of irritation or changes in activity or food or water intake during exposure. In general, sniffing was increased after formaldehyde exposure and movement was decreased (crossed quadrants and climbing) in both male and female rats ($p < 0.05$). Significant reductions in horizontal movements (crossed quadrants) were observed at all dose levels and were characterized by a U-shaped dose response (Figure 4-20). The lowest dose tested (1 ppm) demonstrated a higher level of activity suppression than the two higher doses, but all groups were still suppressed relative to controls. Although female rats displayed a greater level of activity overall, a similar U-shaped dose-response pattern was also observed.

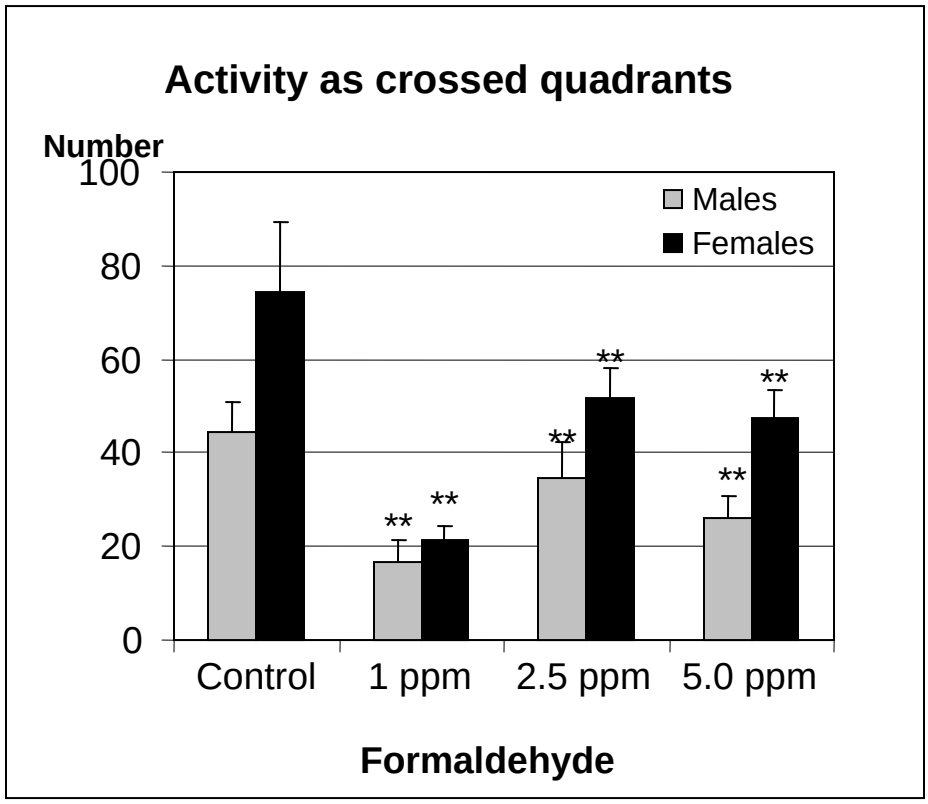


Figure 4-20. Motor activity in male and female rats 2 hours after exposure to formaldehyde expressed as mean number of crossed quadrants ± SEM. Greater reductions were observed in the lowest dose group, a pattern that was evident in both genders. ** = different from control, $p < 0.005$.

Source: Drawn from data reported by Malek et al. (2003a).

Activity in the same apparatus was reassessed 24 hours later. As expected, controls demonstrated habituation to the test apparatus, exhibiting only 20% of the motor activity observed on day 1 (Figure 4-21). In contrast, formaldehyde-treated animals failed to demonstrate the same degree of habituation. Activity levels for males observed on day 2 were 60–80% of the activity levels seen on day 1. Formaldehyde-treated females also failed to habituate and actually demonstrated increases in activity on day 2 relative to day 1 at all formaldehyde exposure levels.

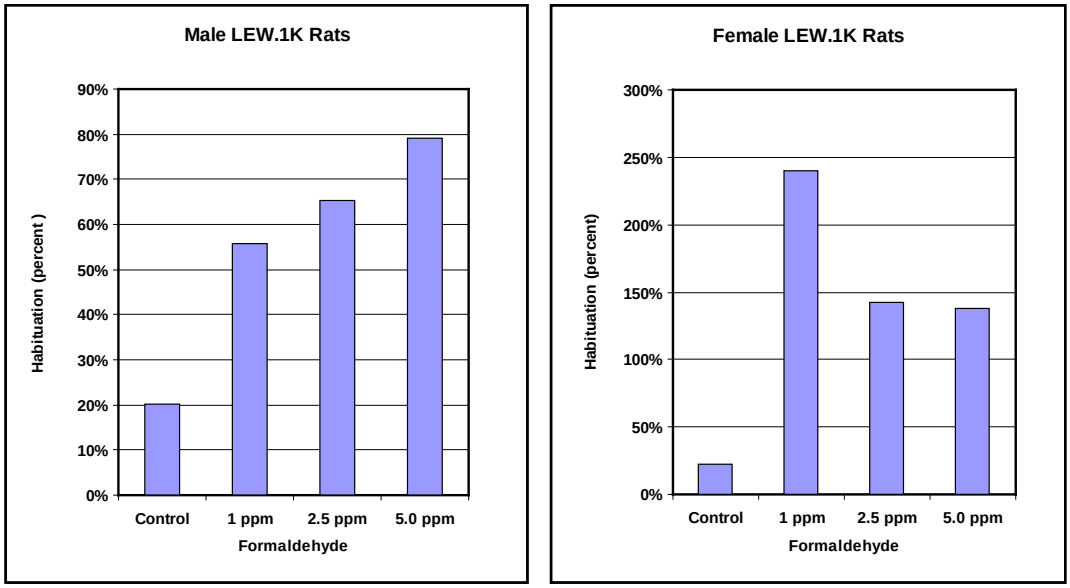


Figure 4-21. Habituation of motor activity was observed in control rats during the second observation period (day 2, 24 hours after formaldehyde exposure).

Note: Habituation is shown here as the percent decrease in number of crossings between sessions from day 1 to day 2. The degree of habituation was reduced in male rats exposed to formaldehyde (left panel) since their activity was closer to 100% of that seen on day 1. Females (right panel) had increased activity on day 2 (greater than 100% of activity on day 1), which is a sensitization rather than habituation.

Source: Drawn from data reported by Malek et al. (2003a).

A follow-up study by Malek et al. (2003b) further expanded the dose-response analysis for acute formaldehyde exposure. As described above, male and female LEW.1K rats (10 per sex per group) were exposed at 0, 0.1, 0.5, or 5 ppm (0, 0.123, 0.615, or 6.15 mg/m^3) formaldehyde for 2 hours. Formaldehyde levels were checked nine times per hour during the exposure periods, and mean values were found to be 0.13 ± 0.04, 0.48 ± 0.05, and 5.18 ± 0.66 ppm. Open field behavior was evaluated for each animal 2 hours after formaldehyde exposure. The number of crossed quadrants for both controls and a 5 ppm group were generally comparable with those observed in the first study, although female values were somewhat lower. Horizontal movement was decreased by formaldehyde exposure in a dose-dependent manner with significant reductions in motor activity as low as 0.1 ppm in males and 0.5 ppm in females (Figure 4-22). The consistency of the findings across studies and between genders provides

greater confidence in the effects of low-level formaldehyde exposure on this standard test of neurotoxicity.

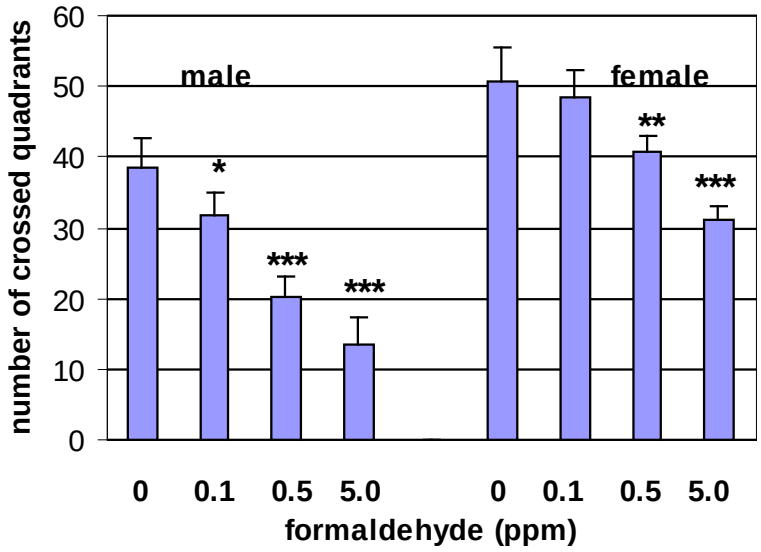


Figure 4-22. Motor activity was reduced in male and female LEW.1K rats 2 hours after termination of 10-minute formaldehyde exposure.

Note: Values are means ± SDs. * = different from control, $p < 0.05$. ** = different from controls, $p < 0.01$. *** = different from controls, $p < 0.001$.

Source: Drawn from data reported in Malek et al. (2003b).

Malek et al. (2004) also assessed the capacity of formaldehyde to induce persistent behavioral deficits in mice. Groups of 20 male AB mice received a single 2-hour exposure to 0, 1.1, 2.3, or 5.2 ppm (0, 1.3, 2.8, or 6.4 mg/m^3) formaldehyde prior to being tested 2 and 24 hours after exposure for a series of behavioral responses, including ambulation (crossed squares), grooming, sniffing, rearing, wall climbing, and defecation. Even though there were no clinical signs of toxicity in any of the exposed groups, a number of behavioral anomalies were apparent in response to formaldehyde exposure, some of which persisted for at least 24 hours, as indicated in Tables 4-54 and 4-55.

Table 4-54. Fluctuation of behavioral responses when male AB mice inhaled formaldehyde in a single 2-hour exposure: effects after 2 hours

Open field parameter	Formaldehyde concentration (ppm)[a]			
	0	1.1	2.3	5.2
No. of crossed inner squares	34.10 ± 7.51	25.30 ± 5.03[b]	21.20 ± 3.41[b]	16.10 ± 5.37[b]
No. of crossed peripheral squares	56.65 ± 9.68	59.55 ± 9.75	49.70 ± 13.24	29.15 ± 7.47[b]
Total no. of crossed squares	90.75 ± 11.08	84.85 ± 9.96	71.10 ± 13.91[b]	44.20 ± 7.42[b]
Air sniffing	19.35 ± 2.5	21.50 ± 4.26	16.35 ± 3.84[c]	8.10 ± 1.77[b]
Floor sniffing	20.95 ± 3.72	26.50 ± 4.64[b]	21.35 ± 4.77	22.80 ± 4.02
Grooming	7.95 ± 2.26	7.10 ± 3.19	7.05 ± 2.48	6.55 ± 2.06
Rearing	17.85 ± 2.56	13.90 ± 3.19[b]	11.30 ± 2.30[b]	9.95 ± 1.61[b]
Wall climbing	13.20 ± 3.09	14.55 ± 2.74	13.95 ± 2.31	13.95 ± 1.82
No. of excreted fecal boli	0.65 ± 0.81	0.75 ± 0.85	0.80 ± 0.77	0.90 ± 1.12

[a]Values are means ± SDs.
[b]Statistical significance of differences from controls ($p < 0.005$).
[c]Statistical significance of differences from controls ($p < 0.05$).

Source: Malek et al. (2004).

Table 4-55. Fluctuation of behavioral responses when male AB mice inhaled formaldehyde in a single 2-hour exposure: effects after 24 hours

Open field parameter	Formaldehyde concentration (ppm)[a]			
	0	1.1	2.3	5.2
No. of crossed inner squares	10.40 ± 2.35	9.55 ±1.73	9.10 ± 1.25	9.70 ± 1.13
No. of crossed peripheral squares	42.80 ± 9.27	44.85 ± 14.60	44.95 ± 16.56	41.10 ± 9.08
Total no. of crossed squares	53.20 ± 8.67	54.40 ± 14.77	54.05 ± 15.81	50.80 ± 9.15
Air sniffing	13.65 ± 2.81	13.30 ± 3.21	12.65 ± 2.70	12.30 ± 4.14
Floor sniffing	21.55 ± 3.47	15.85 ± 3.94[b]	13.25 ± 4.17[b]	17.65 ± 3.13[b]
Grooming	8.35 ± 2.56	13.95 ± 2.21[b]	10.20 ± 3.33[c]	11.90 ± 3.26[b]
Rearing	18.30 ± 4.23	12.40 ± 2.23[b]	12.25 ± 2.17[b]	12.00 ± 3.32[b]
Wall climbing	9.25 ± 2.38	8.70 ± 1.98	8.20 ± 2.14	9.90 ± 2.27
No. of excreted fecal boli	0.80 ± 0.83	1.20 ± 0.83	1.60 ± 0.94[c]	1.20 ± 0.89

[a]Values are means ± SDs.
[b]Statistical significance of differences from controls ($p < 0.005$).
[c]Statistical significance of differences from controls ($p < 0.05$).

Source: Malek et al. (2004).

Usanmaz et al. (2002) assessed spontaneous locomotor activity (SLMA) in Balb/c mice (4–14 per group, sex unspecified) after both acute and subchronic formaldehyde exposures. Prior to the acute exposure, mice were acclimated to the exposure chamber for 4 days but exposed only to clean air. On the fifth day, mice (six/group, sex unspecified) were exposed for 3 hours at 0, 1.8, 3.2, 4.5, 6.4, 9.7, or 14.8 ppm (0, 2.2, 3.9, 5.5, 7.9, 11.9, or 18.2 mg/m^3) formaldehyde. Mice were removed from the exposure chamber, and SLMA behavior was

evaluated by direct observation for 5 minutes. In addition to horizontal and vertical movement, wet dog shake (WDS) behavior was noted. In a separate trial, Balb/c mice (six/group, sex unspecified) were exposed to 8.2 ppm formaldehyde for 1 week, 2 ppm formaldehyde for 2 weeks, or 3.3 ppm formaldehyde for 3 weeks (3 hours/day, 5 days/week) compared with controls exposed only to air. SLMA behavior was observed for 5 minutes after the last exposure. Mice exposed to 8.2 ppm formaldehyde for 1 week, 3.3 ppm formaldehyde for 2 weeks, and 2 ppm formaldehyde for 3 weeks lost weight over the course of the treatment ($p < 0.05$). All other treatment groups had weight gain similar to control mice.

As shown in Figure 4-23, acute 3-hour formaldehyde exposures resulted in a dose-dependent decrease in SLMA. Decreases in horizontal activity were significant for the three highest dose groups (6.4, 9.7, and 14.8 ppm), and decreases in vertical activity were significant for all six formaldehyde treatment groups. SLMA was similarly decreased following subchronic exposures (data not shown here). Although the experimental protocol included longer exposures and a slightly longer observation period (5 versus 3 minutes) than in Malek et al. (2003a, b), the results are consistent, indicating decreased activity in formaldehyde-exposed animals several hours after exposure was ended.

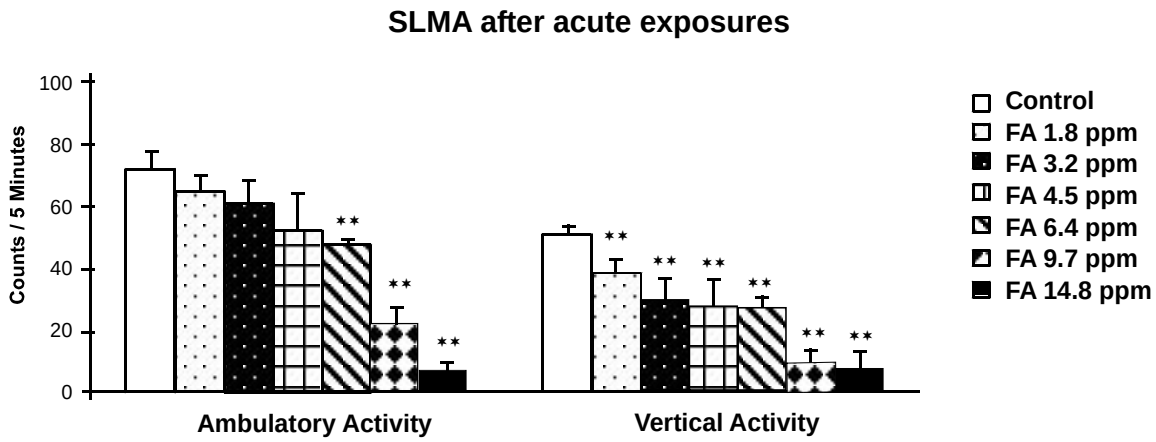


Figure 4-23. The effects of the acute formaldehyde (FA) exposures on the ambulatory and vertical components of SLMA.

Note: FA = formaldehyde exposure concentration. ** = $p < 0.01$ from controls.

Source: Usanmaz et al. (2002).

Usanmaz et al. (2002) noted an increase in WDS, after the acute exposures, as a possible preconvulsive effect. However, the mice were only observed for 5 minutes, and it is unclear how

the researchers distinguished between a WDS due to an irritating odor and a preconvulsive movement. No other study has noted convulsive effects from formaldehyde exposure in any species. A second set of trials was reported in the same paper that sought to evaluate formaldehyde effects on CNS excitability. Balb/c mice (six/group, sex unspecified) were exposed to 0, 1.8, 6.4, or 14.8 ppm (0, 2.2, 7.9, or 18.2 mg/m^3) formaldehyde for 3 hours. Subchronic exposures were at 2 ppm (2.5 mg/m^3) formaldehyde for 3 weeks or 3.3 ppm (4.1 mg/m^3) formaldehyde for 2 weeks. Seizures were induced by I.P. injection of pentilenetetrazole (PTZ), and the incidence, severity, and course of induced seizures were recorded. The PTZ injection induced seizures in 83, 88, and 91% of controls, with 16, 38, and 67% mortality in controls in the three trials. Mortality was highly variable in treatment groups as well. The authors report that PTZ-induced seizures were decreased in incidence by acute formaldehyde exposure in a dose-dependent fashion with only 33% of mice exposed to 14.8 ppm formaldehyde experiencing seizures versus 91% in control mice ($p < 0.05$ at the highest dose only). However, the methodology for observing and scoring seizures is unclear. Additionally, there was high mortality and high variability of results for the three similarly treated control groups. Therefore, it is difficult to assess data quality and interpret these findings.

Boja et al. (1985) exposed male Sprague-Dawley rats to air or to formaldehyde at 5, 10, or 20 ppm for 3 hours on 2 consecutive days. On the second day, half the rats received the same exposure as the previous day, while half the rats were switched (e.g., half those rats receiving air the first day received formaldehyde the second day, and half those receiving formaldehyde the first day received air the second day), for a total of four possible exposure combinations. During the exposure period, activity levels were monitored by observation, once per minute for the first hour and once every 5 minutes for the second hour. At the end of the second exposure session, rats were sacrificed and brains removed for neurochemical analysis (see Section 4.2.1.6.1.5).

Behavioral results were described in detail only for control and 5 ppm groups. During the first exposure session, activity levels of formaldehyde-exposed animals were significantly decreased (approximately 50% of control levels). On the second day of exposure, those animals previously exposed to formaldehyde exhibited partial recovery, those experiencing their first formaldehyde exposure behaved similarly to those initially exposed on the first day, and those animals exposed to formaldehyde for a second time had a greater decrease in activity than during the first exposure (to approximately 30% of control levels). The authors stated that a similar effect was seen in animals exposed at 10 ppm but that results at 20 ppm were not interpretable (data were not presented). Overall, the decreased activity seen in this study is consistent with effects seen by other authors.

Senichenkova (1991) exposed pregnant female rats to 0 or 0.5 mg/m^3 (0 or 403 ppb) formaldehyde on gestation days (GDs) 1–19 for 4 hours/day. Reproductive aspects of this study will be discussed in the reproduction section; however, results from behavioral assessments conducted on the neonates are discussed here. The author stated that maturation of motor reflexes (assessed as surface righting and pendular reflex), open field behavior, and maze learning ability were assessed. Detailed descriptions of procedures and results were not provided for all assessments, but it was stated that motor reflex development did not differ in treated and control animals. Open field motor activity assessments in 40-day-old (juvenile) offspring revealed an increase in squares visited and an increased frequency of rearing on the second and third days of testing, indicating a lack of habituation in the offspring of formaldehyde-treated dams; similar levels of activity by both measures were found on the first test day. Counts of defecation and urination were increased on all 3 days of testing. Increased exploratory behavior, described as increased impulses, was also noted in a learning task (not otherwise described), but the author stated that learning rate and ability of the formaldehyde-treated group was not different from controls (no data were provided).

Mobility and neuromuscular excitability (not otherwise described) in offspring of female white rats were also evaluated by Sheveleva (1971). Dams were exposed to 0.005 or 0.0005 mg/L (approximately 4,000 or 400 ppb, respectively) formaldehyde on GDs 1–19. Spontaneous mobility (over 15 minutes) and neuromuscular excitability were evaluated in offspring at 1 or 2 months of age (other results from this study are discussed under developmental toxicity, above). At 1 month, spontaneous mobility was reduced at the low dose in males (52% of control levels; $p < 0.01$) but not at the high dose, and at both doses in females (to 64 and 56% of control levels at the mid dose and high dose, respectively; $p < 0.02$). At two months, there was a dose-related increase in activity for both sexes, statistically significant ($p < 0.001$) in high-dose females only (391% of control levels).

4.2.1.6.1.3. ***Learning and memory.*** The effects of repeated formaldehyde exposures on learning were investigated by Malek et al. (2003c), using a labyrinth swim maze. In this task, animals are required to make a series of consecutive right or left turns to gain access to an escape platform (Malek et al., 2003c). Adult male and female LEW.1K rats (15/sex/group) were exposed to 0, 0.1, 0.5, or 5.4 ppm (0, 0.123, 0.615, or 6.64 mg/m^3) formaldehyde 2 hours/day for 10 consecutive days. Formaldehyde levels were checked eight times throughout the 2-hour exposure periods. Mean formaldehyde levels of 0.1 ± 0.02, 0.5 ± 0.1, and 5.4 ± 0.65 ppm were achieved. Body weight was measured on days 1, 5, and 10 of the experiment. Two days prior to beginning the formaldehyde exposures, all subjects were given an acclimation trial in which they

were individually placed into the water-filled basin at the start position and allowed to navigate to the escape platform with manual assistance to learn the correct route. Thereafter, the water labyrinth test was run on each day of formaldehyde treatment, 2 hours after completion of each daily exposure. Time taken to complete the test and errors made were recorded for each rat (Table 4-56). An error was defined as swimming toward the start position or circling in the same position without moving forward toward the escape platform. Rats were sacrificed at the end of the experiment, and tissues were taken from the lung, heart, thymus, kidney, liver, pancreas, skeletal muscle, and spleen. Tissues were fixed and prepared for histologic examination by light microscope. No differences were noted in food consumption or body weight gain for either male or female rats (Malek et al., 2003c). No treatment-related differences in organ pathology were reported (with the possible exception of focal microatelectasis (lung collapse at the microscopic level) seen in two to three animals in each formaldehyde-exposed group but not control animals).

Table 4-56. Effects of formaldehyde exposure on completion of the labyrinth test by male and female LEW.1K rats

Male rats	Swimming time (sec)			Error rate (mean)		
	Day 1	Day 6	Day 10	Day 1	Day 6	Day 10
Control	105	12.2	6.33	7.4	0.5	0.0
0.1 ppm[a]	100	12.9	6.07	7.7	5.0[c]	3.2[c]
0.5 ppm	97	16.7[c]	7.60[b]	7.6	4.4[c]	1.8[c]
5.4 ppm	105	25.7[c]	10.9[c]	7.7	5.0[c]	2.8[c]
Female rats	**Swimming time (sec)**			**Error rate (mean)**		
	Day 1	Day 6	Day 10	Day 1	Day 6	Day 10
Control	103	12.5	6.47	7.9	0	0.0
0.1 ppm	96	12.3	7.53	7.1	5.2[c]	3.0[c]
0.5 ppm	97	14.6[c]	7.60[b]	8.0	4.6[c]	2.2[c]
5.4 ppm	98	23.5[c]	9.73[c]	7.9	5.2[c]	2.6[c]

[a]Rats were exposed to formaldehyde for 2 hours/day, for 10 consecutive days.
[b]Different from control, $p < 0.05$.
[c]Different from control, $p < 0.005$.

Source: Malek et al. (2003c).

A clear learning curve was evident in control animals, with rats completing the task in less time and with fewer errors over days (Table 4-56). Although the number of errors decreased with increasing experience in all groups, error rates in formaldehyde-exposed rats at all doses were consistently higher than those observed in controls, starting on day 3 (Figure 4-24). All control animals performed without errors by day 6, whereas all treated animals were still making two to three errors on day 10, the final day of testing. Time required (latency) to complete the maze was also reduced over days. Although this measure of performance was not as sensitive as

error rate, formaldehyde-induced deficits were still evident in the 0.5 and 5.4 ppm exposure groups of both sexes.

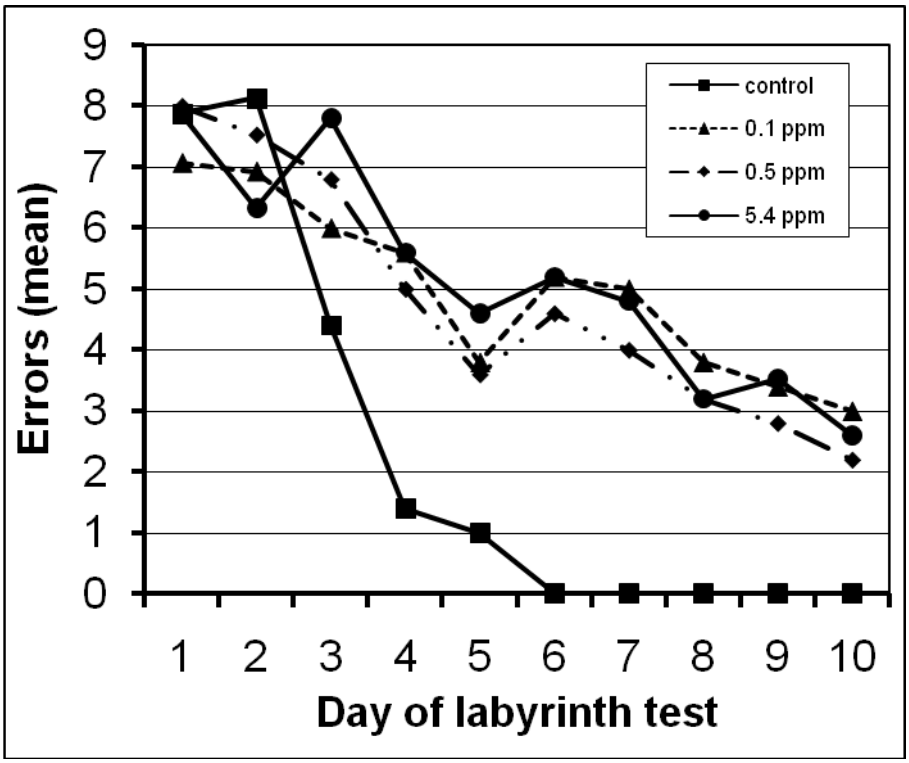


Figure 4-24. Effects of formaldehyde exposure on the error rate of female LEW.1K rats performing the water labyrinth learning test.

Source: Drawn from data reported in Malek et al. (2003c).

Impaired performance on formaldehyde-treated subjects cannot be attributed to alterations in swimming ability, since latencies to complete the maze were identical for 0 and 0.1 ppm groups, yet acquisition of the task was still impaired in the 0.1 ppm group based on number of errors committed (see Figure 4-24). This study reports an adverse effect level of 0.1 ppm for increased error rate in the labyrinth water test, and all dose groups were equally impaired across a broad range of exposures, 0.1–5.4 ppm. An independent estimate of swimming speed was not included, so motor competency could not be directly evaluated. However, comparable latency scores and error rates at the beginning of testing across all groups and latency scores that track together over days suggest that impaired swimming ability does account for the observed differences in latency, which are most likely reflective of the increased

number of errors in treated animals (errors usually increase the distance traveled and thus time required for completion of the trial).

Pitten et al. (2000) evaluated the effects of very brief formaldehyde exposures (10 minutes) but prolonged duration (90 days) on previously learned performance in a land version of the labyrinth maze. Adult male and female Wistar rats (13/group) were trained on the task for 14 days, two trials/day. Animals were required to make a series of five left or right turns from the entrance of the maze to retrieve a piece of cheese placed in the goal box at the opposite end. Animals were guided by the experimenter through the maze during this acclimation phase until all subjects were able to retrieve the food without aid. After animals were trained (but prior to formaldehyde exposure), performance was assessed once daily for 11 days, and the latency to complete the maze as well as the number of errors committed when traversing from the entrance to the goal box was recorded. Animals were then assigned to one of three dose groups (five to eight/sex/group) such that task performance was equivalent across groups prior to commencement of formaldehyde exposures. Animals were exposed to 0 ppm, 2.6 ppm (0.25% formaldehyde solution to yield 3.06 ± 0.77 mg/m^3), or 4.6 ppm (0.70% formaldehyde solution to yield 5.55 ± 1.27 mg/m^3) formaldehyde 10 minutes/day, 7 days/week for 90 days. Animals were assessed for performance in the maze every seventh day, at least 22 hours after the exposure on the previous day. At the end of the 90-day exposure period, monitoring of maze performance continued once every 10 days for an additional 30 days. All rats were sacrificed at the end of the postexposure trials and tissue sections were prepared for histologic examination by light microscopy, including liver, trachea, lung, kidney, heart, spleen, pancreas, testicle, and brain. No treatment-related changes in food or water consumption weight gain or in histologic samples obtained at the termination of the experiment were observed.

Pitten et al. (2000) reported that no gender differences existed as a function of formaldehyde treatment; therefore, data were presented by combining sexes. Control rats showed no change in error rate but a slight decrease in running time through the maze during the course of the experiment. The formaldehyde-exposed groups began with a similar performance level and error rate as controls, but their performance degraded over the course of formaldehyde exposure. By the fourth week of exposure, increased numbers of errors were evident in both exposed groups relative to controls. This trend reached statistical significance at the 12-week time point, with a greater than twofold increase in number of errors ($p < 0.05$). Formaldehyde-treated rats also tended to have increased run times through the maze ($p = 0.04$), but no difference was seen by formaldehyde concentration. By 4 weeks after termination of exposure, no statistical differences among the three groups were evident, but the tendency for the two exposed groups to make more errors and have longer latencies remained. Since Pitten et al.

(2000) tested animals after the task was acquired, these results indicate deficits in the retention of a previously learned task.

Lu et al. (2008) evaluated the effects of formaldehyde on performance of mice in a Morris water maze. Kunming mice (five males/group) were exposed to formaldehyde at 0.2, 1, or 3 mg/m^3 6 hours/day for 7 days (measured concentrations: 0.2 $\pm$ 0.01, 0.99 $\pm$ 0.04, and 3.03 $\pm$ 0.16 mg/m^3). Mice were trained to locate a hidden platform in a large, circular tank (106 cm diameter, 31 cm deep). Each animal received four training trials per day, beginning 30 minutes after the end of exposure. During training, latency to locate the platform was recorded for each trial, with a maximum of 60 seconds, after which the animal was guided to the platform. After the last day of training, an additional trial was conducted with the platform removed (the probe trial); time spent in each maze quadrant was measured to determine the time the animal spent searching for the platform in the correct area of the maze. Performance in the water maze, measured as mean escape latency across the seven training trials, was significantly impaired in the 3 mg/m^3 group. No significant difference was seen at 1 mg/m^3, although there appeared to be an increased latency during the second day of testing. During the probe trial, control animals spent significantly more time in the correct quadrant, but neither formaldehyde-exposed group did so. Results of this study indicate deficits in learning and retention of the Morris water maze following formaldehyde exposure, with greater effects seen in the higher dose group.

Apfelbach and Reibenspies (1991) published a brief report of formaldehyde effects on olfactory learning. Ferrets were exposed to 0.25 ppm (0.31 mg/m^3) formaldehyde gas continuously for 6 months. A Y-shaped maze was used to test odor detection, discrimination between odors, and odor threshold. Ferrets were conditioned to distinguish ethyl acetate (0.1 vol%) from clean air. Untreated ferrets achieved 75% success after an average of 110 trials. However, formaldehyde-treated ferrets required on average 320 trials to reach a 75% success rate. A 90% success rate was achieved by untreated ferrets after 420 trials. However, this level of success was not reached in formaldehyde-treated ferrets.

The same researchers also tested olfactory function in formaldehyde-treated ferrets, as summarized in Section 4.2.1.7 (Apfelbach et al., 1992). A decrease in olfactory discrimination and a reduction in the percentage of olfactory cells in the olfactory epithelium were reported after 3–12 months exposure to 0.25 or 0.5 ppm formaldehyde. Decreased olfactory sensitivity in rats exposed to 0.25 or 0.5 ppm formaldehyde has also been reported by the same researchers (Weiler and Apfelbach, 1992; Apfelbach and Weiler, 1991), and Weiler and Apfelbach (1992) reported in an abstract that shifts in olfactory thresholds were greater when exposure was initiated at PND 30 than at adult ages. Given the documented changes in olfactory thresholds,

observed changes in olfactory learning would likely be confounded by the potential for decreased olfactory function by formaldehyde exposures, and definitive conclusions regarding formaldehyde effects specific to learning cannot be made based on these studies.

4.2.1.6.1.4. ***Neurosensitization.*** Sorg et al. (1996) studied the potential for formaldehyde exposure to induce sensitization in the CNS, possibly through the limbic pathways in the brain. The authors hypothesized that multiple chemical sensitivity (MCS) has an onset and progression similar to CNS sensitization and may, therefore, be a similar process. These experiments were conducted to test this hypothesis and to determine whether formaldehyde exposure could be used as a model for MCS. Behavioral sensitization can be initiated by psychostimulants (e.g., cocaine) and manifest as increased locomotor activity upon subsequent challenge with the stimulant.

Sorg et al. (1996) evaluated cross-sensitization of cocaine-induced increases in activity from an initial formaldehyde exposure. Female Sprague-Dawley rats (eight to nine) were exposed to 0 or 11 ppm (0 or 13.5 mg/m^3) formaldehyde 1 hour/day for 7 days. Locomotor activity was measured (by photocell) after saline injection (1 day postexposure) and after cocaine injection (2 days postexposure). A similar protocol was conducted on days 36 and 37 postexposure. Motor activity levels following saline injection were similar for controls and formaldehyde-treated rats. However, formaldehyde exposure initiated sensitization to cocaine as evidenced by a greater increase in locomotor activity in mice treated with formaldehyde followed by cocaine ($p < 0.05$) with an average count of crossed grids greater than 40,000 (2 hours) in treated animals compared with 25,000 (2 hours) in controls. The cross-sensitization was transient, with no treatment effects on cocaine-induced activity either 29 or 37 days postexposure. When examining individual data, the authors suggested that the formaldehyde-treated groups in both cases have a cluster of high responders (HRs), suggesting some animals may have been more sensitive. A second group of similarly treated female rats was pretested for locomotor activity and divided into subgroups of HRs or low responders (LRs). They were then given a panel of neurobehavioral tests: anxiety (elevated plus maze, day 11); memory (passive avoidance training, day 12; passive avoidance test, day 19); and nociceptive test (day 20). Trunk blood corticosterone levels were determined during stress on day 35 postexposure. No significant treatment differences were found in the passive avoidance test, nociception, or corticosterone levels (basal or stress induced). On the elevated plus maze, a two-way ANOVA indicated no overall formaldehyde treatment effects, but the HR rats had higher open arm time ratios (indicating greater anxiety) regardless of treatment. Within the treatment groups, the difference in behavior between HR and LR subgroups was only significant for the formaldehyde-

treated rats ($p < 0.05$). Authors suggested that cross-sensitization to cocaine-induced locomotor activity was caused by enhanced dopamine transmission within the mesolimbic system (ventral tegmental area to nucleus accumbens projection) following repeated formaldehyde exposure. A critical role of the hypothalamic-pituitary-adrenal (HPA) axis has also been implicated in cross-sensitization.

Sorg et al. (1998) and Sorg and Hochstatter (1999) further explored formaldehyde-induced behavioral sensitization using the cocaine model. In contrast to the results following exposure to 11 ppm (Sorg et al., 1996), rats exposed to only 1 ppm for 7 days showed no cross-sensitization to cocaine injection. However, animals exposed to 1 ppm formaldehyde for 4 weeks exhibited increased cocaine-induced vertical activity (with no difference in horizontal activity) for 4–6 weeks after cessation of exposure. Activity levels of formaldehyde-exposed rats were approximately threefold those of control rats 3–4 days postexposure and still 1.5-fold control levels at 4–6 weeks postexposure ($p < 0.05$).

Sorg et al. (2001) examined changes in corticosterone levels in rats with and without formaldehyde treatment. Basal corticosterone levels in trunk blood were established in naïve male Sprague-Dawley rats taken directly from their home cage immediately prior to sacrifice. In an acute trial, male rats were exposed to 0, 0.7, or 2.4 ppm (0, 0.86, or 2.96 mg/m^3) formaldehyde for either 20 or 60 minutes, and trunk blood was collected for corticosterone analysis. Therefore, these rats were challenged with a new environment (the exposure chamber) in the presence or absence of formaldehyde. In a separate trial, basal and challenged corticosterone levels were measured after repeated exposure (1 hour/day, 5 days/week for 2 or 4 weeks). Basal corticosterone levels were measured in trunk blood immediately after removing the animal from its home cage. Challenged corticosterone levels were measured after rats were placed into the exposure chamber for a final 20-minute exposure. Body weight was measured at the beginning of each week of exposure and was unchanged by formaldehyde treatment.

Corticosterone levels were increased over basal levels when rats were placed in the exposure chamber for 20 minutes (Figure 4-25, panel a) but returned to basal levels after 60 minutes in the exposure chamber (not shown). This response may reflect the stress of the new environment and acclimatization after 60 minutes in the chamber. Corticosterone levels were the same in the presence or absence of formaldehyde, indicating no treatment effect.

Panel a Panel b Panel c

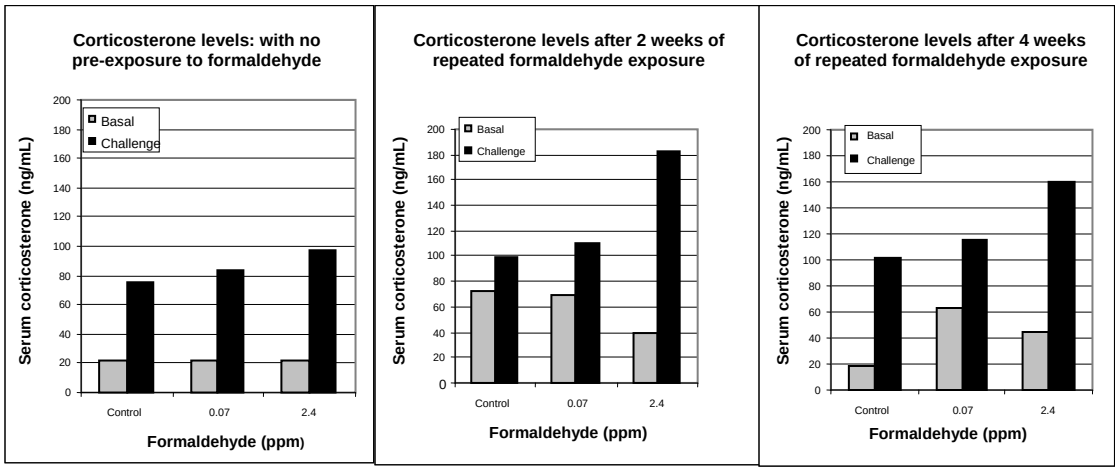


Figure 4-25. Basal and stress-induced trunk blood corticosterone levels in male LEW.1K rats after formaldehyde inhalation exposures.

Note: Panel a: no pretreatment, corticosterone levels after 20-minute formaldehyde exposure. Panels b and c show both basal and induced corticosterone levels after a 2- or 4-week pretreatment to formaldehyde 1 hour/day. Challenge to induce corticosterone was a 20-minute reexposure at the formaldehyde level tested.

Source: Sorg et al. (2001).

Control animals exhibited an increase in basal corticosterone after 2 weeks, which returned to naïve levels after 4 weeks (Figure 4-25, panels b and c). Formaldehyde-treated rats demonstrated a comparable increase in basal corticosterone levels at 2 weeks, but these levels did not return to naïve levels at 4 weeks as seen with controls. Control and 0.7 ppm exposed rats showed a similar response to challenge (the final 20-minute exposure). However, rats exposed to 2.4 ppm were hyperresponsive, with exaggerated corticosterone levels during this final exposure. Differences in basal corticosterone levels after formaldehyde exposure and the hyperresponsiveness seen in animals exposed at 2.4 ppm provide evidence of formaldehyde-induced perturbations of the HPA axis. Authors suggested that elevated corticosterone levels induced by repeated formaldehyde exposures may contribute to the cross-sensitization to cocaine-induced motor activity.

Formaldehyde-induced changes in the HPA axis may contribute to behavioral effects of formaldehyde exposure reported by Sorg et al. (2004) and Sorg and Hochstatter (1999). The authors also reported an enhanced conditioning to odor in animals previously exposed to

repeated formaldehyde. Male and female Sprague-Dawley rats (60–80 days of age) were exposed at 1 ppm (1.23 mg/m^3) formaldehyde 1 hour/day, 5 days/week for 4 weeks (Sorg and Hochstatter, 1999). Two weeks after exposure ended, rats were trained to the conditioned fear task. Rats were conditioned to a fear response by either odor only or odor associated with footpad shock. Orange-oil extract was used as the odor conditioned stimulus (CS). One day after conditioning, rats were reintroduced into the environment without an odor cue, and time spent motionless in the freezing posture (freezing) was observed. On day 2 after conditioning, rats were placed in a novel environment, and time spent in the freezing posture was evaluated in the absence and then the presence of odor. This was repeated on day 12 after conditioning to measure the loss of the freezing response to the conditioned odor.

Both treated and exposed rats showed similar responses on reintroduction into the conditioning environment in the absence of an odor cue on day 1 (Sorg and Hochstatter, 1999). As expected, rats conditioned with a footpad shock demonstrated greater time motionless than odor-trained only rats, and there was no difference between control and formaldehyde-treated rats. However, in the presence of odor on days 2 and 12, formaldehyde-exposed rats who were conditioned with odor associated with foot shock spent significantly more time freezing than odor-only trained rats ($p < 0.05$); control animals on those days showed no difference in time freezing in the presence and absence of odor. The authors concluded that the formaldehyde-treated rats had more difficulty than controls in extinguishing the fear response to the conditioned odor, and speculated that an enhancement of the fear-conditioned response by formaldehyde pretreatment supports the hypothesis that sensitization may include effects through the limbic system of the brain.

In a second experiment, adult male and female Sprague-Dawley rats were exposed at 0 or 2 ppm (2.45 mg/m^3) formaldehyde 1 hour/day, 5 days/week for 4 weeks (Sorg et al., 2004). Two to 3 weeks after exposure ended, rats were trained to the conditioned fear task. Rats were given a foot shock either associated with an odor (paired group) or unassociated with an odor (unpaired group). Orange-oil extract was used as the odor CS. After training, freezing behavior was assessed (1) in the same context in the absence of odor (1 day), (2) in a new context in the presence and absence of the CS (5 consecutive days), and (3) in another novel context in the presence and absence of the CS.

Formaldehyde-exposed male rats demonstrated increased conditioned fear response to an odor CS (orange oil) paired with foot shock with no change in the degree of conditioning to the context. For female rats, formaldehyde exposure did not affect the percent of time spent freezing, either in the conditioning context or the novel context in the absence of the conditioned odor. In contrast, male rats spent an increased time freezing in a novel context in the presence of

odor, indicating a greater conditioned fear response to the olfactory cue ($p < 0.05$). This is in agreement with the previous study where formaldehyde effects were seen in the presence of the conditioning odor but not the environment (Sorg and Hochstatter, 1999). However, in this study female rats did not exhibit a similar enhancement of fear conditioning to the olfactory CS.

The authors suggested that repeated exposure to low levels of formaldehyde acts as a stressor in much the same way as inescapable foot shock, with resulting sensitized responses within the olfactory/limbic pathways (Sorg et al., 2004). This interpretation is consistent with work described above in which augmented basal corticosterone levels following repeated formaldehyde exposures were demonstrated. However, while the fear conditioning in the present study and cross-sensitization to cocaine described above (Sorg and Hochstatter, 1999) occurred 3–4 weeks after termination of exposure to formaldehyde, the duration of corticosterone elevation induced by repeated exposure to formaldehyde has not been determined. It is possible that augmentation of corticosterone levels following formaldehyde exposure results from direct action of formaldehyde on the HPA axis. Experiments designed to compare HPA activation following standard stressors (repeated inescapable foot shock or restraint stress), stress induced by other irritants (chemicals with strong irritant odors but no CNS action), and repeated formaldehyde exposures are necessary to dissociate primary from secondary action of formaldehyde on CNS function in this paradigm. It is also possible that enhanced conditioning to an odor stimulus results from formaldehyde-induced increases in airway irritation, rendering the conditioned odor stimulus a more salient cue, producing a conditioned response that is not extinguished as readily as in air-exposed controls. However, damage of the nasal mucosa and lesions would be expected to be minimal at 1 ppm formaldehyde exposures and most likely resolved 2 weeks after exposure was ended (see Section 4.2.1.2). Therefore, a more salient cue for fear conditioning to odor due to physical irritation is not likely. Alternatively, formaldehyde may act to up regulate olfactory activity, producing a stronger sense of odor during conditioning.

4.2.1.6.1. 5. ***Neurochemistry and neuropathology.*** Several studies that were focused on general toxicity or carcinogenicity of formaldehyde also assessed histopathology in exposed animals, including pathological evaluation of the brain. In all cases, details of the pathological evaluation were not provided. Reported results stated that no significant lesions were seen on unspecified tissues (Appelman et al., 1988; Maronpot et al., 1986; Kerns et al., 1983) or that an increase in relative brain weight (data not provided) was considered of no toxicological significance (Woutersen et al., 1987). The absence of procedural information, or specific reported results, limits the utility of this information.

Boja et al. (1985) measured changes in several neurotransmitters (norepinephrine, dopamine, 5-hydroxytryptamine) and their major metabolites (3,4-dihydroxyphenylacetic acid [DOPAC] and 5-hydroxyindoleacetic acid [5-HIAA]) following one or two 3-hour exposures to formaldehyde at 0, 5, 10, or 20 ppm. Animals were sacrificed immediately following the second exposure, and brains were immediately removed, frozen, and sectioned. Regions of interest were analyzed by high-pressure liquid chromatography with electrochemical detection. Authors stated that neurotransmitter concentrations were measured in multiple brain regions, but results were reported only for the 5 ppm exposure and only for the hypothalamus. No change was seen in concentrations of norepinephrine or 5-hydroxytryptamine for any exposure paradigm. For those animals exposed twice to formaldehyde, there was a slight (statistically significant) increase in dopamine and a larger (approximately fourfold) increase in 5-HIAA. DOPAC was increased (approximately 30%) in animals receiving formaldehyde during the second exposure only.

Recent work by Hayashi et al. (2004) indicates that formaldehyde exposure increases the activity of periglomerular (PG) cells in the main olfactory bulb. Tyrosine hydroxylase activity was measured as a marker for activity of olfactory function. The authors surmised that expression levels of this enzyme are useful markers since it has been reported that the protein is up regulated after sensory stimulation and is down regulated by odor deprivation or when the olfactory epithelium is removed (Cho et al., 1996; Stone et al., 1991; McLean and Shipley, 1988; Baker et al., 1983). Eight-week-old female C3H/HeN mice were exposed at 0, 0.08, 0.4, or 2 ppm (0, 0.1, 0.49, or 2.45 mg/m^3) formaldehyde 16 hours/day for 1 day or 12 weeks (5 days/week). Formaldehyde exposure did not affect body weight. Mice were sacrificed 24 hours after exposure; the brains were removed, fixed, and prepared for sectioning. One side of the olfactory bulb was sliced into 40 µm-thick serial frontal sections and immuno-stained for tyrosine hydroxylase activity. The number of tyrosine hydroxylase-positive PG cells was determined by examining digital photomicrographs of three tissue sections, averaging the counts from 10–15 glomeruli per section.

Neither the size of the olfactory bulb (rostrocaudal, dorsoventral, and mediolateral lengths) nor the total number of PG cells was changed by formaldehyde exposure. The number of tyrosine hydroxylase-positive PG cells per glomerulus was unchanged by a single formaldehyde exposure but increased after 12 weeks of repeated exposures. The increases were similar among treatment groups: 5.54 ± 0.31 at 0.80 ppm, 5.18 ± 0.60 at 0.4 ppm, and 6.0 ± 0.83 at 2 ppm or 196, 167, and 196% of controls, respectively. As an indicator of activity, it is not unexpected that the enzyme was up regulated after repeated exposure to an odorous compound. Hayashi et al. (2004) hypothesize that the increased tyrosine hydroxylase activity is an indication of increased sensitivity and, therefore, may be a model for MCS. However, it is unknown if the

increase in enzyme activity after repeated exposures is transient or could result in sensitization. Tyrosine hydroxylase is the first enzyme in the dopamine synthetic pathway, but the role of dopamine in PG cells is not known. Further research would be needed to understand the potential for formaldehyde to act as a sensitizing agent in this model.

In an abstract, Kakeyama et al. (2004) outline the results of experiments to address the effects of subchronic exposure to low levels of formaldehyde on changes in neurotransmitter-related mRNA expressions in mice forebrains. An unstated number of female C3H/He mice were exposed 16 hours/day, 5 days/week to 400 ppb (0.49 mg/m^3) formaldehyde for 12 weeks. The authors used RT-PCR methodologies to quantify mRNA encoding for the glutamate receptor subunits GluR1 and GluR2, the dopamine receptor D1, and the serotonin receptor 5-HT1A in the neocortex, hippocampus, amygdala, and hypothalamus. Raised levels of mRNA expression were observed for GluR1 in the neocortex and hippocampus; GluR1, GluR2, and the dopamine receptor D1 in the amygdala; and the serotonin receptor 5-HT1A in the hypothalamus. Reduced mRNA expression was observed for GluR2 in the hippocampus and neocortex. When other mice were subjected to a radiofrequency-induced lesion of the hippocampus then exposed to formaldehyde for 12 weeks as before, the altered expression of GluR1 and GluR2 in the neocortex was abolished. However, the increment of mRNA expression of 5-HT1A in the hypothalamus was further enhanced. In demonstrating that formaldehyde affects neocortical GluR1 and GluR2 mRNA expressions through a hippocampal function, Kakeyama et al. (2004) concluded that subchronic exposure to low concentrations of formaldehyde can affect neural transmission in the forebrain.

Fujimaki et al. (2004b) examined the effects of formaldehyde on NGF in the brain and hippocampus. Ten female C3H/HeN mice/group were exposed to 0, 80, 400, or 2,000 ppb (0, 0.1, 0.5, or 2.45 mg/m^3) formaldehyde 16 hours/day, 5 days/week for 12 weeks. Some groups of mice received the same treatment after I.P. injection of 10 µg of OVA and 2 mg alum prior to the commencement of formaldehyde exposure. For this subgroup, booster injections of OVA were administered on days 21, 42, 63, and 77 during the formaldehyde exposure regimen. Quantitative measures of NGF and BDNF in homogenates of whole brain and hippocampus were obtained by ELISA and mRNA determination. The amount of NGF protein in whole brains remained unchanged in the non-immunized mice. However, brain NGF levels were significantly increased in OVA-immunized mice exposed to 80 and 400 ppb (but not 2,000 ppb) formaldehyde (Figure 4-26). This result was confirmed by parallel increases in the concentrations of hippocampal NGF mRNA that were produced in immunized mice exposed to formaldehyde at the same concentrations. However, there were no comparable increases in the amounts of brain-derived neurotrophic factor in either immunized or non-immunized mice. In

discussing the mechanisms potentially associated with their results, Fujimaki et al. (2004b) considered it likely that low-level exposure to formaldehyde could enhance NGF production through the stimulation of the HPA axis together with immunization.

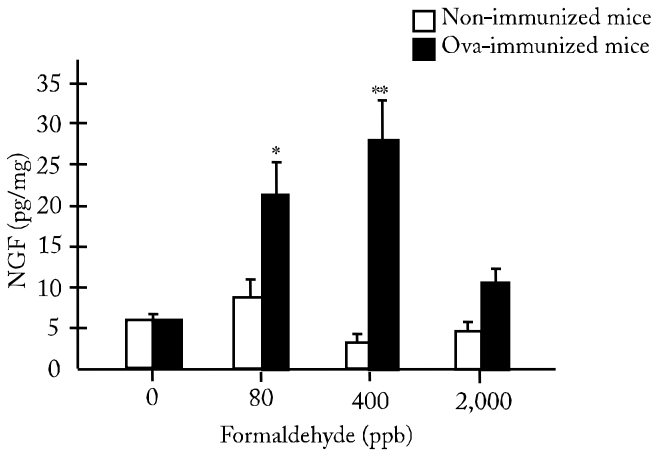


Figure 4-26. NGF production in the brains of formaldehyde-exposed mice.

Note: Female C3H mice were exposed to formaldehyde 16 hours/day, 5 days/week for 12 weeks. NGF in homogenates of whole brain and hippocampus were measured by ELISA. Values are means ± SEM (n = 5–6). * = $p < 0.05$ and ** = $p < 0.01$ versus control mice, as calculated by the authors.

Source: Redrawn from Fujimaki et al. (2004b).

The enhancement of NGF in the brains of immunized mice exposed to formaldehyde gave rise to the suggestion that NGF may promote the survival of hippocampal neurons when challenged with formaldehyde. To examine whether or not apoptosis plays a role in this process, Tsukahara et al. (2006) measured the effects of formaldehyde on apoptotic mechanisms regulating the survival and death of cells and on N-methyl-D-aspartate (NMDA) receptors. Female C3H/HeN mice (13/group) were exposed to 0 or 400 (393 ± 34) ppb (0 or 490.8 μg/m^3) formaldehyde 16 hours/day, 5 days/week for 12 weeks. Seven control and formaldehyde-treated mice were immunized with 10 μg OVA plus 2 mg aluminum hydroxide prior to exposure. Subsequently, these mice received OVA via aerosol as a booster during weeks 3, 6, 9, and 11. Hippocampi were dissected from all animals 1 day after the final exposure and homogenized in hypotonic buffer. The 12,000 rpm supernatants were analyzed by Western blotting for the presence of the proteins BCl-2 (which inhibits apoptosis) and Bax (which opposes BCl-2 action and promotes apoptosis) and the NMDA receptor subtypes 2A and 2B (NR2A and NR2B).

Immunohistochemical analysis was also carried out for the presence of active caspase-3, an apoptosis marker.

The levels of NR2A and NR2B were unaffected by exposure to formaldehyde in either immunized or non-immunized mice. Likewise, the number of caspase-3 immunoreactive cells did not change as a result of formaldehyde exposure. However, when measured amounts of Bcl-2 and Bax were normalized to the amount of β-tubulin, the ratio Bcl-2/Bax was significantly increased in immunized mice exposed to formaldehyde. Non-immunized mice did not show this apparently compound-related response. Consistent with the concept that the proportions of Bcl-2 and Bax are critical for the regulation of cell survival and death, the authors interpreted their data as an indication that changes to the ratio of Bcl-2/Bax expressions might be an important adaptive response to the effects of formaldehyde, such that the antiapoptotic changes might contribute to the protection of hippocampal neurons from the pernicious effects of formaldehyde exposure itself.

The same research group used the immunized mouse model to determine whether formaldehyde exposure affected mRNA expression of genes related to synaptic plasticity (Ahmed et al., 2007). Ten female C3H/HeN mice were exposed to 0 or 400 ppb formaldehyde 16 hours/day, 5 days/week for 12 weeks. All mice were immunized with 10 µg OVA plus 2 mg aluminum hydroxide prior to initial formaldehyde exposure then treated in weeks 3, 6, 9, and 11 with aerosolized OVA as a booster. Five treated and control animals were I.P. injected with 1 mg/kg MK-801, a noncompetitive NMDA receptor agonist before the last formaldehyde exposure. At term, hippocampi were dissected and frozen at −80°C until processing. At that point, total mRNA was extracted and first strand cDNA was synthesized by using reverse transcriptase. Expression levels of various proteins/receptors, including NMDA NR2A and NR2B receptor subunits, dopamine D1 and D2 receptors, cyclic AMP responsive element-binding proteins (CREB-1 and CREB-2), and the transcription factors FosB and ΔFosB were determined by using the PCR. The expression level of each mRNA species was expressed relative to the sample's content of 18S rRNA. The total protein lysate was also assayed for pCREB by Western blotting.

In the first of a sequence of histograms, Ahmed et al. (2007) demonstrated a significant increase in mRNA expression of NR2A as a result of formaldehyde exposure. However, this effect was abolished in animals treated with MK-801. A similar trend in the mRNA expression of NR2B in response to formaldehyde exposure did not achieve statistical significance. MK-801 treatment significantly reduced receptor in mRNA expression in the presence of formaldehyde. The authors provided data showing an increased expression of dopamine D1 and D2 receptor mRNA response to formaldehyde, in both cases abolished by treatment with MK-801. The

expression of CREB-1 mRNA also conformed to the pattern of being increased as a result of formaldehyde exposure but abolished by MK-801. However, the expression of CREB-2 and FosB/ΔFosB was unaffected by formaldehyde. When normalized to the amount of β-tubulin, there were no significant effects of formaldehyde exposure and MK-801 treatment on the protein levels of pCREB. Finally, there was no significant difference in the expression of transient receptor potential vanilloid receptor (TRPV1) between control and formaldehyde-exposed mice, and MK-801 itself did not significantly alter the mRNA level of TRPV1. In seeking to explain their results, the authors speculated that low-level exposure of immunized mice to formaldehyde had an effect on hippocampal synaptic plasticity at the mRNA level, as evidenced by the enhancement of mRNA for NR2A, the dopamine D1 and D2 receptors, and CREB1, with up regulation compensating for the sustained levels of enhanced protein expression under low-level formaldehyde exposure. The interpretation of these changes in NR2A mRNA, in the context of the results of Tsukahara et al. (2006), showing no change in NR2A and NR2B protein expression, was not discussed.

4.2.1.6.1.6. ***Neurogenesis.*** Two papers have examined the effects of subacute exposure to formaldehyde on the overall size (volume) of discrete cellular areas of the hippocampus in neonatal rats. The researchers also used an optical fractionator counting method to derive a plausible estimate of cell number. Aslan et al. (2006) studied the effects of formaldehyde exposure on the number and volume of granular cells in the hippocampal dentate gyrus. Sarsilmaz et al. (2007) examined the impact of postnatal formaldehyde exposure on brain hemisphere volume and on the size and cell number of pyramidal cells in the cornu ammonis region of the hippocampus. The in-life phase was the same in each study, featuring the exposure of 10 neonatal male Wistar rats/group to 0, 6, and 12 ppm (0, 7.36, and 14.7 mg/m^3) formaldehyde 6 hours/day, 5 days/week for 30 days. Five rats/group were sacrificed at that point (PND 30), while the rest were maintained without further treatment until PND 90.

For both pyramidal and granular areas, a much lower number of cells was seen on PND 90 versus PND 30 ($p < 0.001$). This response was evident irrespective of the amount of exposure to formaldehyde and is consistent with normal brain development. Compound-specific effects of formaldehyde on the volume and number of granular and pyramidal cells varied by dose and over the two time points. There was a small increase in the volume of the granular cell layer of the dentate gyrus in rats sacrificed on PND 30 ($p < 0.001$) in response to increasing formaldehyde concentration (Aslan et al., 2006), with no significant change in neuron number; the increased volume (now accompanied by an increase in neuron number) was still evident at the low-exposure level on PND 90 ($p < 0.01$) but not at the high dose. Brain hemisphere volume

was decreased at both concentrations on PND 30 ($p < 0.01$) but was increased at both concentrations ($p < 0.01$, with a larger magnitude of effect at 6 ppm) on PND 90 (Sarsilmaz et al., 2007). In the hippocampal cornu ammonis region, the volume of the pyramidal cell layer on PND 30 was increased in low-dose animals ($p < 0.001$) but decreased in high dose animals ($p < 0.001$) as compared with control values; neither group was significantly different from controls on PND 90. There was a dose-related decrease in total neuron number in the cornu ammonis on PND 30 ($p < 0.01$ for both doses); on PND 90 the decrease in neuron number remained statistically significant in both treatment groups ($p < 0.01$), but there was no longer any difference in the magnitude of the effect between doses (Sarsilmaz et al., 2007).

In a third study from the same laboratory, Songur et al. (2008) used the same exposure paradigm to evaluate changes in oxidant and antioxidant systems in the cerebellum of perinatally exposed rats. Exposure was carried out as in Aslan et al. (2006) and Sarsilmaz et al. (2007), described above. On PND 30 or 90, cerebellums from seven male rats per group were evaluated for levels of malondialdehyde (MDA), NO, superoxide dismutase activity (SOD), and glutathione peroxidase (GPX) activity. Dose-related increases in NO (approximately 20–80%), MDA (100–160%), and GPX (25–60%) and dose-related decreases in SOD (20–30%) were seen on PND 30. In general, the magnitude of change from control levels was maintained on PND 90, with the exception of MDA levels in 6 ppm animals, which appeared to approach control levels at 90 days. The authors stated that these findings indicated that formaldehyde exposure may cause neurotoxicity via the production of oxidative damage in the brain. Persistence of the effect to the 90-day time point (30 days after cessation of exposure) supports the possibility that formaldehyde may cause long-lasting or permanent changes in the brain following early life exposure. These results are consistent with the earlier studies by Aslan et al. (2006) and Sarsilmaz et al. (2007), finding permanent changes in brain structure (although in a different brain region) following early life exposure.

4.2.1.6.1.7. ***Summary of formaldehyde effects on neurobehavioral and neuropathological measures, following exposure via inhalation.*** As has been demonstrated in mice (Wood and Coleman, 1995), it is possible that rats experience respiratory tract irritation during low-level formaldehyde exposure. Perturbations in nervous system function reported with formaldehyde exposure include reductions in motor activity, lack of habituation, impairment in acquisition of a new learning task, deficits in retention of a previously learned task, increases in corticosterone levels, sensitization to cocaine-induced locomotor activity, and enhanced fear conditioning using an olfactory CS (Table 4-57). Many of these effects were observed at exposure levels at or below 1 ppm, and some persisted days to weeks after termination of exposure.

Malek et al. (2004, 2003a, b) detected behavioral changes in rats and mice tested 2 to 24 hours postexposure. The mechanism of these behavioral changes is unknown, and available data do not allow dissociation of direct effects on the nervous system and behavioral responses to the irritant effects of formaldehyde (control experiments [e.g., using a different aversive odor with or without irritant properties] were not included). Given that behavioral changes were observed hours to days after cessation exposure (i.e., beyond the time required for formaldehyde-induced irritation to subside), it is unlikely that these behavioral changes were caused by formaldehyde-induced irritation. Similarly, although it is possible that systemic effects of formaldehyde exposure might cause reduced motor activity during or immediately following exposure, it is unlikely that these effects can account for the differences in responses of male rats 24 hours after exposure (Malek et al., 2003a). Furthermore, a follow-up study demonstrated reduced motor activity in animals 2 hours after a 2-hour exposure to much lower levels of formaldehyde (0.1 ppm), which fall well below the levels identified by Wood and Coleman (1995) as the AC_{50} for formaldehyde in mice (Malek et al., 2003b).

Two studies reported significant reductions in learning or retention following brief periods of repeated exposure to low levels of formaldehyde (Malek et al., 2003c; Pitten et al., 2000) (Table 4-57). Malek et al. (2003c) reported an increased number of errors in acquiring a water maze task; testing took place daily 2 hours after termination of a 2-hour exposure. The work of Pitten et al. (2000) revealed that brief exposures over many weeks led to increases in errors performing a previously learned task and that the magnitude of the effect increased over the course of the exposure period. Testing occurred remote from the time of exposure (22 hours after the previous exposure), and the deficits appeared to persist for several weeks after exposure terminated, minimizing the possibility that these effects were related to irritant properties of formaldehyde. Although the exposure levels were moderately high (2.6–4.6 ppm) and continued over several months, the duration of a single exposure event was very brief (10 minutes).

Sorg and Hochstatter (1999) and Sorg et al. (2004, 2001) suggest that behavioral sensitization associated with low-level formaldehyde exposure was linked to alterations in HPA control of corticosterone. Cross-sensitization to the locomotor activity-enhancing properties of cocaine and changes in response to a conditioned fear paradigm were observed in animals exposed several weeks earlier to repeated low-level formaldehyde. Direct activation of mesolimbic dopamine pathways or activation of conditioned fear response in the amygdala by formaldehyde could underlie these behavioral effects; these observations were also seen by study authors as consistent with a formaldehyde-induced stress response.

Table 4-57. Summary of neurological and neurobehavioral studies of inhaled formaldehyde in experimental animals

Species	No./ group	Treatment[a]	Observations	LOAEL/ NOAEL	Reference
Irritant detection threshold					
Male Swiss mice	8	0, 1, 1.8, 3, 5.6, or 10 ppm 60-second exposure episode to determine irritant response	*Sensitivity of mice to acute formaldehyde levels* determines the median concentration at which 50% of exposures were terminated by the subject (AC_{50}) decreased upon repeat exposure. AC_{50} = 3.63 for first series, AC_{50} = 1.88 ppm for second series. Time to exposure termination decreased with increasing formaldehyde concentration. Time to termination was decreased in repeat exposures.	NA[b]	Wood and Coleman (1995)
Motor activity and habituation					
Male and female LEW.1K rats	10/sex	0, 1, 2.5, or 5 ppm for 2 hours	*Reduced horizontal activity*: Number of crossed quadrants reduced 2 hours after exposure at all doses for males and females. *Reduced habituation*: Exposed rats exhibited greater activity than controls when reintroduced into the testing environment 24 hours later (males and females, all doses).	LOAEL = 1 ppm 2 hours	Malek et al. (2003a)
Male and female LEW.1K rats	10/sex	0, 0.1, 0.5, or 5 ppm for 2 hours	*Reduced horizontal activity*: Number of crossed quadrants reduced 2 hours after exposure at all doses for males and females.	LOAEL = 0.1 ppm 2 hours in males	Malek et al. (2003b)
Male AB mice	5–7/sex	0, 1.1, 2.3, or 5.2 ppm for 2 hours	*Reduced horizontal activity*: Number of crossed quadrants reduced 2 hours after exposure at all doses.	LOAEL = 1.1 ppm 2 hours	Malek et al. (2004)
Balb/c mice	6	0, 1.8, 3.2, 4.5, 6.4, 9.7, or 14.8 ppm for 3 hours	*Reduced horizontal and vertical activity*: Dose-dependent decreases in crossed quadrants and rearing. Significant for males at 1.8 ppm and greater ($p < 0.01$). Significant for females at 6.4 ppm or greater ($p < 0.01$).	LOAEL = 1.8 ppm 3 hours in males	Usanmaz et al. (2002)
Balb/c mice	6	3.3 ppm for 2 weeks or 2 ppm for 3 weeks 3 hours/day, 5 days/week	*Reduced horizontal and vertical activity* decreases in crossed quadrants and rearing. 3.3 ppm (2 weeks) and 2 ppm (1 week) ($p < 0.01$, $p < 0.05$).	LOAEL = 2 ppm 3 weeks	Usanmaz et al. (2002)
Sprague-Dawley rats	8	0, 5, 10, or 20 ppm; 3 hours/day for 1 or 2 days	*Reduced activity levels on both days.* Decreases seen at 5 and 10 ppm; data reported only for 5 ppm group	LOAEL = 5 ppm, 3 hours	Boja et al. (1985)

Table 4-57. Summary of neurological and neurobehavioral studies of inhaled formaldehyde in experimental animals

Species	No./ group	Treatment[a]	Observations	LOAEL/ NOAEL	Reference
Rats		0 or 0.5 mg/m^3 (0.4 ppm) on GDs 1–19, 4 hours/day	*Increased motor activity on the 2nd and 3rd days of testing (reduced habituation) in offspring exposed in utero.* Increased number of squares entered ($p < 0.01$) and frequency of rearing ($p < 0.05$).	LOAEL = 0.4 ppm, gestational	Senichenkova (1991)
Rats	15	0, 0.005, or 0.0005 mg/L (approximately 4 or 0.4 ppm), GDs 1–19	*Changes in motor activity at one and two months in offspring exposed in utero.* Decreased spontaneous mobility at 1 month in both sexes, increased activity at 2 months in both sexes.	LOAEL = 0.4 ppm, gestational	Sheveleva (1971)
Learning and memory					
Adult male and female LEW.1K rats	15/sex	0, 0.1, 0.5, or 5.4 ppm 2 hours for 10 consecutive days	*Impairment in acquisition of a new task*: Male and female rats at all formaldehyde exposures had significantly more errors in completing a water labyrinth ($p < 0.01$). Male and female rats had longer times to completion of the maze at 0.5 and 5.4 ppm ($p < 0.05$, $p < 0.01$).	LOAEL = 0.1 ppm 2 hours/ 10 days	Malek et al. (2003c)
Adult male and female Wistar rats	5–8/ sex	0, 2.6, 4.6 ppm 10 minutes/day for 90 consecutive days	*Deficit in the retention of a learned task*: Male and female rats committed significantly more errors ($p < 0.05$) and took more time to complete the land maze in across the course of the experiment ($p < 0.04$).	LOAEL = 2.6 ppm 10 minutes/ 90 days	Pitten et al. (2002)
Ferrets		0.25 ppm	*Impairment in acquisition of a new task*: Exposed ferrets only achieved a 75% success rate in training to discriminate odors in a Y-maze versus 90% success rate in controls. Note: The results are confounded with other effects on the olfactory epithelium. The same researchers also reported a decrease in olfactory sensitivity and a reduction in percentage of olfactory cells in similarly treated animals.	None established	Apfelbach and Reibenspies (1991) (abstract only)
Male juvenile and adult rats	5/group	0.25, 0.5 ppm	*Decreases in olfactory thresholds, in juvenile animals but not in adults* ($p < 0.002$).	LOAEL = 250 ppm in juveniles	Weiler and Apfelbach (1992) (abstract only)

Table 4-57. Summary of neurological and neurobehavioral studies of inhaled formaldehyde in experimental animals

Species	No./ group	Treatment[a]	Observations	LOAEL/ NOAEL	Reference
Neurosensitization endpoints					
Female Sprague-Dawley rats	8–9	0 or 11 ppm 1 hour/day, 7 days	*Increase in cocaine-induced activity*: Increased quadrants crossed after cocaine injection 1 and 2 days after exposure ($p = 0.05$ and $p < 0.04$, respectively). No change in corticosterone levels 28 days postexposure. No change in nociceptive or passive avoidance test or plus-maze (21, 20, and 13 days postexposure, respectively) (21 days).	LOAEL = 11 ppm/ 7 days (unbounded)	Sorg et al. (1996)
Female and male Sprague-Dawley rats	Various up to 24/group	11 ppm, 1 hour/day, 7 days 1 ppm, 1 hour/day, 7 days 1 ppm, 1 hour/day, 5 days/week, 4 weeks	*Increase in cocaine-induced activity*: Increase in rearing after cocaine injection 1 day after exposure but not 4–6 weeks after exposure; 11 ppm for 7 days or 1 ppm for 4 weeks. No change in rats exposed at 1 ppm for 1 week.	LOAEL = 1 ppm 4 weeks NOAEL = 1 ppm 7 days	Sorg and Hochstatter (1999)
Female and male Sprague-Dawley rats	Various up to 24/group	1 ppm, 2 hours/day, 5 days/week, 4 weeks	*Increased conditioned fear response* in formaldehyde-treated, foot-shock-conditioned rats, twofold ($p < 0.05$).	LOAEL = 1 ppm 4 weeks	Sorg and Hochstatter (1999)
Male Sprague-Dawley rats	4–9 or 16	0, 0.7, or 2.4 ppm for 20 or 60 minutes 0, 0.7, or 2.4 ppm 1 hour/day, 5 days/week for 2 or 4 weeks	No change in corticosterone in acute (20- and 60-minute) exposures. *Increase in basal corticosterone*: 0.7 ppm for 2 or 4 weeks. *Hyperresponsive corticosterone response to environment*: 2.4 ppm for 2 or 4 weeks.	LOAEL = 0.7 ppm/ 2 weeks NOAEL = 0.7 ppm/ 20 minutes	Sorg et al. (2001)
Female and male Sprague-Dawley rats	4–9 or 16	0 or 2 ppm 1 hour/day, 5 days/week for 4 weeks	*Increased conditioned fear response* to an olfactory cue in formaldehyde-treated, foot-shock-conditioned male rats. Measured as increased time freezing when presented with a novel environment ($p < 0.05$). *No effect in female rats*.	LOAEL = 2 ppm/ 4 weeks NOAEL = 2 ppm/ 4 weeks	Sorg et al. (2004)

Table 4-57. Summary of neurological and neurobehavioral studies of inhaled formaldehyde in experimental animals

Species	No./ group	Treatment[a]	Observations	LOAEL/ NOAEL	Reference
Neurochemistry and neuropathology					
8-week-old female C3H/ HeN mice	5	0, 0.08, 0.4, or 1 ppm 16 hours/day, 5 days/week for 1 day or 12 weeks	No change in size of main olfactory bulb by several measures. No change in numbers of PG cells. No change in tyrosine hydroxylase immunopositive PG cells after 1 day. *Increase in tyrosine hydroxylase-immunopositive PG cells* after 12 weeks to 196, 167, and 196% of controls at 0.08, 0.40, and 1 ppm, respectively.	LOAEL = 0.08 ppm/ 12 weeks	Hayashi et al. (2004)
Adult male Sprague-Dawley rats	8	0, 5, 10, 20 ppm 3 hours/day, 1 or 2 days	No change in norepinephrine or 5-hydroxytryptamine in hypothalamus. Increase in DOPAC in hypothalamus after one exposure. Increase in dopamine and 5-HIAA in hypothalamus after two exposures.	LOAEL = 5 ppm/ 3 hours	Boja et al. (1985)
Neurogenesis					
Neonatal Wistar rats	5	0, 6, or 12 ppm, 6 hours/day, 5 days/week for 30 days	*Changes in volume of granular cell layer of the dentate gyrus in the hippocampus at postnatal days 30 and 90* ($p < 0.001$)	LOAEL = 6 ppm/ 30 days	Aslan et al. (2006)
Neonatal Wistar rats	5	0, 6, or 12 ppm, 6 hours/day, 5 days/week for 30 days	*Decreases in brain hemisphere volume at PND 30* ($p < 0.01$) *Changes in volume and cell numbers in the CA region of the hippocampus on PND 30* ($p < 0.01$)	LOAEL = 6 ppm/ 30 days	Sarsilmaz et al. (2007)
Neonatal Wistar rats	7	0, 6, or 12 ppm, 6 hours/day, 5 days/week for 30 days	*Changes in oxidant and antioxidant systems in cerebellum on PNDs 30 and 90* ($p = 0.017–0.001$). Increases in MDA, NO, and GSH-Px and decreases in SOD at both time points.	LOAEL = 6 ppm/ 30 days	Songur et al. (2008)

[a]Treatment is given as the formaldehyde concentration in air (ppm) with the length of exposure each day and the duration of treatment in days, as available.
[b]NA = not available.

Limited data regarding possible neurochemical changes in the brains of formaldehyde-exposed, immunized mice (Ahmed et al., 2007; Fujimaki et al., 2004b; Hayashi et al., 2004; Kakeyama et al., 2004) and rats (Boja et al., 1985) provided conflicting information, and the implications of these data regarding possible formaldehyde neurotoxicity are difficult to determine.

In developmental exposure paradigms, changes in brain structure (Sarsilmaz et al., 2007; Aslan et al., 2006), brain chemistry (Songur et al., 2008), and motor activity (Senichenkova, 1991; Sheveleva, 1971) were seen following neonatal or in utero exposure to formaldehyde. In addition, Weiler and Apfelbach (1992) found juvenile animals to be more sensitive to formaldehyde-induced changes in olfactory thresholds when compared with adult animals. These studies raise concern about possible long-lasting neurological effects of early exposure to formaldehyde. It is important to note, however, that exposure levels in these studies were higher (250–6,000 ppb) than those producing the behavioral effects in adults described above.

Overall, available data provide substantial evidence of behavioral effects, including motor activity changes and changes in learning and retention, following repeated exposure to relatively low levels of formaldehyde. These effects were seen in multiple laboratories, in studies conducted by different authors, and using different behavioral paradigms. These conclusions are also supported by more limited data, indicating possible developmental effects on the nervous system, including changes in brain structure and in the behavior of offspring; the developmental findings are less robust since they were seen only in individual laboratories and occurred following exposure to higher concentrations of formaldehyde. Studies evaluating developmental neurotoxicity at lower doses, comparable to those used in the adult studies, were not available. None of the available data provide sufficient information to allow a determination of the mechanism for these behavioral changes, although it is unlikely that they are attributable to the irritant properties of formaldehyde. The data regarding behavioral sensitization provide some support for a stress-related mechanism for those findings, but the applicability of this mechanism to the behavioral changes seen in the other studies, including the learning deficits, has not been evaluated.

4.2.1.6.2. ***Oral exposure.*** Available data regarding neurotoxic effects of formaldehyde exposure following oral exposure are very limited. Several chronic or subchronic oral toxicity studies evaluated changes in brain weight or histopathology in rats or dogs following repeated oral exposures to formaldehyde at doses as high as 300 mg/kg-day, administered in drinking water (Til et al., 1989, 1988; Tobe et al., 1989; Johannsen et al., 1986). Although data were not

presented in the publications, all stated that no changes in brain weight or pathology were seen in the standard evaluations performed in these studies.

Two studies evaluated changes in behavior following exposure to formaldehyde in drinking water (Venkatakrishna-Bhatt et al., 1997; Venkatakrishna-Bhatt and Panchal, 1992). Venkatakrishna-Bhatt and Panchal (1992) evaluated changes in performance on a conditioned avoidance task in adult male albino rats (five/group). Animals were exposed to formaldehyde in drinking water (10 mg/mL) or by I.P. injection (10 mg/kg) for 60 days. Although it was stated that water consumption was recorded, the data were not presented, and thus actual exposure levels cannot be documented. Prior to the initiation of exposure, rats were trained on the conditioned avoidance task (climbing a wooden pole in response to a warning buzzer, thus avoiding electric shock from a floor grid). Rats were trained to a predetermined performance criterion (not described); animals not achieving the criterion were removed from study. Training and testing conditions (e.g., retest interval and duration of sessions) were not well described. Data were presented as percent response in behavioral performance (apparently separately for the escape or avoidance aspects of the task) or percentage decrease in response. No control data were presented, and pretreatment performance was not described. Figures presented performance at 10-day intervals, starting with day 0, with each data point stated to represent the mean for five experimental sets; again, the interval between experimental sets and the number of trials per set was not specified. Although the authors concluded that a deficit in performance was demonstrated, the data as presented were difficult to interpret and the conclusion could not be verified based on the data as presented.

Venkatakrishna Bhatt and Panchal (1992) examined changes in performance on a conditioned avoidance response, presumably using a procedure similar to the one described above. Albino rats (sex not specified, five/group) were exposed to formaldehyde in drinking water at 0.2 or 0.5 mg/mL for 90 days. As described above, rats were trained in performing the task prior to the start of exposure. Venkatakrishna-Bhatt Bhatr and Panchal (1992) stated that there was a dose-related deterioration of performance, but no data were presented to support these conclusions.

In summary, available data are insufficient to conduct a reliable assessment of neurotoxic effects of formaldehyde following oral exposure. Limited data suggest a lack of overt neuropathological changes at doses up to 300 mg/kg-day (Til et al., 1989, 1988; Tobe et al., 1989; Johannsen et al., 1986), but detailed information regarding the types of neuropathological evaluations performed in those studies is not available, and thus no firm conclusions can be drawn regarding the potential for neuropathological effects. The two available studies evaluating

behavioral changes are not considered to provide useful information, and thus effects on nervous system function could not be evaluated.

4.2.1.6.3. ***Summary.*** Overall, there is strong evidence that formaldehyde exposure via inhalation may cause adverse effects on nervous system function in experimental animals at relatively low levels of exposure (LOAELs as low as 100 ppb). Although human data regarding neurotoxicity following formaldehyde inhalation are limited, available data provide support that the types of effects seen in humans are similar to those found in animal studies. Evidence from available human controlled inhalation exposure studies indicates that humans may be affected at doses similar to those used in animal studies; however, the human data are extremely limited.

There are insufficient data to evaluate the potential for neurotoxicity following oral exposure to formaldehyde. Limited evaluations of brain weight or histopathology in available chronic or subchronic oral studies found no evidence of formaldehyde-induced changes (Til et al., 1989, 1988; Tobe et al., 1989; Johannsen et al., 1986). However, reliable studies examining nervous system function or focused studies of neuropathology following oral exposure to formaldehyde are not available.

4.2.1.6.4. ***Other considerations.*** Major data gaps were found regarding the evaluation of changes in nervous system structure or function following formaldehyde exposure by both the inhalation or oral routes.

With respect to inhalation exposure, none of the available human studies resulted in data sufficient to conduct a reliable dose-response assessment for changes in nervous system function. Most of the available animal inhalation studies used short exposure durations (acute or short-term), precluding a reliable evaluation of neurotoxicity following chronic exposure. Available data for neurodevelopmental exposures are also quite limited, consisting of evaluation of neuropathology in only one brain region and functional evaluations focused only on changes in motor activity.

Major data gaps also exist regarding neurotoxicity following oral exposure, with no relevant human data and extremely limited animal data. Available oral exposure studies were insufficient to permit a reliable evaluation of the potential for neurotoxicity following oral exposure to formaldehyde.

4.2.1.7. *Reproductive and Developmental Toxicity*

The potential for developmental and reproductive effects after formaldehyde exposure by the inhalation route has generally been considered low, since formaldehyde, as a reactive gas, is

not expected to penetrate past the POE (NEG, 2003; IPCS, 2002; Collins et al., 2001). Nevertheless, a number of animal studies have demonstrated effects of formaldehyde on pre- and postnatal development and on the reproductive system. For example, developmental toxicity was observed in two studies that evaluated a standard battery of developmental endpoints resulting from inhalation exposure on GDs 6–10 (Martin, 1990; Saillenfait et al., 1989). Similarly, oral exposures resulted in developmental effects when administered during comparable gestational windows (Marks et al., 1980; Hurni and Ohder, 1973). There have also been reports that identified developmental effects at lower-level formaldehyde exposures that were administered throughout gestation (Senichenkova and Chebotar, 1996; Senichenkova, 1991; Kitaev et al., 1984; Sheveleva, 1971; Gofmekler and Bonashevskaya, 1969; Gofmekler, 1968; Pushkina et al., 1968). Postnatal functional consequences of developmental exposures have also been identified (Sarsilmaz et al., 2007; Aslan et al., 2006; Weiler and Apfelbach, 1992; Senichenkova, 1991; Sheveleva, 1971). Additionally, a number of studies suggest that formaldehyde adversely affects the male reproductive system after both inhalation and oral exposures (Xing et al., 2007; Zhou et al., 2006; Özen et al., 2005, 2002; Sarsilmaz et al., 1999; Chowdhury et al., 1992; Cassidy et al., 1983; Guseva, 1972). This section reviews the available published studies assessing reproductive and developmental endpoints of formaldehyde.

4.2.1.7.1. ***Inhalation studies addressing developmental and reproductive toxicity.*** Saillenfait et al. (1989) reported a comprehensive and well-documented developmental study in Sprague-Dawley rats. Pregnant rats were exposed beginning on GD 6 in order to cover critical stages of development (e.g., implantation and major organogenesis). Female Sprague-Dawley rats (25/group) were exposed to 0, 5, 10, 20, or 40 ppm (0, 6.15, 12.3, 24.6, or 49.2 mg/m^3) formaldehyde 6 hours/day on GDs 6–20. The onset of pregnancy was determined by the presence of sperm in a vaginal smear. Dams were exposed to formaldehyde in a dynamic flow chamber, and formaldehyde concentrations were determined to be 0, 5.17 ± 0.51, 9.92 ± 0.88, 20.04 ± 0.88, and 38.96 ± 3.70 ppm. Dams were weighed on GDs 0, 6, and 21 and sacrificed on day 21. Upon examination, uterine weights, fetal weights, sex ratio, number of implantation and resorption sites, and live and dead fetuses were recorded. Fetuses were examined for external malformations and cleft palate. One-half of viable fetuses were sectioned to assess soft-tissue alterations. The other half were fixed, stained with alizarin red S, and examined for skeletal alterations.

Body weight gain of dams and body weight of male and female fetuses were reduced by exposure to 40 ppm formaldehyde to 49, 78, and 81% of control values, respectively ($p < 0.01$) (Saillenfait et al., 1989). Reduced weight gain in dams remained significantly decreased when

uterine weight was accounted for ($p < 0.01$). Mean fetal weight of male pups was reduced at maternal exposures of 20 and 40 ppm formaldehyde (5.53 and 4.42 g versus 5.61 g in controls). Decreased fetal body weight in females was only seen at 40 ppm (4.27 g versus 5.24 g in controls). All other pregnancy endpoints were unchanged by formaldehyde exposure (e.g., uterine weight, implantation and resorption sites, live fetuses, dead fetuses, and sex ratios). No major malformations were noted in fetuses. Some minor soft tissue and skeletal anomalies, such as dilated ureter, missing sternebrae, extra fourteenth rib, and rudimentary thirteenth rib (statistics not given), were reported. However, these effects occurred at similar frequencies in control and treatment groups. The incidence of delayed ossification of the thoracic vertebrae was 8.7% in fetuses from the 40 ppm exposure group versus 1.8% in controls. However, this difference was not statistically significant. Overall, from these results formaldehyde was neither lethal to embryos nor teratogenic, only exhibiting fetotoxic effects at exposures of 20 ppm and above. These are levels where there was a significant decrease in fetal body weight.

Martin (1990) conducted a similar study exposing pregnant rats during similar stages of development. Mated female Sprague-Dawley rats (25/group) were exposed to 2, 5, or 10 ppm (2.46, 6.15, or 12.3 mg/m^3) formaldehyde 6 hours/day on GDs 6–15. The study included two control groups: dams placed in the exposure chambers once a day but exposed only to clean air and dams fed and housed similarly to the experimental groups but never put into the inhalation chambers. The method of formaldehyde vapor generation and details of the exposure chamber were not described. Mean formaldehyde exposure concentrations were reported as 1.88, 4.88, and 9.45 ppm (variability not given, analytical method not discussed). Food consumption and body weight were recorded. On GD 20, rats were sacrificed, and the following pregnancy parameters were recorded: live fetuses, dead fetuses and resorptions, number of corpora lutea, fetal weights, sex ratios, and preimplantation and postimplantation losses. Fetuses were examined for major malformations, minor external and visceral anomalies, and minor skeletal anomalies (details not given). Weight gain and food consumption in dams were said to be reduced at 10 ppm ($p < 0.05$). Formaldehyde exposure of the dams at 5 and 10 ppm led to an increased incidence of reduced ossification of the pubic and ischial bones in fetuses on GD 20 ($p < 0.05$). Reduced ossification correlated with lower fetal weights, and the author considered both of these findings a result of larger litter size and, therefore, not related to formaldehyde exposure. However, no tables presenting the data or statistical analysis were provided. All other pregnancy parameters and fetal anomalies were described as unaffected by formaldehyde exposure. However, without data presented for the assessed endpoints, background rates of malformations, trends in the data, and variability, it is difficult to evaluate the Martin (1990)

comparisons. However, the author's observations of reduced fetal weight and increased incidence of reduced ossification are consistent with the results of Saillenfait et al. (1989).

Kilburn and Moro (1985) studied similar endpoints but included formaldehyde exposure during earlier gestational windows. The study report, only available in abstract form and not found as a subsequent published article, does not provide many methodological details. Female rats (number and strain not reported) were exposed to 0 or 30 ppm (0 or 37.2 mg/m^3) formaldehyde 8 hours/day during GDs 3–17, 3–12, 8–12, or 9–11. A second experiment included pair-fed controls for dams exposed to 30 ppm formaldehyde during GDs 3–17, 3–12, and 8–12. The authors reported reductions in fetal and maternal weight gain that were greater than decreases in pair-fed controls. Fetal anomalies were noted after 15 days of gestational exposure (e.g., altered organ size and undescended testes). Although the report indicates some maternal toxicity and fetotoxic effects (for example, stunted growth), lack of study details and clear reporting make this report of negligible utility in human health risk assessment.

There are several early studies that examined developmental effects of formaldehyde exposure administered throughout gestation (Gofmekler and Bonashevskaya, 1969; Gofmekler, 1968; Pushkina et al., 1968). It is unclear if these reports represent the same or overlapping experimental groups. They were performed in the same laboratory and are reported with a similar level of detail. The source of formaldehyde, method of vapor generation, exposure conditions (dynamic versus static), confirmation of exposure concentrations, study design, and data presentation details were not provided. Absence of such critical information detracts from the quality of these studies as a coherent record of experimental information, and, thus, these findings can only be utilized qualitatively in the formaldehyde risk assessment.

In the Gofmekler (1968) study, female rats (36, strain not specified) were continuously exposed at 0, low, or high formaldehyde concentrations beginning 10–15 days prior to mating (target concentrations of 0, 0.01, or 0.81 ppm formaldehyde [0, 0.01, or 1 mg/m^3]). The author reported a 14–15% increase in pregnancy duration and a decrease in litter size (data not shown). However, males and females were mated 6–10 days, and no information was provided on how mating and conception were confirmed. No external malformations were attributed to formaldehyde exposure. Concentration-dependent increases in pup body weight and decreases in lung and liver weight were attributed to formaldehyde exposure. Pup weights were increased from 5.6 g in controls to 6.0 and 6.3 g in groups 1 and 2, respectively ($p < 0.01$ and $p < 0.001$). Formaldehyde exposure increased pup adrenal weight in both groups and pup thymus and kidney weight in group 2 only (Table 4-58).

Table 4-58. Effects of formaldehyde on body and organ weights in rat pups from dams exposed via inhalation from mating through gestation

Exposure (ppm)[a]	Body weight (g)	Relative organ weights (mg/10 g body weight)					
		Thymus	Heart	Lung	Liver	Adrenals	Kidney
0	5.6	26	61.4	287.1	587.7	3.2	51.4
0.01	6.0[b]	25.1	61.5	230.2[c]	557.7[d]	4.2[c]	53.4
0.81	6.3[c]	31.7[c]	64.5	223.2[c]	550.8[b]	3.8[d]	55.7[b]

[a]Dams were exposed to formaldehyde continuously from 10–15 days prior to mating. Exposure concentrations were not validated.
[b]Different from controls, $p < 0.01$.
[c]Different from controls, $p < 0.001$.
[d]Different from controls, $p < 0.05$.

Source: Gofmekler (1968).

In a study by Gofmekler and Bonashevskaya (1969), the researchers evaluated organ histopathology in pups from similarly treated dams. Pregnant female albino rats were continuously exposed at two formaldehyde concentrations (groups 1 and 2, as described above). Adult males were similarly exposed during mating. Offspring were examined for malformations, and the organs were fixed and sectioned for histopathologic examination, including hematoxylin and eosin staining, Brachet stain for RNA, and Feulgen stain for DNA. Liver and kidney sections were also stained with Schiff's reagent (which reacts with aldehydes), with Sudan III for lipids, and Pearl's stain for iron. Placenta, uterus, and ovaries from the dams and testes of the males were sectioned, stained, and evaluated. The authors stated that formaldehyde induced no external anomalies (reported elsewhere, but no reference given). The authors also noted involution of lymphoid tissue and changes in liver, mild hypertrophy of Kupffer cells, and numerous extramedullary myelopoietic centers in pups from dams in group 2. Pups from both treatment groups showed reduced glycogen content in the myocardium and the presence of iron in Kupffer cells. There was a localized increase in positive reaction to Schiff's reagent in the basement membrane and intertubular connective tissue of the kidneys. The authors suggested that this was an indication of functional alterations in the renal tubular apparatus. All other tissues examined and histochemical staining indicated no differences due to formaldehyde exposure.

Researchers in the same laboratory (Pushkina et al., 1968) studied the effects of formaldehyde exposure on vitamin C (ascorbic acid, an antioxidant) and nucleic acid levels in dams and fetuses as general measures of toxicity. Female white rats (n = 160) were continuously exposed at two formaldehyde concentrations (groups 1 and 2, as described above) from 20 days prior to mating (6–10 days) and then throughout gestation. Dams were sacrificed and ascorbic acid and nucleic acid levels determined in harvested organs (methods referenced but not

described). No visible malformations in pups were noted. Formaldehyde exposure increased fetal body weight and organ weight in both groups (data not given). There was an average of 11.3 fetuses per litter for control dams versus 9.8 and 8.6 for groups 1 and 2, respectively. The authors reported that formaldehyde exposure decreased DNA levels and increased RNA levels in organs (further details not provided). Formaldehyde exposure resulted in lower vitamin C levels in the whole fetus (76 and 75% of controls) and in maternal liver specimens (82 and 88% of controls) for exposure groups 1 and 2, respectively ($p < 0.05$). In contrast, vitamin C was higher in fetal liver (127% of controls) in group 1 ($p < 0.05$). The significance of these differences is unknown. The authors considered the results as general measures of biochemical changes and therefore toxic.

The reports of Gofmekler and Bonashevskaya (1969), Gofmekler (1968), and Pushkina et al. (1968) lack key methodological details. As discussed above, exposure conditions and actual formaldehyde concentrations cannot be validated. Although methods were not thoroughly detailed, results were reported in data tables with statistics and detailed descriptions of observed pathological changes. However, without validation of exposure concentrations, these findings can only be considered qualitatively.

In another study, Sheveleva (1971) exposed female mongrel (i.e., not a homogeneous genetic strain) white rats to 0, 0.0005, or 0.005 mg/L (0, 0.5, or 5 mg/m^3) (0, 0.4, or 4 ppm) formaldehyde on GDs 1–19 (where GD 1 was defined as the day that spermatozoa were detected in vaginal smears) for 4 hours/day. In each group, 15 dams were terminated on GD 20 for evaluation of ovarian corpora lutea, uterine implantation sites, pre- and postimplantation loss, number of live fetuses, fetal length and weight, and external examination for malformations. Additionally, in each group, six dams were allowed to deliver their litters. Developmental landmarks were monitored (i.e., ear and eye opening, incisor eruption, emergence of hair coat), and the pups were further evaluated at 1 and 2 months of age for body weight, threshold of neuromuscular excitability, total oxygen consumption in 1 hour per 100 g of weight, and spontaneous mobility over 10 minutes. Maternal toxicity (recorded on GD 17) included significantly ($p < 0.05$) decreased leukocyte counts in both treated groups and a number of additional findings at 0.005 mg/L (i.e., significant reductions in the threshold of neuromuscular excitability, rectal temperature, and blood hemoglobin level) as well as an increase in spontaneous mobility over 15 minutes. (It is noted that a significant reduction in blood hemoglobin was also observed by Sanotskii et al. [1976] in pregnant rats, following 20 days of gestational inhalation exposure for 4 hours/day to formaldehyde at 6 mg/m^3 [4.83 ppm].) Fetal examinations on GD 20 identified a 50–70% increase in mean preimplantation loss in both formaldehyde-exposed groups. When pups were 1 month of age, a reduction in spontaneous

mobility was noted in both treated groups; in pups at 2 months of age, an increase in mobility was observed in the 0.005 mg/L group. Also, when pups were 2 months of age, there were alterations in hemoglobin levels and leukocyte counts in both treated groups. Detailed descriptions of some study methodologies (particularly in regard to neurological and behavioral assessments) were not provided in the published paper.

Based on a review of the work by Gofmekler (1968) and various epidemiologic studies available at the time, Kitaev et al. (1984) hypothesized that formaldehyde may exert toxic effects in the early days of gestation. To study embryotoxic effects of formaldehyde inhalation exposures, mature female Wistar rats (five to nine per group) were exposed to 0.41 or 1.22 ppm (0.5 or 1.5 mg/m^3) formaldehyde 4 hours/day, 5 days/week for 4 months (Kitaev et al., 1984). Rats were exposed in dynamic flow chambers and formaldehyde levels measured gravimetrically (but not reported). Females were mated on day 120 of exposure and mating confirmed by the presence of sperm in a vaginal smear. Embryos were harvested on the second or third day of pregnancy (GD 2 or 3) and examined by both light and phase contrast microscopy for changes in morphology (i.e., evidence of embryonic degeneration). Additionally, maternal weight gain and organ weights (ovaries, uterus, and adrenal glands) and blood samples (HCT, Hb, and TP) were monitored as indicators of general toxicity. These parameters were unchanged by formaldehyde exposure. Formaldehyde exposure at 1.22 ppm for 4 months resulted in an increased number of degenerating embryos on GD 3 (14.9 versus 4.4% in controls) and a smaller increase of 10.2% (versus 5.1% in controls; statistical significance not assessed) on GD 2. Indications of degeneration included reduced size and changes in appearance (granulation of the ooplasm, wrinkling and degradation of nuclear material). However, it is unclear if litter effects were accounted for in the statistical analyses, and it is unknown how the affected embryos were distributed between litters. For dams exposed to 0.41 ppm formaldehyde, the number of degenerated embryos was not increased on day 2 (3.8 versus 5.1% in controls) but was increased on day 3 (9.1 versus 4.4% in controls; again, unknown if statistically significant) after maternal exposure to 1.22 ppm formaldehyde. This observation may be coincidental since it was seen in dams sacrificed on GD 2 but not in those sacrificed on GD 3. Kitaev et al. (1984) considered these findings to indicate that repeated exposure to formaldehyde over a 4-month period can disturb reproductive function, resulting in adverse effects early in embryonic development.

To further explore the effects of inhalation exposures to formaldehyde on reproductive function, Kitaev et al. (1984) conducted a second series of experiments on 200 similarly treated female rats. After 4 months of repeated formaldehyde exposure at 0.41 or 1.22 ppm as described above, organ weights (ovaries and uterus) and blood levels of gonadotropic hormones and progesterone were determined. However, the day and time of hormone measurement were not

given in the report, and normal diurnal variations in these hormones could affect the reported findings if time of day was not accounted for. The length of the estrous cycle was unchanged during exposure. Formaldehyde exposure modulated gonadotropin levels and relative ovarian weight, suggesting low-level effects on the female rat reproductive system prior to mating (Kitaev et al., 1984). Ovarian weight and blood levels of luteinizing hormone (LH) were both significantly increased after exposures at 0.41 ppm formaldehyde but remained at control levels in rats exposed at 1.22 ppm. Blood levels of follicle-stimulating hormone (FSH) were increased approximately 66% from control after 1.22 ppm formaldehyde exposure ($p < 0.05$). Progesterone levels were unchanged by formaldehyde treatment. Kitaev et al. (1984) suggested a role of the hypothalamus-pituitary system based on increased ovary weight, a greater number of degenerated embryos, and increased LH in rats exposed at 0.41 ppm. They postulated that these effects were not seen at 1.22 ppm due to a toxic effect exhibited as embryonic degeneration, thus the absence of a dose-response did not alter the interpretation of the validity of the adverse response. The study NOAEL was not determined, and the study LOAEL was 0.4 ppm (0.5 mg/m^3), based upon increased early embryo loss and on maternal outcomes (increased ovarian weight and increased blood LH levels) following 4 months of formaldehyde treatment. For the finding of increase blood FSH levels, the endpoint NOAEL was 0.4 ppm (0.5 mg/m^3) and the LOAEL was 1.2 ppm (1.5 mg/m3).

Senichenkova and Chebotar (1996) and Senichenkova (1991) examined reproductive and developmental effects of daily formaldehyde exposure on GDs 1–19 of pregnancy, including the potential effect of formaldehyde exposure on development early in gestation. Additionally, since anemia adversely affects fetal development, Senichenkova and Chebotar (1996) also examined formaldehyde effects in iron-deficient dams to determine whether co-exposure further compromises fetal development. In both studies, female white rats were exposed to 0 or 0.41 ppm formaldehyde (0 or 0.5 mg/m^3), 4 hours/day on GDs 1–19. Formaldehyde concentrations in the dynamic exposure chambers were measured gravimetrically to confirm the exposure concentration but were not reported (methods not provided). It is unclear if gravimetric measurements would be sensitive or accurate enough to validate these low-exposure concentrations without a better understanding of the methodology. This uncertainty in exposure conditions should be considered in evaluating the reported results.

Mongrel female white rats were exposed at a target concentration of 0 or 0.41 ppm (0 or 0.5 mg/m^3) formaldehyde 4 hours/day on GDs 1–19 (Senichenkova, 1991). On GD 20, a subset of the dams was sacrificed and examined for number of corpora lutea, implantation and resorption sites, live/dead fetuses, and fetal weights. Fetuses were examined for gross pathology of the internal organs and skeleton (details not given). Blood pH, partial pressure of CO_2, and

partial pressure of oxygen were measured in both dams and embryos. The remaining dams were brought to term to study postnatal effects of formaldehyde exposure. Rat pups were observed on PNDs 1–25 for viability, physical development, and maturation rate of motor reflexes. Behavior of juvenile offspring (PND 40) was studied in an open field test, and maze learning was tested at sexual maturity. In a follow-up report, Senichenkova and Chebotar (1996) present blood chemistry data, pregnancy outcome, and developmental data for similarly treated dams and their pups in a chemical model of iron deficiency. Intraperitoneal injections of the iron-chelating agent bipyridyl were given on GDs 12–15 at the threshold embryotoxic dose (1 mL, 25% solution). On day 20, the dams were sacrificed and dams and fetuses examined as described above. In addition to blood pH, partial pressure of carbon dioxide and partial pressure of oxygen, acid metabolic products (not detailed), and true bicarbonates were reported for maternal and fetal blood. A review of the data from these reports indicates there may be an overlap of the study groups. Neither paper presents the entire data set; thus, for tranparency and brevity, the following text discusses the combined findings from both studies as if they were a single study

Formaldehyde exposure did not affect such indicators of pregnancy outcome as number of corpora lutea, implantation and resorption sites, and live and dead fetuses, all of which were unchanged (Senichenkova and Chebotar, 1996; Senichenkova, 1991). Although fetal weight was slightly increased by formaldehyde exposure, 2.35 versus 2.24 g in controls ($p < 0.001$), neither fetal length nor bone length were changed (femur and humerus) (Senichenkova and Chebotar, 1996; Senichenkova, 1991). Often, increased fetal weight is the result of early physical development, and other signs of development, such as ossification, would be expected to be enhanced as well. The average number of bone centers per limb was increased by formaldehyde exposure from 2.45 and 2.66 to 2.78 and 2.91 in controls for metacarpal and metatarsal bone centers, respectively ($p < 0.05$) (Senichenkova, 1991); these findings were consistent with increased growth and weight. In contrast, Senichenkova (1991) reported a decrease in the number of embryos with ossification centers in the hyoid bone (100% in controls versus 91% for formaldehyde exposure, $p < 0.05$), consistent with the results of Saillenfait et al. (1989) and Martin (1990). However, litter size, a factor influencing fetal weight, was not provided, and it is unclear if Senichenkova (1991) took litter size into account in the analysis.

Senichenkova and Chebotar (1996) reported increased blood acidosis and decreased blood alkaline reserves (bicarbonates and total CO_2) in formaldehyde-treated dams and their embryos ($p < 0.05$). However, this finding should be considered in light of the fact that chronic blood acidosis may increase bone remodeling and decrease bone density in adults. It is unknown if the reported blood acidosis could reduce ossification rates in developing embryos. A better

understanding of exposure conditions and the acid metabolic products measured is needed to determine the biological relevance of the reported changes in blood acid balance.

Iron deficiency, induced by injections of bipyridyl (an iron-chelating agent), was found to be fetotoxic. Iron-deficient dams with no formaldehyde exposure had higher rates of postimplantation death than controls (12.6 ± 5.5 versus 4.8 ± 1.3%). Formaldehyde exposure in conjunction with iron deficiency increased postimplantation death to 23.1 ± 5.9%. Fetal weight and litter size were not reported for the bipyridyl treatment groups, but bipyridyl treatment in conjunction with formaldehyde resulted in a decreased number of metatarsal bone centers (2.21 ± 0.12 versus 2.72 ± 0.08 in controls; $p < 0.001$). This decrease was also significant when compared with formaldehyde or bipyridyl alone ($p < 0.02$). However, all pregnancy outcome parameters were not reported for the bipyridyl treatment.

Fetal anomalies were reported after formaldehyde exposure and were increased by iron deficiency. The incidence of litters with internal organ anomalies was increased from 1.4% in controls to 14.2% in formaldehyde-treated dams (Senichenkova, 1991). Undescended testes were the predominant anomaly described: 20.8% in litters from formaldehyde-treated dams versus 1.2% in controls ($p < 0.05$) (Senichenkova, 1991). Similar findings were reported by Senichenkova and Chebotar (1996). Bipyridyl treatment in conjunction with formaldehyde exposure increased the overall incidence of fetal anomalies (13.8 ± 2.1% in controls versus 6.6 ± 1.8% with iron deficiency alone) (Senichenkova and Chebotar, 1996). However, there are discrepancies between the two papers in the reporting of the anomalies, and it is unclear whether the experimental groups overlap between papers, where some parameters are identical (which would lead to double counting of the same animal, including identical SDs) and others are different. Additionally, the reporting is unclear with respect to the basis of the incidence rates reported (for example, overall incidence versus incidence within litter or incidence of litters with anomalies). Unclear reporting, together with some of the uncertainties regarding exposure conditions, suggests that the data may be of limited quality to support risk assessment.

In the second phase of the studies, pups were delivered and postnatal development was assessed (Senichenkova, 1991). Eruption of the upper and lower incisors was delayed in pups from formaldehyde-treated dams, occurring on PND 14 versus PND 12 in controls ($p < 0.01$). All other measures of physical postnatal development were unchanged by formaldehyde. To evaluate postnatal functional outcomes following in utero exposure to formaldehyde, an open field test was conducted in juvenile rats on 3 consecutive days (PNDs 40–42). Juvenile rats from formaldehyde-treated dams exhibited increased mobility (crossed squares), rearings, and defecations/urinations compared with control rats on the second and third open field tests

($p < 0.05$). There were no differences seen in the maze-learning test assessed in mature offspring of formaldehyde-treated dams (Senichenkova, 1991).

Additional assessments of formaldehyde exposure on neurological development are described above in the section on neurological and behavioral toxicity in animal studies (Section 4.2.1.6). In brief, studies conducted by Sarsilmaz et al. (2007) and Aslan et al. (2006) exposed 10 neonatal male Wistar rats/group to 0, 6, or 12 ppm (0, 7.36, or 14.7 mg/m^3) formaldehyde 6 hours/day, 5 days/week for 30 days. At that time, five rats/group were killed and subjected to neuropathological examination; the remaining rats were maintained until PND 90, at which time they were killed and evaluated. Aslan et al. (2006) examined the number and volume of granular cells in the hippocampal dentate gyrus, while Sarsilmaz et al. (2007) examined the size and number of the pyramidal cells in the cornu ammonis of the hippocampus. In both studies, lower numbers of cells were observed in both treated groups at PND 90 as compared with PND 30. Although the effects of treatment on the volume and number of granular and pyramidal cells were somewhat inconsistent, a significant decrease in the number of neurons in the pyramidal cell layer of the hippocampal cornu ammonis was observed at both PNDs 30 and 90 (Sarsilmaz et al., 2007).

One other study reported effects on nervous system function following exposure to formaldehyde during postnatal development. An abstract by Weiler and Apfelbach (1992) described a study in which juvenile rats (strain not specified) were exposed to 0.25 ppm (0.31 mg/m^3) formaldehyde from PNDs 30–160 or adult rats were exposed to 0.5 ppm (0.62 mg/m^3) formaldehyde for 90 days. Decreased olfactory sensitivity (i.e., increased olfactory thresholds) was observed and was greater when the exposure was initiated in the young rats, as compared with the adults.

Evaluation of offspring following prenatal, perinatal, and/or juvenile inhalation exposures to formaldehyde have also been reported by Kum et al. (2007), Sandikci et al. (2007), and Songur et al. (2005). Kum et al. (2007) exposed female Sprague-Dawley rats (six dams/group) and their offspring to 0 or 6 ppm (0 or 7.4 mg/m^3) formaldehyde for 8 hours/day in separate groups with exposures starting on GD 1, on postparturition day 1, or in offspring at 4 weeks of age and continuing for 6 weeks. In another group, exposures were initiated in adult rats. Mean body and liver weights were significantly decreased in the offspring exposed in utero and in early postnatal life, and mean liver weights were also significantly decreased in rats with juvenile exposures. However, neither body weight nor liver weight was affected in the group with exposure initiating at an adult age, suggesting a life-stage-related susceptibility to formaldehyde-induced hepatic toxicity. Evaluation of biomarkers of oxidative stress revealed significantly increased catalase (CAT) and MDA in the livers of offspring that were exposed prenatally,

significantly decreased GSH levels in the livers of offspring that were exposed in early postnatal life, and significantly decreased SOD levels in the livers of offspring that were exposed starting at 4 weeks of age. No biomarkers were altered in the livers of rats exposed to formaldehyde only as adults.

A similar study design was used by Sandikci et al. (2007) to examine the effects of 0 or 6 ppm (0 or 7.4 mg/m^3) formaldehyde on bronchus associated lymphoid tissue (BALT) following pre- and perinatal, juvenile, or adult exposures of 6 weeks duration in Sprague-Dawley rats (six/group). The presence of the lysosomal enzyme alpha-naphthylacetate esterase (ANAE) served as a marker of T-lymphocytes in peripheral blood and tissue sections. Significant increases in ANAE-positive T-lymphocytes were found in BALT in all but the in utero exposed groups as compared with control; this outcome is consistent with the postnatal development of BALT in the rat. In peripheral blood, ANAE-positive lymphocyte ratios were significantly ($p <$ 0.001) increased as compared with controls at all life stages tested.

Songur et al. (2005) examined the effect (and reversibility) of formaldehyde exposures during the early postnatal period on zinc, copper, and iron levels and activity of SOD in the lung tissue of Wistar rats. Litters (12–14/group) were exposed to 0, 6, or 12 ppm (0, 7.4, or 14.9 mg/m^3) formaldehyde 6 hours/day, 5 days/week for 30 days. Trace element and biochemical analyses were conducted on PND 30 or 90. Decreased SOD activity, decreased levels of copper and iron levels, and increased zinc levels were observed in the lungs of treated groups following 30 days of treatment and at 90 days (i.e., 60 days posttreatment). Survival was not affected in neonatal rats. Clinical observations during treatment included evidence of respiratory irritation and toxicity. Body weight and food and water consumption were also nonsignificantly decreased as compared with controls.

There are several reports in the literature regarding formaldehyde effects after inhalation exposure on the male reproductive system in animals (Galalipour et al., 2007; Zhou et al., 2006; Özen et al., 2005, 2002; Sarsilmaz et al., 1999; Woutersen et al., 1987; Maronpot et al., 1986; Guseva, 1972). The earliest report examined the effect of simultaneous exposures to formaldehyde from air and water (Guseva, 1972). Male rats (n = 12, strain not specified) were co-exposed to formaldehyde in air and drinking water 4 hours/day, 5 days/week for 6 months. There were three exposure levels in the experiment of different air and drinking water concentrations: (1) 0.41 ppm (0.5 mg/m^3) formaldehyde in air and 0.1 mg/L in water; (2) 0.20 ppm (0.25 mg/m^3) formaldehyde in air and 0.01 mg/L in water; or (3) 0.10 ppm (0.12 mg/m^3) formaldehyde in air and 0.005 mg/L in water. Reproductive function was assessed by mating two females per male. The time for the onset of pregnancy and the number of pregnancies per treatment group were recorded. A subset of dams was sacrificed on GD 20 of pregnancy, and

the number and weight of fetuses was determined. Postnatal development of the remaining dams was tracked (e.g., times of eye opening and development of hair coat). Nucleic acid levels were determined in the testes of formaldehyde-exposed rats. Gonadotropic response was assessed by injecting an emulsion of pituitaries from exposed male rats into unexposed infantile females and measuring the weight ratios of the uterus and ovaries. Formaldehyde exposure reduced nucleic acid levels in testes to 88 and 92% of controls in groups 1 and 2, respectively. No other formaldehyde-induced differences were found.

Woutersen et al. (1987) and Maronpot et al. (1986) examined tissue sections from testes and ovaries of exposed animals as part of studies primarily addressing respiratory tract toxicity (see Section 4.2.1.2.2.4 for complete study details). Maronpot et al. (1986) exposed female and male B6C3F1 mice to 0, 2, 4, 10, 20, and 40 ppm (0, 2.45, 4.91, 12.3, 24.5, and 49.1 mg/m^3) formaldehyde 6 hours/day, 5 days/week for 13 weeks. Decreased weight gain due to formaldehyde exposure was seen in both male and female mice. Additionally, there was 80% mortality at the highest exposure (40 ppm). The authors reported endometrial hypoplasia and lack of ovarian luteal tissue in females exposed to 40 ppm, but no compound-related changes were observed in testes sectioned and viewed by light microscopy.

In a study by Appleman et al. (1988), male Wistar rats (40/group) with undamaged or damaged (via bilateral intranasal electrocoagulation) nasal mucosa were exposed for 13 or 52 weeks to 0, 0.1, 1, or 10 ppm (0, 0.124, 1.24, or 12.4 mg/m^3) formaldehyde 6 hours/day, 5 days/week. At study termination, mean body weight was decreased, but relative testes weight was increased in the 10 ppm group (interpreted as a non-adverse outcome that was associated with the decreased body weight). No treatment-related histopathologic findings were reported for male reproductive organs (although it is not clear to what extent they were evaluated since the primary focus of the study was on the nasal epithelium).

Following up on earlier reports of decreased Leydig cell quality in rats administered I.P. injections of formaldehyde (Chowdhury et al. [1992], described in Section 5.2.1.8.3), Sarsilmaz et al. (1999) studied the effects of formaldehyde inhalation on Leydig cells. Adult male Wistar rats (30) were exposed to 0, 10, or 20 ppm (0, 12.3, or 24.6 mg/m^3) formaldehyde 8 hours/day, 5 days/week for 4 weeks. Animals were observed daily and weighed weekly. Rats were sacrificed on day 29 and autopsied, and testes were weighed, fixed, and sectioned for histologic examination. Signs of irritation from formaldehyde exposure were noted (frequent eye blinking, excessive licking, increased frequency of nose cleaning, interrupted breathing, and sneezing). Body weight gain was reduced by formaldehyde exposure from 17.7% gain in control rats to 4.66 and 2.63% in rats exposed at 10 and 20 ppm, respectively ($p < 0.001$). As shown in Table 4-59, relative testes weights were unaffected (reported as proportions but more likely to be

percentages), although trends and numerical differences were similar to those reported by Özen et al. (2002). Sarsilmaz et al. (1999) found that both Leydig cell quantity and the percentage of cells with normal nuclei were reduced by formaldehyde treatment. Although the dose-dependent reduction in Leydig cell quantity was statistically significant at both exposure levels, the study authors considered the data to be within the normal range.

Table 4-59. Formaldehyde effects on Leydig cell quantity and nuclear damage in adult male Wistar rats

Inhalation exposure[a]	Relative testes weight[b,c,d]	Leydig cell quantity[c,e,f]	Appearance of nucleus[e,g]			
			Normal	Pyknotic	Karyorectic	Karyolytic
Control	0.93 (0.03)	47.27 (7.8)	98	2	0	0
10 ppm	0.92 (0.06)	45.04 (7.8)[h]	92	2	4	2
20 ppm	0.89 (0.06)	44.36 (7.5)[i]	76	9	10	5

[a]Rats were exposed 8 hours/day, 5 days/week for 4 weeks.
[b]Stated to represent the ratio of the last day's testicle weight to the body weight but more likely to be the percent of body weight.
[c]Cells within 100 defined areas.
[d]n = 10.
[e]For each exposure group, 100 defined locations were assessed.
[f]n = 100.
[g]Values presented as percentage of cells.
[h]Different from control ($p < 0.05$), as reported by the authors.
[i]Different from control ($p < 0.01$), as reported by the authors.

Source: Sarsilmaz et al. (1999).

It was hypothesized that decreased Leydig cell quality may have been the result of oxidative stress induced by formaldehyde exposure. Özen et al. (2002), in the same laboratory, investigated changes in testicular iron, copper, and zinc levels as measures of oxidative stress and damage. Adult male albino Wistar rats (seven/group) were exposed at 0, 10, or 20 ppm (0, 12.2, or 24.4 mg/m^3) formaldehyde 8 hours/day, 5 days/week for either 4 or 13 weeks. Rats were observed daily and weighed once a week. Rats were sacrificed at the end of the exposure period and autopsied, and the testes were removed and weighed. Zinc, copper, and iron levels were determined in testes tissue by using atomic absorption spectrophotometry. Both weight gain and relative testes weight were decreased in a concentration-dependent and duration-dependent manner (Table 4-60). Both zinc and copper levels in rat testes were reduced in a concentration- and duration-dependent manner by formaldehyde exposure. For example, zinc was reduced from 277 to 107 mg/kg after a 4-week × 20 ppm exposure and from 260 to 95 mg/kg after a 12-week × 20 ppm exposure ($p < 0.001$) (Table 4-60). Iron levels in testes were

increased from 30 to 39 mg/kg after a 4-week 20 ppm exposure ($p < 0.01$) and from 33 to 58 mg/kg after 12 weeks at 20 ppm ($p < 0.05$). The authors suggested that alterations in trace element levels in the testes were consistent with oxidative damage and may have contributed to changes in Leydig cell function. These researchers also reported alterations in trace metals in lung tissue from Wistar rats exposed to formaldehyde 8 hours/day, 5 days/week for 4 or 13 weeks. Iron levels were increased at 5 ppm for 13 weeks and 10 ppm for either 4 or 13 weeks. Zinc levels decreased for all formaldehyde exposures. In both cases, the authors attributed elevated iron levels to oxidative stress. In addition to citing the role of zinc as a cofactor of cytoplasmic Cu-Zn-SOD, the authors suggested that zinc may have been consumed by increased FALDH activity. Although oxidative stress and increased FALDH activity may be relevant to the POE and therefore impact the lung, it is less clear how these changes would occur in the testes. Taken together, the reports of Özen et al. (2002) and Sarsilmaz et al. (1999) suggested a LOAEL of 10 ppm 8 hours/day for 4 weeks for changes in Leydig cell quantity and quality, decreased testes weight, and changes in trace metal content (zinc, copper, and iron).

Table 4-60. Formaldehyde effects on adult male albino Wistar rats

Inhalation exposure[a]	Weight gain (%)	Testes weight (%)	Zinc (mg/kg)	Copper (mg/kg)	Iron (mg/kg)
4 Weeks					
Control	19.1 (2.7)	0.94 (0.03)	277 (16)	6.4 (0.42)	30 (2.7)
10 ppm	5.8 (2.4)[b]	0.92 (0.02)[c]	132 (8.9)[b]	4.2 (0.33)[b]	35 (2.8)[d]
20 ppm	2.4 (0.6)[b]	0.91 (0.01)[c]	107 (6.9)[b]	3.3 (0.27)[b]	39 (3.1)[d]
13 Weeks					
Control	55.9 (2.3)	0.91 (0.01)	269 (15)	6.0 (0.34)	33 (2.6)
10 ppm	34.7 (3.5)[b]	0.84 (0.03)[b]	112 (8.1)[b]	3.6 (0.30)[b]	52 (3.5)[b]
20 ppm	20.8 (1.4)[b]	0.82 (0.03)[b]	95 (6.4)[b]	1.9 (0.17)[b]	58 (3.0)[b]

[a]Formaldehyde exposure was 8 hours/day, 5 days/week for either 4 or 13 weeks. Values are means ± SDs of seven animals..
[b]Different from control, $p < 0.001$, as calculated by the authors.
[c]Different from control, $p < 0.05$, as calculated by the authors.
[d]Different from control, $p < 0.01$, as calculated by the authors.

Source: Özen et al. (2002).

In another study that assessed testicular toxicity (Özen et al., 2005), male Wistar rats (18/group) were exposed by inhalation to 0, 5, or 10 ppm (0, 6.2, or 12.4 mg/m^3) formaldehyde 8 hours/day, 5 days/week for 91 days. In-life observations in exposed rats included clinical signs of respiratory irritation and decreased mean food and water consumption. At study termination, serum testosterone levels and mean seminiferous tubule diameters were significantly decreased from control in a dose-responsive manner (Table 4-61). Immunohistochemical staining of testis

tissues showed increased localization of heat shock protein (Hsp) 70 in the cytoplasm of spermatogonia, spermatocytes, and spermatids of treated animals compared with controls (not shown here).

Table 4-61. Formaldehyde effects on testosterone levels and seminiferous tubule diameters in Wistar rats following 91 days of exposure

Inhalation exposure[a]	Testosterone levels (ng/dL)	Seminiferous tubule diameters (µm)
	n = 6	n = 100
Control	406.54 ± 16.82	259.22 ± 16.18
10 ppm	244.01 ± 23.86[b]	236.17 ± 13.09[c]
20 ppm	141.30 ± 08.56[b]	233.24 ± 10.13[c]

[a]Formaldehyde exposure was 8 hours/day, 5 days/week for 91 weeks. Values are means ± SEMs.
[b]Different from control, $p < 0.0001$, by one-way ANOVA, as calculated by the authors.
[c]Different from control, $p < 0.001$, by one-way ANOVA, as calculated by the authors.

Source: Özen et al. (2005).

Zhou et al. (2006) investigated the effect of formaldehyde on the testes and the protective effect of vitamin E against oxidative damage by formaldehyde in adult male rats. In this study, adult male Sprague-Dawley rats (10/group) were treated for 2 weeks in the following groups: (1) control rats were administered physiological saline by oral gavage, (2) rats were administered physiological saline by gavage and exposed to 10 mg/m^3 (8.05 ppm) formaldehyde by inhalation for 12 hours/day, and (3) rats were administered daily gavage doses of 30 mg/kg vitamin E and exposed to 10 mg/m^3 (8.05 ppm) formaldehyde by inhalation for 12 hours/day. Formaldehyde treatment resulted in significantly decreased ($p < 0.05$) mean testis weight. Histopathologic findings in treated rats included atrophy of seminiferous tubules, decreased spermatogenic cells, and seminiferous cells that were "disintegrated" and shed into the lumina, which was azoospermic. Interstitial tissue was edematous with vascular dilatation and hyperemia. In the formaldehyde-treated group, epididymal sperm count and percentage of motile sperm were significantly decreased, and the percentage of abnormal sperm was increased ($p < 0.05$), as compared with control. Evaluation of biochemical markers in testes tissue showed the activities of testicular SOD, GPX, and GSH were decreased; MDA levels were significantly increased as compared with control. All observed effects of formaldehyde treatment (i.e., decreased testes weight, biochemical alterations, histopathologic effects, and sperm count, motility, and morphology findings) were attenuated by administration of 30 mg/kg-day vitamin E.

In a study by Golalipour et al. (2007), testicular changes of increasing severity with increasing duration were reported. A total of 28 Wistar rats, aged 6-7 weeks old, were divided into four groups including three FA treatment groups (4 hours of exposure/day, 4 days/week for 18 weeks; 2 hours of exposure/day, 4 days/week for 18 weeks; 4 hours of exposure/day, 2 days/week for 18 weeks) and one untreated control. The three FA-treated groups were exposed via inhalation to formaldehyde. The mean concentration of FA vapor, based on three measurements during the study (stated as the beginning, during, and end of the study period), was reported as 1.5 ppm. At the end of the study period, the rats were sacrificed by ether anesthesia and subsequent cervical dislocation. The left testis was dissected from each rat and a specimen was taken from each testis. Tissues were fixed, embedded, sectioned (at 4 μm), and stained with hematoxylin and eosin. Using a light microscope, a histopathological examination was performed on the testes tissues, including morphometric evaluation of the diameter and height of 20 seminiferous tubules/testis. Golalipour et al. (2007) reported an FA exposure frequency (or duration)-dependent increase in testicular germ cells and seminiferous tubule defects. In the most frequent duration treatment group, a severe decrease in germ cells in >85% of the seminiferous tubules and arrested spermatogenesis were observed. In the mid-level frequency of duration treatment group, a decrease in the number of germ cells and an increased thickness of the basement membrane of 75% of the tubules was observed. In the lowest level duration treatment group, a disruption in the Sertoli and germinal cell arrangement, and increased spacing between germ cells was observed. Further, the seminiferous tubule diameter (STD) and seminiferous epithelial height (SEH) was most decreased among the treatment groups (exhibiting the greatest decrease in the group with the greatest hours and days of exposure) compared to the control (Table 4-62). The results of this study are consistent with the findings of other studies of male reproductive system outcomes with inhalation FA exposure (e.g., Özen et al., 2005 and Zhou et al., 2006).

Xing et al. (2007) also studied the effects of formaldehyde on sperm development and reproductive capacity in adult male mice. In this study, male mice (12/group, strain not specified) were exposed to 0, 21, 42, or 84 mg/m^3 (0, 16.9, 33.8, or 67.6 ppm) formaldehyde via inhalation for 13 weeks at 2 hours/day, 6 days/week. The males were mated to untreated females in a dominant lethal protocol, and sperm morphology was assessed at study termination. The percent abnormal sperm was increased significantly ($p < 0.05$) in all treated groups, as was the rate of resorptions ($p < 0.01$) (Table 4-63). The mean number of live fetuses/litter was decreased in all treated groups, with statistical significance achieved at 84 mg/m^3. Although this study did not assess the number of corpora lutea per dam, thereby precluding the calculation of

preimplantation loss, it is nevertheless indicative of formaldehyde-induced sperm morphology changes and dominant lethal effects in male mice.

Table 4-62. Effects of formaldehyde exposure on seminiferous tubule diameter and epithelial height in Wistar rats following 18 weeks of exposure

Inhalation exposure[a]	Seminiferous tubule diameters (µm)	Seminiferous tubule height (µm)
	n = 7	n = 7
Control	252.12 ± 4.82	82.77 ± 2.00
1.5 ppm, 4 h/d, 4 d/w	204.55 ± 3.29[b]	65.26 ± 1.43[b]
1.5 ppm, 2 h/d, 4 h/w	232.45 ± 2.42[b]	69.46 ± 1.78[b]
1.5 ppm, 2 h/d, 2 d/w	238.94 ± 4.37[b]	72.80 ± 2.03[b]

[a] Values are means ± SEMs.
[b] Different from control, $p < 0.05$, as calculated by the authors.

Source: Golalipour et al. (2007).

Table 4-63. Incidence of sperm abnormalities and dominant lethal effects in formaldehyde-treated mice

Dose (mg/m^3)	Sperm abnormalities		Reproductive capacity	
	Total abnormal sperm heads	Aberration rate (%)	Mean live fetuses/litter	Resorption rate (%)
0	391	3.53 ± 0.98	11.00 ± 1.01	2.273
21	568	5.48 ± 1.45	10.67 ± 1.16	9.380[b]
42	849	6.15 ± 1.36	9.63 ± 2.83	10.390[b]
84	974	9.24 ± 2.13[a]	9.04 ± 2.98[a]	12.440[b]

[a]Significantly different from controls ($p < 0.05$), as calculated by the authors.
[b]Significantly different from controls ($p < 0.01$), as calculated by the authors.

Source: Xing et al. (2007).

4.2.1.7.2. ***Oral exposure studies addressing developmental and reproductive toxicity.*** No contemporary testing guideline studies, such as a prenatal developmental toxicity study or two-generation reproductive toxicity study, have been performed by the oral route for formaldehyde. However, a number of studies have evaluated developmental toxicity and reproductive parameters in rats, mice, and dogs.

Hurni and Ohder (1973) tested the developmental toxicity of formaldehyde administered as a 40% w/v solution containing 11–14% w/w methanol in 9–10 pregnant beagle dogs that

received the compound in their diet on GDs 4–56. Commercial grade formaldehyde (as a 40% solution) was sprayed on the pellets prior to feeding. Each animal was allotted a diet of 300 g of chow (reduced to 200 g 1 week prior to term) that was promptly consumed (within 5–10 minutes) before the formaldehyde volatilized appreciably. The concentrations of formaldehyde in the chow were 0, 125, or 375 ppm, equivalent to doses of 0, 3.1, or 9.4 mg/kg-day, respectively. Dams were allowed to deliver normally and weight gain, gestation length, number of litters, litter size, number of live pups, number of pups surviving through weaning, and pup weights weekly for the first 8 weeks were monitored as indices of the potential reproductive/developmental toxicity of formaldehyde. There were no formaldehyde-related effects in any of the parameters other than progressive pup weights, which were lower by group in litters of dams exposed to formaldehyde (Table 4-64). A developmental impact of formaldehyde was evident in this strain of dog under the conditions of the experiment. At birth, mean pup body weights were 4 and 8.4% less than control for the low- and high-dose groups, respectively; at 8 weeks of age, the pup weight decrements were 8.3% for the low dose and 12.5% for the high dose, as compared with control, and established a LOAEL of 125 ppm. The contribution of methanol, which is a developmental toxin (Deglitz et al., 2004; Rogers et al., 2004) to these outcomes is not known. No internal or skeletal malformations were observed in any of the 264 live-born and 20 still-born pups.

Table 4-64. Body weights of pups born to beagles exposed to formaldehyde during gestation

Time (weeks)	Formaldehyde concentration in chow (ppm)		
	0	125	375
	Average body weight (g)		
0	321	308	294
1	547	526	467
2	818	755	706
3	1,078	987	944
4	1,264	1,247	1,166
5	1,601	1,512	1,429
6	2,020	1,816	1,741
7	2,449	2,263	2,145
8	2,957	2,712	2,587

Source: Hurni and Ohder (1973).

Marks et al. (1980) conducted a developmental toxicity study of formaldehyde in CD-1 mice in which 29–35 pregnant animals were gavaged on GDs 6–15 with aqueous formaldehyde (containing 10–15% methanol) at 74, 148, and 185 mg/kg-day. Seventy-six controls were gavaged with water alone. All dams were sacrificed on GD 18, and the numbers of implantation

sites in each uterine horn were counted. The high dose of formaldehyde was toxic to the dams, as indicated by the deaths of 22 of 34 mice before GD 18. Thus, the dose of 148 mg/kg-day was a NOAEL for maternal toxicity in this study. However, it is unclear to what extent an estimated concurrent dose of up to 75 mg/kg-day methanol may have contributed to this toxic response. To assess the developmental toxicity of formaldehyde, live fetuses were weighed individually, sexed, and examined for external, visceral, and skeletal malformations. Fetuses of surviving high-dose dams and of those of other groups did not show an increased incidence of malformations. Therefore, Marks et al. (1980) concluded that formaldehyde did not induce fetal abnormalities and that the 185 mg/kg-day dose level was a NOAEL for the developmental toxicity of formaldehyde, nor were fetotoxic effects of methanol apparent under the study experimental conditions.

Seidenberg and Becker (1987) and Seidenberg et al. (1986) included formaldehyde (purity not indicated) in a survey of the behavior of potential toxicants in a developmental toxicity screening assay (Chernoff and Kavlock, 1982). The protocol featured the administration of a borderline toxic dose to 26–30 pregnant ICR/SIM mice on GDs 8−12. Dams were allowed to deliver, and the neonates were examined, counted, and weighed on PNDs 1 and 3. The selected formaldehyde dose of 540 mg/kg-day was fatal for 11/30 dams, but the average weight gain among surviving dams was little changed compared with controls (3.9 ± 2.3 versus 4.0 ± 1.0 g). Similarly, there were no changes in perinatal responses in the neonates of exposed dams compared with controls. For example, the average values for number of neonates/litter, percent survival, and fetal weights on PNDs 1 and 3 were closely similar to those of controls.

Evidence of toxicity to the male reproductive system has been observed following oral administration of formaldehyde in a 40% w/v solution containing 11–14% w/w methanol. Cassidy et al. (1983) administered single oral doses of 100 or 200 mg/kg to five male Wistar rats/group. Testes from these animals and 20 controls were excised and examined for spermatogenic abnormalities 11 days after dosing. A significant (19%) increase in testicular sperm head counts was observed in rats exposed to 200 mg/kg-day formaldehyde as compared with controls (Table 4-65). The percentage of abnormal sperm heads was also significantly increased (5%) in the 200 mg/kg-day dose group compared with controls. These data suggest that formaldehyde can induce morphologic abnormalities in the germ cells of male experimental animals at dose levels that did not significantly affect testis weights. The contribution of methanol to these outcomes is unknown.

Table 4-65. Testicular weights, sperm head counts, and percentage incidence of abnormal sperm after oral administration of formaldehyde to male Wistar rats

Dose (mg/kg)	Mean testes weight (g)	Mean sperm heads × 10^6/g testis	Abnormal sperm heads (%)
0	3.30	175	4.76
100	3.27	166	5.22
200	3.16	209[a]	9.77[a]

[a]Significantly different from controls ($p < 0.001$), as calculated by the authors.

Source: Cassidy et al. (1983).

Postmortem evaluation of the reproductive organs was conducted in a number of oral studies that ranged between 4 weeks and 2 years in duration (Til et al., 1989, 1988; Tobe et al., 1989; Johanssen et al., 1986). Johannsen et al. (1986) administered 0, 50, 100, or 150 mg/kg-day formaldehyde in the drinking water to Sprague-Dawley rats (15/sex/group) for 91 days and 0, 50, 75, or 100 mg/kg-day formaldehyde in basal diet to beagle dogs (4/sex/group) for 91 days; the study reported no treatment-related effects on absolute or relative gonad weights or histopathology for either species. In a 4-week drinking water study conducted by Til et al. (1988), formaldehyde was administered to Wistar rats (10/sex/treated group) at nominal levels of 0, 25, and 125 mg/kg-day; gonad organ weights and histopathology were not affected by treatment. Tobe et al. (1989) conducted a chronic (24-month) study in Wistar rats (20/sex/group), with drinking water concentrations of 0, 0.02, 0.1, or 0.5%. According to the study report, gonad weights were measured and histopathology was conducted, but no treatment-related findings were noted. In a chronic (105-week) study (Til et al., 1989) in Wistar rats (70/sex/group), formaldehyde was administered in the drinking water at mean actual levels of 0, 1.2, 15, or 82 mg/kg-day to males and 0, 1.8, 21, or 109 mg/kg-day to females; serial sacrifices were conducted at 53, 79, and 105 weeks of study. At study termination (105 weeks), mean testes weights were 30% increased ($p < 0.01$) in high-dose males as compared with controls, and histopathology evaluation revealed Leydig cell tumors in treated males (incidences of 0/50, 3/50, 3/50, and 2/50 for the control through high-dose groups, respectively; historical control tumor incidence data were not provided). The study authors did not judge these findings to be treatment related. By design, none of the subacute to chronic studies included measures of reproductive function (e.g., estrous cyclicity, sperm measures, or reproductive performance). With the exception of Til et al. (1989), detailed mean organ weight and histopathology incidence data were not provided in the published reports, and Til et al. (1988) only included tumor (not non-tumor) data.

4.2.1.7.3. ***Intraperitoneal studies addressing developmental and reproductive toxicity.*** Other studies in which formaldehyde was administered by I.P. injection have confirmed the potential effects on the male reproductive system.

Chowdhury et al. (1992) administered I.P. injections of 0, 5, 10, or 15 mg/kg-day formaldehyde to Charles foster adult male rats (10/group) for 30 days. On study day 31, blood was collected for serum testosterone measurements and the rats were sacrificed. The testes were removed, weighed, fixed in Bouin's solution, and processed for histopathology. The study authors reported adverse findings in all treated groups, including significant ($p < 0.01$) mean body weight gains, serum testosterone levels, and testes weights. Histopathologic evaluation revealed normal spermatogenic processes and Leydig cells in control animals. However, in treated rats, gradual cellular degeneration in seminiferous tubules and in Leydig cells was observed. Marked nuclear damage was noted in the 10 and 15 mg/kg-day groups, with significantly ($p < 0.001$) decreased Leydig cell populations and nuclear diameters observed in all treated groups. Additionally, a decrease in 3β-Δ^5-hydroxy steroid dehydrogenase was noted in the Leydig cell region of treated rat testes.

In a 30-day study performed by Majumder and Kumar (1995), 10 mg/kg-day formaldehyde was administered I.P. to eight male Wistar rats. All animals were sacrificed at term, and the testes, prostate, seminal vesicles, and epididymides were excised and weighed. With the use of methodologies that were not described in the report other than by reference to the *Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction* (WHO, 1987), sperm counts, motility, and viability were compared with those of 10 controls (injected I.P. with water alone). As shown in Table 4-66, striking reductions in sperm count and motility were noted in formaldehyde-treated rats compared with controls. Sperm viability was also significantly reduced by formaldehyde treatment, though to a lesser overall extent than sperm count and motility.

Table 4-66. Effect of formaldehyde on spermatogenic parameters in male Wistar rats exposed intraperitoneally

Parameters	Control (n = 10)	Treated (n = 8)
Sperm count (10^6/mL)	46.30 ± 5.01	20.40 ± 2.01[a]
Sperm viability (%)	87.10 ± 0.83	72.60 ± 2.32[a]
Sperm motility (%)	75.00 ± 10.90	22.00 ± 6.40[a]

[a]Significantly different from controls ($p < 0.0001$), as calculated by the authors.

Source: Majumder and Kumar (1995).

Majumder and Kumar (1995) also carried out an in vitro experiment in which sperm from normal rats were incubated with different concentrations of aqueous formaldehyde at concentrations ranging from 125 pg/mL to 2.5 µg/mL. Viability of control sperm remained close to 80% for a period of 1 hour, whereas the presence of formaldehyde dose-dependently reduced viability. Thus, only 50% spermatozoa were viable for 30 minutes in the presence of 5 ng/mL formaldehyde compared with 6 minutes in the presence of 500 ng/mL. Sperm motility also was sensitive to the presence of formaldehyde. Less than 10% of sperm was motile for 10 minutes in the presence of 125 pg/mL formaldehyde. The authors of the study considered their data to be good evidence that functional parameters of spermatozoa, such as viability and motility, can be adversely affected by exposure to formaldehyde. Moreover, they suggested that the cumulative effects of I.P. administration of formaldehyde on the male rat reproductive system raise an alert that formaldehyde might impair the reproductive health of males who are occupationally exposed to the compound.

Odeigah (1997) conducted two short-term in vivo assays to examine sperm head abnormalities and dominant lethal mutations. In the sperm assessment, five daily I.P. injections of 0, 0.125, 0.25, or 0.5 mg/kg formaldehyde were administered to male albino rats (six/group; strain not specified). The rats were killed 3 weeks after the last injection, and epididymal sperm counts and abnormalities were assessed. A dose-related decrease in sperm count was observed, and significantly increased incidences of sperm head abnormalities were found at all treatment levels (Table 4-67).

Table 4-67. Incidence of sperm head abnormalities in formaldehyde-treated rats

Dose (mg/kg)	Total abnormal sperm heads	Frequency (%) ± SEM
0	90	1.50 ± 0.11
0.125	184	3.09 ± 0.16 [a]
0.25	436	7.27 ± 0.30 [b]
0.5	514	8.57 ± 0.33 [b]

[a]Significantly different from controls ($p < 0.05$), as calculated by the authors.
[b]Significantly different from controls ($p < 0.001$), as calculated by the authors.

Source: Odeigah (1997).

In the dominant lethal assay (Odeigah, 1997), five daily I.P. injections of 0, 0.125, 0.25, or 0.5 mg/kg formaldehyde were administered to male rats (5 control rats, 12/treated group). Subsequently, each male was mated with two untreated virgin females per week for 3 consecutive weeks. The females were killed 13 days after the midpoint of the mating period

and evaluated for live and dead uterine implants. In general, the number of live embryos was decreased with treatment, and the number of dead implants was increased (Table 4-68). Additionally, there was a reduction in fertile matings in females mated 1–7 days after the males had been treated. This study did not assess the number of corpora lutea and therefore precluded the determination of preimplantation loss. Nevertheless, it is indicative of dominant lethal effects on the male germ cells.

Table 4-68. Dominant lethal mutations after exposure of male rats to formaldehyde

Dose (mg/kg)	Time of mating (days)	Fertile matings[a] (%)	Implants per female[a] (mean ± SE)	Live embryos per female (mean ± SE)	Dead implants per female (mean ± SE)	Dominant lethal mutation index[b]
0	0–21	96.67 (29)	7.86 ± 0.2 (29)	7.43 ± 0.3	0.43 ± 0.8	0
0.125	1–7	75.0 (18)	7.18 ± 0.3 (18)	5.95 ± 0.2	1.23 ± 0.5	19.92
	8–14	79.17 (19)	7.38 ± 0.5 (19)	6.30 ± 0.5	1.08 ± 0.3	15.21
	15–21	91.67 (22)	7.68 ± 0.2 (22)	6.89 ± 0.3	0.79 ± 0.5	7.27
0.25	1–7	33.33 (8)	5.75 ± 0.3 (8)	2.05 ± 0.3	3.70 ± 0.4	72.41
	8–14	50.0 (12)	6.60 ± 0.2 (12)	3.91 ± 0.2	2.69 ± 0.2	47.38
	15–21	87.5 (21)	7.25 ± 0.4 (21)	6.63 ± 0.3	0.62 ± 0.5	10.77
0.5	1–7	25.0 (6)	5.05 ± 0.03 (6)	1.10 ± 0.5	3.95 ± 0.22	85.20
	8–14	29.17 (7)	5.27 ± 0.01 (7)	1.50 ± 0.6	3.77 ± 0.28	79.81
	15–21	83.33 (20)	7.08 ± 0.04 (20)	5.79 ± 0.4	1.29 ± 0.17	22.07

[a]Number of females with implants presented in parentheses.
[b]Dominant lethal mutation index:

$$\text{Index} = 1 - \frac{\text{(Live implants experiment group per female)}}{\text{(Live implants of control group per female)}} \times 100$$

Source: Odeigah (1997).

4.2.1.7.4. ***Dermal exposure studies addressing developmental and reproductive toxicity.*** In a study designed to assess the embryotoxic effects of dermal exposure to formaldehyde, Overman (1986) applied 0.5 mL of a 37% formaldehyde solution directly to the dorsal skin of female Syrian golden hamsters (four–six/group) on GDs 8, 9, 10, or 11 for 2 hours. To prevent grooming during the treatment period, the animals were anesthetized with Nembutal. At the end of the 2-hour treatment period, the application site was washed thoroughly to remove any remaining formaldehyde. The dams were terminated on GD 15 (i.e., one day prior to expected delivery, since the typical gestation period for the Syrian golden hamster is 16–18 days). The fetuses were removed and fixed in either Bouin's solution or 95% ethanol for visceral or skeletal evaluation, respectively. The uteri were examined for implantation sites. Fixed fetuses were

weighed, measured (crown-rump), and examined for external abnormalities; fetuses that had been placed in Bouin's fixative were evaluated for visceral anomalies by using a free-hand sectioning technique, and those that were placed in ethanol were macerated, stained, and cleared for skeletal examination. In this study, the dams exhibited signs of dermal irritation and irritability, but the author reported no treatment-related effects on maternal body weight gain. The percent of resorption sites was increased (although not significantly) in treated litters as compared with control (0, 4.2, 8.1, 4.6, and 3.2% resorbed implantation sites for control and GDs 8, 9, 10, and 11 treatment groups, respectively). No treatment-related effects on fetal weight, length, or visceral or skeletal development were observed.

4.2.1.7.5. ***Summary of reproductive and developmental effects.*** Formaldehyde exposures up to 40 ppm 6 hours/day from GDs 6–15 or 6–20 did not result in external or internal malformations (Martin, 1990; Saillenfait et al., 1989). Martin (1990) reported delayed skeletal ossification and dose-dependent decreases in fetal body weight at 5 ppm. Formaldehyde exposure at 40 ppm to pregnant female Sprague-Dawley rats reduced fetal body weights in male and female progeny and in male pups of dams exposed to 20 ppm formaldehyde (Saillenfait et al., 1989). Based on these studies (Table 4-69), the LOAEL for developmental effects in rats is 5 ppm, with a NOAEL of 2 ppm for decreased fetal weight and delayed skeletal ossification, based on inhalation exposures during GDs 6–20.

Developmental studies during earlier gestational windows of inhalation exposure to formaldehyde have reported additional adverse health effects, including delayed ossification, changes in relative organ weight, undescended testes, biochemical changes (e.g., ascorbic acid and nucleic acids), and blood acidosis (Senichenkova and Chebotar, 1996; Senichenkova, 1991; Kilburn and Moro, 1985; Gofmekler and Bonashevskaya, 1969; Gofmekler, 1968; Pushkina et al., 1968). Kitaev et al. (1984) hypothesized that formaldehyde may affect reproductive function by stimulating the hypothalamo-pituitary-gonadal (HPG) axis based on their observations of increased ovary weight, increased number of ovulating cells, and changes in blood levels of gonadotropins (LH and FSH). Evidence of preimplantation loss, which may be related to HPG disruption, was observed in this study and by Sheveleva (1971). Additional studies are needed to better understand developmental effects of formaldehyde exposure during early gestational windows.

The prenatal developmental toxicity of oral and dermal exposures to formaldehyde has not been thoroughly studied. Reductions in postnatal growth in beagle pups was observed by Hurni and Ohder (1973) following in utero exposure to 125 ppm maternal dietary formaldehyde during GDs 4–56 in beagle dogs. However, gavage dosing during gestation of mice to overtly

maternally toxic doses (Seidenberg and Becker, 1987; Marks et al., 1980) (Table 4-70) and dermal application during gestation to hamsters at a dose that caused dermal irritation and irritability (Overman, 1986) did not result in any observed fetal toxicity (Table 4-71).

Few studies identified effects on maternal toxicity or female reproductive capacity. As summarized in Table 4-69, exposure of rat dams at 10–40 ppm formaldehyde during pregnancy has been shown to result in significantly decreased weight gain (Martin, 1990; Saillenfait et al., 1989; Kilburn and Moro, 1985). Maronpot et al. (1986) reported endometrial hypoplasia with a lack of ovarian luteal tissue in female rats exposed at 40 ppm but not at 20 ppm. Changes in LH and FSH levels were reported in dams exposed to 0.41 ppm formaldehyde by Kitaev et al. (1984), establishing an unbounded LOAEL for maternal toxicity.

Studies designed to assess male reproductive system endpoints in rats following repeated inhalation exposures to formaldehyde have shown concentration-dependent decreases in Leydig cell number and quality, effects on seminiferous tubules, decreases in testes weight, alterations in sperm measures, decreased testosterone levels, alterations in trace metals in the testes, and/or dominant lethal effects (Zhou et al., 2006; Özen et al., 2005, 2002; Zhou et al., 2006Sarsilmaz et al., 1999) (Table 4-72). Based on available studies, the LOAEL for changes in the male reproductive system in rats following 5 days/week of inhalation exposures is 5 ppm for 3 months of daily exposures and 10 ppm for 4 weeks of daily exposures; these dose levels are unbounded. Abnormal sperm were also noted in mice at an inhalation dose of 16.9 ppm 2 hours/day, 6 days/week for 13 weeks (Xing et al., 2007), but, in contrast, Maronpot et al. (1986) reported no histologic abnormalities in male mice after formaldehyde exposures at 40 ppm 6 hours/day, 5 days/week for 13 weeks. Varied results among studies may be due to species differences or differences in methods. Although several oral subchronic and chronic studies with formaldehyde did not identify effects on the testes (Tobe et al., 1989; Til et al., 1988; Johanssen et al., 1986), Cassidy et al. (1983) observed spermatogenic abnormalities after a single oral dose of 200 mg/kg to rats, and a chronic drinking water study in rats (Til et al., 1989) reported low incidences of Leydig cell tumors in all treated groups, compared with none in control (Table 4-73). Additionally, studies utilizing I.P. injection of formaldehyde in rats have demonstrated testes and sperm anomalies (Majumder and Kumar, 1995; Chowdhury et al., 1992) and dominant lethal effects (Odeigah, 1997) (Table 4-74).

Table 4-69. Summary of reported developmental effects in formaldehyde inhalation exposure studies

Species, strain, sex	n/Group	Dose; time of treatment[a]	Reported study findings[b]		LOAEL/NOAEL[c]		Reference
			Maternal	Offspring	Maternal	Offspring	
Rat, strain NR, female	12	0, 0.01, or 0.81 ppm (reported as 0.012, and 1 mg/m^3)[d]; continuous dosing 10–15 days prior to mating and during gestation	At 0.01 and 0.81 ppm: ↑ pregnancy duration (dose-dependent data not shown)[e]	Fetuses: At 0.01 and 0.81 ppm: ↓ fetuses/dam (dose dep., data not shown)[e] ↑ body wt (dose dep., stat. sig.) ↓ lung and liver wt (dose dep., stat. sig.) ↑ adrenal wt (dose dep., stat. sig.) At 0.81 ppm: ↑ thymus and kidney wt (stat. sig.)	L: 0.01 ppm N: ND	L: 0.01 ppm N: ND	Gofmekler (1968)
Rat, "albino" strain NR, female	12	0, 0.01, or 0.81 ppm (reported as 0.012, and 1 mg/m^3)[d]; continuous dosing 10–15 days prior to mating and during gestation	NE	Age of assessment NR. At 0.81 ppm: histologic effects in liver (e.g., ↑ extramedullary hematopoietic centers), kidney (e.g., ↑ polymorphism of renal epithelial cell nuclei) and thymus	NE[e]	N: 0.01 ppm L: 0.08 ppm	Gofmekler and Bonashevskaya (1969)[f,h]
Rat, strain NR, male	4	Inhalation and drinking water co-exposure: 0; 0.10 ppm plus 0.005 mg/L water; 0.20 ppm plus 0.01 mg/L water; or 0.41 ppm plus 0.1 mg/L water; all treatments 4 hours/day, 5 days/week for 6 months	No effects	No effects	ND[g]	ND	Guseva (1972)

Table 4-69. Summary of reported developmental effects in formaldehyde inhalation exposure studies

Species, strain, sex	n/Group	Dose; time of treatment[a]	Reported study findings[b]		LOAEL/NOAEL[c]		Reference
			Maternal	Offspring	Maternal	Offspring	
Rat, strain NR, female	NR	Expt 1: 0 or 30 ppm[d] Expt 2: 0, pair-fed control (15, 10, or 5 days), or 30 ppm; 8 hours/day for 15 days (GDs 3–17), 10 days (GDs 3–12), 5 days (GDs 8–12), or 3 days (GDs 9–11)	At 30 ppm 50% mortality (10 and 15 day exp) ↓ wt gain (duration dep.; 3, 5, 10, and 15 day exp.) ↓ wt of liver, kidney, spleen and thymus ↑ wt of lung and adrenal[e]	Fetuses: At 30 ppm ↓ fetal wt and growth (duration dep., 10 and 15 day exp.) ↑ dev. defects (undescended testes, large hearts, small thymuses, small lungs)[e]	N: ND L: 30 ppm[e]	N: ND L: 30 ppm[e]	Kilburn and Moro (1985)[f] Ab
Rat, Wistar, female	Embryo dev expt: 5–9/group (42 adult animals); maternal effects: NR (200 adult females total) [i]	0, 0.4, or 1.2 ppm (converted from reported 0, 0.5 or 1.5 mg/m^3); 4 hours/day, 5 days/week for 4 months; exposed females mated to unexposed males on 120th day exp.	At 0.4 ppm: ↑ wt of ovaries (stat. sig[e]) ↑ LH level (stat. sig.[e]) At 1.2 ppm: ↑ FSH level in blood (stat. sig.; nonsig. at 0.4 ppm[e])	At 0.4 ppm: ↑ no. of embryos and 2 blastomere stage embryos (stat. sig. in 2nd day preg.) At 1.2 ppm: ↑ no. degenerating embryos (stat. sig. in 3rd day preg.)	L: 0.4 ppm N: ND	L: 0.4 ppm N: ND	Kitaev et al. (1984)[g]
Rat, Sprague-Dawley, female and offspring of both sexes	25	0 (air control group), 0 (room control group), 2, 5, or 10 ppm; 6 hours/day GDs 6–15. Exposed females mated to unexposed males	At 10 ppm: ↓ food consumption (stat. sig.) ↓ wt gain (stat. sig.)	At 5 and 10 ppm: Fetuses: ↑ incidence of reduced ossification of pubic and ischial bones (stat. sig. compared with air control group) ↓ fetal wts (nonsig.) ↑ litter size (nonsig.)	L: 10 ppm N: 5 ppm	L: 5 ppm N: 2 ppm	Martin (1990)[e,f]

Table 4-69. Summary of reported developmental effects in formaldehyde inhalation exposure studies

Species, strain, sex	n/Group	Dose; time of treatment[a]	Reported study findings[b]		LOAEL/NOAEL[c]		Reference
			Maternal	Offspring	Maternal	Offspring	
Rat, "white" strain NR, female and offspring of both sexes	5–12 (NR for formalde-hyde only)	0, 0.01, or 0.81 ppm (reported as 0.012 and 1 mg/m^3)[d]; continuous 10–15 days prior to mating through gestation	At 0.01 and 0.81: ↓ vit. C level in liver (stat. sig.) ↓ vit. C level in placenta (nonsig.)	Fetuses: At 0.01 and 0.81 ppm: ↓ fetuses/female[e] ↑ body wt and organ wt (data not shown[e]) ↓ vit. C level in whole fetus (stat. sig.) At 0.01 ppm: ↓ vit. C level in fetal liver (stat. sig.)	L: 0.01 ppm N: ND	L: 0.01 N: ND	Pushkina et al. (1968)[f]
Rat, Sprague-Dawley, female and offspring of both sexes	25	0 (air control), 5, 10, 20, 40 ppm; 6 hours/day, GDs 6–20. Exposed females mated to unexposed males.	GD 21 dams: At 5 ppm: ↑ absolute body wt gain (5 ppm only) At 40 ppm: ↓ body wt gain GDs 6–21 (stat. sig.) ↓ absolute body wt gain (stat. sig., dose-dependent trend 20 and 40 ppm)	GD 21 fetuses: At 20 and 40 ppm: ↓ fetal body wt, male (stat. sig.) At 40 ppm: Delayed ossification of thoracic vertebrae (stat. sig., trend 20 ppm) ↑ unossified sternebrae (nonsig. at 40 ppm) ↓ fetal body wt, female (stat. sig.)	L: 40 ppm N: 20 ppm	L: 20 ppm N: 10 ppm	Saillenfait et al. (1989)

Table 4-69. Summary of reported developmental effects in formaldehyde inhalation exposure studies

Species, strain, sex	n/Group	Dose; time of treatment[a]	Reported study findings[b]		LOAEL/NOAEL[c]		Reference
			Maternal	Offspring	Maternal	Offspring	
Rat, mongrel white, female and offspring of both sexes	NR[i]	0 or 0.41 ppm (reported as 0.5 mg/m^3) formaldehyde (also a 3rd group of gasoline exposure, not described in this table); 4 hours/day GDs 1–19	Dams GD 20: ↓ corpora lutea (nonsig.), embryos dead before implantation (not stat. sig.), and implanted embryos (nonsig.) ↑ blood pCO_2 (stat. sig.)	Fetuses (GD 20): Stat. sig. findings include ↑ fetal wt ↑ litters w/internal organ anomalies ↓ fetuses w/ossification centers in hyoid bone ↑ metacarpal bone centers ↑ metatarsal bone centers ↑ developmental defects ↑ blood pCO_2 and pO_2 Pups: ↓ pup wt Dev. delays (data not shown)	L: 0.41 ppm N: ND	L: 0.41 ppm N: ND	Senichenkova (1991)
Mouse, mongrel, female and offspring of both sexes	NR (254 dams) [i]	0 + ethyl alcohol; 0.41 ppm formaldehyde; 0.41 ppm formaldehyde + bipyridyl; 4 hours/day GDs 1–19. Induced maternal iron deficiency anemia by I.P. bipyridyl injections on GDs 12–15; controls injected w/25% ethyl alcohol.	Dams GD 20: formaldehyde alone: ↑ blood pCO_2 (stat. sig.) formaldehyde + bipyridyl: ↓ blood acid metabolic products (stat. sig.) ↓ blood true bicarbonates and CO_2 conc. (stat. sig.)	Fetuses (GD 20): Formaldehyde alone: ↑ cryptorchidism Formaldehyde + bipyridyl: ↑ birth defects (stat. sig.) ↓ dev. delay (stat. sig.) ↓ blood acid-base measures of embryos (stat. sig.)	L: 0.41 ppm N: ND	L: 0.41 ppm N: ND	Senichenkova and Chebotar (1996)

Table 4-69. Summary of reported developmental effects in formaldehyde inhalation exposure studies

Species, strain, sex	n/Group	Dose; time of treatment[a]	Reported study findings[b]		LOAEL/NOAEL[c]		Reference
			Maternal	Offspring	Maternal	Offspring	
Rat, mongrel, white, female and offspring of both sexes	15/group terminated GD 20, 6/group littered	0, 0.0005, or 0.005 mg/L (0, 0.4, or 4 ppm), GDs 1–19, 4 hours/day	At 0.4 ppm: ↓ leukocyte counts At 4 ppm: ↓ leukocyte counts; reduced threshold of neuromuscular excitability, ↓ rectal temperature, ↓ blood hemoglobin; ↑ spontaneous mobility	At 0.4 ppm: ↑ preimplantation loss; at 1 mo. of age, ↓ spontaneous mobility; at 2 mo. of age, ↓ hemoglobin levels and leukocyte counts At 4 ppm: ↑ preimplantation loss; at 1 and 2 mo. of age, ↓ spontaneous mobility; at 2 mo. of age, ↓ hemoglobin levels and leukocyte counts	L: 0.4 ppm N: ND	L: 0.4 ppm N: ND	Sheveleva (1971)
Rat, Sprague-Dawley, female and offspring of both sexes	6 dams	0 or 6 ppm 8 hours/day, 6 weeks, starting at GD 1, PND 1, 4 weeks of age, or adult age	NE	In offspring exposed in utero and during early postnatal life: ↓ mean BW and liver weight; ↑ markers of oxidative stress In offspring exposed as juveniles: ↓mean liver weight; ↑ markers of oxidative stress In offspring exposed only as adults: no effect	NE	L: 6 ppm N: ND	Kum et al. (2007)
Rat, Sprague-Dawley, female and offspring of both sexes	6 dams	0 or 6 ppm 8 hours/day; 6 weeks, starting at GD 1, PND 1, 4 weeks of age, or adult age	NE	In offspring exposed in early postnatal life, as juveniles, or as adults, ↑ ANAE-positive T-lymphocytes in BALT In all exposure initiation groups, ↑ ANAE-positive lymphocyte ratios	NE	L: 6 ppm N: ND	Sandikci et al. (2007)

Table 4-69. Summary of reported developmental effects in formaldehyde inhalation exposure studies

Species, strain, sex	n/Group	Dose; time of treatment[a]	Reported study findings[b]		LOAEL/NOAEL[c]		Reference
			Maternal	Offspring	Maternal	Offspring	
Rat, Wistar, female and offspring of both sexes	12–14 dams	0, 6, or 12 ppm, 6 hours/day; 5 days/week, 30 days	NE	At 6 and 12 ppm, at postnatal days 30 and 90: respiratory irritation and toxicity; decr. BW, FC, WC; ↓ SOD activity, ↓ levels of copper and iron levels in lungs, ↑ zinc levels in lungs	NE	L: 6 ppm N: ND	Songur et al. (2005)

ND: not determined; NE: not evaluated; NR: not reported; Ab: abstract only; wt: weight; stat. sig.: statistically significant; p: pressure; l: length;
To convert concentrations in air (at 25°C) from mg/m^3 to ppm: 1 ppm = 1.23 mg/m^3; 1 mg/m^3 = 0.813 ppm.

[a]Treatment is given as the formaldehyde concentration in air (ppm) with the length of exposure each day and the duration of treatment in days, as available.
[b]Studies with negative findings are included.
[c]L: LOAEL; N: NOAEL.
[d]Exposure concentrations not validated; details of formaldehyde vapor generation not reported; exposure during gestation not well characterized in study report.
[e]No statistics provided.
[f]Lack of study details.
[g]See Table 4-72 for reproductive effects.
[h]Gofmekler and Bonashevskaya (1969) seem to report on different findings from the same study (i.e., same animals) as Gofmekler (1968).
[i]Number/group not clear from study report.

Table 4-70. Summary of reported developmental effects in formaldehyde oral exposure studies

Species, strain, sex	n/ Group	Dose; time of treatment	Reported developmental effects [a]		LOAEL/NOAEL [b]		Reference
			Maternal	Offspring	Maternal	Offspring	
Dog, beagle, female and pups	9–10	0, 125, or 375 ppm, dietary, GDs 4–56	No effects	At 125 and 375 ppm: ↓ birth wt and wt gain through postnatal week 8	L: ND N: 375 ppm	L: 125 ppm N: ND	Hurni and Ohder (1973)
Mouse, CD-1, female	29–35 total	0, 74, 148, or 185 mg/kg-day, GDs 6–15 (aqueous formaldehyde solution contained 10–15% methanol)	At 185 mg/kg-day: Mortality	No effects at GD 18	L: 185 mg/kg-day N: 148 mg/kg-day	L: ND N: 185 mg/kg-day	Marks et al. (1980)
Mouse, ICR/SIM, female	26–30 total	0 or 540 mg/kg-day, GDs 8–12	At 540 mg/kg-day: Mortality	No effects in pups on PND 1 and 3	L: 540 mg/kg-day N: ND	L: ND N: 540 mg/kg-day	Seidenberg and Becker (1987)

ND: not determined.
[a]Studies with negative findings are included.
[b]L: LOAEL; N: NOAEL.

Table 4-71. Summary of reported developmental effects in formaldehyde dermal exposure studies

Species, strain, sex	n/ Group	Dose; time of treatment[a]	Reported developmental effects		LOAEL/NOAEL[a]		Reference
			Maternal	Offspring	Maternal	Offspring	
Hamster, Syrian golden , female	4–6	0 or 37%; 0.5 mL applied to dorsal skin (hair clipped) for 2 hours then washed; GDs 8, 9, 10, or 11	Signs of dermal irritation and irritability	At all GDs of treatment, ↑ percent resorptions (not sig.)	L: 37% N: ND	L: 37% N: ND	Overman (1986)

ND, not determined
[a]L: LOAEL; N: NOAEL.

Table 4-72. Summary of reported reproductive effects in formaldehyde inhalation studies

Species, strain, sex	n/ Group	Dose; time of treatment[a]	Reported reproductive effects[b]	LOAEL/ NOAEL[c]	Reference
Rat, strain NR, male	4	Inhalation plus drinking water co-exposure: 0; 0.10 ppm plus 0.005 mg/L water; 0.20 ppm plus 0.01 mg/L water; or 0.41 ppm plus 0.1 mg/L water; all treatments; 4 hours/day, 5 days/week for 6 months. Exposed males mated to unexposed females	At 0.20 ppm + 0.01 mg/L water and 0.41 ppm + 0.1 mg/L water: ↓ nucleic acid in testes (dose dep.; data not shown; stat. sig.)	L: 0.20 ppm + 0.01 mg/L water N: ND	Guseva (1972)[d,e]
Rat, Wistar, female	NR (200 female)	0, 0.4, or 1.2 ppm ; 4 hours/day, 5 days/week for 4 months. Exposed females mated to unexposed males on 120th day of exposure.	At 0.4 ppm: ↑ wt of ovaries (stat. sig.[e]) ↑ LH level in blood (stat. sig. at 0.4 ppm[f]) At 1.2 ppm: ↑ FSH level in blood (stat. sig., dose dep. trend[f])	L: 0.4 ppm [h] N: ND [h]	Kitaev et al. (1984)[e]
Mouse, B6C3F1, male and female	10	0, 2, 4, 10, 20, or 40 ppm; 6 hours/day, 5 days/week for 13 weeks	Males and females: At 20 ppm:↓ wt gain At 40 ppm: ↑ mortality (13 weeks exp.); ↓ wt loss Females: At 40 ppm: ↑ Uterine endometrial and ovarian hypoplasia	L: 20 ppm N: ND	Maronpot et al. (1986)
Rat, albino Wistar, male	7	6 groups: 0, 10, or 20 ppm; 8 hours/day, 5 days/week for 4 weeks (subacute) or 13 weeks (subchronic)	At 10 and 20 ppm (both durations): ↓ wt gain (stat. sig., dose dep.) ↓ relative testes wt (stat. sig., dose and conc. dep.) ↓ zinc and copper in testes (stat. sig., dose and conc. dep.) ↑ iron in testes (stat. sig., dose and conc. dep.) No effect on testes wt.	L: 10 ppm N: ND	Özen et al. (2002)[f]
Rat, Wistar, male	18	0, 5, or 10 ppm; 8 hours/day, 5 days/week, 91 days	At 5 and 10 ppm: clinical signs of respiratory irritation, ↓ BW, FC, WC; ↓serum testosterone; ↓ mean seminiferous tubule diameters; ↑ localization of heat shock protein 70 in cytoplasm of spermatogonia, spermatocytes, and spermatids	L: 5 ppm N: ND	Özen et al. (2005)

Table 4-72. Summary of reported reproductive effects in formaldehyde inhalation studies

Species, strain, sex	n/ Group	Dose; time of treatment[a]	Reported reproductive effects[b]	LOAEL/ NOAEL[c]	Reference
Rat, albino Wistar, male	10	0, 10, or 20 ppm 8 hours/day, 5 days/week for 4 weeks	Dose NR: Irritation: standing hair, interrupted breathing, ↑ eye blinking, licking, nose cleaning, and sneezing. At 10 and 20 ppm: ↓ Body wt gain (dose dep.; stat. sig.) ↓ Leydig cell quantity (stat. sig.) ↑ Nuclear damage of Leydig cells (dose dep.; stat. sig.)	L: 10 ppm N: ND	Sarsilmaz et al. (1999)
Mouse, strain not specified, male	12	0, 21, 42, or 84 mg/m^3 (0, 16.9, 33.8, or 67.6 ppm); 2 hours/day, 6 days/week; 13 weeks. Exposed males mated to unexposed females.	In all treated groups: ↑ percentage abnormal sperm, ↑ resorption rate, and ↓ live fetuses	L: 16.9 ppm N: ND	Xing et al. (2007)
Rat, Wistar, male	7	0 or 1.5 ppm, 18 weeks FA exposures: (1) 4 hours/day, 4 days/week (2) 2 hours/day; 4 days/week (3) 2 hours/day, 4 days/week	In all treated groups: sig. ↓ seminiferous tubular diameter and epithelial height. Other effects in exposure groups: (1) sig. ↓ germ cells; arrested spermatogenesis (2) ↓ cells, increased thickness in basal membrane (3) ↑ spaces between germ cells; disrupted association between Sertoli and germinal cells	L: 1.5 ppm N: ND	Golalipour et al. (2007)
Rat, Sprague-Dawley, male	10	(1) 0 (gavage saline); (2) 10 mg/m^3 (8.05 ppm), 12 hours/day, 2 weeks; or (3) 10 mg/m^3 (8.05 ppm), 12 hours/day, 2 weeks, plus 30 mg/kg-day vitamin E orally	At 10 mg/m^3: sig. ↓ testis weight, atrophy of seminiferous tubules, ↓ spermatogenic cells, disintegrated and sloughed seminiferous cells; edematous interstitial tissue with vascular dilatation and hyperemia; ↓ epididymal sperm count and percentage motile sperm, ↑ percentage abnormal sperm; ↓ SOD, GSH-Px, GSH and ↑ MDA in testes; vitamin E attenuated all effects	L: 8.05 ppm N: ND	Zhou et al. (2006)
Rat, Wistar, male	40	0, 0.1, 1, or 10 ppm; 6 hours/day, 5 days/week, 13 or 52 weeks	No effects: testis weight; histopathologic findings[g]	L: ND N: 10 ppm	Appleman et al. (1986)

[a]Treatment is given as the formaldehyde concentration in air (ppm) with the length of exposure each day and the duration of treatment in days.
[b]Studies with negative findings are included.
[c]L: LOAEL; N: NOAEL.
[d]Guseva (1972) was a drinking water and inhalation study.
[e]Developmental effects shown in Table 4-69.
[f]Statistics not provided in study report.

[g]Focus of study was not the reproductive system; only reproductive system findings are addressed in the table; NOAEL and LOAEL in table are based only on reproductive system findings.
[h] For increased FSH, the LOAEL was 1.2 ppm (1.5 mg/m^3) and the NOAEL was 0.4 ppm (0.5 mg/m^3).

ND: not determined; NR: not reported.
To convert concentrations in air (at 25°C) from mg/m^3 to ppm: 1 ppm = 1.23 mg/m^3; 1 mg/m^3 = 0.813 ppm.

Table 4-73. Summary of reported reproductive effects in formaldehyde oral studies

Species, strain, sex	n/ Group	Dose; time of treatment	Reported reproductive effects[a]	LOAEL/ NOAEL[b]	Reference
Rat, Wistar, male	5 (20 control)	0, 100, or 200 mg/kg, single gavage dose	At 200 mg/kg: ↑ (19%) testicular sperm head counts (stat. sig.) ↑ (5%) abnormal sperm head (stat. sig.)	L: 200 mg/kg N: 100 mg/kg	Cassidy et al. (1983)
Rat, Sprague-Dawley, both sexes	15	0, 50, 100, or 150 mg/kg-day, drinking water; 91 days	No effects: absolute or relative gonad weights; histopathologic findings of reproductive organs[c]	L: ND N: 150 mg/kg-day	Johanssen et al. (1986)
Dog, beagle, both sexes	4	0, 50, 75, or 100 mg/kg-day, dietary; 91 days	No effects: absolute or relative gonad weights; histopathologic findings of reproductive organs[c]	L: ND N: 100 mg/kg-day	
Rat, Wistar, both sexes	10	0, 25, or 120 mg/kg-day, drinking water, 4 weeks	No effects: gonad weights; histopathologic findings of reproductive organs[c]	L: ND N: 120 mg/kg-day	Til et al. (1988)
Rat, Wistar, both sexes	70	0, 1.2, 15, or 82 mg/kg-day (males), 0, 1.8, 21, or 109 mg/kg-day (females),[d] drinking water; 105 weeks	In all treated groups: Leydig cell tumors observed at 105 weeks of study[c] At 82 mg/kg-day: ↑ mean testes weights	L: 1.2 mg/kg-day N: ND	Til et al. (1989)
Rat, Wistar, both sexes	20	0, 0.02, 0.1, or 0.5% in drinking water; 24 months	No effects: gonad weights; histopathologic findings of reproductive organs[c]	L: ND N: 0.5%	Tobe et al. (1989)

ND, not determined.
[a]Studies with negative findings are included.
[b]L: LOAEL; N: NOAEL.
[c]Focus of study was not the reproductive system; only reproductive system findings are addressed in the table; NOAEL and LOAEL in table are based only on reproductive system findings.
[d]Actual concentrations.

Table 4-74. Summary of reported reproductive effects in formaldehyde intraperitoneal studies

Species, strain, sex	n/ Group	Dose; time of treatment[a]	Reported reproductive effects[a]	LOAEL/ NOAEL[b]	Reference
Rat, Charles foster, male	10	0 or 5 mg/kg-day; 30 days	↓ body weight gain ↓ Leydig cell population and cell nuclear diameter ↓ serum T levels ↓ testes weights cellular degeneration of seminiferous tubules	L: 5 mg/kg-day N: ND	Chowdhury et al. (1992)
Rat, Wistar, male	8	0 or 10 mg/kg-day; 30 days	↓ sperm count, motility and sperm viability	L: 10 mg/kg-day N: ND	Majumder and Kumar (1995)
Rat, "albino" strain NR, male	6	0, 0.125, 0.250, or 0.60 mg/kg-day; 5 days	At all treatment levels: ↓ sperm count and ↑ sperm head abnormalities (3 weeks after the last injection)	L: 0.125 mg/kg-day N: ND	Odeigah (1997)
Rat, "albino" strain NR, male	12	0, 0.125, 0.250, or 0.60 mg/kg-day; 5 days. Exposed males mated to unexposed females.	At all treatment levels (at GD 13): Delayed time to mating ↓ mean no. implants and live embryos ↑ dead implants and dominant lethal index following mating to untreated females	L: 0.125 mg/kg-day N: ND	

[a]Studies with negative findings are included.
[b]L: LOAEL; N: NOAEL.

ND: not determined; NR: not reported; T: testosterone.

4.2.2. Carcinogenic Potential: Animal Bioassays

Chronic animal studies (inhalation and oral exposures) chronicle tumor incidence in a variety of rodent models. Study descriptions are provided above in detail (Section 4.2.1, Table 4-34). The study results are evaluated here for both routes of exposure in context of how they inform the carcinogenic potential for the three major affected systems: respiratory tract, GI tract, and LHP system. Experimental design and implementation must be carefully considered when interpreting study results. For example, some key factors involved in evaluating cancer bioassays include study size, organs/tissues examined, and study length (especially for late-in-life tumors).

4.2.2.1. *Respiratory Tract*

In the respiratory tract, only nasal tumors are considered formaldehyde induced in rodent studies. The majority of studies were conducted using rats (F344, Wistar, or Sprague-Dawley), and all studies of 18 months or greater in mice and rats show evidence of formaldehyde-induced nasal carcinogenicity. The nasal tumors are primarily SCCs, although papillomas, polypoid adenoma, adenocarcinoma, fibrosarcoma, and esthesioneuroepithelioma have been reported (Kamata et al., 1997; Monticello et al., 1996; Morgan et al., 1986a, b; Takahashi et al., 1986; Sellakumar et al., 1985; Kerns et al., 1983; Albert et al., 1982). Although hyperplasia, dysplasia, and squamous metaplasia of the respiratory epithelium have been observed beyond the nasal cavity, other respiratory tract tumors have not been significantly increased by formaldehyde exposure alone.

Increased tumor incidence and decreased latency are correlated with increasing formaldehyde exposure concentration. Reviewing data from the only lifelong inhalation study with multiple exposure groups, SCC is first noted at 8 and 9 months for high exposed (15 ppm) female and male F344 rats autopsied as “early deaths” prior to the 12 month sacrifice, with an incidence of 43% over the course of the study (unadjusted for mortality) (Kerns et al., 1983). In contrast only two SCCs were found in male and female rats sacrificed after 24 months of exposure (incidence of SCC 2.5% at 24 months) (Kerns et al., 1983). In a follow-up study by Monticello et al. (1996), the incidence of SCC in rats exposed at 15 ppm was 47% with the first tumor noted at 12 months. The incidence of SCC in male rats exposed at 10 ppm was 22% with the first SCC noted at 18 months. Moreover, of 90 rats exposed at 6 ppm for 20 months only one SCC was noted. No SCCs were detected in rats exposed at 0.7 or 2 ppm formaldehyde. These incidence rates are not mortality adjusted and include animals from each scheduled sacrifice (3, 6, 12, and 18 months). In a lifelong study of male Sprague-Dawley rats exposed at 10 ppm formaldehyde, the cumulative nasal tumor incidence was calculated as a function of time of

exposure (Figure 4-27) (Sellakumar et al., 1985). After 2 years of exposure, the probability of nasal carcinoma was greater than 60%.

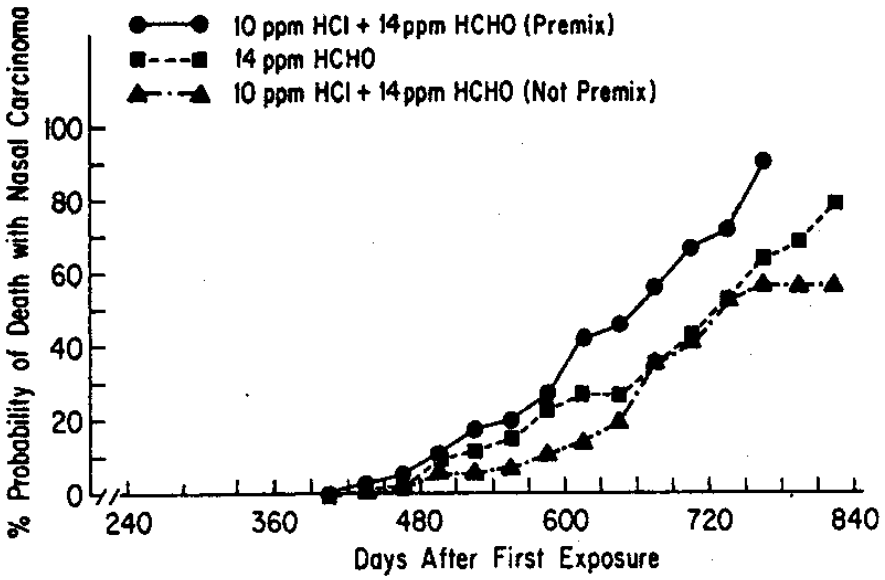


Figure 4-27. Mortality corrected cumulative incidences of nasal carcinomas in the indicated exposure groups.

Source: Sellakumar et al. (1985).

There is some evidence that less-than-lifetime exposure to formaldehyde can induce nasal tumors over an extended observation period. Two studies, both in male Wistar rats, report nasal tumors in response to less-than-lifetime exposures (Woutersen et al., 1989; Feron et al., 1988). A 13-week exposure at 20 ppm resulted in four nasal tumors (three SCCs), a cystic SCC of the nasolacrimal duct, and an epithelial tumor on the mandible, for a total of six tumors observed over 30 months of observation (Feron et al., 1988). No tumors were noted in 13-week controls. A limited number of formaldehyde-related tumors were noted due to 4 or 8 weeks of exposure followed by 30 months of observation. Although the tumor incidence of these less-than-lifetime exposures is low, this is consistent with the 2-year bioassays in Wistar rats. Wistar rats are more resilient to formaldehyde-induced nasal toxicity than F344 or SD rats (Section 4.2.1), and only 1 of 26 (4%) Wistar rats exposed at 10 ppm for 28 months developed SCC (Woutersen et al., 1989) versus 22% in F344 rats (Monticello et al., 1996).

Woutersen et al. (1989) also examined the effect of severe nasal damage from electrocoagulation on formaldehyde-induced SCC in Wistar rats. Nasal tumors were noted in formaldehyde-exposed rats without damaged noses (exposed for only 3 months and observed for

25 months). However, the low incidence of tumors in each treatment group (1/26, 2/60, 2/60, 1/58) indicates these data should be considered suggestive even though no SCCs were noted in control rats with or without damaged noses (n = 83). The studies by Woutersen et al. (1989) did demonstrate a synergistic effect of nasal damage from electrocoagulation and 10 ppm formaldehyde exposure (3 months), where 15/58 rats had SCC versus 1/26 with undamaged noses. The study was originally designed to examine the effect of formaldehyde on the damaged tissue on cancer promotion. However, it is unclear if the synergistic effect of formaldehyde exposure on damaged nasal tissue is an effect of formaldehyde on the damaged cells and joint effects of a mutagen with regenerative proliferation from the nasal damage. It is also possible the damaged nasal passages may alter airflow in the nasal passages, resulting in significantly different flux of formaldehyde into the tissue.

There is a single inhalation study (Dalbey, 1982) that investigates the role of promotion in formaldehyde-induced cancer. Although hamsters exhibit little to no effects of formaldehyde on the nasal mucosa or other respiratory tract tissues (Rusch et al., 1983a, b; Dalbey, 1982), DEN-induced respiratory adenomas were increased with formaldehyde exposure (10 ppm) 48 hours prior to DEN injection (but not by formaldehyde alone or formaldehyde exposure after DEN injection). The number of tracheal tumors per TBA was doubled by formaldehyde exposure. The study authors note that adenomas should be considered independent tumors and that the increase in tracheal tumors is of biological significance even given the incidence of TBAs (77%, DEN alone), was not further increased by formaldehyde exposure. It is of particular interest that a promotion study in hamsters is positive, since so little nasal pathology occurs with formaldehyde exposure. The absence of significant hyperplasia and tissue damage in these animals suggests that formaldehyde may induce subtle changes in the respiratory tract mucosa that permit formaldehyde to act as a tumor promoter.

4.2.2.2. *Gastrointestinal Tract*

As with the respiratory tract, the proximal portion of the GI tract exhibits formaldehyde-induced lesions in the forestomach and glandular stomach (Soffritti et al., 1989; Til et al., 1989; Tobe et al., 1989; Takahashi et al., 1986). However, data are mixed regarding the carcinogenic potential of formaldehyde in the GI tract from oral exposures.

Two independent 2-year cancer bioassays in Wistar rats exposed to formaldehyde in drinking water were both negative; they reported no tumors found at the 24-month sacrifice (Til et al., 1989; Tobe et al., 1989). Til et al. (1989) exposed rats to a range of formaldehyde doses (0, 1.2, 15, or 82 mg/kg-day) and evaluated 44–49 animals per sex per dose group at 24 months of exposure. No formaldehyde-related tumors were found. A smaller study by Tobe et al.

(1989) failed to note any tumors after a 2-year exposure at 0, 10, 50 or 300 mg/kg-day (eight rats per sex per treatment group).

In contrast, two lifelong studies in male and female Sprague-Dawley rats provide support for formaldehyde-induced GI tract tumors (Soffritti et al., 1989). Both stomach and intestinal tumors are rare; low background rates are expected in this colony of Sprague-Dawley rats. These studies demonstrate an increase in tumors (although rare) correlated with exposure to formaldehyde and significantly increased susceptibility to early-lifetime exposure. The authors provide a detailed report of the background rates of various stomach and intestinal neoplasia for male (n = 2,677) and female (n = 2,582) rats (Soffritti et al., 1989). From this background pool, the total incidence of benign and malignant tumors in the stomach and intestine combined is only 1.4% (combining all sites and locations). The majority of tumors are located in the stomach (1% benign, 0.2% malignant). Usually, a very large study population is needed to detect increases in rare tumor types. In this study, the study size of each treatment group was relatively small. Thus, only a few TBAs are responsible for the observed increases. Additionally, a clear dose-response relationship is not evident (perhaps due to the low incidence) despite the fact that the greatest tumor incidence was observed in the highest treatment group. As presented above, apparent increases in both stomach and intestinal neoplasia are noted in formaldehyde-treated rats (ranging from 1 to 6% by type). When summed across the GI tract, tumor incidence in the highest treatment group was 8% versus 1.4% in historical controls. Despite the limitations of group size and lack of dose response, the findings do support the carcinogenic potential for formaldehyde administered orally. Moreover, these findings are not inconsistent from Tobe et al. (1989) and Til et al. (1989) because the study design is significantly different.

The second study reported by Soffritti et al. (1989) demonstrates early lifetime susceptibility with GI tumor incidence of 21.6% in females (n = 37) and 13.9% in males (n = 36) after exposure to formaldehyde. Sprague-Dawley rats were exposed to formaldehyde in drinking water for 2 years (0 or 2,500 mg/L). Exposures began on GD 12 in the offspring. The most common tumor detected was intestinal leiomyosarcoma (13.5% in female offspring) with a background rate of 0.04% in female rats in the colony.

Soffritti et al. (1989) stands alone in supporting formaldehyde-induced GI tumors. These findings are largely attributed to a unique study design that included lifelong observation, neonatal exposure, examination of individual tumor types as well as combined rare tumor types for analysis, and summation of tumors across locations. The study design results in a more sensitive assay for rare tumors. Thus, Soffritti et al. (1989) utilized a more appropriate design and analysis for detecting rare tumors and should not be compared with the results by Tobe et al. (1989) and Til et al. (1989).

There is evidence that formaldehyde may act as a tumor promoter by the oral route as well as the inhalation route (discussed above). Takahashi et al. (1986) reported an increase in MNNG-initiated GI cancers with formaldehyde exposure (29.4 versus 13.3% TBA in controls); the greatest difference in tumor-containing versus non-tumorigenic mice was associated with adenocarcinoma in the glandular stomach (23.5 versus 3.3% in controls). Additionally, forestomach papillomas and preneoplastic hyperplasia in the glandular stomach were increased with formaldehyde exposure alone.

The data indicate carcinogenic potential from formaldehyde ingestion in drinking water. Formaldehyde may act in part as a tumor-promoting agent and shows clear increased susceptibility from early lifetime exposures.

4.2.2.3. *Lymphohematopoietic Cancer*

The majority of chronic animal bioassays do not report either leukemia or lymphoma, but many of these studies did not have adequate study length or study design to detect these malignancies. Many studies focused the histopathology on the nasal passages and respiratory tract (Monticello et al., 1996; Holmström et al., 1989a; Woutersen et al., 1989; Appleman et al., 1988; Dalbey, 1982; Horten et al., 1963). Kamata et al. (1997) did examine additional organs, but there were only five animals at each sacrifice. Similarly, the oral study by Takahashi et al. (1989) focused on the stomach and intestines. Tobe et al. (1989) only included 20 Wistar rats per group with interim sacrifices. Therefore, few studies can inform the carcinogenic potential of formaldehyde on the LHP system. Table 4-75 lists the chronic bioassays that have the potential to detect LHP malignancies.

Soffritti et al. (1989) first published an observation of formaldehyde-induced leukemia in animal studies. These study results have been criticized for their combination of lymphatic leukemia and lymphoma. However, this classification is consistent with the current WHO classification of lymphoid malignancies in humans where adult B- and T-cell leukemias and lymphomas are considered the same disease (Harris, 2000a). Although there may be a slight vehicle effect, a dose response is still readily apparent among the formaldehyde-treated groups (Figure 4-28). In contrast, the 2-year bioassay in Wistar male in female rats (Til et al., 1989) was clearly negative for leukemia and lymphoma with only four TBAs in all treatment groups sacrificed at 24 months. Moreover, the drinking water levels were similar at the highest dose of both Til et al. (1989) and Soffritti et al. (1989). However, the two study designs differ in length, which may have influenced results since leukemia is a late-in-life malignancy in rodents. Two-year survival in the Soffritti et al. (1989) study varied between 50 and 60%. These animals were available to develop leukemia after the 2-year window of the Til et al. (1989) study. Any

potential role of strain differences is unknown. Overall the results of Soffritti et al. (1989) are strong since they indicate an exposure-response relationship in a lifelong study appropriate for late-in-life malignancies. Unlike the GI tract tumors, increased LHP malignancies were not associated with early-life exposure to formaldehyde in drinking water.

Table 4-75. Summary of chronic bioassays which address rodent leukemia and lymphoma

Study	Histopathology	Endpoint	Results	Comments
Drinking water exposure				
Soffritti et al. (1986)	*Male and female Sprague-Dawley rats*			
	Complete histopathology	Lymphocytic leukemia and lymphosarcoma	Increased, showing a dose-response	Lifelong study High exposure of 1,500 mg/L in water
Til et al. (1989)	*Male and female Wistar Rats*			
	Complete histopathology in control and high-dose group (15 ppm)	Lymphoma, leukemia	No increase (three lymphomas and one leukemia found in 200 animals at the 2-year sacrifice)	2-year bioassay High exposure of approximately 1,900 mg/L (82 mg/kg for males and 109 mg/kg for females)
Inhalation exposures				
Sellakumar et al. (1985); Albert et al. (1982)	*Male rats, Sprague-Dawley*			
	Necropsy focused on respiratory tract: also liver, spleen, kidney, and testes and organs demonstrating gross pathology	Lymphoma	No increase	Lifelong study, high mortality at 24 months (>80%)
Battelle, Columbus Laboratories (1981)	*Male rats, F344*			
	Complete histopathology in controls and high-dose group (15 ppm)	Leukemia, all	No increase	Extended study, high mortality
	Female rats, F344			
	Complete histopathology in controls and high-dose group (15 ppm)	Leukemia, all	Increase in mortality-adjusted incidence; $p = 0.0056$[a]	Extended study, high mortality. Apparent elevation in 2 and 6 ppm treatment groups as well; statistical comparison to controls is problematic.
	Female mice, C57BL/6xC3HF1			
	All organs in controls and high-dose group (15 ppm)	Lymphoma, all	26% increase in 15 ppm group, 16% in controls; $p = 0.0617$	Extended study. All mice included in statistics conducted by BattelleColumbus Laboratories.

[a]Original statistical analysis provided by Battelle Columbus Laboratories. Significance set at $p < 0.0167$. Analysis of adjusted data where time to lesion and survivorship were considered (Cox [1972] and Tarone [1975]).

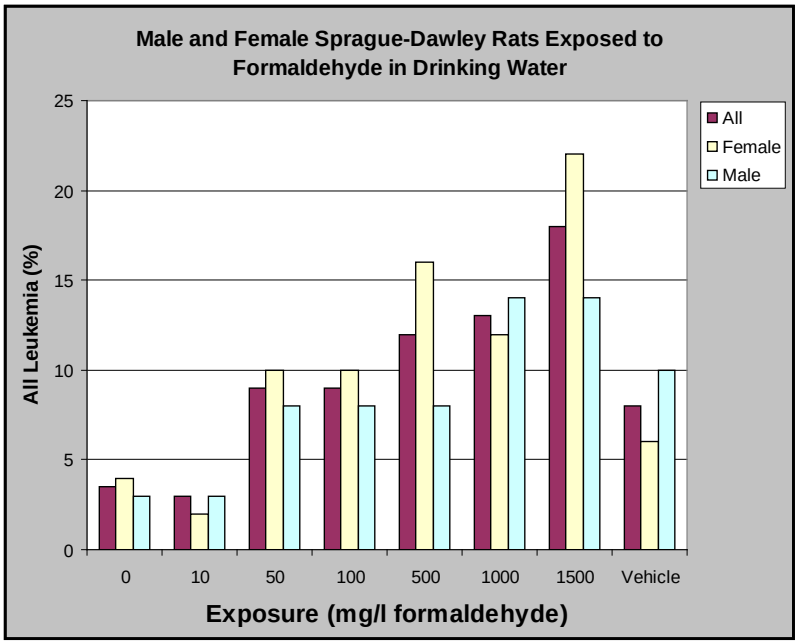


Figure 4-28. Leukemia incidence in Sprague-Dawley rats exposed to formaldehyde in drinking water for 2 years.

Note: Animals were observed until natural death. The vehicle control contained the level of methanol in drinking water for the high-dose group (1,500 mg/L).

Source: Soffritti et al. (1989).

Sellakumar et al. (1985) conducted a lifelong inhalation study in male Sprague-Dawley rats exposed at 10 ppm formaldehyde. Organs outside of the respiratory tract were routinely examined (liver, kidneys, and testes), including any organ exhibiting gross pathology, so there was some ability to detect leukemia and lymphoma. However, spleen, thymus, and lymph nodes were not routinely examined, limiting detection of smaller lesions. Although Sellakumar et al. (1985) was a lifelong study, there was a high mortality rate at 2 years (>80% from the figure), again limiting the power of this study to detect late-in-life malignancies. Nonetheless, this study did not indicate formaldehyde-induced lymphoma or leukemia.

The largest and most comprehensive study of carcinogenic health effects from formaldehyde inhalation exposures is the study conducted at the Battelle Columbus Laboratory (1981) and reported by Kerns et al. (1980) and Swenberg et al. (1983). Although the summary reports of this study do not discuss leukemia or lymphoma rates, mouse lymphoma and rat leukemia were selected by the study pathologist and biostatistician for analysis (Battelle Columbus Laboratories, 1981). Statistical analysis performed by Battelle, which accounted for

time to lesion and survivorship rates, indicated a statistically significant increase in female rat leukemia ($p = 0.0003$) and near significant increase in female mouse lymphoma ($p = 0.06$). No trend analysis could be conducted since only gross pathology was conducted on mid-dose mice and rats (2 and 6 ppm). EPA has further analyzed these data to better understand the significance of these findings.

As noted in the study description (Section 4.2.1.2.3), both male and female rats at the highest exposure (15 ppm) exhibited significant early deaths due to nasal lesions (see Figure 4-29). No female rats in the highest exposure group remained after the 24-month sacrifice (which included only 14 animals). Nine male rats were examined after 24 months. Since the first leukemia in rats was noted at 21 months, the early deaths prior to that time reduced the number of animals in which the leukemia could have been observed. Unadjusted data do not show an increase in female rat leukemia, but, when data are adjusted to account for the early deaths, dramatically different results are apparent. In the unadjusted data, leukemia incidence is expressed as the number of cases over total animals examined (including early deaths and interim sacrifices). By using this methodology, there is a lower incidence of leukemia in high exposed animals, and a slightly higher incidence of leukemia in the mid-exposed groups compared with controls (Figure 4-30, panel A). However, when the leukemia incidence rates are calculated only for female rats that survived to at least 21 months (the first case noted), formaldehyde-induced increases in leukemia are evident in all treatment groups relative to controls (Figure 4-30, panel B). These results are consistent with the original statistical analysis conducted by the researchers at the Battelle Columbus Laboratories (1981). Although elevated in all treatment groups, no exposure-response relationship is evident. The lack of a dose-response relationship may in part be due to the fact that no animals in the high-exposure group survived past 24 months. Additionally, the 6 ppm (mid-exposure) group had significant early deaths between 21 to 24 months compared with the 2 ppm and control groups. A similar reanalysis of data from male rats did not reveal any relationship between formaldehyde treatment and leukemia incidence.

Male and female mice exposed to formaldehyde for 24 months did not experience the same rate of formaldehyde-related mortality (Kerns et al., 1983). However, significant early deaths were observed in male mice due to infighting. Therefore, the data for male mice may not inform incidence of late-in-life tumor, such as lymphoma. As discussed above in the full study description, full histopathology, including spleen, liver, thymus, and lymph nodes, was only done on the control and high-exposure group (15 ppm) mice. When comparing unadjusted incidence lymphoma-bearing mice, there is a clear elevation lymphoma in formaldehyde-exposed female mice (28%) over controls (22%) (Figure 4-31).

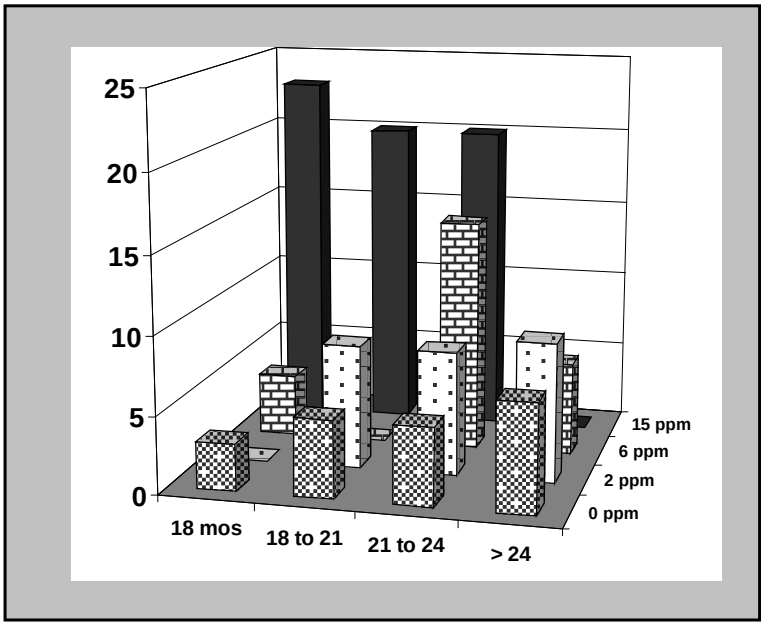


Figure 4-29. Unscheduled deaths in female F344 rats exposed to formaldehyde for 24 months.

Source: Battelle Columbus Laboratories (1981).

Panel A: Unadjusted Data **Panel B: Adjusted Data**

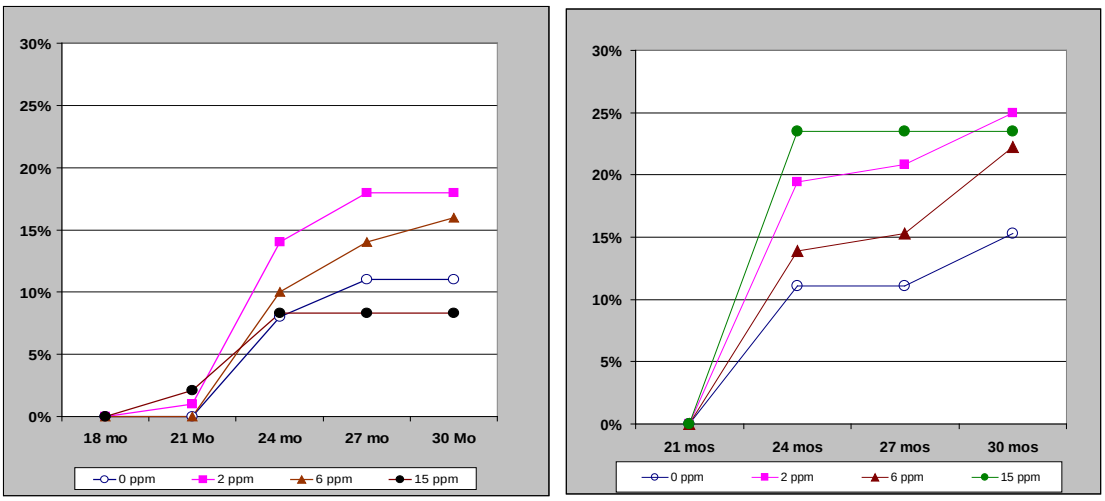


Figure 4-30. Cumulative leukemia incidence in female F344 rats exposed to formaldehyde for 24 months.

Note: Panel A shows the unadjusted data where incidence rates include all scheduled sacrifices and early deaths up to the time point shown. Panel B shows incidence of leukemia only in rats who survived at least 21 months.

Source: Battelle Columbus Laboratories (1981).

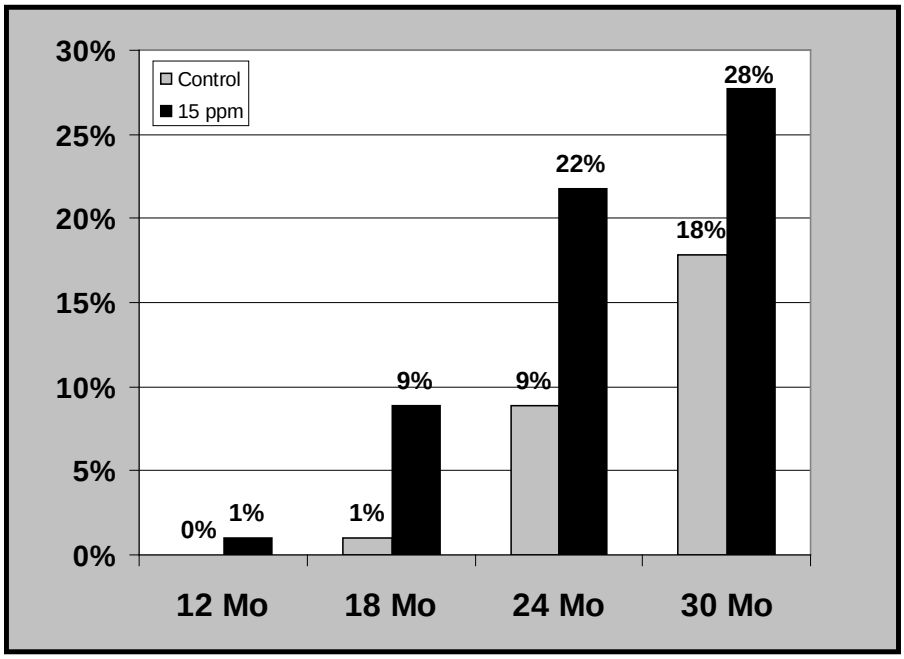


Figure 4-31. Cumulative incidence of tumor bearing animals for lymphoma in female mice exposed to formaldehyde for 24 months.

Note: Mice from the 6-month interim sacrifice are not included since only nasal passages were examined ($p < 0.05$).

Source: Battelle Columbus Laboratories (1981).

Although results are somewhat mixed between studies, there is evidence in the animal bioassays for formaldehyde-induced LHP malignancies. Differences in study design may account in part for mixed results. The lifelong drinking water study by Soffritti et al. (1989) may have allowed for malignancies to develop late in life, whereas the drinking water study by Til et al. (1989) sacrificed all animals at 24 months. Even though the exposure levels were similar, the studies are not directly comparable. Similarly it is hard to directly compare results from the two major inhalation studies in rats. Although Sellakumar et al. (1985) is a lifelong study, the mortality for rats was greater than 80% at 2 years. Additionally, the pathology examination was much less rigorous than in the Battelle Columbus Laboratories (1981) study, perhaps missing smaller lesions. Therefore, the increase in formaldehyde-induced leukemia seen in female F344 rats late-in-life (Battelle Columbus Laboratories [1981]) may reflect a more sensitive study design. Finally, strain differences may account for different susceptibility as well. The two positive rat studies, by different routes of exposure, along with a positive result for

formaldehyde-induced mouse lymphoma, make a substantive case for the potential of formaldehyde-induced LHP malignancies.

4.2.2.4. *Summary*

Formaldehyde is toxic at the POE. Similar lesions, including increased cell proliferation, DPX, and focal lesions, are noted in the GI tract or URT, depending on route of exposure. Similarly, formaldehyde-induced tumors are noted at the POE for both routes of exposure. Additionally, data exist for both routes of exposure to indicate that formaldehyde may act in part as a tumor promoter.

When evaluating the studies with adequate study design to assess LHP malignancies, results are mixed by strain, sex, route of exposure, and length of study. The three positive studies (Soffritti et al., 1986; Battelle Columbus Laboratories, 1981) had the best histopathologic examinations and greater sensitivity for detection of late-in-life tumors. Based on these results, sufficient evidence is available in animal studies to support formaldehyde-induced LHP malignancies.

4.3. GENOTOXICITY

Formaldehyde has been extensively studied for its mutagenic and genotoxic activity in a variety of assay systems. The first reported mutagenic activity of formaldehyde was when Rapoport (1946) described the induction of sex-linked recessive lethals in drosophila larvae fed on a medium containing formalin. A variety of genotoxic and mutagenic effects have been subsequently demonstrated in both in vitro and in vivo test systems, including the formation of DPXs, point mutations, DNA strand breaks, increased MNs, and CAs (Auerbach et al 1977; Ma and Harris, 1988; Conaway et al 1996; IARC 1995; 2006).

In this section, reactions of formaldehyde with cellular macromolecules, such as DNA and proteins, and formaldehyde-induced clastogenicity are described. In addition, the evidence for formaldehyde-induced mutations is considered in the context of the current EPA cancer guidelines (U.S. EPA, 2005a). Particular emphasis is given to the genotoxic effects of formaldehyde in humans, described in Section 4.3.4.2.

4.3.1. Formaldehyde-DNA Reactions

Formaldehyde is a reactive chemical and interacts with DNA in several ways, forming DPXs, DNA adducts, and DNA-DNA cross-links (DDXs) (Fennell, 1994; Casanova et al., 1989; Heck and Casanova, 1987; Casanova-Schmitz et al., 1984a, b; Casanova-Schmitz and Heck, 1983; Ohba et al., 1979; Dönecke, 1978; Brutlag et al., 1969). Formaldehyde also may facilitate the formation of adducts between other chemicals (endogenous or xenobiotic) and DNA

(Fennell, 1994; Koppel et al., 1991; Casanova et al., 1989; Heck and Casanova, 1987; Lam et al., 1985; Casanova-Schmitz et al., 1984a; Casanova-Schmitz and Heck, 1983; Ohba et al., 1979; Dönecke, 1978; Brutlag et al., 1969).

The high reactivity of formaldehyde results in little specificity in reaction sites, indicating that a range of adducts and cross-links might be expected. However, the spectrum of formaldehyde-DNA reaction products is difficult to quantify in vivo as many of these are labile and difficult to measure directly (Fennell, 1994; Casanova et al., 1989). Additionally, formaldehyde is metabolically incorporated into nucleic acids, and therefore DNA and RNA assays incorporating radiolabeled formaldehyde need careful interpretation to distinguish between covalently bound and metabolically incorporated formaldehyde (Casanova et al., 1989; Heck and Casanova, 1987; Casanova-Schmitz et al., 1984a, b; Casanova-Schmitz and Heck, 1983). Hence, reports of formaldehyde-DNA reactivity in cell-free system results may not provide a useful measure of exposure (Fennell, 1994). Besides, the question of biological relevance must also be considered. On the other hand, methods used to extract and measure DNA-formaldehyde reaction products after in vivo exposures should be evaluated to ensure that formaldehyde reaction products are neither created nor removed during sample preparation (Fennell, 1994; Casanova et al., 1989).

4.3.1.1. DNA-Protein Cross-Links (DPXs)

Evidence from numerous experimental models, ranging from cell-free systems to single cells and in vivo animal and human exposures, suggests that formaldehyde reacts readily with DNA forming DPXs (Reviewed in Conaway et al 1996; IARC 2006). As shown in Table 4-76, cross-links between histones and DNA have been demonstrated in isolated chromatin samples on exposure to formaldehyde from earlier studies (Ohba et al., 1979; Dönecke, 1978; Brutlag et al., 1969). Several in vitro studies demonstrated induction of DPX by formaldehyde exposure in bacteria (Wilkins and McLeod 1976), yeast (Magana-Schwencke and Ekert, 1978) and mammalian cells including animal cells (Chinese hamster ovary cells, Chinese hamster V79 lung epithelial cells, mouse leukemia L1210 cells, mouse hepatocytes, rat Yoshida lymphsarcoma cells, rat C18 tracheal epithelial cells, rat hepatocytes, rat nasal, tracheal epitheial cells and aortic endothelial cells) and human cells (lung and bronchial epithelial cells, fibrobasts, white blood cells, peripheral blood lymphocytes, Epstein-Barr Virus-Burkitt's lymphoma cells, Jurkat E6-1 cells, HeLa cells, lymphoblastoid cells, gastric mucosal cells and whole blood) as summarized in Table 4-76.

Ross and Shipley (1980) showed that formaldehyde induces SSBs and DPXs; SSBs are formed at concentrations >200 µM and a reduction of radiation-induced breaks (indirect measure

of DPXs) at 50 µM. The authors used a [^{14}C]-thymidine-incorporated mouse L1210 cell line to monitor formaldehyde-induced DNA strand breaks and DPXs. They exposed cells to varying concentrations of formaldehyde for 2.5 hours. An alkaline-elution technique in the presence or absence of proteinase K was used to measure strand breaks. In order to detect DPXs, some cells were exposed to 300 R of X-rays immediately after formaldehyde treatment. Formaldehyde-induced DPXs were repaired 24 hours after the compound was removed from the culture (Ross and Shipley, 1980).

Casanova-Schmitz and Heck (1983) have shown that homogenates of rat nasal mucosa incubated with formaldehyde in vitro followed by extraction with a strong aqueous-immiscible organic solvent demonstrated increased DPX formation in DNA obtained after enzymatic proteolysis from the aqueous-organic interface, termed as "interfacial DNA". In the same study, they have shown that DNA isolated from the nasal, but not olfactory, mucosa of rats exposed to formaldehyde (2, 6, 15, and 30 ppm 6 hours/day for 2 days) via inhalation showed significant increase in DPXs in the interfacial DNA ≥6 ppm, which was shown to be linear in the exposure range of 2–30 ppm (2.45–36.8 mg/m^3). However, DNA in the aqueous phase did not show DPX formation. Thus, the cross-linked DNA that could be extracted from the interface after proteolysis was considered to be supporting evidence of chemically induced DPX formation. The inability of this study to detect DPXs at lower levels of formaldehyde exposure is likely be due to the protective mechanism of GSH, which catalyzes the conversion of formaldehyde to formate.

So, in a later study, Casanova and Heck (1987) reported that GSH depletion caused an increase in DPX formation in the interfacial DNA in the nasal mucosa of F344 rats when a dual-isotope (^{3}H/^{14}C) method was used. The dual isotope method helps in making the distinction between metabolic incorporation and covalent binding of formldehyde. Oxidatoin by removal of one hydrogen atom is required for metabolic incorporation of formaldehyde into cellular macromolecules, but not in the formation of DNA adducts or DNA-protein crosslinks. Thus, the ratio of ^{3}H/^{14}C of DNA containing DPX will be higher than the macromoleucules where formaldehyde is metabolically incorporated. However, the authors further demonstrated that, when the double isotope method was used, the ^{3}HCHO is oxidized significantly more slowly than H^{14}CHO under these conditions, resulting in an overestimate of the concentration of cross-links due to an isotopic effect on the oxidation of ^{3}HCHO catalyzed by formaldehyde dehydrogenase (FDH). Besides, this method leaves residual formaldehyde that is likely to form DNA adducts by reacting with deoxyribonucleosides in the DNA hydrolysates (Heck and Casanova, 1987).

To overcome this, Casanova et al. (1989) used an improved method, which is based not on the analysis of residual formaldehyde bound to deoxyribonucleosides in DNA hydrolysates but on the determination of the total ^{14}C-formaldehyde bound to DNA. This study showed that formaldehyde was exclusively bound to interfacial DNA, indicating the formation of DPXs. Hydrolysis of DPXs in different samples quantitatively released formaldehyde. Besides, DPX formation was detectable at all concentrations of exposure to formaldehyde (0.3–10 ppm for 6 hours). Overall, these studies clearly show that formaldehyde induces DPXs in nasal epithelial cells of rodents. However, there are no published rodent studies that assess DPXs beyond the nasal passages of the URT.

Formaldehyde-induced DPXs were also found in the nasal mucosa and extra-nasal tissues of rhesus monkeys exposed to 0, 0.71, 2, or 6 ppm (0, 0.86, 2.45, or 7.36 mg/m^3) formaldehyde 6 hours/day for 3 days (Casanova et al., 1991). These data were used as a basis for cross-species prediction of formaldehyde-induced DPXs in humans. The presence of DPXs in rhesus monkeys confirms formaldehyde‘s DNA reactivity as a general effect. Additionally, DPXs were detected in the larynx/trachea/carina (pooled sample) and in intrapulmonary airways of monkeys exposed to 2 or 6 ppm formaldehyde. These data demonstrate direct effects of formaldehyde on DNA in tissues that correspond to observed tumor sites in humans (nasal and nasopharynx).

Bermudez and Delehanty (1986) observed the formation of DPXs, scheduled (S) and unscheduled DNA synthesis (UDS), and synthesis of RNA when cultured F344 rat nasal epithelial cells from the naso- and maxillary turbinates were incubated with formaldehyde. Unscheduled and scheduled DNA synthesis was stimulated (0.05–0.1 mM) and then inhibited (0.1–1 mM), depending on the formaldehyde concentration. Experiments by Cosma et al. (1988) and Cosma and Marchok (1988) showed the induction of DPXs and DNA SSBs in cultured C18 rat tracheal epithelial cells exposed to 200 μM formaldehyde for 90 minutes (Cosma et al., 1988; Cosma and Marchok, 1988).

Several human cells (epithelial cells, fibroblasts, buccal cells) or cell lines (lymphoblastoid cells) exposed to formaldehyde have been shown to form DPX (Craft et al 1987; Costa et al 1997; Emri et al 2004; Li et al 2004).

Craft et al. (1987) detected DPXs by alkaline elution in TK6 human lymphoblastoid cells immediately after a 2-hour exposure (zero time) to 0, 15, 50, 75, 100, 150, 300, and 600 μM formaldehyde with a significant nonlinear increase in DPXs above 50 μM concentration, which correlated with the onset of cytotoxicity, but DPXs were completely removed in cultures held for 24 hours before processing. In the zero-time sample, significant increases in DPXs were first observed at 50 μM and increased linearly up to 150 μM. In cells held for 24 hours, there was no detectable increase in DPXs.

However, Costa et al. (1997) detected DPXs with paraformaldehyde (which dissociates to release formaldehyde) at doses that were cytotoxic (>0.003%) but could not discriminate between the DPX-inducing and cytotoxic effects of this chemical in EBV, human Burkitt's lymphoma cells (Costa et al., 1997). Grafström et al. (1983) reported that the number of DPXs induced by 100 μM formaldehyde in vitro in human epithelial cells and fibroblasts of bronchial origin was similar and that the frequency of these cross-links was proportional to the concentration of formaldehyde. Besides the bronchial epithelial cells and fibroblasts, the authors also noted that formaldehyde exposure resulted in DPXs and DNA SSBs in skin fibroblasts and DNA excision repair-deficient skin fibroblasts. However, formaldehyde was only moderately cytotoxic to normal bronchial epithelial cells and fibroblasts at concentrations that induced substantial DNA damage. Repair of the formaldehyde-induced DNA SSBs and DPXs appeared to be inhibited by the continued presence of formaldehyde in the culture medium (Grafström et al., 1984).

Emri et al. (2004) detected a significant increase in DPX formation in primary human skin fibroblasts and keratinocytes at 8 hours of exposure in vitro to formaldehyde at 25 μM with an approximately linear increase up to 100 μM. These cells were exposed to 0, 12.5, 25, 50, and 100 μM formaldehyde for 8 hours and then exposed to 250 μM methyl methane sulfonate (MMS) for 2.5 hours. The induction of DPX formation was measured by the ability of formaldehyde to reduce DNA migration in the comet assay induced by MMS in this study (Emri et al., 2004).

Li et al. (2004) measured DNA damage in primary human buccal cells by using the comet assay. The appearance of SSBs, suggesting compound-induced fragmentation of DNA, occurred at formaldehyde concentrations of 5 and 7.5 μM. At higher concentrations, the response slope decreased, indicating DPXs or DDXs (Li et al., 2004). The same laboratory reported similar findings in primary human peripheral blood lymphocytes and HeLa cells (Liu et al., 2006). Peak response for SSBs was seen at 10 μM in both cells, with higher concentrations resulting in cross-link formation. SSBs in HeLa cells induced by 10 μM formaldehyde were repaired by 60 minutes after cells were washed to remove formaldehyde.

Schmid and Speit (2007) tested formaldehyde for its ability to induce DPXs in blood cultures. They used an indirect method to monitor DPX formation in which the extent of DNA migration in the comet assay in response to γ radiation was compared in formaldehyde-treated cultures versus controls. A concentration of 25 μM was required for DPX formation, and repair of these lesions was rapid, with DPXs induced by concentrations of formaldehyde up to 100 μM and completely removed after 8 hours.

Table 4-76. Formaldedyde-DNA reactions (DPX formation)

Species/Strain	Cell/Strain	Result	References
DNA Interaction			
DPX formation in vitro			
In vitro	Nucleohistone	+	Brutlag et al 1969
In vitro	Nucleohistone	+	Doenecke 1978
In vitro	Nucleohistone	+	Ohba et al 1979
Bacteria		+	Wilkins and McLeod 1976
Yeast	*Saccharomyces cerevisiae*	+	Magana-Schwencke and Ekert 1978
Hamster/Chinese	Ovary cells	+	Marinari et al 1984
Hamster/Chinese	Ovary cells	+	Zhitkovich and Costa 1992
Hamster/Chinese	Ovary cells	+	Olin et al 1996
Hamster/Chinese	Ovary cells	+	Garcia et al 2009
Hamster/Chinese	V79 lung epithelial cells	+	Swenberg et al 1983
Hamster/Chinese	V79 lung epithelial cells	+	Merk and Speit 1998
Hamster/Chinese	V79 lung epithelial cells	+	Merk and Speit 1999
Hamster/Chinese	V79 lung epithelial cells	+	Speit et al 2007
Mouse	Leukemia L1210 cells	+	Ross and Shipley, 1980
Mouse	Leukemia L1210 cells	+	Ross et al 1981
Mouse	Hepatocytes	+	Casanova and Heck 1997
Mouse	Hepatocytes	+	Casanova et al 1997
Rat	Yoshida lymphosarcoma cells	+	O'Connor and Fox 1987
Rat	C18 tracheal epithelial cell line	+	Cosma and Marchok 1988
Rat/F344	Hepatocytes	+	Casanova and Heck 1997
Rat/F344	Nasal mucosa	+	Casanova-Schmitz and Heck 1983
Rat/F344	Nasal epithelium	+	Bermudez et al., 1986
Rat/F344	Primary tracheal epithelial cells	+	Cosma et al., 1988
Rats/Wistar	Aorta endothelial cells	+	Lin et al 2005
Human	Lung/bronchial epithelial cells	+	Fornace et al 1982
Human	Bronchial cell	+	Grafstrom et al., 1983
Human	Bronchial/ Skin fibroblast	+	Grafstrom et al., 1984
Human	Lung/bronchial epithelial cells	+	Grafstrom et al 1984
Human	Lung/bronchial epithelial cells	+	Saladino et al 1985
Human	Lung/bronchial epithelial cells	+	Grafstrom et al 1986
Human	Foreskin fibroblasts	+	Snyder and Van Houten 1986
Human	Lung/bronchial epithelial cells	+	Grafstrom 1990
Human	Bronchial/Skin fibroblasts	+	Olin et al 1996
Human	White blood cell	+	Shaham et al., 1996
Human	EBV-Burkitt's lymphoma cells	+[A]	Costa et al., 1997
Human	Gastric mucosa cells	+	Blasiak et al 2000
Human	Peripheral lymphocyte	+	Quievryn and Zhitkovich 2000
Human	Fibroblast cells	+	Speit et al 2000
Human	Lymphocyte	+	Andersson et al., 2003

Human	Primary skin fibroblasts and keratinocytes	+	Emri et al 2004
Human	Buccal cells	+	Li et al 2004
Human	Jurkat E6-1 cells	+	Saito et al 2005
Human	Peripheral lymphocyte	+	Liu et al 2006
Human	HeLa cells	+	Liu et al 2006
Human	Whole blood	+	Schmid and Speit 2007
Human	Lung/bronchial epithelial cells	+	Speit et al 2008
Human	Lymphoblastoid/TK6	+	Craft et al., 1987
DPX formation in vivo			
Rat/F344	Nasal mucosa	+	Casanova-Schmitz and Heck 1983
Rat/F344	Nasal mucosa	+	Casanova-Schmitz et al 1984b
Rat/F344	Nasal mucosa	+	Lam et al 1985
Rat/F344	Nasal mucosa	+	Heck et al 1986
Rat/F344	Nasal mucosa	+	Heck Hd and Casanova 1987
Rat/F344	Tracheal implants	+	Cosma et al., 1988
Rat/F344	Nasal mucosa	+	Casanova et al 1989
Rat/F344	Nasal mucosa	+	Casanova et al 1994
Rhesus monkeys	Nasal, larynx, trachea, and carina	+	Casanova et al 1991
Human	White blood cell	+	Shaham et al., 1996
Human	Peripheral lymphocyte	+	Shaham et al., 1997
Human	Peripheral lymphocyte	+	Shaham et al., 2003

'+' indicates a positive test result
'-' indicates a negative test result
[A] indicates that DNA-protein cross-links formed at cytotoxic concentrations

4.3.1.2. DNA Adducts

In addition to the formation of DPX, there is evidence that formaldehyde forms hydroxymethyl (hm) DNA adducts in vitro in a variety of cell-free systems (Zhong and Que Hee, 2004a; Cheng et al., 2003; Kennedy et al., 1996; Fennell, 1994; Beland et al., 1984) and nasal epithelial cells (Zhong and Que Hee 2004b). In cell-free systems, formaldehyde directly reacts with DNA forming hmDNA adducts (Cheng et al., 2003; Kennedy et al., 1996; Fennell, 1994; McGhee and von Hippel, 1977a, b, 1975a, b).

Beland et al. (1984) first reported hmDNA adducts in Chinese hamster ovary (CHO) cells incubated with 1 mM of radiolabeled formaldehyde. After a 2-hour incubation, small amounts of N^6-hmdA were detected with concomitant metabolic incorporation of formaldehyde. Various forms of hmDNA adducts, including N^6-hydroxymethyldeoxyadenosine (N^6-hmdA), N^4-hydroxymethyldeoxycytidine (N^4-hmdC), and N^2-hydroxymethyldeoxyguanosine (N^2-hmdG), were detected by high performance liquid chromatography (HPLC) following in vitro reaction between formaldehyde and calf thymus DNA or individual deoxynucleotides.

^{32}P-postlabeling studies allowed for much greater analytical sensitivity but did not confirm the level of N^6-sulfomethyldeoxyadenosine found by HPLC. However, either estimate of adduct formation is much less than the estimate of DPX formation (120 pmol/mg DNA) in similarly treated rat nuclei (Heck and Casanova, 1987).

Casanova et al. (1989) demonstrated that detection of hmDNA adduct formation was sensitive to the methodology used, particularly the buffer used for sample preparation. Specifically, Tris buffer can prevent hmDNA adduct formation due to the abundance of formaldehyde-reactive primary amine sites in the buffer. In contrast, the tertiary amine sites that predominate in Bis-Tris buffer do not react with formaldehyde.

Zhong and Que Hee (2004a) observed hmDNA adducts (N^6-hmdA, N^2-hmdG, and N^4-hmdC) in placental DNA exposed to 100 ppm formaldehyde in vitro for 20 hours at 37°C followed by hydrolysis of formaldehyde-reacted DNA using bis-Tris buffer. However, deoxythymidine did not form hydroxymethyl derivatives in this study (Zhong and Que Hee, 2004a). On the other hand, the same investigators were able to detect N^6-hmdA and N^2-hmdG adducts in human nasal epithelial cells cultured in the presence of 0, 10, 25, 50, 100, 250, 400, or 500 µg/mL formaldehyde and using Tris buffer during hydrolysis of adducted DNA. The toxicity threshold for <90% viability appeared to be between 100 and 250 µg/mL initial formaldehyde culture concentration, and even at 500 µg/mL concentration it was not toxic, with a viability was 70% in this study (Zhong and Que Hee, 2004b).

The only report of formaldehyde-induced hmDNA adducts in vivo is a recent study (Wang et al., 2007), showing an indirect evidence of formation of formaldehyde-induced N^6-hmdA in hepatic and pulmonary DNA from rats exposed to *N*-nitrosodimethylamine and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone.

Since the formaldehyde adducts are labile, Fennel (1994) developed a method by derivatizing them with sodium bisulfite to their sulfomethyl form, whereby he detected N^6-sulfomethyldeoxyadenosine (SOMedA) and N^2-sulfomethyldeoxyguanosine by using HPLC. However, the levels of SOMedA in DNA isolated following incubation of radiolabeled formaldehyde with isolated rat hepatic nuclei were similar to those in control nuclei. And in human TK6 lymphoblastoid cells treated with formaldehyde, detection of SOMedA adducts was precluded by additional radioactive sports. These observations suggest that N^6-sulfomethyldeoxyadenosine adducts are formed at very low levels in formaldehyde-incubated rat nuclei and that measurement of hydroxymethyldeoxyadenosine would not provide a useful measure of formaldehyde exposure (Fennell, 1994).

Table 4-77. Formaldedyde-DNA reactions (DNA adduct formation)

Species/Strain	Cell/Strain	Result	References
DNA Interaction			
DNA Adduct Formation in vitro			
Cell-free system	Deoxyribonucleosides	+	Cheng et al 2008
Cell-free system	Guanosine	+	Kennedy et al 1996
Cell-free system	Guanosine	+	Cheng et al 2003
Placental DNA	In vitro	+	Zhong and Que Hee 2004a
Calf thymus	In vitro	+	Beland et al 1984
Calf thymus DNA	In vitro	+	Von Hippel and Wang 1971
Cell-free system	In vitro	+	McGhee and von Hippel 1975a
Cell-free system	In vitro	+	McGhee and von Hippel 1975b
Cell-free system	In vitro	+	McGhee and von Hippel 1977a
Cell-free system	In vitro	+	McGhee and von Hippel 1977b
Cell-free system	In vitro	+	Fennell 1994
Cell-free system	In vitro	+	Cheng et al 2003
Rat	Nuclei	+	Fennell 1994
Rats	Nasal epithelial cells	+	Casanova et al 1989
Hamster	CHO cells	+	Beland et al 1984
Rat	Nuclei	+	Heck Hd and Casanova 1987
Human	Nasal epithelial cells	+	Zhong and Que Hee 2004b
DNA adduct formation in vivo			
Drosophila	Larvae	+	Alderson, 1985
Rats	Indirect evidence	+	Wang et al 2007

'+' indicates a positive test result
'-' indicates a negative test result

4.3.1.3. DNA-DNA Cross-Links (DDXs)

Formaldehyde, besides forming DPXs and DNA adducts, has also been shown to form DDX in vitro. Li et al. (2004) showed that formaldehyde induces DNA strand breaks at low-exposure concentration and DDXs and DPXs at higher concentrations in buccal cells. The authors also showed that formaldehyde induces DDXs in human peripheral blood lymphocytes exposed in vitro when the concentration was more than 25 μM. However, the formation of DDXs has not been demonstrated in vivo, and the relevance of these modifications in formaldehyde-induced genotoxicity is not known at the moment.

Overall, formaldehyde forms predominantly DPXs that are detected in cell-free systems and single cells in vitro and in animal and human tissues in vivo. In rodents, DPXs are formed in nasal epithelia but not in extra-nasal passages, which are completely removed within a day after formation. The DPXs are detected in nasal and extra-nasal tissues of monkeys, suggestive of

direct effects of formaldehyde in tissues that correspond to observed tumor sites (nasal and nasopharynx) in humans. Besides, this is used as a basis for cross-species comparison with humans. Formaldehyde-DNA adducts are labile, constituting a minor fraction of the DNA-reaction products. DPXs but not DNA adducts appear to play an important role in the genotoxicity of formaldehyde.

4.3.1.4. Single Strand Breaks

Formaldehyde has been shown to induce DNA single strand breaks in a number of mammalian cell systems in vitro as well as in vivo as shown in Table 4-78. Additionally, there is some evidence that DNA single strand breaks (SSBs) may be induced directly by formaldehyde reactivity (Grafström et al., 1984).

Table 4-78. Formaldehyde-DNA interactions (single strand breaks)

Species/Strain	Cell/Strain	Result	References
DNA single strand breaks (in vitro)			
Hamster/Chinese	V79 lung epithelial cells	-	Speit et al 2007a
Mouse	Leukemia L1210 cells	(+)	Ross and Shipley, 1980
Mouse	Leukemia L1210 cells	-	Ross et al 1981
Rat	Hepatocytes	+	Demkowicz-Dobrzanski and Castonguay 1992
Rat	Yoshida lymphosarcoma cells	+	O'Connor and Fox 1987
Rat/F344 trachea	Epithelial cell/ Primary culture	+	Cosma et al., 1988
Human	Bronchial cell/Skin fibroblast	+	Grafstrom et al., 1984
Human	Peripheral blood lymphocytes	+	Liu et al 2006
Human	HeLa cells	+	Liu et al 2006
Human	Skin fibroblast	+	Snyder and Van Houten, 1986
Human	Lung/bronchial epithelial cells	+	Saladino et al 1985
Human	Lung/bronchial epithelial cells	+	Grafstrom et al 1984
Human	Lung/bronchial epithelial cells	+	Grafstrom 1990
Human	Lung/bronchial epithelial cells	+	Fornace et al 1982
Human	Lung/bronchial epithelial cells	+	Vock et al 1999
Human	Skin keratinocytes/fibroblasts	-	Emri et al 2004
DNA single strand breaks (in vivo)			
Mouse	Liver (maternal)	+	Wang and Liu 2006
Mouse	Liver (fetal)	+	Wang and Liu 2006
Rats/Spraque-Dawley	Lung cells	+	Sul et al 2007
Rat	Lymphocytes	+	Im et al 2006
Rat	Lymphocytes	+	Im et al 2006

'no' indicates test was not done in vivo; '+' indicates a positive test result; 'yes' indicates test was done in vivo ; - indicates a negative test result; (+) indicates a weak positive test result.

4.3.1.5. Other Genetic Effects of Formaldehyde in Mammalian Cells

Formaldehyde induces several other genetic and related effects in mammalian cells which are evaluated by in vitro assays such as unscheduled DNA synthesis (UDS), DNA repair inhibition and cell transformation as summarized in Table 4-79.

UDS, which represents DNA repair activity has been reported in nasal epithelial cells of F344 rats (Bermudez and Allen 1984; Bermudez and Delahanty 1986), rat hepatocytes (Williams et al 1989) and Syrian hamster embryo cells (Hamaguchi and Tsutsui 2000). UDS was observed in HeLa cells (Martin et al 1978), but not in human bronchial epithelial cells (Doolittle et al 1985) upon formaldehyde exposure. These studies suggest that following formaldehyde-induced DNA damage was followed by DNA repair.

Studies involving human bronchial epithelial cells and skin fibroblasts or keratinocytes (Grafstrom et al 1984; Emri et al 2004), DNA repair proficient or –deficient cell lines (e.g. xeroderma pigmentosum) or cell lines hypersensitive to DNA-DNA crosslinks (e.g. Fanconi's anemia) (Speit et al 2000) it has been shown that formaldehyde causes DNA repair inhibition at a concentration range of 0.125 mM to 10 mM). Emri et al (2004) have shown that DNA repair was inhibited in human keratinocytes and fibroblasts after irradiation with UVB and UVC, but not UVA follwed by treatment with low concentrations of formaldehyde (10 μM). They observed that DNA SSB induced by UVB or UVC irradiation alone were repaired within 3-6 hours of exposure, while cells with UV irradiation followed by formaldehyde exposure still had the strand breaks at the same timepoints suggesting that formaldehyde is likely to contribute to UV-induced carcinogenesis.

4.3.2. In Vitro Clastogenicity

Clastogenic effects, including increased MNs, CAs, and SCEs are also reported in a range of in vitro study systems as shown in Table 4-80.

Miyachi and Tsutsui (2005) measured the induction of sister chromatid exchanges (SCEs) in Syrian hamster embryo (SHE) cells. Cells were exposed to 0, 3.3, 10, and 33 μM formaldehyde for 24 hours. SCE levels after 3.3 μM formaldehyde were not different from controls, but significant increases were observed at both 10 and 33 μM. Toxicity as measured by reduced cloning efficiency was seen only at 33 μM (Miyachi and Tsutsui, 2005). The same laboratory used SHE cells to measure the induction of CAs (Hikiba et al., 2005). Cells were exposed to 0, 33, 66, and 99 μM formaldehyde for 24 hours prior to staining for analysis and the percentages of aberrant metaphases were 0, 6, 6, and 71, respectively. The aberrations were predominantly chromosome gaps and chromosomal breaks and exchanges. The relative colony-

forming efficiency remained high (at least 85%) for the concentrations of formaldehyde used in the experiment (Hikiba et al., 2005).

Table 4-79. Other genetic effects of formaldehyde in mammalian cells

Species/Strain	Cell/Strain	Result	References
Unschedule DNA synthesis (UDS)			
Rat/F344	Nasal epithelial cells	+	Bermudez and Allen 1984
Rat/F344	Nasal epithelial cells	+	Bermudez and Delehanty 1986
Rat	Hepatocytes	+	Williams et al 1989
Hamster/Syrian	Embryo cells	+	Hamaguchi and Tsutsui 2000
Human	HeLa cells	+	Martin et al 1978
Human	Bronchial epithelial cells	-	Doolittle et al 1985
DNA repair inhibition			
Human	Bronchial epithelial cells/skin fibroblasts	+	Grafstrom et al 1984
Human	Normal fibroblasts (MRC5CV), XPA cell line, & FA cell line	+	Speit et al 2000
Human	Skin fibroblasts/keratinocytes	+	Emri et al 2004

XPA, xeroderma pigmentosum, complementation group A (deficient in NER pathway)
FA, Fanconi's anemia (cell line has genetic defect leading to hypersensitivity to DNA-DNA cross links; NER, nucleotide excision repair

Schmid and Speit (2007) observed that SCEs were induced in lymphocytes of whole blood cultures at a formaldehyde concentration of 200 µM, an effect apparently associated with cytotoxicity. This was indicated by a concomitant reduction in the proliferative index. These authors also observed the formation of MNs in their cultures. This effect was statistically significant at a formaldehyde concentration of 300 µM and above. However, MN formation was confined to those cultures in which formaldehyde treatment commenced 44 hours after the start of the culture. This prompted the conclusion that the level of DPX formation would need to be high for MN formation and that the cells must be exposed after the first mitosis. In examining MN formation more closely, Schmid and Speit (2007) used the FISH technique, employing a "biotin-labeled pan-centromeric chromosome paint specific for all human centromeres." Indicative that formaldehyde was inducing a clastogenic (rather than aneugenic) effect, 81% of MNs in binucleated cells were centromere-negative.

In summary, formaldehyde forms MNs, SCEs, and CAs in isolated animal and human cells following in vitro exposure (Table 4-80).

4.3.3. In Vitro Mutagenicity

Mutations may occur during repair of formaldehyde-induced DNA damage (DPXs, DNA adducts, SSBs, or clastogenic effects) or as a result of replication errors during mitogenesis. The in vitro evidence for formaldehyde-induced mutations is strengthened by examining the correlation between these genotoxic and clastogenic events and induction of mutations. Therefore studies are presented with respect to relevance to one or more of the following lines of evidence for mutagenicity recommended for consideration in the EPA guidance (U.S. EPA, 2005a): (1) that the chemical is DNA reactive and/or has the ability to bind to DNA, (2) that the chemical generates positive results in in vitro mutagenic test systems (specifically gene mutations and CAs), and (3) that the chemical induces indications of genetic damage in in vivo tests (specifically gene mutations and CAs). Numerous studies have demonstrated formaldehyde-induced DNA mutations under a variety of experimental conditions (reviewed in IARC 1995, 2006; Ma and Harris 1988; Auerbach et al. 1977; Conaway et al 1996; NTP 2009).

4.3.3.1. Mutagenicity in Bacterial Systems

A number of research reports describe the mutagenicity of formaldehyde in bacterial test systems using reverse and forward mutation assays as well as specific strains detecting deletions, insertions and point mutations. Among the bacterial strains, *Salmonella typhimurium* TA102 and the *Escherichia coli* strains containing an AT base pair at the primary reversion site are often used to detect oxidative compounds, cross-linking agents and hydrazines. In an early National Toxicology Program (NTP) collaborative study with three laboratories, formaldehyde consistently tested positive for mutagenicity in *Salmonella typhimurium* strain TA100 in the presence of a rat or hamster liver S9 activating system (Haworth et al., 1983). Formaldehyde was mutagenic with and without metabolic activation in a number of other studies using in reverse mutation assays with *S. typhimurium* strains TA98, TA100, TA102, TA104, TA2638, and TA2638a and *E. coli* strains WP2 (pkM101), WP2 *uvr*A (pkM101), and hrs/r30R (Ryden et al., 2000; Dillon et al., 1998; Watanabe et al., 1996; Le Curieux et al., 1993; O'Donovan and Mee, 1993; Zielenska and Guttenplan, 1988; Schmid et al., 1986; Connor et al., 1983, 1985; Orstavik and Hongslo, 1985; Takahashi et al., 1985; Fiddler et al., 1984; Frei et al., 1984; Donovan et al., 1983), while other studies (Muller et al., 1993; Jung et al., 1992; Wilcox et al., 1990; Marnett et al., 1985) show both positive and negative results. These results are summarized in Table 4-81 and some of the studies are described in greater detail.

Table 4-80. In vitro clastogenicity of formaldehyde.

Species	Cell/Tissue origin	Without activation	With activation	References
Cytogenetic Assays				
Chromosomal aberratoins (CA)				
Hamster/Chinese	Ovary cells	(+)	(+)	Galloway et al., 1985
Hamster/Chinese	Ovary cells	-	ND	Dresp and Bauchinger, 1988
Hamster/Chinese	Ovary cells	+	ND	Natarajan et al 1983
Mouse	Lymphoma cells	+	ND	Speit and Merk 2002
Hamster/Syrian	Embryo cells	+	ND	Hikiba et al 2005
Hamster/Syrian	Embryo cells	+	ND	Hagiwara et al 2006
Hamster/Chinese	Ovary cells	+	ND	Garcia et al 2009
Hamster/Chinese	Lung fibroblasts	+	ND	Ishidate Jr et al 1981
Human	Lymphocytes	+	+	Schmid et al 1986
Human	Lymphocytes	+	ND	Miretskaya and Shvartsman 1982
Human	Lymphocytes	+	ND	Dresp and Bauchinger, 1988
Human	Fibroblasts	+	ND	Levy et al 1983
Micronucleus (MN)				
Hamster/Chinese	V79 lung epthelial cells	+	ND	Speit et al 2007b
Hamster/Chinese	V79 lung epthelial cells	+	ND	Merk and Speit 1998
Human	Whole blood cultures	+	ND	Schmid and Speit 2007
Human	Human MRC5CV (normal) and XP(Repair-deficient) and FA (repair-deficient) cell lines	+[a]	ND	Speit et al 2000
Sister Chromatid Exchange (SCE)				
Hamster/Chinese	Ovary cells	(+)	(+)	Galloway et al., 1985
Hamster/Chinese	Ovary cells	+	ND	Natarajan et al 1983
Hamster/Chinese	Ovary cells	+	ND	Garcia et al 2009
Hamster/Chinese	Ovary cells	+	ND	Obe and Beek 1979
Hamster/Syrian	Embryo cells	+	ND	Miyachi and Tsutsui 2005
Hamster/Chinese	V79 lung epithelial cells	+	(+)	Basler et al. 1985
Hamster/Chinese	V79 lung epithelial cells	+	ND	Speit et al 2007b
Hamster/Chinese	V79 lung epithelial cells	+	ND	Merk and Speit 1998, 1999
Hamster/Chinese	V79 lung epithelial cells	+	ND	Neuss and Speit 2008
Human	A549 lung epithelial cells	+	ND	Neuss and Speit 2008
Human	A549 + V79 (co-cultivated)	+[c]	ND	Neuss and Speit 2008
Human	A549 + V79 (co-cultivated)	-[d]	ND	Neuss and Speit 2008
Human	Lymphocytes	+[b]	ND	Garry et al., 1981
Human	Lymphocytes	+	ND	Krieger and Garry 1983
Human	Lymphocytes	+	ND	Schmid et al 1986
Human	Lymphocytes	+	ND	Obe and Beek 1979
Human	Whole blood cultures	+	ND	Schmid and Speit 2007

Premature chromosome Condensation (PCC)				
Hamster/Chinese	Ovary cells	+	ND	Dresp and Bauchinger, 1988

'+' indicates a positive test result
'ND' indicates test was not done
- indicates a negative test result
(+) indicates a weak positive test result
[a] MN frequency increased in repair-deficient cell lines compared to normal cell lines
[b] indicates SCE with significant loss of cell viability
[c] A549 cells exposed for 1 h with formaldehyde then co-cultivated with V79 cells
[d] A549 cells exposed for 1 h with formaldehyde, cells washed and then co-cultivated with V79 cells
XP, xeroderma pigmentosum; FA = Fanconi's anemia.

Table 4-81. Summary of mutagenicity of formaldehyde in bacterial systems

Species	Strain	Metabolic activation		References
		+S9	-S9	
Mutagenicity Assays				
Reverse Mutation				
S. typhimurium	TA98, 100, 1535, 1537, 1538	-	-	De Flora, 1981
S. typhimurium	TA100	ND	(+)	Couch et al., 1982
S. typhimurium	TA100	+	-	Haworth et al., 1983
S. typhimurium	TA1535, 1537	-	-	Haworth et al., 1983
S. typhimurium	TA98	(+)	-	Haworth et al., 1983
S. typhimurium	TA98, TA100	+	+	Connor et al., 1983*
S. typhimurium	UTH8414, UTH8413	-	-	Connor et al., 1983*
S. typhimurium	TA97, 98, 100	+	+	Donovan et al., 1983
S. typhimurium	TA102	+	+	De Flora et al., 1984
S. typhimurium	TA100	+	ND	Frei et al., 1984
S. typhimurium	TA100	ND	+	Fiddler et al., 1984
S. typhimurium	TA100	+	(+)	Connor et al., 1985
S. typhimurium	TA98	(+)	-	Connor et al., 1985
S. typhimurium	UTH8414, UTH8413	-	-	Connor et al., 1985
S. typhimurium	TA100	(+)	-	Ashby et al., 1985**
S. typhimurium	TA97, 98, 1535, 1537, 1538	-	-	Ashby et al., 1985**
S. typhimurium	TA98, 100, 102	ND	(+)	Takahashi et al., 1985
E. coli	WP2, WP2 *uvrA*	ND	+	Takahashi et al., 1985
E. coli	H/R30R, HS30RuvrA	ND	+	Takahashi et al., 1985
E. coli	NG30*recA, 016polA*	ND	-	Takahashi et al., 1985
S. typhimurium	TA97, 98, 100	ND	-	Marnett et al., 1985
S. typhimurium	TA102, 104	ND	+	Marnett et al., 1985
S. typhimurium	TA98, 100	+	+	Oerstavik and Hongslo, 1985
S. typhimurium	TA100	+	+	Schmid et al., 1986
S. typhimurium	TA104	+	ND	Zielenska and Guttenplan, 1988
S. typhimurium	TA102	ND	-	Wilcox et al., 1990
E. coli	WP2 *uvrA* /(pKM101)	ND	+	Wilcox et al., 1990
E. coli	WP2 (pKM101)	ND	-	Wilcox et al., 1990
S. typhimurium	TA102	+	ND	Jung et al., 1992
S. typhimurium	TA102	ND	+	Le Curieux et al., 1993
S. typhimurium	TA102	+	ND	Muller et al., 1993
S. typhimurium	TA98, 100, 102	ND	+	O'Donovan and Mee, 1993
S. typhimurium	TA1535, 1537, 1538	ND	-	O'Donovan and Mee, 1993
E. coli	WP2 (pKM101),	ND	+	O'Donovan and Mee, 1993
E. coli	WP2uvrA (pKM101)			O'Donovan and Mee, 1993
E. coli	K12 (AB1157)(WT)	ND	+	Graves et al., 1994
E. coli	K12 (AB1886)/(uvrA), K12(AB2480)/(recA/uvrA)	ND	-	Graves et al., 1994
S. typhimurium	TA102, 2638	ND	+	Watanabe et al., 1996
E. coli	WP2 (pKM101), WP2uvrA (pKM101)	ND	+	Watanabe et al., 1996

S. typhimurium	TA1535[A]	-	-	Sarrif et al., 1997
S. typhimurium	TA1537[A]	+	+	Sarrif et al., 1997
S. typhimurium	TA98, 100[A]	+	-	Sarrif et al., 1997
S. typhimurium	TA97[A]	ND	+	Sarrif et al., 1997
S. typhimurium	TA1535, 1537[B]	-	-	Sarrif et al., 1997
S. typhimurium	TA98[B]	+	+	Sarrif et al., 1997
S. typhimurium	TA100[B]	-	+	Sarrif et al., 1997
S. typhimurium	TA100[C]	+	+	Sarrif et al., 1997
S. typhimurium	TA100, 104[B]	+	+	Dillon et al., 1998
E. coli (Lac+ reversion)	WP3101P, WP3106P	+		Ohta et al 1999
S. typhimurium	TA102, 2638[B]	ND	+	Ryden et al., 2000
Forward Mutation				
S. typhimurium	TM677	ND	(+)	Couch et al., 1982
S. typhimurium	TM677	+	+	Donovan et al., 1983
S. typhimurium	TM677	+	+	Temcharoen and Thilly, 1983
E. coli	D494*uvrB*	+		Bosworth et al 1987
Deletion Mutation				
E.coli	GP120, GP120A 7-2, 33694	ND	+[D]	Crosby et al., 1988
Point Mutation				
E.coli	GP120, GP120A 7-2, 33694	ND	+	Crosby et al., 1988
Insertion Mutation				
E.coli	GP120, GP120A 7-2, 33694	ND	+	Crosby et al., 1988

'+' indicates a positive test result
'ND' indicates test was not done
'-' indicates a negative test result
(+) indicates a weak positive test result
* indicates the use of formalin in mutagenicity assay
** indicates the use of hexamethylmelamine (HEMLA), a formaldehyde-releasing compound, in mutagenicity assay
[A] indicates use of the Standard Plate Method
[B] indicates use of the Preincubation Plate Method
[C] indicates use of the Suspension Method
[D] indicates loss of DNA

Formaldehyde has been shown to be mutagenic in forward mutation assays using *S. typhimurium* (Couch et al 1982; Donovan et al 1983; Temcharoen and Thilly 1983) as well as in *E. coli* (Bosworth et al 1987). Temcharoen and Thilly (1983) examined the toxicity and mutagenicity of *S. typhimurium* strain TM677, using forward mutation to 8-azaguanine resistance, and have shown that formaldehyde induced both toxicity and mutagenicity at minimum concentrations of 0.17 mM (–S9) and 0.33 mM (+S9). It has also been shown that

formaldehyde formed as an intermediate by oxidation at the methyl group of N-nitrodimethylamine, a biologically active N-nitramine of environmental significance, is mutagenic to *S. typhimurium* TA100 strain at low concentrations and toxic above 2 μmol/plate (Frei et al., 1984).

Bosworth et al (1987) developed a forward mutation assay in *E. coli* D494 *uvr*B strain transformed with a multi-copy mutator plasmid pGW1700, in which mutations are scored by an increase in ampicillin-resistant colonies after exposure of bacterial cells during the logarithmic growth by the test chemicals. This assay is more sensitive to base-pair substitutions, but less sensitive to frameshift mutations compared to Salmonella/miceosome-based assays. In this assay, the authors (Bosworth et al 1987) observed positive curvilinear response to formaldehyde exposure. Crosby et al (1988) used four *E. coli* strains GP120, GP120A, 7-2, and 33694 containing the xanthine guanine phosphoribosyl transferase (*gpt*) gene (which detects point mutations, deletions and insertions) tested the mutagenicity of formaldehyde by exposing for 1 hour at 4 and 40 mM concentrations. They observed 41% large insertions, 18% large deletions and 41% point mutations. However, at 40 mM dose there were 92% point mutations, a majority of them (62%) being transition mutations at a single AT base pair in the *gpt* gene. In the same study they observed frameshift mutations in E. coli that was transformed with naked pSV2gpt plamid DNA exposed to 3.3 or 10 mM formaldehyde. Thus, the mutation pattern appear to differ depending on the concentration of formaldehyde exposure to the bacterial strain as well as the nature of DNA.

Formaldehyde has also been shown to induce primary DNA damage in *E. coli* and mutagenic activity in the Ames fluctuation test in *S. typhimurium* TA100, TA102, or TA98 strains (Le Curieux et al., 1993).

O'Donovan and Mee (1993) observed clear mutagenicity by the pre-incubation exposure method in *S. typhimurium* TA98, TA100, and TA102 strains and both *E. coli* WP2(pKM101) and WP2uvrA(pKM101) strains, while the standard plate-incorporation assays showed consistent mutagenicity only with TA100 and WP2*uvr*A(pKM101) strains and no evidence of mutagenicity in TA1535, TA1537, or TA1538 strains using either method of exposure in the absence of metabolic activation. The *S. typhimurium* and *E. coli* strains used in this study are histidine and tryptophan auxotrophs, with an AT base pair at the critical mutation site within the *his*G and *trp*E genes, respectively, with an intact excision repair system facilitating the detection of cross-linking agents and both strains carrying the mutator plasmid, pKM101, which enhances error-prone repair. These salmonella strains detect frameshift (TA98 and TA1537) and base-pair substitutions (TA100, TA102, and TA1535), while the *E. coli* strains detect base-pair substitutions (WP2*uvr*A). These findings are consistent with the suggestion that formaldehyde

induces excision-repairable lesions in bacteria and indicate that the presence of the R-factor plasmid may be required for the expression of its mutagenicity in excision repair-deficient salmonella (O'Donovan and Mee, 1993).

Dillon et al. (1998) employed salmonella strains TA100, TA102, and TA104 because of the latter two strains being more sensitive to oxidative mutagens. Formaldehyde was clearly mutagenic between 6 and 50 μg/plate in all three strains with and without metabolic activation using Aroclor-induced S9 from male F344 rats or male B6C3F1 mice, except for an equivocal response in TA102 with mouse S9 (Dillon et al., 1998). Using a set of six tester strains (WP3101–WP3106) of *E. coli*, each reversible by a mutation involving a single DNA base pair substitution, Ohta et al. (1999) determined that formaldehyde preferentially induced GC to TA transversion mutations. Ryden et al. (2000) demonstrated a statistically significant increase in the number of revertants in *S. typhimurium* TA102 (2.5-fold) and TA2638a (3-fold) strains by formaldehyde at ≥17 μg/plate compared with solvent controls.

In summary, formaldehyde induces mutations in several bacterial strains containing an AT base pair at the primary reversion site that are used to detect oxidative compounds and cross-linking agents without metabolic activation by exogenous enzyme-activating systems. This evidence is strengthened by examining the correlation between genotoxic and clastogenic events and mutation induction.

4.3.3.2. Mutagenicity in Non-Mammalian Cell Systems

Formaldehyde has been shown to be mutagenic in several non-mammalian systems also. It has been shown to cause gene conversion, strand breaks, crosslinks, homozygosis and related damage in yeasts (*Saccharomyces cervisiae*), forward and reverse mutations in molds (*Neurospora crassa*), micronuclei formation in spiderworts (*Tradescantia pallida*), DNA damage and mutations in several plants, genetic cross-over or recombination, sex-linked recessive lethal mutations, dominant lethal mutations, heritable translocations and gene mutations in insects (*Drosophila melanogaster*) and recessive lethal mutations in nematodes (*Caenorhabditis elegans*), but failed to show micronuclei formation in newt larvae (*Pleurodeles waltl*) (Reviewed in Conaway 1996; IARC 2006).

4.3.3.3. Mutagenicity in Mammalian Cell Systems

Several studies demonstrated the mutagenicity of formaldehyde in mammalian cells. In its report, the Federal Panel on Formaldehyde underlined the role of formaldehyde as an inducer of gene mutations and CA in a variety of test systems (Report of the Federal Panel on Formaldehyde, 1982). Results from several studies are summarized in Table 4-82.

Snyder and Van Houten (1986) demonstrated that formaldehyde increases the levels of misincorporation of bases into synthetic polynucleotides catalyzed by *E. coli* DNA polymerase I, indicating that the mutagenicity of formaldehyde may be due to covalent alteration of DNA bases. They have also shown that formaldehyde-induced DNA damage in human fibroblasts was not susceptible to repair by the typical "long patch" excision repair mechanism.

Craft et al. (1987) measured the induction of mutations at the thymidine kinase (*tk*) locus or at the ouabain resistance (*Oua*^{r}) locus in TK6 human lymphoblastoid cells. The *tk* mutations can result from a variety of mutational events, including base pair substitution, small and large deletions, and chromosome exchange events, while mutations to *Oua*^{r} require specific base pair substitutions. Single treatment of formaldehyde (0, 15, 30, 50, 125, and 150 μM) for 2 hours resulted in a nonlinear increase in *tk* mutagenesis with increasing slope >125 μM (Figure 4-32). To explore a dose-response effect, cells were also exposed as follows: three treatments of 50 μM for 2 hours or five treatments of 30 μM or 10 treatments of 15 μM for 2 hours (treatments were spaced 2–4 days apart) with multiple treatments causing an increase in *tk* mutations, although their combined effect was less than a single treatment of equivalent C × t (150 μM for 2 hours). Lymphoblasts given four treatments of 150 μM formaldehyde for 2 hours failed to induce mutations at the *Oua*^{r} locus. Dose-response increases were seen in all exposure scenarios, with 30 μM being the level of statistical significance. There was little indication of a dose-response effect until the cumulative concentration was greater than 100 μM. Formaldehyde-induced DPXs were no longer evident after 24 hours of exposure; mutants induced in the TK6 lymphoblast cell line showed a similar dose-response curve to the DPXs measured immediately after exposure ended (Craft et al., 1987).

The same group also studied mutations induced at the X-linked hypoxanthine-guanine phosphoribosyl transferase (HPRT) locus by eight repetitive treatments of 150 μM formaldehyde in TK6 human lymphoblast cell line by Southern blot analysis, wherein half (14/30) of induced mutants contained partial or complete deletions with most of the partial deletions showing unique deletion patterns, while only a third (5/15) of spontaneous mutants had partial or complete deletions, indicating that formaldehyde can induce large losses of DNA in human lymphoblast cells (Crosby et al., 1988).

Table 4-82. Mutagenicity in mammalian cell systems.

Species/Strain	Cell/Strain	*In Vivo test*	Without activation		References
Mutagenicity Assays					
Dominant Lethal Mutation					
Rat/Albino	Spermatocyte, Live implants	yes	+	ND	Odeigah, 1997
Mouse	Dominant lethal	yes	-		Epstein and Shafner 1968
Mouse	Dominant lethal	yes	-		Epstein et al 1972
Mouse	Dominant lethal	yes	(+)		Fontignie-Houbrechts 1981
Rat	Dominant lethal	yes	(+)		Kitaeva et al 1990
Deletion Mutation					
Hamster/Chinese	V79 cells *Hprt* locus)	no	-		Merk and Speit 1998
Hamster/Chinese	V79 cells *Hprt* locus)	no	-		Merk and Speit 1999
Hamster/Chinese	V79/HPRT	no	+	ND	Grafstrom et al., 1993
Hamster/Chinese	Ovary HPRT	no	-	+	Graves et al., 1996
Mouse	Lymphoma L5178Y cells (Tk $^{+/-}$ locus)	no	+		Macerer et al 1996
Mouse	Lymphoma L5178Y cells	no	+	ND	Speit and Merk 2002
Human	Bronchial cell	no	+		Grafstrom et al., 1983
Human	Bronchial fibroblasts/epithelial cells (HPRT locus)	no	+		Grafstrom et al 1985
Human	Bronchial fibroblasts/epithelial cells (HPRT locus)	no	+		Grafstrom 1990
Human	Lymphoblast/HPRT	no	+[a]	ND	Crosby et al., 1988
Human	Lymphoblast/tk	no	+		Craft et al 1987
Human	Peripheral lymphocytes	yes	+	ND	Shaham et al 2003
Human	Lymphoblast (TK6)	no	+		Goldmacher and Thilly 1983
Point Mutation					
Hamster/Chinese	Ovary HPRT	no	+	ND	Graves et al., 1996
Mouse	Lymphoma cell/ TK+/-	no	+	+	Blackburn et al., 1991
Mouse	Lymphoma cell/ TK+/-	no	+	ND	Wangeheim and Bolcsfoldi, 1988
Human	Lymphoblast/TK6	no	+	ND	Liber et al., 1989
Insertion Mutation					
Hamster/Chinese	Ovary HPRT	no	+	ND	Graves et al., 1996
Heritable Mutation					
Mouse	Heritable mutation	yes	+		Liu et al 2009
DNA Repair enzyme activity					
Human	Peripheral lymphocyte	yes	-		Hayes et al., 1997

Cell Transformation					
Mouse	C3H10T1/2 cells		+[b]		Ragan and Boreiko 1981
Mouse	Embryo fibroblast/C3H/10T1/2	no	[+]	ND	Boreiko et al., 1983
Mouse	Embryo fibroblast/C3H/10T1/2	no	[+]	ND	Frazelle et al., 1983
Hamster	Kidney cell/BHK-21/cI.13	no	+	+	Plesner and Hansen, 1983
p53 mutation and/or p53 protein expression					
Rats/F344	Nasal squamous cell carcinomas	yes	+[c]		Recio et al 1992
Rats/F344	Nasal tumor cell lines	No	+		Bermudez et al 1994
Rats/F344	Nasal squamous cell carcinomas	Yes	+[d]		Wolf et al 1995
Human	Peripheral blood lymphocytes	yes	+		Shaham et al 2003

'no' indicates test was not done in vivo
'+' indicates a positive test result
'ND' indicates test was not done
'yes' indicates test was done in vivo
- indicates a negative test result
(+) indicates a weak positive test result
[+] indicates positive test result after TPA or N-methyl-N-nitro-N-nitrosoguanidine promoter treatment
[a] indicates loss of DNA
[b] Positive only in the presence of 12-O-tetradecanoylphorbol 13-acetate (TPA)
[c] p53 mutations
[d] p53 mutated protein detected by immunohistochemistry

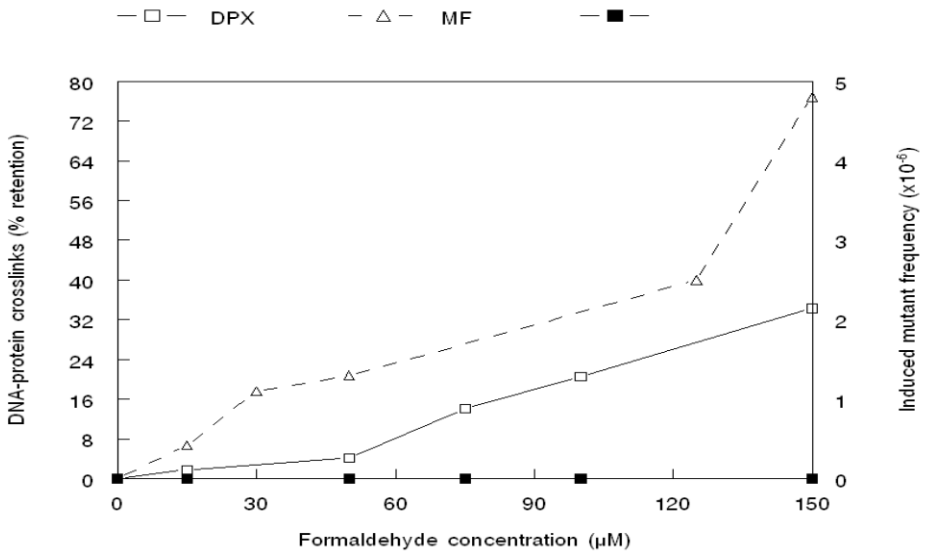


Figure 4-32. DNA-protein cross-links (DPX) and thymidine kinase (*tk*) mutants in TK6 human lymphoblasts exposed to formaldehyde for 2 hours.

Note: ☐ DPXs immediately after exposure, ■DPXs 24 hours after exposure, △ *tk* mutants. Relative survival was 100% at 0, 15, 30, and 50 μM, 30% at 125 μM, and 20% at 150 μM.

Source: Adapted from Craft et al. (1987).

Liber et al. (1989) followed up the findings of Crosby et al. (1988) by performing Southern blot, Northern blot, and DNA sequence analysis on the 16 induced and 10 spontaneous human lymphoblast mutants not showing deletions. Northern blot analysis showed that the point mutations fell into four categories: normal size and amount of RNA, normal size but reduced amounts of RNA, reduced size and amounts of RNA, and no RNA. Sequence analysis of recombinant DNAs from *hprt* mRNA in formaldehyde-induced mutants showed a preferential AT to CG transversion at a specific site, with other changes represented to a lesser degree (Liber et al., 1989).

Even in CHO cells formaldehyde has been shown to induce *hprt* mutations involving mostly single-base pair transversions mostly occurring at AT sequences, including three AT to TA at position 548 of exon 8 and two AT to CG and one GC to TA transversion at other sites

(Graves et al., 1996). In another study, formaldehyde-induced forward mutations to trifluorothymidine resistance in mouse lymphoma L5178Y $tk^{\pm}$ cells both in the absence and presence of rat liver S9 (higher concentrations required for effect with S9). Both toxicity and mutagenicity were abolished when FADH was incorporated in the exposure medium (Blackburn et al., 1991).

Formaldehyde-induced DPXs are removed in part through spontaneous hydrolysis and in part due to active repair processes (Quievryn and Zhitkovich, 2000). Inhibition of specific proteosomes in XP-A cells inhibited DPX repair, thereby supporting the role of enzymatic degradation (Quievryn and Zhitkovich, 2000). The half-life of formaldehyde-induced DPXs in human cell lines was consistent with the findings of Craft et al. (1987), ranging from 11.6 to 13 hours (Quievryn and Zhitkovich, 2000). In the same report, removal of DPXs from human peripheral lymphocytes was much slower, with a half-life of 18.1 hours. This difference was primarily in slower active repair of DPXs, with a $t_{1/2}$ of 66.6 hours for human lymphocytes versus 23.3 hours for human cell lines (Quievryn and Zhitkovich, 2000).

Since DPX repair involves proteolytic removal of proteins from the DNA, Speit et al. (2000) hypothesized that single peptides or small peptide chains cross-linked to the DNA are critical to formaldehyde-induced mutation. However, these authors did not find significant difference in the induction and repair of DPXs in normal and DNA repair-deficient cell lines but observed increased susceptibility of the repair-deficient cell lines to formaldehyde-induced MN induction. In this study, a normal human cell line (MRC5CV1), a xeroderma pigmentosum cell line deficient in nucleotide excision repair (NER), and a Fanconi anemia cell line, which has a genetic defect leading to hypersensitivity towards DDXs, were exposed to 125, 250, and 500 μM formaldehyde for 2 hours. The authors suggest that more than one repair pathway is involved in the repair of cross-links and that the altered NER pathway has more severe consequences to formation of CAs than disturbed cross-link repair (Speit et al., 2000).

The correlation of early DPX formation and mutation is at first counterintuitive since the cross-linking of protein to DNA inhibits DNA replication. Without active DNA replication, formaldehyde-DNA adducts and DPXs would not induce replication error and would be unlikely to result in a change in DNA sequence or mutation. Recent evidence indicates that residual peptides and short polypeptides that remain cross-linked to DNA after DPX removal may in fact be the cause of DPX-associated, formaldehyde-induced mutation (Speit et al., 2000).

A study by Merk and Speit (1998) indicated that formaldehyde-induced DPXs did not result in direct gene mutations in the *hprt* locus of V79 Chinese hamster cells, leading the authors to speculate that formaldehyde was not mutagenic. Since, the *hprt* locus in the V79 Chinese hamster cell line is primarily sensitive to point mutations and other studies show the

formation of deletion mutations by formaldehyde at the same locus in human lymphoblasts (Crosby et al., 1988), Merk and Speit (1998) concluded that the *hprt* mutation assay is insensitive to deletion mutations.

Later, using the mouse lymphoma assay, Speit and Merk (2002) demonstrated that exposure to formaldehyde for 2 hours was mutagenic in a concentration-dependent manner in the L5178Y mouse lymphoma cells, which was mainly contributed by a strong increase in small colony mutants, suggestive of CAs (Speit and Merk, 2002). Detailed analysis of both spontaneous and formaldehyde-induced lesions indicates that recombination or deletion of DNA from the *tk* locus was primarily responsible for the loss of heterogeneity, thereby leading to the observed mutant phenotype. Therefore, it is believed that formaldehyde is mutagenic in the L5178Y cell mouse lymphoma system by a clastogenic mechanism rather than through point mutations. This finding is consistent with that of Craft et al. (1987), who demonstrated formaldehyde mutagenicity at the *tk* locus of TK6 cells, and also with the findings of Grafström et al. (1984), who demonstrated increased SSB formation in formaldehyde-exposed cell lines.

Formaldehyde has also been shown to induce cell transformation in mouse embryo fibroblasts (Ragan and Boreiko 1981; Boreiko et al 1983; Frazelle et al 1983) . At low concentrations of 0.017 mM formaldehyde has shown to cause cell transformation in C3H10T1/2 mouse cells (Ragan and Boreiko 1981) and hamster kidney cells in vitro (Plenser and Hansen 1983).

More recently, Shaham et al. (2003) examined the frequency of DPXs and the incidence of mutant versus wild type p53 tumor suppressor genes in the peripheral blood lymphocytes of a cohort of workers exposed to formaldehyde. The adjusted mean levels of DPXs were greater in the lymphocytes of exposed subjects compared with those of unexposed subjects, and exposure to formaldehyde increased the likelihood of their having a higher level of pantropic p53 (>150 pg/mL). The authors speculated on a possible causal relationship between DPXs and mutations in p53. Recio et al (1992) demonstrated point mutations in the p53 tumor suppressor gene in 45% (5 out of 11) of the primary nasal squamous cell carcinomas (SCCs) obtained from F344rats that were chronically exposed to 15 ppm formaldehyde for 2 years (Recio et al ., 1992).

In summary, the results of in vitro experiments demonstrate the mutagenicity of formaldehyde. Mutagenicity is observed below levels of significant cytolethality in mammalian cell lines. Formaldehyde is clearly a DNA-reactive genotoxicant inducing lesions (DPXs) that show clastogenicity (SSBs, MNs, etc.). The experiments by Speit and Merk (2002) explore mechanistic links between DPXs, clastogenicity, and the observed locus-specific mutations in the mouse lymphoma in vitro testing system.

4.3.4. In Vivo Mammalian Genotoxicity

4.3.4.1. *Genotoxicity in Laboratory Animals*

As discussed above, formaldehyde is clearly reactive at the POE in animal studies, resulting in increased DPXs in the nasal mucosa. Despite formaldehyde's reactivity and mutagenicity in isolated mammalian cells, clear evidence of mutagenicity does not emerge from animal bioassays (Table 4-83).

In a chromosomal analysis study (Fontignie-Houbrechts, 1981), formaldehyde given I.P. at 50 mg/kg to male Q strain mice and analyzed 8–15 days after treatment did not induce any chromosomal lesions in spermatocytes. Also, in another study from the same group (Fontignie-Houbrechts et al., 1982), formaldehyde (30 mg/kg) given along with hydrogen peroxide (90 mg/kg) as a mixture to male Q strain mice failed to produce significant increases in chromosomal lesions in the spermatogonia.

In a different study Natarajan et al. (1983) failed to detect significant differences in MN induction in bone-marrow cells or CAs in spleen cells of male and female CBA mice given I.P. 6.25, 12.5, and 25 mg/kg formaldehyde compared with saline-treated controls. However, the same study showed a positive induction of MNs and CAs in vitro. The authors suggest that the lack of genotoxicity in vivo may be due to the inability of formaldehyde to reach the target cells in sufficient quantity to induce biological effects.

Kligerman et al. (1984) also found no difference in the incidence of SCEs or chromosome breakage in the peripheral lymphocytes of male and female F344 rats exposed to formaldehyde in air at 0.5, 6, or 15 ppm (0.61, 7.36, or 18.4 mg/m^3) 6 hours/day for 5 days. However, in a different study (Migliore et al., 1989), clastogenic effects, such as increased MNs and CAs, were reported in GI epithelial cells of male Sprague-Dawley rats after oral exposures to 200 mg/kg formaldehyde. In this study, micronucleated cells and nuclear anomalies were increased in a time-dependent manner in the stomach, duodenum, ileum, and colon of rats, and the mitotic index was unchanged for these cells compared with controls at 16, 24, and 30 hours. These clastogenic effects were seen without regenerative cell proliferation, supporting formaldehyde-induced mutations as primary effects of formaldehyde rather than secondary to regenerative cell proliferation.

Kitaeva et al. (1990) observed cytopathological and cytogenetic effects of formaldehyde chronic inhalation in 0.5 and 1.5 mg/m^3 doses in the female rat's germ and marrow cells, where formaldehyde-induced harmful effects were seen in germ cells at <1.5 mg/m^3 doses, while the reliable clastogenic and cytogenetic effects on the marrow cells were induced even at the 0.5 mg/m^3 dose, suggesting differences among effects of small doses of formaldehyde on different cell systems.

Table 4-83. Genotoxicity in laboratory animals.

Species/Strain	Cells/Organ/Tumor	Result	References
Cytogenetic Assays			
Chromosomal aberratoins (CA)			
Mice/Q strain	Spermatocyte	-	Fontignie-Houbrechts et al., 1981
Mice/Q strain	Spermatogonia	-	Fontignie-Houbrechts et al., 1982
Mice/CBA	Polychromatic erythrocytes	-	Natarajan et al., 1983
Mice/CBA	Spleen cells	-	Natarajan et al., 1983
Rats/F344	Lymphocytes	-	Kligerman et al 1984
Rats/Spraque-Dawley	Gastric epithelial cells	+	Migliore et al 1989
Rats/Wistar	Bone marrow	+	Kitaeva et al 1990
Rats/Spraque-Dawley	Bone marrow	-	Dallas et al 1992
Rats/Spraque-Dawley	Pulmonary lavage cells	+	Dallas et al 1992
Rats/F344	Peripheral blood cells	-	Speit et al 2009
Micronucleus (MN)			
Mouse/NMRI	Bone marrow	-	Gocke et al 1981
Mice/CBA	Femoral polychromatic erythrocyte and spleen cell	-	Natarajan et al., 1983
Rats/Spraque-Dawley	Gastric epithelial cells	+	Migliore et al 1989
Sister Chromatid Exchange (SCE)			
Rats/F344	Lymphocyte	-	Kligerman et al 1984
Rats/F344	Peripheral blood cells	-	Speit et al 2009

'+' indicates a positive test result
- indicates a negative test result

Dallas et al. (1992) observed a slight increase (7.6 and 9.2%) in CAs in pulmonary lavage cells from male Sprague-Dawley rats exposed to 15 ppm (18.4 mg/m^3) formaldehyde in air 6 hours/day, 5 days/week for 1 or 8 weeks by inhalation compared with corresponding controls (3.5 and 4.8%), respectively. However, the small study, limited as it was to five animals/group, showed statistically significant increase at the highest dose tested (15 ppm) but not at lower doses (0.5 and 3 ppm). In the same study, no clastogenic effects were seen in bone marrow, which is consistent with formaldehyde acting primarily at the site of first contact.

Speit et al (2009) investigated the genotoxicity of formaldehyde in peripheral blood samples of Fischer-344 rats exposed to 0 to 15 ppm formaldehyde by whole-body inhalation for 4 weeks (6 h/day, 5 days/week). In this study, the authors found no significant increase in the genotoxic assays such as comet assay with or without gamma-irradiation of blood samples (DNA migration as determined by tail movement or intensity), sister chromatid exchange (SCE) assay

and micronucleus test (MNT) compared to controls. However, rats given 50 mg/kg methylmethane sulfonate (MMS) by gavage for 4 hrs (positive control for Comet and SCE assays) or 10 mg/kg cyclophosphamide (CP) given twice orally (positive control for MNT) induced significant increase in genotoxicity in this study. The lack of genotoxicity in this study is not surprising since earlier studies by Casanova-Schimitz et al (1984a) have shown that formaldehyde does not cause toxicity to bone marrow possibly due to the inability of this chemical to reach the bone marrow. Although MMS and CP used in this study were positive in the genotoxicity assays, the data from positive controls can not be used for validation since the exposure routes of formaldehyde (inhalation) and the positive controls (oral) were different.

No animal studies have examined clastogenic effects of formaldehyde in nasal or respiratory epithelial cells. Therefore, it is unknown whether similar changes would occur in response to exposure to formaldehyde via inhalation. However, the negative finding in bone marrow cannot be considered definitive evidence on the question of the mutagenic potential of formaldehyde for cells present at the POE. With weak positive results in pulmonary lavage cells and clear clastogenicity in GI epithelial cells below exposures that trigger regenerative cell proliferation, the existing evidence, however incomplete, supports the concept of genotoxic action of formaldehyde at the POE.

4.3.4.2. *Genotoxicity in Humans*

The majority of the studies on the effects of formaldehyde in exposed humans have measured various cytogenetic endpoints, such as MNs, SCEs, or CAs in nasal and oral mucosal cells (considered to be in direct contact with formaldehyde) as well as peripheral lymphocytes. Since genotoxicity at the proximal sites (oral, nasal) can be readily linked to the reactive nature of formaldehyde, these studies are discussed first, noting where researchers also collected blood lymphocyte samples. A subsequent discussion is focused on results in blood lymphocytes. Finally, the few studies that measured DPXs in exposed humans are discussed. Table 4-89 provides a summary of human cytogenetic studies of formaldehyde.

4.3.4.2.1. ***Nasal, buccal, and oral mucosal cells.*** Epithelial cells of the URT and oral cavity are potential targets of formaldehyde's DNA reactivity and genotoxicity. Several studies indicate that formaldehyde exposure results in measurable increases in SCEs, MN formation, and DPXs in nasal, buccal, and oral mucosal cells; however, these genotoxic effects vary with the type of exposure. Study quality, sample size, availability of exposure measurements, and assay methodology may in part contribute to variability in study findings. The studies fall into three

general categories: workers (industrial or professional), students and staff attending anatomy and mortuary science courses, and subjects in a controlled clinical trial.

Ballarin et al. (1992) observed significantly higher frequency of micronucleated cells in a formaldehyde exposed group in a plywood factory compared with controls (0.9 ± 0.47 versus 0.25 ± 0.22, $p < 0.01$). In this study, the frequency of MNs and cytology of respiratory nasal mucosal cells was examined in 15 nonsmokers exposed to levels of formaldehyde that ranged between 0.1 and 0.39 mg/m^3 (~0.32 ppm) for an average of 6.8 years. Exposed subjects were compared with age- and sex-matched controls.

Ye et al. (2005) reported significant increases in MNs per thousand cells in nasal mucosal cells for 18 nonsmoking workers (2.70 ± 1.50) in a formaldehyde manufacturing plant in the Hugei province of China as compared with controls (1.25 ± 0.41). In addition, higher frequencies of SCEs in peripheral lymphocytes of workers were also reported (8.24 ± 0.89 versus 6.38 ± 0.41). In this study, the average age of workers was 29 ± 6.8 years, the average duration at work was 8.5 years (range 1–15 years), and the reported 8-hour TWA was 0.985 mg/m^3 (0.8 ppm). The control group consisted of 23 undergraduate students with an average age of 19 ± 2.3 years. The 8-hour TWA in the student dormitories was 0.011 mg/m^3 (9 ppb). A group of 16 waiters with an average exposure duration of only 12 weeks and an 8-hour TWA of 0.107 mg/m^3 (90 ppb) was also included in the study. The incidence of MNs and SCEs in the waiters was the same as that in controls. Overall, results from this study suggest that the genotoxic potential of high-level formaldehyde exposure may have occupational risks in long-term exposure.

However, in a different study, Speit et al. (2007b) showed that formaldehyde did not induce MNs in exfoliated buccal mucosa cells of humans exposed up to a maximum of 1 ppm and a cumulative exposure of 13.5 ppm-hours over 2 weeks. In this study, volunteers exposed to formaldehyde in closely controlled conditions (4 hours/day for 10 days) with a complex exposure schedule, amounting to a cumulative total of 13.5 ppm-hours (16.6 mg/m^3-hours), were used. Samples of the buccal mucosa were taken from subjects 1 week before the start of the experiment, at the start of the experiment, at the conclusion of the series of exposures, and at 7, 14, and 21 days after the completion of exposure. Thus, the subjects served as their own controls. Two thousand cells per data point were assessed for the frequency of MNs on slides that were coded by an independent quality assurance organization. As shown in Table 4-84, the frequency of MN formation was statistically unchanged from that in controls. The apparent slight increase in subjects evaluated at the conclusion of exposure was caused by frequencies of MNs in two subjects (5.0 and 4.5 MNs per 1,000 cells). The data as reported show a high

variability, where the SD approaches or exceeds the mean for each sample point, suggestive of data with an asymmetrical distribution.

Table 4-84. MN frequencies in buccal mucosa cells of volunteers exposed to formaldehyde

Sampling point	Group	MN/1000 cells (± SD)
Control data		
1 week before exposure	1	0.95 ± 0.67
Immediately before exposure series	2	0.86 ± 0.84
Test data		
Immediately after exposure series	3	1.33 ± 1.45
7 days after exposure	4	0.94 ± 0.73
14 days after exposure	5	0.85 ± 0.86
21 days after exposure	6	0.44 ± 0.38[a]

[a]Statistically significantly different from control values ($p < 0.05$), as calculated by the authors.

Source: Speit et al. (2007b).

The best evidence of formaldehyde-induced clastogenic changes in peripheral lymphocytes is found in studies of anatomy class and mortuary class students. Since genetic damage accumulates with age, the studies in younger adults, where cells are analyzed before and after exposure, may have greater sensitivity and fewer confounding factors.

Suruda et al. (1993) showed a 12-fold increase in the MN frequency of epithelial cells from the buccal area of the mouth in mortuary science students exposed to embalming fluids containing formaldehyde following an 85-day exposure period (Table 4-85). Overall, students were exposed to 0.33 ppm (0.4 mg/m^3) formaldehyde as an 8-hour TWA on days when embalming was performed (an average of 6.9 embalmings). Blood, oral, and nasal samples were collected pre- and postexposure. As shown in Table 4-85, nasal epithelial MNs increased by 22% (frequency of micronucleated lymphocytes increased by 28%). By contrast, SCE frequency decreased by 7.5% after formaldehyde exposure.

Table 4-85. MN and SCE formation in mortuary science students exposed to formaldehyde for 85 days

Sampling point	Buccal mucosa (MN/1,000)	Nasal epithelium (MN/1,000)	Blood (MN/1,000)	Blood (SCEs/cell)
Before course	0.046 ± 0.17	0.41 ± 0.52	4.95 ± 1.72	7.72 ± 1.26
After course	0.60 ± 1.27[a]	0.50 ± 0.67	6.36 ± 2.03[a]	7.14 ± 0.89

[a]Statistically significant ($p < 0.05$), as calculated by the authors.
Source: Suruda et al. (1993).

Another group (Titenko-Holland et al., 1996) also reported a significant increase in MN frequency of buccal, but not nasal, epithelial cells from mortuary students exposed to embalming fluid. In this study, 28 out of 35 students were sampled before and after a 90-day embalming class. The mean formaldehyde exposure for the subjects providing data on buccal cell MNs was 14.8 ± 7.2 ppm-hours (18.2 ± 8.8 mg/m^3-hours) for the entire 90-day period and 16.5 ± 5.8 ppm-hours (20.3 ± 7.1 mg/m^3-hours) for students providing data on nasal cell MNs. Cells were recorded as having either whole chromosomes with centromeres (MN^+) or acentric fragments and no centromeres (MN^-). Cells with multiple nuclei were present only in samples taken after exposure to embalming fluid. There was a ninefold increase in the MN frequency in buccal cells ($p < 0.5$) and only a twofold increase ($p > 0.05$) in nasal cells. In addition, there was a twofold increase in the MN^+ frequency in buccal cells (Table 4-86). The authors suggested that chromosomal breakage appears to be the primary mechanism of MN formation.

Table 4-86. Incidence of MN formation in mortuary students exposed to formaldehyde for 90 days

Sampling point	Buccal cells (n = 19)			Nasal epithelial cells (n = 13)		
	Total MN	MN^+	MN^-	Total MN	MN^+	MN^-
Pre-exposure	0.6 ± 0.5	0.4 ± 0.4	0.1 ± 0.2	2.0 ± 1.3	1.2 ± 1.3	0.5 ± 0.5
Postexposure	2.0 ± 2.0[a]	1.1 ± 1.3	0.9 ± 1.1[a]	2.5 ± 1.3	1.0 ± 0.8	1.0 ± 0.6[a]
p value	0.007	0.08	0.005	0.20	0.31	0.03

[a]Statistically significant at the level shown, as calculated by the authors.

Source: Titenko-Holland et al. (1996).

Ying et al. (1997), however, observed higher frequencies of MNs in the nasal exfoliative cells (3.85 ± 1.48 versus 1.20 ± 0.676, paired t-test, $p < 0.001$) and oral exfoliative cells (0.857 ± 0.558 versus 0.568 ± 0.317, $p < 0.001$) after formaldehyde exposure, although there was no significant increase in the frequency of lymphocyte MNs ($p > 0.05$) in students exposed to formaldehyde in anatomy classes (three classes per week for 3 hours over an 8-week duration). In this study, blood samples and nasal swabs were collected before and after the study. The TWA concentration of formaldehyde in anatomy laboratories and student dormitories was 0.508 ± 0.299 mg/m^3 and 0.012 ± 0.0025 mg/m^3, respectively, suggesting that nasal mucosa cells exposed through respiration are the primary target of formaldehyde-induced genotoxicity.

In a different study (Ying et al., 1999), however, the same group showed that exposure to formaldehyde affected the composition of lymphocyte subsets (B cells, total T cells, T helper-inducer cells, T cytotoxic-suppressor cells), but no significant difference was reported between lymphocyte proliferation rate and SCEs at the given levels and durations of formaldehyde

exposure. This study involved 23 nonsmoking students exposed to 0.508 ± 0.299 mg/m^3 formaldehyde for a period of 8 weeks (3 hours , 3 times per week).

Burgaz et al. (2002) reported significantly ($p < 0.05$) higher mean MN frequencies in buccal mucosal cells from shoe workers as well as anatomy and laboratory workers (0.62 ± 0.45% and 0.71 ± 0.56%, respectively) compared with unexposed controls (0.33 ± 0.30%). In this study, the measured air concentrations of formaldehyde in the breathing zone of the anatomy and pathology laboratory workers were between 2 and 4 ppm (2.5 and 5 mg/m^3). MN count per 3,000 cells was measured in buccal smears from shoe workers and from anatomy and pathology staff, and eighteen male university staff were used as controls.

In a critical review, Speit and Schmid (2006) examined data from studies that have reported the formation of MNs in nasal or buccal cells of persons either environmentally or occupationally exposed to formaldehyde. The authors identified a number of issues relating to study design, exposure regimen, and confounding factors, including MN levels in nasal and buccal cells well below established background levels, reports limited by the number of cells observed, variation in standard techniques, and non-concordance between buccal and nasal findings. However, the authors concluded that, despite these limitations, the weight of evidence supports the finding that formaldehyde may be genotoxic in human cells in direct contact with formaldehyde.

4.3.4.2.2. ***Peripheral blood lymphocytes.*** Mature lymphocytes are present at the POE as intraepithelial lymphocytes and within germinal centers in the mucosa. Because more lymphocytes may be available in the nasal mucosa than the oral mucosa, mouth versus nose breathing may contribute to variability in findings. Since some of the lymphocytes traffic around the body, it is reasonable to find clastogenic effects in these relatively long-lived cells reflected in peripheral blood lymphocytes. Thus, lymphocytes proliferating in response to antigen would be more vulnerable to DNA reactivity of formaldehyde and to the clastogenic effects in general.

A cytogenetic evaluation by Fleig et al. (1982) of 15 employees exposed for an average of 28 years in a formaldehyde manufacturing plant revealed no statistically significant increase in the frequency of CAs in peripheral blood lymphocytes compared with a matched control group. Likewise, in a different study (Thomson et al., 1984), no compound-related differences were evident in the frequency of CAs and MNs in lymphocytes from six pathology workers and five unexposed controls.

Bauchinger and Schmid (1985) observed an increased incidence of CAs (dicentric and ring chromosomes) in the peripheral lymphocytes of 20 male paper mill workers and supervisors exposed to formaldehyde (average exposure of 14.5 years) compared with unexposed workers.

When workers and supervisors were analyzed separately, significant increases were only seen for supervisors. The average length of exposure for supervisors (n = 11) and workers (n = 9) was 18.9 years and 7.2 years, respectively. Information regarding formaldehyde concentrations for the two groups was not provided. However, the incidence of SCEs among workers was actually slightly lower than among the 20 controls. In contrast, the frequency of SCEs in peripheral lymphocytes of 18 nonsmoking formaldehyde workers was increased over controls (8.24 ± 0.89 versus 6.38 ± 0.41) (Ye et al., 2005) (described in Section 4.3.4.2.1).

Vargová et al. (1992) observed that the percentage of aberrant cells and number of breaks per cell in the peripheral blood lymphocytes of formaldehyde-exposed workers was 3.08 and 0.045 versus 3.6 and 0.080 in controls in a pressed board factory, respectively, suggesting both groups to be at an increased risk. However, normal unexposed population had only 1–2% aberrant cells. The authors also noted that the mitotic index was significantly decreased in exposed workers compared with controls.

Kitaeva et al. (1996) evaluated the genotoxic effects of formaldehyde among 15 industrially exposed workers and 8 academic laboratory instructors and observed an increase in the frequencies of CAs and MNs in the lymphocytes of exposed subjects compared with 6 unexposed controls.

Shaham et al. (1996, 1997) found significantly higher levels of DPXs and SCEs in peripheral blood lymphocytes of workers occupationally exposed to formaldehyde (physicians and technicians) compared with unexposed control workers. The authors also observed a linear relationship between years of exposure to formaldehyde and levels of DPXs and SCEs.

Formaldehyde-induced genotoxicity has also been reported in peripheral blood lymphocytes of anatomy class students and mortuary workers. Vasudeva and Anand (1996) did not observe significant differences in the incidences of CAs between the formaldehyde exposed students and the matched, unexposed controls. In this study, peripheral blood lymphocytes from 30 medical students exposed to formaldehyde in a gross anatomy laboratory for 15 months with average exposures of less than 1 ppm (1.23 mg/m^3) formaldehyde were used.

He et al. (1998) used the cytokinesis-blocked MN (CBMN) assay to detect the frequency of micronucleated peripheral lymphocytes in 13 students exposed to formaldehyde during a 12-week (10 hours/week) anatomy class. Sampling of breathing zone air showed a mean concentration of 2.37 ppm (3.17 mg/m^3). Ten students from the same school, without exposure to formaldehyde, were used as controls. CAs and SCEs were observed in both groups, and there were significant increases ($p < 0.01$) in the frequencies of micronucleated cells and CAs in the formaldehyde-exposed group compared with the control group.

In a study involving 97 plasticware workers (34 males and 63 females) exposed to 0.5 to 0.9 mg/m3 formaldehyde, 4.4 to 6.2 mg/m3 styrene and 0.5 to 0.75 mg/m3 phenol for 2 months to 25 years, Lazutka et al (1999) observed significantly higher CAs than controls (non-exposed donors matched by age and similar smoking habits as the exposed workers). Although workers with short and long exposures showed significant increases in the frequency of CAs, the cytogenetic damage did not increase with exposure duration.

Sari-Minodier et al. (2001), using the CBMN assay in anatomy/pathology laboratory workers, reported higher frequency of micronucleated peripheral blood lymphocytes than in matched controls.

Shaham et al. (2002) observed a mean number of 0.27 SCEs per chromosome in the peripheral lymphocytes of an exposed cohort compared with 0.19 in controls ($p < 0.01$). This study involved 90 individuals employed in 14 hospital pathology laboratories and 52 unexposed controls.

Yu et al. (2005) reported dose-dependent increase in MNs and comet assay parameters (olive tail moment and comet tail length) in peripheral lymphocytes in 151 workers from two plywood factories compared with 112 unexposed controls. The TWA exposure level in the working environment was 0.1–7.88 mg/m^3 (0.08–6.42 ppm) formaldehyde compared with a background level of <0.01 mg/m^3 (<0.008 ppm) formaldehyde applicable to controls. In the comet assay, the authors observed olive tail moments averaging 0.93 (0.78–1.1), 3.03 (2.49–3.67), and 3.95 (3.53–4.43) for control, low-, and high-exposure individuals, respectively. For the same subjects, comet tail lengths were 6.78 (6.05–7.6), 11.25 (10.12–12.5), and 12.59 (11.8–13.43), respectively. In the CBMN assay, MNs/100 cells were 0.27 ± 0.13, 0.41 ± 0.25, and 0.65 ± 0.36, respectively, for control, low-, and high-exposure individuals.

In a population of 18 workers exposed to formaldehyde at a plant in China, with a mean employment of 8.5 years (range 1 to 15 years), Ye et al (2005) examined nasal and lymphocytes for cytogenetic effects. This study also included a second group of 16 waiters who worked in a newly fitted ball room for 12 weeks with a low level exposure to formaldehyde from building material, tobacco smoke and furniture and a group of 23 college students as a control group. The background indoor air conentraton of 0.009 ppm formaldehyde was reported in students' dorms. Significantly increased frequencies of MNs in the nasal mucosal cells and SCEs in peripheral blood lymphocytes were reported for the workers, but not the waiters in this study.

Orsière et al. (2006) reported no apparent effect on the DNA damage in peripheral blood lymphocytes as assessed by a chemiluminescence microplate assay in pathology and anatomy laboratory workers (n = 59) before and after a 1-day exposure to formaldehyde. This study had 59 exposed workers and 37 controls. However, with the CBMN assay, the authors reported

statistically significant differences in the frequency of binucleated micronucleated cells (1.69 ± 0.93 versus 1.11 ± 0.6%) in exposed versus control subjects. Discrimination between clastogenic and aneugenic events by using FISH with a pan-centromeric DNA probe resulted in a higher rate of binucleated micronucleated cells (1.91 ± 1.01 versus 1.19 ± 0.56% in controls) and showed that the frequency of centromeric nuclei was higher in the exposed group than in controls, though not significantly. Among the centromeric MNs, the frequency of MNs with only one centromere (C1+MN) was significantly greater in pathologists/anatomists than in controls (1.1 ± 0.62 versus 0.31 ± 0.24%, $p < 0.001$). The authors interpreted their data on monocentromeric nuclei in anatomists/pathologists as an indication that formaldehyde exposure might be associated with aneugenic (rather than clastogenic) events.

Based on pooled analysis of two reports (Iarmarcovai et al., 2006a, b) (Table 4-87), MN frequency ratios in the peripheral lymphocytes of cancer patients, welders, and anatomists/pathologists were significantly increased compared with the corresponding controls. The data were taken from three biomonitoring studies by using CBMN/FISH. The incidence of MNs was scored and then evaluated further for the presence of centromere-negative MNs (C−MNs), centromere-positive MNs (C+MNs), and, for the latter case, those containing a single centromere (C1+MNs) and those containing two or more centromeres (Cx+MNs). Applying their findings to considerations of the aneugenic mechanism of action of formaldehyde, the authors hypothesized that the use of centromeric signals enables the identification of endpoints representing impaired chromosomal migration (with C1+MN formation) or centrosome amplification (with Cx+MN formation).

Table 4-87. Multivariate repression models linking genomic instability/occupational exposures to selected endpoints from the MN assay

Study populations	Number	MN[a]	C−MN	C+MN	C1+MN	Cx+MN
Cancer patients versus controls	10/10	**1.85 (1.18−2.87)**	**2.05 (1.07−3.94)**	**1.81 (1.02−3.21)**	1.68 (0.80−3.53)	1.28 (0.63–2.59)
Welders versus controls	27/30	**1.37 (1.09−1.72)**	1.39 (0.99−1.95)	**1.37 (1.03−1.83)**	1.10 (0.80−1.53)	1.31 (0.99−1.74)
Pathologists/anatomists versus controls	18/18	1.28 (0.86−1.90)	0.79 (0.46−1.36)	**1.65 (1.05−2.59)**	**3.29 (2.04−5.30)**	0.68 (0.38−1.20)

[a]Bolded values indicate statistical significance ($p < 0.05$).

Source: Iarmarcovai et al. (2006b).

Recently, Costa et al. (2008) observed a significant increase in the genotoxicity of formaldehyde-exposed pathological anatomy laboratory workers (n = 30) compared with controls (n = 30) in cytogenetic assays. In this study, the authors evaluated the level of exposure

to formaldehyde near the breathing zone of workers, and TWA of exposure was calculated for each subject, giving a mean level of exposure to be 0.44 ± 0.08 ppm (range: 0.04–1.58 ppm). As compared with control subjects, peripheral blood lymphocyte cultures of formaldehyde exposed workers showed significant increases in MN frequency (5.47 ± 0.76 versus 3.27 ± 0.69; p = 0.003), SCEs (6.13 ± 0.29 versus 4.49 ± 0.16; $p < 0.05$), and comet assay as determined by tail length (TL) (60.00 ± 2.31 versus 41.85 ± 1.97; $p < 0.05$). In addition, Costa et al. (2008) observed a positive correlation between formaldehyde exposure levels and MN frequency (r = 0.384; p = 0.001) and TL (r = 0.333; p = 0.005) (Table 4-88). However, polymorphic genes of xenobiotic metabolizing and DNA repair enzymes did not show any significant effect on the genotoxic endpoints. This is the lowest level of exposure to formaldehyde in the studies observed so far, wherein a clear indication of genotoxic effects of formaldehyde was demonstrated.

Table 4-88. Genotoxicity measures in pathological anatomy laboratory workers and controls

	MN assay	SCEs	Comet assay
	Mean MN ± SEM (range)	**Mean SCE ± SEM (range)**	**Mean TL (μM) ± SEM (range)**
Controls (n = 30)	3.27 ± 0.69 (0–17)	4.49 ± 0.16 (3.10–3.06)	41.85 ± 1.97 (28.85–66.52)
Exposed (n = 30)	5.47 ± 0.76 (1–17)	6.13 ± 0.29 (3.64–8.80)	60.00 ± 2.31 (33.76–99.09)
p value	0.003	<0.05	<0.05

Source: Costa et al. (2008).

4.3.5. Summary of Genotoxicity

Formaldehyde's genotoxicity has been demonstrated in a variety of in vitro and in vivo test systems measuring a variety of genetic endpoints. Formaldehyde forms predominantly DPXs that are detected in cell-free systems and single cells in vitro. DPXs are formed in nasal epithelia but not in extra-nasal passages of rodents, which are completely removed within a day after formation. In vivo data in human and mammalian cells demonstrate that formaldehyde is genotoxic at the site of first contact, including cells of the mouth or the nose. DPXs are also detected in nasal and extra-nasal tissues of monkeys, suggestive of direct effects of formaldehyde in tissues that correspond to observed tumor sites (nasal and nasopharynx) in humans. In addition, this is used as a basis for cross-species comparison with humans. Formaldehyde-DNA adducts are labile and constitute a minor fraction of the DNA-reaction products and are less likely to play an important role in the genotoxicity of formaldehyde.

Formaldehyde clastogenicity has been demonstrated by the induction of SCEs, SSBs, MNs, and CAs in cultured mammalian cells. Formaldehyde induces mutations in salmonella and escherichia bacterial strains that contain an AT base pair at the primary reversion site that is used to detect oxidative compounds and cross-linking agents without metabolic activation by exogenous enzyme-activating systems. Formaldehyde induces mutations in cultured mammalian cells at levels that do not cause significant toxicity. Despite formaldehyde's reactivity and mutagenicity in isolated mammalian cells, clear evidence of mutagenicity does not emerge from animal bioassays.

Formaldehyde exposure causes differential induction of MNs in human nasal epithelial and buccal epithelial cells, which is significant in industrial exposure workers and students working in anatomy or mortuary science, respectively. However, recent data and data from larger studies support a finding of increased MNs in blood lymphocytes, although the issue remains controversial because of issues relating to study design, exposure regimen, and confounding factors, including MN levels in nasal and buccal cells well below established background levels, reports limited by the number of cells observed, variation in standard techniques, and non-concordance between buccal and nasal findings (Speit and Schmid, 2006). Several clastogenic effects, such as induction of MNs, SCEs, and CAs, were seen in human peripheral blood lymphocytes; however, the data are not very clear. Formaldehyde exposure also caused p53 mutations in rat nasal carcinomas with the expression of mutant p53 protein.

Overall, induction of DPXs as a predominant lesion in vitro and in vivo, clastogenicity, and mutagenicity with locus-specific mutations in nonhuman and human cells supports the concept of genotoxic action of formaldehyde at the POE.

A summary of the genotoxicity of formaldehyde in humans is presented in Table 4-89.

4.4. SYNTHESIS AND MAJOR EVALUATION OF NONCARCINOGENIC EFFECTS

The adverse health effects due to formaldehyde exposure have been extensively studied in humans and in animal models. Studies of human exposure include occupational exposures, environmental exposures, and clinical studies of intentionally exposed subjects (Section 4.1). Occupational exposures are primarily due to inhalation and dermal contact. Animal studies are available for a variety of routes of exposure, including inhalation, oral, dermal, and intravenous and I.P. injections (Section 4.2). Additionally, as discussed in Chapter 3, in vitro studies address biological activity and the metabolic fate of formaldehyde.

Table 4-89. Summary of human cytogenetic studes

Study population	N	Exposure time (years)		Formaldehyde concentration (ppm)		Cytogenetic observations			Reference
		Range	Mean	Range	Mean (TWA)	CAs	SCEs	MNs	
Analyses of nasal and/or buccal cells									
Plywood workers Age and sex matched controls	15 15	2–19	6.8	0.32–0.83	(1)			+ nasal	Ballarin et al. (1992)
Male mortuary science students Female mortuary science students	22 7	Buccal and nasal swabs taken before and after first 9 weeks of embalming course		0.1–4.3	1.4			+ buccal – nasal – buccal – nasal	Suruda et al. (1993)
Mortuary science students[a]	28	Buccal and nasal swabs taken before and after first 9 weeks of embalming course		0.1–4.3	1.4			+buccal[b] – nasal	Titenko-Holland et al. (1996)
Female anatomy faculty Male anatomy faculty Controls (Females)	8 5 7	NA	23.6 25.6	NA	NA			+ buccal – buccal	Kitaeva et al. (1996)
Anatomy students	25	Buccal and nasal swabs taken before and after 8-week anatomy course		0.06–1.06	(0.508)			+ buccal + nasal	Ying et al. (1997)
Anatomy/ pathology staff Controls (University staff)	28 18	1–13	4.70	2–4	NA			+ buccal	Burgaz et al. (2002)
Workers at a formaldehyde plant Controls	18 23	1–15	8.5		0.8			+ nasal	Ye et al. (2005)
Volunteers	21	10 days		13.5 ppm-hours				– buccal	Speit et al. (2007b)
Analyses of peripheral lymphocytes									
Manufacturing workers Age and sex matched controls	15 15	23–35	28		<5 1971 <1 later	–			Fleig et al. (1982)
Pathology workers Controls	6 5	4–11			0.9–5.8			–	Thomson et al. (1984)

Table 4-89. Summary of human cytogenetic studes

Study population	N	Exposure time (years)		Formaldehyde concentration (ppm)		Cytogenetic observations			Reference
		Range	Mean	Range	Mean (TWA)	CAs	SCEs	MNs	
Anatomy students[c]	8	10-week class		1.08–1.99[d] 0.08–0.6[e]	1.2 0.3		+		Yager et al. (1986)
Papermakers Controls	20 20	2–30	14.4	<3	NA	+[f]	–		Bauchinger and Schmid (1985)
Wood workers Controls	25 19	<5 to <16		0.45–8.6	NA	–			Vargová et al. (1992)
Male embalming students Female embalming students	22 7	Blood sampled before and after first 9 weeks of embalming course		0.15–4.3	1.4		– –	+ –	Suruda et al. (1993)
Manufacturing workers Anatomy faculty Controls	15 8 6		10 17	Up to 4 NA	NA	+ ND		+	Kitaeva et al. (1996)
Medial students Controls	30 30	Sampled near end of 15-month term		<1	NA -	–			Vasudeva and Anand (1996)
Anatomy students Controls (students)	13 10	12-week class			2.37[g]	+	+	+	He at al. (1998)
Physicians Technicians Controls (age matched/unexposed)	6 7 20	2-24 2-25	10 15	3.1-2.8	1.6		+ +		Shaham et al 1997
Anatomy students	23-25	Blood samples taken before and after 8-week anatomy course		0.06–1.06	(0.508)	-	–	–	Ying et al. (1999, 1997)
Female anatomy/pathology lab workers Controls (Women)	10 27	1–16	8.9	1.2–15.1	NA			+	Sari-Minodier et al. (2001)
Hospital pathology workers[h] Controls	90 52	1–39	15.4	0.04–0.7[i] 0.72-5.6	0.4 2.24		+[j] +		Shaham et al. (2002)

Table 4-89. Summary of human cytogenetic studes

Study population	N	Exposure time (years)		Formaldehyde concentration (ppm)		Cytogenetic observations			Reference
		Range	Mean	Range	Mean (TWA)	CAs	SCEs	MNs	
Workers at a formaldehyde plant	18	1–15	8.5		0.8		+	–	Ye et al. (2005)
Controls	23								
Workers at two plywood factories	151	ND			0.08–6.42			+	Yu et al. (2005)
Controls	112								
Pathology or anatomy workers	59	ND		<0.1–20.4[k]	2[k]			+	Orsière et al. (2006)
Controls	37								
Pathologists	18	ND		0.4–7.0[k]	2.3[k]			+	Iarmarcovai et al. (2006a, b)
Controls	18								
Pathological anatomy lab workers	30	0.5–27	11	0.04–1.58	0.44		+	+	Costa et al. (2008)
Controls (21 females and 9 males)	30								
Plasticware workers	97	2 mo to 25		0.5-0.9		+			Lazutka et al 1999
Controls (non-exposed donors)	90	yrs		mg/m3					
Wood workers	40					+			Chebotarev et al 1986
Controls	22	NR		NR					
School children (1984)	20				0.26	+			Neri et al 2006
School children (1985)	16				0.11	+			
School children (1986)	18				0.03				
Controls (1984)	17				0				
Preschool controls (1984)	24				0				
Preschool children (1984)	13			0.17-0.3					
Phenolformaldehyde resin workers	31	0.33-30 yr			0.41	+			Suskov and Sazonova 1982
Controls	74				0				

[a]Same population in Suruda et al. (1993) but different slides used. Nineteen complete slide sets for buccal analysis and 13 complete slide sets for nasal epithelial cell analysis.
[b]Not dose related; both low- and high-exposure groups had same SCE increase.
[c]Each student sampled before and after 10-week anatomy class.
[d]Breathing zone samples.

[e]Room air samples.
[f]Increase only in 11 supervisors. See text for details.
[g]Average breathing zone during dissection procedure.
[h]Exposed and controls from 14 hospitals.
[i]Low- and high-exposure groups established but numbers not provided.
[j]Not dose related; both low and high groups had same SCE increase.
[k]Described as "mean concentrations for sampling times of 15 minutes."
CAs = chromosomal aberrations; SCEs = sister chromatid exchanges; MNs = micronuclei; TWA = time-weighted average; ND = not determined; NA = not applicable.

Taken together, the human and animal studies support numerous health effects, not only at the POE as expected for a reactive gas but also on pulmonary function, neurobehavioral function, reproduction, development, immunomodulation, and sensitization (atopy, asthma). The discussion below provides a description of the adverse effects seen in each area, summarizing the data for both human and animal studies. MOA data are discussed where information regarding formaldehyde's biological activity may be linked to the observed adverse health effects.

4.4.1. Sensory Irritation

Sensory irritation of the eyes, nose, and throat is reported in humans upon direct contact with formaldehyde gas during inhalation exposures (Holmström and Wilhelmsson, 1988; Ritchie and Lehman, 1987) and includes irritation resulting from acute exposures (Lang et al., 2008; Yang et al., 2001; Krakowiak et al., 1998; Kulle, 1993; Green et al., 1989, 1987; Kulle et al., 1987; Sauder et al., 1987, 1986; Schachter et al., 1987, 1986; Witek et al., 1987; Day et al., 1984; Bender et al., 1983; Weber-Tschopp et al., 1977). Controlled exposures in inhalation chambers confirm the specificity of these responses to formaldehyde exposure and allow for assessment of these symptoms through both subjective and objective measures (Kulle, 1993; Holness and Nethercott, 1989; Green et al., 1987; Kulle et al., 1987; Sauder et al., 1986; Weber-Tschopp et al., 1977). Eye irritation may be reported as itching, burning, and general discomfort. Tearing, redness of the eyes, and increased blink frequency are observed and may be quantified in exposure under controlled conditions (Lang et al., 2008; Yang et al., 2001; Andersen and Molhave, 1983; Weber-Tschopp et al., 1977; Schuck et al., 1966). Eye irritation appears to be the most sensitive endpoint in most individuals and may be observed after short exposures (195 minutes at 0.5 ppm: Lang et al. [2008]; 30 seconds at 1.65 ppm: Yang et al. [2001]).

Itching, burning, and discomfort of the nose, which may be accompanied by increased mucous production (runny nose), are reported by individuals exposed via inhalation (Krakowiak et al., 1998; Kulle, 1993; Green et al., 1987; Kulle et al., 1987; Weber-Tschopp et al., 1977). Throat irritation may also be described subjectively as itching and burning and is often accompanied by a cough (Krakowiak et al., 1998). Symptoms of eye and mucous membrane irritation are also reported in numerous rodent studies and support the health effects reported in humans (see Section 4.1.1.1). Although dermal contact may result in dermatitis and an apparent hypersensitivity reaction, symptoms do not present upon contact as sensory irritation. There are no human or animal data that assess sensory irritation from oral exposures.

The time to onset of sensory irritation symptoms and severity of the sensory irritation are a function of both the air concentration and duration of exposure. Additionally, nose and throat irritation becomes more prominent at higher exposures and longer duration of exposure (Kulle,

1993; Kulle et al., 1987). Controlled human laboratory exposures (Yang et al., 2001; Kulle, 1993; Kulle et al., 1987; Cain et al., 1986; Andersen and Molhave, 1983) provide more direct exposure-response evidence for sensory irritation. These studies are limited to healthy nonsmoking individuals. Two studies (Cain et al., 1986; Andersen and Molhave, 1983) document discomfort and irritation of the eye in response to acute exposures as low as 0.25 ppm. Dose-response relationships are reported in a number of different ways: as an incidence of the reported symptom among subjects, as a score for severity of the symptom, or in some cases as a subjective measure, such as blink frequency for eye irritation.

Symptoms of sensory irritation, including eye irritation (burning watering, increased blinking), nasal irritation (rhinitis, itching/burning), throat/respiratory tract irritation (wheezing, coughing, phlegm production), have been reported in numerous worker cohorts. Occupational exposure environments include hospital and medical settings, students, and industrial workers (Takahashi et al., 2007; Takigawa et al., 2005; Krakowiak et al., 1998; Akbar-Khanzadeh et al., 1994; Uba et al., 1989; Horvath et al., 1988; Schachter et al., 1987). Formaldehyde levels often vary in a work environment and peak as well as average exposures may be used to report occupational exposures. Although sensitive individuals often remove themselves from an irritating workplace (the HWE), eye, nose, and throat symptoms are still reported in this environment. Among workers in a plant where formaldehyde resins were used, those exposed to an average of 210 ppb formaldehyde reported increased symptoms above those in the control population (Holmström and Wilhelmsson, 1988).

These effects have been noted in students, particularly medical students, who are exposed to formaldehyde in cadaver labs. In a study of 24 formaldehyde-exposed anatomy students (personal breathing zone samples 0.73 ppm, range 0.49–0.93) (Kriebel et al., 1993), eye, nose, and throat irritation was present when comparing rates of irritation from the end or middle of class to before the start of class. Takahashi et al. (2007) showed that 143 medical students reported various symptoms (including eye and throat irritation) and that the percentage of students reporting symptoms increased between the beginning (measured after the first day of class) and the end of the course (2 months later). After the first day of class, approximately 35% of students reported eye soreness and about 15% reported throat irritation.

Sensory irritation has also been noted in occupational settings. Horvath et al. (1988) compared irritation symptoms between 109 workers at a particleboard manufacturing plant and 264 workers at food plants. Eye, nose, and throat irritation were more common among the group in a particleboard manufacturing facility, exposed to a mean concentration of 0.40 mg/m^3. Similarly, Alexandersson and Hedenstierna (1988) reported that the frequency of eye, nose, and throat irritation was significantly greater (65.8%) in 38 workers exposed to formaldehyde and

solvents in lacquers as compared with 18 nonexposed individuals working at the same factory (16.7%). Holmström and Wilhelmsson (1988) conducted a study at a chemical plant and reported nasal and eye discomfort in 64 and 24%, respectively, of workers (n = 70) exposed to formaldehyde (range 0.05–0.50 mg/m^3 with a mean of 0.26 mg/m^3) versus 25 and 6%, respectively, in nonexposed desk clerks (n = 36). Holness and Nethercott (1989) reported significant increases in eye irritation (42 versus 21%) and nose irritation (44 versus 16%) among 84 funeral service workers (active embalmers, >10-year experience) as compared with 38 students and individuals from a service organization. The exposure concentration in both groups was 0.36 and 0.02 ppm, respectively.

Reports of similar symptoms are correlated to indoor residential exposures, providing exposure-response relationships for the general population in low-level chronic exposure scenarios. Ritchie and Lehnen (1987) surveyed residents in 2,000 homes classified as having formaldehyde concentration <0.1 ppm, 0.1–0.3 ppm, and >0.3 ppm. A LOAEL of 200 ppb was established from the results of Ritchie and Lehnen (1987). Liu et al. (1991) report irritant effects associated with formaldehyde exposure in mobile homes, where formaldehyde concentrations ranged from the 0.01 ppm detection limit to 0.46 ppm. Eye irritation (60%), nose/throat irritation (10–20%), or headache (<10%) were reported in residents.

MOA

The mucosae of the URT, oral cavity pharynx, and upper airways are complex tissues, where epithelial and goblet cells predominate. In addition, the nasal mucosa is highly enervated. The main nerves include the trigeminal nerve and olfactory sensory cells (olfactory epithelium, the vomeronasal organ, and the organ of Masera) (Feron et al., 2001). A possible MOA for sensory irritation includes formaldehyde-induced stimulation of the trigeminal nerve (though whether formaldehyde acts as a direct agonist is unknown). Trigeminal nerve stimulation in the nasal passages transmits signals to the CNS, which then sends efferent signals back to the nasal tissues, causing sensory irritation, and possibly systemically via vagal nerve stimulation, resulting in more systemic effects.

Animal studies are potentially useful models for understanding mechanisms of toxicity, especially where sufficient human data do not exist. While experimental animal studies provide a model of secondary effects, rodents also demonstrate RB, an effect not seen in humans. Thus, species that exhibit bradypnea (like mice and rats) may not be appropriate for assessing respiratory endpoints. The mechanism underlying RB includes formaldehyde binding to the sensory nerve endings of the trigeminal nerve, where signals travel to the CNS. The vagus nerve transmits the efferent signal to produce smooth muscle contraction. The animals become

inactive, their core temperatures decrease by several degrees C, and their respiratory rates and minute volumes decrease. However, this is not to say that trigeminal nerve stimulation is not an appropriate potential mechanism of action in other species or in humans. Since trigeminal nerve stimulation has been independently confirmed in species without RB, this mechanism may be a viable explanation for the observed effects.

4.4.2. Pulmonary Function

Workers chronically exposed to formaldehyde have exhibited signs of reduced lung function, such as BC, inflammation, and chronic obstructive lung disease. Lung function deficits have been reported in pre- versus post-shift measurements and as a result of chronic exposures (Pourmahabadian et al., 2006; Herbert et al., 1994; Malaka and Kodama, 1990; Alexandersson and Hedenstierna, 1989; Alexandersson et al., 1982). Decreases in spirometric values, including VC, FEV, FVC, and FEV/FVC, have been reported. Chronic studies (Pourmahabadian et al., 2006; Herbert et al., 1994; Malaka and Kodama, 1990; Alexandersson and Hedenstierna, 1989; Alexandersson et al., 1982) also report increased respiratory symptoms, including cough, increased phlegm, asthma, chest tightness, and chest colds, in exposed workers.

Students have also shown decrements in lung function that are associated with exposure to formaldehyde in laboratories. Kriebel and colleagues (1993) observed a 2% decrement in PEF in healthy students attending anatomy classes once per week and a 7.3% decrement in PEF in students with histories of asthma. The strongest pulmonary response was observed when examining the average cross-laboratory decrement in PEF in the first 2 weeks of the study (formaldehyde geometric average concentration of 0.73 ppm). These findings were corroborated by Kriebel et al. (2001) in which a similar study design was applied to another class of anatomy students.

Similarly, Akbar-Khanzadeh et al. (1994) compared pre- and postexposure pulmonary function among students before and after working 3 hours in a laboratory (n = 34). On average, FVC decreased by 1.4%, FEV_3 decreased by 1.2%, FEV_1/FVC increased by 1.6%, and $FVC_{-25–75\%}$ increased 2.5%. These average percent changes in the control group are –0.3%, 1.30%, 2.31%, and 0.6% but were not statistically significant. In a follow-up study, Akbar-Khanzadeh and Mlynek (1997) recorded FEV values in 50 exposed students and 36 controls and reported a larger increase in lung function among controls when compared with cases after 1–3 hours of exposure that persisted after 3 hours after exposure termination. In a similar study, Fleisher (1987) reported that approximately 8% of students reported experiencing shortness of breath during the laboratory with formaldehyde exposure, but none of the students reported shortness of

breath in the laboratory session with no exposure. However, no objective measurements of formaldehyde exposure were used.

Unlike the study by Kriebel et al. (1993), Uba et al. (1989) did not find a change in pulmonary function over the course of the 7 months in a study of 96 anatomy laboratory students. These negative findings may be attributed to differential cross-shift exposures and to significant differences in FVC on exposed days.

Deficits in pulmonary function have been reported in occupational or residential exposure studies (Khamgaonkar and Fulare, 1991; Krzyzanowski et al., 1990; Malaka and Kodama, 1990; Alexandersson and Hedenstierna, 1989; Kilburn et al., 1985; Alexandersson et al., 1982). Krzyzanowski et al. (1990) documented a significantly decreased PEFR in children (298 children) who resided in homes with an average formaldehyde concentration of 26 ppb (maximum sample value of 140 ppb). Among adults, there was a statistically significant nonlinear relationship with decreased morning PEFR for formaldehyde concentration <40 ppb (Krzyzanowski et al., 1990). Similarly, Malaka and Kodama (1990) reported that an average 8-hour TWA formaldehyde exposure of 1.13 ppm from area samples was associated with statistically significant decrements in FEV_1, FEV_1/FVC, and $FEF_{25–75\%}$ compared with a referent population. Alexandersson and Hedenstierna (1989) investigated not only the acute effects of exposure across shift but also measured effects of exposure among some of the same workers that had been studied 5 years earlier (Alexandersson et al., 1982). Statistically significant decreases ($p < 0.01$) in FEV_1/FVC and $FEF_{25–75\%}$ were noted over the intervening five years in nonsmokers after correcting for aging. Similar decrements have been documented in laboratory workers in India (Khamgaonkar and Fulare, 1991) and in factory workers (Kilburn et al., 1985).

Alexandersson et al. (1982) reported only slight deficits in lung function 1 day following occupational formaldehyde exposure in a carpentry shop in Sweden, where the measured formaldehyde level was 0.36 ppm (0.47 mg/m^3). In this case, subjects were compared with 20 nonexposed workers.

Other studies have found no association between formaldehyde and lung function (Ostojic et al., 2006; Holness and Nethercott, 1989; Holmström and Wilhelmsson, 1988; Horvath et al., 1988). Ostojic et al. (2006) used an interesting measurement, “diffusing lung capacity” instead of decrements in FEV1 or similar measurements. Similarly, Nunn et al. (1990) assessed the decrease in FEV_1 with age and showed no association between formaldehyde exposure and decreased FEV_1. Franklin et al. (2000) did not report an association between FVC or FEV and the indoor concentrations of formaldehyde in children (ages 6–13), although there were signs of lower airway inflammation as measured by levels of exhaled NO (Franklin et al., 2000). Similarly, Main and Hogan (1983) did not observe differences between FEV_1 or FVC at the end

of the 34 months between mobile home trailer workers compared with controls who did not work in trailers. The average exposure was reported as ranging from 0.12 to 1.6 ppm.

Occupational studies share certain limitations, including the potential for confounding by occupational co-exposures. Also, studies that did not report pre-shift pulmonary function as a percentage of expected function are less useful to assess potential chronic effects because, post hoc, it is difficult to calibrate for cross-study comparison due to lack of data on important pulmonary function determinants, such as age, gender, smoking status, height, and year of birth.

Controlled human studies and studies in nonhuman primates also document changes in formaldehyde-induced pulmonary dysfunction. Acute exposures of healthy non-asthmatic volunteers resulted in transient decreases in pulmonary function (e.g., decreased FEV_1, FVC_1, FEV_3, specific airway conductance) (Green et al., 1987; Sauder et al., 1986). Green et al. (1987) noted differential responsiveness in formaldehyde-exposed subjects; some were responders while others were nonresponders. This differential response suggests susceptibility in certain subjects (Green et al., 1987).

Several animal studies document increased airway resistance and BC following inhalation exposure to formaldehyde (Nielson et al., 1999; Swiecichowski et al., 1993; Biagini et al., 1989; Amdur et al., 1960). A study using cynomolgus monkeys (Biagini et al., 1989) demonstrated that methacholine-induced BC can be similarly induced by acute formaldehyde exposure (10 minutes at 2.5 ppm). Thus, formaldehyde exposure simulated BC observed after methacholine challenge, but these effects may not occur by a similar MOA. Similar results were reported in guinea pigs (Swiecichowski et al., 1993; Amdur et al., 1960), rats (Ohtsuka et al., 1997), and mice (Nielson et al., 1999).

Deficits in pulmonary function have been documented in occupational as well as controlled chamber human studies and have been corroborated in animal studies exposed to formaldehyde. However, some of these deficits are slight or transient. Some studies did not identify a statistically significant decrease in pulmonary function, and others did not observe any change at all. Pulmonary function alterations appear to be specifically tied to exposure regimen and may be reversible but remain, nevertheless, an important symptom often associated with exposure to formaldehyde.

MOA

Formaldehyde-induced inflammation of the airways may contribute to observed decreases in measures of pulmonary function. Even short-term inflammatory reactions could reduce the effective diameter of the conductive airways, resulting in lower respiratory volumes in a number of functional tests. Formaldehyde-induced trigeminal nerve stimulation contributes to

airway inflammation, which in turn would reduce airway function. Chronic exposures may result in increased sensitization or chronic inflammatory responses, which could contribute to the effects seen in the worker and residential populations.

Formaldehyde-induced pulmonary function deficits may also be in part a result of smooth muscle contraction in repose to trigeminal nerve stimulation. Trigeminal nerve stimulation transmits signals to the CNS. The resulting efferent signal from the vagal nerve produces smooth muscle contraction and may result in decreased pulmonary function. Efferent signaling has also resulted in release of substance P and other neuromodulatory compounds, which may contribute to BC and sensitization of pulmonary responses (asthma, atopy).

4.4.3. Hypersensitivity and Atopic Reactions

Sensitization to inhalational chemical exposure may manifest as an allergic or asthmatic response that is characterized by BC or BHR. This sensitization may be a result of immune involvement, as in the case of hypersensitivity, or a neurogenic sensitization, where a chemical may directly stimulate inflammation. Asthma is a specific manifestation of IgE-mediated hypersensitivity, characterized by BHR and airway inflammation, resulting in lower airway obstruction (Fireman, 2003; Kuby, 1991).

A variety of hypersensitivity reactions have been reported following exposure to formaldehyde. Rashes and skin reactions have been reported in some individuals after dermal exposures to formaldehyde. Increased expression of Th-2 cytokines in the lymph nodes of mice given dermal applications of formaldehyde does indicate the involvement of an immune component to the observed sensitization (Dearman et al., 2005; Hilton et al., 1998; Arts et al., 1997). However, the response does not appear to be IgE mediated (Arts et al., 1997; Lee et al., 1984). Gorski et al. (1992) observed an increase in formaldehyde-mediated neutrophil burst in dermatitis patients exposed in a controlled chamber study and suggests a putative role of oxidative stress and reactive oxygen species (ROS).

Inhalation exposure has been associated with increased asthmatic responses in asthmatics in occupational settings. While few available case reports of bronchial asthma suggest direct respiratory tract sensitization to formaldehyde gas (Lemiere et al., 1995; Burge et al., 1985; Hendrick et al., 1982; Hendrick and Lane, 1977, 1975), a greater body of human data provides evidence of an association between formaldehyde exposure and exacerbation of asthmatic responses in compromised individuals (Kriebel et al., 1993) and particularly in children (Rumchev et al., 2002; Garrett et al., 1999; Krzyzanowski et al., 1990). Increased asthma incidence reported after inhalation exposure to formaldehyde led to a NOAEL of 30 ppb

(Rumchev et al., 2002). An increased frequency of respiratory symptoms associated with asthmatic responses and formaldehyde exposure led to a LOAEL of 30 ppb (Garrett et al., 1999).

Exacerbation of response after formaldehyde exposure has been demonstrated in animal studies as well. Sadakane et al. (2002) demonstrated that formaldehyde exposure exacerbated sensitization and challenge with Der f and suggested that formaldehyde exposure may aggravate eosinophilic infiltration and goblet cell proliferation that accompanies allergic responses. Several animal studies report increased airway resistance and BC due to inhalation exposures to formaldehyde (Nielsen et al., 1999; Swiecichowski et al., 1993; Biagini et al., 1989; Amdur, 1960). Changes in pulmonary resistance were observed as early as 10 minutes after exposure (Biagini et al., 1989), and reported effect levels ranged from 0.3 to 13 ppm. BHR is commonly associated with allergic Type I hypersensitivity reactions but is not sufficient to demonstrate that an agent induces Type 1 hypersensitivity.

MOA

The MOA underlying this response has not been elucidated. Formaldehyde-induced IgE production has been reported in some studies (Vandenplas et al., 2004; Wantke et al., 1996a). Other studies suggest that this effect does not appear to be immunogenic in nature (Fujimaki et al., 2004; Lee et al., 1984). Although formaldehyde exposure has been reported to alter cytokine levels and immunoglobulins in some experimental systems (Fujimaki et al., 2004a; Ohtsuka et al., 2003), these immunomodulatory effects do not support immunogenically mediated type 1 hypersensitivity.

These decrements may be mediated via neurogenic potentiation (Sadakane et al., 2002; Riedel et al., 1996; Tarkowski and Gorski, 1995). Tarkowski and Gorski (1995) suggest that formaldehyde may increase permeability of respiratory epithelium and destruction of immunologic barriers. Tachykinin NK1 receptor and various neuropeptides (NGF and substance P) have been implicated in formaldehyde-induced sensitization and lend weight of evidence to a neurogenic MOA (Van Schoor et al., 2000; Ito et al. 1996).

4.4.4. Upper Respiratory Tract Histopathology

Several studies in occupational workers have reported increased squamous cell metaplasia and reduced mucociliary clearance in nasal and buccal swabs from humans occupationally exposed to formaldehyde (Holmström et al., 1989; Holmström and Wilhelmsson, 1988). Evidence of genotoxic effects include increased MNs and CAs in nasal and buccal epithelial cells from both workers and students exposed to formaldehyde (Ying et al., 1997;

Titenko-Holland et al., 1996; Suruda et al., 1993) and suggest a potential association between genotoxicity and altered histopathology.

Numerous animal experimental studies in multiple strains of rats, mice, hamsters, rabbits, and monkeys describe formaldehyde-induced URT pathology (Fló-Neyret et al., 2001; Roemer et al., 1993; Reuzel et al., 1990; Monticello et al., 1989; Zwart et al., 1988; Wilmer et al., 1987; Morgan et al., 1986b, 1983; Swenberg et al., 1986; Buckley et al., 1984). Effects are first observed in the anterior respiratory mucosa and progress through the nasal passages with increasing exposure concentration and time. The first observed effect includes damage to the mucociliary apparatus of the nasal passages in response to formaldehyde. Studies conducted both in vivo and in vitro demonstrate that formaldehyde disrupts mucus flow and ciliary beat that are dependent on concentration and duration of exposure. Mucociliary apparatus deficits have been recorded even after 18 hours of recovery following formaldehyde exposure. The breakdown of the mucociliary apparatus may allow for increased infection and allow the underlying epithelium to come into contact with exogenous chemicals.

Formaldehyde is highly reactive and may impact all cells in the nasal mucosa, including epithelial cells (ciliated, columnar, and cuboidal), goblet cells, sensory neurons, and intraepithelial lymphocytes. The histologic changes of these processes have been described in all laboratory animals examined and progress from the anterior nares to the posterior regions of the nasal passages, including the ETs and olfactory epithelium if the concentration and duration of exposure are great enough.

Humans and nonhuman primates have significantly less complex nasal passages than rodents. Formaldehyde has lower peak flux in human nasal tissues compared with rodents, which are obligate nose breathers, but will penetrate more deeply into the human respiratory tract than in rodents, since humans lack the autonomic RA response. Additionally, humans may switch to mouth breathing in the presence of an irritant gas, thus bypassing the sensitive nasal passages and increasing the tissue dose in the mouth and throat. These differences have been demonstrated by using nonhuman primates where, at comparable concentrations, tissue pathology and increased cell proliferation progressed further into the respiratory tract than in rodents (Monticello et al., 1989). Nonhuman primates share common structural respiratory components and patterns of breathing and do not have a reflex autonomic apnea response.

Despite the anatomical and physiological differences in breathing patterns and different exposure parameters between humans and rodents, similar toxic effects are reported in tissues at the POE in humans and laboratory animals. Several occupational studies have reported increased squamous cell metaplasia in nasal and buccal samples in response to formaldehyde exposure (Ballarin et al., 1992; Boysen et al., 1990; Holmström et al., 1989), paralleling the

histologic effects seen in experimental animal studies. A few human epidemiology studies suggest increased NPCs (see Section 4.5) as well as oral/buccal tumors in response to formaldehyde exposure (Shangina et al., 2006; Laforest et al., 2000).

The observed formaldehyde-induced URT toxicity is related to its high reactivity and solubility. Moreover, additional interspecies differences in the surface area and configuration of the nasal passages and upper airways will influence the areas of high formaldehyde flux in POE tissues.

MOA

Formaldehyde-induced damage to the mucociliary apparatus of the nasal passages may occur because formaldehyde may disrupt mucus flow and ciliary beat that is dependent on concentration and duration of exposure. Formaldehyde reacts with the mucosal glycoproteins and thus may contribute directly to the breakdown of the mucus layer. As formaldehyde reaches the cells of the pseudostratified epithelium in the nasal passages, it exerts a range of effects from direct damage to cell membrane, intracellular proteins, and DNA to alterations in GSH pools and increased ROS. Adaptive effects include increased mucus flow and goblet cell proliferation as well as the transition of respiratory epithelium to more insensitive cuboidal cells. With continued exposure at sufficient concentration, squamous metaplasia develops, creating a protective layer of keratinized cells. Gradually, this damage exceeds the cell's ability to compensate for and repair damage; chronic nasal lesions develop, and the cells die both through general necrosis as well as programmed cell death, depending on the severity of the cellular damage (Monticello et al., 1989; Swenberg et al., 1983).

Genotoxic effects have been reported in nasal and buccal lesions taken from both workers and students exposed to formaldehyde (Ying et al., 1997; Titenko-Holland et al., 1996; Suruda et al., 1993). MN formation occurs in the more sensitive pseudostratified epithelium of the nasal passages, nasopharynx, and upper airways, since there is only one layer of epithelial cells that are constantly regenerating. However, the genotoxicity observed in buccal cells is more difficult to explain, since buccal basal cells are usually covered by protective keratinized cell layers. Cuts, sores, or other buccal lesions would increase basal epithelial cells' vulnerability to direct exposure to formaldehyde.

4.4.5. Toxicogenomic and Molecular Data That May Inform MOAs

Over the past several years, studies have begun to examine the effects of formaldehyde exposure on gene and protein expression. These include studies on in vivo and in vitro changes in the global expression of mRNA (transcriptomics) and proteins (proteomics) in the tissues and

cells of humans and rodents exposed to formaldehyde. Currently (2009), nine "-omics" studies from five research groups are available. These studies are summarized in Section 5.2 and are evaluated and discussed in the context of their relevance to informing MOAs and the dose-response characterization briefly here.

In 2002, EPA released the *Interim Policy on Genomics* (U.S. EPA, 2002c), which addresses how to use genomic data in regulatory decision making. Although the policy encourages research in genomics, it places limits on its use, stating that genomic data alone are not sufficient as a basis for decision making. These data thus cannot currently be utilized as the critical effect in a chemical risk assessment but can be utilized in a weight-of-evidence approach on a case-by-case basis. The Science Policy Council developed a white paper entitled *Potential Implications of Genomics for Regulatory and Risk Assessment Applications at EPA* (U.S. EPA, 2004). This report described three areas where genomic data might be applied in risk assessment at EPA: MOA analysis, susceptible population, and mixtures assessments. The genomic data on formaldehyde thus may be applied to a discussion of MOA.

Toxicogenomics studies have investigated the gene and protein expression changes resulting from formaldehyde exposure in a variety of respiratory tissues, including nasal tissue (Andersen et al., 2008; Thomas et al., 2007; Hester et al., 2005, 2003), and, in lung tissue (Lee et al., 2008, 2007; Sul et al., 2007; Im et al., 2006) used human tracheal cell lines to study genomic changes after exposure to formaldehyde in vitro. Unfortunately, these studies are not directly comparable because different gene chip technology platforms were used in different tissues, in both in vivo and in vitro study designs. In general, the gene and protein expression changes reflect changes in apoptotic pathway genes, oxidative stress, and tissue remodeling. Andersen et al. (2008) concluded that there was a threshold level where exposure to formaldehyde (6 ppm) does not elicit changes in nasal epithelium of F344 rats. Overall, Andersen et al. (2008) concluded that genomic changes were no more sensitive than tissue responses and that formaldehyde, being an endogenous chemical, is well handled until some threshold is achieved when toxicity rapidly ensues with genomic and histologic changes. At about 6 ppm, this largely involves tissue remodeling (and protection), but regenerative hyperplasia occurs at higher doses. Andersen et al. (2008) conclude that there is a threshold where exposure to formaldehyde does not elicit changes in F344 nasal epithelial tissue over the duration examined in this study (i.e., 15 days). Andersen et al. (2008) argue that this is consistent with bioassays that indicate no tumor formation in rodents below 6 ppm formaldehyde.

The primary conclusion in the Andersen et al. (2008) paper is that genomic changes, including those suggestive of mutagenic effects, did not temporally precede or occur at lower doses than phenotypic changes in the tissue. The authors stated as follows:

"The genomic signatures related to these transitions are for cell membrane and extracellular components, then inflammation and cellular stress, and eventually apoptosis. Importantly, these hierarchical models indicate that the tissue responses at low dose concentrations are qualitatively different from those at high concentrations and linear extrapolations or extrapolations that specify similar modes of action at high and low doses would be inappropriate."

4.4.6. Noncancer Modes of Actions

Noncancer health effects of interest span numerous organ systems and include reproductive and developmental effects, neurological/neurobehavioral effects, and a complex interaction between inflammation and immune and adverse pulmonary function. To date, no -omics studies have examined changes in reproductive tissue or altered gene expression in developing animals. In regard to neurological/behavioral effects, one study (Lu et al. [2008], described in Section 4.1.1.6) has reported elevations in the mRNA for NMDA receptor subunits in brain homogenates following exposure to 2.4 ppm. Hester et al. (2003) reported a significant increase in NMDA receptor subunit transcripts, along with other neuropeptide genes, in nasal tissue. Together, these changes may relate to formaldehyde-induced sensory irritation and, perhaps, changes throughout the brain.

In regard to inflammatory, immune, and pulmonary effects, transcript and protein changes in rodent tracheal tissue and lung tissue indicate that exposure to 3 to 38 ppm formaldehyde results in genes involved in oxidative stress and cell proliferation and may additionally increase airway ADH3 levels (Lee et al., 2008; Sul et al., 2007; Yi et al., 2007; Im et al., 2006; Yang et al., 2005). Together, these data provide evidence for adverse pulmonary effects that may exacerbate or facilitate asthma.

In lung tissue, Yang et al. (2005) identified three proteins up regulated and one protein down regulated following 15 days of exposure to about 28 ppm formaldehyde. None of the proteins corresponded with transcript changes reported by Sul et al. (2007). Interestingly, Sul et al. (2007) reported that only two transcripts were significantly up regulated in the lung in response to 5–10 ppm formaldehyde, while 19 were down regulated. In this regard, it is worth considering that changes in proteins may not relate to their regulation but rather to their overall percent composition in a cell (relative to other protein changes) before and after exposure. In addition to transcript changes, Sul and colleagues (2007) reported DNA damage and lipid peroxidation and noted that the observed down regulation of GR would facilitate oxidative stress, while the down regulation of phospholipase A2 (PLA2) might represent a mechanism for mitigating lipid peroxidation. It is worth noting that an increase in either of these genes could also be argued to support similar conclusions (i.e., that GR is up regulated to increase GSH levels and that PLA2 up regulation explains lipid peroxidation); this highlights the problem with

interpreting these data. Nevertheless, these studies indicate adverse effects in the rodent lung in response to 5–30 ppm formaldehyde.

In a hypothesis-driven study by Yi et al. (2007), formaldehyde exposure was shown to increase lung ADH3 levels. Several studies indicate that allergic responses and hyperreactivity are uncoupled and may relate to ADH3 expression and activity. Que et al. (2005) has shown that, in a rodent asthma model, ADH3 knockout mice exhibit similar signs of inflammation but are protected from bronchoconstriction. Lino dos Santos Franco et al. (2006) provided evidence, in rodents, that formaldehyde may induce inflammatory responses (e.g., leukocyte infiltration in the lung) through neurogenic mechanisms but that bronchial tone is mediated by NO. The latter effect is likely to be mediated by S-nitrosoglutathione GSNO and thus influenced by ADH3. Interestingly, the single nucleotide polymorphisms (SNPs) that Wu et al. (2007) reported as linked to other polymorphisms in the promoter region was the one demonstrating protection against asthma. Hedberg et al. (2001) reported that at least one SNP in the promoter region reduced ADH3 expression. Together, these data indicate that reduced ADH3 expression might lower GSNO turnover and bronchial tone, thereby reducing signs of asthma. In this regard, it is conceivable that wheezing and bronchoconstriction are the symptoms that lead to medical intervention and not the inflammation per se. Thus, while ADH3 polymorphisms may not cause asthma, ADH3 polymorphisms may influence hyperresponsivity and the likelihood of asthma diagnosis. This is discussed further in Section 4.6 on susceptible populations. Formaldehyde has been shown to accelerate GSNO breakdown (Staab et al., 2008a; Yi et al., 2007); thus, pulmonary responses to formaldehyde may represent a balance between mechanisms that induce NO (i.e., inflammation) and those that terminate GSNO (i.e., ADH3).

In regard to -omics changes in blood samples, the apparent limited distribution of formaldehyde may suggest that these changes are secondary to effects at sites of contact but could also indicate systemic distribution. As noted elsewhere in this report, bradypnea can induce changes in dosimetry as well as changes in core body temperature and blood gases (hypoxia itself induces hypothermia in rodents, and thus the reduction in minute volume and gas changes may both contribute to hypothermia). These physiological responses (hypothermia and hypoxia) surely induce changes in gene expression. Observed gene and protein changes in the blood following formaldehyde exposure could also relate to irritation and inflammation at sites of contact. In this regard, Im et al. (2006) reported changes in cytokines indicative of Th-2 responses. Altogether, the authors identified 32 proteins altered in the plasma of rats exposed to formaldehyde. Although no coherent mechanisms are apparent from these changes, the authors posited that they could serve as biomarkers for formaldehyde exposure. The concordance of such changes across species remains to be demonstrated.

Li et al. (2007) identified dose-response relationships for six genes in human blood samples that were putatively associated with formaldehyde exposure. Three of these genes are reported to inhibit apoptosis and were posited as supporting in vitro data by Tyihak et al. (2001); however, Li and colleagues (2007) did not report any increase in blood cell count or in Hs 680.Tr cell counts in vitro (i.e., these changes were not phenotypically linked to changes in cell kinetics or hematology). However, these findings are not inconsistent with those of Hester et al. (2003) that indicated no significant increase (or decrease) in nine genes involved in apoptotic pathways. Finally, serum and glucocorticoid-induced protein kinase 1 (SGK1) was elevated in blood samples and was posited to relate to possible inflammatory and immune responses.

4.4.7. Immunotoxicity

Results from studies that evaluated the immunotoxicity of formaldehyde are mixed. For example, most human studies that investigated systemic immune effects by measuring increases in formaldehyde-specific IgE are negative (Doi et al., 2003; Kim et al., 2001; Palcynski et al., 1999; Krakowiak et al., 1998; Wantke et al., 1996; Grammer et al., 1990). Vandenplas et al. (2004) reported a transiently positive increased formaldehyde-specific IgE titer in occupationally exposed workers. In contrast, Thrasher et al. (1990, 1988, 1987) reported positive formaldehyde-specific IgE titers in small (six to eight person) case studies of exposed workers, and Carraro et al. (1997) reported elevated IgE titers in smokers. Grammer et al. (1990) did not report any differences in albumin IgE in formaldehyde-exposed workers compared with controls. In a residential study, Pross et al. (1986) found that formaldehyde insulation in homes had no effect on tested human immunologic parameters.

One study suggests that immune system parameters are perturbed by formaldehyde exposure. Lyapina et al. (2004) reported decreased immune resistance in all 29 workers exposed to formaldehyde. This effect was associated with decreased neutrophil respiratory burst activity. A LOAEL of 700 ppb was established.

Results from animal studies are mixed as to whether formaldehyde causes immunotoxicity. Leach et al. (1983) reported systemic immunomodulation in F344 rats that was attributed to formaldehyde exposure, but the formaldehyde effects on measures of humoral and cell-mediated immunity were not confirmed in B6C3F1 mice (Dean et al., 1984). Jakab et al. (1992) detected no differences in phagocytic ability of alveolar MPs from mice after formaldehyde exposure. Formaldehyde-exposed rats that were injected with pneumococcus antigen or tetanus toxoid produced antibodies in amounts similar to nonexposed, infected control animals (Holmström et al., 1989b).

However, specific immune parameters appear to be affected by formaldehyde exposure. For example, increased host resistance and hydrogen peroxide release from peritoneal MPs were reported and confirmed (Adams et al., 1987; Dean et al., 1984) and suggest a putative role for ROS. Increased host resistance may be mediated by formaldehyde-induced chronic inflammation and respiratory mucosal damage that causes an up regulation in MPs and therefore increases host immunity. Jakab et al. (2002) reported reduced pulmonary bacterial resistance in mice after exposure to formaldehyde, as determined by increased bacterial loading. This result contrasts with Dean et al. (1984) and is attributed to differential exposure regimens and experimental design.

Mode of action

Circulating immune cells present in the mouth and upper airways, such as intraepithelial lymphocytes, direct a local inflammatory response but may also contribute to systemic responses through secreted cytokines and soluble factors released into the bloodstream (Togias, 1999).

Altered host resistance and hydrogen peroxide release from peritoneal MPs were reported and confirmed (Adams et al., 1987; Dean et al., 1984) and suggest a putative role for ROS. Indeed, increased neutrophilic ROS have been associated with formaldehyde-induced dermatitis (Gorski et al., 1992), and, conversely, decreased neutrophil respiratory burst activity has been shown in workers with history of formaldehyde-induced respiratory tract inflammation (Lyapina et al., 2004). Oxidative stress may occur directly as a result of formaldehyde exposure or as a secondary consequence to inflammatory responses.

4.4.8. Effects on the Nervous System

There is considerable evidence that formaldehyde exposure causes adverse effects on the nervous system following inhalation at relatively low exposure levels but little or no information regarding a possible mechanism of action for these effects. Data regarding adverse effects on the nervous system following oral exposure are very limited, reflecting a data gap in this area. Relevant data in animals and humans for several types of neurological endpoints, following inhalation exposure, are summarized below.

4.4.8.1. *Irritant Threshold Detection*

Humans are exquisitely sensitive to the irritant properties of formaldehyde, as has been discussed previously (see Section 4.1.1.1). Animal data confirm the irritant properties of this compound at very low concentrations (Wood and Coleman, 1995).

4.4.8.2. *Behavioral Effects*

Limited data in humans, as well as more robust data in animals, provide evidence of behavioral changes following exposure to formaldehyde at levels as low as 0.1 ppm. Studies in animals have found effects that persist for days to weeks after termination of exposure. In spite of significant limitations, the available human data are consistent with the animal findings.

Several types of behavior have been evaluated in animals following formaldehyde exposures. The most consistent findings, demonstrated by multiple laboratories and in multiple species, have been changes in motor activity, habituation, and learning/memory task performance. Motor activity and habituation have been evaluated under a variety of exposure conditions, using both rats and mice. Consistent decreases in activity have been seen in adult animals (Malek et al., 2004, 2003 a, b; Usanmaz et al., 2002). Senichenkova (1991) and Sheveleva (1971) also found changes in motor activity in offspring following in utero exposure, including decreased habituation in juvenile rats exposed in utero. Decreased performance in learning and/or memory paradigms have been seen in multiple laboratories, also in both rats and mice (Lu et al., 2008; Malek et al., 2003c; Pitten et al., 2000).

Data from controlled human exposures are very limited, but studies have shown decreased performance in addition tasks and reaction time tasks following acute exposures to formaldehyde (Lang et al., 2008; Bach et al., 1990). In contrast, Andersen and Molhave (1983) indicated they found no change in performance on several types of tasks (including addition, multiplication, and card punching) following acute exposure to volunteers, but supporting data were not provided.

Data for humans are also available from epidemiology studies of individuals who were occupationally exposed to formaldehyde. A variety of neurobehavioral deficits, including lack of concentration and loss of memory, disturbed sleep, impaired balance and dexterity, and changes in mood, were identified (Kilburn et al., 1987, 1985). However, most of the individuals evaluated in these studies were also occupationally exposed to other solvents, raising questions regarding possible confounding of the results due to multiple exposures. In addition, the formaldehyde exposure information provided in the studies is not sufficient to permit a reliable dose-response assessment for the effects identified. The types of effects seen in humans in the available epidemiology studies are, however, consistent with those seen in available animal studies.

In general (and noting the differences in exposure paradigms and types of tasks), behavioral effects in animals and humans appear to occur at similar exposure levels. Animal studies demonstrated LOAELs as low as 100 ppb following acute or repeated exposures (Malek et al., 2003b, c); human controlled exposure studies have found effects in that same range, with

LOAELs of approximately 300 ppb following acute exposures (Lang et al., 2008; Bach et al., 1990).

4.4.8.3. *Neurochemistry, Neuropathology, and Mechanistic Studies*

Limited data are available regarding neurochemical and neuropathological sequelae of formaldehyde exposure. Studies from one laboratory (Sorg et al., 2004, 2001) have suggested that behavioral sensitization to formaldehyde is linked to alterations in HPA control of corticosterone and changes in mesolimbic dopamine pathways. Neurochemical changes in response to formaldehyde exposure have also been documented in other laboratories (Fujimaki et al, 2004b; Hayashi et al., 2004). Some of these data appear to be conflicting, and there are no definitive data supporting a specific mechanism for formaldehyde effects on the nervous system at this time. Neuropathological data are also limited, although data from one laboratory (Sarsilmaz et al., 2007; Aslan et al., 2006) suggest a concern for changes in brain structure in neonatal rats following exposure at 6 or 12 ppm. No human data are available that address these endpoints. However, a prospective cohort study of nearly one million people followed for 15 years reported strongly significant dose-response associations between death from ALS and exposure to formaldehyde of a known duration, with longer exposures associated with greater risk (Weisskopf et al., 2009). This large, well-designed prospective cohort study strongly supports the causal association of neuropathological effects in humans following long-term formaldehyde exposure.

4.4.8.4. *Summary*

Overall, there is strong evidence that formaldehyde exposure via inhalation may cause adverse effects on nervous system function in experimental animals at relatively low levels of exposure (LOAELs as low as 100 ppb). Although human data regarding neurotoxicity following formaldehyde inhalation are limited, available data provide support that the types of effects seen in humans are similar to those found in animal studies. Evidence from available human controlled inhalation exposure studies indicates that humans may be affected at doses similar to those used in animal studies; however, the human data are extremely limited.

There are insufficient data to evaluate the potential for neurotoxicity following oral exposure to formaldehyde. Limited evaluations of brain weight or histopathology in available chronic or subchronic oral studies found no evidence of formaldehyde-induced changes (Til et al., 1989, 1988; Tobe et al., 1989; Johannsen et al., 1986). However, reliable studies examining nervous system function or focused studies of neuropathology following oral exposure to formaldehyde are not available.

4.4.8.5. *Data Gaps*

Major data gaps were found regarding the evaluation of changes in nervous system structure or function following formaldehyde exposure by both the inhalation or oral routes.

With respect to inhalation exposure, none of the available human studies resulted in data sufficient to conduct a reliable dose-response assessment for changes in nervous system function. Most of the available animal inhalation studies used short exposure durations (acute or short-term), precluding a reliable evaluation of neurotoxicity following chronic exposure. Available data for neurodevelopmental exposures are also quite limited, consisting of evaluation of neuropathology in only one brain region and functional evaluations focused only on changes in motor activity.

Major data gaps also exist regarding neurotoxicity following oral exposure, with no relevant human data and extremely limited animal data. Available oral exposure studies were insufficient to permit a reliable evaluation of the potential for neurotoxicity following oral exposure to formaldehyde.

4.4.9. Reproductive and Developmental Toxicity

A number of studies have been identified that indicate an effect of formaldehyde exposure on reproductive and developmental outcomes. Human data are described in Section 4.1.1.7, and animal studies are addressed in Section 4.2.1.7 of this document.

4.4.9.1. *Spontaneous Abortion and Fetal Death*

Several epidemiologic studies reported increases in risk of spontaneous abortion following maternal occupational formaldehyde exposure (Taskinen et al., 1999, 1994; John et al., 1994; Seitz and Baron, 1990; Axelsson et al., 1984). While these finding have been questioned (Collins et al., 2001b), upon careful examination, none of the principal biases in epidemiologic studies, including information bias, selection bias, and confounding, appear to be more likely causes of these reported findings than the conclusion that they may reflect an underlying causal process. While each of these occupational studies focused on women who were co-exposed to formaldehyde and other chemicals, the occupational groups were quite different and had different sets of co-exposures. The woodworkers in the Taskinen et al. (1999) study were potentially co-exposed to organic solvents related to painting and lacquering, dusts, and phenols, none of which was shown to be an independent predictor of adverse risk. The cosmetologists studied by John et al. (1994) were co-exposed to hair dyes, bleach, alcohol-based disinfectants, and chemicals specific to services, such as fingernail sculpturing, but, in analyses that were specifically adjusted for other work exposures and their potentially confounding effects, the

investigators reported an increased risk for the use of formaldehyde-based disinfectants. The laboratory workers studied by Axelsson et al. (1984) were potentially co-exposed to a wide range of solvents, but the miscarriage rate was highest among those exposed to formaldehyde. For a potential confounder to entirely explain an observed effect of another exposure, it must be more strongly associated with the adverse outcome. It does not appear that the collective results of formaldehyde exposures associated with increased risk of spontaneous abortion—often in spite of exposures being crudely measured—can be explained by information bias or confounding.

Taken together, these findings are consistent with an adverse effect of formaldehyde exposure on the risk of pregnancy loss. The single study with the strongest quantitative assessment of that risk is Taskinen et al. (1999), and the results presented are of sufficient quality to support quantitative risk assessment by using the LOAEL/NOAEL approach.

This study was a well-designed population-based case-control study that appears to have been well executed and appropriately analyzed. The study population of Finnish women was well defined and adequately selected to allow for meaningful comparisons of health effects between individuals with different levels of exposure to formaldehyde. The participation rate was 64%, which is low enough to raise a concern about the potential for selection bias. However, the authors noted that selection bias has not influenced the results of other reproductive epidemiology studies reporting results on smoking, irregular menstruation, and earlier miscarriages, which are known to lengthen the time to pregnancy (Bolumar et al., 1996; Sallmén et al., 1995; Baird and Wilcox, 1985). Furthermore, there is no evidence to support conjecture that an individual's decision to participate in this study would be differential with respect to their workplace formaldehyde exposures while being nondifferential with respect to the other exposures of interest, including organic solvents, wood dust, and phenols. Since the women who chose to participate in this study were not likely to be aware of the specific hypotheses under investigation nor could they have known the formaldehyde exposures that were independently estimated by an industrial hygienist, selection bias is not a likely explanation for the findings of adversity.

Data on pregnancy history, including spontaneous abortions, were collected by questionnaire. Spontaneous abortion is the most common adverse outcome of pregnancy (Klein et al., 1989), and retrospective self-report of spontaneous abortion has been found to match well with prospectively collected reproductive histories (Wilcox and Horney, 1984). Many spontaneous abortions, however, are missed with self-reporting, with the magnitude likely exceeding 25%, but only rarely do women self-report false positive events (Wilcox and Horney, 1984). The effect of such an undercount is to cause a bias towards the null when the likelihood of undercounting is unrelated to formaldehyde exposure. The implication is that the observed

association of increased risk of spontaneous abortion associated with occupational exposure to formaldehyde may be an underestimation of the true risk.

The findings by Taskinen et al. (1999) of reduced fertility and increased risk of spontaneous abortion are internally consistent and coherent with other reports of increased risk of pregnancy loss associated with exposure to formaldehyde (John et al., 1994; Taskinen et al., 1994; Seitz and Baron, 1990; Axelsson et al., 1984). Absent evidence of alternative explanation for these findings, it is concluded that exposure to formaldehyde is associated with pregnancy loss and diminished fertility.

In animal studies, Sheveleva (1971) noted an increase in preimplantation loss in rats exposed to 0.04 and 0.4 ppm formaldehyde by inhalation on GDs 1–19, and Kitaev et al. (1984) observed evidence of degeneration in harvested embryos on GD 2, following 4 months of maternal inhalation exposure to 0.41 ppm formaldehyde in rats. In a second series of tests reported in Kitaev et al. (1984), female rats were exposed to 0.41 and 1.22 ppm formaldehyde for 4 months to test the hypothesis that the embryo degeneration could have been the result of disrupted reproductive hormone levels in the dams. Ovarian weight and blood levels of LH were increased at 0.41 ppm (but not at 1.22 ppm), and significantly increased levels of FSH were observed at 1.22 ppm. Kitaev et al. (1984) proposed that effects at the 0.41 ppm might be related to disruption of the hypothalamic-pituitary axis and that at the higher exposure level (1.22 ppm) frank toxic effects to the embryo were observed. The increased FSH levels at 1.22 ppm may also be indicative of hormonal perturbations induced by formaldehyde exposure that could affect pregnancy maintenance in humans. The finding of treatment-related increased preimplantation loss in rats appears to support the evidence of spontaneous abortion in the epidemiologic data. In addition, a dominant lethal study in rats by Odeigah (1997) identified significant postimplantation loss following pre-mating I.P. formaldehyde exposures to males, suggesting a potential MOA involving germ cell toxicity. Nevertheless, a number of developmental toxicity studies in rats did not report treatment-related embryolethality following gestation exposures to formaldehyde. These included inhalation studies by Martin (1990), Saillenfait et al. (1989), and Kilburn and Moro (1985), a series of studies by Gofmekler and Bonashevskaya (1969), Gofmekler (1968), and Pushkina et al. (1968), and studies by Senichenkova and Chebotar (1996) and Senichenkova (1991). It is noted, however, that, to the extent that these studies evaluated embryonic or fetal death, the observations were conducted late in gestation and the studies may not have been designed to detect the preimplantation losses as observed in Kitaev et al. (1984) and Sheveleva (1971). Additionally, a number of the reports for these studies did not include sufficient details to engender a high degree of confidence in the reported results. Fetal death was

also not observed in oral studies with formaldehyde in beagle dogs (Hurni and Ohder, 1973) and rats (Seidenberg and Becker, 1987).

4.4.9.2. *Congenital Malformations*

The effect of occupational exposures to formaldehyde on the incidence of congenital malformations was examined by Dulskiene and Gražulevičiene (2005), Taskinen et al. (1994), and Hemminki et al. (1985). Results were mixed.

In animal studies, the most frequently observed structural anomaly noted following inhalation exposures to formaldehyde during gestation was a delay in fetal (i.e., 1st stage) testes descent (Senichenkova and Chebotar, 1996; Senichenkova, 1991; Kilburn and Moro, 1985), although similar findings were not reported by Saillenfait et al. (1989) or Martin (1990) in what appear to be well-conducted prenatal developmental toxicity studies. No study in the available database specifically examined the 2nd stage of postnatal testes descent in pups. Thus, there is no evidence to determine if the observed effect represented a developmental delay or if it was related to disruptions in male reproductive tract ontogeny, which is dependent on normal levels of fetal testicular testosterone and on the expression of insulin-like hormone-3 (insl3) in fetal Leydig cells (Klonisch et al., 2004). Senichenkova (1991) observed an increased incidence of other organ anomalies following formaldehyde exposure during gestation; however, the anomalies are not characterized in the report. Alterations on fetal organ weights and/or size were noted in several studies (Kilburn and Moro, 1985; Gofmekler, 1968), but it is difficult to ascertain if these findings represented agenesis, hypoplasia, or evidence of systemic organ toxicity. Histopathologic evaluation of pup tissues following maternal gestational exposures to 0.01 and 0.81 ppm formaldehyde was conducted by Gofmekler and Bonashevskaya (1969), revealing reduced glycogen content in the myocardium, the presence of iron in hepatic Kupffer cells, and a positive Schiff reaction in the basement membrane (indicating functional alterations in the renal tubule) at both exposure levels.

4.4.9.3. *Low Birth Weight and Growth Retardation*

A population-based study (Gražulevičiene et al., 1998) reported an association between atmospheric formaldehyde exposure and low birth weight, with an adjusted OR of 1.37 (95% CI: 0.90–2.09).

A number of inhalation studies in rats identified reduced fetal weight as an adverse outcome of in utero formaldehyde exposure and are supportive of the association noted in humans. In a study that exposed pregnant rats to formaldehyde during GDs 6–20, Saillenfait et al. (1989) observed significantly decreased male and female fetal rat weights (78 and 81% of

control values, respectively) at 40 ppm formaldehyde. In a study that exposed the dams from GDs 6–15, Martin (1990) found decreased fetal weights at exposure levels of 5 and 10 ppm. In both studies, observations of reduced or delayed skeletal ossification (i.e., the thoracic vertebrae in Saillenfait et al. [1989] and the pubes and ischia bones in Martin [1990]) were consistent with the fetal weight deficits. Kilburn and Moro (1985) also reported fetal body weight decreases in rats at an inhalation exposure level of 30 ppm. Conversely, increased fetal body weight as compared with controls (generally considered to be non-adverse) was noted by Gofmekler (1968) and Pushkina et al. (1968) at maternal formaldehyde exposure levels of 0.1 and 0.81 ppm administered for approximately 2–3 weeks prior to mating and then throughout gestation. Increased fetal weight was also noted in rats by Senichenkova (1991) and Senichenkova and Chebotar (1996) following maternal exposures to 0.41 ppm formaldehyde throughout gestation.

Studies that assessed the effects of oral administration of formaldehyde on development are quite limited. The only oral study identified that found a treatment-related effect on offspring growth was a study using beagle dogs (Hurni and Ohder, 1973). In this study, formaldehyde was administered at doses of 3.1 or 9.4 mg/kg-day in the feed during gestation, and pup weight decrements at postnatal week 8 were 6.3 and 12% in the low- and high-dose groups, respectively.

4.4.9.4. *Functional Developmental Outcomes (Developmental Neurotoxicity)*

Indications of effects on the developing nervous system were observed in several rodent studies, although no similar epidemiologic findings in children were identified. These studies (Sarsilmaz et al., 2007; Aslan et al., 2006; Weiler and Apfelbach, 1992; Senichenkova, 1991; Sheveleva, 1971) are described in detail in Section 4.2.1.6. In the studies by Aslan et al. (2006) and Sarsilmaz et al. (2007), neonatal rats were exposed to formaldehyde for 30 days at 6,000 or 12,000 ppb. Decreases in the volume of discrete areas of the brain were observed at both exposure levels in both studies, and, additionally, decreased cell numbers were noted in a region of the hippocampus in the Sarsilmaz et al. (2007) study. Weiler and Apfelbach (1992) exposed juvenile rats to 0.25 ppm formaldehyde for 130 days or adult rats to 0.5 ppm formaldehyde for 90 days. Olfactory thresholds measured in this study were significantly higher in the rats that had been exposed as juveniles than in those that had been exposed only as adults. Sheveleva (1971) observed alterations in spontaneous mobility in 1- and 2-month-old pups from dams that had been exposed to formaldehyde at 0.04 or 0.4 ppm throughout gestation. In the Senichenkova (1991) study, maternal rats were exposed to 400 ppb formaldehyde during GDs 1–19, and functional observational testing was conducted on the juvenile offspring. Changes in open-field motor activity, exploratory activity, and habituation were observed in the offspring.

4.4.9.5. *Male Reproductive Toxicity*

A number of laboratory animal studies have reported effects of formaldehyde exposure on male reproductive system endpoints. These effects include decreased testes weight, changes in Leydig cell quantity and quality, degeneration of seminiferous tubules, decreased testosterone levels, alterations in biomarkers of toxicity in the testes, and alterations in sperm measures (Galilapour et al., 2007; Xing et al., 2007; Zhou et al., 2006; Özen et al., 2005, 2002; Sarsilmaz et al., 1999; Odeigah, 1997; Majumder and Kumar, 1995; Chowdhury et al., 1992; Til et al., 1989, 1988; Tobe et al., 1989; Johanssen et al., 1986; Maronpot et al., 1986; Cassidy et al., 1983; Appelman et al., 1982; Guseva, 1972). Following concurrent exposures to formaldehyde in air and drinking water for 6 months, Guseva (1972) found decreases in testicular nucleic acid levels. In a study conducted by Chowdhury et al. (1992), rats were administered I.P. injections of formaldehyde for 30 days, and evidence of decreased testes weight, serum testosterone levels, and Leydig cell quality was observed. Sarsilmaz et al. (1999) followed up on these findings (exposing male rats to formaldehyde via inhalation at 10 and 20 ppm for 4 weeks) and found decreases in Leydig cell quantity and the percentage of cells with normal nuclei. Hypothesizing that the reported decreases in Leydig cell quality may have been the result of oxidative stress and damage, Özen et al. (2002) evaluated biomarkers of such changes and found that testicular zinc and copper levels were decreased and iron levels were increased following exposures of adult male rats to 10 and 20 ppm formaldehyde for 4 or 13 weeks. Additionally, relative testes weight was decreased in a dose- and duration-dependent manner, although this effect had not been observed by Sarsilmaz et al. (1999). Özen et al. (2005) noted decreased serum testosterone levels, decreased seminiferous tubule diameters, and increased levels of heat shock protein in spermatogonia, spermatocytes, and spermatids of rats following 91 days of exposure to 10 ppm formaldehyde. A study by Golalipour et al. (2007) observed decreased numbers of testicular germ cells, altered sprmatogenesis, and reduced seminiferous tubular diameter and epithelial height in rats following 18 weeks of formaldehyde inhalation exposure; the severity of the seminiferous tubule pathology was positively correlated to the number of hours/week of exposure. Zhou et al. (2006) found decreased testis weight, atrophy of seminiferous tubules, edematous interstitial tissue, and alteration of epididymal sperm count, morphology, and motility in rats after 2 weeks of formaldehyde exposure at 8 ppm. Abnormal sperm were also observed in mice by Xing et al. (2007) after 13-weeks of inhalation exposure at 16.9 ppm, and Cassidy et al. (1983) reported sperm abnormalities in rats following a single oral dose of 200 mg/kg. Additionally, Majumder and Kumar (1995) observed significantly reduced sperm count, motility, and viability following 30 days of I.P. injection of 10 mg/kg-day formaldehyde to male rats. Also in this study, the ability of formaldehyde to affect sperm parameters was confirmed with in

vitro testing. A study conducted by Odeigah (1997) demonstrated epididymal sperm count and morphology abnormalities following five I.P. doses of ≥0.125 mg/kg formaldehyde and additionally identified dominant lethal effects (decreased live embryos and increased dead implants) following mating of treated male rats with untreated females.

Although Til et al. (1989) reported low incidences of Leydig cell tumors in formaldehyde-treated rats in a chronic drinking water study, no alterations in testes weight or histopathologic lesions of the testes were observed in subchronic inhalation studies conducted by Appleman et al. (1982) or Maronpot et al. (1986) or in subchronic or chronic oral studies by Johanssen et al. (1986), Til et al. (1988), or Tobe et al. (1989).

No epidemiologic studies have identified an association between formaldehyde exposure and alterations in the male reproductive system (e.g., see Ward et al. [1984]).

4.4.9.6. *Female Reproductive Toxicity*

The available database for the assessment of the effects of formaldehyde exposure on the female reproductive system was limited. In addition to the findings of spontaneous abortions, as described above, Taskinen et al. (1999) examined fecundability in a cohort of healthy female wood-processing industry workers and found that conception was significantly delayed in women who were occupationally exposed to formaldehyde. The FDR, a ratio of average incidence densities of pregnancies for exposed female employees compared with unexposed female employees, was lower in women exposed to mean formaldehyde levels of approximately 0.33 ppm (range: 0.15–1.00 ppm) compared with controls (adjusted FDR = 0.64 [95% CI: 0.28-0.92]). An FDR <1.0 is indicative of delayed conception, which is an indicator of reduced fertility. The subfertility observed in this study is supportive of the association observed between formaldehyde exposure and spontaneous abortion, since subclinical pregnancy losses are increased in women with compromised fertility (Gray and Wu, 2000; Hakim et al., 1995), and both spontaneous abortion and subfertility can be related to exposure to environmental toxicants (Correa et al., 1996).

As described above, formaldehyde exposures to female rats resulted in decreased ovarian weight and altered LH and FSH levels (Kitaev et al., 1984). Maronpot et al. (1986) reported endometrial hypoplasia and lack of ovarian luteal tissue in female mice exposed to 40 ppm formaldehyde for 13 weeks. Additionally, it is noted that, in developmental toxicity studies that included repeated exposures of dams before mating and/or during gestation, reports of adverse pregnancy outcomes were few. Gofmekler (1968) reported an increase in pregnancy duration and decrease in litter size; however, this finding was not observed in other studies.

With the exception of spontaneous abortion and increased time to pregnancy, associations of formaldehyde exposure with adverse female reproductive system outcomes were not observed in the available epidemiologic data.

4.4.9.7. *Mode of Action*

A strong case cannot be made for any one MOA that explains one or more of the reproductive and developmental outcomes observed in formaldehyde epidemiologic or toxicology studies. This is due to a number of issues, including the following: (1) inconsistencies in study findings for the toxicology studies, which may be explained by study quality issues (see detailed descriptions of studies in Sections 4.1 and 4.2); (2) few studies that allow for comparisons because no study was performed with the same study design (e.g., stage of exposure, dose, species, and strain); (3) few mechanistic studies available to test hypothesized MOAs; and (4) a bias that is pervasive in the formaldehyde literature that outcomes observed beyond the POE (the nose) are not expected from inhalation exposure, which is the route of exposure for most of the developmental and reproductive studies. This discussion presents putative MOAs that have been hypothesized by study authors and the studies that support the hypothesized MOAs. The four hypothesized MOAs are not mutually exclusive. They could be acting alone for certain endpoints (in which case the others are not operable) or in concert for certain endpoints.

The focus of this discussion is on analyzing possible MOAs for the developmental and reproductive outcomes that were noted most consistently, across toxicology studies and, in some cases, across human and animal studies. These outcomes include developmental delays, fetal loss, and sperm quality and quantity effects.

An endocrine-disrupting MOA is supported by some of the reproductive and developmental epidemiology and toxicology studies. For example, the decreases in fetal body weight (Martin, 1990; Saillenfait et al., 1989), delayed ossifications (Senichenkova and Chebotar, 1996; Senichenkova, 1991; Martin, 1990; Saillenfait et al., 1989), and delayed eruption of incisors (Senichenkova, 1991) noted in rats after gestational exposure to formaldehyde are consistent with developmental delays. In turn, developmental delays can result from effects on the hypothalamic-gonadal-pituitary axis in the dam that cause hormonal level changes in the pup; however, hormone levels in pups were not measured. Kilburn and Moro (1985) also observed organ size changes and undescended testes after developmental formaldehyde exposure. These outcomes can also be explained by an endocrine MOA. There are three studies that directly tested for changes in hormones after formaldehyde exposure. Kitaev et al. (1984) observed ovarian weight and serum LH and FSH increases after inhaled

formaldehyde in adult female rats. Chowdhury et al. (1992) assessed serum testosterone levels in adult rats after formaldehyde IP exposure and found significant decreased testosterone and testes weights and a decrease in 3-β,Δ-5-hydroxy steroid dehydrogenase in Leydig cells, suggesting that formaldehyde affects steroidogenesis. Özen et al. (2002) also reported significant serum testosterone level decreases as well as decreased mean seminiferous tubule diameters. Furthermore, the steroidogenesis MOA leading to reduced testosterone is also consistent with the sperm quality and quantity decrements observed in the studies by Özen et al. (2002), Sarsilmaz et al. (1999), and Odeigah (1997) studies.

In human studies, delayed time to pregnancy and increased incidence of spontaneous abortion (Taskinen et al., 1999), consistent with some study findings from the toxicology literature, could also be explained by an endocrine MOA. Alterations in hormone levels could lead to pregnancy maintenance problems. Extrapolating the Chowdhury et al. (1992) results of the steroidogenesis MOA to females, formaldehyde exposure could affect progesterone levels required for pregnancy. However, progesterone levels were unchanged in the female rat in the one study that assessed progesterone (Kitaev et al., 1984). Consistent with an endocrine mediated MOA, Maronpot et al. (1986) observed endometrial hypoplasia and lack of ovarian luteal tissue in females exposed to formaldehyde.

A second hypothesized MOA for some of the developmental and reproductive outcomes is genotoxicity of the gametes. Such an MOA could explain pregnancy loss in humans (Taskinen, et al., 1999) and preimplantation loss in animal studies (Xing et al., 2007; Kitaev et al., 1984; Sheveleva, 1971) and fetal viability (e.g., litter size decreases) after formaldehyde exposure. Consistent with male gamete genotoxicity, Odeigah (1997) and Xing et al. (2007) observed reduced fertile matings and live embryos, and increased dead implants in a dominant lethal study.

Oxidative stress/damage is another MOA that is consistent with testicular toxicity, sperm effects, and reduced embryo viability. Özen et al. (2002) investigated the mechanism of oxidative stress being responsible for the testes quality effects by assessing testicular iron, copper, and zinc levels. Zinc and copper levels were reduced in the rat testes, consistent with an oxidative stress MOA. Özen et al. (2002) also reported increased iron levels and decreased zinc levels in the lung, consistent with oxidative stress. Another study (Zhou et al., 2006) that investigated the oxidative stress MOA in the testes observed significant changes in oxidative stress biochemical markers (decreases in SOD, GPX, GSH, and an increase in MDA levels). The authors also assessed the protective effect of coadministration with vitamin E, an antioxidant, on decreased testes weight, biochemical alterations, histopathologic effects, and on sperm count, motility, and morphology. The study of Pushkina et al. (1968) found reduced

levels of Vitamin C, another antioxidant, in the fetus and maternal liver after formaldehyde exposure.

The MOAs proposed are not mutually exclusive and in fact could interact with one another. For example, an endocrine MOA could lead to oxidative stress, and that oxidative stress could lead to genotoxicity.

4.4.9.8. *Data Gaps*

The inhalation developmental toxicity studies conducted on formaldehyde and described in Section 4.2.1.7 comprise an adequate assessment of prenatal developmental toxicity for application to inhalation reference concentration determination. The assessments of postnatal developmental toxicity and of reproductive function following inhalation of formaldehyde are less complete. It is notable that, although the database contains some studies that assess various aspects of reproductive organ system toxicity, particularly in males, there is no assessment of multigenerational reproductive function, such as would be evaluated in a two-generation reproductive toxicity study, nor is there an assessment of potential reproductive effects of formaldehyde exposure in human males.

Adequate assessments of developmental and reproductive toxicity following oral exposures to formaldehyde have not been conducted.

4.5. SYNTHESIS AND EVALUATION OF CARCINOGENICITY

4.5.1. Cancers of the Respiratory Tract

Epidemiologic studies of formaldehyde-exposed workers provide sufficient evidence of a causal association between formaldehyde exposure and upper respiratory tract (URT) cancers (e.g., nasopharyngeal cancer (NPC; Section 4.1.2.1.1), nasal and paranasal cancers (Section 4.1.2.1.2), and other upper respiratory tract cancers (Section 4.1.2.1.3)). In addition, the observational evidence from epidemiologic studies reporting associations between formaldehyde exposure and increased risk of NPC supports a conclusion of a causal association for this specific cancer. However, epidemiologic studies of rare outcomes such as NPC, which has an expected incidence of 1 per 100,000 people per year in the United States, do not typically have great statistical power to rule out the null hypothesis (i.e. no association). However, the weight of evidence of the several studies reviewed in Section 4.1.2.1.1 provide an accumulation of consistent observational evidence sufficient to exclude chance as an explanation for the association. Additionally, there is insufficient evidence of consistent confounding or other bias across the studies that were considered; thus, confounding and bias were also ruled out as explanations for the observed association. The lack of a convincing and consistent alternative

hypothesis of causation − in spite of repeated examinations − further supports the conclusion that the association between formaldehyde exposure and increased risk of NPC is causal.

The single strongest cohort study, Hauptmann et al. (2004), shows a statistically significant exposure-response relationship between formaldehyde exposure and URT cancers. Hauptmann et al. (2004) demonstrated significant excess risk of NPC in exposed workers based on U.S. population death rates (standardized mortality ratio [SMR] = 2.1; 95% confidence index [CI] 1.05–4.21) in a large cohort of 25,619 industrial workers. In addition to the SMR based on an external comparison population, relative risks (RRs) were presented based on internal comparisons of workers in order to minimize potential selection bias due to the well known healthy worker effect. Statistically significant exposure-response relationships between increased formaldehyde exposure and increased risk of NPC were reported for two different metrics of exposure (peak and cumulative exposure). Relative risks for NPC were also elevated for increased duration of exposure to formaldehyde and for the average intensity of exposure. These analyses controlled for potential confounders including calendar year, age, sex, race, and pay category. While exposure measurement error is likely to be present in any epidemiologic study, there was no evidence of any differential measurement error that could have produced the observation of a spurious association. Any non-differential measurement error would likely have attenuated the effect of formaldehyde was smaller than that which would otherwise have been observed in the absence of measurement error.

The case-control studies similarly also report associations between formaldehyde exposure and cancer mortality for NPC. Although other risk factors for NPC (e.g., Epstein-Barr Virus) and the predominant NPC histological sub-type (SCC versus undifferentiated) vary significantly across the world, case-control studies consistently provide evidence of an association between occupational exposure to formaldehyde and NPC (Vaughn et al., 1986a; Vaughn et al., 2000; Roush et al., 1987; Hildesheim et al., 2001; West et al., 1993). In their more recent study, Vaughn et al. (2000) used worker histories to estimate each individual worker's formaldehyde exposure. Workers with more than 1.10 ppm-years of cumulative exposure were found to be at significantly higher risk of NPC, with an odds ratio (OR) of 3.0 (95% CI 1.3-6.6) (Vaughn et al., 2000). Two different exposure metrics, duration of exposure and cumulative exposure, were positively associated with increased risk of NPC, with a significant test for trend (p = 0.014 and 0.033, respectively). The OR also increased in magnitude as the probability of "Ever" having occupational exposure increased, from OR = 1.6 among those whose exposure was judged to be "Possible, probable or definite" to OR = 13.3 among those with "Definite" exposure (p–trend < 0.001).

NPC histological sub-type analysis indicates that these associations held for both SCC and epithelial NPC, but not for the undifferentiating and nonkeritinizing NPC (Vaughn et al., 2000). However, formaldehyde exposure is also associated with risk of NPC in Taipei, Taiwan, where greater than 90% of the cases had nonkeritinizing and undifferentiated carcinomas and less than 10% of the cases were diagnosed as having SCCs (Hildesheim et al., 2001). These reported associations were strengthened by considering higher probability of exposure (RR = 2.6; 95% CI 1.1-6.3), greater intensity of exposure (RR = 2.1; 95% CI 1-4.2) and EBV seropositive cases (RR = 2.7; 95% CI 1.2-5.9) (Hildesheim et al., 2001). Case-control studies have also linked residential exposure to NPC, specifically for years of residence in mobile homes (Vaughn et al., 1986b) and the use of mosquito coils in the Philippines (West et al., 1993). Independent testing of 6 brands of East Asian mosquito coils evaluated the emission rates of carbonyl compounds in the mosquito smoke and reported that formaldehyde and acetaldehyde had the highest emission rates. Among the three experiments on each of the six brands, the range of formaldehyde concentrations was from 0.87 $\mu g/m^3$ (1 ppb) to 25 $\mu g/m^3$ (31 ppb).

As a group, other URT sites of direct contact with formaldehyde upon inhalation (i.e., salivary gland, mouth, nasal cavity and larynx) also showed evidence of a trend in increasing relative risks with increasing average intensity and peak exposure in the Hauptmann et al. (2004) cohort study, although these trends did not reach the level of statistical significance. The results from other cohort studies and case-control studies are mixed (between positive associations and null findings) for associations between formaldehyde exposure and specific cancers of the URT (IARC, 2006). For rare cancers, extremely large cohorts would be needed to have the statistical power to detect an association for tumors defined by individual sites (e.g., mouth, salivary gland, hypopharnyx). Results vary in the smaller cohort studies, where a single case may result in an elevated risk but taken together the evidence is considered suggestive (Section 4.1.2). Case-control studies have been useful to better understand potential associations between formaldehyde exposure and rare cancers of the URT. Luce et al. (2002) evaluated pooled data from 12 case-control studies and demonstrated a statistically significant increased risk between formaldehyde exposure and sinonasal cancer. A case-control study by Gustavsson et al. (1998) suggested an association between formaldehyde exposure and oral squamous cell carcinoma (SCC), esophageal, and laryngeal cancers, with odds ratios (ORs) of 1.28, 1.90, and 1.45, respectively. However, the individual ORs were not statistically significant. Hypopharyngeal cancer was linked with formaldehyde exposure with an OR of 3.78 (95% CI 1.50-9.49) in another case-control study (Laforest et al., 2000). While the data on site-specific cancers of the URT is somewhat sparse, they are consistent with a carcinogenic hypothesis and in their large cohort study, Hauptmann and colleagues (2004) concluded that in spite of the small numbers of

deaths from cancers of the URT, the positive associations with average intensity and peak exposure were consistent with the carcinogenicity of formaldehyde at these sites of first contact.

Supporting animal evidence

Inhalation exposure:

Animal studies, primarily rodent bioassays, strongly support the causal relationship between of formaldehyde exposure and URT carcinogenicity. Formaldehyde-induced cancers are primarily seen in the nasal passages (Kerns et al., 1983; Monticello et al., 1996; Tobe at al., 1985; Kamata et al, 1997; Sellakumar et al., 1985), but it should be noted that rodents, unlike humans, are obligate nose breathers and have convoluted nasal turbinates. Chronic animal studies (inhalation and oral exposures) report tumor incidence in a variety of rodent models. Study descriptions are provided above in detail (Section 4.2.1, Table 4-34). The study results are evaluated here for both routes of exposure in the context of how they inform the carcinogenic potential at the portal of entry, specifically the URT.

In rodent studies of the respiratory tract, only nasal tumors are considered to be induced by formaldehyde. Repeated exposures to 10-15 ppm formaldehyde result in gross nasal lesions and high incidence of nasal tumors (See Table 4-38, Section 4.2.1). Although increased cell proliferation, squamous metaplasia, dysplasia and focal necrotic lesions have been noted in the larynx and trachea in some studies, no tumors in these locations have been reported in the rodent studies. The majority of studies were conducted using rats (F344, Wistar, or Sprague-Dawley), and all studies of 18 months or greater in mice and rats show evidence of formaldehyde-induced nasal carcinogenicity. The nasal tumors are primarily SCCs, although papillomas, polypoid adenomas, adenocarcinomas, fibrosarcomas, and esthesioneuroepitheliomas have been reported (Kamata et al., 1997; Monticello et al., 1996; Morgan et al., 1986a, b; Takahashi et al., 1986; Sellakumar et al., 1985; Kerns et al., 1983; Albert et al., 1982). Although hyperplasia, dysplasia, and squamous metaplasia of the respiratory epithelium have been observed beyond the nasal cavity, other respiratory tract tumors have not been reported to be significantly increased by formaldehyde exposure alone.

Increased tumor incidence and decreased latency are correlated with increasing formaldehyde exposure concentration. Reviewing data from the only lifelong inhalation study (i.e., until "natural death") with multiple exposure groups, nasal SCCs occurred much earlier in the high-exposure animals. For example, tumors are first noted at 8 and 9 months following exposure for high-exposed (15 ppm) female and male F344 rats versus tumors not arising until 24 months in low-exposed rats (2 ppm) (Kerns et al., 1983). In a follow-up study by Monticello et al. (1996), the incidence of SCC in rats exposed at 15 ppm was 47%, with the first tumor noted

at 12 months. The incidence of SCC in male rats exposed at 10 ppm was 22%, with the first tumors observed at 18 months after exposure. Moreover, of the 90 rats exposed at 6 ppm for 20 months, only one SCC was noted. No SCCs were detected in rats exposed to 0.7 or 2 ppm formaldehyde. These incidence rates are not mortality-adjusted (see Chapter 5, section 5.3.4) and include animals from each scheduled sacrifice (3, 6, 12, and 18 months). In a lifelong study of male Sprague-Dawley rats exposed to 10 ppm formaldehyde, the cumulative nasal tumor incidence was calculated as a function of time of exposure (Sellakumar et al., 1985). After 2 years of exposure, the adjusted probability of nasal carcinoma was greater than 60%.

There is some evidence that less-than-lifetime exposure to formaldehyde can induce nasal tumors over an extended observation period. Two studies, both in male Wistar rats, report nasal tumors in response to less-than-lifetime exposures (Woutersen et al., 1989; Feron et al., 1988). A 13-week exposure at 20 ppm followed by an observation period of 30 months (inclusive of exposure) in Wistar rats resulted in six nasal tumors including three nasal SCCs, one cystic SCC of the nasolacrimal duct, one carcinoma in situ and an ameloblastoma, while no tumors were noted in the corresponding air-exposed controls (Feron et al., 1988). A limited number of formaldehyde-related tumors were noted from 4 or 8 weeks of exposure followed by 30 months of observation. Although the tumor incidence of these less-than-lifetime exposures is low, this is consistent with the 2-year bioassays in Wistar rats. Wistar rats are more resilient to formaldehyde-induced nasal toxicity than F344 or SD rats (Section 4.2.1), and only 1 of 26 (4%) Wistar rats exposed at 10 ppm for 28 months developed SCC (Woutersen et al., 1989) versus 22% in F344 rats (Monticello et al., 1996).

The specificity of formaldehyde-induced tumors in the nasal passages of rodents is believed, at least in part, to be a function of tissue dose. Computational fluid dynamics (CFD) modeling used to predict formaldehyde tissue flux during inhalation exposures suggests that at comparable concentrations, tissue flux in the nasal passages of rodents is more intense than for non-human primates and humans. Modeling predicts a different pattern of formaldehyde flux into URT tissues of rodents compared to humans, where formaldehyde penetrates more deeply into the respiratory tract of primates than rodents even considering nose-only breathing for primates (See Section 3.4). Humans will generally switch to mouth breathing when sensing an irritating smell and during physical exertion, resulting in direct exposures to the mouth and greater tissue flux in tissues beyond the bypassed nasal passages. Therefore, species differences in tissue dose may contribute to formaldehyde-induced tumors in humans beyond the nasal passages, which are not evident in rodent bioassays.

Oral Exposure:

Consistent with the observed carcinogenic activity of formaldehyde on tissues at the portal of entry (POE) from inhalation exposure, there is evidence to support POE effects from oral exposures as well – further strengthening the overall weight of evidence of formaldehyde's carcinogenicity. As with the respiratory tract, the proximal portion of the gastrointestinal (GI) tract exhibits formaldehyde-induced lesions specifically in the forestomach and glandular stomach (Soffritti et al., 1989; Til et al., 1989; Tobe et al., 1989; Takahashi et al., 1986). However, data are mixed regarding the carcinogenic potential of formaldehyde in the GI tract from oral exposures.

Two independent 2-year cancer bioassays in Wistar rats exposed to formaldehyde in drinking water were both negative (Til et al., 1989; Tobe et al., 1989). Til et al. (1989) exposed rats to a range of formaldehyde doses (0, 1.2, 15, or 82 mg/kg-day) and evaluated 44–49 animals per sex per dose group at 24 months of exposure. No formaldehyde-related tumors were found. A smaller study by Tobe et al. (1989) failed to note any tumors after a 2-year exposure at 0, 10, 50 or 300 mg/kg-day (eight rats per sex per treatment group.)

In contrast, two studies that included lifelong observation in male and female Sprague-Dawley rats provide support for formaldehyde-induced GI tract tumors – one study where exposure commenced at 7 weeks of age and a second study conducted with breeder rats (25 weeks of age) and their offspring (Soffritti et al., 1989). These studies demonstrate an increase in GI tumors (although rare) correlated with exposure to formaldehyde and significantly increased susceptibility to early-lifetime exposure. The authors provide a detailed report on the background rates of various stomach and intestinal neoplasia for male (n = 2,677) and female (n = 2,582) rats within the colony (Soffritti et al., 1989). From this background pool, the total incidence of benign and malignant tumors in the stomach and intestine combined is only 1.4% (combining all sites and locations), with the majority of tumors located in the stomach (1% benign, 0.2% malignant). In comparison to colony-specific background rates, apparent increases in both stomach and intestinal neoplasia are noted in formaldehyde-treated rats (ranging from 1 to 6% by type in rats exposed beginning week 7). When summed across the GI tract, tumor incidence in the highest treatment group was 8% versus 1.4% in historical controls. Elevations of individual tumors or a clear dose-response relationship are difficult to discern for rare cancers where there are only 50 animals per group. Despite the limitations of group size and lack of a dose–response relationship, the findings do support the carcinogenic potential for formaldehyde administered orally.

The second study reported by Soffritti et al. (1989) in Sprague-Dawley rats demonstrates early lifetime susceptibility, with GI tumor incidences of 21.6% in female (n = 37) and 13.9% in

male (n = 36) offspring after exposure to formaldehyde beginning *in utero*, versus 5.6% in the adult breeders. Rats were exposed to formaldehyde in drinking water for 2 years (0 or 2,500 mg/L). Exposures began on gestational day 12 in the offspring. The most common tumor detected was intestinal leiomyosarcoma (13.5% in female offspring), which has a background rate of 0.04% in female rats in the colony. The incidence of GI tumors in the adult breeders (n = 18 per sex) was due to one adenocarcinoma in a male rat and one pallipoma/acanthoma in a female rat. Although severely limited by study size, their occurrence is consistent with the observation of formaldehyde-induced tumors, given the low background rates for this colony.

The Soffritti et al. (1989) studies stand alone in observing formaldehyde-induced GI tumors. These findings are largely attributed to a unique study design that included lifelong observation (i.e., until "natural death"), neonatal exposure, examination of individual tumor types as well as combined rare tumor types for analysis, and summation of tumors across locations. The study design results in a more sensitive assay for rare tumors as well as tumors with a long latency. Thus, Soffritti et al. (1989) utilized a more appropriate design and analysis for detecting rare tumors, and these findings are not diminished by the null results of Tobe et al. (1989) and Til et al. (1989).

There is evidence that formaldehyde may act as a tumor promoter by the oral route as well as the inhalation route (discussed above). Takahashi et al. (1986) reported an increase in N-Methyl-N'-Nitro-N-Nitrosoguanidine (MNNG)-initiated GI cancers in mice with formaldehyde exposure (29.4%, versus 13.3% in controls); the greatest difference in tumor response was associated with adenocarcinoma of the glandular stomach (23.5%, versus 3.3% in controls). Additionally, forestomach papillomas and preneoplastic hyperplasia in the glandular stomach were increased with formaldehyde exposure alone.

4.5.2 Lymphohematopoietic Malignancies

4.5.2.1. *Background*

Lymphohematopoietic (LHP) cancers include neoplasms of both lymphoid and myeloid cell origins. Cancers of the immune system are described as leukemia if they primarily involve cells from peripheral blood and bone marrow at diagnosis and lymphomas if they constitute a solid tumor (Robbins, 2004). Some forms of leukemia which present as an immature immune cell phenotype are believed to arise from lymphomyeloid stem cells or progenitor cells normally found in the bone marrow (e.g., acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML)) (Greaves, 2004). However, multiple myeloma, lymphomas and some leukemias may arise from mature functional lymphocytes present outside of the bone marrow (Greaves, 2004; see Figure 4-33). Therefore, the use of the general term ‘leukemia’ is not

restricted to cancers from a transformed stem cell or progenitor cell in the bone marrow but also applies to cancers which arise from differentiated cells (e.g., mature lymphocytes). Epidemiologic studies have reported that formaldehyde exposure is associated with both leukemia and lymphomas (Chapter 4.1.2.2.1). Specific neoplasms reported to be associated with formaldehyde exposure include myeloid leukemia, Hodgkin's lymphoma, and multiple myeloma.

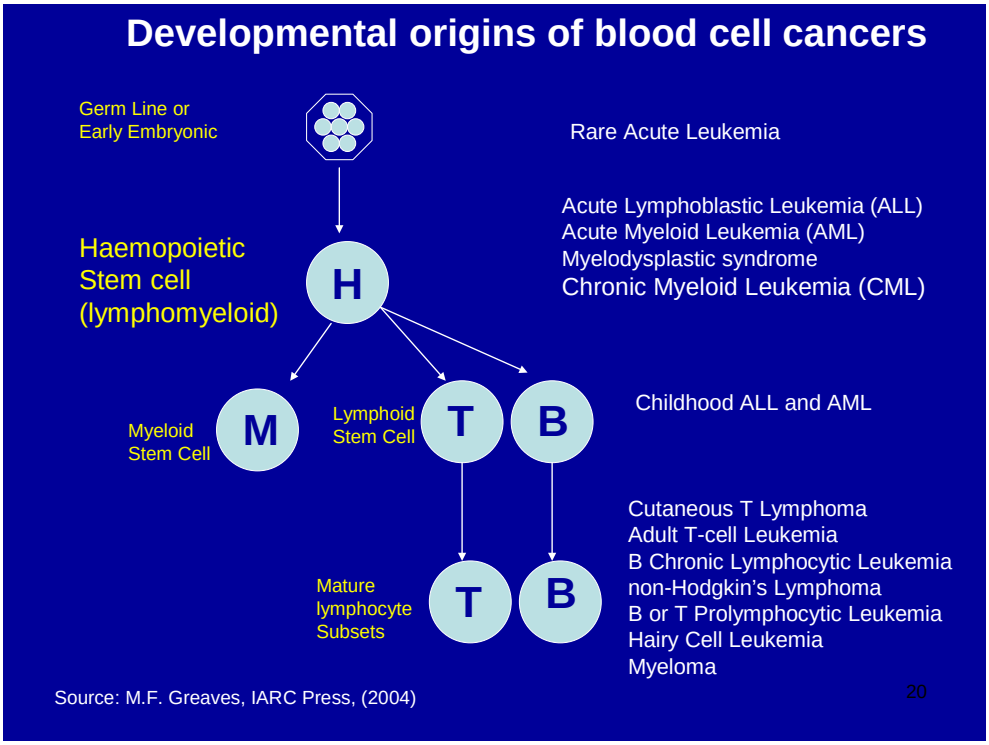


Figure 4-33: Developmental origins for cancers of the lymphohematopoietic system (Adapted from Greaves (2004).

When evaluating cancers of the LHP system, epidemiologic analysis often groups many of these cancers together. In part, this may be done to increase the statistical strength of the analysis. Additionally, since there is a potential for disease misclassification, grouping these diseases is preferred by some researchers, especially when analyzing older mortality data. Historically, misclassification may have been due to factors such as poor histopathology and diagnosis of late-stage disease, where cell line of origin may have been hard to distinguish. Without the cell surface markers and molecular tools used today to classify cells, diagnosis was accomplished primarily based on histology. However, as the cancers progress, the leukemic stem cells may present as less mature cells. For example, poor health surveillance may allow chronic myeloid leukemia (CML) to remain undiagnosed until the blast crisis, often seen late in

the disease. This blast crisis presents as a leukemia of relatively immature myeloid cells and may have been mistaken for AML without the more recent techniques available for classifying disease. Since cancers of the LHP system may present with cell surface markers for multiple cell lines, classification remains problematic in some cases.

Although often grouped for analysis, the LHP cancers represent many distinct malignancies which may arise from discrete cell types in different tissues throughout the body (Greaves, 2004). The World Health Organization (WHO) has developed a classification system for both lymphoid and myeloid leukemia defined by a combination of morphology, immunophenotype, genetic features and clinical features (Harris et al., 2000a; Harris et al., 2000b). The historical nomenclature and International Classification of Disease (ICD) codes used in epidemiologic studies do not correspond to these new classification systems. For example, both chronic lymphocytic leukemia (CLL) and B-cell lymphomas arise from similar cell types in the periphery, yet epidemiologic studies have considered them independently even though they are currently considered to be the same disease in the new WHO classification system − which would diminish the statistical power to detect an association. Careful re-analysis of epidemiologic data addresses evidence for the class as a whole (all LHP cancers) but also various subclasses (e.g. myeloid versus lymphoid).

Therefore, the following analysis first examines the epidemiologic evidence for all LHP cancers as a class, then all leukemia, to best take advantage of the majority of publications available in assessing the weight of evidence for the carcinogenicity of formaldehyde. The subsequent analysis by sub-type draws upon the available evidence for specific diseases or groups of diseases (e.g., myeloid leukemia, Hodgkin's lymphoma, non-Hodgkin's lymphoma). Although fewer data are available for the sub-type analysis, these data help clarify which cancers may contribute to the consistent observation of an association between formaldehyde exposure and all LHP cancers. Novel combinations of phenotypic sub-types are presented where they are etiologically relevant. The sub-type analysis frames the subsequent mode of action (MOA) analysis.

4.5.2.2. *All LHP Malignancies*

Epidemiologic studies involving formaldehyde-exposed workers provide sufficient evidence of a causal association between formaldehyde exposure and all LHP malignancies (Section 4.1.2.1.5). Positive associations between formaldehyde exposure and LHP cancers have been reported for chemical workers (Wong et al., 1983; Bertazzi et al., 1986), embalmers (Walrath and Fraumeni, 1983, Walrath and Fraumeni, 1984; Hayes et al., 1990), anatomists and pathologists (Harrington and Shannon 1975; Hall et al., 1991; Levine at al 1984; Stroup et al., 1986; Matanoski et al., 1989) (Table 4-90). However, clear associations (in terms of overall

standardized mortality ratios [SMRs] or proportional mortality ratios [PMRs]) were not reported in analyses for garment workers, iron-foundry workers, and a large US industrial cohort (Pinkerton et al., 2004; Andjelkovich et al., 1995; Beane Freeman et al., 2009; Marsh et al., 1996), although associations were observed in some of these studies when exposure-response relationships were considered. Several published meta-analyses are available which more formally assess the strength of association between formaldehyde exposure and mortality from all LHP cancers. Pooled SMRs indicate stronger associations for professional workers (embalmers, anatomists and pathologists) than industry workers (Table 4-91). Bosetti et al. (2008) found similar relationships, with a pooled SMR of 1.31 (95% CI 1.16-1.47) for 'professionals' (i.e. embalmers, anatomists and pathologists) versus a pooled estimate of 0.85 (95% CI 0.74-0.96) for industrial workers. A recent meta-analysis by Zhang et al. (2009) reports a summary relative risk (RR) of 1.25 (95% CI 1.09-1.43) for both professional and industry workers for all LHP cancers (ICD 9 codes 200-209). These researchers identified 19 cohort study analyses, including cohort study updates. Zhang et al. (2009) used the reported RR from the highest exposure category to increase statistical power and reduce uncertainty regarding confounding or other bias. Although study selection was controversial, e.g., the inclusion of multiple reports from a single cohort and the use of one cohort where only a portion of the workers were formaldehyde-exposed, this meta-analysis is generally supportive of an association between formaldehyde and LHP malignancies.

The apparent differences by industry/profession may reflect many influences, including exposure potential and demographic characteristics. External analysis (use of the general population for comparison) relies on the assumption that cancer incidence rates are expected to be similar between the general population and the study population in the absence of exposure. The 'healthy worker effect' is well known, and there may be differences in the magnitude of this selection bias by industry or profession. For instance, LHP cancer incidence and mortality have many risk factors including socioeconomic status. Therefore, the consistent positive findings in professional workers versus mixed results in industrial workers could be influenced by the appropriateness of the comparison to the general population − that is, a differential extent of selection bias. Interestingly, salaried workers, but not the hourly workers, in an Italian plastic manufacturing plant had elevated SMRs for LHP cancers (1.69 (95% CI 1.07-2.53) and 0.93(95% CI 0.62-1.35), respectively) (Dell and Teta, 1995). Without knowledge of which worker group is most similar to the comparison population with respect to LHP cancers mortality, one cannot discern if this potential effect of demographic variability accentuates effects in professional/salaried workers or obscures the effects in industrial/hourly-wage workers.

Table 4-90. Summary of cohort and case-control studies which evaluated the incidence of all LHP cancers in formaldehyde-exposed populations (ICD-8 Codes: 200-209) and all leukemias (ICD-8 Codes: 204-207). (See Table 4-6 for complete study details and findings)

Study population	Study details	All LHP cancers	Leukemia	Reference
SMR Analysis[1]				
Pathologists and technicians (n=2,079)	Years of study 1955-1973	**2.0** (p<0.01) {pathologists} **0.5** {technicians}	**0.6** {pathologists} **0.5** {technicians}	Harrington and Shannon, 1975
Pathologists and technicians (n=2,720)	1974-1980	**NR**	0.91 (0.05-4.29) men 9.26(0.47-43.9) women	Harrington and Oakes, 1984
Pathologists (n=4,512)	Years of study 1974-1987	**1.44** (0.69-2.63)	1.52 (0.41-3.89)	Hall et al., 1991
Ontario Undertakers (n=1,477)	Mortality from 1950-1977	**1.24**	1.60	Levine et al.,1984
Male Anatomists (n=2,327)	Mortality from 1925-1979	**1.20** (0.7-2.0)	1.5 (0.7-2.7)	Stroup et al., 1986
Male pathologists (n=4,485)	Mortality through 1977	**NR**	1.06	Logue et al, 1986
Male pathologists (n=6,111)	Participants from 1912-1950 membership rolls. Mortality followed through 1978.	**1.25** (0.95-1.62)	1.35 (0.92-1.92)	Matanoski et al., 1989
Chemical industry workers, men (n=14,014)	Mortality from 1941-2000	**NR**	0.91(0.62-1.39)	Coggon et al., 2003
Chemical workers (n=2,026)		**1.36** (0.5 – 2.95)		Wong et al., 1983
Industrial workers (n=25,619)	Mortality followed through 2004	**0.94** (0.84-1.06)	1.02 (0.85-1.59)	Beane-Freemen et al., 2009
Industrial workers (n=7,328)		**0.89**		Marsh et al., 1996 {Subset of NCI cohort reported by Hauptmann et al., 2003}
Garment workers (n=11,098)	Mortality followed through 1998	**0.97** (0.74-1.26)	1.09 (0.70-1.62)	Pinkerton et al., 2004
Resin plant workers (n=1,330)	Employed between 1959-1980 Mortality through 1986	**2.01**	NR	Bertazzi et al., 1986
Plastic manufacturing		**1.69** (salaried workers)	**1.98** (salaried workers)	Dell and Teta, 1995

(n=5,932)		**0.93** (hourly workers)	**0.98** (hourly workers)	
PMR Analysis[1]				
Embalmers, New York (n=1,132)	Licensed between 1925-1980	**1.15**	1.32	Walrath and Fraumeni, 1983
Embalmers, CA (n=1,007)	Licensed between 1925-1980	**1.22**	1.75 (p<0.05) 1.24 (<20 years) 2.21 (P<0.05) (>20 years)	Walrath and Fraumeni, 1984
Embalmers, U.S. (n=4,046)		**1.39** (1.15-1.63) White **1.31** (1.06-1.59) Nonwhite **2.41** (1.35-3.97)	**1.52** [2] (0.98-2.35) White **1.44** (p<0.05) Nonwhite **2.72** (p<0.05)	Hayes et al., 1990
Case-Control Studies[1]				
American cancer Society Cancer Prevention Study II: (n=362,828 men)	Results for men reporting formaldehyde exposure, and occupations related to formaldehyde exposure	1.22 (0.84-1.77) (formaldehyde exposed) 3.44 (1.11-10.68) {formaldehyde exposure and occupation}	0.96 (0.54-1.71) (formaldehyde exposed) 5.79 (1.44-23.25) {formaldehyde exposure and occupation}	Stellman et al., 1998
White men diagnosed with leukemia (Iowa and Minnesota) (n=622)	Recruited in 1980-1983	NR	1.0 (0.7-1.4) Low 0.7 (0.2-2.6) High	Blair et al., 1993

Notes:
1. Relative risk estimate (SMR, PMR, or OR) presented with 95% confidence intervals, where available.
2. PMR for leukemia for the total group calculated from the published data for lymphatic leukemia (204, myeloid leukemia (205), and other/unspecified (206, 207).

The only study which has data to inform the effects of either exposure level or the appropriateness of an external comparison group on the association between formaldehyde exposure and all LHP cancer mortality is the National Cancer Institute (NCI) cohort study of industrial workers (Blair et al., 1986; Beane Freeman et al., 2009), which presents relative rates based on internal comparisons for 3 different exposure metrics. Although SMR analysis with an external comparison group did not indicate increased mortality from all LHP cancers (0.94, CI 0.84-1.06, for the exposed workers), internal analysis using the low-exposed workers as the comparison group demonstrates positive exposure-response relationships for increased mortality

from all LHP malignancies cancers and peak exposure across the study periods (1965-2004) (Figure 4-34A and 4-34B) (Beane Freeman et al., 2009), with a statistically significant trend ($p < 0.05$) for every year since 1977. These results, indicating a positive exposure-response relationship among plant workers, who most likely have similar demographic characteristics, are noteworthy given the apparent lack of association when SMRs for the same cohort are calculated against mortality rates for the general population. The lack of an apparent association with SMRs may be attributable to the healthy worker effect and/or some other difference between the exposed workers and the general population.

Although the association between formaldehyde exposure and all LHP cancer mortality in industrial and professional cohorts is mixed, the strength of the internal analysis of the NCI cohort, in the absence of positive SMRs compared to the general population, suggests that SMR analyses may not be the most appropriate methodology for assessing LHP cancer mortality. Given the potential for demographic differences between an industrial workforce and the general population, the results of the internal analysis of the NCI industrial cohort provide a higher quality analysis – and therefore should be given significantly more weight than SMR analyses of industrial workers that could not distinguish their findings from the null. Given the consistency and strength of the positive associations for all LHP cancers cancer mortality in professional cohorts (embalmers, anatomists and pathologists) taken together with the strong positive results of the NCI cohort, human epidemiologic evidence are sufficient to conclude that there is a causal association between formaldehyde exposure and mortality from all LHP malignancies (as a group).

4.5.2.3. *All Leukemia*

Like the analysis of all LHP cancers, an analysis of all leukemia combines diseases which differ significantly in cell of origin and etiology, including acute and chronic forms of both myeloid and lymphatic leukemia. This class also includes other leukemia (e.g., erythraemia) and a general category of ‘other and unspecified leukemia’ (ICD-8 207). Regardless, there is some utility in evaluating the all leukemia mortality data because many studies provided results for this grouping. Also, the diagnosis of leukemia versus solid LHP tumors is fairly distinct thereby limiting misclassification of the endpoint.

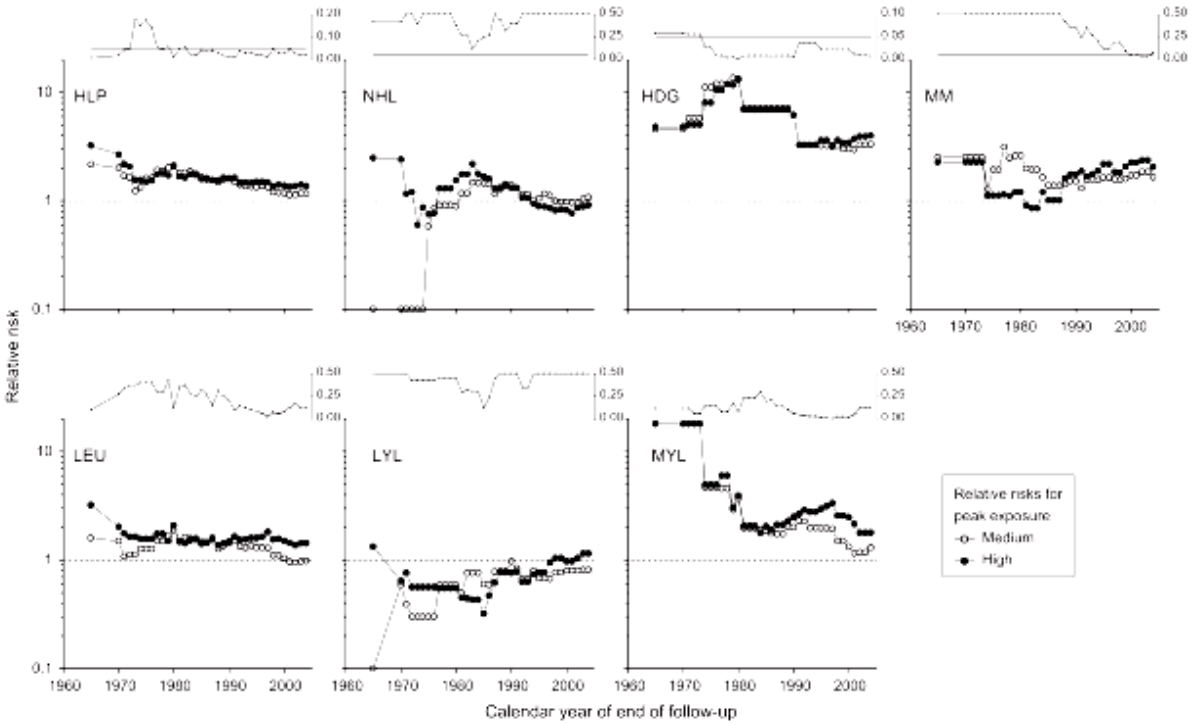


Figure 4-34A. Association between peak formaldehyde exposure and the risk of lymphohematopoietic malignancy.

Relative risks for medium-peak (2.0 to <4.0 ppm) and high-peak (≥4.0 ppm) formaldehyde exposure categories compared with the low exposed category (>0 to <2.0 ppm) and *P* values for trend tests among the exposed person-years for lymphohematopoietic malignancies are shown by year of end of follow-up, 1965-2004. Values plotted at 0.1 represent RR = 0 due to no cases in the exposure category values plotted at 20 represent RR = infinity due to no cases in the referent category. The **small graphs** above the relative risk plots represent the exposure-response trend *P* values based on two-sided likelihood ratio tests (1 df) of zero slope for continuous formaldehyde exposure among exposed person-years only. The **points** represent the relative risk estimates based on the cumulative number of cases and person-years accrued from the start of the study to that point in time and for 2004 are equivalent to the relative risk estimates presented in Table 2. HLP = lymphohematopoietic malignancies, NHL = non-Hodgkin lymphoma, HDG = Hodgkin lymphoma, MM = multiple myeloma, LEU = leukemia, LYL = lymphatic leukemia, MYL = myeloid leukemia, RR = relative risk.

Source: Beane Freeman, et al. (2009)

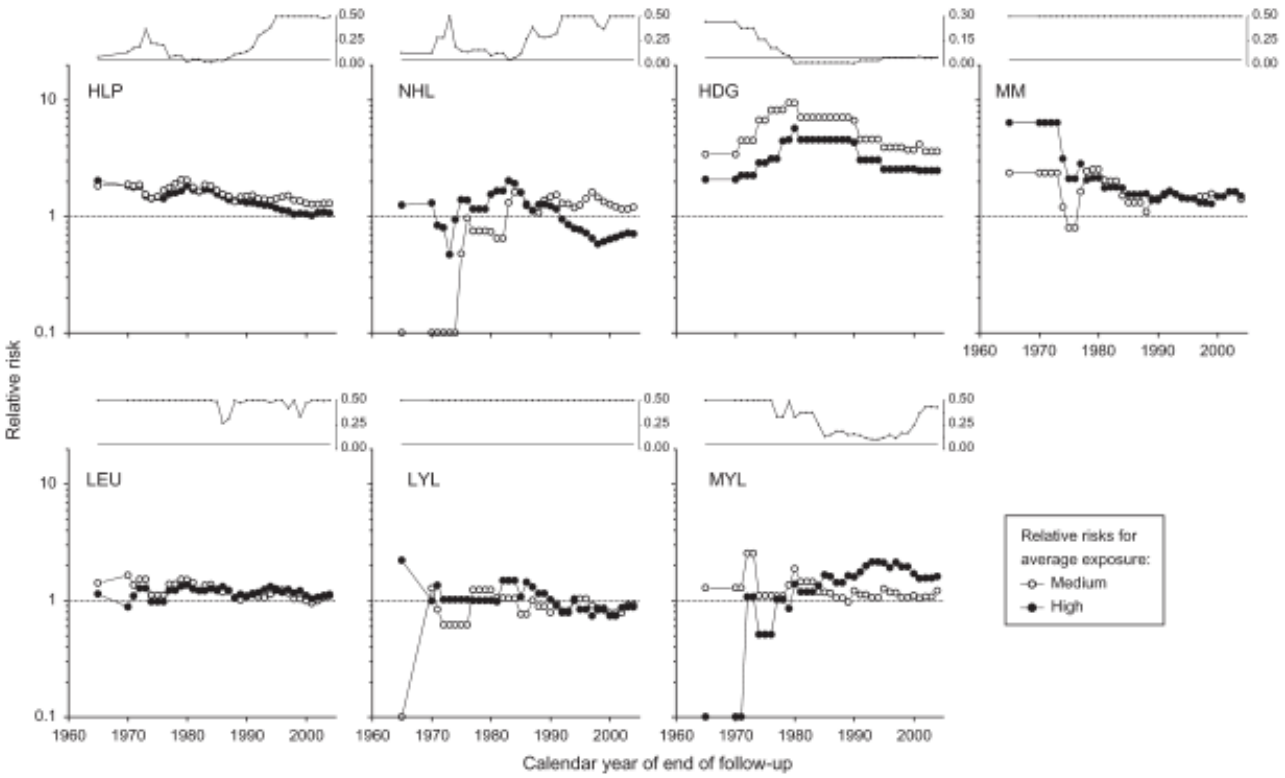


Figure 4-34B. Association between average intensity of formaldehyde exposure and the risk of lymphohematopoietic malignancy.

Relative risks for medium (0.5 – 0.9 ppm) and high (≥ 1.0 ppm) average-intensity formaldehyde exposure categories compared with the low exposed category (0.1 – 0.4 ppm) and *P* values for trend tests among the exposed person-years for lymphohematopoietic malignancies by year of end of follow-up, 1965 – 2004. Values plotted at 0.1 represent RR •• = due to no cases in the exposure category. The **small graphs** above the relative risk plots represent the exposure – response trend *P* values based on two-sided likelihood ratio tests (1 *df*) of zero slope for continuous formaldehyde exposure among exposed person-years only. The **points** represent the relative risk estimates based on the cumulative number of cases and person-years accrued from the start of the study to that point in time and for 2004 are equivalent to the relative risk estimates presented in Table 3 . HLP = lymphohematopoietic malignancies, NHL = non-Hodgkin lymphoma, HDG = Hodgkin lymphoma, MM = multiple myeloma, LEU = leukemia, LYL = lymphatic leukemia, MYL = myeloid leukemia; RR = relative risk.

Source: Beane Freeman, et al. (2009)

Although results are mixed across the studies (Table 4-90), an association between formaldehyde exposure and leukemia mortality is supported by cohort analyses of embalmers, pathologists and anatomists (Hayes et al., 1990; Walrath and Fraumeni, 1983; Walrath and Fraumeni 1984; Hall et al., 1991; Levine et al., 1984; Stroup et al., 1986; Matanoski et al., 1989). Formaldehyde exposure and formaldehyde-related occupation are associated with leukemia diagnosis in a case-control study (RR = 5.79 (95% CI 1.44-23.25), but not formaldehyde exposure alone (RR = 0.96; 95% CI 0.54-1.71) (Stellman et al., 1998).

In contrast, SMR analyses of the industrial cohorts do not indicate a similar association (Coggon et al., 2000; Beane Freeman et al., 2009, Pinkerton et al, 2004). Although the SMR analysis provided for the NCI cohort does not indicate a positive association for all leukemia using an external reference group (Beane Freeman et al., 2009), the SMR for exposed versus unexposed workers within the cohort suggests all leukemia is elevated 2.1-fold with this internal comparison (95% CI 0.99-4.56)[4]. A positive exposure-response relationship further strengthens the association of formaldehyde exposure to leukemia mortality (Beane Freeman et al., 2009). Where the referent group is defined as 'low exposed' individuals, leukemia is elevated in the highest peak exposure category (RR = 1.42; 95% CI 0.92-2.18) compared to both the referent group and the unexposed category (RR = 0.59; 95% CI 0.25-1.36), and there is a statistically significant trend across all groups (p = 0.02). Categorical analysis for the average intensity and cumulative exposure metrics suggests greater mortality in the high-exposure groups versus the 'low exposed' individuals (RR = 1.10 [95% CI 0.68-1.78] and 1.11 [0.7-1.74]. respectively), but analysis of individual results across the exposure-response range indicates cumulative exposure is a better predictor (p = 0.08 for trend across all exposed and unexposed.)

Several meta-analyses have been conducted for formaldehyde exposure and leukemia which indicate a positive association. Collins et al. (2004) report an overall RR for 18 available studies of 1.1 (CI 1.0-1.2), suggesting an association of leukemia with formaldehyde exposure. This association was stronger for both pathologists/anatomists (1.4; CI 1.0-1.9) and embalmers (RR = 1.6; 1.2-2.0) than for industrial workers (RR = 0.9; 0.8-1.0). Study design also impacted the apparent strength of association, with stronger associations seen in case--control studies (RR = 2.4; 0.9-6.5) versus cohort studies (RR = 1.0; 0.9-1.2). Bosetti et al. (2008) reported an association between formaldehyde exposure and leukemia mortality with a pooled RR of 1.39 (95% CI 1.15-1.68) for 8 groups of professional workers. In the same analysis, the pooled RR for the 4 industrial cohorts was 0.90 (0.75-1.07). Zhang et al. (2009) reported a pooled RR of

[4] $Var\left(\ln\left(\frac{SMR_{Exposed}}{SMR_{Non\ exposed}}\right)\right) = \frac{1}{Obs_{Exposed}} + \frac{1}{Obs_{Non\ exposed}}$

1.54 (95% CI 1.18-2.00) for all cohorts identified in their meta-analysis, although this pooled RR should be considered with some caution, as myeloid leukemia alone was included in the analysis where available (Zhang et al., 2009).

4.5.2.4. *Subtype Analysis*

Given the associations discussed above between formaldehyde exposure and both all LHP cancers and all leukemia, further analysis is needed to examine if the observed increase in all LHP cancers is primarily a reflection of increased leukemia, or if other types of LHP cancers may be elevated as well. Although analysis of mortality data by sub-type may provide a better understanding of the specific disease associations, there are potential pitfalls as well. Chief among these concerns are the potential for disease misclassification (especially in studies with older mortality data) and lack of statistical power as the number of observed cases is reduced by considering sub-types. Case control studies by design address specific diseases and are well-suited for sub-type analysis, but often provide little exposure information. The following analysis will draw from the available data to examine which forms of LHP malignancies may be associated with formaldehyde exposure.

There has been speculation that the association between formaldehyde exposure and increases in all LHP cancers and all leukemia are driven by increased myeloid leukemia (Pyatt et al., 2008; Heck and Casanova 2004; Golden et al., 2006). If this were the case, then mortality from LHP cancers other than myeloid should not be elevated, once the excess mortality from myeloid leukemia is accounted for. Only 2 studies provide the data to evaluate this hypothesis – both conducted by the NCI (Hayes et al., 1990 and Beane Freeman et al., 2009). From the published data, crude mortality statistics can be calculated for alternative disease groupings (Table 4-91). In the NCI embalmer study (Hayes et al., 1990), only myeloid leukemia was statistically elevated in the subtype analysis. For the NCI industrial cohort (Beane Freeman et al., 2009), elevations were also seen for Hodgkin lymphoma relative to the referent group. In both cases, the association between formaldehyde exposure and LHP malignancies remains when myeloid leukemia is dropped from the analysis. Further, similar associations are found when all leukemia and myeloproliferative diseases are dropped from the analysis and only solid tumors of lymphoid origin are included (lymphosarcoma and reticulosarcoma, Hodgkin lymphoma, non-Hodgkin lymphoma and multiple myeloma). These reanalyses illustrate the need for a more careful sub-type analysis to assess the potential for associations between formaldehyde exposure and various forms of LHP cancers.

Table 4-91. Secondary analysis of published mortality statistics to explore alternative disease groupings within the broad category of all lymphohematopoietic malignancies

	ICD-8 Codes	U.S. embalmers (Whole cohort) (Hayes et al., 1990) PMR (95% CI)	U.S. industry (peak exposure metric: >4 ppm vs. >0 to ≤2 ppm) (Beane Freeman et al., 2009) Relative risk (95% CI)
All Lymphohematopoietic Malignancies	200-209	1.39 [a] (1.15-1.67) [b]	1.37 [c] (1.03-1.81) [c]
Alternative Disease Groupings			
Exclude Myeloid Leukemia	200-204, 206-209	1.35 [a] (0.99-1.85) [b]	1.31 [d] (0.97-1.75) [d,e]
Solid tumors of lymphoid origin (Lymphosarcoma and reticulosarcoma, Hodgkin lymphoma, non-Hodgkin lymphoma and multiple myeloma)	200-203	1.24 [a] (0.84-1.84) [b]	1.33 [d] (0.93-1.90) [d,e]

[a] $PMR = \frac{Obs}{Exp}$

[b] $Var(\log PMR) = \frac{1}{Obs} + \frac{1}{Exp}$

[c] See Table 2 of Beane Freeman et al. (2009)

[d] $RR = \frac{Deaths_{Comparison\ Group} / Person-Time_{Comparison\ Group}}{Deaths_{referent\ Group} / Person-Time_{referent\ Group}} = \left[\frac{108-19}{103-14}\right] \times \left[\frac{PT_{referent\ Group}}{PT_{Comparison\ Group}}\right]$

$where \left[\frac{PT_{referent\ Group}}{PT_{Comparison\ Group}}\right] \cong \left[\frac{108^c}{103^c \times 1.37^c}\right] = 0.765$

[e] $Var(\log RR) = \frac{1}{Deaths_{Comparison\ Group}} + \frac{1}{Deaths_{referent\ Group}}$

4.5.2.5. *Myeloid Leukemia*

The associations between myeloid leukemia and formaldehyde exposure are strong and consistent (Table 4-92). Of the four studies which formally assess myeloid leukemia mortality, all are positive, including cohorts of both professional and industrial workers (Beane Freeman et al., 2009; Hayes et al., 1990; Pinkerton at al., 2003; Stroup et al., 1986). Although few cases exist for further subtype analysis, the available data indicate either no differences in SMRs for acute myeloid leukemia (AML) versus chronic myeloid leukemia (CML) (Hayes et al., 1990; Pinkerton et al., 2003) or suggest CML is more prominent (Blair et al., 2000; Stroup et al., 1986).

Table 4-92. Summary of studies which provide mortality statistics for myeloid leukemia sub-types.

Study population				Reference
	Myeloid Leukemia	**Acute Myeloid Leukemia**	**Chronic Myeloid Leukemia**	
SMR Analysis[1]				
Garment workers (n=11,098)	1.44 (0.08-2.37)	1.34 (0.61-2.54)	1.39 (0.38-3.56)	Pinkerton at al., 2003
Anatomists (n=2,317)	NR	NR	8.8*	Stroup et al., 1986
Industrial workers (n=25,619)	0.90 (0.67-1.21) SMR Ratio 1.38 (0.65-2.97) (exposed/unexposed)	NR	NR	Beane Freeman et al., 2009
PMR Analysis[1]				
Embalmers, U.S. (N=4,046)	1.57 (1.01-2.34)	1.52 (0.85-2.52)	1.84 (0.79-3.62)	Hayes et al., 1990
Case-Control Studies[1]				
White men diagnosed with leukemia (Iowa and Minnesota) (n=622)	NR	Low: 0.9 (0.5-1.6) High: NR	Low: 1.3 (0.6-3.1) High: 2.9 (0.3-24.5)	Blair et al., 1993

* Leukemia SMR 1.5 (0.7-2.7) {5 of 10 deaths due to myeloid}; Chronic Myeloid Leukemia (CML) SMR of 8.8
1. Relative risk estimate (SMR, PMR, or OR) presented with 95% confidence intervals, where available.

Walrath and Fraumeni (1983, 1984) note that AML is prominent in their analyses of New York and California licensed embalmers; however, they do not provide PMR analyses for CML. Walrath and Fraumeni (1983 and 1984) report leukemia cell types - for both studies the majority of myeloid leukemia are acute (5/6 and 4/6, respectively, for New York State and California embalmers). However, PMRs cannot be calculated for AML versus CML in this paper, as comparison rates are not available from the 1920's through the 1960's - the timeframe with the majority of deaths. The authors do contrast the observed rate of AML in the cohort to the background rate for AML in white men in the 1970s - but given the potential misclassification of late stage CML as AML, especially historically, this may not be an appropriate comparison. Additionally, one would expect older data to over-represent AML rather than CML due to diagnosis in the early decades of CML in the blast crisis as AML. Therefore, although these studies support an association between formaldehyde exposure and myeloid leukemia in general

(Walrath and Fraumeni, 1983; 1984), the reported AML and CML subtype information does not allow a satisfactory sub-type analysis for myeloid leukemia.

4.5.2.6. *Solid Tumors of Lymphoid Origin*

Multiple myeloma, Hodgkin lymphoma, non-Hodgkin lymphoma, lymphosarcoma, reticulosarcoma, and other lymphomas may all be derived from immune cells outside of the bone marrow compartment, in peripheral blood, in the gut and respiratory mucosa and immune tissues at the POE (e.g., lymph nodes, mucosa-associated lymphoid tissue (MALT), gut-associated lymphoid tissue (GALT) (Greaves, 2004). The only meta-analysis to specifically address lymphoid malignancies found evidence for increased lymphoma (Hodgkin lymphoma (pooled RR = 1.23; 95% CI 0.67-2.29) and multiple myeloma (1.31; 1.02-1.67), but not for non-Hodgkin lymphoma (1.08; 0.86-1.35) (Zhang et al., 2009). As seen in Table 4-93 below, individual study results are mixed for these lymphoid cell-line malignancies, as they are for all LHP cancers and all leukemia above. Although these tumors are from mature lymphocytes, there is still variability in the etiology, natural history and risk factors for the many sub-types which are included in these categories.

There is evidence for an exposure response relationship for both Hodgkin lymphoma and multiple myeloma in the NCI industrial cohort among exposed workers (Beane-Freeman et al., 2009). Clear exposure response relationships for Hodgkin lymphoma are defined with all three metrics of exposure, peak average intensity and cumulative exposure ($p=0.01$, $p=0.05$ and $p=0.08$ respectively for mortality through 2004). These associations have been evident from first follow-up through the current publication, and statistically significant for the majority of the follow-up period demonstrating that this is a strong and consistent finding in the NCI cohort (Figure 4-34 A&B) (Beane-Freeman et al., 2009). Although the overall SMR for multiple myeloma does not indicate an association, trends across time indicate consistent elevation of multiple myeloma mortality with both peak and average intensity of exposure, where the statistical strength of the association with peak exposure increases with follow-up (Figure 4-34 A&B).

Table 4-93. Summary of mortality statistics for Hodgkin lymphoma, lymphoma and multiple myeloma from cohort analyses of formaldehyde exposed workers.

Study population	Hodgkin Lymphoma	Non-Hodgkin Lymphoma	Multiple Myeloma	Reference
SMR Analysis[6]				
Pathologists (n=2,079)	1.4	2.0 (p<0.05)	NR[1]	Harrington and Shannon, 1975
Pathologists (n=4,512)	1.21 (0.03-6.71)	1.44 (0.69-2.63)	NR	Hall et al., 1991
Male Anatomists (n=2,327)	–[2]	0.7 (.1-2.5)[3] 2.0 (0.7-4.4)[4]	NR	Stroup et al., 1986
Male pathologists (n=6,111)	0.36 (0.04-1.31)	1.31 (0.66-2.35)[3] 1.54 (0.82-2.63)[4]	NR	Matanoski et al., 1989
Chemical workers (n=2,026)	2.94 (0.33-10.63)	NR	NR	Wong et al., 1983
British Chemical plants (n=14,014)	0.36 (0.01-2.01)	0.89 (0.41-1.70)	1.18 (0.48-2.44)	Coggon et al., 2003
Swedish workers- abrasive production plant (n=911)	NR	2.0 (0.2-7.2)	4.0 (0.5-14)	Edling et al., 1987a
Industrial workers (n=25,619)	1.42 (0.96-2.10)	0.86 (0.70-1.05)	0.94 (0.71-1.25)	Beane Freemen et al., 2009
PMR Analysis[6]				
Embalmers, New York (n=1,132)	0.87 (p<0.05)	1.08[3] 1.22[4]	NR	Walrath and Fraumeni, 1983
Embalmers, CA (n=1,007)	–[2]	3.10[3] 1.33[4]	NR	Walrath and Fraumeni, 1984
Embalmers, U.S. N=4,046	0.72 (0.15-2.10)	1.26 (0.87-1.76) 1.12 (0.58-1.96)[3] 1.35 (0.84-2.01)[4]	1.37 (0.84-2.12	Hayes et al., 1990
Case-Control Studies[6]				
Women in Connecticut (n=601)	NR	1.3 (1.0-1.7)	NR	Wang et al., 2009
White men, Iowa and Minnesota (n=622)	NR	1.2 (0.9-1.7)	NR	Blair et al., 1993
ACS Cancer Prevention Study II (n=128)	NR	NR	1.8 (0.6-5.7)	Boffetta et al., 1999
Men, ACS Cancer Prevention Study II (n=45,399)	NR	0.92 (0.5-1.68) 2.88 (0.40-10.5)[5]	0.74 (0.27-2.02)	Stellman et al., 1998
Danish workers (n=1,098)	NR	NR		Heineman et al., 1992
Danish women (607)	NR	NR	1.6 (0.4-5.3)	Pottern et al., 1992

Notes :
1: NR is not reported
2. "—" no cases observed.
3. Lymphosarcoma and reticulosarcoma only
4. "other lymphoma"
5. Formaldehyde exposure in a wood-related occupation. RR for wood-related occupation alone was not elevated 0.97 (0.55-1.73)
6. Relative risk estimate (SMR, PMR, or OR) presented with 95% confidence intervals, where available

4.5.2.7. *Supporting Evidence from Animal Bio-Assays for Formaldehyde-Induced Lymphohematopoietic Malignancies*

Chronic animal studies provide supporting evidence for formaldehyde-induced leukemia and lymphoma (Soffritti et al., 1989; Battelle Laboratories, 1981). Although the majority of chronic animal bioassays do not report either leukemia or lymphoma, it should be noted that many studies focused primarily on respiratory tract and did not provide routine examination of other organs, limiting the detection of leukemia and lymphoma (Horten et al., 1963; Holmstrom et al., 1989; Wouterson et al., 1989; Appleman et al., 1988; Monticello et al., 1996; Dalbey, 1982). Kamata et al. (1986) did examine additional organs, but there were only 5 animals at each sacrifice. Similar issues are seen with some of the drinking water studies, where Takahashi et al. (1986) focus on the stomach and intestines, and the study in Wistar rats by Tobe et al. (1989) only included 20 animals per sex per exposure group with interim sacrifices. Therefore, few studies remain to be explored which are informative about the carcinogenic potential of formaldehyde on the LHP system. Table 4-94 lists the studies from the chronic bioassays which have the potential to detect LHP malignancies.

Soffritti et al. (1989) were the first to publish a finding of formaldehyde-induced leukemia in an animal bio-assay. Sprague-Dawley rats (50 per each sex) were exposed to formaldehyde in drinking water at 0, 10, 50, 100, 500, 1000, 1500 mg/L given *ad libitum*. Lymphoblastic leukemia and lymphosarcomas were the most common lesion reported, and exhibited an apparent dose-dependent increase in both male and female rats (Table 4-95). All hemolymphoreticular cancers summed together reflected similar trends, but the other types did not show similar relationship with exposure (e.g. immunoblastic lymphosarcoma). A subsequent publication of full study results reports all lesions together as lymphoma and leukemia, providing less-specific information (Soffritti et al 2002.) Additionally, there is a large discrepancy in reported lesions between the two study reports where nearly double the incidence is reported in 2002. Dr. Soffritti explains the increase in reported LHP malignancies as a result of tabulating the full histopathological findings in the 2002 report (personal communication, 23 July 2009). Although the second report from Soffritti et al. (2002) have been broadly criticized on both of these points (summing of dissimilar lesions and discrepancies in reporting), these errors do not iupune the original report. The 1989 report does distinguish between different sub-types providing positive results for lymphoblastic leukemia/lymphosarcoma and histological examinations were consistently conducted between treatment groups – even if further results were published at a later timepoint. Therefore, the findings of Soffritti et al. (1989) are considered as supportive of the biological plausibility of formaldehyde-induced lymphohematopoietic malignancies.

Table 4-94: Summary of chronic bioassays which address rodent leukemia and lymphoma

Study	Histopathology	Endpoint	Results	Comments
Drinking Water Exposure				
Male and Female Sprague-Dawley Rats				
Sofritti et al. 1986	Complete histopathology	Lymphocytic leukemia and lymphosarcoma	Increased, showing a dose-response	Life-long study High exposure of 1,500 mg/l in water
Male and Female Wistar Rats				
Til et al, 1989	Complete histopathology in control and high dose group (15 ppm)	Lymphoma, leukemia	No increase (3 lymphomas and 1 leukemia found in 200 animals at the 2 yr sacrifice)	2-year bioassay High exposure of approximately 1900 mg/l (82mg/kg for males and 109 mg/kg for females)
Inhalation Exposures				
Male rats, Sprague-Dawley				
Sellakumar et al., 1985 ; Albert et al., 1982	Necropsy focused on respiratory tract : also liver, spleen, kidney and testes and organs demonstrating gross pathology	Lymphoma	No increase	Life-long study – high mortality at 24 months (>80%)
Male rats, F344				
Batelle, Columbus Laboratories, 1981	Complete histopathology in control and high dose group (15 ppm)	Leukemia, all	No increase	Extended study – high mortality
Female Rats, F344				
Batelle, Columbus Laboratories, 1981	Complete histopathology in control and high dose group (15 ppm)	Leukemia, all	Increased in mortality adjusted incidence. P=0.0056[1]	Extended study – high mortality Apparent elevation in 2 ppm and 6 ppm treatment groups as well (fig5-xx)– statistical comparison to controls is problematic
Female mice - C57BL/6xC3HF1				
Batelle, Columbus Laboratories, 1981	All organs in control and high dose group (15 ppm)	Lymphoma, all	26% in FA-exposed (15ppm) 16% in control P=0.0617	Extended study All mice included in statistics conducted by Batelle Lab.

1. Original statistical analysis provided by Battelle, Columbus Laboratories. Significance set at P<0.0167. Analysis of adjusted data where time to lesion and survivorship were considered, Cox (1972, Tyrone (1975).

Table 4-95. Incidence of lymphoblastic leukemia and lymphosarcoma orally dosed in Sprague-Dawley rats

	Lymphoblastic leukemia and lymphosarcoma	
	Tumor bearing animals (%)	
	Male	Female
control	3	1
vehicle	8	2
10	0	2
50	4	6
100	8	4
500	8	4
1000	12	10
1500	22	10

Source: Soffritti et al. (1989)

The two-year bioassay by Til et al. (1989) in male and female Wistar rats indicates no increase in leukemia and lymphoma, with only 4 tumor-bearing animals in all treatment groups sacrificed at 24 months. The drinking water levels were similar at the highest dose of both studies. The major difference in study design is length, which may have influenced results, as leukemia is a late-life malignancy in rodents. Two-year survival in the Soffritti et al. (1989) study varied between 50-60%. These animals would be available to develop leukemia after the two-year window of the Til et al (1989) study. Any potential role of strain differences is unknown. Overall, the results of Soffritti et al. (1986) are strong, showing an exposure-response relationship, in a lifelong study, appropriate for late-life malignancies. Unlike the GI tract tumors, early-life exposure to formaldehyde in drinking water did not increase LHP malignancies (Soffritti et al., 1989).

The largest and most comprehensive study of health effects from formaldehyde inhalation exposures is the study reported by Kerns et al. (1983) and Swenberg et al. (1980) conducted at the Columbus Laboratory of Battelle Corporation (1981). Although the summary reports of this study do not discuss leukemia or lymphoma rates, mouse lymphoma and rat leukemia were selected by the study pathologist and biostatistician for analysis (Battelle Laboratory, 1981). Statistical analysis performed by Battelle Laboratories which accounted for time-to-lesion and survivorship rates did indicate a statistically significant increase in female rat leukemia (P = 0.0003) and a nearly significant increase in female mouse lymphoma ($P = 0.06$). No trend analysis could be performed, as only gross pathology was conducted on mid-dose mice

and rats (2 and 6 ppm, respectively). EPA has further analyzed these data to better understand the significance of these findings. The percentage of lymphoma--bearing female mice increased from 18% in control mice to 28% in mice exposed at 15 ppm for 24 months (6 hr/day, 5 days/wk) ($P<0.05$). Female rat leukemia was similarly elevated to 26%, 22% and 24% by inhalation exposure to 15 ppm, 6 ppm and 2 ppm, respectively, versus 16% in controls when early deaths prior to the first observed leukemia are removed from the analysis (21 months). In contrast, leukemia was not elevated in formaldehyde-exposed male F344 rats within the same study.

Differences in study design may account in part for mixed results. The lifelong study by Soffritti et al. (1989 may have allowed for detection of malignancies developing late in life, whereas the other drinking water study by Til et al. (1989) sacrificed all animals at 24 months. Even though the exposure levels were similar, the studies are not directly comparable. Likewise, it is hard to directly compare results from the two major inhalation studies in rats. Although a life-long study, the mortality for rats in the Sellakumar et al. (1985) study was greater than 80% at 2 years. Additionally, the pathology examination was much less rigorous than in the Battelle Laboratory study, perhaps missing smaller lesions. Therefore, the increase in formaldehyde-induced leukemia seen in female F344 rats late in life (Battelle laboratories, 1981) may be reflecting a more sensitive study design. Finally, strain differences may account for different susceptibilities as well. In summary, the available evidence from chronic animal studies supports the biological plausibility of the formaldehyde-induced LHP malignancies observed in epidemiologic studies. The two positive rat studies, by different routes of exposure, along with a positive result for formaldehyde-induced mouse lymphoma make a substantive case for formaldehyde-induced LHP malignancies.

The epidemiologic studies provide sufficient evidence to conclude that there is a causal association between formaldehyde exposure and lymphohematopoietic malignancies. When data are evaluated for all leukemia together, again there is sufficient evidence to establish a causal association, with consistent positive results in individual studies as well as 3 independent pooled analyses. Mortality from myeloid leukemia, as well as mortality attributed to “other and unspecified leukemia” is consistently elevated where reported. In addition, strong evidence for a causal relationship between formaldehyde exposure and Hodgkin lymphoma is provided by the consistent associations seen between formaldehyde exposure and Hodgkin lymphoma in the NCI industrial cohort, with elevations observed across decades of follow-up and significant exposure-response relationships for all three exposure metrics examined in the most recent follow-up (Beane Freeman et al., 2009).

4.5.3 Carcinogenic Mode(s) of Action

The US EPA 2005 Guidelines for Carcinogen risk Assessment recommend a Mode of Action (MOA) analysis when data are available for evaluation. The purpose of this MOA analysis is to determine if sufficient data exist to adequately inform the exposure-response relationship for cancer below the range of observed data in either human or animal studies. Since the majority of the data supporting the carcinogenicity of formaldehyde comes from animal bio-assays and epidemiological studies of workers, EPA must extrapolate from the observed risk of cancer mortality/incidence in those studies to levels considered protective of human health for lifelong environmental exposures. In this context, the US EPA cancer guidelines provide a framework to review MOA information for relevant data to establish an MOA informing appropriate low-dose extrapolation.

The supporting data for the MOA evaluation of formaldehyde are complex, and presented across multiple sections of a large document; therefore, this section includes a brief summary of the biological actions of formaldehyde and key mechanistic data which are believed to be relevant to the MOA evaluation (Section 4.5.3.1). This information is not intended as a stand-alone description of the evidence for a particular mechanism, but is intended to highlight the major supporting arguments and direct the reader to text providing more detailed discussion.

The summary of data discussed below combines what is known about the human cancer of concern (nasopharyngeal cancer, sinonasal cancer, leukemia and other lymphohematopoietic cancers) with the potential formaldehyde-specific mechanisms of action to postulate carcinogenic modes of action for each cancer or group of cancers (Section 4.5.3.2 and 4.5.3.2). The resulting evaluation provides multiple possible MOAs for formaldehyde-induced cancers where some key mechanistic events may be commonly at work in different tissues, and some key events may be more relevant to a specific tissue/cancer type. Each of these MOAs is evaluated with respect to its relevance to human cancer, and the overall weight of evidence for its relevance to formaldehyde-related human cancer.

Overall, multiple MOAs considered relevant to humans are presented for each cancer type. Although some MOAs may have a greater level of supporting evidence, this reflects in part how well a particular mechanism or key event may have been studied. For example, there are a large number of studies across many testing systems, and levels of biological organization to support the mutagenicity of formaldehyde. In contrast other likely MOAs, such as viral reactivation, have little direct mechanistic evidence, but the available evidence is supportive.

The MOAs considered most relevant to upper respiratory tract cancers (e.g. NPC and sinonasal cancer) are: 1) direct mutagenicity; 2) inhibition of DNA repair mechanisms; 3) formaldehyde-induced cell proliferation; 4) cytotoxicity-induced cell proliferation; 5) tumor

promotion activity; and 6) localized immunosuppression/viral reactivation (Section 4.5.3.2). The majority of these MOAs would apply equally to immune cells present at the site of first contact and may also contribute to those lymphohematopoietic cancers which arise from peripheral immune cells (e.g. Hodgkins lymphoma, multiple myeloma and some forms of leukemia). Additional MOAs are considered, specifically for formaldehyde-induced leukemia are: 1) damage of a circulating hematopoietic stem cell or progenitor cell at the site of first contact; and 2) bone marrow toxicity (Section 4.5.3.3).

In summary – no single MOA is singled out as the best explanation for cancer resulting from formaldehyde exposure. Only one MOA - cytotoxicity induced cell proliferation – suggests an exposure threshold below which the MOA would not be active. However this MOA is the least applicable to humans and other MOAs are considered operative at exposures below exposures associated with cytotoxicity-induced cell proliferation. Therefore, multiple MOAs are considered supported by formaldehyde-specific mechanistic information which provide biological plausibility for the cancers observed in formaldehyde exposed populations.

4.5.3.1 *Mechanistic Data for Formaldehyde*

4.5.3.1.1 DNA Reactivity/Genotoxicity/Mutagenicity

An agent's genotoxic potential and ability to induce mutations is a key consideration in assessing a carcinogenic MOA, as cancer results from a series of genetic and epigenetic alterations affecting genes that control cell growth, division and differentiation (Hanahan and Weinberg, 2000; Vogelstein et al., 1988; Kinzler and Vogelstein, 2002). The US EPA *Cancer guidelines* suggest several lines of evidence which are key to evaluating a mutagenic MOA: 1) Is the chemical under study DNA-reactive and/or has the ability to bind to DNA; 2) Does the chemical generate positive results in in vitro mutagenic test systems (specifically gene mutations and chromosomal aberrations); and 3) Does the chemical induce manifestations of genetic damage in in vivo tests (specifically gene mutations and chromosomal aberrations) and 4) Does the chemical have properties and structure-activity relationships (SAR) similar to known mutagens (US EPA, 2005). As reviewed in Section 4.3 above, there is adequate evidence for formaldehyde-induced genotoxicity and mutagenicity for consideration of these key events in formaldehyde's carcinogenic MOA.

Formaldehyde induces a variety of genotoxic and mutagenic events when tested both in vitro and in vivo systems including DNA-protein crosslinks (DPC or DPX), point mutations, DNA single strand breaks (SSB) and chromosomal aberrations (CAs) (See Section 4.3). Formaldehyde, as a reactive chemical, also forms DNA adducts and DNA-DNA crosslinks (DDC) and may act to form adducts between other chemicals and DNA (Brutlag et al., 1969;

Donecke, 1978; Ohba et al., 1979; Fennel, 1999; Casanova-Schmitz and Heck, 1983; Casanova-Schmitz and Heck, 1984; Heck and Casanova, 1987 and Casanova et al., 1989). The high reactivity of formaldehyde results in little specificity indicating that a range of adducts and crosslinks might be expected.

Numerous studies have shown that formaldehyde induces genotoxic and mutagenic effects under a variety of experimental conditions (see section 4.3 for a detailed discussion, also reviewed by IARC 2006; Ma and Harris 1988 and Auerbach et al, 1977). As discussed, formaldehyde is known to directly react with DNA forming DPC and DNA adducts. Mutations may occur during repair of formaldehyde-induced DNA damage, or as a result of replication errors during mitogenesis. Additionally, there is some evidence that DNA single strand breaks (SSB) may be induced directly by formaldehyde reactivity (Grafstrom et al, 1984). Clastogenic effects including increased micronuclei (MN), chromosomal aberrations (CAs) and sister chromatid exchanges (SCEs) are also reported in a range of in vitro study systems.

Formaldehyde caused a concentration-dependent icrease in calstogenicity (e.g. MN) in human cell lines deficient in either DNA nucleotide excision repair (NER) or DDC repair systems even though there is no change seen in DPC induction or removal between these cell lines (Speit et al., 2000). These data suggest that alteration of DNA repair, not DPC removal, contributes to formaldehyde-induced clastogenicity. Since DPC repair involves proteolytic removal of proteins from the DNA, the authors hypothesize that single peptides or small peptide chains cross-linked to the DNA as in the case of DPC are critical to formaldehyde-induced mutations.

Formaldehyde-induced MN and CAs are associated to concentration-dependent mutagenic effects in L5178Y mouse lymphoma cells (Speit and Merk, 2002). Detailed analysis of both spontaneous and formaldehyde-induced lesions indicate that recombination or deletion of DNA from the thymidine kinase (*tk*) locus was primarily responsible for the loss of heterogeneity leading to the observed mutant phenotype. Therefore, it is believed that formaldehyde is mutagenic by a clastogenic mechanism, rather than through point mutations in the L5178Y mouse lymphoma cell system. This finding is consistent with Craft et al. (1987) who demonstrated formaldehyde-induced mutagenicity in the *tk* locus of TK6 human lymphoblastoid cells, while Graftsrom et al, (1984) demonstrated increased SSBs in formaldehyde-exposed human cell lines. The elegant series of experiments by Speit and Merk provide the possible links between DPC, clastogenicity and locus-specific mutations firmly demonstrating formaldehyde-induced mutations in the in vitro mouse lymphoma testing system.

Formaldehyde is genotoxic at the portal of entry (POE) in animal studies, resulting in increased DPC formation in the nasal mucosa as discussed above. However, there are no animal

studies which directly examine the mutagenicity in nasal or respiratory epithelial cells in the early stages of exposure. It is likely that the mutations are seen in advanced stage of the tissue around the transformation stage with formaldehyde exposure. With weak positive results in pulmonary lavage cells (Dallas et al., 1992) and clastogenicity demonstrated in gastro-intestinal epithelial cells of rats (Migliore et al., 1989) , below exposure levels which trigger regenerative cell proliferation, the existing evidence, although thin, supports clastogenic effects of formaldehyde.

Clastogenic effects are consistently reported in humans exposed to formaldehyde in the industrial workplace or during anatomy or mortuary classes (See section 4.3 for a full discussion). Increased micronuclei have been reported in nasal epithelial cells from industry workers (Ballarin et al., 1992; Ye et al., 2005), buccal epithelial cells from anatomy and mortuary science students and/or staff (Kitaeva et al., 1996; Titenko-Holland et al., 1996; Burgaz et al., 2001; 2002 compared to corresponding controls). Comparisons of micronuclei in nasal and buccal cells of anatomy students before and after classes where they are exposed to formaldehyde indicate an increase in clastogenicity (Ying et al., 1997). An examination of exfoliated buccal and nasal cells in mortuary students indicates greater increases in centromere-negative micronuclei, suggesting the effects are due to chromosome breakage or clastogenicity rather than aneuploidy (Titenko-Holland et al., 1996). Micronuclei were also increased in a dose-dependent manner in buccal cells as well as peripheral blood lymphocytes (PBLs) in mortuary students during the course of an embalming class; however, SCEs were reduced in post-exposure samples (Suruda et al., 1993). Buccal, oral and nasal cells present at the portal of entry may be directly exposed to formaldehyde and thus reports of clastogenic effects are consistent with direct interaction of formaldehyde at the POE. There is some supporting evidence for the mutagenicity of formaldehyde in human populations. Shaham et al. (2003) reported a increase in mutant *p*53 protein in the PBLs of individuals with mean formaldehyde exposure duration of 16 years. Additionally there was is a significant association between mutant *p*53 protein and DPC in this study suggesting a relationship between the formaldehyde's genotoxic effects. More recently, Zhang et al., (2010) have reported aneuploidy in circulating hematopietic stem cells in formaldehyde exposed workers with increases in both monosomy7 and trisomy 8.

In summary, there are several lines of evidence supporting mutagenic effects of formaldehyde exposure:

1) Formaldehyde directly interacts with DNA generating DPC,

2) DPC in tissues at the POE exhibit a dose-response relationship to formaldehyde exposure,

3) Formaldehyde-induced DPC are associated with formaldehyde-induced MN and CAs,
4) Mutations induced by formaldehyde due to small deletions and rearrangements in DNA in various experimental systems are consistent with formaldehyde's observed clastogenic effects (MN and CAs),
5) Formaldehyde-induced mutations and clastogenic effects occur at levels below where significant cytotoxicity is detected, and
6) Formaldehyde exposure has been correlated to similar increased MN and CAs in human buccal and oral cells corresponding to sites where formaldehyde-induced tumors arise.

4.5.3.1.2 Inhibition of DNA repair

Studies indicate that formaldehyde exposure may inhibit DNA repair mechanisms directly (See Section 4.3.1.5). Graftsrom (1985) first documented formaldehyde effects on DNA repair mechanisms, reporting that formaldehyde treatment of human bronchial fibroblasts in vitro inhibited repair of O6-methyl-guuanine adducts induced by N-methyl-Nitrosurea (NMU). Inhibition of DNA repair in human keratinocytes and fibroblasts cultured at 10 µM formaldehyde affected repair of DNA single strand breaks from ultraviolet light but was specific to UVB and UVC, not impacting repair of single strand breaks from UVA (Emri et al., 2004).

To determine if formaldehyde may have similar effects in exposed humans, Hayes et al., (1997) assessed the activity O6-alkylguanine-DNA alkyltranferase (AGT) an enzyme critical in repairing DNA damage induced by alkylating agents in formaldehyde-exposed mortuary students previously shown to have increased micronuclei in both buccal cells and peripheral lymphocytes (Suruda et al., 1993). AGT activity was lower in mortuary students with prior embalming exposures versus students with no prior exposure ($p=0.08$). Seventeen of 23 students had lower AGT activity after the 9 week course ($p<0.05$) with a larger proportion of naïve students demonstrating decreased activity (7 of 8) versus previously exposed students (10 of 15). Although detailed exposure measurements were taken for each student, the changes in AGT activity were not correlated to cumulative exposure (ppm-hrs).

4.5.3.1.3 Protein to protein cross-links

Formaldehyde is a reactive molecule that is likely to interact with both low molecular weight cellular components (e.g., reduced glutathione [GSH]) as well as high molecular weight cellular components. Unlike nuclear DNA, which has additional membrane barriers to exposure (i.e., nucleus), extracellular and intracellular proteins, are obvious primary targets for interacting with formaldehyde. Formaldehyde is a well-known cross-linking agent that is used in the

fixation of tissues, inactivation of toxins and viruses (e.g. preparation of vaccines), and study of protein-protein interactions (Metz et al., 2006). Using several identical synthetic polypeptides differing on one amino acid, Metz et al (2004) have shown that formaldehyde initially reacts with the primary amino and thiol groups of amino acids forming unstable methylol adducts, which later are partially dehydrated forming labile Schiff bases that are capable of forming crosslinks with other amino acid residues, such as arginine, asparagine, glutamine, histidine, tryptophan, and tyrosine through methylene bridges, but not between two primary amino groups. The same group (Metz et al 2006) has also shown that formaldehyde forms seven intramolecular crosslinks in proteins with defined structure, such as insulin, involving arginine, tyrosine and lysine and the N-terminus of insulin was converted to a imidazolidinone adducts similar to that observed with the synthetic peptide (Metz et al 2004). (Figure 3-1 provides a general reaction scheme for formaldehyde-mediated modifications of amino acids.)

4.5.3.1.4 Break-down of the mucociliary apparatus

The mucociliary apparatus of the upper respiratory tract is the first line of defense against airborne toxicants. Comprised of a thick mucus layer (epiphase), hydrophase and ciliated epithelium, the mucociliary apparatus may entrain, neutralize and remove particulates and airborne chemicals from inspired air (Figure 4-4). Formaldehyde reacts with the components of the mucous layer (proteins, glycoprotein, and lipids), crosslinking proteins. Formaldehyde exposure induces slowing of the mucous flow, stiffing and breaking up of the mucous layer and eventual mucostasis where gaps have been observed exposing the underlying hydrophase and epithelium. Although ciliary beat first increases in response to formaldehyde exposure, perhaps to compensate for reduced flow of the epiphase, ciliastasis ensues with both higher levels of exposure, and increased duration of exposure. Altered ciliary has been noted in as little as 15 minutes of exposure (1.25 ppm) with functional deficits in the mucociliary apparatus at 30 minutes. Altered ciliary beat has been reported at the lowest concentration tested (0.5ppm) for a single 6 hour exposure. Severity of effects increase with both duration and level of exposure (see section 4.2.1.2.1).

4.5.3.1.5 Induced cell proliferation

There are several reports apparently demonstrating formaldehyde-induced proliferation in cells below cytotoxic levels of exposure. This phenomenon has been reported from studies involving both *in vitro* and *in vivo* exposures. Tyihak et al. (2001) demonstrated significantly increased cell proliferation in both HT-29 human colon carcinoma and human umbilical vein endothelial cell (HUVEC) lines treated with 0.1mM (the lowest dose) formaldehyde compared

to untreated controls (P<0.0001). This effect was quantified as both an increase in cell number over time (Figure 4-35), and an increase in the percentage of cells undergoing mitosis at each time point. The authors also report a significant (P<0.01) inhibition of apoptosis in formaldehyde-treated cells as compared to untreated cells (data not shown here). In a novel system using xenotransplanted human tracheobronchial epithelial cells, formaldehyde was shown to induce increased cell proliferation at doses below those required for a "massive toxic effect" (Ura et al., 1989).

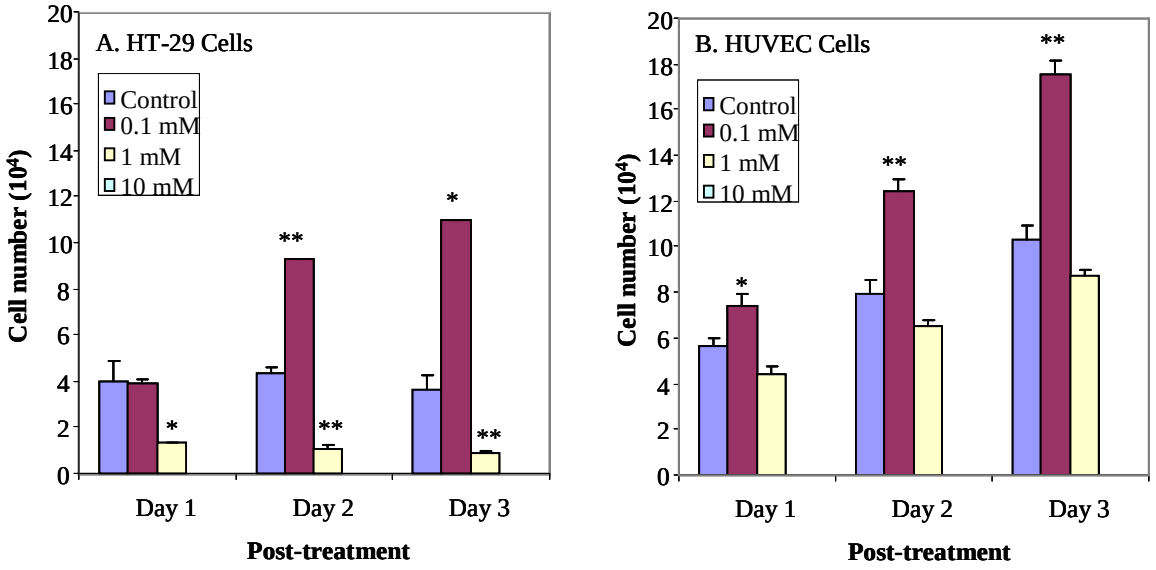


Figure 4-35. Effect of various doses of formaldehyde on cell number in (A) HT-29 human colon carcinoma cells and in (B) human umbilical vein epithelial cells (HUVEC).

Values are average of three samples + SD; * $P < 0.01$ and ** $P < 0.0001$ compared to corresponding controls.

Source: Tyihak et al 2001.

Some animal studies have demonstrated increased cell proliferation after formaldehyde exposures by both inhalation and ingestion (See section 4.2.1). However, whether sustained increases in cell proliferation over baseline rates are observed upon exposure to sub-cytotoxic doses of formaldehyde remains unclear. Several of the inhalation studies demonstrate increased cell proliferation in the nasal epithelium at formaldehyde exposures levels that were sub-cytotoxic—i.e. in the absence of significant cell death. Acute formaldehyde exposures (1 to 3 days) induced increased cell proliferation at discrete locations in the nasal mucosa, where cell

proliferation was measured as a labeling index (percentage of cells pulse-labeled with tritiated-thymidine. Reuzel et al. (1990) reported increased cell proliferation in the nasal passages including the nasoturbinates, maxilloturbinates septum and lateral wall in male Wistar rats exposed at 3 ppm, but not at 0.3 or 1 ppm, formaldehyde for 22 hours/day for 3 days. Zwart et al. (1988) reported increased cell proliferation after exposure to 1 or 3 ppm formaldehyde, 6 hours/day for 3 days or 13 weeks in male and female albino Wistar rats. These increases were transient at level 3 but sustained at level 2 of the nose and were not correlated with cytotoxicity. In contrast, Wilmer et al. (1989), from the same group of investigators and using a similar exposure regimen, reported no increase in cell proliferation after repeated 8–hour exposures at 1 or 2 ppm formaldehyde for 3 days or 4 weeks. Swenberg et al. (1986) demonstrated a transient increase in cell proliferation after a single 8–hour exposure to 0.5 or 2 ppm formaldehyde in male F344 rats but no increases after 3 days or repeated 8–hour exposures. The authors suggest that adaptive responses of the nasal mucosa contribute to the transient nature of formaldehyde-induced cell proliferation. After a series of acute studies at various formaldehyde concentrations, Swenberg and coworkers concluded that, in addition to cell proliferation being concentration-, dose- and time-dependent, the response varies by species and by location of exposure in the nose (Swenberg et al., 1983, Swenberg et al, 1986).

Other methods of quantifying cell proliferation in the nasal mucosa have demonstrated formaldehyde-induced cell proliferation at similar low exposure concentrations. Roemer et al. (1992) measured cell proliferation by flow-cytometry in epithelial cells harvested from the nose and trachea of male Sprauge-Dawley rats exposed to 2 ppm formaldehyde for 6 hours/day for 1 or 3 days and found increased cell proliferation after the 1-day exposure. These increases were transient and were not evident after 3 days of exposure. Cassee and Feron (1994) identified proliferating cells by staining for the presence of proliferating cell nuclear antigen (PCNA). Formaldehyde exposure at 3.6 ppm for 6 consecutive periods of 12 hours (8-hour exposures followed by 4-hour-periods of non-exposure) over three days, qualitatively increased the expression of PCNA in respiratory epithelium at levels 2 and 3 of the nose in albino male Wistar rats (Cassee and Feron, 1994). Hyperplasia, squamous metaplasia and frank necrosis were also reported for these tissues.

Monticello et al. (1990, 1991, 1996) conducted *in vivo* cell proliferation studies in which they exposed F344 rats for short durations (1, 4, 9 and 42 days) as well as much longer durations (13, 26, 52 and 78 weeks) to exposure concentrations of 0, 0.7, 2.0, 6.0, 10.0 and 15.0 ppm. These data are unique in that they also included low exposure concentrations. The authors reported statistically significant increases in cell proliferation only at 6.0 ppm and higher exposure concentrations in the short duration study and only at 10.0 ppm and higher

concentrations in the longer duration study. These data have undergone considerable statistical analysis in several papers as well as in this document. Conolly et al. (2002, 2003) and Gaylor and Conolly (2004) interpreted these data, when combined, as indicating a non-monotonic behavior at low dose. In other words, formaldehyde was judged to result in a reduction in cell proliferation at low dose in comparison to baseline rates, with increased proliferation effect kicking in only at exposures that were cytotoxic. However, as shown in Appendix 5-3 and in Subramaniam et al. (2008), Crump et al. (2008), analysis of the individual animal data shows considerable uncertainty and variability, both quantitative and qualitative, in the interpretation of these cell proliferation data. (For example, even the control data vary over an order of magnitude in some cases. See Figures 5-22 and 5-23 in Appendix 5-3.) These analyses (which were based on the replicate animal data used in the above studies) considered regional formaldehyde dose to the tissue (flux), nasal site and duration of exposure, as well as the number of cells at a given site. The overall conclusion in Section 5.3.3 (and detailed in Appendix 5-3) is that the cell proliferation dose-response at low dose could be reasonably described by both monotonic (with and without a threshold) and non-monotonic curves.

Only one study, by Monticello et al. (1989), quantified cell proliferation in primates after formaldehyde exposure; this study, reported an 18-fold increase in cell proliferation in the nasal epithelium (respiratory and transitional), larynx, trachea and carina of male Rhesus monkeys exposed to 6 ppm formaldehyde compared to controls (See section 4.2.1 for detailed study description). The authors also noted that increased cell proliferation was seen in locations with minimal histological changes, indicating proliferation may be a more sensitive predictor of adverse health effects of formaldehyde exposure.

4.5.3.1.6 Cytolethality and resulting regenerative cell proliferation

The toxic and cytolethal effects of formaldehyde exposure at the POE are well documented after both inhalation and oral exposures (See Section 4.2.1). The nature and progression of tissue injury has been best documented in rodent inhalation assays. Early effects on the nasal mucosa include altered ciliary beat and mucus flow, hyperplasia and metaplasia of nasal epithelium (Morgan et al., 1986a; Morgan et al., 1986b; Monteiro-Riviere and Popp, 1986; Maronpot et al 1986; Rusch et al., 1983 and Monticello et al., 1986). These first changes may be considered adaptive responses. Squamous epithelium may thicken and transitional epithelium may change to squamous epithelium as evidenced by squamous hyperplasia, squamous metaplasia and thickening of the epithelium in these anterior portions of the nose. Tissue damage may be transient at lower formaldehyde exposures as these changes serve to protect tissue from formaldehyde's reactivity. However, higher formaldehyde concentrations can

overwhelm these adaptive responses and result in gross tissue damage. Frank necrosis and focal erosions have been reported in time- and concentration-dependent manner in rodent bioassays.

Both adaptive changes and cytolethality are associated with cell proliferation. However, where adaptive changes are successful, e.g. prevent continued toxic insult to the tissue, cell proliferation is transient. Exposure regimens where the adaptive changes are not adequate to protect the tissue, would result in continued cytoxicity and cell death. Sustained damage to the epithelium would result in sustained cell proliferation to compensate for cell death. A series of rodent bioassays present convincing evidence that chronic inhalation exposures 6 hours a day, 5 days a week at 6, 10 and 15 ppm formaldehyde do result in sustained damage to the nasal epithelium, sustained cell proliferation and tumor development (Kerns et al., 1983, Morgan et al., 1986; Monticello et al., 1990; Monticello et al., 1991 and Monticello et al., 1996). Work by Monticello and coworkers demonstrate that chronic repeated inhalation exposures at 6, 10 or 15 ppm formaldehyde result in sustained cell proliferation at the lateral meatus, mid-septum and maxilloturbinates of rat nasal passages (Monticello et al., 1991 and Monticello et al., 1996).

4.5.3.1.7 Evidence for promotion

There is some evidence, although mixed, that formaldehyde may promote tumor development by other carcinogens, and known initiating agents by various routes of exposure. Formaldehyde exposure in drinking water (0.5% formalin) increased glandular stomach adenocarcinomas in male Wistar rats after initiation with 100 mg/L, N-methyl-N'-nitrosoguanidine (MNNG), compared to MNNG-only-treated rats (Takahashi et al., 1986). In white non-inbred rats, inhalation exposures (3, 30 or 300 ug/m^3 formaldehyde 7hr/day, 5 days/week for 1 year) increased tumor multiplicity per animal and decreased latency of benzo[a]pyrene induced tumors in white non-inbred rats (Yanysheva et al., 1998.) Similarly, formaldehyde skin application decreased tumor latency, in 7,12-dimethylbenz(a)anthacene (DMBA) initiated hairless Oslo mice (Iversen, 1986). Although formaldehyde exposure also increased the tumor multiplicity in Syrian golden hamsters where diethylnitrosamine (DEN) (0.25mg I.P.) was the tumor initiator, positive results were only reported for the exposure regimen where hamsters were exposed to formaldehyde via inhalation 48 hours prior to DEN injection, and then one a week thereafter for life. However, formaldehyde did not increase the number of tumors per tumor bearing animals when only administered beginning one week after all DEN injections. In contrast, bladder cancer was not enhanced by intravesical instillation of 0.5ml of 0.3% formalin, one week after instillation of N-methyl-N-nitrosourea (MNU) in male Fisher rats (Homma et al., 1986).

The observed promotion activity of formaldehyde has been tested in several systems, by different routes of exposure. By several routes of exposure, formaldehyde enhanced tumor development at a site where formaldehyde did not induce tumors alone, without the initiating agent (Takahashi et al., 1986, Yanysheva et al., 1998 and Iversen, 1986). Promotion activity in these studies was evidenced by increased in tumor bearing animals (oral route), increase in tumors per animal (inhalation routes) and decreased tumor latency compared to those animals only exposed to the initiating agent (inhalation route) (Takahashi et al., 1986, Yanysheva et al., 1998 and Iversen, 1986). Although these experiments do not indicate how formaldehyde acts as a promoter in these systems, it is possible formaldehyde-induced mutation, increased cell proliferation or other toxic action could enhance tumor development from another agent.

4.5.3.1.8 Localized Immunosuppression

Formaldehyde exposure has induced localized immune suppression in experimental animals (Dean et al., 1984) and in exposed workers (Lyapina et al., 2004). Repeated inhalation exposures in rodents depopulated the URT and pulmonary tissues of resident macrophages, resulting in a transient decrease in POE host defenses (Admas et al., 1987). After cessation of exposure, the mononuclear phagocyte (MP) populations were replenished and there was a subsequent increase in host defense representing both increased MP numbers and increased bacteriocidal activity of the MPs. These data suggest that peak exposures of formaldehyde may present localized immunosuppression for components of the mononuclear phagocyte system (MPS) in tissues at the site of first contact.

A number of studies have evaluated the ability of formaldehyde to induce systemic immunotoxic effects in humans (Ohtani et al., 2004a, b; Erdei et al., 2003; Thrasher et al., 1990, 1987; Pross et al., 1987). Some studies have reported altered innate immune responses associated with formaldehyde exposure (Erdei et al., 2003), while others have noted adaptive immune response suppression associated with formaldehyde exposure (Thrasher et al., 1990, 1987) and changes associated with alterations to a predominant T—lymphocyte helper 2 (Th2) pattern (Ohtani et al., 2004a, b). In contrast, Pross et al. (1987) did not observe formaldehyde-associated changes in systemic immune function.

Numerous studies have reported increased respiratory tract infections in formaldehyde exposed individuals both in occupational and residential environments (Lyapina et al., 2004; Krzyzanowski et al., 1990; Holness and Nethercott, 1989). Incidences of physician-diagnosed chronic bronchitis were more prevalent in children (under age 15) living in homes with higher formaldehyde (>60 ppb) readings in the kitchen ($p < 0.001$) but this effect was more pronounced ($p < 0.001$) in children simultaneously exposed to environmental tobacco smoke (Kryzanowski

et al., 1990). The prevalence of chronic cough was also increased in adults living in homes with measurable levels of formaldehyde, but data were not shown. Holness and Nethercott (1989) assessed chronic bronchitis in 87 funeral workers, where the average formaldehyde exposure was reported at 0.38 ± 0.19 ppm. Chronic bronchitis was observed in 20 funeral workers (n = 87) exposed to formaldehyde compared with 3 cases of chronic bronchitis in nonexposed referent controls (n = 38). A statistically significant association of self-reported chronic bronchitis and decreased resistance to URT infection was reported in formaldehyde exposed workers compared with controls (p = 0.02) (Lyapina et al., 2004). Of the workers, 41% had a history of chronic respiratory infection and frequent long-lasting infectious inflammatory relapses (group 1a). Another group (group 1b) consisted of 17 exposed workers, 12 of whom had no history of recurrent viral infections of the URT. There was a statistically significant association of frequency and duration of inflammatory relapses between groups 1a and 1b.

Lyapina et al. (2004) also reported effects of formaldehyde exposure on neutrophil respiratory burst activity (NRBA), the capacity of polymorphonuclear leukocytes to produce reactive oxygen radicals in response to chemical or microbial stimuli using flow cytometry. A suite of hematological tests and flow cytometric analysis for respiratory burst activity were performed. Although no significant difference was observed in the spontaneous and stimulated NRBA (median percentage of oxidizing cells) between the 29 exposed workers with URT inflammation and the healthy controls (0.83 versus 1.35, respectively), a separate comparison of the NRBA of 12 workers with chronic, repeating URT infections and 17 workers with short, infrequent episodes of URT inflammations was significant (0.45 versus 1.00, p = 0.037). When the NRBA of the group with chronic URT infections (n = 12) was separately compared with that of the healthy controls (n = 21), the results were also significant (0.45 versus 1.35, p = 0.012). Individuals with chronic URT infections have reduced NRBA that could be due to formaldehyde exposure. Neutrophils respond to tissue damage or local invasion of microorganisms and act to phagocytize foreign cells. If neutrophilic activity is hampered or altered by formaldehyde exposure, then the ability to fight infection will be diminished, leading to prolonged infection. However, no dose-response pattern of formaldehyde exposure could be determined from this study.

4.5.3.1.9 Potential for systemic transport of formaldehyde

In aqueous solution formaldehyde exists in equilibrium with it's hydrated form methanediol (CH_2OH_2) ($K_d = 5.5x10^{-4}$). The equilibrium favors methanediol at physiological temperature and pH (>99.9%) and is readily reversible. In biological systems, as free formaldehyde is removed from aqueous solution through binding with serum proteins and

cellular components, the equilibrium is reestablished by dehydration of methanediol to free formaldehyde. The reversible nature of this hydration reaction describes how a pool of free formaldehyde may be sustained in biological systems.

There is strong and consistent evidence in biological testing systems in vitro that treating cells with formaldehyde in an aqueous media results in significant cytoxicity, cell proliferation, clastogenic effects and clear evidence of mutational events (Section 4.3). Similarly, animal bioassays where formaldehyde is administered in drinking water report portal of entry toxicity including hyperplasia, increased cell proliferation, focal lesions and tumors (Section 4.2.1). It should be noted that URT tissues are covered by an aqueous mucous layer, through which formaldehyde must pass to react the cellular components of the URT. It has been postulated that formaldehyde transports through this mucous layer and the underlying tissues as methanediol (Georgieva et al., 2003).

The dynamic equilibrium between the hydrated and unhydrated forms of formaldehyde in biological systems is well understood. Since the hydration reaction favors methanediol, it is expected that exogenous formaldehyde which reaches the blood will primarily exist as methanediol and is subject to physiological elimination. As free, unhydrated formaldehyde continues to react with serum proteins and cellular components, the blood levels of methanediol are expected to reduce as it is dehydrated to maintain equilibrium. Although some attempts to measure significant changes in free formaldehyde levels in blood after inhalation exposure have not been successful, the half-life in blood has been measured after i.v. injection at approximately 2 minutes (McMartin et al., 1979). Additionally, the detection of antibodies to formaldehyde-hemoglobin adducts and formaldehyde-albumin adducts in exposures workers, smokers and laboratory animals exposed via inhalation provides direct evidence that formaldehyde is able to react with serum albumin and hemoglobin in biological systems (Thrasher et al., 1990, Grammer et al., 1990; Grammer et al., 1993; Dykewicz et al., 1991; Varro et al., 1997 and Li et al., 2007). These data support the hypothesis that exogenous formaldehyde may reach and transport through the blood. If so, formaldehyde (or methanediol) may reach sites distal to the portal of entry.

4.5.3.2 Mode of Action Evaluation for Upper Respiratory Tract Cancer (Nasopharyngeal Cancer, Sino-nasal)

From the above discussion, it can be seen that numerous mechanisms of action for formaldehyde-induced cancer can be reasonably supported based on various known biological actions of formaldehyde (e.g., mutation, cell proliferation, cytotoxicity, and regenerative cell proliferation). Additionally, alternative actions, such as immunosuppression or viral reactivation, are possible, although less data exist to evaluate these MOAs. Rather than a single

MOA, it is plausible that a combination of these factors contribute to cancer incidence in an exposed population. Considering multiple factors may help to better understand the biological and mechanistic basis for the increases in cancer incidence observed in exposed human populations. Unlike animal bioassays, human epidemiological studies may reflect not only the effects of the agent of concern but also numerous other risk factors (e.g., viral status, diet, smoking, etc.). Additionally, human studies may be impacted by biological human variability across individuals, cancer biology (sub-types), wide variability in exposure regimens in human populations, etc. Therefore, if the purposes of exploring the carcinogenic MOA of an agent are to better understand the relevance of a given carcinogen to human populations and to inform the exposure-response analysis, then discussions of MOAs which recognize the interaction of an agent with human variability and various risk factors is an appropriate analysis.

a) **Direct mutagenicity of formaldehyde in cells at the site of first contact:** Mutations, the permanent heritable changes in the genome of the cell, are a primary mechanism for the activation of oncogenes or the inactivation of tumor suppressor genes. Mutagenicity is the most widely recognized determinant of chemical-induced carcinogenicity, and it is difficult to set aside the relevance of direct formaldehyde-induced mutations from its demonstrated carcinogenicity. Formaldehyde-induced mutation in mucosal cells of the URT, throat and buccal cavity may serve to initiate cells, or provide subsequent mutageic events to already initiated cells. Since the mucosal cells have proliferative capacity, and cell proliferation is a normal tissue function, mutations may be fixed and passed to daughter cells due to baseline cell proliferation of the tissue.

   Relevance to humans: This MOA is relevant to humans. The well-documented DNA reactivity (e.g. DPC and DNA adducts) and clastogenicity of formaldehyde in the URT of laboratory animals is a direct effect of formaldehyde on tissues of first contact. As this is a direct acting agent - no distribution or metabolism is required for the genotoxic action – there is little expected species variability. As discussed in chapter 3, there are species differences in flux of formaldehyde into the respiratory mucosal tissues, but this introduces species differences in dosimetry – not mechanism. Finally, the clastogenic effects in nasal and buccal epithelial cells in formaldehyde- exposed workers confirms the direct genotoxic effects of formaldehyde at the first site of contact in humans.

b) **Decrease in DNA repair function within cells at the site of first contact:** A decrease in DNA repair capacity in these tissues by formaldehyde may increase total mutations over time due to either endogenous or exogenous sources of mutation. Although there

are only a few studies which have explored the potential for formaldehyde to reduce DNA repair capacity, the evidence is positive, both in vitro testing systems, and in one study of occupationally exposed humans (Grafstrom 1985; Hayes et al., 1997).

Relevance to humans: This MOA is considered relevant to humans. The general population is exposed to various carcinogens, many with mutagenic potential, at sites of first contact including; air pollution, tobacco products, nitrosamines and viruses. Additionally, there are endogenous sources of DNA damage and mutagenicity in humans (e.g. lipid peroxidation, oxidative stress). The demonstration of reduced DNA repair activity (O6-alkylguanine-DNA alkyltransferase activity) in formaldehyde-exposed mortuary students suggests this toxic action of formaldehyde is possible in humans.

c) **Formaldehyde-induced cell proliferation:** Formaldehyde-induced cell proliferation in the oral and respiratory mucosa may be considered a key event in conjunction with the genotoxic effects, and induced mutational events observed with formaldehyde exposure. This MOA is intended to describe events which occur below exposure levels which induce cell death and mucosal lesions. Therefore this MOA is comprised of two key events:

   a. Formaldehyde-induced genotoxicity or mutation
   b. Formaldehyde-induced cell proliferation

DNA replication during cell proliferation may serve to translate DNA damage or a formaldehyde-related DNA lesion into a permanent change in the sequence of nucleic acids during replication of the DNA– e.g. ‘fix’ a mutation from DNA damage. Additionally formaldehyde-induced cell proliferation may provide an opportunity for initiated cells to proliferate, increasing the potential for additional mutation events and transformation. The increased cell proliferation observed in the mucosal tissues in direct contact with formaldehyde during inhalation exposures may serve to amplify the risk of cell transformation from mutation alone. Researchers have noted that increased cell proliferation may be transient in some locations as adaptive responses compensate (Swenberg 1983). However, evidence in both monkeys and rodents indicate that increased cell proliferation in repeated exposures across time do result in sustained cell proliferation. Data in Rhesus monkeys indicates increased cell proliferation is observed beyond the nasal cavities to the larynx, trachea and carnia (first tracheal branching)(Monticello et al., 1989). Additionally, the authors note that cell proliferation

is a more sensitive indicator of effects on the epithelium, observed even when minimal histological changes were present.

Human Relevance: Both formaldehyde-induced mutation and cell proliferation are direct effects on the oral and nasal mucosa, well documented in rodent models with supporting evidence in human epidemiological studies. Therefore both key events are relevant to humans. As noted above, there are species differences in localized flux of formaldehyde into the tissues of the oral and respiratory tract based on structural differences in the airways, as well as breathing patterns. Although these differences may effects the dosimetry of the formaldehyde absorption into the tissues, this only influences the magnitude of response at any given location. Data from exposed Rhesus monkeys which documents formaldehyde-induced cell proliferation in tissues beyond the nasal cavity, and tissues with minimal histological changes supports a role for cell-proliferation in the observed cancers in humans, which occur beyond the nasal cavities, and in tissues without formaldehyde-related focal lesions.

d) **Cytotoxicity-induced cell proliferation (CICP):** Cell death followed by compensatory cell proliferation is a reasonable MOA for agent-induced cancer. It should be noted that the exposure conditions which result in CICP in rodents is known to result in significant DNA reactivity and genotoxicity. Therefore, formaldehyde-induced mutations cannot be excluded from this MOA. The animal bioassays support the carcinogenic potential of formaldehyde in this context (Kerns et al., 1983; Selkemur et al, 1983 and Monticello et al 1986). The majority of squamous cell carcinomas (SCCs) seen in formaldehyde-exposed rats have been localized to the lateral meatus and mid-septum in the nasal passages (Morgan et al., 1986; Monticello et al., 1996), while polyploid adenomas have predominantly been reported at the maxilloturbinates (Morgan et al., 1986; Monticello et al., 1996). Morgan et al. (1986) speculated that the maxilloturbinate was less susceptible to SCC due to metabolic differences. However, Monticello et al. (1996) later suggested that the smaller population of cells available at the maxilloturbinate accounted for fewer SCCs observed at that site. Regardless, for those locations where SCCs do arise in rats chronically exposed to formaldehyde, a clear temporal relationship can be demonstrated for dose regimens capable of producing sustained epithelial damage and sustained cell proliferation to eventual tumor development. Conversely, tumors are not observed in these rodent models at those sites in the nasal passages without sustained cell proliferation.

Relevance to humans: Human exposure to formaldehyde would most likely involve chronic exposures to indoor levels of formaldehyde, and episodic exposures in the environment or from an occupational exposure (See review in Chapter 2). An exposure scenario parallel to that used in chronic rodent bioassays is unlikely (e.g. 2-15 ppm 6-8 hours/day, 5 days/week for 10-30 months). Exposure conditions are difficult to assess especially in retrospective studies. However, only the most extreme industrial work conditions would result in human exposures similar to those that produce sustained compensatory cell proliferation in animal studies (i.e. 6-15 ppm 6 hrs/day, 5 days per week). Gross tissue lesions as reported in rodents from repeated chronic exposures at 6 and 10 ppm formaldehyde have not been reported from workplace exposure, and only minor histopathological changes have been noted (Boysen et al., 1990 and Holmstrom and Wilhelmsson et al., 1989). It is possible that workers were episodically exposed to formaldehyde levels which resulted in cell death and focal or gross lesions requiring cell proliferation for tissue remodeling or repair. However, it is unexpected that these conditions would be relevant to human environmental exposures. Therefore, although regenerative cell proliferation is retained as a reasonable MOA for formaldehyde carcinogenicity in experimental animals, it is unclear whether it is relevant to the extrapolation of health risks to formaldehyde exposures in the general environment.

e) **Promotion:** Several animal studies indicate that formaldehyde exposure may promote tumor formation due to other carcinogenic or initiating agents. There are positive data by several routes of exposure (oral, dermal and inhalation) and promotion has been reported as an increase in tumor bearing animals, an increase in tumors multiplicity or a decrease in tumor latency with formaldehyde exposure in conjunction with the initiating agent compared to tumors from the initiating agent along, or formaldehyde alone. The specific key events which may explain this promotion effect are unknown but may include several of the mechanisms discussed as potential MOAs for formaldehyde: mutagenicity, mitogenesis, co-carcinogenicity, immunosuppression. Promotion is considered here as a separate MOA, since these activities are noted for experimental conditions and tumor sites where formaldehyde did not induce tumors in the absence of the initiating agent.

Relevance to humans: Although the human epidemiologic literature doesn't address issues of tumor promotion, the nature of the cancers of concern indicate that chemical promotion may be relevant to cancer incidence for these sites. Many of the risk factors for NPC and other mouth and oral and URT cancers include direct mutagens (e.g.

smoking, dietary nitrosamines) where a promoting agent would be expected to increase cancer incidence with these other risk factors. Additionally, the well known viral risk factors for cancers of the mouth and URT also suggest a role for promoting agents to human cancer incidence. Although only tangential evidence, this does suggest that the promoting activity of a chemical agent, would be relevant to the agent's carcinogenicity at these sites. Therefore, the potential for formaldehyde to act as a promoter with other initiators – is considered relevant to formaldehyde's carcinogenic MOA.

f) Increased URT infections / viral reactivation: Inhalation exposure to formaldehyde has been shown to decrease the defenses of the body against infection through two mechanisms: 1) damage to the protective mucous barrier and function of the mucociliary apparatus; and 2) localized immunosuppression. These effects have been demonstrated in both exposed humans and controlled animal experiments. Additionally, increased respiratory tract infections are associated with formaldehyde exposure in several populations. Common viral agents (e.g. Epstein barr virus) are known risk factors for NPC, sinonasal cancers and other URT cancers. Although direct evidence does support increased URT infections due to formaldehyde exposure, and URT infections are considered risk factors for URT cancers, direct evidence for formaldehyde-related infections leading to cancer is lacking. There is however one epidemiological study which finds the association between formaldehyde and NPC is strengthened in Epstein barr virus sero-positive cases versus sero-negative cases. These data suggest a possible role for formaldehyde in infection, viral reactivation, or co-carcinogenicity with a viral agent.

Relevance to humans: The potential role of increased URT infections and immunosuppression at the portal of entry is considered to relevant to humans. Data in humans are available to support both key events in this MOA. Additionally, epidemiological studies are conducted in human populations where individuals may be exposed to various viral agents across the study period. Therefore, toxic actions by formaldehyde which may increase URT infections, or viral-reactivation at the site of first contact, could be acting in conjunction with viral agents to contribute, in part, to observed associations between formaldehyde exposure and increased URT cancer.

Summary and integration of key events:

Each of the hypothesized MOAs discussed above to better understand the carcinogenic potential of formaldehyde is supported by formaldehyde-specific evidence, either in animal

studies, human studies or both. For those key events studied in animal models such as cell proliferation, genotoxicity, degradation of the mucociliary apparatus and CICP, supporting evidence is available in more than one species, multiple strains (e.g. rats) and has been reported by multiple researchers. Therefore the overall database supporting these key events in laboratory studies, and their corresponding MOAs is fairly large. In contrast, some key events relevant to humans, but less studied in animal models may have a small supporting database (e.g. increased respiratory tract infections). These alternative MOAs are retained as potentially relevant to the carcinogenic action of formaldehyde as the intent of this discussion is to identify modes of action will may contribute to the observation of increased upper respiratory tract cancers in exposed human populations. It is noted that additional study is needed to better understand the range of effects formaldehyde may have at sites of first contact in humans.

The MOAs which include genotoxicity, mutation, decreased DNA repair, increased cell proliferation and CICP are interrelated. Conditions which provide both a source of cell proliferation and increased mutation would be expected to increase neoplastic transformation. Formaldehyde acts on the target tissue, the respiratory epithelium, to induce each of these events. However, these key events operate across different exposure ranges and present different exposure response relationships. For example, formaldehyde-induced mutations would be expected across the exposure range, where any incremental increase in genotoxicity and formaldehyde-related mutation would contribute to background levels, with the potential to increase cancer risk incrementally. In contrast, focal and gross lesions to the respiratory mucosa due to cytolethality are not observed unless exposure concentrations are sufficient to provide localized tissue doses (flux) required to result in cell death and related compensatory cell proliferation. Since tissue dose (flux) is dependent on not only exposure concentration but also duration of exposure and location in the respiratory tract (Section 3.4), and varies by species, correlation of exposure concentrations to tissue responses directly are complex. Exposure response relationships for the key events (cell proliferation, genotoxicity, degradation of the mucociliary apparatus and CICP) are reported by exposure concentration, not tissue flux, which would be a more biologically relevant measure.

Although the tissue dose-response relationships for formaldehyde induced mutation, mitogenesis and cytolethality are different, the effects at the tissue level cannot be easily disaggregated. At any given exposure concentration, target cells in the respiratory tract will experience different effective tissue concentrations of formaldehyde. Measurement of cell proliferation, DNA protein crosslinks or genotoxicity may require examining a population of cells which would have been subject to different flux rates of formaldehyde (See chapter 3). Similarly, when evaluating the tumor dose response, cells within the target tissue will represent a

range of target tissue formaldehyde concentrations. Therefore, an integrated MOA scheme is hypothesized where key events may influence the observed tumor response differentially across the exposure response range (Figure 4-36). This schematic illustrates the potential for genotoxicity and formaldehyde-induced mutation to occur where tissue dose (flux of formaldehyde into the tissue is minimal). Where tissue dose is increased, formaldehyde-induced cell proliferation is observed in addition to genotoxicity. As tissue dose increases and formaldehyde effects on the respiratory mucosa are more severe, gross pathology including focal and gross lesions due to cell death are noted. Therefore, several of the MOAs presented above may be operative and relevant to human exposures at exposure levels resulting in minimal tissue flux – a) direct formaldehyde genitoxicity and resulting mutation, b) inhibition of DNA repair and c) formaldehyde induced cell proliferation in conjunction with mutation. CICP, which involves localized and gross tissue lesions would be operative at higher exposure levels. There is little data to inform the dose range over which the remaining hypothesized MOAs may operate (promotion and increased respiratory tract infections/viral action).

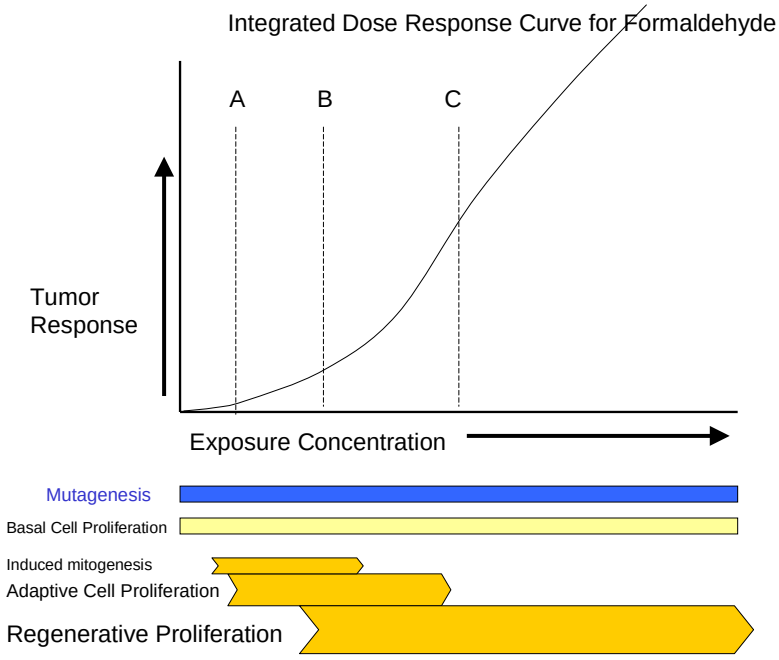


Figure 4-36: Integrated MOA scheme for respiratory tract tumors

4.5.3.3 Mode(s) of Action for Lymphohematopoietic Malignancies

4.5.3.3.1 MOA evaluation for Leukemia

Leukemia may arise from stem cells and progenitor cells in the bone marrow (e.g. acute and chronic myeloid leukemia) or from mature lymphocytes (e.g. chronic lymphatic leukemia,

hairy cell leukemia) (Figure 4-33, Section 4.5.2). Although there is a consistent association between formaldehyde exposure and forms of leukemia when considered as group of diseases (Table 4-91, Section 4.5.2), the strongest and most consistent associations are seen specifically with myeloid leukemia. Little evidence supports an association between formaldehyde exposure and other specific leukemia subtypes, although two studies support a strong association between formaldehyde and "other leukemia and unspecified leukemia (ICD-9 code 207). Therefore, this MOA evaluation will focus on mechanisms which may impact all forms of leukemia (e.g. bone marrow toxicity) or those specific to myeloid leukemia. The mechanistic data supporting the key events in this analysis are presented in section 4.5.3.1.

a) **Direct effects of formaldehyde on a circulating stem cell or progenitor cell present at the portal of entry:** Hematopoietic stem cells do circulate throughout the body and can be harvested from peripheral blood. Formaldehyde exhibits a range of toxic effects at the site of first contact including genotoxic effects believed to be mediated by direct DNA reactivity (Section 4.3). Formaldehyde is known to directly react with blood components in formaldehyde exposed humans and animals resulting in both hemoglobin and albumin adducts (Thrasher et al., 1990, Grammer et al., 1990; Grammer et al., 1993; Dykewicz et al., 1991; Varro et al., 1997 and Li et al., 2007). Therefore, it has been hypothesized that formaldehyde could react with DNA in circulating hematopoietic stem cells (Zhang et al., 2009) resulting in heritable mutations which may contribute to leukemia incidence. Recently Zhang et al. (2010) have tested the hypothesis that exogenous formaldehyde may damage circulating stem cells. Clastogenic effects were found in circulating hematopoietic stem cells cultured from formaldehyde exposed workers. The reported aneuploidy was demonstrated as significant increases in both monosomy 7 and trisomy 8. These specific chromosomal changes are consistent with those reported for agent-induced myeloid leukemia (Zhang et al., 2010).

   Relevance to Humans: This hypothesized MOA is considered relevant to humans. Supporting evidence is found in humans for formaldehyde direct reactivity with blood proteins (e.g. albumin and hemoglobin) as well as clastogenic effects in circulating hematopoietic stem cells in formaldehyde exposed workers.

b) Bone marrow toxicity: Direct bone marrow toxicity is the most studied leukemogenic action for an endogenous agent (e.g. benzene, ionizing radiation). It is believed that an agent which exerts its toxicity on the bone marrow, resulting in translocations and heritable mutations in hematopoietic stem cells may cause leukemia. It has been

hypothesized that formaldehyde may transport to the bone marrow in its hydrated form (methandiol) and react with cellular proteins, and DNA casuing direct effects on components of the bone marrow. Pancytopenia (a reduction in blood borne cells formed in the bone marrow) is a symptom of direct bone marrow toxicity and is observed with other leukemogenic agents (e.g. benzene, ionizing radiation). A recent review of 8 published studies of formaldehyde exposed workers in China by Tang et al. (2009) indicates 7 of the studies provide evidence of reduced white blood cell counts, platelet levels and hemoglobin levels associated with formaldehyde exposure. A study of occupationally exposed nurses provided a correlation between decreased white blood cell counts and formaldehyde exposure (Kuo et al., 1997). A recent study by Zhang et al. (2010) provides the best evidence for bone marrow toxicity, where they report not only a reduction in white blood cell counts, but reductions in cell counts of all the blood cells, as well as increased mean cell volume. Although these reductions did not meet the clinical definition of pancytopenia (when averaged across the study population), reduction of all blood borne cells formed in the bone marrow is consistent with the bone marrow toxicity associated with pancytopenia seen with other leukemogens (Zhang et al., 2010).

Relevance to Humas: This hypothesized MOA is considered relevant to humans. Supporting evidence is found in humans for bone marrow toxicity in formaldehyde exposed workers.

4.5.3.3.2 MOA evaluation for Lymphomas (e.g. Hodgkin lymphoma, Multiple myeloma)

The general MOA for formaldehyde is based on direct chemical reactivity and toxic effects at the portal of entry (POE). Formaldehyde is directly and indirectly genotoxic, and reacts with cellular proteins and DNA in cells which it comes into contact. Additionally, immunosuppression, viral reactivation and promotion effects are relevant to lymphoma and related malignancies. Therefore, the key events for the adult cell lymphoid cancers would include these actions. Lymphoid tumors (e.g. lymphocytic leukemia, B-cell lymphoma, mantle cell lymphoma [a rare form on non-Hodgkin lymphoma] and myeloma) may arise from cells present at the portal of entry (POE) (Figure 4-33). The location and function of mature lymphocytes contribute to their vulnerability to transformation by agents at the POE. Therefore, a brief summary of the immuno-biology of these cells is provided in order to provide context for the MOA evaluation:

Location: Lymphocytes are present in the oral and respiratory tract epithelium, as well as in cell aggregates and tertiary immune structures (e.g. germinal centers) in the mucosal

tissues (Zuercher and Cebra, 2002; Zuercher et al., 2002; Wu et al 1997 and Kupper et al., 1990). These mucosa-associated lymphoid tissues (MALT) provide the opportunity for formaldehyde to directly interact with components of the immune system present at the POE (Wu et al., 1997, Claeys et al. 1996, Park et al., 2003 and Fujimura 2000). Intraepithelial lymphocytes are present in the pseudostratified epithelium of the nasopharyngeal passages and there are aggregates of immune cells and germinal cells present in these tissues. Crypts containing mature lymphocytes exist at the surface of the nasal epithelium (Fujimura 2000). Microfold cells or M-cells form the crypts, where the lymphocytes are covered by a thin membrane (Figure 4-37). Functionally, these lymphocytes identify and process foreign antigens at the POE (Fujimura 2000). Therefore the mature lymphocytes within these crypts, exposed to exogenous agents, are involved in active immune responses to foreign antigens.

Clonal Expansion: Mature lymphocytes (both B and T-cells) clonally expand their populations in response to an exogenous antigen when a humoral immune response is stimulated. Therefore cell proliferation is a normal function of these mature lymphocytes and occurs every time there is an infection. Cell proliferation of mature B and T-cells, responsive to a particular antigen, occurs in active germinal centers (including those within the respiratory tract). Cells may be exposed to exogenous agents during the immune response, or cells responding in the germinal center may have previously been in the epithelium or M-cell crypt.

Somatic Hypermutation: Normal immune function includes the process of somatic hypermutation where B-cells undergo DNA rearrangement of the variable region genes to produce novel antibodies specific to a given antigen. This process is key to adaptive immunity and demonstrated by the basic principles of immuno-biology which underlie vaccination theory. Gene sequencing of adult B-cell lymphomas and leukemias indicate that the chromosomal regions involved in somatic hypermutation correspond to known oncogenes in these cancers. The vulnerability of these processes is evidenced by the observation that approximately 90-95% of adult lymphomas and leukemias are of B-cell origin (Gordon et al., 2003). Formaldehyde-induced protein-protein crosslinking could disrupt cell processes including somatic hypermutation and cell mitosis, resulting in agent-induced translations similar to those found in spontaneous B-cell malignancies.

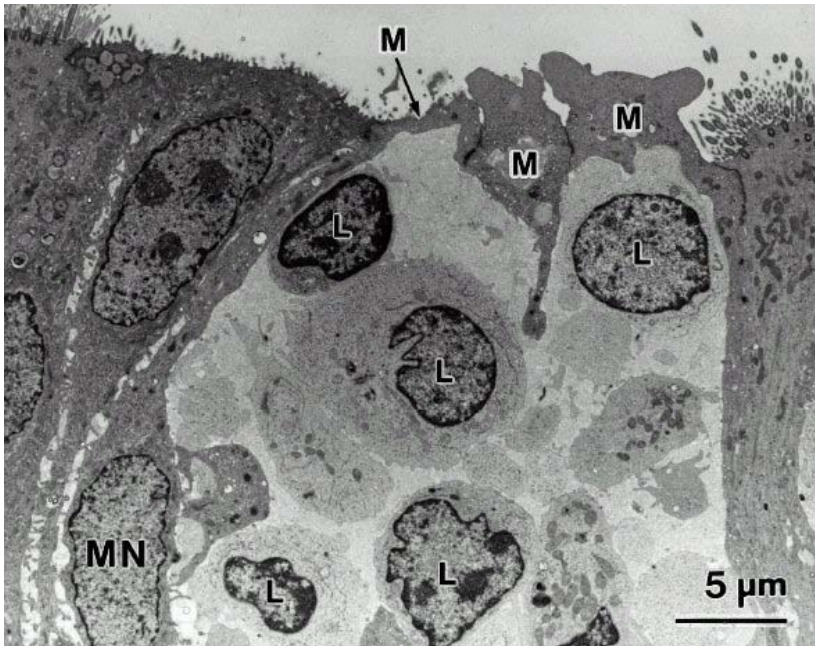


Figure 4-37. Location of intra-epithelial lymphocytes along side epithelial cells in the human adenoid. (Source Fujimura et al., 2000)

a) Direct or indirect formaldehyde-induced mutation in cells at the site of first contact: Immune cells including intraepithelial lymphocytes, and cells in mucosal associated lymph tissue (MALT) are collocated with the epithelial cells from which URT cancers arise (Figure 4-37). Therefore the direct and indirect mutagenic potential for formaldehyde is equally applicable to components of the immune system present in these tissues. Mutations, the permanent heritable changes in the genome of the cell, are a primary mechanism for the activation of oncogenes or the inactivation of tumor suppressor genes. Mutagenicity is the most widely recognized determinant of chemical-induced carcinogenicity, and it is difficult to set aside the relevance of direct formaldehyde-induced mutations from its demonstrated carcinogenicity. Formaldehyde-induced mutation in immune cells present at the site of first contact, may initiate cells or or provide subsequent mutagenic events to already initiated cells. The competence of our immune system relies on the proliferation of peripheral lymphocytes in response to immune challenge (infection). Additionally, heritable changes to the variable gene regions in B-cells generated during somatic hyper-mutation are essential to adaptive immunity (e.g. immunization) demonstrating that permanent heritable changes in the DNA of peripheral B-cells are passed to daughter cells and retained in the body for decades. Any agent-induced mutations would be similarly propagated and retained with the potential to contribute to the transformation of mature lymphocytes.

Relevance to humans: This MOA is relevant to humans. The well-documented DNA reactivity (e.g. DPC and DNA adducts) and clastogenicity of formaldehyde at the POE in laboratory animals is a direct effect of formaldehyde on tissues of first contact, and these mechanisms are considered relevant to humans. As with epithelial cells, clastogenic effects in peripheral lymphocytes are documented in formaldehyde-exposed students and workers, confirming the genotoxic effects of formaldehyde in immune cells, from which lymphomas and related diseases may arise (Section 4.5.2Figure 4-33).

b) **Formaldehyde-induced protein-protein crosslinks may disrupt somatic-hypermutation:** Although not as well studied as DNA-protein crosslinks, formaldehyde also formes crosslinks between amino acids on proteins (Section 4.5.3.1.3 for details). Specific oncogenes for malignancies which arise from mature B-cells are linked to errors in the process of somatic hyper-mutation (Greaves et al. 2004). If formaldehyde creates protein crosslinks in competent B-cells which effect the process of DNA rearrangement, formaldehyde may generate translocations and related oncogenes similar to those observed in observed in spontaneous B-cell malignancies.

Relevance to humans: This hypothesis has not been tested in either exposed human or animal test systems. However, the link between somatic-hypermnutation and B-cell oncogenes is well established and perturbation of this process by an exogenous agent is a reasonable extension of the current understanding of the etiology of B-cell malignancies.

c) Increased URT infections / viral reactivation: Inhalation exposure to formaldehyde has been shown to decrease the defenses of the body against infection through two mechanisms: 1) damage to the protective mucous barrier and function of the mucociliary apparatus; and 2) localized immunosuppression (Section 4.5.3.1). These effects have been demonstrated in both exposed humans and controlled animal experiments. Additionally, increased respiratory tract infections are associated with formaldehyde exposure in several populations. Common viral agents (e.g. Epstein barr virus) are known risk factors for malignancies which arise from mature lymphocytes. Thus, increased URT infections or viral reactivation due to formaldehyde exposure may influence the incidence of these cancers.

Relevance to humans: The potential role of increased URT infections and immunosuppression at the portal of entry is considered to be relevant to humans. Data in humans are available to support both key events in this MOA. Additionally, co-exposure to infectious agents (including viruses) would be expected in participants in an

epidemiological study, suggesting an MOA which acted in conjunction with infectious agents may be relevant to agent-induced cancer. Therefore, toxic actions by formaldehyde which may increase URT infections, or viral-reactivation at the site of first contact, could be acting in conjunction with viral agents to contribute, in part, to observed associations between formaldehyde exposure and increased lymphoma and related diseases.

4.5.3.3.3 Summary and evaluation of hypothesized MOA(s) for Lymphohematopoietic Malignancies

The well-documented direct toxic action of formaldehyde on cells at the site of first contact is a general effect based on the reactivity of formaldehyde with cellular components (e.g. proteins and DNA) (Section 4.5.3.1). As a general effect, it is reasonable that these toxic effects would be relevant to all cells which come into contact with formaldehyde. The current debate regarding the biological plausibility of formaldehyde-induced lymphohematopoeitic malignancies centers around a perspective that the diseases within this general grouping are systemic cancers arising only out of bone marrow toxicity (Heck et al., 2006, Pyatt et al., 2008) and that it is implausible for formaldehyde to induce bone marrow toxicity. The above MOA evaluation expands the current debate by considering the impact of POE toxicity on elements of the immune system and cancers might arise from these cells (Section 4.5.3.3.2) and by presenting data which support the observation that formaldehyde is associated with bone marrow toxicity and damage to circulating stem cells in exposed humans (Section 4.5.3.3.1).

As significant increases in free formaldehyde in peripheral blood from exogenous exposure has not been detected (Heck et al., 1987), it has been hypothesized that formaldehyde does not transport and therefore cannot exert toxic effects outside of the tissues at the site of first contact (Heck et al., 2006, Pyatt et al., 2008). In contrast to this hypothesis, effects are seen in formaldehyde-exposed humans which indicate systemic effects on the hematopoietic system including reduced white blood cell counts, clastogenic effects in peripheral blood lymphocytes and aneuploidy in circulating stem cells (Tang et al., 2008, Zhang et al., 2010 and Section 4.5.3.1). These observed effects in humans are consistent with agent-induced bone marrow toxicity and are observed with other well-studied exogenous leukemogens (e.g. benzene and ionizing radiation.) It is unknown if formaldehyde is distributed systemically to exert its effects directly on cells in the bone marrow or if damage to circulating stem cells or progenitor cells would be sufficient to result in the observed effects in humans (Zhang et al., 2010). Additional research is needed to better determine the potential for systemic transport of formaldehyde considering both detection of its hydrated form (methylene glycol) as well as formaldehyde

protein adducts (e.g. FA-GSH and FA-albumin). Similarly the results of Zhang et al. (2010) need to be extended (analysis for additional chromosomal aberrations) and repeated. Although further evidence is needed to better understand the hypothesized mechanisms for formaldehyde-induced effects on hematopoietic stem cells, the observed hematotoxic effects in humans cannot be set aside. Therefore, however unlikely, the current data support the biological plausibility of formaldehyde effects on the hematopoietic system.

4.5.4. Hazard Characterization FOR Formaldehyde Carcinogenicity

Formaldehyde is Carcinogenic to Humans by the Inhalation Route of Exposure

Based on the results from a large and well-followed longitudinal cohort study of industrial workers and several case-control studies, the epidemiologic evidence is sufficient to characterize the association between formaldehyde exposure and nasopharyngeal cancer (NPC) as causal in humans (Hauptmann et al., 2004; Hildesheim et al., 2001; Vaughan et al., 2000). As a group, upper respiratory tracts sites of direct contact with formaldehyde upon inhalation (i.e., salivary gland, mouth, nasopharynx, nasal cavity and larynx) also showed sufficient evidence of a causal association. Case-control studies have demonstrated associations between formaldehyde exposure and rare cancers of the URT. Luce et al. (2002) evaluated pooled data from 12 case-control studies and demonstrated a statistically significant increased risk between formaldehyde exposure and sinonasal cancer. Hypopharyngeal cancer was linked with formaldehyde exposure with an OR of 3.78 (95% CI 1.50-9.49) in another case-control study (Laforest et al., 2000). Hauptmann and colleagues (2004) concluded that in spite of the small numbers of deaths from cancers of the URT, the positive associations with average intensity and peak exposure were consistent with the carcinogenicity of formaldehyde at these sites of first contact. The finding that formaldehyde inhalation causes nasal squamous cell carcinoma in animals (Section 4.2.1.2) further supports the determination of a causal association of formaldehyde exposure and increased risk of upper respiratory tract cancer in humans. Both humans and animals developed tumors within the upper respiratory tract, the POE site expected to receive direct exposure to formaldehyde.

Overall, there is a consistent association between formaldehyde exposure and various forms of lymphohematopoietic (LHP) cancers, with all leukemias, myeloid leukemia specifically, Hodgkin lymphoma and multiple myeloma demonstrating the greatest strength and consistency of results. Where exposure-response data exist, exposure-response trends have been seen for all LHP malignancies, all leukemia, myeloid leukemia and Hodgkin lymphoma (Pinkerton et al., 2004; Beane Freeman et al., 2009). Taken together, the data demonstrate a consistent association, across various worker populations, with the expected temporal association

to exposure and defined exposure–response relationships in two different worker cohorts. The strongest associations tend to be with myeloid leukemia and Hodgkin lymphoma. The criterion of reasonable biological plausibility is easily met for the majority of the diseases which contribute to an observation of all LHP cancers, specifically the cancers derived from mature lymphocytes. The potential for formaldehyde-induced LHP cancer is further supported by the results of animal bioassays, where formaldehyde-induced leukemia and lymphoma had been demonstrated in 3 independent studies in two species (rats and mice) and both sexes.

4.6. SUSCEPTIBLE POPULATIONS

"Susceptible subpopulations" is used here to refer to factors, such as life stage, genetics, health status, etc., that may predispose individuals to greater response to an exposure. This greater response could be achieved either through differences in exposure to the chemical or differences in underlying toxicokinetic (TK) and toxicodynamic (TD) differences between the susceptible and other populations. For example, life stages may include the developing individual before and after birth up to maturity (e.g., preconception, embryo, fetus, young child, adolescent), adults, or aging individuals. Another susceptibility factor is genetics. Specifically, susceptible subpopulations may also include people with specific genetic polymorphisms that render them more vulnerable to a specific agent or people with specific diseases or pre-existing conditions (e.g., asthmatics). The term may also refer to gender differences, lifestyle choices, or nutritional state (USEPA, 2002, Section 4.3.2.3).

A discussion of a comprehensive list of all possible susceptibility factors affecting exposure and response to formaldehyde, or any chemical, is not possible. Therefore, the discussion of susceptibility factors focuses on 1) factors hypothesized to be of importance to formaldehyde; and 2) factors for which there are available formaldehyde data. A partial list of these factors includes gender, genetic polymorphisms, preexisting disease status, nutritional status, diet, and previous or concurrent exposures to other chemicals. Qualitatively, the presence of multiple susceptibility factors will increase the variability that is seen in a population response to formaldehyde toxicity.

4.6.1. Life Stages

Individuals at different life stages are physiologically, anatomically, and biochemically different. Examples include physiological changes that occur through the lifespan (Selevan et al., 2000). They may also have distinctive exposure pathways (i.e., transplacental, breast milk ingestion), and exhibit differences in behavior (U.S. EPA, 2006b; NRC, 1993). Early life stages (i.e., during development, prior to mature adulthood) and the later life stages (i.e., aging) differ

greatly from mature adulthood in body composition, organ function, and many other physiological parameters that can impact the TK and/or TD of chemicals and their metabolites (ILSI, 2003). This section presents and evaluates the pertinent published literature available to assess whether and how individuals of differing life stages may respond differently to formaldehyde.

4.6.1.1. *Early Life Stages*

4.6.1.1.1. Factors influencing exposure and dosimetry

For all life stages, the primary exposure routes for formaldehyde include inhalation and, in some cases, ingestion (see Chapter 5). Some exposure scenarios may be child specific. For example, to the extent that the presence of baby furniture produced with formaldehyde in a child's house contributes to greater concentrations in a child's room, exposures for very young children in those circumstances may be increased (Environment California, 2008). As with all chemicals, placental transfer and breast milk ingestion are exposure pathways that are unique to early life stages. Studies assessing early life stage exposure pathways to formaldehyde have not been performed. Presumably, unmetabolized formaldehyde reacts too quickly to be effectively transported from mother to fetus by placental transfer; in addition, formaldehyde is not lipophilic and is therefore unlikely to accumulate in breast milk. However, the relevant dose metric for formaldehyde-related effects may vary depending on the specific target of concern (e.g., direct toxicity at the portal of entry versus systemic effects); insufficient information is currently available to determine whether individuals in different life stages are at higher risk for exposure to specific target tissues.

There are some calculations however which shed light on lifestage differences in the inhaled tissue dose at the portal of entry. Using respiratory tract surface areas and ventilation rates reported in the literature and the scheme in USEPA (1994), Ginsberg et al. (2005) calculated that overall extrathoracic absorption of highly reactive and soluble gases is similar in adults and children. These results are in agreement with that of Garcia et al. (2009) who used computational fluid dynamics to study differences in the nasal dosimetry of reactive, water-soluble gases between 5 adults and 2 children, aged 7 and 8 years old. Overall uptake efficiency, average flux (rate of gas absorbed per unit surface area of the nasal lining) and maximum flux levels over the entire nasal lining did not vary substantially between adults and children (1.6-fold difference in average flux and much less in maximum flux). On the other hand, the local flux of formaldehyde varies between the two children by a factor of 2 to 4 at various distances along the septal axis of the nose. The results in Garcia et al. (2009) have been further described and evaluated in Appendix 3-1. Under normal resting breathing conditions, it is expected that very

little formaldehyde is delivered to the lung. However, under higher activity as well as mouth breathing scenarios, both of which appear likely to happen more regularly in children, formaldehyde dose to the lung will be substantial.[5]

The toxicokinetic characteristics of formaldehyde are described in Chapter 3, with absorption and distribution studies discussed in Sections 3.2 and 3.3. Studies to assess differential absorption or distribution of formaldehyde in early life stages have not been performed and represent a significant data gap. The metabolism of formaldehyde is described in section 3.4. Expression of the enzymes that metabolize formaldehyde (ALDH2 and FALDH, and specifically ADH3) is known to be developmentally regulated and thus may alter the TK of formaldehyde in early life stages. ADH3 is ubiquitously expressed and is present in the human fetus, neonate, and 1- to 10-year-old children (Hines and McCarver, 2002; Estonius et al., 1996). During early development in rodents, when neurulation first begins and forms collections of somites along the neural tube, ADH3 activities are significantly lower (at 8–10 and 11–13 somite stages) and suggest a decreased ability to detoxify formaldehyde in the early embryo (Harris et al., 2003). ADH mRNA expression levels appear to be age related, with decreased expression of ADH common in premature neonates and infants up to 5 months old. Thereafter, ADH expression increases and is dependent on body weight (Ginsberg et al., 2004). Benedetti et al. (2007) reported that decreased ADH expression persisted until age 2 to 5 years. Westerlund et al. (2005) tracked the ontogeny of ADH3 specifically and reported that ADH3 expression was ubiquitous in mouse and rat embryos and was the only ADH enzyme to be consistently localized to brain tissue, suggesting a housekeeping function. Thus, neonates and very young children may have a decreased ability to metabolize formaldehyde due to differential expression of ADH3 in development compared that of with adults; however, activity levels of this enzyme and alternate pathways specific to children are not available in the literature.

4.6.1.1.2. Life-stage exposure and adverse health outcomes

In general, exposure to toxic agents during early development (i.e, pre-conception, prenatal stages, or postnatal development) may affect organ development and may also lead to increased disease susceptibility later in life. Following early life stage exposure to formaldehyde, a number of adverse health outcomes have been observed, including alterations in the respiratory, reproductive, and neurological systems. For example, the developing respiratory tract may be more vulnerable to insult compared with an adult respiratory tract, and thus, increase the severity of response. The potential for reproductive and developmental toxicity of

[5] For example, in the case of ozone concentrations of 0.1 ppm, a moderately reactive gas, Ginsberg et al. (2008) predict a 5-fold variation in the dose to the deep lung between quiet and heavy breathing conditions for an 8-year old child.

formaldehyde is discussed in detail in Sections 4.1.1.7 (human studies) and 4.2.1.7 (animal studies), while effects on the nervous system are discussed in Sections 4.1.1.6 and 4.2.1.6 (human and animal studies, respectively). The specific case of formaldehyde exposure and pulmonary effects is discussed in detail in Sections 4.1.1.1 to 4.1.1.4 and 4.2.1.1 to 4.2.1.4. A brief summary of identified effects of formaldehyde that may indicate susceptibility during particular life stages is provided below.

4.6.1.1.2.1. *Pre-conception.*

Exposure prior to conception may damage reproductive organs and/or germ cells that could affect reproduction and/or damage the genetic makeup of the offspring. Effects on reproduction are discussed in Sections 4.1.1.7 and 4.2.1.8. In summary, an epidemiological study (Taskinen et al., 1999) reported significantly delayed conception among female workers exposed to formaldehyde at average daily ambient formaldehyde levels; these effects could be consistent with adverse effects on either pre-conceptional and/or gestational exposure. One animal study (Maronpot et al., 1986) reported endometrial hypoplasia and lack of ovarian luteal tissue in female mice exposed for 13 weeks to 40 ppm formaldehyde via inhalation, suggesting the potential for treatment-related alterations to the female reproductive system. Since the exposure was to the adult, the findings suggest that preconceptional FA exposure caused female reproductive system effects that in turn could affect pregnancy.

In the rodent study of Kitaev et al. (1984), a three-fold increase in embryo degeneration on gestational days 2–3 was observed after FA exposure to the dams during premating. Since the exposure was to the adult in these three studies, the findings suggest that preconceptional FA exposure caused female reproductive system effects and/or affected the gametes.

4.6.1.1.2.2. *Prenatal.*

A population-based study (Gražulevičiene et al., 1998) found an association between atmospheric formaldehyde exposure and low birth weight, yielding an adjusted OR of 1.37 (95% CI: 0.90–2.09). Three studies (Dulskiene and Gražulevičiene, 2005; Taskinen et al., 1994; Hemminki et al., 1985) that examined the effect of occupational exposures on the incidence of congenital malformation produced mixed results.

Results from Taskinen et al. (1999) support associations between formaldehyde exposure, subfertility, and spontaneous abortion. Subfertility and spontaneous abortion are biologically linked (subclinical pregnancy losses are increased among women with fertility problems) (Gray and Wu, 2000; Hakim et al., 1995), and both subfertility and spontaneous abortion may be related to sensitivity to environmental agents (Correa et al., 1996).

Two experimental animal studies (Martin, 1990; Saillenfait et al., 1989) evaluated a standard battery of developmental endpoints following inhalation exposure on GDs 6–10, but effects were minimal. Similarly, Chernoff and Kavlock (1982), Marks et al. (1980), and Hurni and Ohder (1973) reported minimal reproductive or developmental effects in rodents in studies in which dams were exposed orally during early gestation. When formaldehyde was administered via inhalation throughout gestation in female rats, some developmental effects, including increased pup weight and decreases in lung and liver weight in newborns, were reported at 0.01 and 0.4 ppm (Senichenkova and Chebotar, 1996; Senichenkova, 1991; Kitaev et al., 1984; Gofmekler and Bonashevskaya, 1969; Gofmekler, 1968; Pushkina et al., 1968). Two studies also reported changes in motor activity in offspring of dams exposed via inhalation to 0.4 ppm formaldehyde during gestation (Senichenkova, 1991; Sheveleva, 1971).

4.6.1.1.2.3. ***Postnatal.***

Following early life stage exposure to formaldehyde, a number of adverse postnatal outcomes are possible, including effects on the developing and adult respiratory, reproductive, and neurological systems. The potential for increased risk of childhood cancer is also discussed below.

4.6.1.1.2.3.1. ***Respiratory toxicity.***

Formaldehyde is known to induce changes in pulmonary function and cause pulmonary irritation in human studies (Rumchev et al., 2002; Garrett et al., 1999; Krzyzanowski et al., 1990; Holmström et al., 1989; Holmström and Wilhelmsson, 1988; Ritchie and Lehnen, 1987) and animal studies (Ohtsuka et al., 2003, 1997; Riedel et al., 1996; Swiecichowski et al., 1993; Lee et al., 1984). Exposure to formaldehyde in early life can cause damage to the lungs and permanently influence airway function, resulting in increased vulnerability to toxicants later in life. Thus, young children may demonstrate increased susceptibility to formaldehyde-related health effects. Krzyzanowski et al. (1990) reported an association between physician-diagnosed asthma and chronic bronchitis in children who lived in homes with formaldehyde levels that were higher than 60 ppb, after controlling for socioeconomic status and ethnicity. Rumchev et al. (2002) reported a statistically significant increased risk of asthma with increased residential concentrations of formaldehyde. Garrett et al. (1999) found an increased association between bedroom concentration of formaldehyde and increased risk of atopy in children. These studies suggest that formaldehyde exposure may exacerbate responses in sensitive airways, particularly in children. Exacerbation of response has also been noted in asthmatic adults and will be discussed below.

Another child-specific concern is that respiratory irritation may have greater impact on lung function in children due to their smaller lung size; this is true even if the lung development is normal. Irritation is commonly accompanied by inflammation, which can have a greater impact on children's airways because they are narrower than adult airways. Thus, less inflammation is required for significant airway obstruction in children than in adults.

4.6.1.1.2.3.2. ***Developmental neurotoxicity.***

In neonatal exposure paradigms, changes in brain structure (Sarsilmaz et al., 2007; Aslan et al., 2006), and brain chemistry (Songur et al., 2008) were seen in young rats following inhalation exposures (6000 or 12000 ppb, 5 days per week from postnatal day 0-30). In addition, Weiler and Apfelbach (1992) found juvenile animals to be more sensitive to formaldehyde-induced changes in olfactory thresholds when compared with adult animals (shifts in olfactory thresholds were greater when exposure [at 250 ppb] was initiated at PND 30 than at adult ages). These studies are consistent with the hypothesis that early life exposure to FA can lead to long-lasting neurological effects. Exposure levels in these studies (250–6,000 ppb) were in the same range as those producing the behavioral effects in adults (as low as 100 ppb), but provide limited information regarding relative sensitivity as no NOAELs were identified, and (with the exception of Weiler and Apfelbach), similar parameters were not measured in adult animals using the same exposure paradigms.

4.6.1.2. *Later Life Stages*

In general, older adults may be at risk for increased susceptibility to exposure to environmental chemicals by virtue of their slower metabolism and increased incidence of altered health status (Benedetti et al., 2007; Ginsberg et al., 2005; U.S. EPA, 2005a). Additionally, adverse effects of earlier exposure to some toxicants may be observed in older adults as a result of latency in expression of the effect (Olsen et al., 1997; Sweeney et al., 1986). No studies have examined the differential effects of formaldehyde exposure for elderly adults (>65 years old) as compared to other age groups.

4.6.1.3. *Conclusions on Life-Stage Susceptibility*

In summary, timing both of the exposure and of the assessment of health outcomes may be important for understanding the relative risk of adverse effects from formaldehyde exposure during different life stages. There are known developmental differences in kinetics across life stages, including differences in enzymes involved in formaldehyde metabolism, but the contribution of these differences to formaldehyde-related health effects is unknown. Similarly,

information regarding life-stage differences in respiratory physiology raises possible concern regarding increased exposure to children, but studies for formaldehyde are not available. Available data do support an increased risk for adverse effects on lung function in children. The overall body of evidence shows some support, although minimal, for susceptibility in reproductive or developmental endpoints associated with exposure to formaldehyde. Some studies observed altered development of the nervous system following formaldehyde exposure during early life. Older adults may be at risk for increased susceptibility to formaldehyde because of slower metabolism and clearance rates. Elderly adults have an increased probability of having both altered health status and altered metabolism, which could impact their ability to process and recover from an adverse effect. The available data are consistent with some life-stage susceptibility differences for FA at the level of TD or TK differences, the results are nonetheless inconclusive due to the number of data gaps.

4.6.2. Health/Disease Status

The factor for which we have the greatest evidence is pre-existing disease, and specifically asthma. Numerous studies have assessed the potential for increased susceptibility to formaldehyde in asthmatics. Formaldehyde does not induce airway hyperreactivity directly (Sheppard et al., 1984) and has not been shown to increase airway hyperreactivity in either asthmatics or non-asthmatics (Pazdrak et al., 1993; Harving et al., 1991; Kulle et al., 1987). Significantly decreased forced expiratory volume (FEV_1) measurements were reported among asthmatics in two studies (Casset et al., 2006; Green et al., 1987), while others did not find any significant change in FEV_1 following formaldehyde exposure (Ezratty et al., 2007; Frigas et al., 1984).

A few available case reports of bronchial asthma do suggest direct respiratory tract sensitization to formaldehyde gas (Lemiere et al., 1995; Burge et al., 1985; Hendrick et al., 1982; Hendrick and Lane, 1977, 1975). All cases displayed marked changes in FEV_1 or pulmonary airflow rate in response to acute challenges with formaldehyde gas at exposure levels <3 ppm. Formaldehyde-induced IgE production has been reported in some studies (Vandenplas et al., 2004; Wantke et al., 1996a).

There is a large quantity of human data providing evidence of an association between formaldehyde exposure and increased incidence of asthma or exacerbation of asthmatic responses in compromised individuals. For example, Krzyzanowski et al. (1990) reported an association between physician-diagnosed asthma and chronic bronchitis in children who lived in homes with formaldehyde levels that were higher than 60 ppb, after controlling for socioeconomic status and ethnicity. Rumchev et al. (2002) reported a statistically significant

increased risk of asthma with increased residential concentrations of formaldehyde. Garrett et al. (1999) found an increased association between bedroom concentration of formaldehyde and risk of atopy in children. These studies suggest that formaldehyde exposure may exacerbate responses in sensitive airways, particularly in children. Exacerbation of response has also been noted in adults. Kriebel et al. (1993) reported a greater decrease in peak expiratory flow (PEF) in asthmatic medical students (7.3% decrement) compared with non-asthmatic medical students (2% decrement) after 2 weeks exposure to formaldehyde (average concentration 0.73 ppm) in an anatomy lab. This effect does not appear to be immunogenic in nature (Fujimaki et al., 2004a; Lee et al., 1984).

Several animal studies document increased airway resistance and bronchial constriction following inhalation exposure to formaldehyde (Nielson et al., 1999; Swiecichowski et al., 1993; Biagini et al., 1989; Amdur et al., 1960). Sadakane et al. (2002) demonstrated that formaldehyde exposure exacerbated sensitization and challenge with a common dust mite allergen (Der f) as measured by increased eosinophil infiltration into the interstitium around the bronchi and bronchioles as well as goblet cell proliferation in the bronchial epithelium; they suggested that formaldehyde exposure may aggravate eosinophilic infiltration and goblet cell proliferation that accompanies allergic responses. The MOA underlying this response is unknown. These decrements may occur indirectly in response to formaldehyde and may be mediated via neurogenic potentiation (Sadakane et al., 2002; Riedel et al., 1996; Tarkowski and Gorski, 1995). In particular, Tarkowski and Gorski (1995) suggest that formaldehyde may increase permeability of respiratory epithelium and destruction of immunologic barriers. Thus, the respiratory tract may become vulnerable to inhaled allergens after formaldehyde exposure (Tarkowski and Gorski, 1995).

In summary, the data indicate that formaldehyde exposure can aggravate a type I hypersensitivity response and that this hypersensitivity may in turn increase the susceptibility to FA exposure in these individuals. Formaldehyde exposure may predetermine an asthmatic phenotype or may induce new incidences of asthma via indirect mechanisms, though definitive evidence and a proposed mechanism remain to be determined. Individuals that exhibit chemically induced sensitivity and are exposed acutely or chronically to formaldehyde in residential and occupational settings might exhibit adverse responses at lower concentrations of formaldehyde than the average healthy person.

4.6.3. Nutritional Status

Limited available data indicate that certain types of malnutrition may increase susceptibility to formaldehyde exposure. Senichenkova and Chebotar (1996) reported increased

fetal anomalies in fetuses from iron-deficient pregnant mice after formaldehyde exposure compared with anemic mice that had not been exposed to formaldehyde. Forced iron reduction (induced by addition of bipyridyl treatment in pregnant mice) *in utero* increased the overall incidence of fetal anomalies when paired with formaldehyde exposure (Senichenkova and Chebotar, 1996). The findings are difficult to evaluate due to poor reporting and have not been substantiated by other laboratories.

4.6.4. Gender Differences

Males and females can differ greatly in body composition, organ function, and many other physiological parameters that may influence the toxicokinetics of chemicals and their metabolites in the body (Gochfeld, 2007; Gandhi et al., 2004).

The human epidemiology data set does not support any specific gender susceptibilities for noncancer effects due to formaldehyde exposure. In general, data suggest that nonpregnant women, on a per kg body weight basis, may have slightly lower air intake than men, which would suggest that women may be less susceptible to inhaled pollutants like formaldehyde than men, but this has not been investigated in the available formaldehyde literature.

A few isolated reports have investigated potential gender differences in development of nasal pharyngeal carcinomas following exposure to formaldehyde. One case-control study identified a higher OR for sinonasal adenocarcinomas in women (OR = 6.2 [95% CI: 2.2–19.7]) compared with the OR observed in men (OR = 3.0 [95% CI: 1.5–5.7]) following exposure to formaldehyde (Luce et al., 2002). However, the overall body of evidence remains scant.

There are a few reports concerning differential formaldehyde-induced effects on the male and female reproductive systems. Özen et al. (2002), Sarsilmaz et al. (1999), and Woutersen et al. (1987) reported reduced Leydig cell numbers in adult male rats exposed by inhalation. In female mice, inhalation exposure to formaldehyde resulted in endometrial hypoplasia and lack of ovarian luteal tissue (Maronpot et al., 1986). The clinical significance of these effects in humans is unknown, and due to limited data it is unclear whether the female or male reproductive system is more susceptible to perturbation by formaldehyde.

4.6.5. Genetic Differences

There are some data for polymorphisms in humans that affect formaldehyde TK. As discussed in Section 3.4, the primary metabolizing enzymes of formaldehyde are ALDH2 and ADH3, with the latter enzyme considered more relevant to low exposures. Polymorphisms in ALDH2 have been shown to have implications in human risk assessment, specifically in regard to acetaldehyde metabolism (Ginsberg et al., 2002). Teng et al. (2001) demonstrated the

importance of ALDH2 for formaldehyde metabolism in rat hepatocytes at fairly high formaldehyde concentrations (2.5 mM and greater). Cheng et al. (2008) investigated the relationship between occupational exposure to formaldehyde and genetic polymorphisms of ALDH2 and CYP2E1. There was a positive relationship between the concentration of formic acid in the urine and ALDH2 genotypes ($\chi^2 = 9.241$, $p < 0.05$). Urinary formic acid concentration may be affected by formaldehyde exposure concentration and ALDH2 genotype (Cheng et al., 2008) for individuals that have high exposure levels. Thus, although ALDH2 may not be involved in formaldehyde metabolism if exposure levels are low, polymorphisms of this enzyme may lead to differences in response at higher exposure levels.

Wu et al. (2007) looked for and identified two SNPs in ADH3 among a population of Mexican asthmatic children 4 to 17 years of age. Carrying one or two copies of the minor allele for one SNP resulted in a decreased RR of asthma (RR = 0.66–0.77). For the second SNP, homozygotes for the minor allele had an RR of 1.6 for asthma. The functional characteristics of these SNPs are unknown. Studies evaluating whether any of the polymorphisms affect the expression, regulation, stability, or activity of the enzyme *in vivo* are lacking; therefore, the relative susceptibility of individuals with different polymorphisms cannot be characterized at this time.

One study (Hedberg et al., 2001) identified three polymorphisms in human ADH3 involving four base-pair substitutions in the promoter region of which one (C→T) showed reduced activity (~50–70% of control). Hedberg et al. (2001) reported differences in allele frequencies among Chinese, Spanish, and Swedish groups, consisting of Asian-Caucasian differences and ethnic subgroups among Caucasians. Their results suggest that a small percentage of Caucasians may have decreased ADH3 expression and thus, be more susceptible to formaldehyde exposure. Additional studies to validate these findings have not been performed.

The relative activity level of these enzymes may also impact the metabolism of formaldehyde. In pharmacokinetic studies, deletion of ADH3 increased the sensitivity of mice to formaldehyde (Deltour et al., 1999) and was deleterious to yeast (Achkor et al., 2003). These results suggest that deficiencies in ADH3 may confer an increased susceptibility to formaldehyde toxicity (Teng et al., 2001). The importance of properly functioning enzymes also suggests that genetic differences in ADH3 or ALDH2 may affect the response to formaldehyde exposure. However, comparable human data are not available.

Race/ethnicity may be a surrogate for genetic differences but racial or ethnic groups may also reflect socioeconomic, and/or cultural factors that are distinct from genetics. Possible ethnic differences may be related to genetic polymorphisms of enzymes ALDH2 and ADH3, relevant for formaldehyde metabolism. ALDH2 variants, present primarily in East Asians, are known to

have protective effects against alcoholism but were not found in the people of Indo-Trinidadian descent (Moore et al., 2007) or in American Indians or Alaska natives (Ehlers, 2007). However, there is no direct evidence to associate these variants to differential susceptibility to formaldehyde exposure, nor is there direct evidence to associate these ethnic groups specifically with differential susceptibility to formaldehyde. Further, no studies have specifically assessed ethnic variability in responses to formaldehyde.

There are complex pathways through which genetic polymorphisms in ADH3 can potentially affect differential susceptibility to formaldehyde. Firstly, ADH3 is central to the metabolism of formaldehyde. However, ADH3 itself may indirectly contribute to the adverse effects of formaldehyde on pulmonary physiology (Thompson et al., 2009; Staab et al., 2008a, b; Thompson and Grafström, 2008). Exposure to formaldehyde is itself thought to alter the activity of ADH3 resulting in the perturbation of critical metabolic pathways. ADH3 participates in the oxidation of retinol and long-chain primary alcohols, as well as the reduction of S-nitrosoglutathione (GSNO). The activity of ADH3 toward some of these substrates has been shown to be significantly increased in the presence of formaldehyde. ADH3 has recently also been shown to contribute to NO signaling through its dual role in metabolizing GSNO, an endogenous bronchodilator and reservoir of NO (Staab et al., 2008a; Hess et al., 2005; Jensen et al. 1998). Through its regulatory function on GSNO, ADH3 may thus play a central role in regulating bronchial tone allergen-induced hyperresponsiveness (Gerard, 2005; Que et al., 2005). As concluded by California Environmental Protection Agency (CalEPA) (2008), "the dysregulation of NO by formaldehyde [in this manner] helps to explain the variety and variability in the toxic manifestations following formaldehyde inhalation."

4.6.6. Co-Exposures

4.6.6.1. Cumulative Risk

When considering health risks, it is important to consider the impact of co-exposures to other agents that may interact with the chemical under evaluation. Co-exposure to other pollutants, particularly those that produce some of the same metabolites and similar health effects as formaldehyde, is likely to occur in both occupational and nonoccupational settings.

Due to effects on metabolic enzymes (inducing and/or inhibition) as well as direct effects on organ system function, co-exposures may alter the way in which formaldehyde is metabolized and cleared from the body. Inhibition or induction of the enzymes responsible for metabolism of chemicals may alter susceptibility to toxicity (Lash and Parker, 2001; IARC, 1995; U.S. EPA, 1985a). Smokers may be at increased risk for effects of formaldehyde exposure, because formaldehyde is one of the components of cigarette smoke and is likely to heighten the point-of-

entry effect when combined with occupational or residential exposures to inhaled formaldehyde. However, no evidence is available to evaluate the potential aggregate effects.

4.6.6.2. Aggregate Exposure

In addition, multiple routes of exposure to a single agent may increase the cumulative risk by increasing the overall body burden of the chemical. A human aggregate exposure model developed by McKone and Daniels (1991) incorporated likely exposures from air, water, and soil media through inhalation, ingestion, and dermal contact. The authors hypothesized that the aggregate exposure could be age-dependent but did not present any data for persons of differing life stages. The role of multiple exposures on different genders, genetic susceptibility, or altered health and nutrition status has not been investigated. The available database regarding the potential for multiple routes of exposure (or aggregate exposure) formaldehyde is limited.

Guseva (1972) specifically assessed the reproductive and developmental effects caused by co-exposure to formaldehyde via both inhalation (0.25 mg/m^3) and ingestion (0.01 mg/L) routes in male rats. The authors reported reduced nucleic acid levels in testes to 88 and 92% of controls, which suggests a possible toxic gonadotropic effect. The ability of male rats (receiving combined exposure to formaldehyde at a low concentration level for a long period of time) to reproduce was preserved since all the cohabited females were impregnated. The number and weight of the fetuses and newborn rat pups in the experimental co-exposure groups did not differ substantially from those figures observed in the control group. No developmental defects or anomalies were observed in the offspring for up to 1 month postnatally. Thus, at low exposures, the reproductive effects due to combined ingestion and inhalation exposure are unknown.

4.6.7. Uncertainties of Database

There is a need to better characterize the implications of formaldehyde exposures to susceptible populations. A number of areas where the database is currently insufficient are identified below.

4.6.7.1. *Uncertainties of Exposure*

Although information exists on early life exposure to formaldehyde, a number of uncertainties regarding children's susceptibility remain. First, inhalation is believed to be of most concern for formaldehyde, since formaldehyde vapors are released from insulation or from ambient sources of formaldehyde, including secondary production from other pollutants involved in photo-oxidant reactions. Any additional pathways of exposure for children have not been characterized. Since formaldehyde is nearly ubiquitous in the environment, it is difficult to

quantify the total exposure. Second, children have different respiratory, metabolic, and activity rates compared with healthy adults, potentially influencing ADME and target tissue exposure to formaldehyde. However, studies to identify the specific changes in absorption of formaldehyde and its metabolites across developmental stages and across organs have not been performed. In addition, exposure prenatally may be altered based on whether formaldehyde or its metabolites pass through the placenta, but placental transfer data are not available. Third, no quantitative models have been developed to characterize these differences for formaldehyde. Formaldehyde-specific PBPK models and their validation will aid in understanding the uncertainties associated with formaldehyde exposure in children.

Given the large proportion of time that most individuals in the U.S. spend indoors, exposure scenarios where indoor concentrations to formaldehyde are high (e.g., in homes or in trailers; see section 2.3.1) may play a significant role and may be of particular concern to the elderly or health-impaired individuals who spend relatively more time at home. Further evaluation of the effects of co-exposures and pathways of exposure and aggregate risk is needed. An estimate of the multiple exposure pathways is needed to know where along the dose-response curve to place an incremental exposure to formaldehyde.

4.6.7.2. *Uncertainties of Effect*

Studies specifically designed to evaluate effects after early and later life stage exposure are needed in order to more fully characterize potential life-stage-related differences in formaldehyde toxicity, including the defining of critical windows during development. For example, life-stage-specific neurotoxic and pulmonary effects, particularly in the developing fetus, need further evaluation. The preconceptional period may be a critical window for FA exposure and reproductive and developmental effects, based on rodent studies of reproductive, embryonic and gamete effects. Data specific to the carcinogenic effects of formaldehyde exposure during early life stages do not exist. The reduction in fertility seen in some studies (Gray and Wu, 2000; Taskinen et al., 1999; Hakim et al., 1995) is not adequately described and a well-established MOA has not been identified, but some have been hypothesized including altered sperm quality (Özen et al., 2002; Sarsilmaz et al., 1999; Woutersen et al., 1987). Further, spontaneous abortion/fetal loss occurring early in gestation, prior to maternal knowledge of the pregnancy, can lead to misclassification of the effect as infertility (see Sections 4.1.1.7 and 4.2.1.7).

More research is needed to clarify the role of genetic polymorphisms in formaldehyde metabolism. Similarly, data gaps pertaining to gender differences remain. A potential impact of nutritional status and iron deficiency on formaldehyde toxicity needs further investigation.

A fair body of evidence suggests that asthmatics are more susceptible to formaldehyde exposure than the general population, however the mechanism of action for this increased susceptibility is unknown.

In the studies discussed above, there are a number of examples of studies that assessed multiple susceptibility factors that are worth noting. For example, the Krzyzanowski et al. (1990) study reported asthma and chronic bronchitis cases for two interacting potential susceptible groups, in children and those with high exposure (due to living in homes with formaldehyde levels that were higher than 60 ppb). Similarly, the Garrett et al. (1999) study assessed the same two interacting potential susceptible groups.

The study of Senichenkova and Chebotar (1996) assessed developmental effects in mouse fetuses after *in utero* iron-deficiency and FA exposure. Thus, the study findings must be considered in light of possible interactions between life stage exposure differences and nutritional status differences.

Studies to understand the nature of the interactions between the various susceptibility factors for FA have not been performed.

4.6.8. Summary of Potential Susceptibility

There is some evidence to demonstrate susceptibility for various populations exposed to formaldehyde. Available data are summarized in Table 4-96 where FA susceptibility factors are presented by those with data for increased FA susceptibility and those with data for differences but with an unknown impact on FA susceptibility.

Exposure to FA during early developmental and later life stages may be of concern. However, human exposure to the developing fetus is unknown since it is not known whether formaldehyde or one of its metabolites crosses the placenta. However, there is very limited life-stage-specific information regarding the TK of formaldehyde. Life-stage-specific TK has not been characterized, and, thus, no PBPK models exist to effectively evaluate the risk to early life stages. Children may be more susceptible to noncancer health effects as a result of inhalation exposure to formaldehyde due to increased respiratory rates. There are no studies to evaluate whether formaldehyde exposure in early life (e.g., pregnancy) is associated with an increased risk of childhood cancer.

The weight of evidence supports a plausible association between formaldehyde exposure and aggravated asthmatic responses in humans and this association is corroborated by limited evidence from animal studies. Formaldehyde does not appear to directly induce airway hyperreactivity but may sensitize airways to subsequent exposures. One issue in interpreting the available studies that assessed the relationship between asthma and FA could not distinguish

between the cases of asthma that were due to earlier FA exposure vs. those without a direct link to FA exposure.

No direct link exists between formaldehyde exposure and differential susceptibility in different ethnic groups, although genetic polymorphisms in the enzymes involved with formaldehyde metabolism, ADH3 and ALDH2, provide some support for differential susceptibility to alcoholism in a number of ethnic groups. The evidence for differential gender responses to formaldehyde exposure is equivocal. Co-exposures may result in altered metabolism and clearance, but there is no evidence that co-exposures are a critical part of formaldehyde-mediated differential susceptibility.

Thus, given the available data, increased susceptibility to adverse effects of formaldehyde is most strongly supported for three populations: 1) Preconception and perinatal exposure based on reproductive and developmental effects; 2) children, whose exposure may be higher by virtue of their increased activity level and respiratory rate; and 3) asthmatics who may exhibit exacerbation of response to formaldehyde.

Table 4-96. Available evidence for susceptibility factors of concern for formaldehyde exposure

Factor	Evidence that factors increase susceptibility to FA	Evidence that factors show differences but unknown impact on susceptibility
Life Stage	Developmental effects reported suggesting that critical windows of exposure may be relevant:	
▪ Preconception	▪ Reproductive outcomes (Taskinen et al., 1999; Maronpot et al., 1986) ▪ Embryo effects (Kitaev et al., 1984)	
▪ Prenatal	▪ Structural- and functional developmental outcomes (Martin, 1990; Saillenfait et al., 1989; Sheveleva, 1971; Seninchenkova, 1991)	▪ Possible life stage level differences in some enzymes involved in FA metabolism (Harris et al., 2003; Ginsberg et al., 2004; Westerlund et al., 2005; Benedetti et al., 2007) ▪ Mixed reports of associations between prenatal exposure and developmental outcomes in human studies (positive association: Gražulevičiene et al., 1998)
▪ Postnatal	▪ Lung function outcome (Krzyzanowski et al., 1990; Rumchev et al., 2002; Garrett et al., 1999) ▪ Developmental neurotoxicity (Weiler and Apfelbach, 1992)	▪ Possible life stage level differences in some enzymes involved in FA metabolism (e.g., ↓ADH expression over first 5 months; Ginsberg et al., 2004) ▪ Developmental neurotoxicity (Sarsilmaz et al., 2007; Aslan et al., 2006; Songur et al., 2008)
Disease Status	▪ Bronchial asthma (Lemiere et al., 1995; Burge et al., 1985; Hendrick et al., 1982; Hendrick and Lane, 1977, 1975) ▪ Increased airway resistance and bronchial constriction (Nielson et al., 1999; Swiecichowski et al., 1993; Biagini et al., 1989; Amdur et al., 1960)	▪ Mixed results for forced expiratory volume (FEV1) measures affected by FA exposure in asthmatics (Casset et al., 2006; Green et al., 1987; Ezratty et al., 2007; Frigas et al., 1984)
Nutritional Status/Diet	▪ Iron-deficiency *in utero* (Senichenkova and Chebotar, 1996).	
Genetics ▪ Polymorphisms	▪ For high FA exposure: Urinary formic acid levels affected by ALDH2 genotype (Cheng et al., 2008) ▪ In mice, ADH3 increased sensitivity to FA (Achkor et al., 2003)	▪ Differences among ADH3 alleles and asthma outcome (Wu et al., 2007) ▪ Differences among ethnic groups in ADH3 alleles (Hedberg et al., 2001)
Gender		▪ Gender differences in incidence of nasopharyngeal carcinoma following FA exposure (Luce et al., 2002)

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