4. HAZARD CHARACTERIZATION

This chapter presents the hazard characterization of trichloroethylene (TCE) health effects. Because of the number of studies and their relevance to multiple endpoints, the evaluation of epidemiologic studies of cancer and TCE is summarized in Section 4.1 (endpoint-specific results are presented in subsequent sections). Genotoxicity data are discussed in Section 4.2. Due to the large number of endpoints and studies in the toxicity database, subsequent sections (see Sections 4.3−4.10) are organized by tissue/organ system. Each section is further organized by noncancer and cancer endpoints, discussing data from human epidemiologic and laboratory experimental studies. In cases where there is adequate information, the role of metabolism in toxicity, comparisons of toxicity between TCE and its metabolites, and carcinogenic mode of action (MOA) are also discussed. Finally, Section 4.11 summarizes the overall hazard characterization and the weight of evidence for noncancer and carcinogenic effects.

4.1. EPIDEMIOLOGIC STUDIES ON CANCER AND TRICHLOROETHYLENE (TCE)—METHODOLOGICAL OVERVIEW

This brief overview of the epidemiologic studies on cancer and TCE below provides background to the discussion contained in Sections 4.4−4.10. Over 50 epidemiologic studies on cancer and TCE exposure (see Tables 4-1−4-3) were examined to assess their ability to inform weight of evidence evaluation, i.e., to inform the cancer hazard from TCE exposure, according to 15 standards of study design (see Table 4-4), conduct, and analysis. The analysis of epidemiologic studies on cancer and TCE serves to document essential design features, exposure assessment approaches, statistical analyses, and potential sources of confounding and bias. This analysis, furthermore, supports the discussion of site-specific cancer observations in Sections 4.4−4.9. In those sections, study findings are presented with an assessment and discussion of their observations according to a study's weight of evidence and the potential for alternative explanations, including bias and confounding. Tables containing observed findings for site-specific cancers are also found in Sections 4.4−4.9. Full details of the weight-of evidence-review to identify a cancer hazard and study selections for meta-analysis may be found in Appendix B.

Table 4-1. Description of epidemiologic cohort and proportionate mortality ratio (PMR) studies assessing cancer and TCE exposure

Reference	Description	Study group (*N*) Comparison group (*N*)	Exposure assessment and other information
Aircraft and aerospace workers			
Radican et al. (2008), Blair et al. (1998)	Civilian aircraft-maintenance workers with at least 1 yr in 1952−1956 at Hill Air Force Base, UT. Vital status (VS) to 1990 (Blair et al. 1998) or 2000 (Radican et al., 2008); cancer incidence 1973–1990 (Blair et al., 1998).	14,457 (7,204 ever exposed to TCE). Incidence (Blair et al., 1998) and mortality rates (Blair et al., 1998; Radican et al., 2008) of nonchemical exposed subjects.	Most subjects (*n* = 10,718) with potential exposure to 1 to 25 solvents. Cumulative TCE assigned to individual subjects using JEM. Exposure-response patterns assessed using cumulative exposure, continuous or intermittent exposures, and peak exposure. TCE replaced in 1968 with 1,1,1-trichloroethane and was discontinued in 1978 in vapor degreasing activities. **Median TCE exposures were about 10 ppm for rag and bucket; 100−200 ppm for vapor degreasing.** Poisson regression analyses controlled for age, calendar time, sex (Blair et al., 1998) or Cox proportional hazard model for age and race.
Krishnadasan et al. (2007)	Nested case-control study within a cohort of 7,618 workers employed for between 1950 and 1992, or who had started employment before 1980 at Boeing/Rockwell/ Rocketdyne (Santa Susana Field Laboratory, [the UCLA cohort of Morgenstern et al., 1997]). Cancer incidence 1988–1999.	326 prostate cancer cases, 1,805 controls. Response rate: Cases, 69%; Controls, 60%.	JEM for TCE, hydrazine, PAHs, benzene, mineral oil constructed from company records, walk-through, or interviews. Lifestyle factors obtained from living subjects through mail and telephone surveys. Conditional logistic regression controlled for cohort, age at diagnosis, physical activity, SES and other occupational exposure (benzene, PAHs, mineral oil, hydrazine).

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Table 4-1. Description of epidemiologic cohort and proportionate mortality ratio (PMR) studies assessing cancer and TCE exposure (continued)

Reference	Description	Study group (*N*) Comparison group (*N*)	Exposure assessment and other information
Zhao et al. (2005); Ritz et al. (1999)	Aerospace workers with $\geq$2 yrs of employment at Rockwell/ Rocketdyne (now Boeing) and who worked at Santa Susana Field Laboratory, Ventura, CA, from 1950-1993 (the UCLA cohort of Morgenstern et al. [1997]). Cancer mortality as of December 31, 2001. Cancer incidence 1988–2000 for subjects alive as of 1988.	6,044 (2,689 with high cumulative exposure to TCE). Mortality rates of subjects in lowest TCE exposure category. 5,049 (2,227 with high cumulative exposure to TCE). Incidence rates of subjects in lowest TCE exposure category.	JEM for TCE, hydrazine, PAHs, mineral oil, and benzene. IH ranked each job title ranked for presumptive TCE exposure as high (3), medium (2), low (1), or no (0) exposure for 3 time periods (1951–1969, 1970–1979, 1980–1989). Cumulative TCE score: low (up to 3), medium (over 3 up to 12), high (over 12) assigned to individual subjects using JEM. Cox proportional hazard, controlled for time, since 1st employment, SES, age at diagnosis and hydrazine.
Boice et al. (2006a)	Aerospace workers with $\geq$6 months employment at Rockwell/ Rocketdyne (Santa Susana Field Laboratory and nearby facilities) from 1948–1999 (IEI cohort, IEI [2005]). VS to 1999.	41,351, 1,642 male hourly test stand mechanics (1,111 with potential TCE exposure). Mortality rates of United States population and California population. Internal referent groups including male hourly nonadministrative Rocketdyne workers; male hourly, nonadministrative SSFL workers; and test stand mechanics with no potential exposure to TCE.	Potential TCE exposure assigned to test stands workers only whose tasks included the cleaning or flushing of rocket engines (engine flush) (n = 639) or for general utility cleaning (n = 472); potential for exposure to large quantities of TCE was much greater during engine flush than when TCE used as a utility solvent. JEM for TCE and hydrazine without semiquantitative intensity estimates. Exposure to other solvents not evaluated due to low potential for confounding (few exposed, low exposure intensity, or not carcinogenic). Exposure metrics included employment duration, employment decade, years worked with potential TCE exposure, and years worked with potential TCE exposure via engine cleaning, weighted by number of tests. Lifetable (SMR); Cox proportional hazard controlling for birth year, hire year, and hydrazine exposure.
Boice et al. (1999)	Aircraft-manufacturing workers with at least 1 yr $\geq$1960 at Lockheed Martin (Burbank, CA). VS to 1996.	77,965 (2,267 with potential routine TCE exposures and 3,016 with routine or intermittent TCE exposure). Mortality rates of United States population (routine TCE exposed subjects) and non-exposed internal referents (routine and intermittent TCE exposed subjects).	12% with potential routine mixed solvent exposure and 30% with route or intermittent solvent exposure. JEM for potential TCE exposure on (1) routine basis or (2) intermittent or routine basis without semiquantitative intensity estimate. Exposure-response patterns assessed by any exposure or duration of exposure and internal control group. Vapor degreasing with TCE before 1966 and PCE, afterwards. Lifetable analyses; Poisson regression analysis adjusting for birth date, starting employment date, finishing employment date, sex and race.

Table 4-1. Description of epidemiologic cohort and proportionate mortality ratio (PMR) studies assessing cancer and TCE exposure (continued)

Reference	Description	Study group (*N*) Comparison group (*N*)	Exposure assessment and other information
Morgan et al. (1998)	Aerospace workers with ≥6 months 1950–1985 at Hughes (Tucson, AZ). VS to 1993.	20,508 (4,733 with TCE exposures). Mortality rates of United States population for overall TCE exposure; mortality rates of all-other cohort subjects (internal referents).	TCE exposure intensity assigned using JEM. Exposure-response patterns assessed using cumulative exposure (low versus high) and job with highest TCE exposure rating (peak, medium/high exposure versus no/low exposure). **"High exposure" job classification defined as >50 ppm.** Vapor degreasing with TCE 1952-1977, but limited IH data <1975. Limited IH data before 1975 and medium/low rankings likely misclassified given temporal changes in exposure intensity not fully considered (NRC, 2006).
Costa et al. (1989)	Aircraft manufacturing workers employed 1954–1981at plant in Italy. VS to 1981.	8,626 subjects Mortality rates of the Italian population.	No exposure assessment to TCE and job titles grouped into one of four categories: blue- and white-collar workers, technical staff, and administrative clerks. Lifetable (SMR).
Garabrant et al. (1988)	Aircraft manufacturing workers ≥4 yrs employment and who had worked at least 1 d at San Diego, CA, plant 1958–1982. VS to 1982.	14,067 Mortality rates of United States population.	TCE exposure assessment for 70 of 14,067 subjects; 14 cases of esophageal cancer and 56 matched controls. For these 70 subjects, company work records identified 37% with job title with potential TCE exposure without quantitative estimates. Lifetable (SMR).
Cohorts Identified From Biological Monitoring (U-TCA)			
Hansen et al. (2001)	Workers biological monitored using U-TCA and air-TCE, 1947–1989. Cancer incidence from 1964–1996.	803 total Cancer incidence rates of the Danish population.	712 with U-TCA, 89 with air-TCE measurement records, 2 with records of both types. U-TCA from 1947−1989; air TCE measurements from 1974. Historic median exposures estimated from the U-TCA concentrations were: 9 ppm for 1947 to 1964, 5 ppm for 1965 to 1973, 4 ppm for 1974 to 1979, and 0.7 ppm for 1980 to 1989. Air TCE measurements from 1974 onward were 19 ppm (mean) and 5 ppm (median). **Overall, median TCE exposure to cohort as extrapolated from air TCE and U-TCA measurements was 4 ppm (arithmetic mean, 12 ppm)**. Exposure metrics: year 1st employed, employment duration, mean exposure, cumulative exposure. Exposure metrics: employment duration, average TCE intensity, cumulative TCE, period 1st employment. Lifetable analysis (SIR).

Table 4-1. Description of epidemiologic cohort and proportionate mortality ratio (PMR) studies assessing cancer and TCE exposure (continued)

Reference	Description	Study group (*N*) Comparison group (*N*)	Exposure assessment and other information
Anttila et al. (1995)	Workers biological monitored using U-TCA, 1965–1982. VS 1965–1991 and cancer incidence 1967–1992.	3,974 total (3,089 with U-TCA measurements). Mortality and cancer incidence rates of the Finnish population.	Median U-TCA, 63 µmol/L for females and 48 µmol/L for males; mean U-TCA was 100 µmol/L. Average 2.5 U-TCA measurements per individual. Using the Ikeda et al. (1972) relationship for TCE exposure to U-TCA, **TCE exposures were roughly 4 ppm (median) and 6 ppm (mean).** Exposure metrics: years since 1st measurement. Lifetable analysis (SMR, SIR).
Axelson et al. (1994)	Workers biological monitored using U-TCA, 1955–1975. VS to 1986 and cancer incidence 1958–1987.	1,4,21 males Mortality and cancer incidence rates of Swedish male population.	Biological monitoring for U-TCA from 1955 and 1975. **Roughly ¾ of cohort had U-TCA concentrations equivalent to <20 ppm TCE.** Exposure metrics: duration exposure, mean U-TCA. Lifetable analysis (SMR, SIR).
Other Cohorts			
Clapp and Hoffman (2008)	Deaths between 1969-2001 among employees ≥5 yrs employment duration at an IBM facility (Endicott, NY).	360 deaths Proportion of deaths among New York residents during 1979 to 1998.	No exposure assessment to TCE. PMR analysis.
Sung et al. (2007, 2008)	Female workers 1st employed 1973-1997 at an electronics (RCA) manufacturing factory (Taoyuan, Taiwan). Cancer incidence 1979–2001 (Sung et al., 2007). Childhood leukemia 1979−2001 among first born of female subjects in Sung et al. (2007, 2008).	63,982 females and 40,647 females with 1st live born offspring. Cancer incidence rates of Taiwan population (Sung et al., 2007). Childhood leukemia incidence rates of first born live births of Taiwan population (Sung et al., 2007).	No exposure assessment. Chlorinated solvents including TCE and PCE found in soil and groundwater at factory site. Company records indicated TCE not used 1975–1991 and PCE 1975–1991 and PCE after 1981. No information for other time periods. Exposure-response using employment duration. Lifetable analysis (SMR, SIR) (Chang et al., 2003, 2005; Sung et al., 2007) or Poisson regression adjusting for maternal age, education, sex, and birth year (Sung et al., 2008).
Chang et al. (2005), Chang et al. (2003)	Male and female workers employed 1978–1997 at electronics factory as studied by Sung et al. (2007). VS from 1985–1997 and cancer incidence 1979–1997.	86,868 total Incidence (Chang et al., 2005) or mortality (Chang et al., 2003) rates Taiwan population.	

Table 4-1. Description of epidemiologic cohort and proportionate mortality ratio (PMR) studies assessing cancer and TCE exposure (continued)

Reference	Description	Study group (*N*) Comparison group (*N*)	Exposure assessment and other information
ATSDR (2004)	Workers 1952–1980 at the View-Master factory (Beaverton, OR).	616 deaths 1989–2001 Proportion of deaths between 1989−2001 in Oregon population.	No exposure information on individual subjects. TCE and other VOCs detected in well water at the time of the plant closure in 1998 were TCE, 1,220−1,670 µg/L; 1,1-DCE, up to 33 µg/L; and, PCE up to 56 µg/L. PMR analysis.
Raaschou-Nielsen et al. (2003)	Blue-collar workers employed >1968 at 347 Danish TCE-using companies. Cancer incidence through 1997.	40,049 total (14,360 with presumably higher level exposure to TCE). Cancer incidence rates of the Danish population.	Employers had documented TCE usage. Blue-collar versus white-collar workers and companies with <200 workers were variables identified as increasing the likelihood for TCE exposure. Subjects from iron and metal, electronics, painting, printing, chemical, and dry cleaning industries. **Median exposures to trichloroethylene were 40−60 ppm for the years before 1970, 10−20 ppm for 1970 to 1979, and approximately 4 ppm for 1980 to 1989.** Exposure metrics: employment duration, year 1st employed, and # employees in company. Lifetable (SIR).
Ritz (1999a)	Male uranium-processing plant workers ≥3 months employment 1951–1972 at DOE facility (Fernald, OH). VS 1951–1989, cancer.	3,814 white males monitored for radiation (2,971with potential TCE exposure). Mortality rates of the United States population; Non-TCE exposed internal controls for TCE exposure-response analyses.	JEM for TCE, cutting fluids, kerosene, and radiation generated by employees and industrial hygienists. Subjects assigned potential TCE according to intensity: light (2,792 subjects), moderate (179 subjects), heavy (no subjects). Lifetable (SMR) and conditional logistic regression adjusted for pay status, date first hire, radiation.
Henschler et al. (1995)	Male workers ≥ 1 yr 1956–1975 at cardboard factory (Arnsberg region, Germany). VS to 1992.	169 exposed; 190 unexposed Mortality rates from German Democratic Republic (broad categories) or renal cell carcinoma incidence rates from Danish population, German Democratic, or non-TCE exposed subjects.	Walk-through surveys and employee interviews used to identify work areas with TCE exposure. TCE exposure assigned to renal cancer cases using workman's compensation files. Lifetable (SMR, SIR) or Mantel-Haenszel.

Table 4-1. Description of epidemiologic cohort and proportionate mortality ratio (PMR) studies assessing cancer and TCE exposure (continued)

Reference	Description	Study group (*N*) Comparison group (*N*)	Exposure assessment and other information
Greenland et al. (1994)	Cancer deaths, 1969–1984, among pensioned workers employed <1984 at GE transformer manufacturing plant (Pittsfield, MA), and who had job history record; controls were noncancer deaths among pensioned workers.	512 cases, 1,202 controls. Response rate: Cases, 69%; Controls, 60%.	Industrial hygienist assessment from interviews and position descriptions. TCE (no/any exposure) assigned to individual subjects using JEM. Logistic regression.
Sinks et al. (1992)	Workers employed 1957–1980 at a paperboard container manufacturing and printing plant (Newnan, GA). VS to 1988. Kidney and bladder cancer incidence through 1990.	2,050 total Mortality rates of the United States population, bladder and kidney cancer incidence rates from the Atlanta-SEER registry for the years 1973−1977.	No exposure assessment to TCE; analyses of all plant employees including white- and blue-collar employees. Assignment of work department in case-control study based upon work history; Material Safety Data Sheets identified chemical usage by department. Lifetable (SMR, SIR) or conditional logistic regression adjusted for hire date and age at hire, and using 5- and 10-year lagged employment duration.
Blair et al. (1989)	Workers employed 1942−1970 in U.S. Coast. VS to 1980.	3,781 males of whom 1,767 were marine inspectors (48%). Mortality rates of the United States population. Mortality rates of marine inspectors also compared to that of noninspectors.	No exposure assessment to TCE. Marine inspectors worked in confined spaces and had exposure potential to multiple chemicals. TCE was identified as one of 10 potential chemical exposures. Lifetable (SMR) and directly adjusted relative risks.
Shannon et al. (1988)	Workers employed $\geq$6 mos at GE lamp manufacturing plant, 1960−1975. Cancer incidence from 1964−-1982.	1,870 males and females, 249 (13%) in coiling and wire-drawing area. Cancer incidence rates from Ontario Cancer Registry.	No exposure assessment to TCE. Workers in CWD had potential exposure to many chemicals including metals and solvents. A 1955-dated engineering instruction sheet identified trichloroethylene used as degreasing solvent in CWD. Lifetable (SMR).
Shindell and Ulrich (1985)	Workers employed $\geq$3 months at a TCE manufacturing plant 1957–1983. VS to 1983.	2,646 males and females Mortality rates of the United States population.	No exposure assessment to TCE; job titles categorized as either white- or blue-collar. Lifetable analysis (SMR).

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Table 4-1. Description of epidemiologic cohort and proportionate mortality ratio (PMR) studies assessing cancer and TCE exposure (continued)

Reference	Description	Study group (*N*) Comparison group (*N*)	Exposure assessment and other information
Wilcosky et al. (1984)	Respiratory, stomach, prostate, lymphosarcoma, and lymphatic leukemia cancer deaths 1964–1972 among 6,678 active and retired production workers at a rubber plant (Akron, OH); controls were a 20% age-stratified random sample of the cohort.	183 cases (101 respiratory, 33 prostate, 30 stomach, 9 lymphosarcoma and 10 lymphatic leukemia cancer deaths).	JEM without quantitative intensity estimates for 20 exposures including TCE. Exposure metric: ever held job with potential TCE exposure.

DCE = dichloroethylene, CWD = coiling and wire drawing; DOE = U.S. Department of Energy, IEI = International Epidemiology Institute, JEM = job-exposure matrix, NRC = National Research Council, PCE = perchloroethylene, PMR = proportionate mortality ratio, SIR = standardized incidence ratio, SMR = standardized mortality ratio, SSFL = Santa Susanna Field Laboratory, U-TCA = urinary trichloroacetic acid, UCLA = University of California, Los Angeles, VOCs = volatile organic compounds, VS = vital status.

Table 4-2. Case-control epidemiologic studies examining cancer and TCE exposure

Reference	Population	Study group (*N*) Comparison group (*N*) Response rates	Exposure assessment and other information
Bladder			
Pesch et al. (2000a)	Histologically confirmed urothelial cancer (bladder, ureter, renal pelvis) cases from German hospitals (5 regions) in 1991−1995; controls randomly selected from residency registries matched on region, sex, and age.	1,035cases 4,298 controls Cases, 84%; Controls, 71%	Occupational history using job title or self-reported exposure. JEM and JTEM to assign exposure potential to metals and solvents (chlorinated solvents, TCE, PCE). Lifetime exposure to TCE exposure examined as 30th, 60th, and 90th percentiles (medium, high, and substantial) of exposed control exposure index. Duration used to examine occupational title and job task duties and defined as 30th, 60th, and 90th percentiles (medium, long, and very long) of exposed control durations. Logistic regression with covariates for age, study center, and smoking.
Siemiatycki et al. (1994), Siemiatycki (1991)	Male bladder cancer cases, age 35−75 yrs, diagnosed in 16 large Montreal-area hospitals in 1979−1985 and histologically confirmed; controls identified concurrently at 18 other cancer sites; age-matched, population-based controls identified from electoral lists and random digit dialing (RDD).	484 cases 533 population controls; 740 other cancer controls Cases, 78%; Controls, 72%	JEM to assign 294 exposures including TCE on semiquantitative scales categorized as any or substantial exposure. Other exposure metrics included exposure duration in occupation or job title. Logistic regression adjusted for age, ethnic origin, socioeconomic status, smoking, coffee consumption, and respondent status [occupation or job title] or Mantel-Haenszel stratified on age, income, index for cigarette smoking, coffee consumption, and respondent status (TCE).
Brain			
DeRoos et al. (2001) Olshan et al. (1999)	Neuroblastoma cases in children of <19 yrs selected from Children's Cancer Group and Pediatric Oncology Group with diagnosis in 1992−1994; population controls (RDD) matched to control on birth date.	504 cases 504 controls Cases, 73%; Controls, 74%	Telephone interview with parent using questionnaire to assess parental occupation and self-reported exposure history and judgment-based attribution of exposure to chemical classes (halogenated solvents) and specific solvents (TCE). Exposure metric was any potential exposure. Logistic regression with covariate for child's age and material race, age, and education.

Table 4-2. Case-control epidemiologic studies examining cancer and TCE exposure (continued)

Reference	Population	Study group (*N*) Comparison group (*N*) Response rates	Exposure assessment and other information
Heineman et al. (1994)	White, male cases, age ≥30 yrs, identified from death certificates in 1978−1981; controls identified from death certificates and matched for age, year of death and study area.	300 cases 386 controls Cases, 74%; Controls, 63%	In-person interview with next-of-kin; questionnaire assessing lifetime occupational history using job title and JEM of Gomez et al. (1994). Cumulative exposure metric (low, medium or and high) based on weighted probability and duration. Logistic regression with covariates for age and study area.
Colon and Rectum			
Goldberg et al. (2001), Siemiatycki (1991)	Male colon cancer cases, 35−75 yrs, from 16 large. Montreal-area hospitals in 1979−1985 and histologically confirmed; controls identified concurrently at 18 other cancer sites; age-matched, population-based controls identified from electoral lists and random digit dialing (RDD).	497 cases 533 population controls and 740 cancer controls Cases, 82%; Controls, 72%	In-person interviews (direct or proxy) with segments on work histories (job titles and self-reported exposures); analyzed and coded by a team of chemists and industrial hygienists (294 exposures on semiquantitative scales); potential TCE exposure defined as any or substantial exposure. Logistic regression adjusted for age, ethnic origin, birthplace, education, income, parent's occupation, smoking, alcohol consumption, tea consumption, respondent status, heating source socioeconomic status, smoking, coffee consumption, and respondent status (occupation, some chemical agents) or Mantel-Haenszel stratified on age, income, index for cigarette smoking, coffee consumption, and respondent status (TCE).
Dumas et al. (2000), Simeiatycki (1991)	Male rectal cancer cases, age 35−75 yrs, diagnosed in 16 large Montreal-area hospitals in 1979−1985 and histologically confirmed; controls identified concurrently at 18 other cancer sites; age-matched, population-based controls identified from electoral lists and RDD.	292 cases 533 population controls and 740 other cancer controls Cases, 78%; Controls, 72%	In-person interviews (direct or proxy) with segments on work histories (job titles and self-reported exposures); analyzed and coded by a team of chemists and industrial hygienists (294 exposures on semiquantitative scales); potential TCE exposure defined as any or substantial exposure. Logistic regression adjusted for age, education, respondent status, cigarette smoking, beer consumption and body mass index (TCE) or Mantel-Haenszel stratified on age, income, index for cigarette smoking, coffee consumption, ethnic origin, and beer consumption (TCE).

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Table 4-2. Case-control epidemiologic studies examining cancer and TCE exposure (continued)

Reference	Population	Study group (*N*) Comparison group (*N*) Response rates	Exposure assessment and other information
Fredriksson et al. (1989)	Colon cancer cases aged 30−75 yrs identified through the Swedish Cancer Registry among patients diagnosed in 1980−1983; population-based controls were frequency-matched on age and sex and were randomly selected from a population register.	329 cases 658 controls Not available	Mailed questionnaire assessing occupational history with telephone interview follow-up. Self-reported exposure to TCE defined as any exposure. Mantel-Haenszel stratified on age, sex, and physical activity.
Esophagus			
Parent et al. (2000a), Siemiatycki (1991)	Male esophageal cancer cases, 35−75 yrs, diagnosed in 19 large Montreal-area hospitals in 1979−1985 and histologically confirmed; controls identified concurrently at 18 other cancer sites; age-matched, population-based controls identified from electoral lists and RDD.	292 cases 533 population controls; 740 subjects with other cancers Cases, 78%; controls, 72%	In-person interviews (direct or proxy) with segments on work histories (job titles and self-reported exposures); analyzed and coded by a team of chemists and industrial hygienists (294 exposures on semiquantitative scales); potential TCE exposure defined as any or substantial exposure. Logistic regression adjusted for age, education, respondent status, cigarette smoking, beer consumption and body mass index (solvents) or Mantel-Haenszel stratified on age, income, index for cigarette smoking, coffee consumption, ethnic origin, and beer consumption (TCE).
Lymphoma			
Wang et al. (2009)	Cases among females aged 21 and 84 yrs with NHL in 1996−2000 and identified from Connecticut Cancer Registry; population-based female controls (1) if <65 yrs of age, having Connecticut address stratified by 5-yr age groups identified from random digit dialing or (2) ≥65 yrs of age, by random selection from Centers for Medicare and Medicaid Service files.	601 cases 717 controls Cases, 72%; Controls, 69% (<65 yrs), 47% (≥65 yrs)	In-person interview with using questionnaire assessment specific jobs held for >1 yr. Intensity and probability of exposure to broad category of organic solvents and to individual solvents, including TCE, estimated using JEM (Gomez et al, 1994; Dosemeci et al., 1994) and assigned blinded. Exposure metric of any exposure, exposure intensity (low, medium/high), and exposure probability (low, medium/high). Logistic regression adjusted for age, family history of hematopoietic cancer, alcohol consumption and race.

Table 4-2. Case-control epidemiologic studies examining cancer and TCE exposure (continued)

Reference	Population	Study group (*N*) Comparison group (*N*) Response rates	Exposure assessment and other information
Constantini et al. (2008), Miligi et al. (2006)	Cases aged 20−74 with NHL, including CLL, all forms of leukemia, or multiple myeloma (MM) in 1991−1993 and identified through surveys of hospital and pathology departments in study areas and in specialized hematology centers in 8 areas in Italy; population-based controls stratified by 5-yr age groups and by sex selected through random sampling of demographic or of National Health Service files.	1,428 NHL + CLL, 586 Leukemia, 263, MM 1,278 controls (leukemia analysis) 1,100 controls (MM analysis) Cases, 83%; Controls, 73%	In-person interview primarily at interviewee's home (not blinded) using questionnaire assessing specific jobs, extra occupational exposure to solvents and pesticides, residential history, and medical history. Occupational exposure assessed by job-specific or industry-specific questionnaires. JEM used to assign TCE exposure and assessed using intensity (2 categories) and exposure duration (2 categories). All NHL diagnoses and 20% sample of all cases confirmed by panel of 3 pathologists. Logistic regression with covariates for sex, age, region, and education. Logistic regression for specific NHL included an additional covariate for smoking.
Seidler et al. (2007) Mester et al. (2006) Becker et al. (2004)	NHL and Hodgkin's disease cases aged 18−80 yrs identified through all hospitals and ambulatory physicians in six regions of Germany between 1998 and 2003; population controls were identified from population registers and matched on age, sex, and region.	710 cases 710 controls Cases, 87%; Controls, 44%	In-person interview using questionnaire assessing personal characteristics, lifestyle, medical history, UV light exposure, and occupational history of all jobs held for ≥1 yr. Exposure of *a prior* interest were assessed using job task-specific supplementary questionnaires. JEM used to assign cumulative quantitative TCE exposure metric, categorized according to the distribution among the control persons (50th and 90th percentile of the exposed controls). Conditional logistic regression adjusted for age, sex, region, smoking and alcohol consumption.
Persson and Fredriksson (1999) Combined analysis of NHL cases in Persson et al. (1993), Persson et al. (1989)	Histologicallly confirmed cases of B-cell NHL, age 20−79 yrs, identified in two hospitals in Sweden: Oreboro in 1964−1986 (Persson et al., 1989) and in Linkoping between 1975−1984 (Persson et al., 1993); controls were identified from previous studies and were randomly selected from population registers.	NHL cases, 199 479 controls Cases, 96% (Oreboro), 90% (Linkoping); controls, not reported	Mailed questionnaire to assess self reported occupational exposures to TCE and other solvents. Unadjusted Mantel-Haenszel chi-square.

Table 4-2. Case-control epidemiologic studies examining cancer and TCE exposure (continued)

Reference	Population	Study group (*N*) Comparison group (*N*) Response rates	Exposure assessment and other information
Nordstrom et al. (1998)	Histologically-confirmed cases in males of hairy-cell leukemia reported to Swedish Cancer Registry in 1987−1992 (includes one case latter identified with an incorrect diagnosis date); population-based controls identified from the National Population Registry and matched (1:4 ratio) to cases for age and county.	111 cases 400 controls Cases, 91%; Controls, 83%	Mailed questionnaire to assess self reported working history, specific exposure, and leisure time activities. Univariate analysis for chemical-specific exposures (any TCE exposure).
Fritschi and Siemiatycki, 1996a), Siemiatycki (1991)	Male NHL cases, age 35−75 yrs, diagnosed in 16 large Montreal-area hospitals in 1979−1985 and histologically confirmed; controls identified concurrently at 18 other cancer sites; age-matched, population-based controls identified from electoral lists and RDD.	215 cases 533 population controls (Group 1) and 1,900 subjects with other cancers (Group 2) Cases, 83%; Controls, 71%	In-person interviews (direct or proxy) with segments on work histories (job titles and self-reported exposures); analyzed and coded by a team of chemists and industrial hygienists (294 exposures on semiquantitative scales). Exposure metric defined as any or substantial exposure. Logistic regression adjusted for age, proxy status, income, and ethnicity (solvents) or Mantel-Haenszel stratified by age, body mass index, and cigarette smoking (TCE).
Hardell et al. (1994, 1981)	Histologically-confirmed cases of NHL in males, age 25−85 yrs, admitted to Swedish (Umea) hospital between 1974−1978; living controls (1:2 ratio) from the National Population Register, matched to living cases on sex, age, and place of residence; deceased controls from the National Registry for Causes of Death, matched (1:2 ratio) to dead cases on sex, age, place of residence, and year of death.	105 cases 335 controls Response rate not available	Self-administered questionnaire assessing self-reported solvent exposure; phone follow-up with subject, if necessary. Unadjusted Mantel-Haenszel chi-square.

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Table 4-2. Case-control epidemiologic studies examining cancer and TCE exposure (continued)

Reference	Population	Study group (*N*) Comparison group (*N*) Response rates	Exposure assessment and other information
Persson et al. (1993), Persson et al. (1989)	Histologicallly confirmed cases of Hodgkin's disease, age 20−80 yrs, identified in two hospitals in Sweden: Oreboro in 1964−1986 (Persson et al., 1989) and in Linkoping between 1975−1984 (Persson et al., 1993); controls randomly selected from population registers.	54 cases (1989 study); 31 cases (1993 study) 275 controls (1989 study); 204 controls (1993 study) Response rate not available	Mailed questionnaire to assess self reported occupational exposures to TCE and other solvents. Logistic regression with adjustment for age and other exposure; unadjusted Mantel-Haenszel chi-square.
Childhood Leukemia			
Shu et al. (2004, 1999)	Childhood leukemia cases, <15 yrs, diagnosed between 1989 and 1993 by a Children's Cancer Group member or affiliated institute; population controls (random digit dialing), matched for age, race, and telephone area code and exchange.	1,842 cases 1,986 controls Cases, 92%; controls, 77%	Telephone interview with mother, and whenever available, fathers using questionnaire to assess occupation using job-industry title and self-reported exposure history. Questionnaire included questions specific for solvent, degreaser or cleaning agent exposures. Logistic regression with adjustment for maternal or paternal education, race, and family income. Analyses of paternal exposure also included age and sex of the index child.
Costas et al. (2002), MA DPH (1997)	Childhood leukemia (<19 yrs age) diagnosed in 1969−1989 and who were resident of Woburn. MA; controls randomly selected from Woburn public School records, matched for age.	19 cases 37 controls Cases, 91%; Controls, not available	Questionnaire administered to parents separately assessing demographic and lifestyle characteristics, medical history information, environmental and occupational exposure and use of public drinking water in the home. Hydraulic mixing model used to infer delivery of TCE and other solvents water to residence. Logistic regression with composite covariate, a weighted variable of individual covariates.
McKinney et al. (1991)	Incident childhood leukemia and non-Hodgkin's lymphoma cases, 1974–1988, ages not identified, from three geographical areas in England; controls randomly selected from children of residents in the three areas and matched for sex and birth health district.	109 cases 206 controls Cases, 72%; Controls, 77%	In-person interview with questionnaire with mother to assess maternal occupational exposure history, and with father and mother, as surrogate, to assess paternal occupational exposure history. No information provided in paper whether interviewer was blinded as to case and control status. Matched pair design using logistic regression for univariate and multivariate analysis.

Table 4-2. Case-control epidemiologic studies examining cancer and TCE exposure (continued)

Reference	Population	Study group (*N*) Comparison group (*N*) Response rates	Exposure assessment and other information
Lowengart et al. (1987)	Childhood leukemia cases aged ≤10 yrs and identified from the Los Angeles (CA) Cancer Surveillance Program in 1980−1984; controls selected from RDD or from friends of cases and matched on age, sex, and race.	123 cases 123 controls Cases, 79%; Controls, not available	Telephone interview with questionnaire to assess parental occupational and self-reported exposure history. Matched (discordant) pair analysis.
Melanoma			
Fritschi and Siemiatycki (1996b), Siemiatycki (1991)	Male melanoma cases, age 35−75 yrs, diagnosed in 16 large Montreal-area hospitals in 1979−1985 and histologically confirmed; controls identified concurrently at 18 other cancer sites; age-matched, population-based controls identified from electoral lists and RDD.	103 cases 533 population controls and 533 other cancer controls Cases, 78%; Controls, 72%	In-person interviews (direct or proxy) with segments on work histories (job titles and self-reported exposures); analyzed and coded by a team of chemists and industrial hygienists (294 exposures on semiquantitative scales); potential TCE exposure defined as any or substantial exposure. Logistic regression adjusted for age, education, and ethic origin (TCE) or Mantel-Haenszel stratified on age, income, index for cigarette smoking, and ethnic origin (TCE).
Pancreas			
Kernan et al. (1999)	Pancreatic cancer deaths from 1984−1993 in 24 U.S. states; age-, sex-, race-, and state-matched noncancer deaths, excluding other pancreatic diseases and pancreatitis, controls.	63,097 cases 252,386 population controls Response rates not identified	Exposure surrogate assigned for 111 chlorinated hydrocarbons, including TCE, and 2 broad chemical categories using usual occupation on death certificate and job-exposure-matrix of Gomez et al. (1994). Race and sex-specific mortality odds ratios from logistic regression analysis adjusted for age, marital status, metropolitan area, and residential status.

Table 4-2. Case-control epidemiologic studies examining cancer and TCE exposure (continued)

Reference	Population	Study group (*N*) Comparison group (*N*) Response rates	Exposure assessment and other information
Prostate			
Aronson et al. (1996), Siemiatycki (1991)	Male prostate cancer cases, age 35−75 yrs, diagnosed in 16 large Montreal-area hospitals in 1979−1985 and histologically confirmed; controls identified concurrently at 18 other cancer sites; age-matched, population-based controls identified from electoral lists and RDD.	449 cases 533 population controls (Group 1) and other cancer cases from same study (Group 2) Cases, 81%; Controls, 72%	In-person interviews (direct or proxy) with segments on work histories (job titles and self-reported exposures); analyzed and coded by a team of chemists and industrial hygienists (294 exposures on semiquantitative scales). Logistic regression adjusted for age, ethnic origin, socioeconomic status, Quetlet, and respondent status (occupation) or Mantel-Haenszel stratified on age, income, index for cigarette smoking, ethnic origin, and respondent status (TCE).
Renal Cell			
Charbotel et al. (2006, 2009)	Cases from Arve Valley region in France identified from local urologists files and from area teaching hospitals; age- and sex-matched controls chosen from file of same urologist as who treated case or recruited among the patients of the case's general practitioner.	87 cases 316 controls Cases, 74%; controls, 78%	Telephone interview with case or control, or, if deceased, with next-of-kin (22% cases, 2% controls). Questionnaire assessing occupational history, particularly, employment in the screw cutting jobs, and medical history. Semiquantitative TCE exposure assigned to subjects using a task/TCE-Exposure Matrix designed using information obtained from questionnaires and routine atmospheric monitoring of work shops or biological monitoring (U-TCA) of workers carried out since the 1960s. Cumulative exposure, cumulative exposure with peaks, and TWA. Conditional logistic regression with covariates for tobacco smoking and body mass index.
Brüning et al. (2003)	Histologically-confirmed cases 1992−2000 from German hospitals (Arnsberg); hospital controls (urology department) serving area, and local geriatric department, for older controls, matched by sex and age.	134 cases 401 controls Cases, 83%; Controls, not available	In-person interviews with case or next-of-kin; questionnaire assessing occupational history using job title. Exposure metrics included longest job held, JEM of Pannett et al. (1985) to assign cumulative exposure to TCE and PCE, and exposure duration.. Logistic regression with covariates for age, sex, and smoking.
Pesch et al. (2000b)	Histologically-confirmed cases from German hospitals (5 regions) in 1991−1995; controls randomly selected from residency registries matched on region, sex, and age.	935 cases 4,298 controls Cases, 88%; Controls, 71%	In-person interview with case or next-of-kin; questionnaire assessing occupational history using job title (JEM approach), self-reported exposure, or job task (JTEM approach) to assign TCE and other exposures. Logistic regression with covariates for age, study center, and smoking.

Table 4-2. Case-control epidemiologic studies examining cancer and TCE exposure (continued)

Reference	Population	Study group (*N*) Comparison group (*N*) Response rates	Exposure assessment and other information
Parent et al. (2000b), Siemiatycki (1991)	Male renal cell carcinoma cases, age 35−75 yrs, diagnosed in 16 large Montreal-area hospitals in 1979−1985 and histologically confirmed; controls identified concurrently at 18 other cancer sites; age-matched, population-based controls identified from electoral lists and RDD.	142 cases 533 population controls (Group 1) and other cancer controls (excluding lung and bladder cancers) (Group 2) Cases, 82%; Controls, 71%	In-person interviews (direct or proxy) with segments on work histories (job titles and self-reported exposures); analyzed and coded by a team of chemists and industrial hygienists (about 300 exposures on semiquantitative scales); TCE defined as any or substantial exposure. Mantel-Haenszel stratified by age, body mass index, and cigarette smoking (TCE) or logistic regression adjusted for respondent status, age, smoking, and body mass index (occupation, job title).
Dosemeci et al. (1999)	Histologically-confirmed cases, 1988−1990, white males and females, 20−85 yrs, from Minnesota Cancer Registry; controls stratified for age and sex using RDD, 21−64 yrs, or from HCFA records, 64−85 yrs.	438 cases 687 controls Cases, 87%; Controls, 86%	In-person interviews with case or next-of-kin; questionnaire assessing occupational history of TCE using job title and JEM of Gomez et al. (1994). Exposure metric was any TCE exposure. Logistic regression with covariates for age, smoking, hypertension, and body mass index.
Vamvakas et al. (1998)	Cases who underwent nephrectormy in 1987−1992 in a hospital in Arnsberg region of Germany; controls selected accident wards from nearby hospital in 1992.	58 cases 84 controls Cases, 83%; Controls, 75%	In-person interview with case or next-of-kin; questionnaire assessing occupational history using job title or self-reported exposure to assign TCE and PCE exposure. Logistic regression with covariates for age, smoking, body mass index, hypertension, and diuretic intake.
Multiple or Other Sites			
Lee et al. (2003)	Liver, lung, stomach, colorectal cancer deaths in males and females between 1966−1997 from two villages in Taiwan; controls were cardiovascular and cerebral-vascular disease deaths from same underlying area as cases.	53 liver, 39 stomach, 26 colorectal, 41 lung cancer cases 286 controls Response rate not reported	Residence as recorded on death certificate. Mantel-Haenszel stratified by age, sex, and time period.

Table 4-2. Case-control epidemiologic studies examining cancer and TCE exposure (continued)

Reference	Population	Study group (*N*) Comparison group (*N*) Response rates	Exposure assessment and other information
Siemiatycki (1991)	Male cancer cases, 1979−1985, 35−75 yrs, diagnosed in 16 Montreal-area hospitals, histologically confirmed; cancer controls identified concurrently; age-matched, population-based controls identified from electoral lists and RDD.	857 lung and 117 pancreatic cancer cases 533 population controls (Group 1) and other cancer cases from same study (Group 2) Cases, 79% (lung), 71% (pancreas); Controls, 72%	In-person interviews (direct or proxy) with segments on work histories (job titles and self-reported exposures); analyzed and coded by a team of chemists and industrial hygienists (294 exposures on semiquantitative scales); TCE defined as any or substantial exposure. Mantel-Haenszel stratified on age, income, index for cigarette smoking, ethnic origin, and respondent status (lung cancer) and age, income, index for cigarette smoking, and respondent status (pancreatic cancer).

HCFA = Health Care Financing Administration, JEM = job-exposure matrix, JTEM = job-task-exposure matrix, NCI = National Cancer Institute, PCE = perchloroethylene, RDD = random digit dialing, U-TCA = urinary trichloroacetic acid, UV = ultra-violet.

Table 4-3. Geographic-based studies assessing cancer and TCE exposure

Reference	Description	Analysis approach	Exposure assessment
Broome County, NY Studies			
ATSDR (2006a, 2008)	Total, 22 site-specific, and childhood cancer incidence from 1980−2001 among residents in 2 areas in Endicott, NY.	SIR among all subjects (ATSDR, 2006a) or among white subjects only (ATSDR, 2008) with expected numbers of cancers derived using age-specific cancer incidence rates for New York State, excluding New York City. Limited assessment of smoking and occupation using medical and other records in lung and kidney cancer subjects (ATSDR, 2008).	Two study areas, Eastern and Western study areas, identified based on potential for soil vapor intrusion exposures as defined by the extent of likely soil vapor contamination. Contour lines of modeled VOC soil vapor contamination levels based on exposure model using GIS mapping and soil vapor sampling results taken in 2003. The study areas were defined by 2000 Census block boundaries to conform to model predicted areas of soil vapor contamination. TCE was the most commonly found contaminant in indoor air in Eastern study area at levels ranging from 0.18 to 140 $\mu g/m^3$, with tetrachloroethylene, cis-1,2-dichloroethene, 1,1,1-trichloroethane, 1,1-dichloroethylene, 1,1-dichloroethane, and Freon 113 detected at lower levels. PCE was most common contaminant in indoor air in Western study area with other VOCs detected at lower levels.
Maricopa County, AZ Studies			
Aickin et al. (1992) Aickin (2004)	Cancer deaths, including leukemia, 1966−1986, and childhood (≤19 yrs old) leukemia incident cases (1965−1986), Maricopa County, AZ.	Standardized mortality RR from Poisson regression modeling. Childhood leukemia incidence data evaluated using Bayes methods and Poisson regression modeling.	Location of residency in Maricopa County, AZ, at the time of death as surrogate for exposure. Some analyses examined residency in West Central Phoenix and cancer. Exposure information is limited to TCE concentration in two drinking water wells in 1982.
Pima County, AZ Studies			
AZ DHS (1990, 1995)	Cancer incidence in children (≤19 yrs old) and testicular cancer in 1970−1986 and 1987−1991, Pima County, AZ.	Standardized incidence RR from Poisson regression modeling using method of Aickin et al. (1992). Analysis compares incidence in Tucson Airport Area to rate for rest of Pima County.	Location of residency in Pima, County, AZ, at the time of diagnosis or death as surrogate for exposure. Exposure information is limited to monitoring since 1981 and include VOCs in soil gas samples (TCE, PCE, 1,1-dichloroethylene, 1,1,1-trichloroacetic acid); PCBs in soil samples, and TCE in municipal water supply wells.

Table 4-3. Geographic-based studies assessing cancer and TCE exposure (continued)

Reference	Description	Analysis approach	Exposure assessment
Other			
Coyle et al. (2005)	Incident breast cancer cases among men and women, 1995−2000, reported to Texas Cancer Registry.	Correlation study using rank order statistics of mean average annual breast cancer rate among women and men and atmospheric release of 12 hazardous air pollutants.	Reporting to EPA Toxic Release Inventory the number of pounds released for 12 hazardous air pollutants, (carbon tetrachloride, formaldehyde, methylene chloride, styrene, tetrachloroethylene, trichloroethylene, arsenic, cadmium, chromium, cobalt, copper, and nickel).
Morgan and Cassady (2002)	Incident cancer cases, 1988−1989, among residents of 13 census tracts in Redlands area, San Bernardino County, CA.	SIR for all cancer sites and 16 site-specific cancers; expected numbers using incidence rates of site-specific cancer of a four-county region between 1988−1992.	TCE and perchlorate detected in some county wells; no information on location of wells to residents, distribution of contaminated water, or TCE exposure potential to individual residents in studied census tracts.
Vartiainen et al. (1993)	Total cancer and site-specific cancer cases (lymphoma sites and liver) from 1953−1991 in two Finnish municipalities.	SIR with expected number of cancers and site-specific cancers derived from incidence of the Finnish population.	Monitoring data from 1992 indicated presence of TCE, tetrachloroethylene and 1,1,1,-trichloroethane in drinking water supplies in largest towns in municipalities. Residence in town used to infer exposure to TCE.
Cohn et al. (1994) Fagliano et al. (1990)	Incident leukemia and NHL cases, 1979−1987 ,from 75 municipalities and identified from the New Jersey State Cancer Registry. Histological type classified using WHO scheme and the classification of NIH Working Formulation Group for grading NHL.	Logistic regression modeling adjusted for age.	Monitoring data from 1984−1985 on TCE, THM, and VOCs concentrations in public water supplies, and historical monitoring data conducted in 1978−1984.
Mallin (1990)	Incident bladder cancer cases and deaths, 1978−1985, among residents of 9 NW Illinois counties.	SIR and SMR by county of residence and zip code; expected numbers of bladder cancers using age-race-sex specific incidence rates from SEER or bladder cancer mortality rates of the United States population from 1978−1985.	Exposure data are lacking for the study population with the exception of noting one of two zip code areas with observed elevated bladder cancer rates also had groundwater supplies contaminated with TCE, PCE and other solvents.
Isacson et al. (1985)	Incident bladder, breast, prostate, colon, lung and rectal cancer cases reported to Iowa cancer registry between 1969−1981.	Age-adjusted site-specific cancer incidence in Iowa towns with populations of 1,000−10,000 and who were serviced by a public drinking water supply.	Monitoring data of drinking water at treatment plant in each Iowa municipality with populations of 1,000−10,000 used to infer TCE and other volatile organic compound concentrations in finished drinking water supplies.

GIS = geographic information system, NW = Northwestern, PCE = perchloroethylene, RR = rate ratio, SEER = Surveillance, Epidemiology, and End Results, SIR = standardized incidence ratio, SMR = standardized mortality ratio, VOCs = volatile organic compounds, WHO = World Health Organization.

Table 4-4. Standards of epidemiologic study design and analysis use for identifying cancer hazard and TCE exposure.

Category A: Study Design
Clear articulation of study objectives or hypothesis. The ideal is a clearly stated hypothesis or study objectives and the study is designed to achieve the identified objectives.
Selection and characterization in cohort studies of exposure and control groups and of cases and controls (case-control studies) is adequate. The ideal is for selection of cohort and referents from the same underlying population and differences between these groups to be due to TCE exposure or level of TCE exposure and not to physiological, health status, or lifestyle factors. Controls or referents are assumed to lack or to have background exposure to TCE. These factors may lead to a downward bias including one of which is known as "healthy worker bias," often introduced in analyses when mortality or incidence rates from a large population such as the United States population are used to derive expected numbers of events. The ideal in case-control studies is cases and controls are derived from the same population and are representative of all cases and controls in that population. Any differences between controls and cases are due to exposure to TCE itself and not to confounding factors related to both TCE exposure and disease. Additionally, the ideal is for controls to be free of any disease related to TCE exposure. In this latter case, potential bias is toward the null hypothesis.
Category B: Endpoint Measured
Levels of health outcome assessed. Three levels of health outcomes are considered in assessing the human health risks associated with exposure to TCE: biomarkers of effects and susceptibility, morbidity, and mortality. Both morbidity as enumerated by incidence and mortality as identified from death certificates are useful indicators in risk assessment for hazard identification. The ideal is for accurate and predictive indicator of disease. Incidence rates are generally considered to provide an accurate indication of disease in a population and cancer incidence is generally enumerated with a high degree of accuracy in cancer registries. Death certifications are readily available and have complete national coverage but diagnostic accuracy is reduced and can vary by specific diagnosis. Furthermore, diagnostic inaccuracies can contribute to death certificates as a poor surrogate for disease incidence. Incidence, when obtained from population-based cancer registries, is preferred for identifying cancer hazards.
Changes in diagnostic coding systems for lymphoma, particularly non-Hodgkin's lymphoma. Classification of lymphomas today is based on morphologic, immunophenotypic, genotypic, and clinical features using the World Health Organization (WHO) classification, introduced in 2001, and incorporation of WHO terminology into International Classification of Disease (ICD)-0-3. ICD Versions 7 and earlier had rubrics for general types of lymphatic and hematopoietic cancer, but no categories for distinguishing specific types of cancers, such as acute leukemia. Epidemiologic studies based on causes of deaths as coded using these older ICD classifications typically grouped together lymphatic neoplasms instead of examining individual types of cancer or specific cell types. Before the use of immunophenotyping, these grouping of ambiguous diseases such as non-Hodgkin's lymphoma and Hodgkin's lymphoma may be have misclassified. With the introduction of ICD-10 in 1990, lymphatic tumors coding, starting in 1994 with the introduction of the Revised European-American Lymphoma classification, the basis of the current WHO classification, was more similar to that presently used. Misclassification of specific types of cancer, if unrelated to exposure, would have attenuated estimate of relative risk and reduced statistical power to detect associations. When the outcome was mortality, rather than incidence, misclassification would be greater because of the errors in the coding of underlying causes of death on death certificates (IOM, 2003). Older studies that combined all lymphatic and hematopoietic neoplasms must be interpreted with care.

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Table 4-4. Standards of epidemiologic study design and analysis use for identifying cancer hazard and TCE exposure (continued).

Category C: TCE-Exposure Criteria
Adequate characterization of exposure. The ideal is for TCE exposure potential known for each subject and quantitative assessment [job-exposure-matrix approach] of TCE exposure assessment for each subject as a function of job title, year exposed, duration, and intensity. The assessment approach is accurate for assigning TCE intensity [TCE concentration or a time-weighted-average] to individual study subjects and estimates of TCE intensity are validated using monitoring data from the time period. For the purpose of this report, the objective for cohort and case-controls studies is to differentiate TCE-exposed subjects from subjects with little or no TCE exposure. A variety of dose metrics may be used to quantify or classify exposures for an epidemiologic study. They include precise summaries of quantitative exposure, concentrations of biomarkers, cumulative exposure, and simple qualitative assessments of whether exposure occurred (yes or no). Each method has implicit assumptions and potential problems that may lead to misclassification. Studies in which it was unclear that the study population was actually exposed to TCE are excluded from analysis.
Category D: Follow-up (Cohort)
Loss to follow-up. The ideal is complete follow-up of all subjects; however, this is not achievable in practice, but it seems reasonable to expect loss to follow-up not to exceed 10%. The bias from loss to follow-up is indeterminate. Random loss may have less effect than if subjects who are not followed have some significant characteristics in common.
Follow-up period allows full latency period for over 50% of the cohort. The ideal to follow all study subjects until death. Short of the ideal, a sufficient follow-up period to allow for cancer induction period or latency over 15 or 20 yrs is desired for a large percentage of cohort subjects.
Category E: Interview Type (Case-control)
Interview approach. The ideal interviewing technique is face-to-face by trained interviewers with more than 90% of interviews with cases and control subjects conduced face-to-face. The effect on the quality of information from other types of data collection is unclear, but telephone interviews and mail-in questionnaires probably increase the rate of misclassification of subject information. The bias is toward the null hypothesis if the proportion of interview by type is the same for case and control, and of indeterminate direction otherwise.
Blinded interviewer. The ideal is for the interviewer to be unaware whether the subject is among the cases or controls and the subject to be unaware of the purpose and intended use of the information collected. Blinding of the interviewer is generally not possible in a face-to-face interview. In face-to-face and telephone interviews, potential bias may arise from the interviewer expects regarding the relationship between exposure and cancer incidence. The potential for bias from face-to-face interviews is probably less than with mail-in interviews. Some studies have assigned exposure status in a blinded manner using a job-exposure matrix and information collected in the unblinded interview. The potential for bias in this situation is probably less with this approach than for nonblinded assignment of exposure status.
Category F: Proxy Respondents
Proxy respondents. The ideal is for data to be supplied by the subject because the subject generally would be expected to be the most reliable source; less than 10% of either total cases or total controls for case-control studies. A subject may be either deceased or too ill to participate, however, making the use of proxy responses unavoidable if those subjects are to be included in the study. The direction and magnitude of bias from use of proxies is unclear, and may be inconsistent across studies.

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Table 4-4. Standards of epidemiologic study design and analysis use for identifying cancer hazard and TCE exposure (continued).

Category G: Sample Size
The ideal is for the sample size is large enough to provide sufficient statistical power to ensure that any elevation of effect in the exposure group, if present, would be found, and to ensure that the confidence bounds placed on relative risk estimates can be well characterized.
Category H: Analysis Issues
Control for potentially confounding factors of importance in analysis. The ideal in cohort studies is to derive expected numbers of cases based on age-sex- and time-specific cancer rates in the referent population and in case-control studies by matching on age and sex in the design and then adjusting for age in the analysis of data. Age and sex are likely correlated with exposure and are also risk factors for cancer development. Similarly, other factors such as cigarette smoking and alcohol consumption are risk factors for several site-specific cancers reported as associative with TCE exposure. To be a confounder of TCE, exposure to the other factor must be correlated, and the association of the factor with the site-specific cancer must be causal. The expect effect from controlling for confounders is to move the estimated relative risk estimate closer to the true value.
Statistical methods are appropriate. The ideal is that conclusions are drawn from the application of statistical methods that are appropriate to the problem and accurately interpreted.
Evaluation of exposure-response. The ideal is an examination of a linear exposure-response as assessed with a quantitative exposure metric such as cumulative exposure. Some studies, absent quantitative exposure metrics, examine exposure response relationships using a semiquantitative exposure metric or by duration of exposure. A positive dose-response relationship is usually more convincing of an association as causal than a simple excess of disease using TCE dose metric. However, a number of reasons have been identified for a lack of linear exposure-response finding and the failure to find such a relationship mean little from an etiological viewpoint.
Documentation of results. The ideal is for analysis observations to be completely and clearly documented and discussed in the published paper, or provided in supplementary materials accompanying publication.

Twenty-three of the studies identified in a systematic review were selected for inclusion in the meta-analysis through use of the following meta-analysis inclusion criteria: (1) cohort or case-control designs; (2) evaluation of incidence or mortality; (3) adequate selection in cohort studies of exposure and control groups and of cases and controls in case-control studies; (4) TCE exposure potential inferred to each subject and quantitative assessment of TCE exposure assessment for each subject by reference to industrial hygiene records indicating a high probability of TCE use, individual biomarkers, job exposure matrices, water distribution models, or obtained from subjects using questionnaire (case-control studies); and (5) relative risk estimates for kidney cancer, liver cancer, or lymphoma adjusted, at minimum, for possible confounding of age, sex, and race (see Table 4-5). This evaluation is summarized below, separately for cohort and case-control studies. Appendix C contains a full discussion of the meta-analysis, its analytical methodology, including sensitivity analyses, and findings.

The cohort studies (Wilcosky et al., 1984; Shindell and Ulrich, 1985; Garabrant et al., 1988; Shannon et al., 1988; Blair et al., 1989; Costa et al., 1989; Sinks et al., 1992; Axelson et al., 1994; Greenland et al., 1994; Anttila et al., 1995; Henschler et al., 1995; Ritz, 1999; Blair et al., 1998; Morgan et al., 1998; Boice et al., 1999, 2006; Hansen et al., 2001; Raaschou-Nielsen et al., 2003; Chang et al., 2003; ATSDR, 2004; Chang et al., 2005; Zhao et al., 2005; Krishnadasan et al., 2007; Sung et al., 2007, 2008; Clapp and Hoffman, 2008; Radican et al., 2008) (see Table 4-1), with data on the incidence or morality of site-specific cancer in relation to TCE exposure, range in size (803 [Hansen et al., 2001] to 86,868 [Chang et al., 2003, 2005]), and were conducted in Denmark, Sweden, Finland, Germany, Taiwan, and the United States (see Table 4-1). Three case-control studies nested within cohorts (Wilcosky et al., 1984; Greenland et al., 1994; Krishnadasan et al., 2007) are considered as cohort studies because the summary risk estimate from a nested case-control study, the odds ratio, was estimated from incidence density sampling. This is considered an unbiased estimate of the hazard ratio, similar to a relative risk estimate from a cohort study, if, as is the case for these studies, controls are selected from the same source population as the cases, the sampling rate is independent of exposure status, and the selection probability is proportional to time-at-risk (Rothman et al., 2008). Cohort and nested case-control study designs are analytical epidemiologic studies and are generally relied on for identifying a causal association between human exposure and adverse health effects (U.S. EPA, 2005a).

1 **Table 4-5. Summary of criteria for meta-analysis study selection**
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Decision outcome	Studies	Primary reason(s)
Studies recommended for meta-analysis:		
	Siemiatycki, 1991; Axelson et al., 1994; Hardell, 1994; Greenland et al., 1994; Anttila et al., 1995; Morgan et al., 1998; Nordstrom et al., 1998; Boice et al., 1999, 2006a; Dosemeci et al., 1999; Persson and Fredriksson, 1999; Pesch et al., 2000b; Hansen et al., 2001; Brüning et al., 2003; Raaschou-Nielsen et al., 2003; Zhao et al., 2005; Miligi et al., 2006; Seidler et al., 2007; Charbotel et al., 2006, 2009; Radican et al., 2008 (Blair et al., 1998-incidence); Wang et al., 2009	Analytical study designs of cohort or case-control; Evaluation of incidence or mortality; Adequate selection in cohort studies of exposure and control groups and of cases and controls in case-control studies; TCE exposure potential inferred to each subject and quantitative assessment of TCE exposure assessment for each subject by reference to industrial hygiene records indicating a high probability of TCE use, individual biomarkers, job exposure matrices, water distribution models, or obtained from subjects using questionnaire (case-control studies); Relative risk estimates for kidney cancer, liver cancer, or lymphoma adjusted, at minimum, for possible confounding of age, sex, and race).
Studies not recommended for meta-analysis:		
	ATSDR, 2004; Clapp and Hoffman, 2008; Cohn et al., 1994	Weakness with respect to analytical study design (i.e., geographic-based, ecological or proportional mortality ratio design).
	Wilcosky et al., 1984; Isacson et al., 1985; Shindell and Ullrich, 1985; Garabrant et al., 1988; Shannon et al., 1988; Blair et al., 1989; Costa et al., 1989; AZDHS, 1990, 1995; Mallin, 1990; Aickin et al., 1992; Sinks et al., 1992; Vartiainen et al., 1993; Morgan and Cassady, 2002; Lee et al., 2003; Aickin, 2004; Chang et al., 2003, 2005; Coyle et al., 2005; ATSDR, 2006a, 2008; Sung et al., 2007, 2008	TCE exposure potential not assigned to individual subjects using job exposure matrix, individual biomarkers, water distribution models, or industrial hygiene data from other process indicating a high probability of TCE use (cohort studies).
	Lowengart et al., 1987; Fredriksson et al., 1989; McKinney et al., 1991; Heineman et al., 1994; Siemiatycki et al., 1994; Aronson et al., 1996; Fritchi and Siemiatycki, 1996b; Dumas et al., 2000; Kernan et al., 1999; Shu et al., 1999, 2004; Parent et al., 2000a; Pesch et al., 2000a; DeRoos et al., 2001; Goldberg et al., 2001; Costas et al., 2002; Krishnadasan et al., 2007	Cancer incidence or mortality reported for cancers other than kidney, liver, or lymphoma.
	Ritz, 1999a	Subjects monitored for radiation exposure with likelihood for potential confounding; Cancer mortality and TCE exposure not reported for kidney cancer and all hemato- and lymphopoietic cancer reported as broad category.
	Henschler et al., 1995	Incomplete identification of cohort and index kidney cancer cases included in case series.

While all of these cohort studies are considered in the overall weight of evidence, ten of them met all five meta-analysis inclusion criteria: the cohorts of Blair et al. (1998) and its follow-up by Radican et al. (2008); Morgan et al. (1998), Boice et al. (1999, 2006), and Zhao et al. (2005), of aerospace workers or aircraft mechanics; and Axelson et al. (1994), Anttila et al. (1995), Hansen et al. (2001), and Raaschou-Nielsen et al. (2003) of Nordic workers in multiple industries with TCE exposure; and Greenland et al. (1994) of electrical manufacturing workers. Subjects or cases and controls in these studies are considered to sufficiently represent the underlying population, and the bias associated with selection of referent populations is considered minimal. The exposure-assessment approaches included detailed job-exposure matrix, biomonitroing data, or use of industrial hygiene data on TCE exposure pattens and factors that affect such exposure, with high probability of TCE exposure potential to individual subjects. The statistical analyses methods were appropriate and well documented, the measured endpoint was an accurate indicator of disease, and the follow-up was sufficient for cancer latency. These studies are also considered as high-quality studies for identifying kidney, liver and lymphoma cancer hazard. The remaining cohort studies less satisfactorily meet identified criteria or standards of epidemiologic design and analysis, having deficiencies in multiple criteria (Wilcosky et al., 1984; Shindell and Ulrich, 1985; Garabrant et al., 1988; Costa et al., 1989; Sinks et al., 1992; Henschler et al., 1995; Ritz, 1999; Chang et al., 2003, 2005; ATSDR, 2004; Sung et al., 2007, 2008; Clapp and Hoffman, 2008). Krishnandansen et al. (2007), who reported on prostate cancer, met four of the five meta-analysis inclusion criteria except that for reporting a relative risk estimate cancer of the kidney, liver or lymphoma, the site-specific cancers examined using meta-analysis.

The case-control studies on TCE exposure are of several site-specific cancers, including bladder (Siemiatycki, 1991; Siemiatycki et al., 1994; Pesch et al., 2000a); brain (Heineman et al., 1994; DeRoos et al., 2001); childhood lymphoma or leukemia (Lowengart et al., 1987; McKinney et al., 1991; Shu et al., 1999; 2004; Costas et al., 2002); colon cancer (Siemiatycki, 1991; Goldberg et al., 2001); esophageal cancer (Siemiatycki, 1991; Parent et al., 2000a); liver cancer (Lee et al., 2003); lung (Siemiatycki, 1991); adult lymphoma or leukemia (Hardell et al., 1994 [non-Hodgkin's lymphoma (NHL), Hodgkin lymphoma]; leukemia (Siemiatycki, 1991; Fritschi and Siemiatycki, 1996a; Nordstrom et al., 1998 [hairy cell leukemia]; Persson and Fredriksson, 1999 [NHL]; Miligi et al., 2006 [NHL and chronic lymphocytic leukemia (CLL)]; Seidler et al., 2007 [NHL, Hodgkin lymphoma]; Costantini et al., 2008 [leukemia types, CLL included with NHL in Miligi et al., 2006]); melanoma (Siemiatycki, 1991; Fritchi and Siemiatycki, 1996b); rectal cancer (Siemiatycki, 1991; Dumas et al., 2000); renal cell carcinoma, a form of kidney cancer (Siemiatycki, 1991; Parent et al. (2000b); Vamvakas et al., 1998;

Dosemeci et al., 1999; Pesch et al., 2000b; Brüning et al., 2003; Charbotel et al., 2006); pancreatic cancer (Siemiatyck, 1991); and prostate cancer (Siemiatycki, 1991; Aronson et al., 1996) (see Table 4-2). No case-control studies of reproductive cancers (breast or cervix) and TCE exposure were found in the peer-reviewed literature.

While all of these case-control studies are considered in the overall weight of evidence, thirteen of them met the meta-analysis inclusion criteria identified in Section B.2.9 (Siemiatycki, 1991; Hardell et al., 1994; Nordstrom et al., 1998; Dosemeci et al., 1999; Persson and Fredriksson, 1999; Pesch et al., 2000b; Brüning et al., 2003; Miligi et al., 2006; Charbotel et al., 2006, 2009; Seidler et al., 2007; Constantini et al., 2008, Wang et al., 2009). They were of analytical study design, cases and controls were considered to represent underlying populations and selected with minimal potential for bias; exposure assessment approaches included assignment of TCE exposure potential to individual subjects using information obtained from face-to-face, mailed, or telephone interviews; analyses methods were appropriate, well-documented, included adjustment for potential confounding exposures, with relative risk estimates and associated confidence intervals reported for kidney cancer, liver cancer or lymphoma.

These studies were also considered, to varying degrees, as high-quality studies for weight-of evidence characterization of hazard. Both Brüning et al. (2003) and Charbotel et al. (2006, 2009) had *a priori* hypotheses for examining renal cell carcinoma and TCE exposure. Strengths of both studies are in their examination of populations with potential for high exposure intensity and in areas with high frequency of TCE usage and their assessment of TCE potential. An important feature of the exposure assessment approach of Charbotel et al. (2006) is their use of a large number of studies on biological monitoring of workers in the screw-cutting industry a predominant industry with documented TCE exposures as support. Other studies were either large multiple-center studies (Pesch et al., 2000a, b; Miligi et al., 2006; Constantini et al., 2008; Wang et al., 2009) or reporting from one location of a larger international study (Dosemeci et al., 1999; Seidler et al., 2007). In contrast to Brüning et al. (2003) and Charbotel et al. (2006, 2009), two studies conducted in geographical areas with widespread TCE usage and potential for exposure to higher intensity, in these other studies, a lower exposure prevalence to TCE is found (any TCE exposure: 15% of cases [Dosemeci et al.,1999]; 6% of cases [Miligi et al., 2006]; 13% of cases [Seidler et al., 2007]; 13% of cases [Wang et al., 2008]) and most subjects were identified as exposed to TCE probably had minimal contact (3% of cases with moderate/high TCE exposure [Miligi et al., 2006]; 1% of cases with high cumulative TCE [Seidler et al., 2007]; 2% of cases with high intensity, but of low probability TCE exposure [Wang et al., 2008]). This

pattern of lower exposure prevalence and intensity is common to community-based population case-control studies (Teschke et al., 2002).

Thirteen case-control studies did not meet specific meta-analysis inclusion criterion (Siemiatycki et al., 1994; Aronson et al., 1996; Fritchi and Siemiatycki, 1996b; Dumas et al., 2000; Parent et al., 2000a; Goldberg et al., 2001; Vamvakas et al., 1998; Kernan et al., 1999; Shu et al., 1999, 2004; Pesch et al., 2000a; Costas et al., 2002; Lee et al., 2003). Ten of twelve studies reported relative risk estimates for site-specific cancers other than kidney, liver, and lymphomas (Siemiatycki et al., 1994; Aronson et al., 1996; Fritchi and Siemiatycki, 1996b; Kernan et al., 1999; Dumas et al., 2000; Parent et al., 2000a; Pesch et al., 2000a; Goldberg et al., 2001; Shu et al., 1999, 2004; Costas et al., 2002). Vamvakas et al. (1998) has been subject of considerable controversy (Bloemen and Tomenson, 1995; Swaen, 1995; McLaughlin and Blot, 1997; Green and Lash, 1999; Cherrie et al., 2001; Mandel, 2001) with questions raised on potential for selection bias related to the study's controls. This study was deficient in the criterion for adequacy of case and control selection. Brüning et al. (2003), a study from the same region as Vamvakas et al. (1998), is considered a stronger study for identifying cancer hazard since it addresses many of the deficiencies of Vamvakas et al. (1998). Lee et al. (2003) in their study of hepatocellular cancer assigns one level of exposure to all subjects in a geographic area, and inherent measurement error and misclassification bias because not all subjects are exposed uniformly. Additionally, statistical analyses in this study did not control for hepatitis viral infection, a known risk factor for hepatocellular cancer and of high prevalence in the study area.

The geographic-based studies (Isacson et al., 1985; AZ DHS, 1990, 1995; Mallin, 1990; Aicken et al., 1992, 2004; Vartianinen et al., 1993; Cohn et al., 1994, Morgan and Cassady, 2002; ATSDR, 2006, 2008) with data on cancer incidence are correlation studies to examine cancer outcomes of residents in communities with TCE and other chemicals detected in groundwater wells or in municipal drinking water supplies (see Table 4-3). These studies did not meet all five meta-analysis inclusion criteria. The geographic-base studies are not of analytical designs such as cohort and case-control designs. Another deficiency in all studies is their low level of detail to individual subjects for TCE. One level of exposure to all subjects in a geographic area is assigned without consideration of water distribution networks, which may influence TCE concentrations delivered to a home, or a subject's ingestion rate to estimate TCE exposure to individual study subjects. Some inherent measurement error and misclassification bias is likely in these studies because not all subjects are exposed uniformly. Additionally, in contrast to case-control studies, the geographic-based studies, including ATSDR (2008), had limited accounting for other potential risk factors. These studies are of low sensitivity for weight-of evidence characterization of hazard compared to high-quality cohort and case-control studies.

4.2. GENETIC TOXICITY

This section discusses the genotoxic potential of TCE and its metabolites. A summary is provided at the end of each section for TCE or its metabolite for their mutagenic potential in addition to an overall synthesis summary at the end of the genotoxicity section. The liver and kidney are subjects of study for the genotoxic potential of TCE and its metabolites, and are discussed more in-depth in sections 4.4.3, 4.4.7, 4.5.6.2.7, 4.5.7, E.2.3, and E.2.4.

The application of genotoxicity data to predict potential carcinogenicity is based on the principle that genetic alterations are found in all cancers. Genotoxicity is the ability of chemicals to alter the genetic material in a manner that permits changes to be transmitted during cell division. Although most tests for mutagenicity detect changes in DNA or chromosomes, some specific modifications of the epigenome including proteins associated with DNA or RNA, can also cause transmissible changes. Changes that occur due to the modifications in the epigenome are discussed in endpoint-specific Sections 4.3−4.9 as well as Sections E.3.1−E.3.4. Genetic alterations can occur through a variety of mechanisms including gene mutations, deletions, translocations, or amplification; evidence of mutagenesis provides mechanistic support for the inference of potential for carcinogenicity in humans.

Evaluation of genotoxicity data entails a weight of evidence approach that includes consideration of the various types of genetic damage that can occur. In acknowledging that genotoxicity tests are by design complementary evaluations of different mechanisms of genotoxicity, a recent IPCS publication (Eastmond et al., 2009) notes that “multiple negative results may not be sufficient to remove concern for mutagenicity raised by a clear positive result in a single mutagenicity assay.” These considerations inform the present approach. In addition, consistent with U.S. EPA’s *Guidelines on Carcinogenic Risk Assessment and Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens)* (2005a, b), the approach does not address relative potency (e.g., among TCE metabolites, or of such metabolites with other known genotoxic carcinogens) *per se*, nor does it consider quantitative issues related to the probable production of these metabolites *in vivo*. Instead, the analysis of genetic toxicity data presented here focuses on the identification of a genotoxic hazard of these metabolites; a quantitative analysis of TCE metabolism to reactive intermediates, via PBPK modeling, is presented in Section 3.5.

TCE and its known metabolites trichloroacetic acid (TCA), dichloroacetic acid (DCA), chloral hydrate (CH), trichloroethanol (TCOH), S-(1,2-dichlorovinyl)-L-cysteine (1,2-DCVC) and S-dichlorovinyl glutathione (DCVG) have been studied to varying degrees for their genotoxic potential. The following section summarizes available data on genotoxicity for both

1 TCE and its metabolites for each potential genotoxic endpoints, when available, in different
2 organisms.

3 **4.2.1. Trichloroethylene (TCE)**

4 ***4.2.1.1. DNA Binding Studies***

5 Covalent binding of TCE to DNA and protein in cell-free systems has been studied by
6 several investigators. Incubation of ^{14}C-TCE with salmon sperm DNA in the presence of
7 microsomal preparations from B6C3F1 mice resulted in dose-related covalent binding of TCE to
8 DNA. The binding was enhanced when the microsomes were taken from mice pretreated with
9 phenobarbital, which induces cytochrome P450 (CYP) enzymes, suggesting the binding may be
10 related to an oxidative metabolite, or when 1,2-epoxy-3,3,3-trichloropropane, an inhibitor of
11 epoxide hydrolase, was added to the incubations (Banerjee and Van Duuren, 1978). In addition,
12 covalent binding of ^{14}C-TCE with microsomal proteins was detected after incubation with
13 microsomal preparations from mouse lung, liver, stomach and kidney and rat liver (Banerjee and
14 Van Duuren, 1978). Furthermore, incubation of ^{14}C-TCE with calf thymus DNA in the presence
15 of hepatic microsomes from phenobarbital-pretreated rats yielded significant covalent binding
16 (Di Renzo et al., 1982).

17 A number of studies have also examined the role TCE metabolism in covalent binding.
18 Miller and Guengerich (1983) used liver microsomes from control, b-naphthoflavone- and
19 phenobarbital-induced B6C3F1 mice, Osborne-Mendel rats and human liver microsomes.
20 Significant covalent binding of TCE metabolites to calf thymus DNA and proteins was observed
21 in all experiments. Phenobarbital treatment increased the formation of chloral and TCE oxide
22 formation, DNA and protein adducts. In contrast, b-naphthoflavone treatment did not induce the
23 formation of any microsomal metabolite suggesting that the forms of CYP induced by
24 phenobarbital are primarily involved in TCE metabolism while the b-naphthoflavone-inducible
25 forms of CYP have only a minor role in TCE metabolism. TCE metabolism (based on TCE-
26 epoxide and DNA-adduct formation) was 2.5−3-fold higher in mouse than in rat microsomes due
27 to differences in rates and clearance of metabolism (discussed in Section 3.3.3.1). The levels of
28 DNA and protein adducts formed in human liver microsomal system approximated those
29 observed in liver microsomes prepared from untreated rats. It was also shown that whole
30 hepatocytes of both untreated mice and phenobarbital-induced rats and mice could activate TCE
31 into metabolites able to covalently bind extracellular DNA. A study by Cai and Guengerich
32 (2001) postulate TCE oxide (an intermediate in the oxidative metabolism of TCE in rat and
33 mouse liver microsomes) is responsible for the covalent binding of TCE with protein, and to a
34 lesser extent, DNA. The authors used mass spectrometry to analyze the reaction of TCE oxide
35 (synthesized by m-chloroperbenzoic acid treatment of TCE) with nucleosides, oligonucleotides

and protein to understand the transient nature of the inhibition of enzymes in the context of adduct formation. Protein amino acid adducts were observed during the reaction of TCE oxide with the model peptides. The majority of these adducts were unstable under physiological conditions. Results using other peptides also indicate that adducts formed from the reaction of TCE oxide with macromolecules and their biological effects are likely to be relatively short-lived.

Studies have been conducted using *in vitro* and *in vivo* systems to understand the DNA and protein binding capacity of TCE. Binding of TCE was observed in calf thymus DNA. In a study in male mice, after repeated intraperitoneal (i.p.) injections of ^{14}C-TCE, radioactivity was detected in the DNA and RNA of all organs studied (kidney, liver, lung, spleen, pancreas, brain and testis) (Bergman, 1983). However, *in vivo* labeling was shown to be due to metabolic incorporation of C1 fragments, particularly in guanine and adenine, rather than to DNA-adduct formation. In another study (Stott et al., 1982), following i.p. injection of ^{14}C-TCE in male Sprague-Dawley rats (10−100 mg/kg) and B6C3F1 mice (10−250 mg/kg), high liver protein labeling was observed while very low DNA labeling was detected. Stott et al. (1982) also observed very low levels of DNA binding (0.62 ± 0.43 alkylation/10^6 nucleotides) in mice administered 1,200 mg/kg of TCE. In addition, a dose-dependent binding of TCE to hepatic DNA and protein at low doses in mice was demonstrated by Kautiainen et al. (1997). In their dose-response study (doses between 2 μg/kg and 200 mg/kg BW), the highest level of protein binding (2.4 ng/g protein) was observed 1 hour after the treatment followed by a rapid decline, indicating pronounced instability of the adducts and/or rapid turnover of liver proteins. Highest binding of DNA (120 pg/g DNA) was found between 24 and 72 hours following treatment. Dose-response curves were linear for both protein and DNA binding. In this study, the data suggest that TCE does bind to DNA and proteins in a dose-dependent fashion, however, the type and structure of adducts were not determined.

Mazzullo et al. (1992) reported that TCE was covalently bound *in vivo* to DNA, RNA and proteins of rat and mouse organs 22 hours after i.p. injection. Labeling of proteins from various organs of both species was higher than that of DNA. Bioactivation of TCE to its intermediates using various microsomal fractions was dependent on CYP enzyme induction and the capacity of these intermediates to bind to DNA. It appeared that mouse lung microsomes were more efficient in forming the intermediates than rat lung microsomes, although no other species specific differences were found (Mazzullo et al., 1992) This also supports the results described by Miller and Guengerich (1983). The authors suggest some binding ability of TCE to interact covalently with DNA (Mazzullo et al., 1992).

In summary, studies report that TCE exposure *in vivo* can lead to binding to nucleic acids and proteins, and some authors have suggested that such binding is likely due to conversion to one or more reactive metabolites.

4.2.1.2. *Bacterial Systems—Gene Mutations*

Gene mutation studies (Ames assay) in various *Salmonella typhimurium* (*S. typhimurium*) strains of bacteria exposed to TCE both in the presence and absence of stabilizing agent have been conducted by different laboratories (Henschler et al., 1977; Simmon et al., 1977; Waskell, 1978; Baden et al., 1979; Crebelli et al., 1982; Shimada et al., 1985; Mortelmans et al., 1986; McGregor et al., 1989) (see Table 4-6). It should be noted that these studies have tested TCE samples of different purities using various experimental protocols. In all *in vitro* assays, volatization is a concern when TCE is directly administered.

Waskell (1978) studied the mutagenicity of several anesthetics and their metabolites. Included in their study was TCE (and its metabolites) using the Ames assay. The study was conducted both in the presence and absence of S9 and caution was exercised to perform the experiment under proper conditions (incubation of reaction mixture in sealed dessicator vials). This study was performed in both TA98 and TA100 *S. typhimurium* strains at a dose range of 0.5−10% between 4 and 48 hours. No change in revertant colonies was observed in any of the doses or time courses tested. No information either on the presence or absence of stabilizers in TCE obtained commercially nor its effect on cytotoxicity was provided in the study.

In other studies highly purified, epoxide free TCE samples were not mutagenic in experiments with and without exogenous metabolic activation by S9 in *S. typhimurium* strain TA100 using the plate incorporation assay (Henschler et al., 1977). Furthermore, no mutagenic activity was found in several other strains including TA1535, TA1537, TA97, TA98, and TA100using the preincubation protocol (Mortelmans et al., 1986). Simmon et al. (1977) observed a less than 2-fold but reproducible and dose-related increase in *his*+ revertants in plates inoculated with *S. typhimurium* TA100 and exposed to a purified, epoxide-free TCE sample. The authors observed no mutagenic response in strain TA1535 with S9 mix and in either TA1535 or TA100 without rat or mouse liver S9. Similar results were obtained by Baden et al. (1979), Bartsch et al. (1979) and Crebelli et al. (1982). In all these studies purified, epoxide-free TCE samples induced slight but reproducible and dose-related increases in *his*+ revertants in *S. typhimurium* TA100 only in the presence of S9. No mutagenic activity was detected without exogenous metabolic activation or when liver S9 from naïve rats, mice and hamsters (Crebelli et al., 1982) was used for activation. Therefore, a number of these studies showed positive results in TA100 with metabolic activation, but not in other strains or without metabolic activation.

Table 4-6. TCE genotoxicity: bacterial assays

Test system/endpoint	Doses tested	With activation	Without activation	Comments	References
S. typhimurium (TA100)	0.1−10 µL (epoxide-free)	–	–	plate incorporation assay	Henschler et al., 1977
S. typhimurium (TA1535, TA100)	1−2.5% (epoxide-free)	+ (TA100) – (TA1535)			Simmon et al., 1977
S. typhimurium (TA98, TA100)	0.5−10%	–	–	the study was conducted in sealed dessicator vials	Waskell, 1978
S. typhimurium (TA100, TA1535)	1−3% (epoxide-free)	+ (TA100) +/– (TA1535)	–		Baden et al., 1979
S. typhimurium (TA100)	5−20% (v/v)	–	–	negative under normal conditions, but 2-fold increase in mutations in a preincubation assay	Bartsch et al., 1979
S. typhimurium (TA100)	0.33−1.33% (epoxide-free)	+	–		Crebelli et al., 1982
S. typhimurium (TA1535, TA100)	1−5% (higher and lower purity)	– (higher purity) + (lower purity)	–	extensive cytotoxicity	Shimada et al., 1985
S. typhimurium (TA98, TA100, TA1535, TA1537, TA97)	10−1000 µL/plate	–	–	preincubation protocol	Mortelmans et al., 1986
S. typhimurium (TA98, TA100, TA1535)	≤10,000 µg/plate (unstabilized)	–	ND	vapor assay	McGregor et al., 1989
S. typhimurium (TA98, TA100, TA1535)	≤10,000 µg/plate (oxirane-stabilized)	+	+	vapor assay	McGregor et al., 1989
S. typhimurium	≤10,000 µg/plate (epoxybutane stabilized)	ND	+	preincubation assay	McGregor et al., 1989
S. typhimurium	≤10,000 µg/plate (epichlorohydrin stabilized)	ND	+	vapor assay	McGregor et al., 1989
S. typhimurium (YG7108)	1000–3000 µg/plate	ND	+	microcolony assay/ revertants	Emmert et al., 2006
E. coli (K12)	0.9 mM (analytical grade)	+	–	revertants at arg56 but not nad113 or other loci	Greim et al, 1975

ND = not determined .

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1 Shimada et al. (1985) tested a low-stabilized, highly purified TCE sample in an Ames
2 reversion test, modified to use vapor exposure, in *S. typhimurium* TA1535 and TA100. No
3 mutagenic activity was observed—either in the presence or absence of S9 mix. However, at the
4 same concentrations (1, 2.5, and 5%), a sample of lower purity, containing undefined stabilizers,
5 was directly mutagenic in TA100 (>5-fold) and TA1535 (>38-fold) at 5% concentration
6 regardless of the presence of S9. It should be noted that the doses used in this study resulted in
7 extensive killing of bacterial population, particularly at 5% concentration, more than 95%
8 toxicity was observed.
9 A series of studies evaluating TCE (with and without stabilizers) was conducted by
10 McGregor et al. (1989). The authors tested high purity and oxirane-stabilized TCE samples for
11 their mutagenic potential in *S. typhimurium* strains TA1535, TA98, and TA100. Preincubation
12 protocol was used to test stabilized TCE (up to 10,000 μg/plate). Mutagenic response was not
13 observed either in the presence or absence of metabolic activation. When TCE was tested in a
14 vapor delivery system without the oxirane stabilizers, no mutagenic activity was observed.
15 However, TA1535 and TA100 produced a mutagenic response both in the presence and absence
16 of S9 when exposed to TCE containing 0.5-0.6% 1,2-epoxybutane. Furthermore, exposure to
17 epichlorohydrin also increased the frequency of mutants.
18 Emmert et al. (2006) used a CYP2E1-competent bacterial strain (*S. typhimurium*
19 containing YG7108pin3ERb$_5$ plasmid) in their experiments. TCE was among several other
20 compounds investigated and was tested at concentrations of 1,000–3,000 μg/plate. TCE induced
21 toxicity and microcolonies at or above 1,000 μg per plate. A study on *Escherichia coli* (*E. coli*)
22 K12 strain was conducted by Greim et al. (1975) using analytical-grade TCE samples.
23 Revertants were scored at two loci: arg_{56}, sensitive to base-pair substitution and nad_{113}, reverted
24 by frameshift mutagens. In addition, forward mutations to 5-methyltryptophan resistance and
25 galactose fermentation were selected. Approximately 2-fold increase in *arg*+ colonies was
26 observed. No change in other sites was observed. No definitive conclusion can be drawn from
27 this study due to lack of information on reproducibility and dose-dependence.
28 In addition to the above studies, the ability of TCE to induce gene mutations in bacterial
29 strains has been reviewed and summarized by several authors (Fahrig et al., 1995; Crebelli and
30 Carere, 1989; Douglas et al., 1999; Moore and Harrington-Brock, 2000; Clewell and Andersen,
31 2004). In summary, TCE, in its pure form as a parent compound is unlikely to induce point
32 mutations in most bacterial strains. It is possible that some mutations observed in response to
33 exposure to technical grade TCE may be contributed by the contaminants/impurities such as
34 1,2-epoxybutane and epichlorohydrin, which are known bacterial mutagens. However, several

1 studies of TCE reported low, but positive responses in the TA100 strain in the presence of S9
2 metabolic activation, even when genotoxic stabilizers were not present.
3

4 4.2.1.3. *Fungal and Yeast Systems—Gene Mutations, Conversions and Recombination*

5 Gene mutations, conversions, and recombinations have been studied to identify the effect
6 of TCE in fungi and yeast systems (see Table 4-7).
7 Crebelli et al. (1985) studied the mutagenicity of TCE in *Aspergillus nidulans (A.*
8 *nidulans)* both for gene mutations and mitotic segregation. No increase in mutation frequency
9 was observed when *A. nidulans* was plated on selective medium and then exposed to TCE
10 vapors. A small but statistically significant increase in mutations was observed when conidia of
11 cultures were grown in the presence of TCE vapors and then plated on selective media. Since
12 TCE required actively growing cells to exerts its genotoxic activity and previous studies
13 (Bignami et al., 1980) have shown activity in the induction of *methG1* suppressors by
14 trichloroethanol and chloral hydrate, it is possible that endogenous metabolic conversion of TCE
15 into trichloroethanol or chloral hydrate may have been responsible for the positive response.
16 To understand the cytochrome P450 mediated genotoxic activity of TCE, Callen et al.
17 (1980) conducted a study in two yeast strains (D7 and D4) CYP. The D7 strain in it log-phase
18 had a CYP concentration up to 5 times higher than a similar cell suspension of D4 strain. Two
19 different concentrations (15 and 22 mM) at two different time points (1 and 4 hours) were
20 studied. A significant increase in frequencies of mitotic gene conversion and recombination was
21 observed at 15 mM concentrations at 1-hour exposure period in the D7 strain, however, the
22 22 mM concentration was highly cytotoxic (only 0.3% of the total number of colonies survived).
23 No changes were seen in D4 strain, suggesting that metabolic activation via CYP played an
24 important role in both genotoxicity and cytotoxicity. However, marginal or no genotoxic activity
25 was observed when incubation of cells and test compounds were continued for 4 hours in either
26 strain, possibly because of increased cytotoxicity, or a destruction of the metabolic system.
27 Koch et al. (1988) studied the genotoxic effects of chlorinated ethylenes including TCE
28 in various yeast *Saccharomyces cerevisiae* strains. Strain D7 was tested (11.1, 16.6, and 22.2
29 mM TCE) both in stationary-phase cells without S9, stationary-phase cells with S9 and
30 logarithmic-phase cells using different concentrations. No significant change in mitotic gene
31 conversion or reverse mutation was observed in either absence or presence of S9. In addition,
32 there was an considerable increase in the induction of mitotic aneuploidy in Strain D61.M,
33 though no statistical analysis was performed.
34

Table 4-7. TCE genotoxicity: fungal and yeast systems

Test system/endpoint	Doses tested	With activation	Without activation	Comments	References
Gene Conversions					
S. cerevisiae D7 and D4	15 and 22 mM; 1 and 4 h	ND	+ at 1 h, D7 strain; – at 4 h, both D7 and D4	gene conversion; CYP content 5-fold greater in D7 strain; high cytotoxicity at 22 mM	Callen et al., 1980
S. cerevisiae D7	11.1, 16.6, and 22.2 mM	–	–	both stationary and log phase/production of phototropic colonies	Koch et al., 1988
S. pombe	0.2 to 200 mM (“pure” and technical grade)	–	–	forward mutation, different experiments with different doses and time	Rossi et al., 1983
S. cerevisiae D7		+	–		Bronzetti et al., 1980
A. nidulans		no data	+	forward mutation	Crebelli et al., 1985
Recombination					
S. cerevisiae		+	–	gene conversion	Bronzetti et al., 1980
S. cerevisiae D7 and D4	15 and 22 mM; 1 and 4 h	ND	+		Callen et al., 1980
A. nidulans		ND	+	gene cross over	Crebellii et al., 1985
Mitotic aneuploidy					
S. cerevisiae D61.M	5.5, 11.1, and 16.6 mM	+	+	loss of dominant color homolog	Koch et al., 1988

ND = not determined .

Rossi et al. (1983) studied the effect of TCE on yeast species *S. pombe* both using *in vitro* and host mediated mutagenicity studies and the effect of two stabilizers, epichlorohydrin and 1,2-epoxybutane that are contained in the technical grade of TCE. The main goal of this study was to evaluate genotoxic activity of TCE samples of different purity and if the effect is due to the additives present in the TCE or TCE itself. Forward mutations at five loci (*ade 1, 3, 4, 5, 9*) of the adenine pathway in the yeast, strain P1 was evaluated. The stationary-phase cells were exposed to 25 mM concentration of TCE for 2, 4, and 8 hours in the presence and absence of S9. No change in mutation frequency was observed either in pure-grade samples or technical-grade samples either in the presence or absence of S9 at any of the time-points tested. Interestingly, this suggests that the stabilizers used in technical-grade TCE are not genotoxic in yeast. In a follow-up experiment, the same authors studied the effect of different concentrations (0.22, 2.2 and 22.0 mM) in a host mediated assay using liver microsome preparations obtained from untreated mice, from phenobarbital-pretreated and NF-pretreated mice and rats, which also suggested that stabilizers were not genotoxic in yeast. This experiment is described in more detail in Section 4.2.1.4.1.

Furthermore, TCE was tested for its ability to induce both point mutation and mitotic gene conversion in diploid strain of yeast *S. cerevisiae* (strain D7) both with and without a mammalian microsomal activation system. In a suspension test with D7, TCE was active only with microsomal activation (Bronzetti et al., 1980).

These studies are consistent with those of bacterial systems in indicating that pure TCE as a parent compound is not likely to cause mutations, gene conversions, or recombinations in fungal or yeast systems. In addition, the data suggest that contaminants used as stabilizers in technical grade TCE are not genotoxic in these systems, and that the observed genotoxic activity in these systems is predominantly mediated by TCE metabolites.

4.2.1.4. *Mammalian Systems Including Human Studies*

4.2.1.4.1. ***Gene mutations (bacterial, fungal, or yeast with a mammalian host).*** Very few studies have been conducted to identify the effect of TCE, particularly on gene (point) mutations using mammalian systems (see Table 4-8). An overall summary of different endpoints using mammalian systems will be provided at the end of this section. In order to assess the potential mutagenicity of TCE and its possible contaminants, Rossi et al. (1983) performed genotoxicity tests using two different host mediated assays with pure- and technical-grade TCE. Male mice were administered with one dose of 2 g/kg of pure or technical grade TCE by gavage. Following the dosing, for the intraperitoneal host–mediated assay, yeast cell suspensions (2×10^9 cells/mL) were inoculated into the peritoneal cavity of the animals. Following 16 hours, animals were

Table 4-8. TCE genotoxicity: mammalian systems—gene mutations and chromosome aberrations

Test system/endpoint	Doses tested	With activation	Without activation	Comments	References
Gene mutations (forward mutations)					
Schizosaccharomyces pombe	2 g/kg, 4 and 16 h	ND	–	Host-mediated: intravenous and intraperitoneal injections of yeast cells	Rossi et al., 1983
Gene mutations (mutations frequency)					
lac Z transgenic mice	0, 203, 1,153, or 3,141 ppm	No base changes or small deletions	No base changes or small deletions	Lung, liver, bone marrow, spleen, kidney, testicular germ cells used	Douglas et al., 1999
Chromosomal aberrations*					
CHO	745−14,900 µg/mL	ND	–	8−14 h	Galloway et al., 1987
	499−14,900 µg/mL	–	ND	2 h exposure	Galloway et al., 1987
C57BL/6J mice	5, 50, 500, or 5,000 ppm (6 h)	-	NA	Splenocytes	Kligerman et al., 1994
S-D rats	5, 50, 500, or 5,000 ppm (6 h, single and 4-d exposure)	-	NA	Peripheral blood lymphocytes	Kligerman et al., 1994

*It should be noted that results of most chromosomal aberration assays report the combined incidence of multiple effects, including chromatid breaks, isochromatid or chromosome breaks, chromatid exchanges, dicentric chromosomes, ring chromosomes, and other aberrations.

ND = not determined, NA = not applicable.

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sacrificed and yeast cells were recovered to detect the induction of forward mutations at five loci (*ade 1, 2, 4, 5, 9*) of the adenine pathway. A second host-mediated assay was performed by exposing the animals to 2 g/kg of pure or technical grade TCE and inoculating the cells into the blood system. Yeast cells were recovered from livers following 4h of exposure. Forward mutations in the five loci (*ade 1,2,4,5,9*) were not observed in host-mediated assay either with pure or technical-grade TCE. Genotoxic activity was not detected when the mutagenic epoxide stabilizers were tested for mutagenicity independently or in combination. To confirm the sensitivity of the assay, the authors tested a positive control—N-nitroso-dimethyl-nitrosamine (1 mg/kg) and found a mutation frequency of more than 20 times the spontaneous level. The authors suggest that the negative result could have been due to an inadequate incubation time of the sample with the yeast cells.

Male and female transgenic *lac Z* mice were exposed by inhalation to an actual concentrations of 0, 203, 1,153, and 3,141 ppm TCE, 6 hours/day for 12 days (Douglas et al., 1999). Following 14 and 60 days of last exposure, animals were sacrificed and the mutation frequencies were determined in various organs such as bone marrow, kidney, spleen, liver, lung, and testicular germ cells. No statistically significant increases in base-changes or small-deletions were observed at any of the doses tested in male or female lung, liver, bone marrow, spleen, and kidney, or in male testicular germ cells when the animals were sampled 60 days after exposure. In addition, statistically significantly increased gene mutations were not observed in the lungs at 14 days after the end of exposure (Douglas et al., 1999). The authors acknowledge that *lacZ* bacteriophage transgenic assay does not detect large deletions. The authors also acknowledge that their hypothesis does not readily explain the increases in small deletions and base-change mutations found in the *von Hippel-Lindau* tumor suppressor gene in renal cell carcinomas of the TCE-exposed population. DCA, a TCE metabolite has been shown to increase *lacI* mutations in transgenic mouse liver, however, only after 60-weeks–of-exposure to high concentration (>1,000 ppm) in drinking water (Leavitt et al., 1997). DCA induced relatively small increase in *lac I* mutations when the animals were exposed for 60 weeks, a significantly longer duration than the TCE exposure in the Douglas et al. (1999) study (<2 weeks). Because a relatively small fraction of TCE is metabolized to DCA (see Section 3.3), the mutagenic effect of DCA is unlikely to have been detected in the experiments in Douglas et al. (1999). GSH conjugation, which leads to the production of genotoxic metabolites (see Section 4.2.5), constitutes a relatively small (and relatively uncertain) portion of TCE metabolism in mice, with little data on the extent of renal DCVC bioactivation versus detoxification in mice (see Sections 3.3 and 3.5). In addition, statistically significantly increased kidney tumors have not been reported in mice with TCE treatment, and the increased incidence of kidney tumors in rats, while considered

1 biologically significant, are quite low and not always statistically significant (see Section 4.4).
2 Therefore, although Douglas et al. (1999) did not detect increased mutations in the kidney, these
3 results are not highly informative as to the role of mutagenicity in TCE-induced kidney tumors,
4 given the uncertainties in the production in genotoxic GSH conjugation metabolites in mice and
5 the low carcinogenic potency of TCE for kidney tumors in rodents relative to what is detectable
6 in experimental bioassays..
7
8 **4.2.1.4.2.** ***von Hippel-Lindau (VHL) gene mutations.*** Studies have been conducted to
9 determine the role of VHL gene mutations in renal cell carcinoma, with and without TCE
10 exposure, and are summarized here. Most of these studies are epidemiologic, comparing VHL
11 mutation frequencies of TCE-exposed to nonexposed cases from renal cell carcinoma
12 case-control studies, or to background mutation rates among other renal cell carcinoma case
13 series (described in Section 4.4.3). Inactivation of the VHL gene through mutations, loss of
14 heterozygosity and imprinting has been observed in about 70% of renal clear cell carcinomas
15 (Alimov et al., 2000; Kenck et al., 1996). Recent studies have also examined the role of other
16 genes or pathways in renal cell carcinoma subtypes, including c-myc activation and vascular
17 endothelial growth factor (VEGF) (Furge et al., 2007; Toma et al., 2008).
18 Several studies have examined the role of *VHL* gene inactivation in renal cell carcinoma,
19 including a recent study that measured not only mutations but also promoter hypermethylation
20 (Nickerson et al., 2008). This study focused on kidney cancer regardless of cause, and found that
21 91% of cc-renal cell carcinoma (RCC) exhibited alterations of the *VHL* gene, suggesting a role
22 for *VHL* mutations as an early event in cc-RCC. A recent analysis of current epidemiological
23 studies of renal cell cancer suggests *VHL* gene alterations as a marker of cc-RCC, but that
24 limitations of previous studies may make the results difficult to interpret (Chow and Devesa,
25 2008). Conflicting results have been reported in epidemiological studies of *VHL* mutations in
26 TCE-exposed cases and are described in detail in Section 4.5.2. Both Brüning et al. (1997) and
27 Brauch et al. (1999, 2004) associated increased *VHL* mutation frequency in TCE-exposed renal
28 cell carcinoma cases. The two other available studies of Schraml et al. (1999) and
29 Charbotel et al. (2007) because of their limitations and lower mutation detection rate in the case
30 of Charbotel et al. (2007) neither add nor detract to the conclusions from the earlier studies.
31 Additional discussion of these data are in Section 4.4.3.
32 Limited animal studies have examined the role of TCE and *VHL* mutations, although
33 Mally et al. (2006) have recently conducted both *in vitro* and *in vivo* studies using the Eker rat
34 model (see Section 4.4.6.1.1). The Eker rat model (*Tsc-2*$^{+/-}$) is at increased risk for the
35 development of spontaneous renal cell carcinoma and as such has been used to understand the

mechanisms of renal carcinogenesis (Stemmer et al., 2007; Wolf et al., 2000). One study has demonstrated similar pathway activation in Eker rats as that seen in humans with *VHL* mutations leading to renal cell carcinoma, suggesting *Tsc-2* inactivation is analogous to inactivation of *VHL* in human renal cell carcinoma (Liu et al., 2003). In Mally et al. (2006), male rats carrying the Eker mutation were exposed to TCE (0, 100, 250, 500, or 1,000 mg/kg body weight [BW] by gavage, 5 days a week) for 13 weeks to determine the renal effects (additional data from this study on *in vitro* DCVC exposure are discussed below, Section 4.2.5). A significant increase in labeling index in kidney tubule cells was observed, however, no enhancement of preneoplastic lesions or tumor incidence was found in Eker rat kidneys compared to controls. In addition, no *VHL* gene mutations in exons 1−3 were detected in tumors obtained from either control or TCE-exposed Eker rats. Although no other published studies have directly examined *VHL* mutations following exposure to TCE, two studies performed mutational analysis of archived formalin-fixed paraffin embedded tissues from renal carcinomas from previous rat studies. These carcinomas were induced by the genotoxic carcinogens potassium bromate (Shiao et al., 2002) or *N*-nitrosodimethylamine (Shiao et al., 1998). Limited mutations in the *VHL* gene were observed in all samples, but, in both studies, these were found only in the clear cell renal carcinomas. Limitations of these two studies include the small number of total samples analyzed, as well as potential technical issues with DNA extraction from archival samples (see Section 4.4.3). However, analyses of *VHL* mutations in rats may not be informative as to the potential genotoxicity of TCE in humans because the *VHL* gene may not be the target for nephrocarcinogenesis in rats to the extent that it appears to be in humans.

4.2.1.4.3. *Chromosomal aberrations.* A few studies were conducted to investigate the ability of TCE to induce chromosomal aberrations in mammalian systems (see Table 4-8). Galloway et al. (1987) studied the effect of TCE on chromosome aberrations in Chinese hamster ovary cells. When the cells were exposed to TCE (499−14,900 μg/mL) for 2 hours with metabolic activation, S9, no chromosomal aberrations were observed. Furthermore, without metabolic activation, no changes in chromosomal aberrations were found when the cells were exposed to TCE concentrations of 745−14,900 μg/mL for 8−14 hours. It should be noted that in this study, liquid incubation method was used and the experiment was part of a larger study to understand the genotoxic potential of 108 chemicals.

Three inhalation studies in mice and rats examined if TCE could induce cytogenetic damage (Kligerman et al., 1994). In the first two studies, CD rats or C57Bl/6 mice, were exposed to 0-, 5-, 500-, or 5,000-ppm TCE for 6 hours. Peripheral blood lymphocytes (PBL) in rats and splenocytes in mice were analyzed for induction of chromosomal aberrations, sister

chromatid exchanges and micronucleus formation. The results of micronucleus and sister chromatid exchanges will be discussed in the next sections (see Sections 4.2.1.4.4 and 4.2.1.4.5). No significant increase in chromosomal aberrations was observed in binucleated peripheral blood lymphocytes. In the third study, the authors exposed the same strain of rats for 6 hours/day over 4 consecutive days. No statistically significant concentration-related increases in chromosomal aberrations were observed. The limited results of the above studies have not reported TCE to cause chromosomal aberrations either in *in vitro* or *in vivo* mammalian systems.

4.2.1.4.4. *Micronucleus induction.* The appearance of micronuclei is another endpoint that can demonstrate the genotoxic effect of a chemical. Several studies have been conducted to identify if TCE can cause micronucleus formation (see Table 4-9).

Wang et al. (2001) investigated micronucleus formation by TCE administered as a vapor in CHO-K1 cells *in vitro*. Cells were grown in culture media with an inner petri dish containing TCE that would evaporate into the media containing cells. The concentration of TCE in cultured medium was determined by gas chromatography. The actual concentration of TCE ranged from 0.8 and 1.4 ppm after a 24-hour treatment. A significant dose-dependent increase in micronuclei formation was observed. A dose-dependent decrease in cell growth and cell number was also observed. The authors did not test if the micronuclei formed was due to direct damage to the DNA or spindle formation.

Robbiano et al. (2004) conducted an *in vitro* study on DNA damage and micronuclei formation in rat and human kidney cells exposed to six carcinogenic chemicals including TCE. The authors examined for the ability of TCE to induce DNA fragmentation and formation of micronuclei in primary cultures of rat and human kidney cells derived from kidney cancer patients with 1−4 mM TCE concentrations. A significant dose-dependent increase in the frequency of micronuclei was obtained in primary kidney cells from both male rats and human of both genders. The authors acknowledge that the significance of the results should be considered in light of the limitations including (1) examination of TCE on cells from only three rats, (2) considerable variation in the frequency of DNA lesions induced in the cells, and (3) the possibility that kidney cells derived from kidney cancer patients may be more sensitive to DNA-damaging activity due to a more marked expression of enzymes involved in the metabolic activation of kidney procarcinogens and suppression of DNA repair processes. Never the less, this study is important and provides information of the possible genotoxic effects of TCE.

Table 4-9. TCE genotoxicity: mammalian systems—micronucleus, sister chromatic exchanges

Test system/endpoint	Doses tested	With activation	Without activation	Comments	References
Micronucleus					
Human hepatoma HepG2 cells	0.5−4 mM, 24 h	NA	+		Hu et al., 2008
Primary cultures of human and rat kidney cells	1.0, 2.0, or 4.0 mM	NA	+	dose-dependent significant increase	Robbiano et al., 2004
Sprague-Dawley rats	3,591 mg/kg	+	-		Robbiano et al., 2004
CHO-K1 cells	0.8−1.4 ppm		+	dose-dependent significant increase	Wang et al., 2001
Male CD-1 mice	457 mg/kg	+	NA	bone marrow, correlated with TCOH in urine	Hrelin et al., 1994
C56BL/6J mice	5, 50, 500, or 5,000 ppm	-	NA	splenocytes	Kligerman et al., 1994
S-D rats	5, 50, 500, or 5,000 ppm	+	NA	dose dependent; peripheral blood lymphocytes	Kligerman et al., 1994
Sister chromatid exchanges					
CHO	0.17%	–	ND	1 h (vapor)	White et al., 1979
CHO	17.9−700 μg/mL	ND	+	25 h (liquid)	Galloway et al., 1987
CHO	49.7−14,900 μg/mL	+	ND	2 h	Galloway et al., 1987
Human lymphocytes	178 μg/mL	ND	+		Gu et al., 1981a, b
S-D rats	5, 50, 500, or 5,000 ppm	-	NA	peripheral blood lymphocytes	Kligerman et al., 1994
Peripheral blood lymphocytes from humans occupationally exposed	occupational exposure	-	NA		Nagaya et al., 1989
C57BL/6J mice	5, 50, 500, or 5,000 ppm	-	NA	splenocytes	Kligerman et al., 1994

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In the same study, Robbiano et al. (2004) administered rats a single oral dose of TCE (3,591 mg/kg) corresponding to ½ LD_{50}, which had been pre-exposed to folic acid for 48 hours and the rats were euthanized 48 hours later following exposure to TCE. The frequency of binucleated cells was taken as an index of kidney cell proliferation. A statistically significant increase in the average frequency of micronucleus was observed.

Hu et al. (2008) studied the effect of TCE on micronuclei frequencies using human hepatoma HepG2 cells. The cells were exposed to 0.5, 1, 2, and 4 mM TCE for 24 hours. TCE caused a significant increase in micronuclei frequencies at all concentrations tested. It is important to note that similar concentrations were used in Robbiano et al. (2004).

As described in the chromosomal aberration section (see Section 4.2.1.4.3), inhalation studies were performed using male C57BL/6 mice and CD rats (Kligerman et al., 1994) to determine if TCE could induce micronuclei. In the first and second study, rats or mice respectively, were exposed to 0-, 5-, 500-, or 5,000-ppm TCE for 6 hours. Peripheral blood lymphocytes in rats and splenocytes in mice were cultured and analyzed for induction of micronuclei formation. Bone marrow polychromatic erythrocytes (PCEs) were also analyzed for micronuclei. TCE caused a statistically significant increase in micronuclei formation at all concentrations in rat bone marrow PCEs but not in mice. The authors note that TCE was significantly cytotoxic at the highest concentration tested as determined by significant concentration-related decrease in the ratio of PCEs/normochromatic erythrocytes. In the third study, to confirm the results of the first study, the authors exposed rats to one dose of 5,000 ppm for 6 hours. A statistical increase in bone marrow micronuclei-PCEs was observed confirming the results of the first study.

Hrelia et al. (1994) treated male CD-1 mice with TCE (457 mg/kg BW; i.p.) for 30 hours. Bone marrow cells were harvested for determination of micronuclei frequencies in PCEs. An increase in micronuclei frequency at 30 hours after treatment was observed. Linear regression analysis showed that micronuclei frequency induced by TCE correlated with trichloroethanol concentrations in urine, a marker of TCE oxidative metabolism (Hrelia et al., 1994).

In summary, based on the results of the above studies, TCE is capable of inducing micronuclei in different *in vitro* and *in vivo* systems tested. Specific methods were not used that could definitively identify the mechanism of micronuclei formation. These are important findings that indicate TCE has genotoxic potential as measured by the micronucleus formation.

4.2.1.4.5. *Sister chromatid exchanges (SCEs).* Studies have been conducted to understand the ability of TCE to induce SCEs both *in vitro* and *in vivo* systems (see Table 4-9). White et al. (1979) evaluated the possible induction of SCE in CHO using a vapor exposure procedure by

exposing the cells to TCE (0.17%) for 1 hour in the presence of S9 metabolic activation. No change in SCE frequencies were observed between the control and the treatment group. However, in another study by Galloway et al. (1987) a dose-related increase in SCE frequency in repeated experiments both with and without metabolic activation was observed. It should be noted that in this study, liquid incubation was used, and the exposure times were 25 hours without metabolic activation at a concentration between 17.9 to 700 μg/mL and 2 hours in the presence of S9 at a concentration of 49.7 to 14,900 μg/mL. Due to the difference in the dose, length of exposure and treatment protocol (vapor exposure vs. liquid incubation), no direct comparison can be made. It should also be noted that inadequacy of dose selection and the absence of positive control in the White et al. (1979) makes it difficult to interpret the study. In another study (Gu et al., 1981a), a small but positive response was observed in assays with peripheral lymphocytes.

No statistically significant increase in SCEs was found when male C57Bl/6 mice or CD rats were exposed to TCE at concentrations of 5,500, or 5,000 ppm for 6 hours (Kligerman et al., 1994). Furthermore, in another study by Nagaya et al. (1989), lymphocytes of TCE-exposed workers (n = 22) and matched controls (n = 22) were analyzed for SCEs. The workers had constantly used TCE in their jobs although the exact exposure was not provided. The duration of their employment ranged from 0.7 to 34 years, averaging about 10 years. It should be noted that there were both smokers and non-smokers among the exposed population. If a subject had not smoked for at least 2 years before the samples were taken, then they were considered as non-smokers. There were 8 nonsmokers in the group. If they were classified as smokers, then they smoked between 10−50 cigarettes per day. No significant increase in mean SCE frequencies were found in exposed population compared to controls, though the study is relatively small.

In summary, induction of SCEs have been reported in several, though not all, paradigms of TCE exposure, consistent with the structural damage to DNA/chromosomes indicated by excess micronuclei formation.

4.2.1.4.6. ***Unscheduled DNA synthesis.*** *In vitro* studies are briefly described here, with additional discussion of effects related to TCE-induced unscheduled DNA synthesis in the context of the liver in Section E.2.4.1. Perocco and Prodi (1981) studied unscheduled DNA synthesis in human lymphocytes cultured *in vitro* (see Table 4-10). Three doses of TCE (2.5, 5.0, and 10 μL/mL) were used as final concentrations with and without S9. The results indicate that there was an increase in UDS only in the presence of S9, and in addition, the increase was maximal at the TCE concentration of 5 μL/mL. Three chlorinated ethane and ethylene solvent

products were examined for their genotoxicity in hepatocyte primary culture DNA repair assays using vapor phase exposures. Rat hepatocytes primary cultures were initiated and exposed to low-stabilized or standard stabilized TCE (0.1−2.5%) for 3 or 18 hours. Unscheduled DNA synthesis or DNA repair was not observed using either low or standard stabilized TCE, even at vapor phase doses up to those that produced extensive cell killing after 3 or 18 hour exposure (Shimada et al., 1985). Costa and Ivanetich (1984) examined the ability of TCE to induce unscheduled DNA synthesis hepatocytes isolated from phenobarbital treated rats. The UDS was assessed only at the highest concentration that is tolerated by the hepatocytes (2.8 mM TCE).

These results indicate that TCE stimulated unscheduled DNA synthesis in isolated rodent hepatocytes, and, importantly, in human lymphocytes *in vitro*.

4.2.1.4.7. ***DNA strand breaks.*** DNA damage in response to TCE exposure was studied using comet assay in human hepatoma HepG2 cells (Hu et al., 2008; see Table 4-10). The cells were exposed to 0.5, 1, 2, and 4 mM for 24 hours. TCE increased the DNA migration in a significant dose-dependent manner at all tested concentrations suggesting TCE caused DNA strand breaks and chromosome damage.

TCE (4−10 mmol/kg body wt) were given to male mice by i.p. injection. The induction of single-strand breaks (SSB) in DNA of liver, kidney, and lung was studied by the DNA unwinding technique. There was a linear increase in the level of single strand breaks in kidney and liver DNA but not in lung DNA 1 hour after administration (Walles, 1986).

Robbiano et al. (2004) conducted an *in vitro* study on DNA damage in rat and human kidney cells exposed to six carcinogenic chemicals including TCE in the comet assay. The authors examined the ability of TCE to induce DNA fragmentation in primary cultures of rat and human kidney cells with 1−4 mM TCE concentrations. TCE was dissolved in ethanol with a maximum concentration of 0.3% and the rat cultures were exposed to 20 hours. Primary human kidney cells were isolated from fragments of kidney discarded during the course of surgery for carcinoma of both male and female donors with an average age of 64.2 years and were also exposed to 20 hours. Significant dose-dependent increases in the ratio of treated/control tail length (average 4−7 μM compared to control) was observed as measured by comet assay in primary kidney cells from both male rats and human of both genders.

Table 4-10. TCE genotoxicity: mammalian systems—unscheduled DNA synthesis, DNA strand breaks/protein crosslinks, cell transformation

Test system/endpoint	Doses tested	With activation	Without activation	Comments	References
Unscheduled DNA synthesis					
Rat primary hepatocytes		ND	–		Shimada et al., 1985
Human lymphocytes	2.5, 5, 10 μL/mL	+/–	–	increase was only in certain doses and maximum at 5 μL/mL conc.	Perocco and Prodi, 1981
Phenobarbital induced rat hepatocytes	2.8 mM	ND	+		Costa and Ivanetich, 1984
DNA strand breaks/protein crosslinks					
Primary rat kidney cells	0.5, 1.0, 2.0, 4.0 mM	NA	+	dose-dependent significant increase	Robbiano et al., 2004
Primary cultures of human kidney cells	1.0, 2.0, 4.0 mM	ND	+	dose-dependent significant increase	Robbiano et al., 2004
Sprague-Dawley rats	3,591 mg/kg	+	NA	single oral administration	Robbiano et al., 2004
Sprague-Dawley rats	500, 1,000, and 2,000 ppm	–	NA	comet assay	Clay, 2008
Cell transformation					
BALB/c 3T3 mouse cells	4, 20, 100, 250 μg/mL	NA	+	weakly positive compared to other halogenated compounds tested in the same experiment	Tu et al., 1985
Rat embryo cells		NA	+		Price et al., 1978
Syrian hamster embryo cells	5, 10, 25 μg/mL	NA	–		Amacher and Zelljadt, 1983

ND = not determined, NA = not applicable.

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Clay et al. (2008) studied the DNA damage inducing capacity of TCE using the comet assay in rat kidney proximal tubules. Rats were exposed by inhalation to a range of TCE concentrations (500, 1,000, or 2,000 ppm) for 6 hours/day for 5 days. TCE did not induce DNA damage (as measured by tail length and percent tail DNA and tail movement) in rat kidney proximal tubules in any of the doses tested possibly due to study limitations (small number of animals tested [*n* = 5] and limited exposure time [6 hours/day for only 5 days]). These results are in contrast to the findings of Robbiano et al. (2004) which showed DNA damage and increased micronuclei in the rat kidney 20 hours following a single dose (3,591 mg/kg BW) of TCE. Therefore, based on the above studies, while several studies reported DNA damage induced by TCE. The DNA damage reported by comet assay is consistent with results for other markers of chromosomal damage or DNA structural damage such as excess micronuclei formation and SCE induced by TCE exposure.

4.2.1.4.8. ***DNA damage related to oxidative stress.*** A detailed description of studies related to lipid peroxidation of TCE is presented in conjunction with discussion of liver toxicity (see Section 4.5, E.2.4.3, and E.3).

4.2.1.4.9. ***Cell transformation.*** *In vitro* cell transformation using BALB/c-3T3 cells was conducted using TCE with concentrations varying from 0−250 µg/mL in liquid phase exposed for 72 hours (see Table 4-10). The cytotoxicity of TCE at the concentration tested in the transformation assay was determined by counting cells from duplicate plates of each test conditions at the end of the treatment period. A dose-dependent increase in Type III foci was observed although no statistical analysis was conducted (Tu et al., 1985). In another study by Amacher and Zelljadt (1983), Syrian hamster embryo cells were exposed to 5, 10, or 25 µg/mL of TCE. In this experiment, two different serums (horse serum and fetal bovine serum) were also tested to understand the importance of serum quality in the transformation assay. Preliminary toxicity assay was performed to select dose levels which had 50-90% cell survival. One week after dosing, the cell colonies were fixed and counted for variability determination and examination of individual colonies for the evidence of morphological transformation. No significant change in morphological transformation was obtained. Furthermore, no significant changes were seen in transformation colonies when tested in different serum. However, these studies are of limited use for determining the genotoxic potential of TCE because they did not examine the foci for mutations, for instance in oncogenes or tumor suppressor genes.

4.2.1.5. *Summary*

Evidence from a number of different analyses and a number of different laboratories using a fairly complete array of endpoints suggests that TCE, following metabolism, has the potential to be genotoxic. A series of carefully controlled studies evaluating TCE itself (without mutagenic stabilizers and without metabolic activation) found it to be incapable of inducing gene mutations in most standard mutation bacterial assays (Waskell, 1978; Henschler et al., 1977; Mortelmans et al., 1986; Simmon et al., 1977; Baden et al., 1979; Bartsch et al., 1979; Crebelli et al., 1982; Shimada et al., 1985; Simmon et al., 1977; Baden et al., 1979). Therefore, it appears that it is unlikely that TCE is a direct-acting mutagen, though TCE has shown potential to affect DNA and chromosomal structure. Low, but positive responses were observed in the TA100 strain in the presence of S9 metabolic activation, even when genotoxic stabilizers were not present, suggesting metabolites of TCE are genotoxic. TCE is also positive in some but not all fungal and yeast systems (Crebelli et al., 1985; Koch et al., 1988; Rossi et al., 1983; Callen et al., 1980). Data from human epidemiological studies support the possible mutagenic effect of TCE leading to *VHL* gene damage and subsequent occurrence of renal cell carcinoma. Association of increased *VHL* mutation frequency in TCE-exposed renal cell carcinoma cases has been observed (Brüning et al.,1997; Brauch et al., 1999, 2004).

TCE can lead to binding to nucleic acids and proteins (Di Renzo et al., 1982; Bergman, 1983; Miller and Guengerich, 1983; Mazzullo et al., 1992; Kautiainen et al., 1997), and such binding appears to be due to conversion to one or more reactive metabolites. For instance, increased binding was observed in samples bioactivated with mouse and rat microsomal fractions (Banerjee and VanDuuren, 1978; Di Renzo et al., 1982; Miller and Guengerich, 1983; Mazzullo et al., 1992). DNA binding is consistent with the ability to induce DNA and chromosomal perturbations. Several studies report the induction of micronuclei *in vitro* and *in vivo* from TCE exposure (Kligerman et al., 1994; Hrelia et al., 1994; Wang et al., 2001; Robbiano et al., 2004; Hu et al., 2008). Reports of SCE induction in some studies are consistent with DNA effects, but require further study (White et al., 1979; Gu et al., 1981a, b; Nagaya et al., 1989; Kligerman et al., 1994).

Overall, evidence from a number of different analyses and a number of different laboratories using various genetic endpoints indicates that TCE has a potential to induce damage to the structure of the chromosome in a number of targets but has a more limited ability to induce mutation in bacterial systems.

Below, the genotoxicity data for TCE metabolites TCA, DCA, TCOH, chloral hydrate, DCVC, and DCVG are briefly reviewed. The contributions of these data are 2-fold. First, to the extent that these metabolites may be formed in the *in vitro* and *in vivo* test systems for TCE, they

provide insight into what agent or agents may contribute to the limited activity observed with TCE in these genotoxicity assays. Second, because the *in vitro* systems do not necessarily fully recapitulate *in vivo* metabolism, the genotoxicity of the known *in vivo* metabolites themselves provide data as to whether one may expect genotoxicity to contribute to the toxicity of TCE following *in vivo* exposure.

4.2.2. Trichloroacetic Acid (TCA)

The TCE metabolite TCA has been studied using a variety of genotoxicity assay for its genotoxic potential (see International Agency for Research on Cancer [IARC, 2004] for additional information). Evaluation of *in vitro* studies of TCA must consider toxicity and acidification of medium resulting in precipitation of proteins, as TCA is commonly used as a reagent to precipitate proteins.

4.2.2.1. *Bacterial Systems—Gene Mutations*

TCA has been evaluated in a number of *in vitro* test systems including the bacterial assays (Ames) using different *S. typhimurium* strains such as TA98, TA100, TA104, TA1535, and RSJ100 (Table 4-11). The majority of these studies did not report positive findings for genotoxicity (Waskell, 1978; Shirasu et al., 1976; Nestmann et al., 1980; DeMarini et al., 1994; Rapson et al., 1980; Moriya et al., 1983; Nelson et al., 2001; Kargalioglu et al., 2002) Waskell (1978) studied the effect of TCA (0.45 mg/plate) on bacterial strains TA98 and TA100 both in the presence and absence of S9. The author did not find any revertants at the maximum nontoxic dose tested. Following exposure to TCA, Rapson et al. (1980) reported no change in mutagenic activity in strain TA100 in the absence of S9. DeMarini et al. (1994) performed different studies to evaluate the genotoxicity of TCA, including the Microscreen prophage-induction assay (TCA concentrations 0 to 10 mg/mL) and use of the *S. typhimurium* TA100 strain using bag vaporization technique (TCA concentrations 0−100 ppm), neither of which yielded positive results. Nelson et al. (2001) reported no positive findings with TCA using a *S. typhimurium* microsuspension bioassay (*S. typhimurium* strain TA104) following incubation of TCA for various lengths of time, with or without rat cecal microbiota. Similarly, no activity was observed in a study conducted by Kargalioglu et al. (2002) where *S. typhimurium* strains TA98, TA100, and RSJ100 were exposed to TCA (0.1−100 mM) either in the presence or absence of S9 (Kargalioglu et al., 2002).

1 **Table 4-11.. Genotoxicity of Trichloroacetic acid—bacterial systems**
2

Test system/endpoint	Doses (LED or HID)[a]	Results[b] With activation	Results[b] Without activation	Reference
λ Prophage induction, *E. coli* WP2s	10,000	-	-	DeMarini et al., 1994
SOS chromotest, *Escherichia coli* PQ37	10,000	-	-	Giller et al., 1997
S. typhimurium TA1535, 1536, 1537, 1538, reverse mutation	20 µg/plate	NT	-	Shirasu et al., 1976
S. typhimurium TA100, 98, reverse mutation	450 µg/plate	-	-	Waskell, 1978
S. typhimurium TA100, 1535, reverse mutation	4,000 µg/plate	-	-	Nestmann et al., 1980
S. typhimurium TA1537, 1538, 98, reverse mutation	2,000 µg/plate	-	-	Nestmann et al., 1980
S. typhimurium TA100, reverse mutation	520 µg/plate	NT	-	Rapson et al., 1980
S. typhimurium TA100, 98, reverse mutation	5,000 µg/plate	-	-	Moriya et al., 1983
S. typhimurium TA100, reverse mutation	600 ppm	-	-	DeMarini et al., 1994
S. typhimurium TA100, reverse mutation, liquid medium	1,750	+	+	Giller et al., 1997
S. typhimurium TA104, reverse mutation, microsuspension	250 µg/plate	-	-	Nelson et al., 2001
S. typhimurium TA100, RSJ100, reverse mutation	16,300	-	-	Kargalioglu et al., 2002
S. typhimurium TA98, reverse mutation	13,100	-	-	Kargalioglu et al., 2002
S. typhimurium TA1535, SOS DNA repair		+	-	Ono et al., 1991

3
4 [a]LED, lowest effective dose; HID, highest ineffective dose; doses are in µg/mL for *in vitro* tests unless specified.
5 [b]Results: +, positive; -, negative; NT, not tested.
6
7 Table adapted from IARC monograph (2004) and modified/updated for newer references.
8
9
10 TCA was also negative in other bacterial systems. The SOS chromotest (which measures
11 DNA damage and induction of the SOS repair system) in *E. coli* PQ37, +/- S9 (Giller et al.,
12 1997) evaluated the genotoxic activity of TCA ranging from 10 to 10,000 µg/mL and did not
13 find any response. Similarly, TCA was not genotoxic in the Microscreen prophage-induction
14 assay in *E. coli* with TCA concentrations ranging from 0 to 10,000 µg/mL, with and without S9
15 activation (DeMarini et al., 1994).

However, TCA induced a small increase in SOS DNA repair (an inducible error-prone repair system) in *S. typhimurium* strain TA1535 in the presence of S9 (Ono et al., 1991). Furthermore, Giller et al. (1997) reported that TCA demonstrated genotoxic activity in an Ames fluctuation test in *S. typhimurium* TA100 in the absence of S9 at noncytotoxic concentrations ranging from 1,750 to 2,250 μg/mL. The addition of S9 decreased the genotoxic response, with effects observed at 3,000−7,500 μg/mL. Cytotoxic concentrations in the Ames fluctuation assay were 2,500 and 10,000 μg/mL without and with microsomal activation, respectively.

4.2.2.2. *Mammalian Systems*

4.2.2.2.1. *Gene mutations.* The mutagenicity of TCA has also been tested in cultured mammalian cells (Table 4-12). Harrington-Brock et al. (1998) examined the potential of TCA to induce mutations in L5178Y/$TK^{+/-}$ –3.7.2C mouse lymphoma cells. In this study, mouse lymphoma cells were incubated in culture medium treated with TCA concentrations up to 2,150 μg/mL in the presence of S9 metabolic activation and up to 3,400 μg/mL in the absence of S9 mixture. In the presence of S9, a doubling of mutant frequency was seen at concentrations of 2,250 μg/mL and higher, including several concentrations with survival >10%. In the absence of S9, TCA increased the mutant frequency by 2-fold or greater only at concentrations of 2,000 μg/mL or higher. These results were obtained at ≤11% survival rates. The authors noted that the mutants included both large-colony and small-colony mutants. The small-colony mutants are indicative of chromosomal damage. It should be noted that no rigorous statistical evaluation was conducted on these data.

4.2.2.2.2. *Chromosomal aberrations.* Mackay et al. (1995) investigated the ability of TCA to induce chromosomal damage in an *in vitro* chromosomal aberration assay using cultured human cells. The authors treated the cells with TCA as free acid, both in the presence and absence of metabolic activation. TCA induced chromosomal damage in cultured human peripheral lymphocytes at concentrations (2,000 and 3,500 μg/mL) that significantly reduced the pH of the medium. However, exposure of cells to neutralized TCA did not have any effect even at a cytotoxic concentration of 5,000 μg/mL. It is possible that the reduced pH was responsible for the TCA-induced clastogenicity in this study. To further evaluate the role of pH changes in the induction of chromosome damage, the authors isolated liver-cell nuclei from $B6C3F_1$ mice and suspended in a buffer at various pH levels. The cells were stained with chromatin-reactive (fluorescein isothiocyanate) and DNA-reactive (propidium iodide) fluorescent dyes. A decrease in chromatin staining intensity was observed with the decrease in pH, suggesting that pH changes, independent of TCA exposure, can alter chromatin conformation. It was concluded by

the authors that TCA-induced pH changes are likely to be responsible for the chromosomal damage induced by un-neutralized TCA. In another *in vitro* study, Plewa et al. (2002) evaluated the induction of DNA strand breaks induced by TCA (1−25 mM) in CHO cells and did not observe any genotoxicity.

4.2.2.2.3. ***Micronucleus.*** Relative genotoxicity of TCA was tested in a mouse *in vivo* system (Table 4-12) using three different cytogenetic assay (bone marrow chromosomal aberrations, micronucleus and sperm-head abnormalities) (Bhunya and Behera, 1987) and for chromosomal aberrations in chicken (Bhunya and Jena, 1996). TCA induced a variety of anomalies including micronucleus in the bone marrow of mice and chicken. A small increase in the frequency of micronucleated erythrocytes at 80 μg/mL in a newt (Pleurodeles waltl larvae) micronucleus test was observed in response to TCA exposure (Giller et al., 1997). Mackay et al. (1995) investigated the ability of TCA to induce chromosomal DNA damage in the *in vivo* bone-marrow micronucleus assay in mice. C57BL mice were given TCA intraperitoneally at doses of 0, 337, 675, or 1,080 mg/kg/d for males and 0, 405, 810, or 1,300 mg/kg/d for females for two consecutive days, and bone-marrow samples were collected 6 and 24 hours after the last dose. The administered doses represented 25, 50, and 80% of the median lethal dose, respectively. No treatment-related increase in micronucleated polychromatic erythrocytes was observed.

4.2.2.2.4. ***Other DNA damage Studies.*** DNA unwinding assays have been used as indicators of single strand breaks and are discussed in detail in Section E.2.3. Studies were conducted on the ability of TCA to induce single-strand breaks (Chang et al., 1992; Styles et al., 1991; Nelson and Bull, 1988; Nelson et al., 1989; Table 4-12). Nelson and Bull (1988) evaluated the ability of TCA and other compounds to induce single-strand DNA breaks *in vivo* in Sprague-Dawley rats and $B6C3F_1$ mice. Single oral doses were administered to three groups of three animals, with an additional group as a vehicle control. Animals were sacrificed after 4 hours, and 10% liver suspensions were analyzed for single-strand DNA breaks by the alkaline unwinding assay. Dose-dependent increases in single-strand DNA breaks were induced in both rats and mice, with mice being more susceptible than rats. The lowest dose of TCA that produced significant SSBs was 0.6 mmol/kg (98 mg/kg) in rats but 0.006 mmol/kg (0.98 mg/kg) in mice.

Table 4-12.. TCA Genotoxicity—mammalian systems (both *in vitro* and *in vivo*)

Test system/endpoint	Doses (LED or HID)[a]	Results[b]		Reference
		With activation	Without activation	
Gene mutation, mouse lymphoma L5178Y/TK+/- cells, *in vitro*	3,000	(+)	?	Harrington-Brock et al., 1998
DNA strand breaks, B6C3F1 mouse and Fischer 344 rat hepatocytes, *in vitro*	1,630	NT	-	Chang et al., 1992
DNA strand breaks, human CCRF-CEM lymphoblastic cells, *in vitro*	1,630	NT	-	Chang et al., 1992
DNA damage, Chinese hamster ovary cells, *in vitro*, comet assay	3 mM	NT	-	Plewa et al., 2002
DNA strand breaks, B6C3F1 mouse liver, *in vivo*	1.0, oral, ×1	+		Nelson and Bull, 1988
DNA strand breaks, B6C3F1 mouse liver, *in vivo*	500, oral, ×1	+		Nelson et al., 1989
DNA strand breaks, B6C3F1 mouse liver, *in vivo*	500, oral, 10 repeats	-		Nelson et al., 1989
DNA strand breaks, B6C3F1 mouse liver and epithelial cells from stomach and duodenum, *in vivo*	1,630, oral, ×1	-		Chang et al., 1992
DNA strand breaks, male B6C3F1 mice, *in vivo*	500 (neutralized)	-		Styles et al., 1991
Micronucleus formation, Swiss mice, *in vivo*	125, i.p., ×2	+		Bhunya and Behera, 1987
Micronucleus formation, female C57BL/6JfBL10/Alpk mouse bone-marrow erythrocytes, *in vivo*	1,300, i.p., ×2	-		Mackay et al., 1995
Micronucleus formation, male C57BL/6JfBL10/Alpk mouse bone-marrow erythrocytes, *in vivo*	1,080, i.p., ×2	-		Mackay et al., 1995
Micronucleus formation, *Pleurodeles waltl* newt larvae peripheral erythrocytes, *in vivo*	80	+		Giller et al, 1997
Chromosomal aberrations, Swiss mouse bone-marrow cells *in vivo*	125, i.p., ×1	+		Bhunya and Behera, 1987
Chromosomal aberrations, Swiss mouse bone-marrow cells *in vivo*	100, i.p., ×5	+		Bhunya and Behera, 1987
Chromosomal aberrations, Swiss mouse bone-marrow cells *in vivo*	500, oral, ×1	+		Bhunya and Behera, 1987
Chromosomal aberrations, chicken *Gallus domesticus* bone marrow, *in vivo*	200, i.p., ×1	+		Bhunya and Jena, 1996

Table 4-12. TCA Genotoxicity—mammalian systems (both *in vitro* and *in vivo*) (continued)

Test system/endpoint	Doses (LED or HID)[a]	Results[b]		Reference
		With activation	Without activation	
Chromosomal aberrations, human lymphocytes, *in vitro*	5,000, (neutralized)	-		Mackay et al., 1995
Sperm morphology, Swiss mice, *in vivo*	125, i.p., ×5	+		Bhunya and Behera, 1987

[a]LED, lowest effective dose; HID, highest ineffective dose; doses are in μg/mL for *in vitro* tests; mg/kg for *in vivo* tests unless specified.
[b]Results: + = positive; (+) = weakly positive; - = negative; NT = not tested; ? = inconclusive.

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However, in a follow-up study, Nelson et al. (1989) male B6C3F1 mice were treated with 500 mg/kg TCA, and single strand breaks in whole liver homogenate were examined, and no significant differences from controls were reported. Moreover, in the experiments in the same study with DCA, increased single strand breaks were reported, but with no dose-response between 10 and 500 mg/kg, raising concerns about the reliability of the DNA unwinding assay used in these studies. For further details, see Section E.2.3. In an additional follow-up experiment with a similar experimental paradigm, Styles et al. (1991) tested TCA for its ability to induce strand breaks in male $B6C3F_1$ mice in the presence and absence of liver growth induction. The test animals were given 1, 2, or 3 daily doses of neutralized TCA (500 mg/kg) by gavage and killed 1 hour after the final dose. Additional mice were given a single 500-mg/kg gavage dose and sacrificed 24 hours after treatment. Liver nuclei DNA were isolated, and the induction of single strand breaks was evaluated using the alkaline unwinding assay. Exposure to TCA did not induce strand breaks under the conditions tested in this assay. In a study by Chang et al. (1992), administration of single oral doses of TCA (1 to 10 mmol/kg) to $B6C3F_1$ mice did not induce DNA strand breaks in a dose-related manner as determined by the alkaline unwinding assay. No genotoxic activity (evidence for strand breakage) was detected in F344 rats administered by gavage up to 5 mmol/kg (817 mg/kg).

In summary, although Nelson and Bull (1988) report effects on DNA unwinding for TCE and its metabolites with DCA having the highest activity and TCA the lowest, Nelson et al. (1989), using the same assay, reported no effect for TCA and the same effect at 10 and 500 mg/kg for DCA in mice. Moreover, Styles et al.(1991) did not find a positive result for TCA using the same paradigm as Nelson and Bull (1988) and Nelson et al. (1989). Furthermore, Chang et al (1992) also did not find increased single strand breaks for TCA exposure in rats. (see Section E.2.4.3).

4.2.2.3. *Summary*

In summary, TCA has been studied using a variety of genotoxicity assays, including the recommended battery. No mutagenicity was reported in *S. typhimurium* strains in the presence or absence of metabolic activation or in an alternative protocol using a closed system, except in one study on strain TA100 using a modified protocol in liquid medium. This is largely consistent with the results from TCE, which was negative in most bacterial systems except some studies with the TA100 strain. Mutagenicity in mouse lymphoma cells was only induced at cytotoxic concentrations. Measures of DNA-repair responses in bacterial systems have been inconclusive, with induction of DNA repair reported in *S. typhimurium* but not in *E. coli*. TCA-induced clastogenicity may be secondary to pH changes and not a direct effect of TCA.

4.2.3. Dichloroacetic Acid (DCA)

DCA is another metabolite of TCE that has been studied using a variety of genotoxicity assay for its genotoxic potential (Tables 4-13 and 4-14; see IARC [2004] for additional information).

4.2.3.1. *Bacterial and Fungal Systems—Gene Mutations*

Studies were conducted to evaluate mutagenicity of DCA in different *S. typhimurium* and *E. coli* strains (DeMarini et al., 1994; Giller et al., 1997; Waskell, 1978; Herbert et al., 1980; Fox et al., 1996; Kargalioglu et al., 2002; Nelson et al., 2001; Fox et al., 1996). DCA was mutagenic in three strains of *S. typhimurium*: strain TA100 in three of five studies, strain RSJ100 in a single study, and strain TA98 in two of three studies. DCA failed to induce point mutations in other strains of *S. typhimurium* (TA104, TA1535, TA1537, and TA1538) or in *E. coli* strain WP2uvrA. In one study, DCA caused a weak induction of SOS repair in *E. coli* strain PQ37 (Giller et al., 1997).

DeMarini et al. (1994), in the same study as described in the TCA section of this chapter, also studied DCA as one of their compounds for analysis. In the prophage-induction assay using *E. coli*, DCA, in the presence of S9, was genotoxic producing 6.6−7.2 plaque-forming units (PFU)/mM and slightly less than 3-fold increase in PFU/plate in the absence of S9. In the second set of studies, which involved the evaluation of DCA at concentrations of 0−600 ppm for mutagenicity in *S. typhimurium* TA100 strain, DCA was mutagenic both in the presence and absence of S9, producing 3−5 times increases in the revertants/plate compared to the background. The lowest effective concentration for DCA without S9 was 100 ppm and 50 ppm in the presence of S9. In the third and most important study, mutation spectra of DCA were determined at the base-substitution allele *hisG46* of *S. typhimurium* TA100. DCA-induced revertants were chosen for further molecular analysis at concentrations that produced mutant yields that were 2−5-fold greater than the background. The mutation spectra of DCA were significantly different from the background mutation spectrum. Thus, despite the modest increase in the mutant yields (3−5 times) produced by DCA, the mutation spectra confirm that DCA is mutagenic. DCA primarily induced GC-AT transitions.

Table 4-13.. Genotoxicity of dichloroacetic acid (bacterial systems)

Test system/endpoint	Doses (LED or HID)[a]	Results[b]		Reference
		With activation	Without activation	
λ Prophage induction, *E. coli* WP2s	2,500	+	–	DeMarini et al., 1994
SOS chromotest, *E. coli* PQ37	500	–	(+)	Giller et al., 1997
S. typhimurium, DNA repair-deficient strains TS24, TA2322, TA1950	31,000	–	–	Waskell, 1978
S. typhimurium TA100, TA1535, TA1537, TA1538, reverse mutation		–	–	Herbert et al., 1980
S. typhimurium TA100, reverse mutation	50	+	+	DeMarini et al., 1994
S. typhimurium TA100,TA1535, TA1537, TA98, reverse mutation	5,000	–	–	Fox et al., 1996
S. typhimurium TA100, reverse mutation, liquid medium	100	+	+	Giller et al., 1997
S. typhimurium RSJ100, reverse mutation	1,935	–	+	Kargalioglu et al., 2002
S. typhimurium TA104, reverse mutation, microsuspension	150 µg/plate	–	–	Nelson et al., 2001
S. typhimurium TA98, reverse mutation	10 µg/plate	(+)	–	Herbert et al., 1980
S. typhimurium TA98, reverse mutation	5,160	–	+	Kargalioglu et al., 2002
S. typhimurium TA100, reverse mutation	1,935	+	+	Kargalioglu et al., 2002
E. coli WP2uvrA, reverse mutation	5,000	–	–	Fox et al., 1996

[a]LED, lowest effective dose; HID, highest ineffective dose; doses are in µg/mL for *in vitro* tests unless specified.
[b]Results: + = positive; (+) = weakly positive; – = negative.

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Table 4-14.. Genotoxicity of dichloroacetic acid—mammalian systems

Test system/endpoint	Doses (LED or HID)[a]	Results[b]		Reference
		With activation	Without activation	
Gene mutation, mouse lymphoma cell line L5178Y/TK+/- *in vitro*	5,000	−	−	Fox et al., 1996
Gene mutation, mouse lymphoma cell line L5178Y/TK+/--3.7.2C *in vitro*	400	NT	+	Harrington-Brock et al., 1998
DNA strand breaks and alkali-labile damage, Chinese hamster ovary cells *in vitro* (single-cell gel electrophoresis assay)	3,225 μg/mL	NT	−	Plewa et al., 2002
DNA strand breaks, B6C3F1 mouse hepatocytes *in vitro*	2,580	NT	−	Chang et al., 1992
DNA strand breaks, Fischer 344 rat hepatocytes *in vitro*	1,290	NT	−	Chang et al.,1992
Micronucleus formation, mouse lymphoma L5178Y/TK+/--3.7.2C cell line *in vitro*	800	NT	−	Harrington-Brock et al., 1998
Chromosomal aberrations, Chinese hamster ovary *in vitro*	5,000	−	−	Fox et al.,1996
Chromosomal aberrations, mouse lymphoma L5178Y/Tk+/- -3.7.2C cell line *in vitro*	600	NT	+	Harrington-Brock et al., 1998
Aneuploidy, mouse lymphoma L5178Y/Tk+/--3.7.2C cell line *in vitro*	800	NT	−	Harrington-Brock et al., 1998
DNA strand breaks, human CCRF-CEM lymphoblastoid cells *in vitro*	1,290	NT	−	Chang et al., 1992
DNA strand breaks, male B6C3F1 mouse liver *in vivo*	13, oral, ×1	+		Nelson and Bull, 1988
DNA strand breaks, male B6C3F1 mouse liver *in vivo*	10, oral, ×1	+		Nelson et al., 1989
DNA strand breaks, male B6C3F1 mouse liver *in vivo*	1,290, oral, ×1	−		Chang et al., 1992
DNA strand breaks, male B6C3F1 mouse splenocytes *in vivo*	1,290, oral, ×1	−		Chang et al., 1992
DNA strand breaks, male B6C3F1 mouse epithelial cells from stomach and duodenum *in vivo*	1,290, oral, ×1	−		Chang et al., 1992
DNA strand breaks, male B6C3F1 mouse liver *in vivo*	5,000, dw, ×7−14 d	−		Chang et al., 1992
DNA strand breaks, alkali-labile sites, cross linking, male B6C3F1 mouse blood leukocytes *in vivo* (single-cell gel electrophoresis assay)	3,500, dw, ×28 d	+		Fuscoe et al., 1996

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Table 4-14. Genotoxicity of dichloroacetic acid—mammalian systems (continued)

Test system/endpoint	Doses (LED or HID)[a]	Results[b]		Reference
		With activation	Without activation	
DNA strand breaks, male Sprague-Dawley rat liver *in vivo*	30, oral, ×1	+		Nelson and Bull, 1988
DNA strand breaks, male Fischer 344 rat liver *in vivo*	645, oral, ×1	−		Chang et al., 1992
DNA strand breaks, male Fischer 344 rat liver *in vivo*	2,000, dw, ×30 weeks	−		Chang et al., 1992
Gene mutation, lacI transgenic male B6C3F1 mouse liver assay *in vivo*	1,000, dw, ×60 weeks	+		Leavitt et al., 1997
Micronucleus formation, male B6C3F1 mouse peripheral erythrocytes *in vivo*	3,500, dw, ×9 d	+		Fuscoe et al., 1996
Micronucleus formation, male B6C3F1 mouse peripheral erythrocytes *in vivo*	3,500, dw, ×28 d	−		Fuscoe et al., 1996
Micronucleus formation, male B6C3F1 mouse peripheral erythrocytes *in vivo*	3,500, dw, ×10 weeks	+		Fuscoe et al., 1996
Micronucleus formation, male and female Crl:CD (SD) BR rat bone-marrow erythrocytes *in vivo*	1,100, i.v., ×3	−		Fox et al., 1996
Micronucleus formation, Pleurodeles waltl newt larvae peripheral erythrocytes *in vivo*	80 d	−		Giller et al., 1997

[a]LED, lowest effective dose; HID, highest ineffective dose; doses are in µg/mL for *in vitro* tests; mg/kg for *in vivo* tests unless specified; dw = drinking-water (in mg/L); d = day; w = week; i.v. = intravenous.
[b]Results: + = positive; - = negative; NT = not tested.

Table adapted from IARC monograph (2004) and modified/updated for newer references.

Kargalioglu et al. (2002) analyzed the cytotoxicity and mutagenicity of the drinking water disinfection by-products including DCA in *S. typhimurium* strains TA98, TA100, and RSJ100 +/- S9. DCA was mutagenic in this test although the response was low when compared to other disinfection by-products tested in strain TA100. This study was also summarized in a review by Plewa et al. (2002). Nelson et al. (2001) investigated the mutagenicity of DCA using a *S. typhimurium* microsuspension bioassay following incubation of DCA for various lengths of time, with or without rat cecal microbiota. No mutagenic activity was detected for DCA with *S. typhimurium* strain TA104.

Although limited data, it appears that DCA has mutagenic activity in the *S. typhimurium* strains, particularly TA100.

4.2.3.2. *Mammalian Systems*

4.2.3.2.1. ***Gene mutations.*** The mutagenicity of DCA has been tested in mammalian systems, particularly, mouse lymphoma cell lines *in vitro* (Fox et al., 1996; Harrington-Brock et al., 1998) and lacI transgenic mice *in vivo* (Leavitt et al., 1997). Harrington-Brock et al. (1998) evaluated DCA for it mutagenic activity in L5178Y/TK +/- (-) 3.7.2C mouse lymphoma cells. A dose-related increase in mutation (and cytotoxic) frequency was observed at concentrations between 100 and 800 μg/mL. Most mutagenic activity of DCA at the Tk locus was due to the production of small-colony Tk mutants (indicating chromosomal mutations). Different pH levels were tested in induction of mutant frequencies and it was determined that the mutagenic effect observed was due to the chemical and not pH effects.

Mutation frequencies were studied in male transgenic B6C3F1 mice harboring the bacterial *lacI* gene administered DCA at either 1.0 or 3.5 g/L in drinking water (Leavitt et al., 1997). No significant difference in mutant frequency was observed after 4 or 10 weeks of treatment in both the doses tested as compared to control. However, at 60 weeks, mice treated with 1.0 g/L DCA showed a slight increase (1.3-fold) in the mutant frequency over the control, but mice treated with 3.5 g/L DCA had a 2.3-fold increase in the mutant frequency. Mutational spectra analysis revealed that ~33% had G:C-A:T transitions and 21% had G:C−T:A transversions and this mutation spectra was different than that was seen in the untreated animals, indicating that the mutations were likely induced by the DCA treatment. The authors conclude that these results are consistent with the previous observation that the proportion of mutations at T:A sites in codon 61 of the H-ras gene was increased in DCA-induced liver tumors in B6C3F1 mice (Leavitt et al., 1997).

4.2.3.2.2. *Chromosomal aberrations and micronucleus.* Harrington-Brock et al. (1998) evaluated DCA for its potential to induce chromosomal aberrations in DCA-treated (0, 600, and 800 μg/mL) mouse lymphoma cells. A clearly positive induction of aberrations was observed at both concentrations tested. No significant increase in micronucleus was observed in DCA-treated (0, 600, and 800 μg/mL) mouse lymphoma cells (Harrington-Brock et al., 1998). However, no chromosomal aberrations were found in Chinese hamster ovary cells exposed to DCA (Fox et al., 1996)

Fuscoe et al. (1996) investigated *in vivo* genotoxic potential of DCA in bone marrow and blood leukocytes using the peripheral-blood-erythrocyte micronucleus assay (to detect chromosome breakage and/or malsegregation) and the alkaline single cell gel electrophoresis (comet) assay, respectively. Mice were exposed to DCA in drinking water, available *ad libitum*, for up to 31 weeks. A statistically significant dose-related increase in the frequency of micronucleated PCEs was observed following subchronic exposure to DCA for 9 days. Similarly, a significant increased was also observed when exposed for ≥10 weeks particularly at the highest dose of DCA tested (3.5 g/L). DNA cross-linking was observed in blood leukocytes in mice exposed to 3.5 g/L DCA for 28 days. These data provide evidence that DCA may have some potential to induce chromosome damage when animals were exposed to concentrations similar to those used in the rodent bioassay.

4.2.3.2.3. *Other DNA damage studies.* Nelson and Bull (1988) and Nelson et al. (1989) have been described above in Section 4.2.2.4 and E.2.3, with positive results for DNA unwinding for DCA, though Nelson et al. (1989) reported the same response at 10 and 500 mg/kg in mice, raising concerns about the reliability of the assay in these studies. Chang et al. (1992) conducted both *in vitro* and *in vivo* studies to determine the ability of DCA to cause DNA damage. Primary rat (Fischer 344) hepatocytes and primary mouse hepatocytes treated with DCA for 4 hours did not in induce DNA single strand breaks as detected by alkaline DNA unwinding assay. No DNA strand breaks were observed in human CCRF-CEM lymphoblastoid cells *in vitro* exposed to DCA. Similarly, analysis of the DNA single strand breaks in mice killed 1 hour after a single dose of 1, 5 or 10 mM/kg DCA did not cause DNA damage. None of the Fischer 344 rats killed 4 hours after a single gavage treatment (1−10 mM/kg) produced any detectable DNA damage.

4.2.3.3. *Summary*

In summary, DCA has been studied using a variety but limited number of genotoxicity assays. Within the available data, DCA has been demonstrated to be mutagenic in the *S. typhimurium* assay, particularly in strain TA100, the *in vitro* mouse lymphoma assay and

in vivo cytogenetic and gene mutation assays. DCA can cause DNA strand breaks in mouse and rat liver cells following *in vivo* administration by gavage.

4.2.4. Chloral Hydrate

Chloral hydrate has been evaluated for its genotoxic potential using a variety of genotoxicity assays (Tables 4-15, 4-16, and 4-17). These data are particularly important because it is known that a large flux of TCE metabolism leads to chloral hydrate as an intermediate, so a comparison of their genotoxicity profiles is likely to be highly informative.

4.2.4.1. *DNA Binding Studies*

Limited analysis has been performed examining DNA binding potential of chloral hydrate (Keller and Heck, 1988; Von Tungeln et al., 2002; Ni et al., 1995). Keller and Heck (1988) conducted both *in vitro* and *in vivo* experiments using B6C3F1 mouse strain. The mice were pretreated with 1,500 mg/kg TCE for 10 days and then given 800 mg/kg [^{14}C] chloral. No detectable covalent binding of ^{14}C to DNA in the liver was observed. Another study with *in vivo* exposures to nonradioactive chloral hydrate at a concentration of 1,000 and 2,000 nmol in mice B6C3F1 demonstrated an increase in malondialdehyde-derived and 8-oxo-2'-deoxyguanosine adducts in liver DNA (Von Tungeln et al., 2002). Ni et al. (1995) observed malondialdehyde adducts in calf thymus DNA when exposed to chloral hydrate and microsomes from male B6C3F1 mouse liver.

Keller and Heck (1988) investigated the potential of chloral to form DNA-protein cross-links in rat liver nuclei using concentrations 25, 100, or 250 mM. No statistically significant increase in DNA-protein cross-links was observed. DNA and RNA isolated from the [^{14}C] chloral-treated nuclei did not have any detectable ^{14}C bound. However, the proteins from choral-treated nuclei did have a concentration-related binding of ^{14}C.

Table 4-15.. Chloral hydrate genotoxicity: bacterial, yeast and fungal systems

Test system/endpoint	Doses (LED or HID)[a]	Results[b]		Reference
		With activation	Without activation	
SOS chromotest, *Escherichia coli* PQ37	10,000	-	-	Giller et al., 1995
S. typhimurium TA100, TA1535, TA98, reverse mutation	10,000	-	-	Waskell., 1978
S. typhimurium TA100, TA1537, TA1538, TA98, reverse mutation	1,000	+	+	Haworth et al., 1983
S. typhimurium TA100, reverse mutation	5,000 µg/plate	-	-	Leuschner and Leuschner, 1991
S. typhimurium TA100, reverse mutation	2,000 µg/plate	+	+	Ni et al., 1994
S. typhimurium TA100, reverse mutation, liquid medium	300	+	-	Giller et al., 1995
S. typhimurium TA100, TA104, reverse mutation	1,000 µg/plate	+	+	Beland, 1999
S. typhimurium TA104, reverse mutation	1,000 µg/plate	+	+	Ni et al., 1994
S. typhimurium TA1535, reverse mutation	1,850	-	-	Leuschner and Leuschner, 1991
S. typhimurium TA1535, TA1537 reverse mutation	6,667	-	-	Haworth et al., 1983
S. typhimurium TA1535, reverse mutation	10,000	-	-	Beland, 1999
S. typhimurium TA98, reverse mutation	7,500	-	-	Haworth et al., 1983
S. typhimurium TA98, reverse mutation	10,000 µg/plate	-	+	Beland, 1999
A.nidulans, diploid strain 35X17, mitotic cross-overs	1,650	NT	-	Crebelli et al., 1985
A. nidulans, diploid strain 30, mitotic cross-overs	6,600	NT	-	Kafer, 1986
A. nidulans, diploid strain NH, mitotic cross-overs	1,000	NT	-	Kappas, 1989
A. nidulans, diploid strain P1, mitotic cross-overs	990	NT	-	Crebelli et al., 1991
A. nidulans, diploid strain 35X17, nondisjunctions	825	NT	+	Crebelli et al., 1985
A. nidulans, diploid strain 30, aneuploidy	825	NT	+	Kafer, 1986
A. nidulans, haploid conidia, aneuploidy, polyploidy	1650	NT	+	Kafer, 1986
A. nidulans, diploid strain NH, nondisjunctions	450	NT	+	Kappas, 1989
A. nidulans, diploid strain P1, nondisjunctions	660	NT	+	Crebelli et al., 1991

Table 4-15. Chloral hydrate genotoxicity: bacterial, yeast and fungal systems (continued)

Test system/endpoint	Doses (LED or HID)[a]	Results[b]		Reference
		With activation	Without activation	
A. nidulans, haploid strain 35, hyperploidy	2,640	NT	+	Crebelli et al., 1991
S. cerevisiae, meiotic recombination	3,300	NT	?	Sora and Agostini Carbone, 1987
S. cerevisiae, disomy in meiosis	2,500	NT	+	Sora and Agostini Carbone, 1987
S. cerevisiae, disomy in meiosis	3,300	NT	+	Sora and Agostini Carbone, 1987
S. cerevisiae, D61.M, mitotic chr. malsegregation	1,000	NT	+	Albertini, 1990
Drosophila melanogaster, somatic mutation wing spot test	825		+	Zordan et al., 1994
Drosophila melanogaster, induction of sex-linked lethal mutation	37.2 feed		?	Beland, 1999
Drosophila melanogaster, induction of sex-linked lethal mutation	67.5 inj		-	Beland, 1999

[a]LED, lowest effective dose; HID, highest ineffective dose; doses are in µg/mL for *in vitro* tests; inj = injection.
[b]Results: + = positive; - = negative; NT = not tested; ? = inconclusive.

Table adapted from IARC monograph (2004) and modified/updated for newer references.

Table 4-16.. Chloral hydrate genotoxicity: mammalian systems—all genetic endpoints, *in vitro*

Test system/endpoint	Doses (LED or HID)[a]	Results[b]		Reference
		With activation	Without activation	
DNA-protein cross-links, rat nuclei *in vitro*	41,250	NT	-	Keller and Heck, 1988
DNA single-strand breaks, rat primary hepatocytes *in vitro*	1,650	NT	-	Chang et al., 1992
Gene mutation, mouse lymphoma L5178Y/TK$^{+/-}$, *in vitro*	1,000		(+)	Harrington-Brock et al., 1998
Sister chromatid exchange, CHO cells, *in vitro*	100	+	+	Beland, 1999
Micronucleus formation, (kinetochore-positive), Chinese hamster C1 cells, *in vitro*	165	NT	+	Degrassi and Tanzarella, 1988
Micronucleus formation, (kinetochore-negative), Chinese hamster C1 cells, *in vitro*	250	NT	-	Degrassi and Tanzarella, 1988
Micronucleus formation, (kinetochore-positive), Chinese hamster LUC2 cells, *in vitro*	400	NT	+	Parry et al., 1990
Micronucleus formation, (kinetochore-positive), Chinese hamster LUC2 cells, *in vitro*	400	NT	+	Lynch and Parry, 1993
Micronucleus formation, Chinese hamster V79 cells, *in vitro*	316	NT	+	Seelbach et al., 1993
Micronucleus formation, mouse lymphoma L5178Y/TK$^{+/-}$, *in vitro*	1,300	NT	-	Harrington-Brock et al., 1998
Micronucleus formation, mouse lymphoma L5178Y/TK$^{+/-}$, *in vitro*	500	NT	+	Nesslany and Marzin, 1999
Chromosomal aberrations, Chinese Hamster CHED cells, *in vitro*	20	NT	+	Furnus et al., 1990
Chromosomal aberrations, Chinese Hamster ovary cells, *in vitro*	1,000	+	+	Beland, 1999
Chromosomal aberrations, mouse lymphoma L5178Y/TK +/- cells line, *in vitro*	1,250	NT	(+)	Harrington-Brock et al., 1998
Aneuploidy, Chinese hamster CHED cells, *in vitro*	10	NT	+	Furnus et al., 1990
Aneuploidy, primary Chinese hamster embryonic cells, *in vitro*	250	NT	+	Natarajan et al., 1993
Aneuploidy, Chinese hamster LUC2p4 cells, *in vitro*	250	NT	+	Warr et al., 1993
Aneuploidy, mouse lymphoma L5178Y/TK$^{+/-}$, *in vitro*	1,300	NT	-	Harrington-Brock et al., 1998
Tetraploidy and endoredupliation, Chinese hamster LUC2p4cells, *in vitro*	500	NT	+	Warr et al., 1993
Cell transformation, Syrian hamster embryo cells (24-h treatment)	350	NT	+	Gibson et al., 1995
Cell transformation, Syrian hamster dermal cell line (24-h treatment)	50	NT	+	Parry et al., 1996
DNA single-strand breaks, human lymphoblastoid cells, *in vitro*	1,650	NT	-	Chang et al., 1992

Table 4-16. Chloral hydrate genotoxicity: mammalian systems—all genetic endpoints, *in vitro* (continued)

Test system/endpoint	Doses (LED or HID)[a]	Results[b]		Reference
		With activation	Without activation	
Gene mutation, *tk and hprt* locus, human lymphoblastoid	1,000	NT	+	Beland, 1999
Sister chromatid exchanges, human lymphocytes, *in vitro*	54	NT	(+)	Gu et al., 1981
Micronucleus formation, human lymphocytes, *in vitro*	100	-	+	Van Hummelen & Kirsch-Volders, 1992
Micronucleus formation, human lymphoblastoid AHH-1 cell line, *in vitro*	100	NT	+	Parry et al., 1996
Micronucleus formation, human lymphoblastoid MCL-5 cell line, *in vitro*	500	NT	-	Parry et al., 1996
Micronucleus formation (kinetochore-positive), human diploid LEO fibroblasts, *in vitro*	120	NT	+	Bonatti et al., 1992
Aneuploidy (double Y induction), human lymphocytes, *in vitro*	250	NT	+	Vagnarelli et al., 1990
Aneuploidy (hyperdiploidy and hypodiploidy), human lymphocytes *in vitro*	50	NT	+	Sbrana et al., 1993
Polyploidy, human lymphocytes, *in vitro*	137	NT	+	Sbrana et al., 1993
C-Mitosis, human lymphocytes, *in vitro*	75	NT	+	Sbrana et al., 1993

[a]LED, lowest effective dose; HID, highest ineffective dose; doses are in µg/mL for *in vitro* tests.
[b]Results: + = positive; (+) = weakly positive in an inadequate study; - = negative; NT = not tested.

Table adapted from IARC monograph (2004) and modified/updated for newer references.

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Table 4-17.. Chloral hydrate genotoxicity: mammalian systems—all genetic damage, *in vivo*

Test system/endpoint	Doses (LED or HID)[a]	Results[b]	Reference
DNA single-strand breaks, male Sprague-Dawley rat liver	300, oral	+	Nelson and Bull, 1988
DNA single-strand breaks, male Fischer 344 rat liver	1650, oral	-	Chang et al., 1992
DNA single-strand breaks, male B6C3F1 mouse liver	100, oral	+	Nelson and Bull, 1988
DNA single-strand breaks, male B6C3F1 mouse liver	825, oral	-	Chang et al., 1992
Micronucleus formation, male and female NMRI mice, bone-marrow erythrocytes	500, i.p.	-	Leuschner and Leuschner, 1991
Micronucleus formation, BALB/c mouse spermatids	83, i.p.	-	Russo and Levis, 1992
Micronucleus formation, male BALB/c mouse bone-marrow erythrocytes and early spermatids	83, i.p.	+	Russo and Levis, 1992
Micronucleus formation, male BALB/c mouse bone-marrow erythrocytes	200, i.p.	+	Russo et al., 1992
Micronucleus formation, male F1 mouse bone-marrow erythrocytes	400, i.p.	-	Leopardi et al., 1993
Micronucleus formation, C57B1 mouse spermatids	41, i.p.	+	Allen et al., 1994
Micronucleus formation, male Swiss CD-1 mouse bone-marrow erythrocytes	200, i.p.	+	Marrazini et al., 1994
Micronucleus formation, B6C3F1 mouse spermatids after spermatogonial stem-cell treatment	165, i.p.	+	Nutley et al., 1996
Micronucleus formation, B6C3F1 mouse spermatids after meiotic cell treatment	413, i.p.	-	Nutley et al., 1996
Micronucleus formation, male F1, BALB/c mouse peripheral-blood erythrocytes	200, i.p.	_	Grawe et al., 1997
Micronucleus formation, male B6C3F1 mouse bone-marrow erythrocytes	500, i.p., ×3	+	Beland, 1999
Micronucleus formation, infants, peripheral lymphocytes	50, oral	+	Ikbal et al., 2004
Chromosomal aberrations, male and female F1 mouse bone marrow cells	600, i.p.	-	Xu and Alder, 1990
Chromosomal aberrations, male and female Sprague-Dawley rat bone-marrow cells	1,000, oral	_	Leuschner and Leuschner, 1991
Chromosomal aberrations, BALB/c mouse spermatogonia treated	83, i.p.	-	Russo and Levis, 1992b
Chromosomal aberrations, F1 mouse secondary spermatocytes	82.7, i.p.	+	Russo et al., 1984
Chromosomal aberrations, male Swiss CD-1 mouse bone-marrow erythrocytes	400, i.p.	_	Marrazini et al. 1994
Chromosomal aberrations, ICR mouse oocytes	600, i.p.	-	Mailhes et al., 1993
Micronucleus formation, infants, peripheral lymphocytes	50, oral	+	Ikbal et al., 2004

Table 4-17. Chloral hydrate genotoxicity: mammalian systems—all genetic damage, *in vivo* (continued)

Test system/endpoint	Doses (LED or HID)[a]	Results[b]	Reference
Polyploidy, male and female F1, mouse bone-marrow cells	600, i.p.	-	Xu and Adler, 1990
Aneuploidy F1 mouse secondary spermatocytes	200, i.p.	+	Miller and Adler, 1992
Aneuploidy, male F1 mouse secondary spermatocytes	400, i.p.	_	Leopardi et al., 1993
Hyperploidy, male Swiss CD-1 mouse bone-marrow erythrocytes	200, i.p.	+	Marrazini et al., 1994

[a]LED, lowest effective dose; HID, highest ineffective dose; doses are in mg/kg bw for *in vivo* tests, i.p. = intraperitoneally.
[b]Results: + = positive; - = negative.

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4.2.4.2. *Bacterial and Fungal Systems—Gene Mutations*

Chloral hydrate induced gene mutations in *S. typhimurium* TA100 and TA104 strains, but not in most other strains assayed. Four of six studies of chloral hydrate exposure in *S. typhimurium* TA100 and two of two studies in *S. typhimurium* TA104 were positive for revertants (Haworth et al., 1983; Ni et al., 1994; Giller et al., 1995; Beland, 1999). Waskell (1978) studied the effect of chloral hydrate along with TCE and its other metabolites. Chloral hydrate was tested at different doses (1.0−13 mg/plate) in different *S. typhimurium* strains (TA98, TA100, TA1535) for gene mutations using Ames assay. No revertant colonies were observed in strains TA98 or TA1535 both in the presence and absence of S9 mix. Similar results were obtained by Leuschner and Leuschner (1991). However, in TA100, a dose-dependent statistically significant increase in revertant colonies was obtained both in the presence and absence of S9. It should be noted that chloral hydrate that was purchased from Sigma was re-crystallized from one to six times from chloroform and the authors describe this as crude chloral hydrate. However, this positive result is consistent with other studies in this strain as noted above. Furthermore, Giller et al. (1995) studied chloral hydrate genotoxicity in three short-term tests. Chloral-induced mutations in strain TA100 of *S. typhimurium* (fluctuation test). Similar results were obtained by Haworth et al. (1983). These are consistent with several studies of TCE, in which low, but positive responses were observed in the TA100 strain in the presence of S9 metabolic activation, even when genotoxic stabilizers were not present.

A significant increase in mitotic segregation was observed in *Aspergillus nidulans* when exposed to 5 and 10 mM chloral hydrate (Crebelli et al., 1985). Studies of mitotic crossing-over in *Aspergillus nidulans* have been negative while these same studies were positive for aneuploidy (Crebelli et al., 1985, 1991; Kafer, 1986; Kappas, 1989).

Two studies were conducted in *Saccharomyces cerevisiae* to understand the chromosomal malsegregation as a result of exposure to chloral hydrate (Sora and Agostini, 1987; Albertini, 1990). Chloral hydrate (1-25 mM) was dissolved in sporulation medium and the frequencies of various meiotic events such as recombination, disomy were analyzed. Chloral hydrate inhibited sporulation as a function of dose and increased diploid and disomic clones . Chloral hydrate was also tested for mitotic chromosome malsegregation using *Saccharomyces cerevisiae* D61.M (Albertini, 1990). The tester strain was exposed to a dose range of 1−8 mg/mL. An increase in the frequency of chromosomal malsegregation was observed as a result of exposure to chloral hydrate.

Limited analysis of chloral hydrate mutagenicity has been performed in *Drosophila* (Zordan et al., 1994; Beland, 1999). Of these two studies, chloral hydrate was positive in the

1 somatic mutation wing spot test (Zordan et al., 1994), equivocal in the induction of sex-linked
2 lethal mutation when in feed but negative when exposed via injection (Beland, 1999).
3
4 **4.2.4.3. *Mammalian Systems***
5 **4.2.4.3.1. *Gene mutations.*** Harrington-Brock (1998) noted that chloral hydrate-induced
6 concentration related cytotoxicity in TK+/- mouse lymphoma cell lines without S9 activation. A
7 nonstatistical increase in mutant frequency was observed in cells treated with chloral hydrate.
8 The mutants were primarily small colony TK mutants, indicating that most chloral hydrate-
9 induced mutants resulted from chromosomal mutations rather than point mutations. It should be
10 noted that in most concentrations tested (350−1,600 μg/mL), cytotoxicity was observed. Percent
11 cell survival ranged from 96 to 4%.
12
13 **4.2.4.3.2. *Micronucleus.*** Micronuclei induction following exposure to chloral hydrate is
14 positive in most test systems in both *in vitro* and *in vivo* assays, although some negative tests do
15 also exist (Harrington-Brock et al., 1998; Degrassi and Tanzarella, 1988; Beland, 1999; Lynch
16 and Parry, 1993; Seelbach et al., 1993; Marrazini et al., 1994; Nesslany and Marzin, 1999; Russo
17 and Levis, 1992a, b; Russo et al., 1992; Leopardi et al., 1993; Allen et al., 1994; Nutley et al.,
18 1996; Grawe et al., 1997; Giller et al., 1995; Leuschner and Leuschner, 1991; Van Hummelen
19 and Kirsch-Volders, 1992; Parry et al., 1996; Bonatti et al., 1992; Ikbal et al., 2004). Some
20 studies have attempted to make inferences regarding aneuploidy induction or clastogenicity as an
21 effect of chloral hydrate. Aneuploidy results from defects in chromosome segregration during
22 mitosis and is a common cytogenetic feature of cancer cells (see Section E.3.1.5).
23 Giller et al. (1995) studied chloral hydrate genotoxicity in three short-term tests. Chloral
24 hydrate caused a significant increase in the frequency of micronucleated erythrocytes following
25 *in vivo* exposure of the amphibian *Pleurodeles waltl* newt larvae.
26 Chloral hydrate induced aneuploidy *in vitro* in multiple Chinese hamster cell lines
27 (Warr et al., 1993; Furnus et al., 1990; Natarajan et al., 1993) and human lymphocytes
28 (Vagnarelli et al., 1990; Sbrana et al., 1993) but not mouse lymphoma cells
29 (Harrington-Brock et al., 1998). *In vivo* studies performed in various mouse strains led to
30 increased aneuploidy in spermatocytes (Russo et al., 1984; Liang and Pacchierotti, 1988;
31 Miller and Adler, 1992) but not oocytes (Mailhes et al., 1988) or bone marrow cells (Xu and
32 Adler, 1990; Leopardi et al., 1993).
33 The potential of chloral hydrate to induce aneuploidy in mammalian germ cells has been
34 of particular interest since Russo et al. (1984) first demonstrated that chloral hydrate treatment of
35 male mice results in significant increase in frequencies of hyperploidy in metaphase II cells.

This hyperploidy was thought to have arisen from chromosomal nondisjunction in premeiotic/meiotic cell division and may be a consequence of chloral hydrate interfering with spindle formation (reviewed by Russo et al. [1984] and Liang and Brinkley [1985]). Chloral hydrate also causes meiotic delay, which may be associated with aneuploidy (Miller and Alder, 1992). Chloral hydrate has been shown to induce micronuclei but not structural chromosomal aberrations in mouse bone-marrow cells. Micronuclei induced by nonclastogenic agents are generally believed to represent intact chromosomes that failed to segregate into either daughter-cell nucleus at cell division (Russo et al., 1992; Wang Xu and Adler, 1990). Furthermore, chloral hydrate-induced micronuclei in mouse bone-marrow cells (Russo et al., 1992) and in cultured mammalian cells (Degrassi and Tanzarella, 1988; Bonatti et al., 1992) have shown to be predominantly kinetochore-positive in composition upon analysis with immunofluorescent methods. The presence of a kinetochore in a micronucleus is considered evidence that the micronucleus contains a whole chromosome lost at cell division (Degrassi and Tanzarella, 1988; Hennig et al., 1988; Eastmond and Tucker, 1989). Therefore, both TCE and chloral hydrate appear to increase the frequency of micronuclei.

Allen et al. (1994) treated male C57B1/6J mice were given a single intraperitoneal injection of 0, 41, 83, or 165 mg/kg chloral hydrate. Spermatids were harvested at 22 hours, 11, 13.5, and 49 days following exposure (Allen et al., 1994). Harvested spermatids were processed to identify both kinetochore-positive micronucleus (aneugen) and kinetochore-negative micronucleus (clastogen). All chloral hydrate doses administered 49 days prior to cell harvest were associated with significantly increased frequencies of kinetochore-negative micronuclei in spermatids, however, dose dependence was not observed. This study is in contrast with other studies (Degrassi and Tanzarella, 1988; Bonatti et al., 1992) who demonstrated predominantly kinetochore-positive micronucleus.

The ability of chloral hydrate to induce aneuploidy and polyploidy was tested in human lymphocyte cultures established from blood samples obtained from two healthy nonsmoking donors (Sbrana et al., 1993). Cells were exposed for 72 and 96 hours at doses between 50 and 250 μg/mL. No increase in percent hyperdiploid, tetraploid, or endoreduplicated cells were observed when cells were exposed to 72 hours at any doses tested. However, at 96 hours of exposure, significant increase in hyperdiploid was observed at one dose (150 μg/mL) and was not dose dependent. Significant increase in tetraploid was observed at dose 137 mg/mL, again, no dose dependence was observed.

Ikbal et al. (2004) assessed the genotoxic effects in cultured peripheral blood lymphocytes of 18 infants (age range of 31−55 days) before and after administration of a single dose of chloral hydrate (50 mg/kg of body weight) for sedation before a hearing test for

micronucleus frequency. A significant increase in micronuclei frequency was observed after administration of chloral hydrate.

4.2.4.3.3. *Chromosomal aberrations.* Several studies have included chromosomal aberration analysis in both *in vitro* and *in vivo* systems exposed to chloral hydrate and have resulted in positive in *in vitro* studies—although not all studies had statistically significant increase (Furnus et al., 1990; Beland, 1999; Harrington-Brock et al., 1998).

Analysis of chloral hydrate treated mouse lymphoma cell lines for chromosomal aberrations resulted in a nonsignificant increase in chromosomal aberrations (Harrington-Brock et al., 1998). However, it should be noted that the concentrations tested (1,250 and 1,300 μg/mL) were cytotoxic (with a cell survival of 11 and 7%, respectively). Chinese hamster embryo cells were also exposed to 0.001, 0.002, and 0.003% chloral hydrate for 1.5 hours (Furnus et al., 1990). A nonstatistically significant increase in frequency of chromosomal aberrations was observed only 0.002 and 0.003% concentrations, with the increase not dose-dependent. In this study, it should be noted that the cells were only exposed for 1.5 hours to chloral hydrate and cells were allowed to grow for 48 hours (two cell cycles) to obtain similar mitotic index before analyzing for chromosomal aberrations. No information on cytotoxicity was provided except that higher doses decreased the frequency of mitotic cells at the time of fixation.

In vivo chromosome aberration studies have mostly reported negative or null results (Xu and Adler, 1990; Leuschner and Leuschner, 1991; Russo and Levis, 1992a, b; Liang and Pacchierotti, 1988; Mailhes et al., 1993) with the exception of one study (Russo et al., 1984) in an F1 cross of mouse strain between C57B1/Cne × C3H/Cne.

4.2.4.3.4. *Sister chromatid exchanges (SCEs).* SCEs were assessed by Ikbal et al. (2004) in cultured peripheral blood lymphocytes of 18 infants (age range of 31−55 days) before and after administration of a single dose of chloral hydrate (50 mg/kg of body weight) for sedation before a hearing test. The authors report a significant increase in the mean number of SCEs, from before administration (7.03 ± 0.18 SCEs/cell) and after administration (7.90 ± 0.19 SCEs/cell) , with each of the 18 individuals showing an increase with treatment. Micronuclei were also significantly increased. SCEs were also assessed by Gu et al. (1981a) in human lymphocytes exposed *in vitro* with inconclusive results, although positive results were observed by Beland (1999) in Chinese hamster ovary cells exposed *in vitro* with and without an exogenous metabolic system.

4.2.4.3.5. *Cell Transformation.* Chloral hydrate was positive in the two studies designed to measure cellular transformation (Gibson et al., 1995; Parry et al., 1996). Both studies exposed Syrian hamster cells (embryo and dermal) to chloral hydrate and induced cellular transformation.

4.2.4.4. *Summary*

Chloral hydrate has been reported to induce micronuclei formation, aneuploidy, and mutations in multiple *in vitro* systems and *in vivo*. *In vivo* studies have limited results to an increased micronuclei formation mainly in mouse spermatocytes. CH is positive to in some studies in *in vitro* genotoxicity assays that detect point mutations, micronuclei induction, chromosomal aberrations, and/or aneuploidy. The *in vivo* data exhibit mixed results (Xu and Adler, 1990; Russo et al., 1992; Mailhes et al., 1993; Allen et al., 1994; Alder, 1993; Nutley et al., 1996; Leuschner and Beuscher, 1998). Most of the positive studies show that chloral hydrate induces aneuploidy. Based on the existing array of data, CH has the potential to be genotoxic, particularly when aneuploidy is considered in the weight of evidence for genotoxic potential. Some have suggested that chloral hydrate may act through a mechanism of spindle poisoning and resulting in numerical changes in the chromosomes, but some data also suggest induction of chromosomal aberrations. These results are consistent with TCE, albeit there are more limited data on TCE for these genotoxic endpoints.

4.2.5. Dichlorovinyl Cysteine (DCVC) and S-Dichlorovinyl Glutathione (DCVG)

DCVC and DCVG have been studied for their genotoxic potential; however, since there is limited number of studies to evaluate them based on each endpoint, particularly in mammalian systems, the following section has been combined to include all the available studies for different endpoints of genotoxicity. Study details can be found in Table 4-18.

DCVC and DCVG, cysteine intermediates of TCE formed by the GST pathway, are capable of inducing point mutations as evidenced by the fact that they are positive in the Ames assay. Dekant et al. (1986) demonstrated mutagenicity of DCVC in *S. typhimurium* strains (TA100, TA2638, and TA98) using the Ames assay in the absence of S9. The effects were decreased with the addition of a beta-lyase inhibitor aminooxyacetic acid, suggesting that bioactivation by this enzyme plays a role in genotoxicity. Vamvakas et al. (1987) tested *N*-acetyl-S-(1,2-dichlorovinyl)-L-cysteine) (NAcDCVC) for mutagenicity following addition of rat kidney cytosol and found genotoxic activity. Furthermore, Vamvakas (1988a), in another experiment, investigated the mutagenicity of DCVG and DCVC in *S. typhimurium* strain TA2638, using kidney subcellular fractions for metabolic activation and AOAA (a beta-lyase inhibitor) to inhibit genotoxicity. DCVG and DCVC both exhibited direct-acting mutagenicity,

with kidney mitochondria, cytosol, or microsomes enhancing the effects for both compounds and AOAA diminishing, but not abolishing the effects. Importantly, addition of liver subcellular fractions did not enhance the mutagenicity of DCVG, consistent with *in situ* metabolism playing a significant role in the genotoxicity of these compounds in the kidney.

While additional data are not available on DCVG or NAcDCVC, the genotoxicity of DCVC is further supported by the predominantly positive results in other available *in vitro* and *in vivo* assays. Jaffe et al. (1985) reported DNA strand breaks due to DCVC administered *in vivo*, in isolated perfused kidneys, and in isolated proximal tubules of albino male rabbits. Vamvakas et al. (1989) reported dose-dependent increases in unscheduled DNA synthesis in LLC-PK1 cell clones at concentrations without evidence of cytotoxicity. In addition, Vamvakas et al. (1996) reported that 7-week DCVC exposure to LLC-PK1 cell clones at noncytotoxic concentrations induces morphological and biochemical de-differentiation that persists for at least 30 passages after removal of the compound. This study also reported increased expression of the proto-oncogene *c-fos* in the cells in this system. In a Syrian hamster embryo fibroblast system, DCVC did not induce micronuclei, but demonstrated an unscheduled DNA synthesis response (Vamvakas et al., 1988b).

Two more recent studies are discussed in more detail. Mally et al. (2006) isolated primary rat kidney epithelial cells from *Tsc-2*$^{Ek/+}$ (Eker) rats, and reported increased transformation when exposed to 10 μM DCVC, similar to that of the genotoxic renal carcinogens *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine (Horesovsky et al., 1994). The frequency was variable but consistently higher than background. No loss-of-heterozygosity (LOH) of the *Tsc-2* gene was reported either in these DCVC transformants or in renal tumors (which were not increased in incidence) from TCE-treated Eker rats, which Mally et al. (2006) suggested support a nongenotoxic mechanism because a substantial fraction of spontaneous renal tumors in Eker rats showed LOH at this locus (Kubo et al., 1994, Yeung et al., 1995) and because LOH was exhibited both *in vitro* and *in vivo* with 2,3,4-tris(glutathion-S-yl)-hydroquinone treatment in Eker rats (Yoon et al., 2001). However, 2,3,4-tris(glutathion-S-yl)-hydroquinone is not genotoxic in standard mutagenicity assays (Yoon et al., 2001), and Kubo et al. (1994) also reported that none of renal tumors induced by the genotoxic carcinogen *N*-ethyl-*N*-nitrosourea showed LOH. Therefore, the lack of LOH at the *Tsc-2* locus induced by DCVC *in vitro*, or TCE *in vivo*, reported by Mally et al. (2006) is actually more similar to the response from the genotoxic carcinogen *N*-ethyl-*N*-nitrosourea than the nongenotoxic carcinogen 2,3,4-tris(glutathion-S-yl)-hydroquinone. Therefore, these data do not substantially contradict the body of evidence on DCVC genotoxicity.

Table 4-18. TCE GSH conjugation metabolites genotoxicity

Test system/endpoint	Doses tested	With activation	Without activation	Comments	References
Gene mutations (Ames test)					
S. typhimurium, TA100, 2638, 98	0.1−0.5 nmol	ND	+	DCVC was mutagenic in all three strains of *S. typhimurium* without the addition of mammalian subcellular fractions.	Dekant et al., 1986
S. typhimurium, TA2638	50−300 nmol	+	+	Increase in number of revertants in DCVC alone at low doses; further increase in revertants was observed in the presence of microsomal fractions. Toxicity as indicated by decreased revertants per plate were seen at higher doses.	Vamvakas et al., 1988a
Mutation analysis					
In vitro—rat kidney epithelial cells, LOH in *Tsc* gene	10 μM	NA	-	Only 1/9 transformed cells showed LOH.	Mally et al., 2006
In vitro—rat kidney epithelial cells, *VHL* gene (exons 1−3)	10 μM	NA	-	No mutations in *VHL* gene. Note: *VHL* is not a target gene in rodent models of chemical-induced or spontaneous renal carcinogenesis.	Mally et al., 2006
Unscheduled DNA synthesis					
Porcine kidney tubular epithelial cell line (LLC-PK1)	2.5 μM−5, 10, 15, 24 h; 2.5−100 μM	NA	+	Dose-dependent in UDS up to 24 h tested at 2.5 μM. Also, there was a dose dependent increase at lower conc. Higher concentrations were cytotoxic as determined by LDH release from the cells.	Vamvakas et al., 1989
Syrian hamster embryo fibroblasts		NA	+	Increase in UDS in treatment groups.	Vamvakas et al., 1988b

Table 4-18. TCE GSH conjugation metabolites genotoxicity (continued)

Test system/endpoint	Doses tested	With activation	Without activation	Comments	References
DNA strand breaks					
Male rabbit renal tissue (perfused kidneys and proximal tubules)	0−100 mg/kg or 10 µM to 10 mM	ND	+	Dose dependent increase SB in both i.v. and i.p. injections (i.v. injections were done only for 10 and 20 mg/kg). Perfusion of rabbit kidney (45 min exposure) and proximal tubules (30 min exposure) expt. Resulted in a dose dependent difference in the amount of single strand breaks.	Jaffe et.al., 1985
Primary kidney cells from both male rats and human	1−4 mM; 20 h exposure	NA	+	Statistically significant increase in all doses (1, 2, or 4 mM) both in rats and human cells.	Robbiano, 2004
In vivo—male Sprague-Dawley rats exposed to TCE or DCVC—comet assay	TCE: 500−2,000 ppm, inhalation, 6 h/d, 5 d DCVC: 1 or 10 mg/kg, single oral dose for 16 h	+ (DCVC) – (TCE)	NA	No significant increase in tail length in any of the TCE exposed groups. In Expt. 1. 2 h exposure—1 or 10 mg to DCVC resulted in significant increase with no dose response, but not at 16 h. In Expt. 2. ND for 1 mg, significant increase at 10 mg.	Clay, 2008
Micronucleus					
Syrian hamster embryo fibroblasts		NA	-	No micronucleus formation.	Vamvakas et al., 1988b
Primary kidney cells from both male rats and human	1−4 mM; 20 h exposure	NA	+	Statistically significant increase in all doses (1, 2, and 4 mM) both in rats and human cells.	Robbiano, 2004
Male Sprague-Dawley rats; proximal tubule cells (*in vivo*)	4 mM/kg TCE exposure, single dose	NA	+	Statistically significant increase in the average frequency of micronucleated kidney cells was observed.	Robbiano et al., 1998

Table 4-18. TCE GSH conjugation metabolites genotoxicity (continued)

Test system/endpoint	Doses tested	With activation	Without activation	Comments	References
Cell transformation					
Kidney tubular epithelial cell line (LLC-PK1)	1or 5 µM; 7 wks	NA	+	Induced morphological cell transformation at both concentrations tested. Furthermore, cells maintained both biochemical and morphological alterations remained stable for 30 passages	Vamvakas et al., 1996
Rat kidney epithelial cells (*in vitro*)	10 µM; 24 h exposure, 7 wks post incubation	NA	+	Cell transformation was higher than control, however, cell survival percent ranged from 39−64% indicating cytotoxicity	Mally et al., 2006
Gene expression					
Kidney tubular epithelial cell line (LLC-PK1)	1or 5 µM clones, 30, 60, 90 min	NA	+	Increased *c-fos* expression in 1 and 5 µM exposed clones at three different times tested	Vamvakas et al., 1996
Kidney tubular epithelial cell line (LLC-PK1)		NA	+	Expression of *c-fos* and *c-myc* increased in a time-dependent manner	Vamvakas et al., 1993

i.v. = intravenous, LDH = lactate dehydrogenase, LOH = loss of heterozygosity, ND = not determined, NA = not applicable.

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Finally, Clay (2008) evaluated the genotoxicity of DCVC *in vivo* using the comet assay to assess DNA breakage in the proximal tubules of rat kidneys. Rats were exposed orally to a single dose of DCVC (1 or 10 mg/kg). The animals were sacrificed either 2 or 16 hours after dosing and samples prepared for detecting the DNA damage. DCVC (1 and 10 mg/kg) induced no significant DNA damage in rat kidney proximal tubules at the 16-hour sampling time or after 1 mg/kg DCVC at the 2-hour sampling time. While Clay et al. (2008) concluded that these data were insufficient to indicate a positive response in this assay, the study did report a statistically significant increase in percent tail DNA 2 hours after treatment with 10 mg/kg DCVC, despite the small number of animals at each dose ($n = 5$) and sampling time. Therefore, these data do not substantially contradict the body of evidence on DCVC genotoxicity.

Overall, DCVC, and to a lesser degree DCVG and NAcDCVC, have demonstrated genotoxicity based on consistent results in a number of available studies. While some recent studies (Mally et al., 2006; Clay, 2008) have reported a lack of positive responses in some *in vivo* measures of genotoxicity with DCVC treatment, due to a number of limitations discussed above, these studies do not substantially contradict the body of evidence on DCVC genotoxicity. It is known that these metabolites are formed *in vivo* following TCE exposure, specifically in the kidney, so they have the potential to contribute to the genotoxicity of TCE, especially in that tissue. Moreover, DCVC and DCVG genotoxic responses were enhanced when metabolic activation using *kidney* subcellular fractions was used (Vamvakas et al., 1988a). Finally, the lack of similar responses in *in vitro* genotoxicity assays with TCE, even with metabolic activation, is likely the result of the small yield (if any) of DCVC under *in vitro* conditions, since *in vivo*, DCVC is likely formed predominantly *in situ* in the kidney while S9 fractions are typically derived from the liver. This hypothesis could be tested in experiments in which TCE is incubated with subcellular fractions from the kidney, or from both the kidney and the liver (for enhanced GSH conjugation).

4.2.6. Trichloroethanol (TCOH)

Limited studies are available on the effect of TCOH on genotoxicity (Table 4-19). TCOH is negative in the *S. typhimurium* assay using the TA100 strain (Bignami et al., 1980; DeMarini et al., 1994; Waskell, 1978). A study by Beland (1999) using *S. typhimurium* strain TA104 did not induce reverse mutations without exogenous metabolic activation, however did increase mutant frequency in the presence of exogenous metabolic activation at a dose above 2,500 µg/plate. TCOH has not been evaluated in the other recommended screening assays. Therefore, the database is limited for the determination of TCOH genotoxicity.

Table 4-19.. Genotoxicity of trichloroethanol

Test system/endpoint	Doses (LED or HID)[a]	Results[b]		Reference
		With activation	Without activation	
S. typhimurium TA100, 98, reverse mutation	7,500 μg/plate	-	-	Waskell, 1978
S. typhimurium TA100, reverse mutation	0.5 μg/cm^3 vapor	-	-	DeMarini et al., 1994
S. typhimurium TA104, reverse mutation	2,500 μg/plate	+	-	Beland, 1999
S. typhimurium TA100, 1535 reverse mutation	NA	-	-	Bignami et al., 1980
Sister chromatid exchanges	NA	NA	+	Gu et al., 1981 b

[a]LED, lowest effective dose; HID, highest ineffective dose.
[b]Results: + = positive; - = negative; NA = doses not available, results based on the abstract.

4.2.7. Synthesis and Overall Summary

Trichloroethylene and its metabolites (TCA, DCA, CH, DCVC, DCVG, and TCOH) have been evaluated to varying degrees for their genotoxic activity in several of *in vitro* systems such as bacteria, yeast, and mammalian cells and, also, in *in vivo* systems.

There are several challenges in interpreting the genotoxicity results obtained from TCE exposure. For example, some studies in bacteria should be interpreted with caution if conducted using technical grade TCE since it may contain known bacterial mutagens in trace amounts as stabilizers (e.g., 1,2-epoxybutane and epichlorohydrin). Because of the volatile nature of TCE, there could be false negative results if proper precautions are not taken to limit evaporation, such as the use of a closed sealed system. The adequacy of the enzyme-mediated activation of TCE *in vitro* tests is another consideration. For example, it is not clear if standard S9 fractions can adequately recapitulate the complex *in vivo* metabolism of TCE to reactive intermediates, which in some cases entails multiple sequential steps involving multiple enzyme systems (e.g., CYP, GST, etc.) and interorgan processing (as is described in more detail in Section 3.3). In addition, the relative potency of the metabolites *in vitro* may not necessarily inform their relative contribution to the overall mechanistic effects of the parent compound, TCE. Furthermore, although different assays provided data relevant to different types of genotoxic endpoints, not all effects that are relevant for carcinogenesis are encompassed. The standard battery of prokaryotic as well as mammalian genotoxicity test protocols typically specify the inclusion of significantly cytotoxic concentrations of the test compound.

With respect to potency, several TCE studies have been conducted along with numerous other chlorinated compounds and the results interpreted as a comparison of the group of compounds tested (relative potency). However, for the purposes of hazard characterization, such comparisons are not informative – particularly if they are not necessarily correlated with *in vivo* carcinogenic potency. Also, differentiating the effects of TCE with respect to its potency can be influenced by many factors such as the type of cells, their differing metabolic capacities, sensitivity of the assay, need for greater concentration to show any effect, interpretation of data when the effects are marginal, and gradation of severity of effects.

Also, type of samples used, methodology used for the isolation of genetic material, and duration of exposure can particularly influence the results of several studies. This is particularly true for human epidemiological studies. For example, while some studies use tissues obtained directly from the patients others use formalin fixed tissues sections to isolate DNA for mutation detection. Type of fixing solution, fixation time, and period of storage of the tissue blocks often affect the quality of DNA. Formic acid contained in the formalin solution or picric acid contained in Bouin's solution is known to degrade nucleic acids resulting in either low yield or poor quality of DNA. In addition, during collection of tumor tissues, contamination of neighboring normal tissue can easily occur if proper care is not exercised. This could lead to the 'dilution effect' of the results, i.e., because of the presence of some normal tissue; frequency of mutations detected in the tumor tissue can be lower than expected. Due to some of these technical difficulties in obtaining proper material (DNA) for the detection of mutation, the results of these studies should be interpreted cautiously.

The following synthesis, summary, and conclusions focus on the available studies that may provide some insight into the potential genotoxicity of TCE considering the above challenges when interpreting the mutagenicity data for TCE.

Overall, evidence from a number of different analyses and a number of different laboratories using a fairly complete array of endpoints suggests that TCE, following metabolism, has the potential to be genotoxic. TCE has a limited ability to induce mutation in bacterial systems, but greater evidence of potential to bind or to induce damage in the structure of DNA or the chromosome in a number of targets. A series of carefully controlled studies evaluating TCE itself (without mutagenic stabilizers and without metabolic activation) found it to be incapable of inducing gene mutations in most standard mutation bacterial assays (Waskell, 1978; Henschler et al., 1977; Mortelmans et al., 1986; Simmon et al., 1977; Baden et al., 1979; Bartsch et al., 1979; Crebelli et al., 1982; Shimada et al., 1985; Simmon et al., 1977; Baden et al., 1979). Therefore, it appears that it is unlikely that TCE is a direct-acting mutagen, though TCE has shown potential to affect DNA and chromosomal structure. TCE is also positive in

some but not all fungal and yeast systems (Crebelli et al., 1985; Koch et al., 1988; Rossi et al., 1983; Callen et al., 1980). Data from human epidemiological studies support the possible mutagenic effect of TCE leading to *VHL* gene damage and subsequent occurrence of renal cell carcinoma. Association of increased *VHL* mutation frequency in TCE-exposed renal cell carcinoma cases has been observed (Brüning et al.,1997; Brauch et al., 1999, 2004).

TCE can lead to binding to nucleic acids and proteins (Di Renzo et al., 1982; Bergman, 1983; Miller and Guengerich, 1983; Mazzullo et al., 1992; Kautiainen et al., 1997), and such binding appears to be due to conversion to one or more reactive metabolites. For instance, increased binding was observed in samples bioactivated with mouse and rat microsomal fractions (Banerjee and VanDuuren, 1978; Di Renzo et al., 1982; Miller and Guengerich, 1983; Mazzullo et al., 1992). DNA binding is consistent with the ability to induce DNA and chromosomal perturbations. Several studies report the induction of micronuclei *in vitro* and *in vivo* from TCE exposure (Kligerman et al., 1994; Hrelia et al., 1994; Wang et al., 2001; Robbiano et al., 2004; Hu et al., 2008). Reports of SCE induction in some studies are consistent with DNA effects, but require further study (White et al., 1979; Gu et al., 1981a, b; Nagaya et al., 1989; Kligerman et al., 1994).

TCA, an oxidative metabolite of TCE, exhibits little, if any genotoxic activity *in vitro*. TCA did not induce mutations in *S. typhimurium* strains in the absence of metabolic activation or in an alternative protocol using a closed system (Waskell, 1978; Rapson et al., 1980; DeMarini et al., 1994; Giller et al., 1997; Nelson et al., 2001; Kargalioglu et al., 2002) but a mutagenic response was induced in TA100 in the Ames fluctuation test (Giller et al., 1997). However, *in vitro* experiments with TCA should be interpreted with caution if steps have not been taken to neutralize pH changes caused by the compound (Mackay, 1995). Measures of DNA-repair responses in bacterial systems have shown induction of DNA repair reported in *S. typhimurium* but not in *E. coli*. Mutagenicity in mouse lymphoma cells was only induced at cytotoxic concentrations (Harrington-Brock et al., 1998). TCA was positive in some genotoxicity studies *in vivo* mouse, newt, and chick test systems (Bhunya and Behera, 1987; Bhunya and Jena, 1996; Birner et al., 1994; Giller et al., 1997). DNA unwinding assays have either shown TCA to be much less potent than DCA (Nelson and Bull, 1988) or negative (Nelson et al., 1989; Styles et al., 1991). Due to limitations in the genotoxicity database, the possible contribution of TCA to TCE genotoxicity is unclear.

DCA, a chloroacid metabolite of TCE, has also been studied using different types of genotoxicity assays. Although limited studies are conducted for different genetic endpoints, DCA has been demonstrated to be mutagenic in the *S. typhimurium* assays, *in vitro* (DeMarini et al., 1994; Kargalioglu et al., 2002; Plewa et al., 2002) in some strains, mouse

lymphoma assay, (Harrington-Brock et al., 1998) *in vivo* cytogenetic tests (Leavitt et al., 1997; Fuscoe et al., 1996), the micronucleus induction test, the Big Blue mouse system, and other tests (Bignami et al., 1980; Chang et al., 1989; DeMarini et al., 1994; Leavitt et al., 1997; Fuscoe et al., 1996; Nelson and Bull, 1988; Nelson et al., 1989; Harrington-Brock et al., 1998). DCA can cause DNA strand breaks in mouse and rat liver cells following *in vivo* mice and rats (Fuscoe et al., 1996). Because of uncertainties as to the extent of DCA formed from TCE exposure, inferences as to the possible contribution from DCA genotoxicity to TCE toxicity are difficult to make.

Chloral hydrate is mutagenic in the standard battery of screening assays. Effects include positive results in bacterial mutation tests for point mutations and in the mouse lymphoma assay for mutagenicity at the Tk locus (Haworth et al., 1983). *In vitro* tests showed that CH also induced micronuclei and aneuploidy in human peripheral blood lymphocytes and Chinese hamster pulmonary cell lines. Micronuclei were also induced in Chinese hamster embryonic fibroblasts. Several studies demonstrate that chloral hydrate induces aneuploidy (loss or gain of whole chromosomes) in both mitotic and meiotic cells, including yeast (Singh and Sinha, 1976, 1979; Kafer, 1986; Gualandi, 1987; Sora and Agostini-Carbone, 1987), cultured mammalian somatic cells (Degrassi and Tanzarella, 1988), and spermatocytes of mice (Russo et al., 1984; Liang and Pacchierotti, 1988). Chloral hydrate was negative for sex-linked recessive lethal mutations in *Drosophila* (Yoon et al., 1985). It induces SSB in hepatic DNA of mice and rats (Nelson and Bull, 1988) and mitotic gene conversion in yeast (Bronzetti et al., 1984). Schatten and Chakrabarti (1998) showed that chloral hydrate affects centrosome structure, which results in the inability to reform normal microtubule formations and causes abnormal fertilization and mitosis of sea urchin embryos. Based on the existing array of data, CH has the potential to be genotoxic, particularly when aneuploidy is considered in the weight of evidence for genotoxic potential. Chloral hydrate appears to act through a mechanism of spindle poisoning and resulting in numerical changes in the chromosomes. These results are consistent with TCE, albeit there are limited data on TCE for these genotoxic endpoints.

DCVC, and to a lesser degree DCVG, has demonstrated bacterial mutagenicity based on consistent results in a number of available studies (Dekant et al., 1986; Vamvakas et al., 1987; Vamvakas, 1988a). DCVC has demonstrated a strong, direct-acting mutagenicity both with and without the presence of mammalian activation enzymes. It is known that these metabolites are formed *in vivo* following TCE exposure, so they have the potential to contribute to the genotoxicity of TCE. The lack of similar response in bacterial assays with TCE is likely the result of the small yield (if any) of DCVC under *in vitro* conditions, since *in vivo*, DCVC is likely formed predominantly *in situ* in the kidney (S9 fractions are typically derived from the

1 liver). DCVC and DCVG have not been evaluated extensively in other genotoxicity assays, but
2 the available *in vitro* and *in vivo* data are predominantly positive. For instance, several studies
3 have reported the DCVC can induce primary DNA damage in mammalian cells *in vitro* and *in*
4 *vivo* (Jaffe et al., 1985; Vamvakas et al., 1989; Clay, 2008). Long-term exposure to DCVC
5 induced de-differentiation of cells (Vamavakas et al., 1996). It has been shown to induce
6 expression of the protooncogene *c-fos* (Vamvakas et al., 1996) and cause cell transformation in
7 rat kidney cells (Mally et al., 2006). In LLC-PK1 cell clones, DCVC was reported in induce
8 unscheduled DNA synthesis, but not micronuclei (Vamvakas et al., 1988b). Finally, DCVC
9 induced transformation in kidney epithelial cells isolated from Eker rats carrying the
10 heterozygous *Tsc-2* mutations (Mally et al., 2006). Moreover, the lack of LOH at the *Tsc-2* locus
11 observed in exposed cells does not constitute negative evidence of DCVC genotoxicity, as none
12 of renal tumors induced in Eker rats by the genotoxic carcinogen *N*-ethyl-*N*-nitrosourea showed
13 LOH (Kubo et al., 1994).
14 In support of the importance of metabolism, there is some concordance between effects
15 observed from TCE and those from several metabolites. For instance, both TCE and chloral
16 hydrate have been shown to induce micronucleus in mammalian systems, but chromosome
17 aberrations have been more consistently observed with chloral hydrate than with TCE. The role
18 of TCA in TCE genotoxicity is less clear, as there is less concordance between the results from
19 these two compounds. Finally, several other TCE metabolites show at least some genotoxic
20 activity, with the strongest data from DCA, DCVG, and DCVC. While quantitatively smaller in
21 terms of flux as compared to TCA and TCOH (for which there is almost no genotoxicity data),
22 these metabolites may still be toxicologically important.
23 Thus, uncertainties with regard to the characterization of TCE genotoxicity remain,
24 particularly because not all TCE metabolites have been sufficiently tested in the standard
25 genotoxicity screening battery to derive a comprehensive conclusion. However, the metabolites
26 that have been tested particularly DCVC have predominantly resulted in positive data although
27 to a lesser extent in DCVG and NAcDCVC, supporting the conclusion that these compounds are
28 genotoxic, particularly in the kidney, where *in situ* metabolism produces and/or bioactivates
29 these TCE metabolites.
30
31 **4.3. CENTRAL NERVOUS SYSTEM (CNS) TOXICITY**
32 TCE exposure results in central nervous system (CNS) effects in both humans and
33 animals that can result from acute, subchronic, or chronic exposure. There are studies indicating
34 that TCE exposure results in CNS tumors and this discussion can be found in Section 4.9. The
35 studies discussed in this section focus on the most critical neurological effects that were

extracted from the neurotoxicological literature. Although there are several studies and reports that have evaluated TCE as an anesthetic, those studies were not included in this section because of the high exposure levels in comparison to the selected critical neurological effects described below. The critical neurological effects are nerve conduction changes, sensory effects, cognitive deficits, changes in psychomotor function, and changes in mood and sleep behaviors. The selection criteria that were used to determine study importance included study design and validity, pervasiveness of neurological effect, and for animal studies, the relevance of these reported outcomes in humans. More detailed information on human and animal neurological studies with TCE can be found in Appendix D.

4.3.1. Alterations in Nerve Conduction

4.3.1.1. *Trigeminal Nerve Function: Human Studies*

A number of human studies have been conducted that examined the effects of occupational or drinking water exposures to TCE on trigeminal nerve function (see Table 4-20). Many studies reported that humans exposed to TCE present trigeminal nerve function abnormalities as measured by blink reflex and masseter reflex test measurements (Feldman et al., 1988, 1992; Kilburn and Warshaw, 1993; Kilburn, 2002a; Ruitjen et al., 2001). The blink and masseter reflexes are mediated primarily by the trigeminal nerve and changes in measurement suggest impairment in nerve conduction. Other studies measured the trigeminal somatosensory evoked potential (TSEP) following stimulation of the trigeminal nerve and reported statistically significantly delayed response on evoked potentials among exposed subjects compared to nonexposed individuals (Barret et al., 1982, 1984, 1987; Mhiri et al., 2004). Two studies which also measured trigeminal nerve function did not find any effect (El-Ghawabi et al., 1973; Rasmussen et al., 1993c) but the methods were not provided in either study (El-Ghawabi et al., 1973; Rasmussen et al., 1993c) or an appropriate control group was not included (Rasmussen et al., 1993c). These studies and results are described below and summarized in detail in Table 4-20.

1 **Table 4-20. Summary of human trigeminal nerve and nerve conduction**
2 **velocity studies**
3

Reference	Subjects	Exposure	Effect
Barret et al., 1982	11 workers with chronic TCE exposure Controls: 20 unexposed subjects	Presence of TCE and TCA found through urinalysis. Atmospheric TCE concentrations and duration of exposure not reported in paper.	Following stimulation of the trigeminal nerve, significantly higher voltage stimuli was required to obtain a normal response and there was a significant increase in latency for response and decreased response amplitude.
Barret et al., 1984	188 factory workers. No unexposed controls; lowest exposure group used as comparison	>150 ppm; $n = 54 < 150$ ppm; $n = 134$, 7 h/d for 7 yr	Trigeminal nerve and optic nerve impairment, asthenia and dizziness were significantly increased with exposure.
Barret et al., 1987	104 degreaser machine operators Controls: 52 unexposed subjects Mean age 41.6 yrs	Mean duration, 8.2 yrs, average daily exposure 7 h/d. Average TCOH range = 162−245 mg/g creatinine Average TCA range = 93−131 mg/g creatinine	Evoked trigeminal responses were measured following stimulation of the nerve and revealed increased latency to respond, amplitude or both and correlated with length of exposure ($p < 0.01$) and with age ($p < 0.05$), but not concentration.
El-Ghawabi et al., 1973	30 money printing shop workers Controls: 20 nonexposed males 10 workers exposed to inks not containing TCE	Mean TCE air concentrations ranged from 41 ppm to 163 ppm. Exposure durations: Less than 1 yr: $n = 3$ 1 yr: $n = 1$ 2 yrs: $n = 2$ 3 yrs: $n = 11$ 4 yrs: $n = 4$ 5 yrs or greater: $n = 9$	No effect on trigeminal nerve function was noted.
Feldman et al., 1988	21 Woburn, MA residents; 27 controls	TCE maximum reported concentration in well water was 267 ppb; other solvents also present. Exposure duration ranged from 1−12 yrs.	Measurement of the blink reflex as mediated by the trigeminal nerve resulted in significant increases in the latency of reflex components ($p < 0.001$).
Feldman et al., 1992	18 workers; 30 controls	TCE exposure categories of “extensive”, “occasional,” and “chemical other than TCE” “extensive” = chronically exposed (≥1 yr) to TCE for 5 d/wk and >50% workday. “occupational” = chronically exposed to TCE for 1−3 d/wk and >50% workday.	The blink reflex as mediated by the trigeminal was measured. The “extensive” group revealed latencies greater than 3 SD above the nonexposed group mean on blink reflex components.

Table 4-20. Summary of human trigeminal nerve and nerve conduction velocity studies (continued)

Reference	Subjects	Exposure	Effect
Kilburn and Warshaw, 1993	160 residents living in Southwest Tucson with TCE, other solvents, and chromium in groundwater Control: 113 histology technicians from a previous study (Kilburn et al., 1987; Kilburn and Warshaw, 1992)	>500 ppb of TCE in well water before 1981 and 25 to 100 ppb afterwards Duration ranged from 1 to 25 yrs	Significant impairments in sway speed with eyes open and closed and blink reflex latency (R-1) which suggests trigeminal nerve impairment.
Kilburn, 2002a	236 residents near a microchip plant in Phoenix, AZ Controls: 161 regional referents from Wickenburg, AZ and 67 referents in northeastern Phoenix	<0.2−10,000 ppb of TCE, <0.2−260,000 ppb TCA, <0.2−6,900 ppb 1,1-DCE, <0.2−1,600 1,2-DCE, <0.2−23,000 ppb PCE, <0.02−330 ppb VC in well water Exposure duration ranged from 2 to 37 yrs	Trigeminal nerve impairment as measured by the blink reflex test; both right and left blink reflex latencies (R-1) were prolonged. Exposed group mean 14.2 + 2.1 ms (right) or 13.9 + 2.1 ms (left) versus referent group mean of 13.4 + 2.1 ms (right) or13.5 + 2.1ms (left), $p = 0.0001$ (right) and 0.008 (left).
Mhiri et al., 2004	23 phosphate industry workers Controls: 23 unexposed workers	Exposure ranged from 50−150 ppm, for 6 hr/d for at least 2 yrs Mean urinary trichloroethanol and trichloroacetic acid levels were 79.3 ± 42 and 32.6 ± 22 mg/g creatinine	TSEPs were recorded. Increase in the TSEP latency was observed in 15 out of 23 (65%) workers.
Rasmussen et al., 1993c	96 Danish metal degreasers Age range: 19−68; No unexposed controls; low exposure group used as comparison	Average exposure duration: 7.1 yrs.); range of full-time degreasing: 1 month to 36 yrs. Exposure to TCE or to CFC113 1) Low exposure: $n = 19$, average full-time exposure 0.5 yrs 2) Medium exposure: $n = 36$, average full-time exposure 2.1 yrs 3) High exposure: $n = 41$, average full-time exposure 11 yrs. TCA in high exposure group = 7.7 mg/L (max = 26.1 mg/L)	No statistically significant trend on trigeminal nerve function, although some individuals had abnormal function.

Table 4-20. Summary of human trigeminal nerve and nerve conduction velocity studies (continued)

Reference	Subjects	Exposure	Effect
Ruitjen et al., 1991	31 male printing workers. Mean age 44 yrs; mean duration 16 yrs Controls: 28 unexposed; Mean age 45 yrs	Mean cumulative exposure = 704 ppm × yrs (SD 583, range: 160−2,150 ppm × yrs Mean, 17 ppm at time of study; historic TCE levels from 1976−1981, mean of 35 ppm Mean duration of 16 yrs	Measurement of trigeminal nerve function by using the blink reflex resulted in no abnormal findings. Increased latency in the masseter reflex is indicative of trigeminal nerve impairment.
Triebig et al., 1982	24 workers (20 males, 4 females) occupationally exposed—ages 17−56; Controls: 144 individuals to establish normal nerve conduction parameters; Matched group: 24 unexposed workers (20 males, 4 females)	Exposure duration of 1 month to 258 months (mean 83 months). Air exposures were between 5−70 ppm	No statistically significant difference in nerve conduction velocities between the exposed and unexposed groups.
Triebig et al., 1983	66 workers occupationally exposed Control: 66 workers not exposed to solvents	Subjects were exposed to a mixture of solvents, including TCE	Exposure-response relationship observed between length of solvent exposure and statistically significant reduction in mean sensory ulnar nerve conduction velocities.

DCE = dichloroethylene, PCE = perchloroethylene, SD = standard deviation.

Integrity of the trigeminal nerve is commonly measured using blink and masseter reflexes. Five studies (Barret et al., 1984; Feldman et al., 1988, 1992; Kilburn and Warshaw, 1993; Kilburn, 2002a) reported a significant increase in the latency to respond to the stimuli generating the reflex. The latency increases in the blink reflex ranged from 0.4 ms (Kilburn, 2002a) to up to 3.44 ms (Feldman et al., 1988). The population groups in these studies were exposed by inhalation occupationally (Barret et al., 1984) and through drinking water environmentally (Feldman et al., 1988; Kilburn and Warshaw, 1993; Kilburn, 2002a). Feldman et al. (1992) demonstrated persistence in the increased latency of the blink reflex response. In one subject, exposure to TCE (levels not reported by authors) occurred through a degreasing accident (high and acute exposure), and increased latency response times persisted 20 years after the accident. Another two subjects, evaluated at 9 months and 1 month following a high occupational exposure (exposure not reported by authors), also had higher blink reflex latencies with an average increase of 2.8 ms over the average response time in the control group used in the study. Although one study (Ruitjen et al., 1991) did not find these increases in male

printing workers exposed to TCE, this study did find a statistically significant average increase of 0.32 ms ($p < 0.05$) in the latency response time in TCE-exposed workers on the masseter reflex test, another test commonly used to measure the integrity of the trigeminal nerve.

Three studies (Barret et al., 1982, 1987; Mhiri et al., 2004) adopting TSEPs to measure trigeminal nerve function found significant abnormalities in these evoked potentials. These studies were conducted on volunteers who were occupationally exposed to TCE through metal degreasing operations (Barret et al., 1982, 1987) or through cleaning tanks in the phosphate industry (Mhiri et al., 2004). Barret et al. (1982) reported that in eight of the eleven workers, an increased voltage ranging from a 25 to a 45 volt increase was needed to generate a normal TSEP and two of workers had an increased TSEP latency. Three out of 11 workers had increases in TSEP amplitudes. In a later study, Barret et al. (1987) also reported abnormal TSEPs (increased latency and/or increased amplitude) in 38% of the degreasers that were evaluated. The individuals with abnormal TSEPs were significantly older (45 vs. 40.1 years; $p < 0.05$) and were exposed to TCE longer (9.9 vs. 5.6 years; $p < 0.01$). Mhiri et al. (2004) was the only study to evaluate individual components of the TSEP and noted significant increases in latencies for all TSEP potentials (N1, P1, N2, P2, N3; $p < 0.01$) and significant decreases in TSEP amplitude (P1, $p < 0.02$; N2, $p < 0.05$). A significant positive correlation was demonstrated between exposure duration and increased TSEP latency ($p < 0.02$).

Two studies reported no statistically significant effect of TCE exposure on trigeminal nerve function (El-Ghawabi et al., 1973; Rasmussen et al., 1993). El-Ghawabi et al. (1973) conducted a study on 30 money printing shop workers occupationally exposed to TCE. Trigeminal nerve involvement was not detected, but the authors did not include the experimental methods that were used to measure trigeminal nerve involvement and did not provide any data as to how this assessment was made. Rasmussen et al. (1993c) conducted an historical cohort study on 99 metal degreasers, 70 exposed to TCE and 29 to the fluorocarbon, CFC113. It was reported that 1 out of 21 people (5%) in the low exposure, 2 out of 37 (5%) in the medium exposure and 4 out of 41 (10%) in the high exposure group experienced abnormalities in trigeminal nerve sensory function, with a linear trend test p-value of 0.42. The mean urinary trichloroacetic acid concentration was reported for the high exposure group only and was 7.7 mg/L (maximum concentration, 26.1 mg/L). The trigeminal nerve function findings of high exposure group subjects was compared to that of low exposure group since this study did not include an unexposed or no TCE exposure group, and decreased the sensitivity of the study.

4.3.1.2. *Nerve Conduction Velocity—Human Studies*

Two occupational studies assessed ulnar and median nerve function using tests of conduction latencies (Triebig, 1982, 1983) (see Table 4-20). The ulnar nerve and median nerves are major nerves located in the arm and forearm. Triebig (1982) studied twenty-four healthy workers (20 males, 4 females) exposed to TCE occupationally (5−70 ppm) at three different plants and did not find statistically significant differences in ulnar or median nerve conduction velocities between exposed and unexposed subjects. This study has measured exposure data, but exposures/responses are not reported by dose levels. The Triebig (1983) study is similar in design to the previous study (Triebig, 1982) but of a larger number of subjects. In this study, a dose-response relationship was observed between lengths of exposure to mixed solvents that included TCE (at unknown concentration). A statistically significant reduction in nerve conduction velocities was observed for the medium- and long-term exposure groups for the sensory ulnar nerve as was a statistically significant reduction in mean nerve conduction velocity observed between exposed and control subjects.

4.3.1.3. *Trigeminal Nerve Function: Laboratory Animal Studies*

There is little evidence that TCE disrupts trigeminal nerve function in animal studies. Two studies demonstrated TCE produces morphological changes in the trigeminal nerve at a dose of 2,500 mg/kg/d for 10 weeks (Barret et al., 1991, 1992). However, dichloroacetylene, a degradation product formed during the volatilization of TCE was found to produce more severe morphological changes in the trigeminal nerve and at a lower dose of 17 mg/kg/d (Barret et al., 1991, 1992). Only one study (Albee et al., 2006) has evaluated the effects of TCE on trigeminal nerve function and a subchronic inhalation exposure did not result in any significant functional changes. A summary of these studies is provided in Table 4-21.

Barret et al. (1991, 1992) conducted two studies evaluating the effects of both TCE and dichloroacetylene on trigeminal nerve fiber diameter and internodal length as well as several markers for fiber myelination. Female Sprague-Dawley rats (*n* = 7/group) were dosed with 2,500 mg/kg TCE or 17 mg/kg/d dichloroacetylene by gavage for 5 days/week for 10 weeks. TCE-dosed animals only exhibited changes in the smaller Class A fibers where internode length increased marginally (<2%) and fiber diameter increased by 6%. Conversely, dichloroacetylene-treated rats exhibited significant and more robust decreases in internode length and fiber diameter in both fiber classes A (decreased 8%) and B (decreased 4%).

1 **Table 4-21. Summary of animal trigeminal nerve studies**
2

Reference	Exposure route	Species/strain/ sex/number	Dose level/ exposure duration	NOAEL; LOAEL*	Effects
Barret et al., 1991	Direct gastric administration	Rat, Sprague-Dawley, female, 7/group	0, 2.5 g/kg, acute administration 17 mg/kg dichloroacetylene	LOAEL: 2.5 g/kg	Morphometric analysis was used for analyzing the trigeminal nerve. Increase in external and internal fiber diameter as well as myelin thickness was observed in the trigeminal nerve after TCE treatment.
Barret et al., 1992	Direct gastric administration	Rat, Sprague-Dawley, female, 7/group	0, 2.5 g/kg; 1 dose/d, 5 d/wk, 10 wks 17 mg/kg dichloroacetylene	LOAEL: 2.5 g/kg	Trigeminal nerve analyzed using morphometric analysis. Increased internode length and fiber diameter in class A fibers of the trigeminal nerve observed with TCE treatment. Changes in fatty acid composition also noted.
Albee et al., 1997	Inhalation	Rat, Fischer 344, male, 6	0 or 300-ppm dichloro-acetylene, 2.25 h	LOAEL: 300 ppm dichloro-acetylene	Dichloroacetylene (TCE byproduct) exposure impaired the TSEP up to 4 d postexposure.
Albee et al., 2006	Inhalation	Rat, Fischer 344, male and female, 10/sex/group	0, 250, 800, or 2,500 ppm	NOAEL: 2,500 ppm	No effect on TSEPs was noted at any exposure level.

3
4 *NOAEL = no-observed-adverse-effect level, LOAEL = lowest-observed–adverse-effect-level.
5
6
7 Albee et al. (2006) evaluated the effects of a subchronic inhalation TCE exposure in
8 Fischer 344 rats (10/sex/group). Rats were exposed to 0, 250, 800, and 2,500 ppm TCE for
9 6 hours/day, 5 days/week for 13 weeks. TCE exposures were adequate to produce permanent
10 auditory impairment even though TSEPs were unaffected. While TCE appears to be negative in
11 disrupting the trigeminal nerve, the TCE breakdown product, dichloroacetylene, does impair
12 trigeminal nerve function. Albee et al. (1997) showed that a single inhalation exposure of rats to
13 300-ppm dichloroacetylene, for 2.25 hours, disrupted trigeminal nerve evoked potentials for at
14 least 4 days post exposure.
15
16 **4.3.1.4. *Discussion and Conclusions: Trichloroethylene (TCE)-Induced Trigeminal Nerve*
17 *Impairment***

18 Epidemiologic studies of exposure to TCE found impairment of trigeminal nerve
19 function, assessed by the blink reflex test or the TSEP, in humans exposed occupationally by

inhalation or environmentally by ingestion (see Table 4-20). Mean inhalational exposures inferred from biological monitoring or from a range of atmospheric monitoring in occupational studies was approximately 50 ppm to <150 ppm TCE exposure. Residence location is the exposure surrogate in geographical-base studies of contaminated water supplies with several solvents. Well water contaminant concentrations of TCE ranged from <0.2 ppb to 10,000 ppb and do not provide an estimate of TCE concentrations in drinking water to studied individuals. Two occupational studies, each including more than 100 subjects, reported statistically significant dose-response trends based on ambient TCE concentrations, duration of exposure, and/or urinary concentrations of the TCE metabolite TCA (Barret et al., 1984, 1987). Three geographical-based studies of environmental exposures to TCE via contaminated drinking water are further suggestive of trigeminal nerve function decrements; however, these studies are more limited than occupational studies due to questions of subject selection. Both exposed subjects who were litigants and control subjects who may not be representative of exposed (Kilburn and Warshaw, 1993; Kilburn et al., 2002a); referents in Kilburn and Warshaw (1993) were histology technicians and subjects in a previous study of formaldehyde and other solvent exposures and neurobehavioral effects (Kilburn et al., 1987; Kilburn and Warshaw, 1992). Results were mixed in a number of smaller studies. Two of these studies reported changes in trigeminal nerve response (Mhiri et al., 2004; Barret et al., 1982), including evidence of a correlation with duration of exposure and increased latency in one study (Mhiri et al., 2004). Ruitjen et al. (1991) reported no significant change in the blink reflex, but did report an increase in the latency of the masseter reflex, which also may reflect effects on the trigeminal nerve. Two other studies reported no observed effect on trigeminal nerve impairment, but the authors failed to provide assessment of trigeminal nerve function (El-Ghawabi et al., 1973, Rasmussen et al., 1993c) or there was not a control (nonexposed) group included in the study (Rasmussen et al., 1993c). Therefore, because of limitations in statistical power, the possibility of exposure misclassification, and possible differences in measurement methods, these studies are not judged to provide substantial evidence against a causal relationship between TCE exposure and trigeminal nerve impairment. Overall, the weight of evidence supports a relationship between TCE exposure and trigeminal nerve dysfunction in humans.

Impairment of trigeminal nerve function is observed in studies of laboratory animal studies. Although one subchronic animal study demonstrated no significant impairment of trigeminal nerve function following TCE exposure up to 2,500 ppm (no-observed-adverse-effect level [NOAEL]; Albee et al., 2006), morphological analysis of the nerve revealed changes in its structure (Barret et al., 1991, 1992). However, the dose at which an effect was observed by Barret et al. (1991, 1992) was high (2,500 mg/kg/d—lowest-observed-adverse-effect level

[LOAEL]) compared to any reasonable occupational or environmental setting, although no lower doses were used. The acute or subchronic duration of these studies, as compared to the much longer exposure duration in many of the human studies, may also contribute to the apparent disparity between the epidemiologic and (limited) laboratory animal data.

The subchronic study of Barret et al. (1992) and the acute exposure study of Albee et al. (1997) also demonstrated that dichloroacetylene, a (*ex vivo*) TCE degradation product, also induces trigeminal nerve impairment, at much lower doses than TCE. It is possible that under some conditions, coexposure to dichloroacetylene from TCE degradation may contribute to the changes observed to be associated with TCE exposure in human studies, and this issue is discussed further below in Section 4.3.10.

Overall evidence from numerous epidemiologic studies supports a conclusion that TCE exposure induces trigeminal nerve impairment in humans. Laboratory animal studies provide limited additional support, and do not provide strong contradictory evidence. Persistence of these effects after cessation of exposure cannot be determined since exposure was ongoing in the available human and laboratory animal studies.

4.3.2. Auditory Effects

4.3.2.1. *Auditory Function: Human Studies*

The TCE Subregistry from the National Exposure Registry developed by the ATSDR was the subject of three studies (Burg et al., 1995, 1999; ATSDR, 2003). A fourth study (Rasmussen et al., 1993c) of degreasing workers exposed to either TCE or CFC113 also indirectly evaluated auditory function. These studies are discussed below and presented in detail in Table 4-22.

Burg et al. (1995, 1999) reviewed the effects of TCE on 4,281 individuals (TCE Subregistry) residentially exposed to this solvent for more than 30 consecutive days. Face-to-face interviews were conducted with the TCE subregistry population and self-reported hearing loss was evaluated based on personal assessment through the interview (no clinical evaluation was conducted). TCE registrants that were 9 years old or younger had a statistically significant increase in hearing impairment as reported by the subjects. The relative risk (RR) in this age group for hearing impairments was 2.13 (95% confidence interval [CI]: 1.12−4.06) which decreased to 1.12 (95% CI: 0.52−2.24) for the 10−17 age group and 0.32 (95% CI: 0.10−1.02) for all older age groups. A statistically significant association (when adjusted for age and sex) was found between duration of exposure, in these studies this was length of residency, and reported hearing impairment. The odds ratio (OR) was 2.32 (95% CI: 1.18−4.56) for subjects exposed to TCE >2 years and ≤5 years, 1.17 (95% CI: 0.55−2.49) for exposure >5 years and ≤10 years, 2.46 (95% CI: 1.30−5.02) for exposure durations greater than 10 years.

1 **Table 4-22. Summary of human auditory function studies**

2

Reference	Subjects	Exposure	Effect
ATSDR, 2003	116 children, under 10 yrs of age, residing near 6 Superfund sites. Further study of children in Burg et al. (1995, 1999) Control: 182 children	TCE and other solvents in ground water supplies. Exposures were modeled using tap water TCE concentrations and GIS for spatial interpolation, and LaGrange for temporal interpolation to estimate exposures from gestation to 1990 across the area of subject residences. Control = 0 ppb; low exposure group = 0 < 23 ppb-yrs; and high exposure group = >23 ppb-yrs	Auditory screening revealed increased incidence of abnormal middle ear function in exposed groups as indicated from acoustic reflex test. Adjusted odds ratios for right ear ipsilateral acoustic reflects control, OR: 1.0, low exposure group, OR: 5.1, $p < 0.05$; high exposure group, OR: 7.2, $p < 0.05$. ORs adjusted for age, sex, medical history and other chemical contaminants. No significant decrements reported in the pure tone and typanometry screening.
Burg et al., 1995	From an NHIS TCE subregistry of 4,281 (4,041 living and 240 deceased) residents	Environmentally exposed to TCE and other solvents via well water in Indiana, Illinois, and Michigan	Increase in self-reported hearing impairments for children ≤9 yrs.
Burg et al., 1999	3,915 white registrants Mean age 34 yrs (SD = 19.9 yrs)	Cumulative TCE exposure subgroups: <50 ppb, n = 2,867; 50−500 ppb, n = 870; 500−5,000 ppb, n = 190; >5,000 ppb, n = 35 Exposure duration subgroups: <2 yrs, 2−5 yrs, 5−10 yrs., >10 yrs	A statistically significant association (adjusted for age and sex) between duration of exposure and self-reported hearing impairment was found.
Rasmussen et al., 1993b	96 Danish metal degreasers. Age range: 19−68 yrs; No unexposed controls; low exposed group is referent	Average exposure duration: 7.1 yrs.); range of full-time degreasing: 1 month to 36 yrs. Exposure to TCE or and CFC113 (1) Low exposure: n = 19, average full-time exposure 0.5 yrs (2) Medium exposure: n = 36, average full-time exposure 2.1 yrs (3) High exposure: n = 41, average full-time exposure 11 yrs. Mean U-TCA in high exposure group = 7.7 mg/L (max = 26.1 mg/L);	Auditory impairments noted through several neurological tests. Significant relationship of exposure was found with Acoustic-motor function ($p < 0.001$), Paced Auditory Serial Addition Test ($p < 0.001$), Rey Auditory Verbal Learning Test ($p < 0.001$).

3 NHIS = National Health Interview Survey, U-TCA = urinary trichloroacetic acid.

ATSDR (2003) conducted a follow-up study to the TCE subregistry findings (Burg et al., 1995, 1999) and focused on the subregistry children located in Elkhart, IN, Rockford, IL and Battle Creek, MI using clinical tests for oral motor, speech, and hearing function. Exposures were modeled using tap water TCE concentrations and geographic information system (GIS) for spatial interpolation, and LaGrange for temporal interpolation to estimate exposures from gestation to 1990 across the area of subject residences. Modeled data were used to estimate lifetime exposures (ppb-years) to TCE in residential wells. The median TCE exposure for the children was estimated from drinking water as 23 ppb/year of exposure (ranging from 0−702 ppb/year). Approximately 20% (ranged from 17−21% depending on ipsilateral or contralateral test reflex) of the children in the TCE subregistry and 5−7% in the control group exhibited an abnormal acoustic reflex (involuntary muscle contraction that measures movement of the stapedius muscle in the middle ear following a noise stimulus) which was statistically significant ($p = 0.003$). Abnormalities in this reflex could be an early indicator of more serious hearing impairments. No significant decrements were reported in the pure tone and typanometry screening.

Rasmussen et al. (1993b) used a psychometric test to measure potential auditory effects of TCE exposure in an occupational study. Results from 96 workers exposed to TCE and other solvents were presented in this study. Details of the exposure groups and exposure levels are provided in Table 4-22. The acoustic motor function test was used for evaluation of auditory function. Significant decrements ($p < 0.05$) in acoustic motor function performance scores (average decrement of 2.5 points on a 10-point scale) was reported for TCE exposure.

4.3.2.2. *Auditory Function: Laboratory Animal Studies*

The ability of TCE to permanently disrupt auditory function and produce abnormalities in inner ear histopathology has been demonstrated in several studies using a variety of test methods. Two different laboratories have identified NOAELs following inhalation exposure for auditory function of 1,600 ppm for 12 hours/day for 13 weeks in Long Evans rats ($n = 6−10$) (Rebert et al., 1991) and 1,500 ppm for 18 hours/day, 5 days/week for 3 weeks in Wistar-derived rats ($n = 12$) (Jaspers et al., 1993). The LOAELs identified in these and similar studies are 2,500−4,000 ppm TCE for periods of exposure ranging from 4 hours/day for 5 days to 12 hours/day for 13 weeks (e.g., Muijser et al., 2000; Rebert et al., 1995, 1993; Crofton et al., 1994; Crofton and Zhao, 1997; Fechter et al., 1998; Boyes et al., 2000; Albee et al., 2006). Rebert et al. (1993) estimated acute blood TCE levels associated with permanent hearing impairment at 125 μg/mL by methods that probably underestimated blood TCE values (rats were

anaesthetized using 60% carbon dioxide [CO_2]). A summary of these studies is presented in Table 4-23.

Reflex modification was used in several studies to evaluate the auditory function in TCE-exposed animals (Jaspers et al., 1993; Muijser et al., 2000; Fechter et al., 1998; Crofton and Zhao, 1993; Crofton et al., 1994; Crofton and Zhou, 1997; Boyes et al., 2000; Yamamura et al., 1983). These studies collectively demonstrate significant decreases in auditory function at mid-frequency tones (8−20 kHz tones) for TCE exposures greater than 1,500 ppm after acute, short-term, and chronic durations. Only one study (Yamamura et al., 1983) did not demonstrate impairment in auditory function from TCE exposures as high as 17,000 ppm for 4 hours/day over 5 days. This was the only study to evaluate auditory function in guinea pigs, whereas the other studies used various strains of rats. Despite the negative finding in Yamamura et al. (1983), auditory testing was not performed in an audiometric sound attenuating chamber and extraneous noise could have influenced the outcome. It is also important to note that the guinea pig has been reported to be far less sensitive than the rat to the effects of ototoxic aromatic hydrocarbons such as toluene.

Crofton and Zhao (1997) also presented a benchmark dose for which the calculated dose of TCE would yield a 15 dB loss in auditory threshold. This benchmark response was selected because a 15 dB threshold shift represents a significant loss in threshold sensitivity for humans. The benchmark concentrations for a 15 dB threshold shift are 5,223 ppm for 1 day, 2,108 ppm for 5 days, 1,418 ppm for 20 days and 1,707 ppm for 65 days of exposure. While more sensitive test methods might be used and other definitions of a benchmark effect chosen with a strong rationale, these data provide useful guidance for exposure concentrations that do yield hearing loss in rats.

1 **Table 4-23. Summary of animal auditory function studies**
2

Reference	Exposure route	Species/strain/ sex/number	Dose level/ exposure duration	NOAEL; LOAEL [a]	Effects
Rebert et al., 1991	Inhalation	Rat, Long Evans, male, 10/group	Long Evans: 0, 1,600, and 3,200 ppm; 12 h/d, 12 wks	Long Evans: NOAEL: 1,600 ppm; LOAEL: 3,200 ppm	BAERs were measured. Significant decreases in BAER amplitude and an increase in latency of appearance of the initial peak (P1).
		Rat, F344, male, 4−5/group	F344: 0, 2,000, 3,200 ppm; 12 h/d, 3 wks	F344: LOAEL: 2,000 ppm	
Rebert et al., 1993	Inhalation	Rat, Long Evans, male, 9/group	0, 2,500, 3,000, 3,500 ppm; 8 h/d, 5 d	NOAEL: 2,500 ppm LOAEL: 3,000 ppm	BAERs were measured 1−2 wks postexposure to assess auditory function. Significant decreases in BAERs were noted with TCE exposure.
Rebert et al., 1995	Inhalation	Rat, Long Evans, male, 9/group	0, 2,800 ppm; 8 h/d, 5 d	LOAEL: 2,800 ppm	BAER measured 2−14 days postexposure at a 16 kHz tone. Hearing loss ranged from 55−85 dB.
Crofton et al., 1994	Inhalation	Rat, Long Evans, male, 7−8/group	0, 3,500 ppm TCE; 8 h/d, 5 d	LOAEL: 3,500 ppm	BAER measured and auditory thresholds determined 5−8 wks postexposure. Selective impairment of auditory function for mid-frequency tones (8 and 16 kHz).
Crofton and Zhou, 1997; Boyes et al., 2000	Inhalation	Rat, Long Evans, male, 9−12/group	0, 4,000, 6,000, 8,000 ppm; 6 h	NOAEL: 6,000 ppm LOAEL: 8,000 ppm	Auditory thresholds as measured by BAERs for the 16 kHz tone increased with TCE exposure. Measured 3−5 wks post exposure.
		Rat, Long Evans, male, 8−10/group	0, 1,600, 2,400, and 3,200 ppm; 6 h/d, 5 d	NOAEL: 2,400 ppm LOAEL: 3,200 ppm	
		Rat, Long Evans, male, 8−10/group	0, 800, 1,600, 2,400, and 3,200 ppm; 6 h/d, 5 d/wk, 4 wks	NOAEL: 2,400 ppm LOAEL: 3,200 ppm	
		Rat, Long Evans, male, 8−10/group	0, 800, 1,600, 2,400, and 3,200 ppm; 6 h/d, 5 d/wk, 13 wks	NOAEL: 1,600 ppm LOAEL: 2,400 ppm	

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Table 4-23. Summary of animal auditory function studies (continued)

Reference	Exposure route	Species/strain/ sex/number	Dose level/ exposure duration	NOAEL; LOAEL	Effects
Fechter et al., 1998	Inhalation	Rat, Long Evans, male, 12/group	0, 4,000 ppm; 6 h/d, 5 d	LOAEL: 4,000 ppm	Cochlear function measured 5−7 wks after exposure. Loss of spiral ganglion cells noted. Three wks postexposure, auditory function was significantly decreased as measured by compound action potentials and reflex modification.
Jaspers et al., 1993	Inhalation	Rat, Wistar derived WAG-Rii/MBL, male, 12/group	0, 1,500, and 3,000 ppm; 18 h/d, 5 d/wk, 3 wks	NOAEL: 1,500 ppm	Auditory function assessed repeatedly 1−5 wks postexposure for 5, 20, and 35 kHz tones; no effect at 5 or 35 kHz; decreased auditory sensitivity at 20 kHz, 3,000 ppm.
Muijser et al., 2000	Inhalation	Rat, Wistar derived WAG-Rii/MBL, male, 8	0, 3,000 ppm; 18 h/d, 5 d/wk, 3 wks	LOAEL: 3,000 ppm	Auditory sensitivity decreased with TCE exposure at 4, 8, 16, and 20 kHz tones. White noise potentiated the decrease in auditory sensitivity.
Albee et al., 2006	Inhalation	Rat, Fischer 344, male and female, 10/sex/group	0, 250, 800, 2,500 ppm; 6 h/d, 5 d/wk, 13 wks	NOAEL: 800 ppm LOAEL: 2,500 ppm	Mild frequency specific hearing deficits; focal loss of cochlear hair cells.
Yamamura et al., 1983	Inhalation	Guinea Pig, albino Hartley, male, 7−10/group	0, 6,000, 12,000, 17,000 ppm; 4 h/d, 5 d	NOAEL: 17,000 ppm	No change in auditory sensitivity at any exposure level as measured by cochlear action potentials and microphonics. Study was conducted in guinea pig and species is less sensitive to auditory toxicity than rats. Studies were also not conducted in a sound-isolation chamber and effects may be impacted by background noise.

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4 Brainstem auditory-evoked potentials (BAERs) were also measured in several studies
5 (Rebert et al., 1991, 1993, 1995; Albee et al., 2006) following at exposures ranging from
6 3−13 weeks. Rebert et al. (1991) measured BAERs in male Long Evans rats (*n* = 10) and F344
7 rats (*n* = 4−5) following stimulation with 4, 8, and 16 kHz sounds. The Long-Evans rats were
8 exposed to 0, 1,600, or 3,200 ppm TCE, 12 hours/day for twelve weeks and the F344 rats were

exposed to 0, 2,000, or 3,200 ppm TCE, 12 hours/day for three weeks. BAER amplitudes were significantly decreased at all frequencies for F344 rats exposed to 2,000 and 3,000 ppm TCE and for Long Evans rats exposed to 3,200 ppm TCE. These data identify a LOAEL at 2,000 ppm for the F344 rats and a NOAEL at 1,600 ppm for the Long Evans rats. In subsequent studies Rebert et al. (1993, 1995) again demonstrated TCE significantly decreases BAER amplitudes and also significantly increases the latency of appearance. Similar results were obtained by Albee et al. (2006) for male and female F344 rats exposed to TCE for 13 weeks. The NOAEL for this study was 800 ppm based on ototoxicity at 2,500 ppm.

Notable physiological changes were also reported in a few auditory studies. Histological data from cochleas in Long-Evans rats exposed to 4,000 ppm TCE indicated that there was a loss in spiral ganglion cells (Fechter et al., 1998). Similarly, there was an observed loss in hair cells in the upper basal turn of the cochlea in F344 rats exposed to 2,500-ppm TCE (Albee et al., 2006).

4.3.2.3. *Summary and Conclusion of Auditory Effects*

Human and animal studies indicated that TCE produces decrements in auditory function. In the human epidemiological studies (ATSDR, 2003; Burg et al., 1995, 1999; Rasmussen et al., 1993c) it is suggested that auditory impairments result from both an inhalation and oral TCE exposure. A LOAEL of approximately 23 ppb-years TCE (extrapolated from ≤23 ppb-years group in the ATSDR, 2003) from oral intake is noted for auditory effects in children. The only occupational study where auditory effects were seen reported mean urinary trichloroacetic acid concentration, a nonspecific metabolite of TCE, of 7.7 mg/L for the high cumulative exposure group only (Rasmussen et al., 1993c). A NOAEL or a LOAEL for auditory changes resulting from inhalational exposure to TCE cannot be interpolated from average urinary trichloroacetic acid (U-TCA) concentration of subjects in the high exposure group because of a lack of detailed information on long-term exposure levels and duration (Rasmussen et al., 1993c). Two studies (Burg et al., 1995, 1999) evaluated self-reported hearing effects in people included in the TCE subregistry comprised of people residing near Superfund sites in Indiana, Illinois, and Michigan. In Burg et al. (1995), interviews were conducted with the TCE exposed population and it was found that children aged 9 years or younger had statistically significant hearing impairments in comparison to nonexposed children. This significant increase in hearing impairment was not observed in any other age group that was included in this epidemiological analysis. This lack of effect in other age groups may suggest association with another exposure other than drinking water ; however, it may also suggest that children may be more susceptible than adults. In a follow-up analysis, Burg et al. (1999) adjusted the statistical analysis of the original data (Burg et

al., 1995) for age and sex. When these adjustments were made, a statistically significant association was reported self-reported for auditory impairment and duration of residence. These epidemiological studies provided only limited information given their use of an indirect exposure metric of residence location, no auditory testing of this studied population and self-reporting of effects. ATSDR (2003) further tested the findings in the Burg studies (Burg et al., 1995, 1999) by contacting the children that were classified as having hearing impairments in the earlier study and conducting several follow-up auditory tests. Significant abnormalities were reported for the children in the acoustic reflex test which suggested effects to the lower brainstem auditory pathway with the large effect measure, the odds ratio, was reported for the high cumulative exposure group. Strength of analyses was its adjustment for potential confounding effects of age, sex, medical history and other chemical contaminants in drinking water supplies. The ATSDR findings were important in that the results supported Burg et al. (1995, 1999). Rasmussen et al. (1993b) also evaluated auditory function in metal workers with inhalation exposure to either TCE or CFC113. Results from tasks including an auditory element suggested that these workers may have some auditory impairment. However, the tasks did not directly measure auditory function.

Animals strongly indicated that TCE produces deficits in hearing and provides biological context to the epidemiological study observations. Although there is a strong association between TCE and ototoxicity in the animal studies, most of the effects began to occur at higher inhalation exposures. NOAELs for ototoxicity ranged from 800−1,600 ppm for exposure durations of at least 12 weeks (Albee et al., 2006; Crofton and Zhou, 1997; Boyes et al., 2000; Rebert et al., 1991). Inhalation exposure to TCE was the route of administration in all the animal studies. These studies either used reflex modification audiometry (Jaspers et al., 1993; Crofton et al., 1994; Crofton and Zhou, 1997; Muijser et al., 2000) procedures or measured brainstem auditory evoked potentials (Rebert et al., 1991, 1993, 1995) to evaluate hearing in rats. Collectively, the animal database demonstrates that TCE produces ototoxicity at mid-frequency tones (4−24 kHz) and no observed changes in auditory function were observed at either the low (<4 kHz) or high (>24 kHz) frequency tones. Additionally, deficits in auditory effects were found to persist for at least 7 weeks after the cessation of TCE exposure (Rebert et al., 1991; Jaspers et al., 1993; Crofton and Zhou, 1997; Fechter et al., 1998; Boyes et al., 2000). Decreased amplitude and latency were noted in the BAERs (Rebert et al., 1991, 1993, 1995) suggesting that TCE exposure affects central auditory processes. Decrements in auditory function following reflex modification audiometry (Jaspers et al., 1993; Crofton et al., 1994; Crofton and Zhou, 1997; Muijser et al., 2000) combined with changes observed in cochlear histopathology (Fechter

1 et al., 1998; Albee et al., 2006) suggest that ototoxicity is occurring at the level of the cochlea
2 and/or brainstem.
3

4 4.3.3. Vestibular Function

5 4.3.3.1. *Vestibular Function: Human Studies*

6 The earliest reports of neurological effects resulting from TCE exposures focused on
7 subjective vestibular system symptoms, such as headaches, dizziness, and nausea. These
8 symptoms are subjective and self-reported. However, as they have been reported extensively in
9 the literature, there is little doubt that these effects can be caused by exposures to TCE.,
10 occupational exposures (Grandjean et al., 1955; Liu et al., 1988; Rasmussen et al., 1986; Smith
11 et al., 1970), environmental exposures (Hirsch et al., 1996), and in chamber studies (Stewart et
12 al., 1970; Smith et al., 1970).
13 Kylin et al. (1967) exposed 12 volunteers to 1,000 ppm (5,500 mg/m^3) TCE for two hours
14 in a 1.5 × 2 × 2 meters chamber. Volunteers served as their own controls since 7 of the 12 were
15 pretested prior to exposure and the remaining 5 were post-tested days after exposure. Subjects
16 were tested for optokinetic nystagmus, which was recorded by electronystogmography, that is,
17 "the potential difference produced by eye movements between electrodes placed in lateral angles
18 between the eyes." Venous blood was also taken from the volunteers to measure blood TCE
19 levels during the vestibular task. The authors concluded that there was an overall reduction in
20 the limit ("fusion limit") to reach optokinetic nystagmus when individuals were exposed to TCE.
21 Reduction of the "fusion limit" persisted for up to 2 hours after the TCE exposure was stopped
22 and the blood TCE concentration was 0.2 mg/100 mL.
23

24 4.3.3.2. *Vestibular Function: Laboratory Animal Data*

25 The effect of TCE on vestibular function was evaluated by either (1) promoting
26 nystagmus (vestibular system dysfunction) and comparing the level of effort required to achieve
27 nystagmus in the presence and absence of TCE or (2) using an elevated beam apparatus and
28 measuring the balance. Overall, it was found that TCE disrupts vestibular function as presented
29 below and summarized in Table 4-24.

Table 4-24. Summary of mammalian sensory studies—vestibular and visual systems

Reference	Exposure route	Species/strain/ sex/number	Dose level/ exposure duration	NOAEL; LOAEL	Effects
Vestibular system studies					
Tham et al., 1979	Intravenous	Rabbit, strain unknown, sex unspecified, 19	1−5 mg/kg/min	---	Positional nystagmus developed once blood levels reached 30 ppm.
Tham et al., 1984	Intravenous	Rat, Sprague-Dawley, female, 11	80 μg/kg/min	---	Excitatory effects on the vestibule-oculomotor reflex. Threshold effect at blood (TCE) of 120 ppm or 0.9 mM/L.
Niklasson et al., 1993	Inhalation	Rat, strain unknown, male and female, 28	0, 2,700, 4,200, 6,000, 7,200 ppm; 1 h	LOAEL: 2,700 ppm	Increased ability to produce nystagmus.
Umezu et al., 1997	Intraperitoneal	Mouse, ICR, male, 116	0, 250, 500, or 1,000 mg/kg, single dose and evaluated 30 min postadministration	NOAEL: 250 mg/kg LOAEL: 500 mg/kg	Decreased equilibrium and coordination as measured by the Bridge test (staying time on an elevated balance beam).

Niklasson et al. (1993) showed acute impairment of vestibular function in male- and female-pigmented rats during acute inhalation exposure to TCE (2,700−7,200 ppm) and to tricholoroethane (500−2,000 ppm). Both of these agents were able to promote nystagmus during optokinetic stimulation in a dose related manner. While there were no tests performed to assess persistence of these effects, Tham et al. (1979, 1984) did find complete recovery of vestibular function in rabbits (*n* = 19) and female Sprague-Dawley rats (*n* = 11) within minutes of terminating a direct arterial infusion with TCE solution.

The finding that trichloroethylene can yield transient abnormalities in vestibular function is not unique. Similar impairments have also been shown for toluene, styrene, along with trichloroethane (Niklasson et al., 1993) and by Tham et al. (1984) for a broad range of aromatic hydrocarbons. The concentration of TCE in blood at which effects were observed for TCE (0.9 mM/L) was quite close to that observed for most of these other vestibulo-active solvents.

4.3.3.3. *Summary and Conclusions for the Vestibular Function Studies*

Studies of TCE exposure in both humans and animals reported abnormalities in vestibular function. Headaches, dizziness, nausea, motor incoordination, among other subjective symptoms are reported in occupational epidemiological studies of TCE exposure (Grandjean et al., 1955; Liu et al., 1988; Rasmussen et al., 1986; Smith et al., 1970; Hirsch et al., 1996; Stewart et al.,

1970). One human exposure study (Kylin et al., 1967) found that vestibular function was affected following an acute exposure to 1,000-ppm TCE (LOAEL). Individuals had a decreased threshold to reach nystagmus than when exposed to TCE than to air. Animal studies also evaluated the threshold to reach nystagmus and reported that TCE decreased the threshold to produce nystagmus in rats (LOAEL: 2,700 ppm; Tham et al., 1984; Niklasson et al., 1993) and rabbits (Tham et al., 1983).

4.3.4. Visual Effects

4.3.4.1. *Visual Effects: Human Studies*

Visual impairment in humans has been demonstrated following exposures through groundwater (Kilburn, 2002a; Reif et al., 2003), from occupational exposure through inhalation (Rasmussen et al., 1993b; Troster and Ruff, 1990) and from a controlled inhalation exposure study (Vernon and Ferguson, 1969). Visual functions such as color discrimination and visuospatial learning tasks are impaired in TCE-exposed individuals. Additionally, an acute exposure can impair visual depth perception. Details of the studies are provided below and summarized in Table 4-25.

Geographical-based studies utilized color discrimination and contrast sensitivity tests to determine the effect of TCE exposure on vision. In these studies it was reported that TCE exposure significantly increased color discrimination errors (Kilburn, 2002a) or decreases in contrast sensitivity tests approached statistical significance after adjustments for several possible confounders ($p = 0.06$ or 0.07; Reif et al., 2003). Exposure in Kilburn (2002a) is poorly characterized, and for both studies, TCE is one of several contaminants in drinking water supplies; neither study provides an estimate of an individual’s exposure to TCE.

Rasmussen et al. (1993b) evaluated visual function in 96 metal workers, working in degreasing at various factories and with exposure to TCE or CFC113. Visual function was tested through the visual gestalts test (visual perception) and a visual recall test. In the visual gestalts test, the number of total errors significantly increased from the low group (3.4 errors) to the high exposure group (6.5 errors; $p = 0.01$). No significant changes were observed in the visual recall task. Troster and Ruff (1990) presented case studies conducted on two occupationally exposed workers to TCE. Both patients presented with a visual-spatial task and neither could complete the task within the number of trials allowed suggesting visual function deficits as a measure of impaired visuospatial learning.

1 **Table 4-25. Summary of human visual function studies**
2

Reference	Subjects	Exposure	Effect
Kilburn, 2002a	236 residents near a microchip plant in Phoenix, AZ Controls: 67 local referents from Phoenix, AZ and 161 regional referents from Wickenburg, AZ	TCE, TCA, 1,1-DCE, 1,2-DCE, PCE, and VC detected in well water up to 260,000 ppm; TCE concentrations in well water were 0.2−10,000 ppb. Exposure duration ranged from 2−37 yrs. Exposure duration ranged from 2 to 37 yrs.	Color discrimination errors were increased among residents compared to regional referents ($p < 0.01$). No adjustment for possible confounding factors.
Reif et al., 2003	143 residents of the Rocky Mountain Arsenal community of Denver Referent group at lowest concentration (<5 ppb).	Exposure modeling of TCE concentrations in groundwater and in distribution system to estimate mean TCE concentration by census block of residence. High exposure group >15 ppb. Medium exposure group ≥5 ppb and ≤15 ppb. Low exposure referent group <5 ppb.	Contrast sensitivity test performances (C and D) was marginally statistically significant (p = 0.06 and 0.07, respectively). No significant effects reported for the Benton visual retention test. Significant decrements (p = 0.02) were reported in the Benton visual retention test when stratified with alcohol consumption.
Rasmussen et al., 1993b	96 Danish metal degreasers. Age range: 19−68; no unexposed controls; low exposure group was referent	Average exposure duration: 7.1 yrs); range of full-time degreasing: 1 month to 36 yrs. Exposure to TCE or CFC113. 1) Low exposure: n = 19, average full-time expo 0.5 yrs. 2) Medium exposure: n = 36, average full-time exposure 2.1 yrs. 3) high exposure: n = 41, average full-time exposure 11 yrs. TCA in high exposure group = 7.7 mg/L (max = 26.1 mg/L).	Statistically significant relationship of exposure was found with the Visual Gestalts learning and retention test (cognitive test) indicating deficits in visual performance.
Troster and Ruff, 1990	2 occupationally TCE-exposed workers Controls: 2 groups of n = 30 matched controls; (all age and education matched)	Exposure concentration unknown Exposure duration, 3−8 months.	Both workers experienced impaired visuospatial learning.
Vernon and Ferguson, 1969	8 male volunteers age range 21−30; self controls	0, 100, 300, and 1,000 ppm of TCE for 2 h.	Statistically significant effects on visual depth perception as measured by the Howard-Dolman test. NOAEL: 300 ppm; LOAEL: 1,000 ppm; No significant changes in any of the other visual test measurements.

3
4 DCE = dichloroethylene.
5

In a chamber exposure study (Vernon and Ferguson, 1969), eight male volunteers (ages 21−30) were exposed to 0, 100, 300, and 1,000-ppm TCE for 2 hours. Each individual was exposed to all TCE concentrations and a span of at least three days was given between exposures. When the individuals were exposed to 1,000-ppm TCE (5,500 mg/m^3), significant abnormalities were noted in depth perception as measured by the Howard-Dolman test ($p < 0.01$). There were no effects on the flicker fusion frequency test (threshold frequency at which the individual sees a flicker as a single beam of light) or on the form perception illusion test (volunteers presented with an illusion diagram).

4.3.4.2. *Visual Effects: Laboratory Animal Data*

Changes in visual function have been demonstrated in animal studies during acute (Boyes et al., 2003, 2005) and subchronic exposure (Rebert et al., 1991; Blain et al., 1994). In these studies, the effect of TCE on visual evoked responses to patterns (Boyes et al., 2003, 2005; Rebert et al., 1991) or a flash stimulus (Rebert et al., 1991; Blain et al., 1994) were evaluated. Overall, the studies demonstrated that exposure to TCE results in significant changes in the visual evoked response, which is reversible once TCE exposure is stopped. Details of the studies are provided below and are summarized in Table 4-26.

Boyes et al. (2003, 2005) exposed adult, male Long-Evans rats were to TCE in a head-only exposure chamber while pattern onset/offset visual evoked potentials (VEPs) were recorded. Exposure conditions were designed to provide concentration × time products of 0 ppm/hours (0 ppm for 4 hours) or 4,000 ppm/hours (see Table 4-26 for more details). VEP amplitudes were depressed by TCE exposure during the course of TCE exposure. The degree of VEP depression showed a high correlation with the estimated brain TCE concentration for all levels of atmospheric TCE exposure.

In a subchronic exposure study, Rebert et al. (1991) exposed male Long Evans rats to 1,600- or 3,200-ppm TCE, for 12 weeks, 12 hours/day. No significant changes in flash evoked potential measurements were reported following this exposure paradigm. Decreases in pattern reversal visual evoked potentials (N1P1 amplitude) reached statistical significance following 6, 9, and 12 weeks of exposure. The drop in response amplitude ranged from approximately 20% after 8 weeks to nearly 50% at Week 14 but recovered completely within 1 week postexposure.

Table 4-26. Summary of animal visual system studies

Reference	Exposure route	Species/strain/ sex/number	Dose level/ exposure duration	NOAEL; LOAEL	Effects
Rebert et al., 1991	Inhalation	Rat, Long Evans, male, 10/group	0, 1,600, and 3,200 ppm; 12 h/d, 12 wks	NOAEL: 1,600 ppm	Significant amplitude decreases in pattern reversal evoked potentials (N1P1 amplitude) at 6, 9, and 12 wks.
Boyes et al., 2003	Inhalation	Rat, Long Evans, male, 9−10/group	0 ppm, 4 h; 1,000 ppm, 4; 2,000 ppm, 2 h; 3,000 ppm, 1.3 h 4,000 ppm, 1 h	LOAEL: 1,000 ppm, 4 h	Visual function significantly affected as measured by decreased amplitude (F2) in Fourier-transformed visual evoked potentials. Peak brain TCE concentration correlated with dose response.
Boyes et al., 2005	Inhalation	Rat, Long Evans, male, 8−10/group	0 ppm, 4 h; 500 ppm, 4 h; 1,000 ppm, 4 h; 2,000 ppm, 2 h; 3,000 ppm, 1.3 h 4,000 ppm, 1 h; 5,000 ppm, 0.8 h	LOAEL: 500 ppm, 4 h	Visual function significantly affected as measured by decreased amplitude (F2) in Fourier-transformed visual evoked potentials. Peak brain TCE concentration correlated with dose response.
Blain et al., 1994	Inhalation	Rabbit, New Zealand albino, male, 6−8/group	0, 350, 700 ppm; 4 h/d, 4 d/wk, 12 wks	LOAEL: 350 ppm	Significant effects noted in visual function as measured by ERG and OPs immediately after exposure. No differences in ERG or OP measurements were noted at 6 wks post-TCE exposure.

This transient effect of TCE on the peripheral visual system has also been reported by Blain (1994) in which New Zealand albino rabbits were exposed by inhalation to 350- and 700-ppm TCE 4 hours/day, 4 days/week for 12 weeks. Electroretinograms (ERG) and oscillatory potentials (OPs) were recorded weekly under mesopic conditions. Recordings from the 350- and 700-ppm exposed groups showed a significant increase in the amplitude of the a- and b-waves (ERG). The amplitude of the OPs was significantly decreased at 350 ppm (57%) and increased at 700 ppm (117%). These electroretinal changes returned to preexposure conditions within six weeks after the inhalation stopped.

4.3.4.3. *Summary and Conclusion of Visual Effects*

Changes in visual function are reported in human studies. Although central visual function was not evaluated in the human studies (such as electroretinograms, evoked potential measurements), clinical tests indicated deficits in color discrimination (Kilburn, 2002a), visual depth perception (Vernon and Ferguson, 1969) and contrast sensitivity (Reif et al., 2003). These changes in visual function were observed following both an acute exposure (Vernon and Ferguson, 1969) and residence in areas with groundwater contamination with TCE and other chemicals (Kilburn, 2002a; Reif et al., 2003). The exposure assessment approach of Reif et al., who adopted exposure modeling and information on water distribution patterns, is considered superior to that of Kilburn (2002a) who used residence location as a surrogate for exposure. In the one acute, inhalation study (Vernon and Ferguson, 1969), a NOAEL of 300 ppm and a LOAEL of 1,000 ppm for 2 hours was reported for visual effects. A NOAEL is not available from the drinking water studies since well water TCE concentration is a poor surrogate for an individual's TCE ingestion (Kilburn, 2002a) and limited statistical analysis comparing high exposure group to low exposure group (Reif et al., 2003).

Animal studies have also demonstrated changes in visual function. All of the studies evaluated central visual function by measuring changes in evoked potential response following a visual stimulus that was presented to the animal. Two acute exposure inhalation studies (Boyes et al., 2003, 2005) exposed Long Evans rats to TCE based on a concentration × time schedule (Haber's law) and reported decreases in visual evoked potential amplitude. All of the exposures from these two studies resulted in decreased visual function with a LOAEL of 500 ppm for 4 hours. Another important finding that was noted is the selection of the appropriate dose metric for visual function changes following an acute exposure. Boyes et al. (2003, 2005) found that among other potential dose metrics, brain TCE concentration was best correlated with changes in visual function as measured by evoked potentials under acute exposure conditions. Two subchronic exposure studies (Rebert et al., 1991; Blain et al., 1994) demonstrated visual function changes as measured by pattern reversal evoked potentials (Rebert et al., 1991) or electroretinograms/oscillatory potentials (Blain et al., 1994). Unlike the other three visual function studies conducted with rats, Blain et al. demonstrated these changes in rabbits. Significant changes in ERGs and oscillatory potentials were noted following a 12-week exposure at 350 ppm (LOAEL) in rabbits (Blain et al., 1994) and in rats exposed to 3,200-ppm TCE for 12 weeks there were significant decreases in pattern reversal evoked potentials but no effect was noted in the 1,600-ppm exposure group (Rebert et al., 1991). Both subchronic studies examined visual function following an exposure-free period of either 2 weeks (Rebert et al., 1991) or 6 weeks (Blain et al., 1994) and found that visual function returned to pre-exposure levels and the changes are reversible.

4.3.5. Cognitive Function

4.3.5.1. *Cognitive Effects: Human Studies*

Effects of TCE on learning and memory have been evaluated in populations environmentally exposed to TCE through well water, in workers occupationally exposed through inhalation and under controlled exposure scenarios. Details of the studies are provided in Table 4-27 and discussed briefly below. In the geographical-based studies (Kilburn and Warshaw, 1993; Kilburn, 2002a), cognitive function was impaired in both studies and was evaluated by testing verbal recall and digit span memory among other measures. In Arizona residents involved in a lawsuit (Kilburn and Warshaw, 1993), significant impairments in all three cognitive measures were reported; verbal recall ($p = 0.001$), visual recall ($p = 0.03$) and digit span test ($p = 0.07$), although a question exists whether the referent group was comparable to exposed subjects and the study's lack of consideration of possible confounding exposures in statistical analyses. Significant decreases in verbal recall ability was also reported in another environmental exposure study where 236 residents near a microchip plant with TCE concentration in well water ranging from 0.2−10,000 ppb (Kilburn, 2002a).

Cognitive impairments are assessed in the occupational exposure and case studies (Rasmussen, 1993a, b; Troster and Ruff, 1990). In metal degreasers occupationally exposed to TCE and CFC113, significant cognitive performance decreases were noted in verbal recall testing ($p = 0.03$) and verbal learning ($p = 0.04$; Rasmussen et al., 1993a). No significant effects were found in the visual recall or digit span test for these workers. Troster and Ruff (1990) reported decrements (no statistical analysis performed) in cognitive performance as measured in verbal and visual recall tests that were conducted immediately after presentation (learning phase) and one hour after original presentation (retention/memory phase) for two case studies.

Several controlled (chamber) exposure studies were conducted to cognitive ability during TCE exposure and most did not find any significant decrements in the neurobehavioral measurement. Only Salvini et al. (1971) found significant decrements in cognitive function. Six males were exposed to 110 ppm (550 mg/m^3) TCE for 4-hour intervals, twice per day. Statistically significant results were observed for perception tests learning ($p < 0.001$), mental fatigue ($p < 0.01$), subjects ($p < 0.05$); and choice reaction time (CRT) learning ($p < 0.01$), mental fatigue ($p < 0.01$), subjects ($p < 0.05$). Triebig et al. (1977a, b) exposed 7 total subjects (male and female) to 100 ppm TCE for 6 hours/day, 5 days/week and did not report any decreases in cognition but details on the experimental procedures were not provided. Additionally, Gamberale et al. (1976) found that subjects exposed to TCE as high as 194 ppm for 70 minutes did not exhibit any impairments on a short term memory test in comparison to an air exposure.

1 **Table 4-27. Summary of human cognition effect studies**
2

Reference	Subjects	Exposure	Effect
Kilburn and Warshaw, 1993	170 residents living in Southwest Tucson with TCE, other solvents, and chromium in groundwater. Control: 68 residential referents matched to subjects from 2 previous studies of waste oil and oil refinery exposures.	>500 ppb of TCE in well water before 1981 and 25 to 100 ppb afterwards Exposure duration ranged from 1 to 25 yrs	Decreased performance in the digit span memory test and story recall ability.
Kilburn, 2002a	236 residents near a microchip plant; Controls: 67 local referents from Phoenix, AZ and 161 regional referents from Wickenburg, AZ.	<0.2−10,000 ppb of TCE, <0.2−260,000 ppb TCA, <0.2−6,900 ppb 1,1-DCE, <0.2−1,600 1,2-DCE, <0.2−23,000 ppb PCE, <0.02−330 ppb VC in well water Exposure duration ranged from 2 to 37 yrs. Exposure duration ranged from 2 to 37 yrs	Cognitive effects decreased as measured by lower scores on Culture Fair 2A, vocabulary, grooved pegboard (dominant hand), trail making test, and verbal recall (i.e., memory).
Rasmussen, 1993a, b	96 Danish metal degreasers. Age range: 19−68; No external controls.	Average exposure duration: 7.1 yrs.); range of full-time degreasing: 1 month to 36 yrs 1) Low exposure: $n = 19$, average full-time expo 0.5 yrs 2) Medium exposure: $n = 36$, average full-time exposure 2.1 yrs 3) High exposure: $n = 41$, average full-time exposure 11 yrs. TCA in high exposure group = 7.7 mg/L (max = 26.1 mg/L)	Cognitive impairment (psycho-organic syndrome) prevalent in exposed individuals. The incidence of this syndrome was 10.5% in the low exposure, 39.5% for medium exposure, and 63.4% for high exposure. Age is a confounder. Dose-response with 9 of 15 tests; Controlling for confounds, significant relationship of exposure was found with Acoustic-motor function ($p < 0.001$), Paced Auditory Serial Addition Test ($p < 0.001$), Rey Auditory Verbal-Learning Test ($p < 0.001$), vocabulary ($p < 0.001$) and visual gestalts ($p < 0.001$); significant age effects. Age is a confounder.
Troster and Ruff, 1990	2 occupationally TCE-exposed workers. Controls: 2 groups of $n = 30$ matched controls; (all age and education matched.	Exposure concentration unknown; Exposure duration, 3−8 months	Both TCE cases exhibited significant deficits in verbal recall and visuospatial learning.

1

Table 4-27. Summary of human cognition effect studies (continued)

Reference	Subjects	Exposure	Effect
Triebig, 1976	Controlled exposure study 4 females, 3 males Controls: 4 females, 3 males	0, 100 ppm (550 mg/m^3), 6 h/d, 5 d.	There was no correlation seen between exposed and unexposed subjects for any measured psychological test results. No methods description was provided.
Triebig, 1977a	7 men and 1 woman occupationally exposed with an age range from 23−38 yrs. No control group.	50 ppm (260 mg/m^3). Exposure duration not reported	The psychological tests showed no statistically significant difference in the results before or after the exposure-free time period. No methods description was provided.
Triebig, 1977b	Controlled exposure study on 3 male and 4 female students Control: 3 male and 4 female students	0, 100 ppm (550 mg/m^3), 6 h/d, 5 d	No significantly different changes were obtained. No methods description was provided.
Salvini et al., 1971	Controlled exposure study 6 students, male Self used as control	TCE concentration was 110 ppm for 4-hour intervals, twice per day. 0 ppm control exposure for all as self controls	Statistically significant results were observed for perception tests learning ($p < 0.001$) and CRT learning ($p < 0.01$).
Gamberale et al., 1976	15 healthy men aged 20−31 yrs old Controls**:** Within Subjects (15 self-controls)	0 mg/m^3, 540 mg/m^3 (97 ppm), 1,080 mg/m^3 (194 ppm), 70 min	Repetition of the testing led to a pronounced improvement in performance as a result of the training effect; No interaction effects between exposure to TCE and training.
Stewart et al., 1970	130 (108 males, 22 females); Controls: 63 unexposed men	TCA metabolite levels in urine were measured: 60.8% had levels up to 20 mg/L, and 82.1% had levels up to 60 mg/L	No significant effect on cognitive tests noted, but more effort required to perform the test in exposed group.
Chalupa, 1960	Case study - Six subjects. Average age 38	No exposure data were reported	80% of those with pathological EEG displayed memory loss; 30% of those with normal EEGs displayed memory loss.

2
3 DCE = dichloroethylene, EEG = electroencephalogram.
4
5

6 **4.3.5.2. *Cognitive Effects: Laboratory Animal Studies***

7 Many reports have demonstrated significant differences in performance of learning tasks
8 such as the speed to complete the task. However, there is little evidence that learning and
9 memory function are themselves impaired by exposure. There are also limited data that suggest

1 alterations in the hippocampus of laboratory animals exposed to TCE. Given the important role
2 that this structure plays in memory formation, such data may be relevant to the question of
3 whether TCE impairs memory. The studies are briefly discussed below and details are provided
4 in Table 4-28.
5
6 **Table 4-28. Summary of animal cognition effect studies**
7

Reference	Exposure route	Species/strain/ sex/number	Dose level/ exposure duration	NOAEL; LOAEL	Effects
Kjellstrand et al., 1980	Inhalation	Gerbil, Mongolian, males and females, 12/sex/dose	0, 320 ppm; 9 months, continuous (24 h/d) except 1−2 h/wk for cage cleaning	NOAEL: 320 ppm	No significant effect on spatial memory (radial arm maze).
Isaacson et al., 1990	Oral, drinking water	Rat, Sprague-Dawley, male weanlings, 12/dose	(1) 0 mg/kg/d, 8 wks (2) 5.5 mg/d (47 mg/kg/d*), 4 wks + 0 mg/kg/d, 4 wks (3) 5.5 mg/dd, 4 wks (47 mg/kg/d[b]) + 0 mg/kg/d, 2 wks + 8.5 mg/dd (24 mg/kg/d[b]), 2 wks	NOAEL: 5.5 mg/d, 4 wks—spatial learning LOAEL: 5.5 mg/d—hippocampal demyelination	Decreased latency to find platform in the Morris water maze (Group #3); Hippocampal demyelination observed in all TCE-treated groups.
Kishi et al., 1993	Inhalation	Rats, Wistar, male, number not specified	0, 250,500, 1,000, 2,000, and 4,000 ppm, 4 hours	LOAEL: 250 ppm	Decreased lever presses and avoidance responses in a shock avoidance task.
Umezu et al., 1997	Intra-peritoneal	Mouse, ICR, male, 6 exposed to all treatments (repeated exposure)	0, 125, 250, 500, and 1,000 mg/kg, single dose and evaluated 30 min postadministration	NOAEL: 500 mg/kg LOAEL: 1,000 mg/kg	Decreased response rate in an operant response—condition avoidance task.
Oshiro et al., 2004	Inhalation	Rat, Long Evans, male, 24	0, 1,600, and 2,400 ppm; 6 h/d, 5 d/wk, 4 wks	NOAEL: 2,400 ppm	No change in reaction time in signal detection task and when challenged with amphetamine, no change in response from control.

8
9 *mg/kg/d conversion estimated from average male Sprague-Dawley rat body weight from ages 21−49 days (118 g)
10 for the 5.5 mg dosing period and ages 63−78 days (354 g) for the 8.5 mg dosing period.
11
12
13 Two studies (Kulig et al., 1987; Umezu et al., 1997) reported decreased performance in
14 operant-conditioning cognitive tasks for rodents. Kishi et al. (1993) acutely exposed Wistar rats
15 to TCE at concentrations of 250, 500, 1,000, 2,000, and 4,000 ppm for four hours. Rats exposed

to 250 ppm TCE and higher showed a significant decrease both in the total number of lever presses and in avoidance responses compared with controls. The rats did not recover their pre-exposure performance until about 2 hours after exposure. Likewise, Umezu et al. (1997) reported a depressed rate of operant responding in male ICR strain mice (n = 6, exposed to all TCE doses, see Table 4-28) in a conditioned avoidance task that reached significance with i.p. injections of 1,000 mg/kg. Increased responding during the signaled avoidance period at lower doses (250 and 500 mg/kg) suggests an impairment in ability to inhibit responding or failure to attend to the signal.

Although cognitive impairments are noted, two additional studies indicate no change in cognition with continuous TCE exposure or improvements in cognitive tasks. No decrements in cognitive function as measured by the radial arm maze were observed in Mongolian gerbils exposed continuously by inhalation to 320 ppm TCE for 9 months (Kjellstrand et al., 1980). Improved performance was noted in a Morris swim test for weanling rats orally dosed with 5.5 mg/day for 4 weeks followed by 2 weeks of no exposure and an additional 2 weeks of 8.5 mg/day (Isaacson et al., 1990). This improved performance occurred despite a loss in hippocampal myelination.

4.3.5.3. *Summary and Conclusions of Cognitive Function Studies*

Human environmental and occupational exposure studies suggest impairments in cognitive function. Kilburn and Warshaw (1993) and Kilburn (2002a) reported memory deficits individuals although a question exists whether the referent group was comparable to exposed subjects and these studies lack of consideration of possible confounding exposures in statistical analyses. Significant impairments were found in visual and verbal recall and with the digit span test. Similarly, in occupational exposure studies (Rasmussen et al., 1993a, b; Troster and Ruff, 1990), short term memory tests indicated that immediate memory and learning were impaired in the absence of an effect on digit span performance. In controlled exposure and/or chamber studies, two studies did not report any cognitive impairment (Stewart et al., 1970; Gamberale et al., 1976) and one study (Salvini et al., 1971) reported significant impairments in learning memory and complex choice reaction tasks. All of the controlled exposure studies were acute and/or short-term exposure studies and the sensitivity of test procedures is unknown due to the lack of methodologic information provided in the reports. Despite identified study deficiencies, these studies collectively suggest cognitive function impairment.

The animal studies measured cognitive function through spatial memory and operant responding tasks. In the two studies where spatial memory was evaluated, there was either no effect at 320 ppm TCE (Kjellstrand et al., 1980) or improved cognitive performance in weanling

rats at a dose of 5.5 mg/day for four weeks (Isaacson et al., 1990). Improved cognitive performance was observed in weanling rats (Isaacson et al., 1990) and could be due to continuing neurodevelopment as well as compensation from other possible areas in the brain since there was a significant loss in hippocampal myelination. Significant decreases in operant responding (avoidance/punished responding) during TCE exposure were reported in two studies (Kishi et al., 1993; Umezu et al., 1997). When TCE exposure was discontinued operant responding return to control levels and it is unclear if the significant effects are due to decreased motor function or decreased cognitive ability.

4.3.6. Psychomotor Effects

There is considerable evidence in the literature for both animals and humans on psychomotor testing although human and laboratory animal studies utilize very different measures of motor behavior. Generally, the human literature employs a wide variety of psychomotor tasks and assesses error rates and reaction time in the performance of the task. The laboratory animal data, by contrast, tend to include unlearned naturalistic behaviors such as locomotor activity, gait changes, and foot splay to assess neuromuscular ability.

4.3.6.1. *Psychomotor Effects: Human Studies*

The effects of TCE exposure on psychomotor response have been studied primarily as a change in reaction time (RT) with studies on motor dyscoordination resulting from TCE exposure providing subjective reporting.

4.3.6.1.1. ***Reaction time.*** Several studies have evaluated the effects of TCE on reaction time using simple and choice reaction time tasks (simple reaction time [SRT] and CRT tasks). The studies are presented below and summarized in more detail in Table 4-29.

1 **Table 4-29. Summary of human choice reaction time studies**

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Reference	Subjects	Exposure	Effect
Kilburn, 2002a	236 residents near a microchip plant in Phoenix, AZ Controls: 161 regional referents from Wickenburg, AZ 67 referents from Phoenix, AZ not residing near a plant	0.2−10,000 ppb of TCE, chronic exposure	Simple and choice reaction times were increased in the exposed group ($p < 0.05$).
Kilburn and Warshaw, 1993	160 residents living in Southwest Tucson with TCE and other solvents in groundwater Control: 68 residential referents matched to subjects from 2 previous studies of waste oil and oil refinery exposures	>500 ppb of TCE in well-water before 1981 and 25 to 100 ppb afterwards Exposure duration ranged from 1 to 25 yrs	Mean simple reaction time was 67 milliseconds (msec) longer than the referent group $p < 0.0001$). CRT of the exposed subjects was between 93−100 msec longer in three different trials ($p < 0.0001$) compared to referents.
Reif et al., 2003	143 residents of the Rocky Mountain Arsenal community of Denver Referent group at lowest concentration (<5 ppb)	High exposure group >15 ppb Medium exposure group ≥5 ppb and ≤15 ppb Low exposure referent group <5 ppb	Significant increase in reaction time as measured by the simple reaction time test ($p < 0.04$) in only among subjects who reported alcohol use (defined as having at least one drink per month).
Kilburn and Thornton, 1996	Group A: Registered voters from Arizona and Louisiana with no exposure to TCE: $n = 264$, aged 18−83. Group B volunteers from California $n = 29$ (17 males and 12 females) Group C: exposed to TCE and other chemicals for 5 yrs or more $n = 217$	No exposure or groundwater analyses reported	Significant increase in simple and choice reaction time in exposed group compared to the unexposed populations.
Gamberale et al., 1976	15 healthy men aged 20−31 yrs old Controls**:** Within subjects (15 self-controls)	0 mg/m^3, 540 mg/m^3 (97 ppm), 1,080 mg/m^3 (194 ppm), 70 min.	No change in CRT or SRT. Increase in time required to perform the RT-Addition Test (task for adding numbers) ($p < 0.05$).
Gun et al., 1978	4 female workers from one plant exposed to TCE and 4 female workers from another plant exposed to TCE + nonhalogenated hydrocarbon solvent Control: ($n = 8$) 4 unexposed female workers from each plant	3−419 ppm, duration not specified	TCE-only exposure increased reaction time in comparison to controls. In TCE + solvent group, ambient TCE was lower and mean reaction time shortened in Session 2, then rose subsequently to be greater than at the start.

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Increases in reaction time were observed in environmental exposure studies by Kilburn (2002a), Kilburn and Warshaw (1993), and Kilburn and Thornton (1996) as well as in an occupational exposure study by Gun et al. (1978). All populations except that of Gun et al. (1978) were exposed through groundwater contaminated as the result of environmental spills and the exposure duration was for at least 1 year and exposure levels ranged from 0.2 to 10,000 ppb for the three studies. Kilburn and Warshaw (1993) reported that SRT significantly increased from 281 ± 55 msec to 348 ± 96 msec in individuals ($p < 0.0001$). CRT of the exposed subjects was 93 msec longer ($p < 0.0001$) than referents. Kilburn and Thornton (1996) evaluated SRT and CRT function and also found similar increases in reaction time. The average SRT and CRT for the combined control groups were 276 msec and 532 msec, respectively. These reaction times increased in the TCE exposure group where the average SRT was 334 msec and CRT was 619 msec. Similarly, Kilburn (2002a) compared reaction times between 236 TCE-exposed persons and the 161 unexposed regional controls. SRTs significantly increased from 283 ± 63 msec in controls to 334 ± 118 msec in TCE exposed individuals ($p < 0.0001$). Similarly, CRTs also increased from 510 ± 87 msec to 619 ± 153 msec with exposure to TCE ($p < 0.0001$).

No effect on SRT was reported in a geographical-based study by Reif et al. (2003). SRTs were 301 msec for the lowest exposure group and 316 msec for the highest exposure group ($p = 0.42$). When the SRT data were analyzed individuals that consumed at least on alcoholic drink per month ($n = 80$), a significant increase (18%, $p < 0.04$) in SRT times were observed between the lowest exposure and the highest exposure groups. In TCE exposed individuals who did not consume alcohol ($n = 55$), SRTs decreased from 321 msec in the lowest exposed group to 296 msec in the highest exposed group, but this effect was not statistically significantly different. A controlled exposure (chamber study) of 15 healthy men aged 20−31 years old, were exposed to 0, 540, and 1,080 mg/m^3 TCE for 70 minutes or served as his own control, reported no statistically significant differences with the SRT or CRT tasks. However, in the RT-Addition test the level of performance varied between the different exposure conditions ($F(2.24) = 4.35$; $p < 0.05$) and between successive measurement occasions ($F(2.24) = 19.25$; $p < 0.001$).

4.3.6.1.2. ***Muscular dyscoordination.*** Three studies examined motor dyscoordination effects from TCE exposure using subjective and self-reported individual assessment. Rasmussen et al. (1993c) presented findings on muscular dyscoordination for 96 metal degreasers exposed to either TCE or CFC113. A statistically significant increasing trend of dyscoordination with TCE exposure was observed ($p = 0.01$) in multivariate regression analyses which adjusted for the effects of age, neurological disease, arteriosclerotic disease, and alcohol abuse. Furthermore, a

greater number of abnormal coordination tests were observed in the higher exposure group compared to the low exposure group ($p = 0.003$).

Gash et al. (2008) reported fine motor hand movement times in subjects who had filed workman compensation claims were significantly slower ($p < 0.0001$) than age-matched nonexposed controls. Exposures were based on self-reported information, and no information on the control group is presented. Troster and Ruff (1990) reported a case study conducted on two occupationally exposed workers to TCE. Mild deficits in motor speed were reported for both cases. In the first case, manual dexterity was impaired in a male exposed to TCE (unknown concentration) for eight months. In the second case study where a female was exposed to TCE (low concentration; exact level not specified) for 3 months, there was weakness in the quadriceps muscle as evaluated in a neurological exam and a decreased sensation to touch on one hand. Both Gash et al. (2008) and Troster and Ruff (1990) provide very limited information given their deficiencies related to lack of exposure data, self-reported information, and limited reporting of referents and statistical analysis.

4.3.6.2. *Psychomotor Effects: Laboratory Animal Data*

Several animal studies have demonstrated that TCE exposure produces changes in psychomotor function. At high doses (≥2,000 mg/kg) TCE causes mice to lose their righting reflex when the compound is injected intraperitoneally (Shih et al., 2001; Umezu et al., 1997). At lower exposures (inhalation and oral), TCE produces alterations in neurobehavioral measures including locomotor activity, gait, operant responding, and reactivity. The studies are described in Sections 4.3.6.2.1−4.3.6.2.3 and summarized in Tables 4-30 and 4-31.

4.3.6.2.1. ***Loss of righting reflex.*** Umezu et al. (1997) studied disruption of the righting reflex following acute injection (i.p.) of 2,000, 4,000, and 5,000 mg/kg TCE in male ICR mice. TCE disrupted the righting reflex at doses of 2,000 mg/kg and higher. At 2,000 mg/kg, loss of righting reflex (LORR) was observed in only 2/10 animals injected. At 4,000 mg/kg, 9/10 animals experienced LORR and 100% of the animals experienced LORR at 5,000 mg/kg.

Shih et al. (2001) reported impaired righting reflexes at exposure doses of 5,000 mg/kg (i.p.) in male Mf1 mice. Mice pretreated with dimethyl sulfoxide or disulfuram (CYP2E1 inhibitor) delayed LORR in a dose related manner. By contrast, the alcohol dehydrogenase inhibitor, 4-metylpyradine did not delay LORR that resulted from 5,000 mg/kg TCE. These data suggest that the anesthetic properties of TCE involve its oxidation via CYP2E1 to an active metabolite.

Table 4-30. Summary of animal psychomotor function and reaction time studies

Reference	Exposure route	Species/strain/ sex/number	Dose level/ exposure duration	NOAEL; LOAEL	Effects
Savolainen et al., 1977	Inhalation	Rat, Sprague-Dawley, male, 10	0, 200 ppm; 6 h/d, 4 d	LOAEL: 200 ppm	Increased frequency of preening, rearing, and ambulation. Increased preening time.
Kishi et al., 1993	Inhalation	Rats, Wistar, male, number not specified	0, 250,500, 1,000, 2,000, and 4,000 ppm, 4 hours	LOAEL: 250 ppm	Decreased lever presses and increased responding when lever press coupled with a 10-s electric shock (decreased avoidance response).
Kulig et al., 1987	Inhalation	Rat, Wistar, male, 8/dose	0, 500, 1,000, and 1,500 ppm; 16 h/d, 5 d/wk, 18 wks	NOAEL: 1,500 ppm	No change in spontaneous activity, grip strength, or hindlimb movement.
Moser et al., 1995	Oral	Rat, Fischer 344, female, 8/dose	0, 150, 500, 1,500, and 5,000 mg/kg, 1 dose	NOAEL: 500 mg/kg LOAEL: 1,500 mg/kg	Decreased motor activity; Neuro-muscular and sensorimotor impairment.
			0, 50, 150, 500, and 1,500 mg/kg/d, 14 d	NOAEL: 150 mg/kg/d LOAEL: 500 mg/kg/d	Increased rearing activity and decreased forelimb grip strength.
Bushnell, 1997	Inhalation	Rat, Long Evans, male, 12	0, 400, 800, 1,200, 1,600, 2,000, or 2,400 ppm, 1 h/test day, 4 consecutive test days, 2 wks	NOAEL: 800 ppm LOAEL: 1,200 ppm	Decreased sensitivity and increased response time in the signal detection task.
Shih et al., 2001	Intra-peritoneal	Mouse, MF1, male, 6	0, 5,000 mg/kg, acute	LOAEL: 5,000 mg/kg	Impairment of righting reflex.
Umezu et al., 1997	Intra-peritoneal	Mouse, ICR, male, 10/group	0, 2,000, 4,000, 5,000 mg/kg—loss of righting reflex measure	LOAEL: 2,000 mg/kg— loss of righting reflex	Loss of righting reflex.
		Mouse, ICR, male, 6−10/group	0, 62.5, 125, 250, 500, and 1,000 mg/kg, single dose and evaluated 30 min postadministration	NOAEL: 500 mg/kg LOAEL: 1,000 mg/kg—operant behavior NOAEL: 125 mg/kg LOAEL: 250 mg/kg—punished responding	Decreased responses (lever presses) in an operant response task for food reward. Increased responding when lever press coupled with a 20-V electric shock (punished responding).
Bushnell and Oshiro, 2000	Inhalation	Rat, Long Evans, male, 32	0, 2,000, 2,400 ppm; 70 min/d, 9 d	LOAEL: 2,000 ppm	Decreased performance on the signal detection task. Increased response time and decreased response rate.

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Table 4-30. Summary of animal psychomotor function and reaction time studies (continued)

Reference	Exposure route	Species/strain/ sex/number	Dose level/ exposure duration	NOAEL; LOAEL	Effects
Nunes et al., 2001	Oral	Rat, Sprague-Dawley, male, 10/group	0, 2,000 mg/kg/d, 7 d	LOAEL: 2,000 mg/kg/d	Increased foot splay. No change in any other FOB parameter (e.g., piloerection, activity, reactivity to handling).
Moser et al., 2003	Oral	Rat, Fischer 344, female, 10/group	0, 40, 200, 800, and 1,200 mg/kg/d, 10 d	---	Decreased motor activity; Decreased sensitivity to tail pinch; Increased abnormality in gait; Decreased grip strength; Adverse changes in several FOB parameters.
Albee et al., 2006	Inhalation	Rat, Fischer 344, male and female, 10/sex/group	0, 250, 800, 2,500 ppm; 6 h/d, 5 d/wk, 13 wks	NOAEL: 2,500 ppm	No change in any FOB measured parameter.

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1 **Table 4-31. Summary of animal locomotor activity studies**
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Reference	Exposure route	Species/strain/ sex/number	Dose level/ Exposure duration	NOAEL; LOAEL	Effects
Wolff and Siegmund, 1978	Intra-peritoneal	Mouse, AB, male, 18	0, 182 mg/kg, tested 30 min after injection	LOAEL: 182 mg/kg	Decreased spontaneous motor activity.
Kulig et al., 1987	Inhalation	Rat, Wistar, male, 8/dose	0, 500, 1,000, and 1,500 ppm; 16 h/d, 5 d/wk, 18 wks	NOAEL: 500 ppm LOAEL: 1,000 ppm	No change in spontaneous activity, grip strength or hindlimb movement. Increased latency time in the two-choice visual discrimination task (cognitive disruption and/or motor activity related effect).
Moser et al., 1995	Oral	Rat, Fischer 344, female, 8/dose	0, 150, 500, 1,500, and 5,000 mg/kg, 1 dose	NOAEL: 500 mg/kg LOAEL: 1,500 mg/kg	Decreased motor activity; Neuro-muscular and sensorimotor impairment.
			0, 50, 150, 500, and 1,500 mg/kg/d, 14 d	NOAEL: 150 mg/kg/d LOAEL: 500 mg/kg/d	Increased rearing activity.
Waseem et al., 2001	Oral	Rat, Wistar, male, 8/group	0, 350, 700, and 1,400 ppm in drinking water for 90 d	NOAEL: 1,400 ppm	No significant effect on spontaneous locomotor activity.
	Inhalation	Rat, Wistar, male, 8/group	0, 376 ppm for up to 180 d; 4 h/d, 5 d/wk	LOAEL: 376 ppm	Changes in locomotor activity and vary by timepoint when measured over the 180-d period.
Moser et al., 2003	Oral	Rat, Fischer 344, female, 10/group	0, 40, 200, 800, and 1,200 mg/kg/d, 10 d	___	Decreased motor activity; Decreased sensitivity; Increased abnormality in gait; Adverse changes in several FOB parameters.

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5 **4.3.6.2.2.** ***Activity, sensory-motor and neuromuscular function.*** Changes in sensory-motor
6 and neuromuscular activity was reported in three studies (Kishi et al., 1993; Moser et al., 1995;
7 Moser et al., 2003). Kishi et al. (1993) exposed male Wistar rats to 250, 500, 1,000, 2,000, and
8 4,000 ppm TCE for 4 hours. Rats exposed to 250-ppm TCE showed a significant decrease both
9 in the total number of lever presses and in avoidance responses at 140 minutes of exposure
10 compared with controls. Moser et al. (1995) evaluated the effects of acute and short-term
11 (14 day) administration of TCE in adult female Fischer 344 rats (n = 8−10/dose) on activity
12 level, neuromuscular function and sensorimotor function as part of a larger functional

observational battery (FOB) testing. The NOAEL levels identified by the authors are 500 mg/kg (10% of the limit dose) for the acute treatment and 150 mg/kg (3% of the limit dose) for the 14-day study. In the acute study, TCE produced the most significant effects in motor activity (activity domain), gait (neuromuscular domain), and click response (sensorimotor domain). In the 14-day study, only the activity domain (rearing) and neuromuscular domain (forelimb grip strength) were significantly different ($p < 0.05$) from control animals. In a separate 10-day study (Moser et al., 2003), TCE administration significantly ($p < 0.05$) reduced motor activity, tail pinch responsiveness, reactivity to handling, hind limb grip strength and body weight. Significant increases ($p < 0.05$) in piloerection, gait scores, lethality, body weight loss, and lacrimation was also reported in comparison to controls.

There are also two negative studies which used adequate numbers of subjects in their experimental design but used lower doses than did Moser et al. (2003). Albee et al. (2006) exposed male and female Fischer 344 rats (n = 10/sex) to TCE by inhalation at exposure doses of 250, 800, and 2,500 ppm, for 6 hours/day, 5 days/week, for 13 weeks. The FOB was performed monthly although it is not certain how much time elapsed from the end of exposure until the FOB test was conducted. No treatment related differences in grip strength or landing foot splay were demonstrated in this study. Kulig et al. (1987) also failed to show significant effects of TCE inhalation exposure on markers of motor behavior. Wistar rats (n = 8) exposed to 500, 1,000, and 1,500 ppm, for 16 hours/day, 5 days/week, for 18 weeks failed to show changes in spontaneous activity, grip strength, or coordinated hind limb movement. Measurements were made every three weeks during the exposure period and occurred between 45 and 180 minutes following the previous TCE inhalation exposure.

4.3.6.2.3. ***Locomotor activity.*** The data, with regard to locomotor activity, are inconsistent. Several studies showed that TCE exposure can decrease locomotor activity including Wolff and Siegmund (1978) where AB mice (n = 18) were treated acutely with a dose of 182 mg/kg, i.p. at one of 4 time points during a 24-hour day. Moser et al. (1995, 2003) reported reduced locomotor activity in female Fischer 344 rats (n = 8−10) gavaged with TCE over an acute (LOAEL = 5,000 mg/kg TCE) or subacute period (LOAEL = 500 but no effect at 5,000 mg/kg). In the Moser et al. (2003), it appears that 200-mg/kg TCE yielded a significant reduction in locomotor activity and that the degree of impairment at this dose represented a maximal effect on this measure. That is, higher doses of TCE appear to have produced equivalent or slightly less of an effect on this behavior. While this study identifies a LOAEL of 200-mg/kg TCE by gavage over a 10-day period, this is a much more lower dose effect than that reported in Moser et al. (1995). Both studies (Moser et al., 1995, 2003) demonstrate a depression in motor activity that

occurs acutely following TCE administration. Kulig et al. (1987) demonstrated that rats had increased response latency to a two choice visual discrimination following 1,000- and 1,500-ppm TCE exposures for 18 weeks. However, no significant changes in grip strength, hindlimb movement, or any other motor activity measurements were noted.

There are also a few studies (Fredriksson et al., 1993; Waseem et al., 2001) generally conducted using lower exposure doses that failed to demonstrate impairment of motor activity or ability following TCE exposure. Waseem et al. (2001) failed to demonstrate changes in locomotor activity in male Wistar rats ($n = 8$) dosed with TCE (350, 700, and 1,400 ppm) in drinking water for 90 days. Wistar rats ($n = 8$) exposed to 500, 1,000, and 1,500 ppm for 16 hours/day, 5 days/week, for 18 weeks failed to show changes in spontaneous activity. No changes in locomotor activity were observed for 17-day-old male NMRI mice that were dosed postnatally with 50 or 290 mg/kg/d from Day 10 to 16 (Fredriksson et al., 1993). However, rearing activity was significantly decreased in the NMRI mice at Day 60.

4.3.6.3. *Summary and Conclusions for Psychomotor Effects*

In human studies, psychomotor effects such as reaction time and muscular dyscoordination have been examined following TCE exposure. In the reaction time studies, statistically significant increases in CRT and SRT were reported in the Kilburn studies (Kilburn, 2002a; Kilburn and Warshaw, 1993; Kilburn and Thornton, 1996). All of these studies were geographically based and it was suggested that the results were used for litigation and the differences between exposed and referent groups on other factors influencing reaction speed time may introduce a bias to the findings. Additionally, in these studies exposure to TCE and other chemicals occurred through drinking water for at least 1 year and TCE concentrations in well water ranged from 0.2 ppb to 10,000 ppb. Reif et al. (2003) whose exposure assessment approach included exposure modeling of water distribution system to estimate TCE concentrations in tap water at census track of residence found that residents with drinking water containing TCE (up to >15 ppb—the highest level not specified) and other chemicals did not significantly increase CRTs or SRTs. Inhalation studies also demonstrated increased reaction times. An acute exposure chamber study (Gamberale et al., 1976) tested for CRT, SRT, and RT-addition following a 70-minute exposure to TCE. A concentration-dependent significant decrease in performance was observed with the RT-addition test and not for CRT or SRT tasks. An occupational exposure study on 8 female workers exposed to TCE (Gun et al., 1978) also reported increased reaction time in the females exposed to TCE-only. Muscular dyscoordination for humans following TCE exposure has been reported in a few studies as a subjective observation. The studies indicated that exposure resulted in decreased motor speed and dexterity

(Troster and Ruff, 1990; Rasmussen et al., 1993c) and self-reported faster asymptomatic fine motor hand movements (Gash et al., 2008).

Animal studies evaluated psychomotor function by examining locomotor activity, operant responding, changes in gait, loss of righting reflex, and general motor behavior (see Tables 4-30 and 4-31 for references). Overall, the studies demonstrated that TCE causes loss of righting reflex at injection doses of 2,000 mg/kg or higher (Umezu et al., 1997; Shih et al., 2001). Regarding general psychomotor testing, significant decreases in lever presses and avoidance were observed at inhalation exposures as low as 250 ppm for 4 hours (LOAEL; Kishi et al., 1993). Following subchronic inhalation exposures, no significant changes in psychomotor activity were noted at up to 2,500 ppm for 13 weeks (Albee et al., 2006) or at 1,500 ppm for 18 weeks (Kulig et al., 1987). In the oral administration studies (Moser et al., 1995, 2003), psychomotor effects were evaluated using an FOB. More psychomotor domains were significantly affected by TCE treatment in the acute study in comparison to the 14-day study, but a lower NOAEL (150 mg/kg/d) was reported for the 14-day study in comparison to the acute study (500 mg/kg; Moser et al., 1995). Upon closer examination of the data, a biphasic effect in one measure of the FOB (rearing) was resulting in the lower NOAEL for the 14-day study and doses that were higher and lower than the NOAEL did not produce a statistically significant increase in the number of rears. Therefore, it can be surmised that acute exposure to TCE results in significant changes in psychomotor function. However, there may be some tolerance to these psychomotor changes in increased exposure duration to TCE as evidenced by the results noted in the short-term and subchronic exposure studies.

4.3.7. Mood Effects and Sleep Disorders

4.3.7.1. *Effects on Mood: Human Studies*

Reports of mood disturbance (depression, anxiety) resulting from TCE exposure are numerous in the human literature. These symptoms are subjective and difficult to quantify. Studies by Gash et al. (2008), Kilburn and Warshaw (1993), Kilburn (2002a, 2002b), McCunney et al. (1988), Mitchell et al. (1969), Rasmussen and Sabroe (1986), and Troster and Ruff (1990) reported mood disturbances in humans. Reif et al. (2003) and Triebig (1976, 1977) reported no effect on mood following TCE exposures.

4.3.7.2. *Effects on Mood: Laboratory Animal Findings*

It is difficult to obtain comparable data of emotionality in laboratory studies. However, Moser et al. (2003) and Albee et al. (2006) both report increases in handling reactivity among rats exposed to TCE. In the Moser study, female Fischer 344 rats received TCE by oral gavage

for periods of 10 days at doses of 0, 40, 200, 800, and 1,200 while Albee et al. (2006) exposed Fischer 344 rats to TCE by inhalation at exposure doses of 250, 800, and 2,500 ppm for 6 hours/day, 5 days/week, for 13 weeks.

4.3.7.3. *Sleep Disturbances*

Arito et al. (1994) exposed male Wistar rats to 50-, 100-, and 300-ppm TCE for 8 hour/day, 5 days/week, for 6 weeks and measured electroencephalographic (EEG) responses. EEG responses were used as a measure to determine the number of awake (wakefulness hours) and sleep hours. Exposure to all the TCE levels significantly decreased amount of time spent in wakefulness (W) during the exposure period. Some carry over was observed in the 22 hours post exposure period with significant decreases in wakefulness seen at 100-ppm TCE. Significant changes in W-sleep elicited by the long-term exposure appeared at lower exposure levels. These data seem to identify a low dose effect of TCE and established a LOAEL of 50 ppm for sleep changes.

4.3.8. Developmental Neurotoxicity

4.3.8.1. *Human Studies*

In humans, CNS birth defects were observed in a few studies (ATSDR, 2001; Bove, 1996; Bove et al., 1995; Lagakos et al., 1986). Postnatally, observed adverse effects in humans include delayed newborn reflexes following exposure to TCE during childbirth (Beppu, 1968), impaired learning or memory (Bernad et al., 1987, abstract; White et al., 1997); aggressive behavior (Bernad et al., 1987, abstract); hearing impairment (Burg and Gist, 1999); speech impairment (Burg and Gist, 1999; White et al., 1997); encephalopathy (White et al., 1997); impaired executive and motor function (White et al., 1997); attention deficit (Bernad et al., 1987, abstract; White et al., 1997), and autism spectrum disorder (Windham et al., 2006). The human developmental neurotoxicity studies are discussed in more detail in Section 4.8.2.1.2, and summarized in Table 4-32.

Table 4-32. Summary of human developmental neurotoxicity associated with TCE exposures

Finding	Species	Citations
CNS defects, neural tube defects	Human	ATSDR, 2001
		Bove, 1996; Bove et al., 1995
		Lagakos et al., 1986
Delayed newborn reflexes	Human	Beppu, 1968
Impaired learning or memory	Human	Bernad et al., 1987, abstract
		White et al., 1997
Aggressive behavior	Human	Bernad et al., 1987, abstract
Hearing impairment	Human	Burg and Gist, 1999
Speech impairment	Human	Burg and Gist, 1999
		White et al., 1997
Encephalopathy	Human	White et al., 1997
Impaired executive function	Human	White et al., 1997
Impaired motor function	Human	White et al., 1997
Attention deficit	Human	White et al., 1997
	Human	Bernad et al., 1987, abstract
Autism spectrum disorder (ASD)	Human	Windham et al., 2006

4.3.8.2. *Animal Studies*

There are a few studies demonstrating developmental neurotoxicity following trichloroethylene exposure (range of exposures) to experimental animals. These studies collectively suggest that developmental neurotoxicity result from TCE exposure, however, some types of effects such as learning and memory measures have not been evaluated. Most of the studies demonstrate either spontaneous motor activity changes (Taylor et al., 1985) or neurochemical changes such as decreased glucose uptake and changes in the specific gravity of the cortex and cerebellum (Westergren et al., 1984; Noland-Grebec et al., 1986; Isaacson and Taylor, 1989). In addition, in most of these studies there is no assessment of the exposure to TCE or metabolites in the pups/offspring. Details of the studies are presented below and summarized in Table 4-33.

Taylor et al. (1985) administered TCE to female Sprague-Dawley rats in their drinking water from 14 days before breeding throughout gestation and until pups were weaned at 21 days. Measured TCE concentrations in the dams ranged from 312−646 mg/L, 625−1,102 mg/L, and 1,250−1,991 mg/L in the low, mid, and high-dose groups as measured from the drinking water. Pups were evaluated for exploratory activity at 28, 60, or 90 days. No significant differences were noted between control and treated pups at 28 days. At 60 days, all TCE-treated animals

1 had significantly increased exploratory activity in comparison to age-matched controls, but only
2 the high group had increased activity at 90 days. A significant increase in spontaneous motor
3 activity (as measured by a wheel-running task) was noted in only the high dose TCE
4 (1,250−1,991 mg/L) group during the onset of the darkness period. This study demonstrated that
5 both spontaneous and open field activities are significantly affected by developmental TCE
6 exposure.

7 Spontaneous behavioral changes were also investigated in another study by Fredriksson
8 et al. (1993). Male and female NMRI pups (mice) were orally administered 50 or 290 mg/kg/d
9 for 7 days starting at postnatal Day 10. Spontaneous motor activity was investigated in male
10 mice at ages 17 and 60 days. TCE-treated animals tested at Day 17 did not demonstrate changes
11 in any spontaneous activity measurements in comparison to control animals. Both doses of TCE
12 (50 and 290 mg/kg/d) significantly decreased rearing in 60 day-old male mice.
13 Westergren et al. (1984) examined the brain specific gravity of litters from mice exposed to
14 TCE. NMRI mice (male and female) were exposed to 150-ppm TCE (806.1 mg/m^3) for 30 days
15 prior to mating. Exposure in males continued until the end of mating and females were exposed
16 until the litters were born. Brains were removed from the offspring at either postnatal Days 1,
17 10, 20−22, or 29−31. At postnatal Days 1 and 10, significant decreases were noted in the
18 specific gravity of the cortex. Significant decreases in the specific gravity of the cerebellum
19 were observed at postnatal Day 10 (decrease from 1.0429 ± 0.00046 to 1.0405 ± 0.00030) and
20 20−22 (decrease from 1.0496 ± 0.00014 to 1.0487 ± 0.00060). Cerebellum measurements were
21 not reported for postnatal Day 29−31 animals. Neurobehavioral assessments were not conducted
22 in this study. Additionally, decreased brain specific gravity is suggestive of either decreased
23 brain weight or increased brain volume (probably from edema) or a combination of the two
24 factors and is highly suggestive of an adverse neurological effect. The effects of TCE on the
25 cortical specific gravity were not persistent since cortices from postnatal Day 29−31 animals did
26 not exhibit any significant changes. It is unclear if the effects on the cerebellum were persistent
27 since results were not reported for the postnatal Day 29−31 animals. However, the magnitude of
28 the change in the specific gravity of the cerebellum is decreased from postnatal Day 10 to
29 postnatal Day 20−22 suggesting that the effect may be reversible given a longer recovery period
30 from TCE.

1 **Table 4-33. Summary of mammalian *in vivo* developmental neurotoxicity**
2 **studies—oral exposures**
3

Reference	Species/strain/ sex/number	Dose level/ exposure duration	NOAEL; LOAEL [a]	Effects
Fredriksson et al., 1993	Mouse, NMRI, male pups, 12 pups from 3−4 different litters/group	0, 50, or 290 mg/kg/d PND 10−16	LOAEL: 50 mg/kg/d	Rearing activity sig. ↓ at both dose levels on PND 60.
George et al., 1986	Rat, F334, male and female, 20 pairs/ treatment group, 40 controls/sex	0, 0.15, 0.30, or 0.60% microencapsulated TCE in diet Breeders exposed 1 wk premating, then for 13 wk; pregnant ♀s throughout pregnancy (i.e., 18 wk total)	LOAEL: 0.15%	Open field testing in pups: a sig. dose-related trend toward ↑ time required for male and female pups to cross the first grid in the test device.
Isaacson and Taylor, 1989	Rat, Sprague-Dawley, females, 6 dams/group	0, 312, or 625 mg/L (0, 4.0, or 8.1 mg/d)[b] Dams (and pups) exposed from 14 d prior to mating until end of lactation	LOAEL: 312 mg/L	Sig. ↓ myelinated fibers in the stratum lacunosum-moleculare of pups. Reduction in myelin in the CA1 region of the hippocampus.
Noland-Gerbec et al., 1986	Rat, Sprague-Dawley, females, 9−11 dams/ group	0, 312 mg/L (Avg. total intake of dams: 825 mg TCE over 61 d.) Dams (and pups) exposed from 14 d prior to mating until end of lactation	LOAEL: 312 mg/L	Sig. ↓ uptake of ^{3}H-2-DG in whole brains and cerebella (no effect in hippocampus) of exposed pups at 7, 11, and 16 d, but returned to control levels by 21 d.
Taylor et al., 1985	Rat, Sprague-Dawley, females, no. dams/ group not reported	0, 312, 625, and 1,250 mg/L in drinking water Dams (and pups) exposed from 14 d prior to mating until end of lactation	LOAEL: 312 mg/L	Exploratory behavior sig. ↑ in 60- and 90-d old male rats at all treatment levels. Locomotor activity (measured through the wheel-running tasks) was higher in rats from dams exposed to 1,250 mg/L TCE.
Blossom et al., 2008	Mouse, MRL +/+, dams and both sexes offspring, 8 litters/ group; 3−8 pups/group	Drinking water, from GD 0 to PND 42; 0 or 0.1 mg/mL; maternal dose = 25.7 mg/kg/d; offspring PND 24−42 dose = 31.0 mg/kg/d	LOAEL: 31 mg/kg/d for offspring	Righting reflex, bar holding, and negative geotaxis were not impaired. Significant association between impaired nest quality and TCE exposure. Lower GSH levels and GSH:GSSG ratios with TCE exposure.

4
5 [a] LOEL (lowest-observed-effect level) are based upon reported study findings.
6 [b] Dose conversions provided by study author(s).
7 GD = gestation day.

The effect of TCE on glucose uptake in the brain was evaluated in rat pups exposed to TCE during gestation and through weaning. The primary source of energy utilized in the CNS is glucose. Changes in glucose uptake in the brain are a good indicator for neuronal activity modification. Noland-Grebec et al. (1986) administered 312 mg/L TCE through drinking water to female Sprague-Dawley rats from 2 weeks before breeding and up until pups reached 21 days of age. To measure glucose uptake, 2-deoxyglucose was administered intraperitoneally to male pups at either postnatal Day 7, 11, 16, or 21. Significant decreases in glucose uptake were noted in whole brain and cerebellum at all postnatal days tested. Significant decreases in glucose uptake were also observed in the hippocampus except for animals tested at postnatal Day 21. The observed decrease in glucose uptake suggests decreased neuronal activity.

Female Sprague-Dawley rats (70 days old) were administered TCE in drinking water at a level of either 4.0 or 8.1 mg/day for 14 days prior to mating and continuing up through lactation (Isaacson and Taylor, 1989). Only the male pups were evaluated in the studies. At postnatal Day 21, brains were removed from the pups, sectioned, and stained to evaluate the changes in myelin. There was a significant decrease (40% decrease) in myelinated fibers in the CA1 region of the hippocampus of the male pups. This effect appeared to be limited to the CA1 region of the hippocampus since other areas such as the optic tract, fornix, and cerebral peduncles did not have decreases in myelinated fibers.

Neurological changes were found in pups exposed to TCE in a study conducted by the National Toxicology Program (NTP) in Fischer 344 rats (George et al., 1986). TCE was administered to rats at dietary levels of 0, 0.15, 0.30, or 0.60%. No intake calculations were presented for the rat study and therefore, a dose rate is unavailable for this study. Open field testing revealed a significant ($p < 0.05$) dose-related trend toward an increase in the time required for male and female F1 weanling pups (postnatal day [PND] 21) to cross the first grid in the testing device, suggesting an effect on the ability to react to a novel environment.

Blossom et al. (2008) treated male and female MRL +/+ mice with 0 or 0.1 mg/mL TCE in the drinking water. Treatment was initiated at the time of mating, and continued in the females (8/group) throughout gestation and lactation. Behavioral testing consisted of righting reflex on PNDs 6, 8, and 10; bar-holding ability on PNDs 15 and 17; and negative geotaxis on PNDs 15 and 17. Nest building was assessed and scored on PND 35, the ability of the mice to detect and distinguish social odors was examined with an olfactory habituation/dishabituation method at PND 29, and a resident intruder test was performed at PND 40 to evaluate social behaviors. Righting reflex, bar holding, and negative geotaxis were not impaired by treatment. There was a significant association between impaired nest quality and TCE exposure in tests of nest-building behavior; however, TCE exposure did not have an effect on the ability of the mice

to detect social and nonsocial odors using habituation and dishabituation methods. Resident intruder testing identified significantly more aggressive activities (i.e., wrestling and biting) in TCE-exposed juvenile male mice as compared to controls, and the cerebellar tissue from the male TCE-treated mice had significantly lower GSH levels and GSH:GSSG ratios, indicating increased oxidative stress and impaired thiol status, which have been previously reported to be associated with aggressive behaviors (Franco et al., 2006). Histopathological examination of the brain did not identify alterations indicative of neuronal damage or inflammation.

4.3.8.3. *Summary and Conclusions for the Developmental Neurotoxicity Studies*

Gestational exposure to TCE in humans has resulted in several developmental abnormalities. These changes include neuroanatomical changes such as neural tube defects (ATSDR, 2001; Bove et al., 1995, 1996; Lagakos et al., 1986) and encephalopathy (White et al., 1997). Clinical neurological changes such as impaired cognition (Bernad et al., 1987; White et al., 1997), aggressive behavior (Bernad et al., 1987), and speech and hearing impairment (Burg and Gist, 1999; White et al., 1997) are also observed when TCE exposure occurs *in utero*.

In animal studies, anatomical and clinical developmental neurotoxicity is also observed. Following inhalation exposures of 150 ppm to mice during mating and gestation, the specific gravity of offspring brains was significantly decreased at postnatal time points through the age of weaning; this effect did not persist to 1 month of age (Westergren et al., 1984). In studies reported by Taylor et al. (1985), Isaacson and Taylor (1989), and Noland-Gerbec et al. (1986), 312 mg/L exposures in drinking water that were initiated 2 weeks prior to mating and continued to the end of lactation resulted in (a) significant increase in exploratory behavior at postnatal Days 60 and 90, (b) reductions in myelination in the CA1 hippocampal region of offspring at weaning, and (c) significantly decreased uptake of 2-deoxyglucose in the rat brain at postnatal Day 21. Gestational exposures to mice (Fredriksson et al., 1993) resulted in significantly decreased rearing activity on postnatal Day 60, and dietary exposures during the course of a continuous breeding study in rats (George et al., 1986) found a significant trend toward increased time to cross the first grid in open field testing. In a study by Blossom et al. (2008), male mice exposed gestationally to TCE exhibited lower GSH levels and lower GSH:GSSG ratios which is also observed in mice that have more aggressive behaviors (Franco et al., 2006).

4.3.9. Mechanistic Studies of Trichloroethylene (TCE) Neurotoxicity

4.3.9.1. *Dopamine Neuron Disruption*

There are very recent laboratory animal findings resulting from short-term TCE exposures that demonstrate vulnerability of dopamine neurons in the brain to this chlorinated

hydrocarbon. The key limitation of these laboratory animal studies is that only 1 dosing regimen was included in each study. Moreover, there has been no systematic body of data to show that other chlorinated hydrocarbons such as tetrachloroethylene or aromatic solvents similarly target this cell type. Confidence in the limited data regarding dopamine neuron death and *in vivo* TCE exposure would be greatly enhanced by identifying a dose-response relationship. If indeed TCE can target dopamine neurons it would be anticipated that human exposure to this agent would result in elevated rates of parkinsonism. There are no systematic studies of this potential relationship in humans although one limited report attempted to address this possibility. Difficulties in subject recruitment into that study limit the weight that can be given to the results.

Endogenously formed chlorinated tetrahydro-beta-carbolines (TaClo) have been suggested to contribute to the development of Parkinson-like symptoms (Bringmann et al., 1992, 1995; Reiderer et al., 2002; Kochen et al., 2003). TaClo can be formed endogenously from metabolites of TCE such as trichloroacetaldehyde. TaClo has been characterized as a potent neurotoxicant to the dopaminergic system. Some research groups have hypothesized that Parkinson-like symptoms resulting from TCE exposure may occur through the formation of TaClo, but not enough evidence is available to determine if this mechanism occurs.

4.3.9.1.1. ***Dopamine neuron disruption: human studies.*** There are no human studies that present evidence of this effect. Nagaya et al. (1990) examined serum dopamine β-hydroxylase activity without differences observed in mean activities between control and exposed subjects. In the study, 84 male workers exposed to TCE were compared to 83 male age-matched controls. The workers had constantly used TCE in their jobs and their length of employment ranged from 0.1 to 34 years.

4.3.9.1.2. ***Dopamine neuron disruption: animal studies.*** There are limited data from mice and rats that suggest the potential for TCE to disrupt dopamine neurons in the basal ganglia (see Table 4-34). Gash et al. (2008) showed that TCE gavage in Fischer 344 rats ($n = 9$) at an exposure level of 1,000 mg/kg/d, 5 days/week, for 6 weeks yielded degeneration of dopamine neurons in the substantia nigra and alterations in dopamine turnover as reflected in a shift in dopamine metabolite to parent compound ratios. Guehl et al. (1999) reported similar findings in OF1 mice ($n = 10$) that were injected i.p. with 400 mg/kg/d TCE 5 days/week for 4 weeks. Each of these studies evaluated only a single dose level of TCE so that establishing a dose-response relationship is not possible. Consequently, these data are of limited utility in risk assessment because they do not establish the potency of TCE to damage dopamine neurons. They are important, however, in identifying a potential permanent impairment that might occur following

TCE exposure at relatively high exposure doses. They also identify a potential mechanism by which TCE could produce CNS injury.

Table 4-34. Summary of animal dopamine neuronal studies

Reference	Exposure route	Species/strain/ sex/number	Dose level/ exposure duration	NOAEL; LOAEL	Effects
Guehl et al., 1999	Intraperitoneal Administration	Mouse, OF1, male, 10	0 and 400 mg/kg; 5 d/wk, 4 wks	LOAEL: 400 mg/kg	Significant dopaminergic neuronal death in substantia nigra.
Gash et al., 2008	Oral gavage	Rat, Fischer 344, male, 9/group	0 and 1,000 mg/kg; 5 d/wk, 6 wks	LOAEL: 1,000 mg/kg	Degeneration of dopamine-containing neurons in substantia nigra. Change in dopamine metabolism.

4.3.9.1.3. ***Summary and conclusions of dopamine neuron studies.*** Only two animal studies have reported changes in dopamine neuron effects from TCE exposure (Gash et al., 2008; Guehl et al., 1999). Both studies demonstrated toxicity to dopaminergic neurons in the substantia nigra in rats or mice. LOAELs of 400 mg/kg (mice; Guehl et al., 1999) and 1,000 mg/kg (rats; Gash et al., 2008) were reported for this effect. Dopaminergic neuronal degeneration following TCE exposure has not been studied in humans. However, there were no changes in serum dopamine β-hydroxylase activity in TCE-exposed and control individuals (Nagaya et al., 1990). Loss of dopaminergic neurons in the substantia nigra also occurs in patients with Parkinson's disease and the substantia nigra is an important region in helping to control movements. As a result, loss of dopaminergic neurons in the substantia nigra may be one of the potential mechanisms involved in the clinical psychomotor effects that are observed following TCE exposure.

4.3.9.2. *Neurochemical and Molecular Changes*

There are limited data obtained only from laboratory animals that TCE exposure may have consequences on GABAergic (gamma-amino butyric acid [GABA]) and glutamatergic neurons (Briving et al., 1986; Shih et al., 2001; see Table 4-35). However, the data obtained are limited with respect to brain region examined, persistence of effect, and whether there might be functional consequences to these changes. The data of Briving et al. (1986) demonstrating changes in cerebellar high affinity uptake for GABA and glutamate following chronic low level (50 and 150 ppm) TCE exposure do not appear to be reflected in the only other brain region

evaluated (hippocampus). However, glutamate levels were increased in the hippocampus. The data of Shih et al. (2001) are indirect in that it shows an altered response to GABAergic antagonist drugs in mice treated by acute injection with 250, 500, 1,000, and 2,000 mg/kg TCE. However, these data do show some dose dependency with significant findings observed with TCE exposure as low as 250 mg/kg.

The development and physiology of the hippocampus has also been evaluated in two different studies (Isaacson and Taylor, 1989; Ohta et al., 2001). Isaacson and Taylor (1989) found a 40% decrease in myelinated fibers from hippocampi dissected from neonatal Sprague-Dawley rats ($n = 2-3$) that were exposed to TCE (4 and 8.1 mg/day) *in utero* and during the preweaning period. Ohta et al. (2001) injected male ddY mice with 300 mg/kg TCE and found a significant reduction in response to titanic stimuli in excised hippocampal slices. Both of these studies demonstrated that there is some interaction with TCE and the hippocampal area in the brain.

Impairment of sciatic nerve regeneration was demonstrated in mice and rats exposed to TCE (Kjellstrand et al., 1987). Under heavy anesthesia, the sciatic nerve of the animals was artificially crushed to create a lesion. Prior to the lesion, some animals were pre-exposed to TCE for 20 days and then for an additional 4 days after the lesion. Another set of animals were only exposed to TCE for 4 days following the sciatic nerve lesion. For mice, regeneration of the sciatic nerve in comparison to air-exposed animals was 20 and 33% shorter in groups exposed to 150- and 300-ppm TCE for 4 days, respectively. This effect did not significantly increase in mice pre-exposed to TCE for 20 days, and the regeneration was 30% shorter in the 150-ppm group and 22% shorter in the 300-ppm group. Comparatively, a 10% reduction in sciatic nerve regeneration length was observed in rats exposed to TCE for 20 days prior to the lesion plus the 4 days after the sciatic nerve lesion.

1 **Table 4-35. Summary of neurophysiological, neurochemical, and**
2 **neuropathological effects with TCE exposure**
3

Reference	Exposure route	Species/strain/ sex/number	Dose level/ exposure duration	NOAEL; LOAEL	Effects
Neurophysiological studies					
Shih et al., 2001	Intra-peritoneal	Mouse, MF1, male, 6/group	0, 250 500, 1,000, or 2,000 mg/kg, 15 min; followed by tail infusion of PTZ (5 mg/mL), picrotoxin (0.8 mg/mL), bicuculline (0.06 mg/mL), strychnine (0.05 mg/mL), 4-AP (2 mg/mL), or NMDA (8 mg/mL)	---	Increased threshold for seizure appearance with TCE pretreatment for all convulsants. Effects strongest on the $GABA_A$ antagonists, PTZ, picrotoxin, and bicuculline suggesting $GABA_A$ receptor involvement. NMDA and glycine Rc involvement also suggested.
Ohta et al., 2001	Intra-peritoneal	Mouse, ddY, male, 5/group	0, 300, or 1,000 mg/kg, sacrificed 24 hours after injection	LOAEL: 300 mg/kg	Decreased response (LTP response) to tetanic stimulation in the hippocampus.
Neurochemical studies					
Briving et al., 1986	Inhalation	Gerbils, Mongolian, male and female, 6/group	0, 50, or 150 ppm, continuous, 24 h/d, 12 months	NOAEL: 50 ppm; LOAEL: 150 ppm for glutamate levels in hippocampus NOAEL: 150 ppm for glutamate and GABA uptake in hippocampus LOAEL: 50 ppm for glutamate and GABA uptake in cerebellar vermis	Increased glutamate levels in the hippocampus. Increased glutamate and GABA uptake in the cerebellar vermis.
Subramoniam et al., 1989	Oral	Rat, Wistar, female,	0 or 1,000 mg/kg, 2 or 20 hours 0 or 1,000 mg/kg/d, 5 d/wk, 1 yr	---	PI and PIP2 decreased by 24 and 17% at 2 h. PI and PIP2 increased by 22 and 38% at 20 h. PI, PIP, and PIP2 reduced by 52,23, and 45% in 1 yr study.

1

Table 4-35. Summary of neurophysiological, neurochemical, and neuropathological effects with TCE exposure (continued)

Reference	Exposure route	Species/strain/ sex/number	Dose level/ exposure duration	NOAEL; LOAEL	Effects
Haglid et al., 1981	Inhalation	Gerbil, Mongolian, male and female, 6−7/group	0, 60, or 320 ppm, 24 h/d, 7 d/wk, 3 months	LOAEL: 60 ppm, brain protein changes NOAEL: 60 ppm; LOAEL: 320 ppm, brain DNA changes	(1) Decreases in total brain soluble protein whereas increase in S100 protein. (2) Elevated DNA in cerebellar vermis and sensory motor cortex.
Neuropathological studies					
Kjellstrand et al., 1987	Inhalation	Mouse, NMRI, male	0, 150, or 300 ppm, 24 h/d, 4 or 24 d	LOAEL: 150 ppm, 4 and 24 d	Sciatic nerve regeneration was inhibited in both mice and rats.
		Rat, Sprague-Dawley, female	0, 300 ppm, 24 h/d, 4 or 24 d	NOAEL: 300 ppm, 4 d LOAEL: 300 ppm, 24 d	
Isaacson and Taylor, 1989	Oral	Rat, Sprague-Dawley, females, 6 dams/group	0, 312, or 625 mg/L. (0, 4.0, or 8.1 mg/d) Dams (and pups) exposed from 14 d prior to mating until end of lactation.	LOAEL: 312 mg/L	Significant ↓ myelinated fibers in the stratum lacunosum-moleculare of pups. Reduction in myelin in the hippocampus.

2
3 PTZ = pentylenetetrazole.
4
5
6 There are also a few *in vitro* studies (summarized in Table 4-36) that have demonstrated
7 that TCE exposure alters the function of inhibitory ion channels such as $GABA_A$ and glycine
8 receptors (Krasowski and Harrison, 2000; Beckstead et al., 2000), and serotonin receptors
9 (Lopreato et al., 2003). Krasowski and Harrison (2000) and Beckstead et al. (2000) were able to
10 demonstrate that human $GABA_A$ and glycine receptors could be potentiated by TCE when a
11 receptor agonist was coapplied. Krasowski and Harrison (2000) conducted an additional
12 experiment in order to determine if TCE was interacting with the receptor or perturbating the
13 cellular membrane (bilipid layer). Specific amino acids on the $GABA_A$ and glycine receptors
14 were mutated and in the presence of a receptor agonist (GABA for $GABA_A$ and glycine for
15 glycine receptors) and in these mutated receptors TCE-mediated potentiation was significantly
16 decreased or abolished suggesting that there was an interaction between TCE and these
17 receptors. Lopreato et al. (2003) conducted a similar study with the $5HT_{3A}$ serotonin receptor
18 and found that when TCE was coapplied with serotonin, there was a potentiation in receptor

response. Additionally, TCE has been demonstrated to alter the function of voltage sensitive calcium channels (VSCCs) by inhibiting the calcium mediated-current at a holding potential of -70 mV and shifting the activation of the channels to a more hyperpolarizing potential (Shafer et al., 2005).

Table 4-36. Summary of *in vitro* ion channel effects with TCE exposure

Reference	Cellular system	Neuronal channel/ receptor	Concentrations	Effects
In vitro studies				
Shafer et al., 2005	PC12 cells	VSCC	0, 500, 1,000, 1,500, or 2,000 μM	Shift of VSCC activation to a more hyperpolarizing potential. Inhibition of VSCCs at a holding potential of -70 mV.
Beckstead et al., 2000	*Xenopus* oocytes	Human recombinant: glycine receptor α1, $GABA_A$ receptors, α1β1, α1β2γ2L	0 or 390 μM	50% potentiation of the $GABA_A$ receptors; 100% potentiation of the glycine receptor.
Lopreato et al., 2003	*Xenopus* oocytes	Human recombinant serotonin 3A receptor	0 or 390 μM	Potentiation of serotonin receptor function.
Krasowski and Harrison, 2000	Human embryonic kidney 293 cells	Human recombinant Glycine receptor α1, $GABA_A$ receptors α2β1	Not provided	Potentiation of glycine receptor function with an EC_{50} of 0.65 ± 0.05 mM. Potentiation of $GABA_A$ receptor function with an EC_{50} of 0.85 ± 0.2 mM.

EC_{50} = concentration of the chemical at which 50% of the maximal effect is produced.

4.3.10. Potential Mechanisms for Trichloroethylene (TCE)-Mediated Neurotoxicity

The mechanisms of TCE neurotoxicity have not been established despite a significant level of research on the outcomes of TCE exposure. Results from several mechanistic studies can be used to help elucidate the mechanism(s) involved in TCE-mediated neurological effects.

The disruption of the trigeminal nerve appears to be a highly idiosyncratic outcome of TCE exposure. There are limited data to suggest that it might entail a demyelination phenomenon, but similar demyelination does not appear to occur in other nerve tracts. In this regard, then, TCE is unlike a variety of hydrocarbons that have more global demyelinating action. There are some data from central nervous system that focus on shifts in lipid profiles as well as data showing loss of myelinated fibers in the hippocampus. However, the changes in lipid profiles are both quite small and, also, inconsistent. And the limited data from

hippocampus are not sufficient to conclude that TCE has significant demyelinating effects in this key brain region. Indeed, the bulk of the evidence from studies of learning and memory function (which would be tied to hippocampal function) suggests no clear impairments due to TCE.

Some researchers (Albee et al., 1997, 2006; Barret et al., 1991, 1992; Laureno, 1988, 1993) have indicated that changes in trigeminal nerve function may be due to dichloroacetylene which is formed under nonbiological conditions of high alkalinity or temperature during volatilization of TCE. In experimental settings, trigeminal nerve function (Albee et al., 1997) and trigeminal nerve morphology (Barret et al., 1991, 1992) was found to be more altered following a low exposure to dichloroacetylene in comparison to the higher TCE exposure. Barret et al. (1991, 1992) also demonstrated that TCE administration results in morphological changes in the trigeminal nerve. Thus, dichloroacetylene may contribute to trigeminal nerve impairment may be plausible following an inhalation exposure under conditions favoring its formation. Examples of such conditions include passing through a carbon dioxide scrubber containing alkaline materials, application to remove a wax coating from a concrete-lined stone floor, or mixture with alkaline solutions or caustic (Saunders, 1967; Greim et al., 1984; Bingham et al., 2001). However, dichloroacetylene exposures have not been identified or measured in human epidemiologic studies with TCE exposure, and thus, do not appear to be common to occupational or residential settings (Lash and Green, 1993). Moreover, changes in trigeminal nerve function have also been consistently reported in humans exposed to TCE following an oral exposure (Kilburn, 2002a; across many human studies of occupational and drinking water exposures under conditions with highly varying potentials for dichloroacetylene formation (Barret et al, 1982, 1984, 1987; Feldman et al., 1988). As a result, the mechanism(s) for trigeminal nerve function impairment following TCE exposure is unknown., 1992; Kilburn and Warshaw, 1993; Kilburn, 2002a; Mihri et al., 2004; Ruitjen et al., 1991). The varying dichloroacetylene exposure potential across these studies suggests TCE exposure, which is common to all of them, as the most likely etiologic agent for the observed effects.

The clearest consequences of TCE are permanent impairment of hearing in animal models and disruption of trigeminal nerve function in humans with animal models showing comparable changes following administration of a TCE metabolite. With regard to hearing loss, the effect of TCE has much in common with the effects of several aromatic hydrocarbons including ethylbenzene, toluene, and *p*-xylene. Many studies have attempted to determine how these solvents damage the cochlea. Of the hypotheses that have been advanced, there is little evidence to suggest oxidative stress, changes in membrane fluidity, or impairment of central efferent nerves whose endings innervate receptor cells in the cochlea. Rather, for reasons that are still uncertain these solvents seem to preferentially target supporting cells in the cochlea

1 whose death then alters key structural elements of the cochlea resulting ultimately in hair cell
2 displacement and death. Recently, potential modes of action resulting in ototoxicity have been
3 speculated to be due to blockade of neuronal nicotinic receptors present on the auditory cells
4 (Campo et al., 2007) and potentially changes in calcium transmission (Campo et al., 2008) from
5 toluene exposure. Although these findings were reported following an acute toluene exposure, it
6 is speculated that this mechanism may be a viable mechanism for TCE -mediated ototoxicity.
7 A few studies have tried to relate TCE exposure with selective impairments of dopamine
8 neurons. Two studies (Gash et al., 2008; Guehl et al., 1999) demonstrated dopaminergic
9 neuronal death and/or degeneration following an acute TCE administration. However, the only
10 human TCE exposure study examining dopamine neuronal activity found no changes in serum
11 dopamine β-hydroxylase activity in comparison to nonexposed individuals (Nagaya et al., 1990).
12 It is thought that TaClo, which can be formed from TCE metabolites such as
13 trichloroacetaldehyde, may be the potent neurotoxicant that selectively targets the dopaminergic
14 system. More studies are needed to confirm the dopamine neuronal function disruption and if
15 this disruption is mediated through TaClo.
16 There is good evidence that TCE and certain metabolites such as choral hydrate have
17 CNS depressant properties and may account for some of the behavioral effects (such as
18 vestibular effects, psychomotor activity changes, central visual changes, sleep and mood
19 changes) that have been observed with TCE. Specifically, *in vitro* studies have demonstrated
20 that TCE exposure results in changes in neuronal receptor function for the $GABA_A$, glycine, and
21 serotonin receptors (Krasowski and Harrison, 2000; Beckstead et al., 2000; Lopreato et al.,
22 2003). All of these inhibitory receptors that are present in the CNS are potentiated when
23 receptor-specific agonist and TCE are applied. These results are similar to other anesthetics and
24 suggest that some of the behavioral functions are mediated by modifications in ion channel
25 function. However, it is quite uncertain whether there are persistent consequences to such high
26 dose TCE exposure. Additionally, with respect to the GABAergic system, acute administration
27 of TCE increased the seizure threshold appearance and this effect was the strongest with
28 convulsants that were GABA receptor antagonists (Shih et al., 2001). Therefore, this result
29 suggests that TCE interacts with the GABA receptor and that was also verified *in vitro*
30 (Krasowski and Harrison, 2000; Beckstead et al., 2000).
31 Also, TCE exposure has been linked to decreased sensitivity to titanic stimulation in the
32 hippocampus (Ohta et al., 2001) as well as significant reduction in myelin in the hippocampus in
33 a developmental exposure (Isaacson and Taylor, 1990). These effects are notable since the
34 hippocampus is highly involved in memory and learning functions. Changes in the hippocampal

physiology may correlate with the cognitive changes that were reported following TCE exposure.

4.3.11. Overall Summary and Conclusions—Weight of Evidence

Both human and animal studies have associated TCE exposure with effects on several neurological domains. The strongest neurological evidence of hazard in humans is for changes in trigeminal nerve function or morphology and impairment of vestibular function. Fewer and more limited evidence exists in humans on delayed motor function, and changes in auditory, visual, and cognitive function or performance. Acute and subchronic animal studies show morphological changes in the trigeminal nerve, disruption of the peripheral auditory system leading to permanent function impairments and histopathology, changes in visual evoked responses to patterns or flash stimulus, and neurochemical and molecular changes. Additional acute studies reported structural or functional changes in hippocampus, such as decreased myelination or decreased excitability of hippocampal CA1 neurons, although the relationship of these effects to overall cognitive function is not established. Some evidence exists for motor-related changes in rats/mice exposed acutely/subchronically to TCE, but these effects have not been reported consistently across all studies.

Epidemiologic evidence supports a relationship between TCE exposure and trigeminal nerve function changes, with multiple studies in different populations reporting abnormalities in trigeminal nerve function in association with TCE exposure (Barret et al., 1982, 1984, 1987; Feldman et al., 1988, 1992; Kilburn and Warshaw, 1993; Ruitjen et al., 2001; Kilburn, 2002a; Mhiri et al., 2004). Of these, two well conducted occupational cohort studies, each including more than 100 TCE-exposed workers without apparent confounding from multiple solvent exposures, additionally reported statistically significant dose-response trends based on ambient TCE concentrations, duration of exposure, and/or urinary concentrations of the TCE metabolite TCA (Barret et al., 1984; Barret et al., 1987). Limited additional support is provided by a positive relationship between prevalence of abnormal trigeminal nerve or sensory function and cumulative exposure to TCE (most subjects) or CFC-113 (<25% of subjects) (Rasmussen et al., 1993c). Test for linear trend in this study was not statistically significant and may reflect exposure misclassification since some subjects included in this study did not have TCE exposure. The lack of association between TCE exposure and overall nerve function in three small studies (trigeminal: El-Ghawabi et al., 1973; ulnar and medial: Triebig et al., 1982, 1983) does not provide substantial evidence against a causal relationship between TCE exposure and trigeminal nerve impairment because of limitations in statistical power, the possibility of exposure misclassification, and differences in measurement methods. Laboratory animal studies have also

shown TCE-induced changes in the trigeminal nerve. Although one study reported no significant changes in trigeminal somatosensory evoked potential in rats exposed to TCE for 13 weeks (Albee et al., 2006), there is evidence of morphological changes in the trigeminal nerve following short-term exposures in rats (Barret et al., 1991, 1992).

Human chamber, occupational, geographic based/drinking water, and laboratory animal studies clearly established TCE exposure causes transient impairment of vestibular function. Subjective symptoms such as headaches, dizziness, and nausea resulting from occupational (Granjean et al., 1955; Liu et al., 1988; Rasmussen and Sabroe, 1986; Smith et al., 1970), environmental (Hirsch et al., 1996), or chamber exposures (Stewart et al., 1970; Smith et al., 1970) have been reported extensively. A few laboratory animal studies have investigated vestibular function, either by promoting nystagmus or by evaluating balance (Niklasson et al., 1993; Tham et al., 1979; Tham et al., 1984; Umezu et al., 1997).

In addition, mood disturbances have been reported in a number of studies, although these effects also tend to be subjective and difficult to quantify (Gash et al., 2007; Kilburn and Warshaw, 1993; Kilburn, 2002a, 2002b; McCunney et al., 1988; Mitchell et al., 1969; Rasmussen and Sabroe, 1986; Troster and Ruff, 1990), and a few studies have reported no effects from TCE on mood (Reif et al., 2003; Triebig et al., 1976, 1977a). Few comparable mood studies are available in laboratory animals, although both Moser et al. (2003) and Albee et al. (2006) report increases in handling reactivity among rats exposed to TCE. Finally, significantly increased number of sleep hours was reported by Arito et al. (1994) in rats exposed via inhalation to 50–300-ppm TCE for 8 hours/day for 6 weeks.

Four epidemiologic studies of chronic exposure to TCE observed disruption of auditory function. One large occupational cohort study showed a statistically significant difference in auditory function with cumulative exposure to TCE or CFC-113 as compared to control groups after adjustment for possible confounders, as well as a positive relationship between auditory function and increasing cumulative exposure (Rasmussen et al., 1993b). Of the three studies based on populations from ATSDR's TCE Subregistry from the National Exposure Registry, more limited than Rasmussen et al. (1993b) due to inferior exposure assessment, Burg et al. (1995) and Burg and Gist (1999) reported a higher prevalence of self-reported hearing impairments. The third study reported that auditory screening revealed abnormal middle ear function in children less than 10-years-of-age, although a dose-response relationship could not be established and other tests did not reveal differences in auditory function (ATSDR, 2003a). Further evidence for these effects is provided by numerous laboratory animal studies demonstrating that high dose subacute and subchronic TCE exposure in rats disrupts the auditory system leading to permanent functional impairments and histopathology.

Studies in humans exposed under a variety of conditions, both acutely and chronically, report impaired visual functions such as color discrimination, visuospatial learning tasks, and visual depth perception in subjects with TCE exposure. Abnormalities in visual depth perception were observed with a high acute exposure to TCE under controlled conditions (Vernon and Ferguson, 1969). Studies of lower TCE exposure concentrations also observed visuofunction effects. One occupational study (Rasmussen et al., 1993b) reported a statistically significant positive relationship between cumulative exposure to TCE or CFC-113 and visual gestalts learning and retention among Danish degreasers. Two studies of populations living in a community with drinking water containing TCE and other solvents furthermore suggested changes in visual function (Kilburn et al., 2002a; Reif et al., 2003). These studies used more direct measures of visual function as compared to Rasmussen et al. (1993b), but their exposure assessment is more limited because TCE exposure is not assigned to individual subjects (Kilburn et al., 2002a), or because there are questions regarding control selection (Kilburn et al., 2002a) and exposure to several solvents (Kilburn et al., 2002a; Reif et al., 2003).

Additional evidence of effects of TCE exposure on visual function is provided by a number of laboratory animal studies demonstrating that acute or subchronic TCE exposure causes changes in visual evoked responses to patterns or flash stimulus (Boyes et al., 2003, 2005; Blain et al., 1994). Animal studies have also reported that the degree of some effects is correlated with simultaneous brain TCE concentrations (Boyes et al., 2003, 2005) and that, after a recovery period, visual effects return to control levels (Blain et al., 1994; Rebert et al., 1991). Overall, the human and laboratory animal data together suggest that TCE exposure can cause impairment of visual function, and some animal studies suggest that some of these effects may be reversible with termination of exposure.

Studies of human subjects exposed to TCE either acutely in chamber studies or chronically in occupational settings have observed deficits in cognition. Five chamber studies reported statistically significant deficits in cognitive performance measures or outcome measures suggestive of cognitive effects (Stewart et al., 1970; Gamberale et al., 1976; Triebig et al., 1976, 1977a; Gamberale et al., 1977). Danish degreasers with high cumulative exposure to TCE or CFC-113 had a high risk (OR: 13.7, 95% CI: 2.0–92.0) for psychoorganic syndrome characterized by cognitive impairment, personality changes, and reduced motivation, vigilance, and initiative compared to workers with low cumulative exposure. Studies of populations living in a community with contaminated groundwater also reported cognitive impairments (Kilburn and Warshaw, 1993; Kilburn, 2002a), although these studies carry less weight in the analysis because TCE exposure is not assigned to individual subjects and their methodological design is weaker.

Laboratory studies provide some additional evidence for the potential for TCE to affect cognition, though the predominant effect reported has been changes in the time needed to complete a task, rather than impairment of actual learning and memory function (Kulig et al., 1987; Kishi et al., 1993; Umezu et al., 1997). In addition, in laboratory animals, it can be difficult to distinguish cognitive changes from motor-related changes. However, several studies have reported structural or functional changes in the hippocampus, such as decreased myelination (Issacson et al., 1990; Isaacson and Taylor, 1989) or decreased excitability of hippocampal CA1 neurons (Ohta et al., 2001), although the relationship of these effects to overall cognitive function is not established.

Two studies of TCE exposure, one chamber study of acute exposure duration and one occupational study of chronic duration, reported changes in psychomotor responses. The chamber study of Gamberale et al. (1976) reported a dose-related decrease in performance in a choice reaction time test in healthy volunteers exposed to 100- and 200-ppm TCE for 70 minutes as compared to the same subjects without exposure. Rasmussen et al. (1993c) reported a statistically significant association with cumulative exposure to TCE or CFC-113 and dyscoordination trend among Danish degreasers. Observations in a third study (Gun et al., 1978) are difficult to judge given the author's lack of statistical treatment of data. In addition, Gash et al. (2007) reported that 14 out of 30 TCE-exposed workers exhibited significantly slower fine motor hand movements as measured through a movement analysis panel test. Studies of population living in communities with TCE and other solvents detected in groundwater supplies reported significant delays in simple and choice reaction times in individuals exposed to TCE in contaminated groundwater as compared to referent groups (Kilburn, 2002a; Kilburn and Warshaw, 1993; Kilburn and Thornton, 1996). Observations in these studies are more uncertain given questions of the representativeness of the referent population, lack of exposure assessment to individual study subjects, and inability to control for possible confounders including alcohol consumption and motivation. Finally, in a presentation of 2 case reports, decrements in motor skills as measured by the grooved pegboard and finger tapping tests were observed (Troster and Ruff, 1990).

Laboratory animal studies of acute or subchronic exposure to TCE observed psychomotor effects, such as loss of righting reflex (Umezu et al., 1997; Shih et al., 2001) and decrements in activity, sensory-motor function, and neuromuscular function (Kishi et al., 1993; Moser et al., 1995; Moser et al., 2003). However, two studies also noted an absence of significant changes in some measures of psychomotor function (Kulig et al., 1987; Albee et al., 2006). In addition, less consistent results have been reported with respect to locomotor activity in rodents. Some studies have reported increased locomotor activity after an acute i.p. dosage (Wolff and Siegmund,

1978) or decreased activity after acute or short term oral gavage dosing (Moser et al., 1995, 2003). No change in activity was observed following exposure through drinking water (Waseem et al., 2001), inhalation (Kulig et al., 1987) or orally during the neurodevelopment period (Fredriksson et al., 1993).

Several neurochemical and molecular changes have been reported in laboratory investigations of TCE toxicity. Kjellstrand et al. (1987) reported inhibition of sciatic nerve regeneration in mice and rats exposed continuously to 150-ppm TCE via inhalation for 24 days. Two studies have reported changes in GABAergic and glutamatergic neurons in terms of GABA or glutamate uptake (Briving et al., 1986) or response to GABAergic antagonistic drugs (Shih et al., 2001) as a result of TCE exposure, with the Briving et al. (1986) conducted at 50 ppm for 12 months. Although the functional consequences of these changes is unclear, Tham et al. (1979, 1984) described central vestibular system impairments as a result of TCE exposure that may be related to altered GABAergic function. In addition, several *in vitro* studies have demonstrated that TCE exposure alters the function of inhibitory ion channels such as receptors for $GABA_A$ glycine, and serotonin (Krasowski and Harrison, 2000; Beckstead et al., 2000; Lopreato et al., 2003) or of voltage-sensitive calcium channels (Shafer et al., 2005).

4.4. KIDNEY TOXICITY AND CANCER

4.4.1. Human Studies of Kidney

4.4.1.1. *Nonspecific Markers of Nephrotoxicity*

Investigations of nephrotoxicity in human populations show that highly exposed workers exhibit evidence of damage to the proximal tubule (NRC, 2006). The magnitude of exposure needed to produce kidney damage is not clear. Observation of elevated excretion of urinary proteins in the four studies (Brüning et al., 1999a, b; Bolt et al., 2004; Green et al., 2004) indicates the occurrence of a toxic insult among TCE-exposed subjects compared to unexposed controls. Two studies are of subjects with previously diagnosed kidney cancer (Brüning et al., 1999a; Bolt et al., 2004), subjects in Brüning et al. (1999b) and Green et al. (2004) are disease free. Urinary proteins are considered nonspecific markers of nephrotoxicity and include α1-Microglobulin, albumin, and *N*-acetyl-β-D-glucosaminidase (NAG; Price et al., 1999, 1996; Lybarger et al., 1999). Four studies measure α1-microglobulin with elevated excretion observed in the German studies (Brüning et al., 1999a, b; Bolt et al., 2004) but not Green et al. (2004). However, Green et al. (2004) found statistically significant group mean differences in NAG, another nonspecific marker of tubular toxicity, in disease free subjects. Observations in Green et al. (2004) provide evidence of tubular damage among workers exposed to trichloroethylene at 32 ppm (mean) (range, 0.5−252 ppm). Elevated excretion of NAG as a nonspecific marker of

tubular damage has also been observed with acute TCE poisoning (Carrieri et al., 2007). These and other studies relevant to evaluating TCE nephrotoxicity are discussed in more detail below.

Biological monitoring of persons who previously experienced "high" exposures to trichloroethylene (100−500 ppm) in the workplace show altered kidney function evidenced by urinary excretion of proteins suggestive of renal tubule damage. Similar results were observed in the only study available of subjects with TCE exposure at current occupational limits (NRC, 2006). Table 4-37 provides details and results from these studies. Brüning et al. (1999a) report a statistically significantly higher prevalence of elevated proteinuria suggestive of severe tubular damage ($n = 24$, 58.5%, $p < 0.01$) and an elevated excretion of α1-microglobin, another urinary biomarker of renal tubular function, was observed in 41 renal cell carcinoma cases with prior trichloroethylene exposure and with pending workman's compensation claims compared with the nonexposed renal cell cancer patients ($n = 14$, 28%) and to hospitalized surgical patients $n = 2$, 2%). Statistical analyses did not adjust for differences in median systolic and diastolic blood pressure that appeared higher in exposed renal cell carcinoma cases compared to nonexposed controls. Similarly, severe tubular proteinuria is seen in 14 of 39 workers (35%) exposed to trichloroethylene in the electrical department, fitters shop and through general degreasing operations of felts and sieves in a cardboard manufacturing factory compared to no subjects of 46 nonexposed males office and administrative workers from the same factory ($p < 0.01$) (Brüning et al., 1999b). Furthermore, slight tubular proteinuria is seen in 20% of exposed workers and in 2% of nonexposed workers (Brüning et al., 1999b). Exposed subjects also had statistically significantly elevated levels of α1-microglobulin compared to unexposed controls. Furthermore, subjects with tubular damage as indicated by urinary protein patterns had higher GST-alpha concentrations than nonexposed subjects ($p < 0.001$). Both sex and use of spot or 24-hour urine samples are shown to influence α1-microglobulin (Andersson et al., 2008); however, these factors are not considered to greatly influence observations given only males were subjects and α1-microglobulin levels in spot urine sample are adjusted for creatinine concentration.

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Table 4-37. Summary of human kidney toxicity studies

Subjects	Effect	Exposure	Reference
206 subjects- 104 male workers exposed to TCE; 102 male controls (source not identified)	Increased β2-microglobulin and total protein in spot urine specimen. β2-microglobulin: Exposed, 129.0 ± 113.3 mg/g creatinine (Cr) Controls, 113.6 ± 110.6 mg/g Cr Total protein: Exposed, 83.4 ± 113.2 mg/g creatinine (Cr) Controls, 54.0 ± 18.6 mg/g Cr	TCE exposure was through degreasing activities in metal parts factory or semiconductor industry. U-total trichloro compounds: Exposed, 83.4 mg/g Cr (range, 2−66.2 mg/g Cr. Controls, N.D. 8.4 ± 7.9 yrs mean employment duration.	Nagaya et al., 1989
29 metal workers	NAG in morning urine specimen, 0.17 ± 0.11 U/mmol Cr	Breathing zone monitoring, 3 ppm (median) and 5 ppm (mean).	Seldén et al., 1993
191 subjects- 41 renal cell carcinoma cases pending cases involving compensation with TCE exposure; 50 unexposed renal cell carcinoma cases from same area as TCE-exposed cases; 100 nondiseased control and hospitalized surgical patients	Increased urinary proteins patterns, α1-microglobulin, and total protein in spot urine specimen Slight/severe tubular damage: TCE RCC cases, 93% Nonexposed RCC cases, 46% Surgical controls, 11% $p < 0.01$ α1-microglobulin (mg/g creatinine): Exposed RCC cases, 24.6 ± [SD] 13.9 Unexposed RCC cases, 11.3 ± [SD] 9.8 Surgical controls, 5.5 ± [SD] 6.8	All exposed RCC cases exposed to 'high" and "very high" TCE intensity. 18 yr mean exposure duration.	Brüning et al., 1999a
85 male workers employed in cardboard manufacturing factory (39 TCE exposed, 46) nonexposed office and administrative controls)	Increased urinary protein patterns and excretion of proteins in spot urine specimen Slight/severe tubular damage: TCE exposed, 67% Nonexposed, RCC cases, 9% $p < 0.001$ α1-microglobulin (mg/g creatinine): Exposed, 16.2 ± [SD] 10.3 Unexposed, 7.8 ± [SD] 6.9 $p < 0.001$ GST-alpha (μg/g creatinine): Exposed 6.0 ± [SD] 3.3 Unexposed, 2.0 ± [SD] 0.57 $p < 0.001$ No group differences in total protein or GST-pi	"High" TCE exposure to workers in the fitters shop and electrical department. "Very high" TCE exposure to workers through general degreasing operations in carton machinery section.	Brüning et al., 1999b

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Table 4-37. Summary of human kidney toxicity studies (continued)

Subjects	Effect	Exposure	Reference
99 renal cell carcinoma cases and 298 hospital controls (from Brüning et al. [2003] and alive at the time of interview)	Increased excretion of α1-microglobulin in spot urine specimen Proportion of subjects with α1-microglobulin <5.0 mg/L: Exposed cases, 15% Unexposed cases, 51% Exposed controls, 55% Unexposed controls, 55% $p < 0.05$, prevalence of exposed cases compared to prevalences of either exposed controls or unexposed controls Mean α1-microglobulin: Exposed cases, 18.1 mg/L Unexposed cases, <5.0 mg/L $p < 0.05$	All exposed RCC cases exposed to 'high" and "very high" TCE intensity .	Bolt et al., 2004
124 subjects (70 workers currently exposed to TCE and 54 hospital and administrative staff controls)	Analysis of urinary proteins in spot urine sample obtained 4 d after exposure Increased excretion of albumin, NAG, and formate in spot urine specimen Albumin (mg/g creatinine):[a] Exposed, 9.71 ± [SD] 11.6 Unexposed, 5.50 ± [SD] 4.27 $p < 0.05$ Total NAG (U/g creatinine): Exposed, 5.27 ± [SD] 3.78 Unexposed, 2.41 ± [SD] 1.91 $p < 0.01$ Format (mg/g creatinine): Exposed, 9.45 ± [SD] 4.78 Unexposed, 5.55 ± [SD] 3.00 $p < 0.01$ No group mean differences in GST-alpha, retinol binding protein, α1-microglobulin, β2-microglobulin, total protein, and methylmalonic acid	Mean U-TCA of exposed workers was 64 ± [SD] 102 (Range, 1−505). Mean U-TCOH of exposed workers was 122 ± [SD] 119 (Range, 1−639). Mean TCE concentration to exposed subjects was estimated as 32 ppm (range, 0.5−252 ppm) and was estimated by applying the German occupational exposure limit (maximale arbeitsplatz konzentration, MAK) standard to U-TCA and assuming that the linear relationship holds for exposures above 100 ppm. 86% of subjects with exposure to <50 ppm TCE.	Green et al., 2004

Table 4-37. Summary of human kidney toxicity studies (continued)

Subjects	Effect	Exposure	Reference
101 cases or deaths from end-stage renal disease (ESDR) among male and female subjects in Hill Air Force Base aircraft maintenance worker cohort of Blair et al. (1998)	TCE exposure: Cox Proportional Hazard Analysis: Ever exposed to TCE,[b] 1.86 (1.02, 3.39) Logistic regression:[b] No chemical exposure (referent group): 1.0 <5 unit-year, 1.73 0.86, 3.48) 5−25 unit-year, 1.65 (0.82, 3.35) >25 unit-year, 1.65 (0.82, 3.35) Monotonic trend test, $p > 0.05$ Indirect low-intermittent TCE exposure, 2.47 (1.17, 5.19) Indirect peak/infrequent TCE exposure 3.55 (1.25, 10.74) Direct TCE exposure, "not statistically significant" but hazard ratio and confidence intervals were not presented in paper	Cumulative TCE exposure (intensity × duration) identified using 3 categories, <5 unit-year, 5−25 unit year, >25 unit-year per job exposure matrix of Stewart et al. (1991).	Radican et al., 2006

[a]For a urine sample, 10-17 mg of albumin per g of creatinine is considered to be suspected albuminuria in males (15−25 in females) (De Jong and Brenner 2004).
[b]Hazard ratio and 95% confidence interval.

N.D. = not detectable, SD = standard deviation.

Bolt et al. (2004) measured α1-microglobulin excretion in living subjects from the renal cell carcinoma case-control study by Brüning et al. (2003). Some subjects in this study were highly exposed. Of the 134 with renal cell cancer, 19 reported past exposures that led to narcotic effects and 18 of the 401 controls, experienced similar effects (OR: 3.71, 95% CI: 1.80−7.54) (Brüning et al., 2003). Bolt et al. (2004) found that α1-microglobulin excretion increased in exposed renal cancer patients compared with nonexposed patients controls. A lower proportion of exposed cancer patients had normal α1-microglobulin excretion, less than 5 mg/L, the detection level for the assay and the level considered by these investigators as associated with no clinical or subclinical tubule damage, and a higher proportion of high values, defined as ≥45 mg/L, compared to cases who did not report TCE occupational exposure and to nonexposed controls ($p < 0.05$). Exposed cases, additionally, had statistically significantly higher median concentration of α1-microglobulin compared to unexposed cases in creatinine-unadjusted spot urine specimens ($p < 0.05$). Reduced clearance of creatinine attributable to renal cancer does not

explain the lower percentage of normal values among exposed cases given findings of similar prevalence of normal excretion among unexposed renal cell cases and controls.

In their study of 70 current employees (58 males, 12 females) of an electronic factory with trichloroethylene exposure and 54 (50 males, 4 females) age-matched subjects drawn from hospital or administrative staff, Green et al. (2004) found that urinary excretion of albumin, total NAG and formate were increased in the exposed group compared with the unexposed group.[1] No differences between exposed and unexposed subjects were observed in other urinary proteins, including α1-microglobulin, β2-microglobulin, and GST-alpha. Green et al. (2004) stated that NAG is not an indicator of nephropathy, or damage, but rather is an indicator of functional change in the kidney. Green et al. (2004) further concluded that increased urinary albumin or NAG was not related to trichloroethylene exposure; analyses to examine the exposure-response relationship found neither NAG or albumin concentration correlated to U-TCA or employment duration (years). The National Research Council (NRC, 2006) did not consider U-TCA as sufficiently reliable to use as a quantitative measure of TCE exposure, concluding that the data reported by Green et al. (2004) were inadequate to establish exposure-response information because the relationship between U-TCA and ambient TCE intensity is highly variable and nonlinear, and conclusions about the absence of association between TCE and nephrotoxicity can not be made based on U-TCA. Moreover, use of employment duration does not consider exposure intensity differences between subjects with the same employment duration, and bias introduced through misclassification of exposure may explain the Green et al. (2004) findings.

Seldén et al. (1993) in their study of 29 metal workers (no controls) reported a correlation between NAG and U-TCA ($r = 0.48$, $p < 0.01$) but not with other exposure metrics of recent or long-term exposure. Personal monitoring of worker breath indicated median and mean time-weighted-average TCE exposures of 3 and 5 ppm, respectively. Individual NAG concentrations were within normal reference values. Rasmussen et al. (1993), also, reported a positive relationship ($p = 0.05$) between increasing urinary NAG concentration (adjusted for creatinine clearance) and increasing duration in their study of 95 metal degreasers (no controls) exposed to either TCE (70 subjects) or CFC113(25 subjects). Multivariate regression analyses which adjusted for age were suggestive of an association between NAG and exposure duration ($p = 0.011$). Mean urinary NAG concentration was higher among subjects with annual exposure of >30 hours/week, defined as peak exposure, compared to subjects with annual exposure of less

[1] Elevation of NAG in urine is a sign of proteinuria, and proteinuria is both a sign and a cause of kidney malfunction (Zandi-Nejad et al., 2004). For a urine sample, 10–17 mg of albumin per g of creatinine is considered to be suspected albuminuria in males (15–25 in females) (De Jong and Brenner, 2004).

than <30 hours/week (72.4 ± 44.1 μg/g creatinine compared to 45.9 ± 30.0 μg/g creatinine, $p < 0.01$).

Nagaya et al. (1989) did not observe statistically significant group differences in urinary β2-microglobulin and total protein in spot urine specimens of male degreasers and their controls, nor were these proteins correlated with urinary total trichloro-compounds (U-TTC). The paper lacks details on subject selection, whether urine collection was at start of work week or after sufficient exposure, and presentation of *p*-values and correlation coefficients. The presentation of urinary protein concentrations stratified by broad age groups is less statistically powerful than examination of this confounder using logistic regression. Furthermore, although valid for pharmacokinetic studies, examination of renal function using U-TTC as a surrogate for TCE exposure is uncertain, as discussed above for Green et al. (2004).

4.4.1.2. *End-Stage Renal Disease*

End-stage renal disease is associated with hydrocarbon exposure, a group that includes trichloroethylene, 1,1,1-trichloroethane, and JP4 (jet propellant 4), in the one study examining this endpoint (Radican et al., 2006). Table 4-37 provides details and results from Radican et al. (2006). This study assessed end-stage renal disease in a cohort of aircraft maintenance workers at Hill Air Force Base (Blair et al., 1998) with strong exposure assessment to trichloroethylene (NRC, 2006). Other occupational studies do not examine end-stage renal disease specifically, instead reporting relative risks associated with deaths due to nephritis and nephrosis (Boice et al., 1999, 2006; ATSDR, 2004), all genitourinary system deaths (Garabrant et al., 1988; Costa et al., 1989; Ritz, 1999), or providing no information on renal disease mortality in the published paper (Blair et al., 1998; Morgen et al., 1998; Chang et al., 2003).

4.4.2. Human Studies of Kidney Cancer

Cancer of the kidney and renal pelvis is the 6th leading cause of cancer in the United States with an estimated 54,390 (33,130 men and 21,260 women) newly diagnosed cases and 13,010 deaths (Jemal et al., 2008; Ries et al., 2008). Age-adjusted incidence rates based on cases diagnosed in 2001−2005 from 17 Surveillance, Epidemiology, and End Results (SEER) geographic areas are 18.3 per 100,000 for men and 9.2 per 100,000 for women. Age-adjusted mortality rates are much lower; 6.0 per 100,000 for men and 2.7 for women.

Cohort, case-control, and geographical studies have examined trichloroethylene and kidney cancer, defined either as cancer of kidney and renal pelvis in cohort and geographic based studies or as renal cell carcinoma, the most common type of kidney cancer, in case-control studies. Appendix C identifies these studies' design and exposure assessment characteristics.

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Observations in these studies are presented below in Table 4-38. Rate ratios for incidence studies in Table 4-38 are, generally, larger than for mortality studies.

Additionally, a large body of evidence exists on kidney cancer risk and either job or industry titles where trichloroethylene usage has been documented. TCE has been used as a degreasing solvent in a number of jobs, task, and industries, some of which include metal, electronic, paper and printing, leather manufacturing and aerospace/aircraft manufacturing or maintenance industries and job title of degreaser, metal workers, electrical worker, and machinist (IARC, 1995; Bakke et al., 2007). NRC (2006) identifies characteristics for kidney cancer case-control studies that assess job title or occupation in their Table 3-8. Relative risks and 95% confidence intervals reported in these studies are found in Table 4-39 below.

4.4.2.1. *Studies of Job Titles and Occupations with Historical Trichloroethylene (TCE) Usage*

Elevated risks are observed in many of the cohort or case-control studies between kidney cancer and industries or job titles with historical use of trichloroethylene (Partenen et al., 1991; McCredie and Stewart, 1993; Schlehofer et al., 1995; Mandel et al., 1995; Pesch et al., 2000a; Parent et al., 2000; Mattioli et al., 2002; Brüning et al., 2003; Zhang et al., 2004; Charbotel et al., 2006; Wilson et al., 2008). Overall, these studies, although indicating association with metal work exposures and kidney cancer, are insensitive for identifying a TCE hazard. The use of job title or industry as a surrogate for exposure to a chemical is subject to substantial misclassification that will attenuate rate ratios due to exposure variation and differences among individuals with the same job title. Several small case-control studies (Jensen et al., 1988; Harrington et al., 1989; Sharpe et al., 1989; Aupérin et al., 1994; Vamvakas et al., 1998; Parent et al., 2000) have insufficient statistical power to detect modest associations due to their small size and potential exposure misclassification (NRC, 2006). For these reasons, statistical variation in the risk estimate is large and observation of statistically significantly elevated risks associated with metal work in many of these studies is noteworthy. Some studies also examined broad chemical grouping such as degreasing solvents or chlorinated solvents. Observations in studies that assessed degreasing agents or chlorinated solvents reported statistically significant elevated kidney cancer risk (Asal et al., 1998; Harrington et al., 1989; McCredie and Stewart, 1993; Mellemgaard et al., 1994; Schlehofer et al., 1995; Pesch et al., 2000a; Brüning et al., 2003). Observations of association with degreasing agents together with job title or occupations where TCE has been used historically provide a signal and suggest an etiologic agent common to degreasing activities.

1 **Table 4-38. Summary of human studies on TCE exposure and kidney cancer**
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Exposure group		Relative risk (95% CI)	No. obs. events	Reference
Cohort and PMR studies—incidence				
Aerospace workers (Rocketdyne)				Zhao et al., 2005
	Any exposure to TCE	Not reported		
	Low cum TCE score	1.00[a]	6	
	Med cum TCE score	1.87 (0.56, 6.20)	6	
	High TCE score	4.90 (1.23, 19.6)	4	
	p for trend	*p* = 0.023		
	TCE, 20 yrs exposure lag[b]			
	Low cum TCE score	1.00[a]	6	
	Med cum TCE score	1.19 (0.22, 6.40)	7	
	High TCE score	7.40 (0.47, 116)	3	
	p for trend	*p* = 0.120		
All employees at electronics factory (Taiwan)				Chang et al., 2005
	Males	1.06 (0.45, 2.08) [c]	8	
	Females	1.09 (0.56, 1.91) [c]	12	
	Females	1.10 (0.62, 1.82) [c]	15	Sung et al., 2008
Danish blue-collar worker with TCE exposure				Raaschou-Nielsen et al., 2003
	Any exposure, all subjects	1.2 (0.98, 1.46)	103	
	Any exposure, males	1.2 (0.97, 1.48)	93	
	Any exposure, females	1.2 (0.55, 2.11)	10	
	Exposure lag time			
	20 yrs	1.3 (0.86, 1.88)	28	
	Employment duration			
	<1 yr	0.8 (0.5, 1.4)	16	
	1−4.9 yrs	1.2 (0.8, 1.7)	28	
	≥5 yrs	1.6 (1.1, 2.3)	32	
	Subcohort w/higher exposure			
	Any TCE exposure	1.4 (1.0, 1.8)	53	
	Employment duration			
	1−4.9 yrs	1.1 (0.7, 1.7)[d]	23	
	≥5 yrs	1.7 (1.1, 2.4) [d]	30	
Biologically monitored Danish workers		1.1 (0.3, 2.8)	4	Hansen et al., 2001
	Any TCE exposure, males	0.9 (0.2, 2.6)	3	
	Any TCE exposure, females	2.4 (0.03, 14)	1	
	Cumulative exp (Ikeda)	Not reported		
	<17 ppm-yr			
	≥17 ppm-yr			

Table 4-38. Summary of human studies on TCE exposure and kidney cancer (continued)

Exposure group	Relative risk (95% CI)	No. obs. events	Reference
Mean concentration (Ikeda)	Not reported		
<4 ppm			
4+ ppm			
Employment duration	Not reported		
<6.25 yrs			
≥6.25			
Aircraft maintenance workers from Hill Air Force Base			Blair et al., 1998
TCE subcohort	Not reported		
Males, cumulative exp			
0	1.0[a]		
<5 ppm-yr	1.4 (0.4, 4.7)	9	
5−25 ppm-yr	1.3 (0.3, 4.7)	5	
>25 ppm-yr	0.4 (0.1, 2.3	2	
Females, cumulative exp			
0	1.0[a]		
<5 ppm-yr		0	
5−25 ppm-yr		0	
>25 ppm-yr	3.6 (0.5, 25.6)	2	
Biologically-monitored Finnish workers			Anttila et al., 1995
All subjects	0.87 (0.32, 1.89)	6	
Mean air-TCE (Ikeda extrapolation)			
<6 ppm	Not reported		
6+ ppm	Not reported		
Cardboard manufacturing workers in Arnsberg, Germany			Henschler et al., 1995
Exposed workers	7.97 (2.59, 8.59)[e]	5	
Biologically-monitored Swedish workers			Axelson et al., 1994
Any TCE exposure, males	1.16 (0.42, 2.52)	6	
Any TCE exposure, females	Not reported		
Cardboard manufacturing workers, Atlanta area, GA			Sinks et al., 1992
All subjects	3.7 (1.4, 8.1)	6	
All departments	∞ (3.0, ∞)[f]	5	
Finishing department	16.6 (1.7, 453.1)[f]	3	

Table 4-38. Summary of human studies on TCE exposure and kidney cancer (continued)

Exposure group		Relative risk (95% CI)	No. obs. events	Reference
Cohort and PMR studies—mortality				
Computer manufacturing workers (IBM), NY				
Males		1.64 (0.45, 4.21) [g]	4	Clapp and Hoffman, 2008
Females			0	
Aerospace workers (Rocketdyne)				
	Any TCE (utility/eng flush)	2.22 (0.89, 4.57)	7	Boice et al., 2006
	Any exposure to TCE	Not reported		Zhao et al., 2005
	Low cum TCE score	1.00[a]	7	
	Med cum TCE score	1.43 (0.49, 4.16)	7	
	High TCE score	2.13 (0.50, 8.32)	3	
	p for trend	*p* = 0.31		
	TCE, 20 yrs exposure lag[b]			
	Low cum TCE score	1.00[a]	10	
	Med cum TCE score	1.69 (0.29, 9.70)	6	
	High TCE score	1.82 (0.09, 38.6)	1	
	p for trend	*p* = 0.635		
View-Master employees				ATSDR, 2004
	Males	2.76 (0.34, 9.96)[g]	2	
	Females	6.21 (2.68, 12.23)[g]	8	
United States Uranium-processing workers (Fernald)				Ritz, 1999 (as reported in NRC, 2006)
	Any TCE exposure	Not reported		
	Light TCE exposure, 2-10 yrs duration[d]	1.94 (0.59, 6.44)	5	
	Light TCE exposure, >10 yrs duration[d]	0.76 (0.14, 400.0)	2	
	Mod TCE exposure, >2 yrs duration[d]		0	
Aerospace workers (Lockheed)				Boice et al., 1999
	Routine Exp	0.99 (0.40, 2.04)	7	
	Routine-Intermittent[a]	Not presented	11	
	Duration of exposure			
	0 yrs	1.0	22	
	<1 yr	0.97 (0.37, 2.50)	6	
	1−4 yrs	0.19 (0.02, 1.42)	1	
	≥5 yrs	0.69 (0.22, 2.12)	4	
	p for trend			

Table 4-38. Summary of human studies on TCE exposure and kidney cancer (continued)

Exposure group		Relative risk (95% CI)	No. obs. events	Reference
Aerospace workers (Hughes)				Morgan et al., 1998
	TCE subcohort	1.32 (0.57, 2.60)	8	
	Low intensity (<50 ppm)[e]	0.47 (0.01, 2.62)	1	
	High intensity (>50 ppm)[e]	1.78 (0.72, 3.66)	7	
	TCE subcohort (Cox analysis)			
	Never exposed	1.00[a]	24	
	Ever exposed	1.14 (0.51, 2.58)[h]	8	
	Peak			
	No/Low	1.00[a]	24	
	Med/Hi	1.89 (0.85, 4.23)[h]	8	
	Cumulative			
	Referent	1.00[a]	24	
	Low	0.31 (0.04, 2.36)[h]	1	
	High	1.59 (0.68, 3.71)[h]	7	
Aircraft maintenance workers (Hill AFB, Utah)				Blair et al., 1998
	TCE subcohort	1.6 (0.5, 5.1)[a]	15	
	Males, cumulative exp			
	0	1.0[a]		
	<5 ppm-yr	2.0 (0.5, 7.6)	8	
	5−25 ppm-yr	0.4 (0.1, 4.0)	1	
	>25 ppm-yr	1.2 (0.3, 4.8)	4	
	Females, cumulative exp			
	0	1.0[a]		
	<5 ppm-yr		0	
	5−25 ppm-yr	9.8 (0.6, 157)	1	
	>25 ppm-yr	3.5 (0.2, 56.4)	1	
	TCE subcohort	1.18 (0.47, 2.94)[i]	18	Radican et al., 2008
	Males, cumulative exp	1.24 (0.41, 3.71)[i]	16	
	0	1.0[1]		
	<5 ppm-yr	1.87 (0.59, 5.97[i]	10	
	5−25 ppm-yr	0.31 (0.03, 2.75)[i]	1	
	>25 ppm-yr	1.16 (0.31, 4.32)[i]	5	
	Females, cumulative exp	0.93 (0.15, 5.76)[i]	2	
	0	1.0[a]		
	<5 ppm-yr		0	
	5−25 ppm-yr	2.86 (0.27, 29.85)[i]	1	
	>25 ppm-yr	0.97 (0.10, 9.50)[i]	1	

Table 4-38. Summary of human studies on TCE exposure and kidney cancer (continued)

Exposure group		Relative risk (95% CI)	No. obs. events	Reference
Cardboard manufacturing workers in Arnsberg, Germany				Henschler et al., 1995
	TCE exposed workers	3.28 (0.40, 11.84)	2	
	Unexposed workers	- (0.00, 5.00)	0	
Deaths reported to among GE pension fund (Pittsfield, MA)		0.99 (0.30, 3.32)[f]	12	Greenland et al., 1994
Cardboard manufacturing workers, Atlanta area, GA				Sinks et al., 1992
		1.4 (0.0, 7.7)	1	
U. S. Coast Guard employees				Blair et al., 1989
	Marine inspectors	1.06 (0.22, 3.10)	3	
	Noninspectors	1.03 (0.21, 3.01)	3	
Aircraft manufacturing plant employees (Italy)				Costa et al., 1989
	All subjects	Not reported		
Aircraft manufacturing plant employees (San Diego, CA)				Garabrant et al., 1988
	All subjects	0.93 (0.48, 1.64)	12	
Case-control studies				
Population of Arve Valley, France				Charbotel et al., , 2006, 2007, 2009
	Any TCE exposure	1.64 (0.95, 2.84)	37	
	Any TCE exposure (High confidence exposure)	1.88 (0.89, 3.98)	16	
	Cumulative TCE exposure			
	Referent/nonexposed	1.00[a]	49	
	Low	1.62 (0.75, 3.47)	12	
	Medium	1.15 (0.47, 2.77)	9	
	High	2.16 (1.02, 4.60)[j]	16	
	Test for trend	$p = 0.04$		
	Cumulative TCE exposure + peak			
	Referent/nonexposed	1.00[a]	49	
	Low/medium, no peaks	1.35 (0.69, 2.63)	18	
	Low/medium + peaks	1.61 (0.36. 7.30)	3	
	High, no peaks	1.76 (0.65, 4.73)	8	
	High + peaks	2.73 (1.06, 7.07)[j]	8	
	Cumulative TCE exposure, 10-yr lag			
	Referent/nonexposed	1.00[a]	49	
	Low/medium, no peaks	1.44 (0.69, 2.80)	19	
	Low/medium + peaks	1.38 (0.32, 6.02)	3	
	High, no peaks	1.50 (0.53, 4.21)	7	
	High + peaks	3.15 (1.19, 8.38)	8	
	Time-weighted-average TCE exposure[k]			
	Referent/nonexposed	1.00[a]	46	

Table 4-38. Summary of human studies on TCE exposure and kidney cancer (continued)

Exposure group		Relative risk (95% CI)	No. obs. events	Reference
	Any TCE without cutting fluid	1.62 (0.76, 3.44)	15	
	Any cutting fluid without TCE	2.39 (0.52, 11.03)	3	
	<50 ppm TCE + cutting fluid	1.14 (0.49, 2,66)	12	
	50+ ppm TCE + cutting fluid	2.70 (1.02, 7.17)	10	
Population of Arnsberg Region, Germany				Brüning et al., 2003
	Longest job held-TCE/PERC (CAREX)	1.80 (1.01, 3.20)	117	
	Self-assessed exposure to TCE	2.47 (1.36, 4.49)	25	
	Duration of self-assessed TCE exposure			
	0	1.00[a]	109	
	<10 yrs	3.78 (1.54, 9.28)	11	
	10−20 yrs	1.80 (0.67, 4.79)	7	
	>20 yrs	2.69 (0.84, 8.66)	8	
Population in 5 German Regions				Pesch et al., 2000a
	Any TCE Exposure	Not reported		
	Males	Not reported		
	Females	Not reported		
	TCE exposure (Job Task Exposure Matrix)			
	Males			
	Medium	1.3 (1.0, 1.8)	68	
	High	1.1 (0.8, 1.5)	59	
	Substantial	1.3 (0.8, 2.1)	22	
	Females			
	Medium	1.3 (0.7, 2.6)	11	
	High	0.8 (0.4, 1.9)	7	
	Substantial	1.8 (0.6, 5.0)	5	
Population of Minnesota				Dosemeci et al., 1999
	Ever exposed to TCE, NCI JEM			
	Males	1.04 (0.6, 1.7)	33	
	Females	1.96 (1.0, 4.0)	22	
	Males + Females	1.30 (0.9, 1.9)	55	
Population of Arnsberg Region, Germany				Vamvakas et al., 1998
	Self-assessed exposure to TCE	10.80 (3.36, 34.75)	19	
Population of Montreal, Canada				Siemiatycki et al., 1991
	Any TCE exposure	0.8 (0.4, 2.0)[l]	4	
	Substantial TCE exposure	0.8 (0.2, 2.6)[l]	2	

Table 4-38. Summary of human studies on TCE exposure and kidney cancer (continued)

Exposure group		Relative risk (95% CI)	No. obs. events	Reference
Geographic based studies				
Residents in two study areas in Endicott, NY		1.90 (1.06, 3.13)	15	ATSDR, 2006, 2008
Residents of 13 census tracts in Redlands, CA		0.80 (0.54, 1.12)[m]	54	Morgan and Cassidy, 2002
Finnish residents				Vartiainen et al., 1993
	Residents of Hausjarvi	Not reported		
	Residents of Huttula	Not reported		

1
2 [a]Internal referents, workers not exposed to TCE.
3 [b]Relative risks for TCE exposure after adjustment for 1st employment, socioeconomic status, age at event, and all
4 other carcinogens, including hydrazine.
5 [c]Chang et al. (2005)—urinary organs combined.
6 [d]SIR for renal cell carcinoma.
7 [e]Henschler et al. (1995) Expected number of incident cases calculated using incidence rates from the Danish Cancer
8 Registry.
9 [f]Odds ratio from nested case-control analysis.
10 [g]Proportional mortality ratio.
11 [h]Risk ratio from Cox Proportional Hazard Analysis, stratified by age, sex and decade (Environmental Health
12 Strategies, 1997).
13 [i]In Radican et al. (2008), kidney cancer defined as renal cell carcinoma (ICDA 8 code 189.0) and estimated relative
14 risks from Cox proportional hazard models were adjusted for age and sex.
15 [j]Analyses adjusted for age, sex, smoking and body mass index. The odds ratio, adjusted for age, sex, smoking, body
16 mass index and exposure to cutting fluids and other petroleum oils, for high cumulative TCE exposure was 1.96
17 (95% CI: 0.71, 5.37) and for high cumulative + peak TCE exposure was 2.63 (95% CI: 0.79, 8.83). The odds
18 ratio for, considering only job periods with high confidence TCE exposure assessment, adjusted for age, sex,
19 smoking and body mass index, for high cumulative dose plus peaks was 3.80 (95% CI: 1.27. 11.40).
20 [k]The exposure surrogate is calculated for one occupational period only and is not the average exposure concentration
21 over the entire employment period.
22 [l] 90% confidence interval.
23 [m]99% confidence interval.
24
25 JEM = job-exposure matrix, NCI = National Cancer Institute, PERC = perchloroethylene.

Table 4-39. Summary of case-control studies on kidney cancer and occupation or job title

Case ascertainment area/exposure group	Relative risk (95% CI)	No. exposed cases	Reference
Swedish Cancer Registry Cases			Wilson et al., 2008
Machine/electronics industry	1.30 (1.08, 1.55) [a] [M]	120	
	1.75 (1.04, 2.76) [a] [F]	18	
Shop and construction metal work	1.19 (1.00, 1.40) [a] [M]	143	
Machine assembly	1.62 (0.94, 2.59) [a] [M]		
Metal plating work	2.70 (0.73, 6.92) [a] [M]	4	
Shop and construction metal work	1.66 (0.71, 3.26) [a] [F]	8	
Arve Valley, France			Charbotel et al., 2006
Metal industry	1.02 (0.59, 1.76)	28	
Metal workers, job title	1.00 (0.56, 1.77)	25	
Metal industry, screw-cutting workshops	1.39 (0.75, 2.58)	22	
Machinery, electrical and transportation equipment manufacture	1.19 (0.61, 2.33)	15	
Iowa Cancer Registry Cases			Zhang et al., 2004
Assemblers	2.5 (0.8, 7.6)	5	
>10 yrs employment	4.2 (1.2, 15.3)	4	
Arnsberg Region, Germany			Brüning et al., 2003
Iron/steel	1.15 (0.29, 4.54)	3	
Occupations with contact to metals	1.53 (0.97, 2.43)	46	
Longest job held	1.14 (0.66, 1.96)	24	
Metal greasing/degreasing	5.57 (2.33, 13.32)	15	
Degreasing agents			
Low exposure	2.11 (0.86, 5.18)	9	
High exposure	1.01 (0.40, 2.54)	7	
Bologna, Italy			Mattioli et al., 2002
Metal workers	2.21 (0.99, 5.37)	37	
Printers	1.55 (0.17, 13.46)	7	
Solvents	0.79 (0.31, 1.98) [M]	17	
	1.47 (0.12, 17.46) [F]	3	
Montreal, Canada			Parent et al., 2000
Metal fabricating and machining industry	1.0 (0.6, 1.8)	14	
Metal processors	1.2 (0.4, 3.4)	4	
Printing and publishing industry	1.1 (0.4, 3.0)	4	
Printers	3.0 (1.2, 7.5)	6	
Aircraft mechanics	2.8 (1.0, 8.4)	4	

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Table 4-39. Summary of case-control studies on kidney cancer and occupation or job title (continued)

Case ascertainment area/exposure group		Relative risk (95% CI)	No. exposed cases	Reference
5 Regions in Germany				Pesch et al., 2000a
	Electrical and electronic equipment assembler	3.2 (1.0, 10.3) [M]	5	
		2.7 (1.3, 5.8) [F}	11	
	Printers	3.5 (1.1, 11.2)[M]	5	
		2.1 (0.4, 11.7) [F]	2	
	Metal cleaning/degreasing, job task	1.3 (0.7, 2.3) [M]	15	
		1.5 (0.3, 7.7) [F]	2	
New Zealand Cancer Registry				Delahunt et al., 1995
	Toolmakers and blacksmiths	1.48 (0.72, 3.03)	No info	
	Printers	0.67 (0.25, 1.83)		
Minnesota Cancer Surveillance System				Mandel et al., 1995
	Iron or steel	1.6 (1.2, 2.2)	8	
Rhein-Neckar-Odenwald Area, Germany				Schlehofer et al., 1995
	Metal			
	Industry	1.63 (1.07, 2.48)	71	
	Occupation	1.38 (0.89, 2.12)		
	Electronic			
	Industry	0.51 (0.26, 1.01)	14	
	Occupation	0.57 (0.25, 1.33)	9	
	Chlorinated solvents	2.52 (1.23, 5.16)	27	
	Metal and metal compounds	1.47 (0.94, 2.30)	62	
Danish Cancer Registry				Mellemgaard et al., 1994
	Iron and steel	1.4 (0.8, 2.4) [M]	31	
		1.0 (0.1, 3.2) [F]	1	
	Solvents	1.5 (0.9, 2.4) [M]	50	
		6.4 (1.8, 23) [F]	16	
France				Aupérin et al., 1994
	Machine fitters, assemblers, and precision instrument makers	0.7 (0.3, 1.9)	16	

Table 4-39. Summary of case-control studies on kidney cancer and occupation or job title (continued)

Case ascertainment area/exposure group		Relative risk (95% CI)	No. exposed cases	Reference
New South Wales, Australia				McCredie and Stewart, 1993
	Iron and steel	1.18 (0.75, 1.85) [b]	52	
		2.39 (1.26, 4.52) [c]	19	
	Printing or graphics	1.18 (0.87, 2.08) [b]	29	
		0.82 (0.32, 2.11) [d]	6	
	Machinist or tool maker	1.15 (0.72, 1.86) [b]	48	
		1.83 (0.92, 3.61) [c]	16	
	Solvents	1.54 (1.11, 2.14) [b]	109	
		1.40 (0.82, 2.40) [c]	24	
Finnish Cancer Registry				Partenen et al., 1991
	Iron and metalware work	1.87 (0.94, 3.76)	22	
	Machinists	2.33 (0.83, 6.51)	10	
	Paper and pulp; printing/publishing	2.20 (1.02, 4.72) [M]	18	
		5.95 (1.21, 29.2) [F]	7	
	Nonchlorinated solvents	3.46 (0.91, 13.2) [M]	9	
West Midlands UK Cancer Registry				Harrington et al., 1989
	Organic solvents			
	Ever exposed	1.30 (0.31, 8.50)	3	
	Intermediate exposure	1.54 (0.69, 4.10)	3	
Montreal, Canada				Sharpe et al., 1989
	Organic solvents	1.68 (0.83, 2.22)	33	
	Degreasing solvents	3.42 (0.92, 12.66)	10	
Oklahoma				Asal et al., 1988
	Metal degreasing	1.7 (0.7, 3.8) [M]	19	
	Machining	1.7 (0.7, 4.3) [M]	13	
	Painter, paint manufacture	1.3 (0.7, 2.6) [M]	22	
Missouri Cancer Registry				Brownson, 1988
	Machinists	2.2 (0.5, 10.3)	3	
Danish Cancer Registry				Jensen et al., 1988
	Iron and metal, blacksmith	1.4 (0.7, 2.9) [d]	17	
	Painter, paint manufacture	1.8 (0.7, 4.6)	10	

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2 [a]Renal pelvis, Wilson et al. (2008).
3 [b]Renal cell carcinoma, McCredie and Stewart (1993).
4 [c]Renal pelvis, McCredie and Stewart (1993).
5 [d]Renal pelvis and ureter, Jensen et al. (1988).
6
7 UK = United Kingdom.

4.4.2.2. *Cohort and Case-Controls Studies of Trichloroethylene (TCE) Exposure*

Cohort and case-controls studies that include job-exposure matrices for assigning TCE exposure potential to individual study subjects show associations with kidney cancer, specifically renal cell carcinoma, and trichloroethylene exposure. Support for this conclusion derives from findings of increased risks in cohort studies (Henschler et al., 1995; Raaschou-Nielsen et al., 2003; Zhao et al., 2005) and in case-control studies from the Arnsberg region of Germany (Vamvakas et al., 1998; Pesch et al., 2000a; Brüning et al., 2003), the Arve Valley region in France (Charbotel et al., 2006, 2009), and the United States (Sinks et al., 1992; Dosemeci et al., 1999).

A consideration of a study's statistical power and exposure assessment approach is necessary to interpret observations in Table 4-38. Most cohort studies are underpowered to detect a doubling of kidney cancer risks including the essentially null studies by Greenland et al. (1994), Axelson et al. (1994 [incidence]), Anttila et al. (1995 [incidence]), Blair et al. (1998 [incidence and mortality]), Morgan et al. (1998), Boice et al. (1999) and Hansen et al. (2001). Only the exposure duration-response analysis of Raaschou-Nielsen et al. (2003) had over 80% statistical power to detect a doubling of kidney cancer risk (NRC, 2006), and they observed a statistically significant association between kidney cancer and ≥5-year employment duration. Rate ratios estimated in the mortality cohort studies of kidney cancer (e.g., Garabrant et al., 1988; Sinks et al., 1992; Axelson et al., 1994; Greenland et al., 1994; Blair et al., 1998; Morgan et al., 1998; Ritz, 1999; Boice et al., 1999, 2006) are likely underestimated to some extent because their reliance on death certificates and increased potential of nondifferential misclassification of outcome in these studies, although the magnitude is difficult to predict (NRC, 2006). Cohort or PMR studies with more uncertain exposure assessment approaches, e.g., studies of all subjects working at a factory (Garabrant et al., 1998; Costa et al., 1989; ATSDR, 2004; Sung et al., 2007; Chang et al., 2003, 2005; Clapp and Hoffmann, 2008), do not show association but are quite limited given their lack of attribution of higher or lower exposure potentials; risks are likely diluted due to their inclusion of no or low exposed subjects.

Two studies were carried out in geographic areas with a high frequency and a high degree of TCE exposure and were designed with *a priori* hypotheses to test for the effects of TCE exposure on renal cell cancer risk (Brüning et al., 2003; Charbotel et al., 2006, 2009) and for this reason their observations have important bearing to the epidemiologic evidence evaluation. Both studies found a 2-fold elevated risk with any TCE exposure after adjustment for several possible confounding factors including smoking (2.47, 95% CI: 1.36, 4.49) for self-assessed exposure to TCE (Brüning et al., 2003); high cumulative TCE exposure (2.16, 95% CI: 1.02, 4.60) with a positive and statistically significant trend test, $p = 0.04$, (Charbotel et al., 2006). Furthermore,

renal cell carcinoma risk in Charbotel et al. (2005) increased to over 3-fold (95% CI: 1.19, 8.38) in statistical analyses which considered a 10-year exposure lag period. An exposure lag period is often adopted in analysis of cancer epidemiology to reduce exposure measurement biases (Salvan et al., 1995). Most exposed cases in this study were exposed to TCE below any current occupational standard (26 of 37 cases [70%]) had held a job with a highest time-weighted average (TWA [<50 ppm]) (Charbotel et al., 2009). A subsequent analysis of Charbotel et al. (2009) using an exposure surrogate defined as the highest TWA for any job held, an inferior surrogate given TCE exposures in other jobs were not considered, reported an almost 3-fold elevated risk (2.80, 95% CI: 1.12, 7.03) adjusted for age, sex, body mass index (BMI), and smoking with exposure to TCE in any job to ≥50-ppm TWA (Charbotel et al., 2009).

Zhao et al. (2005) compared 2,689 TCE-exposed workers at a California aerospace company to nonexposed workers from the same company as the internal referent population, and found a monotonic increase in incidence of kidney cancer by increasing cumulative TCE exposure. In addition, a 5-fold increased incidence was associated with high cumulative TCE exposure. This relationship for high cumulative TCE exposure, lagged 20 years, was accentuated with adjustment for other occupational exposures (RR = 7.40, 95% CI: 0.47, 116), although the confidence intervals were increased. An increased confidence interval with adjustments is not unusual in occupational studies, as exposure is usually highly correlated with them, so that adjustments often inflate standard error without removing any bias (NRC, 2006). Observed risks were lower for kidney cancer mortality and because of reliance on cause of death on death certificates are likely underestimated because of nondifferential misclassification of outcome (Percy et al., 1981). Boice et al. (2006), another study of 1,111 workers with potential TCE exposure at this company and which overlaps with Zhao et al. (2005), found a 2-fold increase in kidney cancer mortality (standardized mortality ration [SMR] = 2.22, 95% CI: 0.89, 4.57). This study examined mortality in a cohort whose definition date differs slightly from Zhao et al. (2005), working between 1948−1999 with vital status as of 1999 (Boice et al., 2006) compared to working between 1950−1993 with follow-up for mortality as of 2001 (Zhao et al., 2005), and used a qualitative approach for TCE exposure assessment. Boice et al. (2006) is a study of fewer subjects identified with potential TCE exposure, of fewer kidney cancer deaths [7 deaths; 10 incident cases, 10 deaths in Zhao et al. (2005)], of subjects with more recent exposures, and with a inferior exposure assessment approach compared to Zhao et al. (2005); a finding of a two-fold mortality increase (95% CI: 0.89, 4.57) is noteworthy given the insensitivities.

Zhao et al. (2005) and Charbotel et al. (2006), furthermore, are two of the few studies to conduct a detailed assessment of exposure that allowed for the development of a job-exposure

matrix that provided rank-ordered levels of exposure to TCE and other chemicals. NRC (2006) discussed the inclusion of rank-ordered exposure levels is a strength increasing precision and accuracy of exposure information compared to more inferior exposure assessment approaches in some other studies such as duration of exposure or a grouping of all exposed subjects.

The finding in Raaschou-Nielsen et al. (2003) of an elevated renal cell carcinoma risk with longer employment duration is noteworthy given this study's use of a relatively insensitive exposure assessment approach. One strength of this study is the presentation of incidence ratios for a subcohort of higher exposed subjects, those with at least 1-year duration of employment and first employment before 1980, as a sensitivity analysis for assessing the effect of possible exposure misclassification bias. Renal cell carcinoma risk was higher in this subcohort compared to the larger cohort and indicated some potential for misclassification bias in the grouped analysis. For both the cohort and subcohort analyses, risk appeared to increase with increasing employment duration, although formal statistical tests for trend are not presented in the published paper.

4.4.2.2.1. ***Discussion of controversies on studies in the Arnsberg region of Germany.*** Two previous studies of workers in this region, a case-control study of Vamvakas et al. (1998) and Henschler et al. (1995), a study prompted by a kidney cancer case cluster, observed strong associations between kidney cancer and TCE exposure. A fuller discussion of the studies from the Arnsberg region and their contribution to the overall weight of evidence on cancer hazard is warranted in this evaluation given the considerable controversy (Bloemen and Tomenson, 1995; Swaen, 1995; McLaughlin and Blot, 1997; Green and Lash, 1999; Cherrie et al., 2001; Mandel, 2001) surrounding Henschler et al. (1995) and Vamvakas et al. (1998).

Criticisms of Henschler et al. (1995) and Vamvakas et al. (1998) relate, in part, to possible selection biases that would lead to inflating observed associations and limited inferences of risk to the target population. Specifically, these include (1) the inclusion of kidney cancer cases first identified from a cluster and the omission of subjects lost to follow-up from Henschler et al. (1995); (2) use of a Danish population as referent, which may introduce bias due to differences in coding cause of death and background cancer rate differences (Henschler et al., 1995); (3) follow-up of some subjects outside the stated follow-up period (Henschler et al., 1995); (4) differences between hospitals in the identification of cases and controls in Vamvakas et al. (1998); (5) lack of temporality between case and control interviews (Vamvakas et al., 1998); (6) lack of blinded interviews (Vamvakas et al., 1998); (7) age differences in Vamvakas et al. (1998) cases and controls that may lead to a different TCE exposure potential; (8) inherent deficiencies in Vamvakas et al. (1998) as reflected by its inability to identify other known kidney

cancer risk factors; and, (9) exposure uncertainty, particularly unclear intensity of TCE exposure. Overall, NRC (2006) noted that some of the points above may have contributed to an underestimation of the true exposure distribution of the target population (points 5, 6, and 7), other points would underestimate risk (points 3), and that these effects could not have explained the entire excess risk observed in these studies (points 1, 2, and 4). The NRC (2006) furthermore disagreed with the exposure uncertainty criticism (point 9), and concluded TCE exposures, although of unknown intensity, were substantial and, clearly showed graded differences on several scales in Vamvakas et al. (1998) consistent with this study's semiquantitative exposure assessment.

Brüning et al. (2003) was carried out in a broader region in southern Germany, which included the Arnsberg region and a different set of cases and control identified from a later time period than Vamvakas et al. (1998). The TCE exposure range in this study was similar to that in Vamvakas et al. (1998), although at a lower exposure prevalence because of the larger and more heterogeneous ascertainment area for cases and controls. For "ever exposed" to TCE, Brüning et al. (2003) observed a risk ratio of 2.47 (95% CI: 1.36, 4.49) and a 4-fold increase in risk (95% CI: 1.80, 7.54) among subjects with any occurrence of narcotic symptom and a 6-fold increase in risk (95% CI: 1.46, 23.99) for subjects who had daily occurrences of narcotic symptoms; risks which are lower than observed in Vamvakas et al. (1998). The lower rate ratio in Brüning et al. (2003) might indicate bias in the Vamvakas et al. study or statistical variation between studies related to the broader base population included in Brüning et al. (2003).

Observational studies such as epidemiologic studies are subject to biases and confounding which can be minimized but never completely eliminated through a study's design and statistical analysis methods. While Brüning et al. (2003) overcomes many of the deficiencies of Henschler et al. (1995) and Vamvakas et al. (1998), nonetheless, possible biases and measurement errors could be introduced through their use of prevalent cases and residual noncases, use of controls from surgical and geriatric clinics, nonblinding of interviewers, a 2-year difference between cases and controls in median age, use or proxy or next-of-kin interviews, and self-reported occupational history.

The impact of any one of the above points could either inflate or depress observed associations. Biases related to a longer period for case compared to control ascertainment could go in either direction. Next-of-kin interviewers for deceased cases, all controls being alive at the time of interview, would be expected to underestimate risk if exposures were not fully reported and thus, misclassified. On the other hand, the control subjects who were enrolled when the interviews were conducted might not represent the true exposure distribution of the target population through time and would lead to overestimate of risk. Selection of controls from

clinics is not expected to greatly influence observed associations since these clinics specialized in the type of care they provided (NRC, 2006). Brüning et al. (2003) is not the only kidney case-control study where interviewers were not blinded; in fact, only the study of Charbotel et al. (2006) included blinding of interviewers. Blinding of interviewers is preferred to reduce possible bias. Brüning et al.'s use of frequency matching using 5-year age groupings is common in epidemiologic studies and any biases introduced by age difference between cases and controls is expected to be minimal because the median age difference was 3 years.

Despite these issues, the three studies of the Arnsberg region, with very high apparent exposure and different base populations showed a significant elevation of risk and all have bearing on kidney cancer hazard evaluations. The emphasis provided by each study for identifying a kidney cancer hazard depends on its strengths and weaknesses. Brüning et al. (2003) overcomes many of the deficiencies in Henschler et al. (1995) and Vamvakas et al. (1998). The finding of a statistically significantly approximately 3-fold elevated odds ratio with occupational TCE exposure in Brüning et al. (2003) strengthens the signal previously reported by Henschler et al. (1995) and Vamvakas et al. (1998). A previous study of cardboard workers in the United States (Sink et al., 1992), a study like Henschler et al. (1995) which was prompted by a reported cancer cluster, had observed association with kidney cancer incidence, particularly with work in the finishing department where TCE use was documented. Henschler et al. (1995), Vamvakas et al. (1998) and Sinks et al. (1992) are less likely to provide a precise estimate of the magnitude of the association given greater uncertainty in these studies compared to Brüning et al. (2003). For this reason, Brüning et al. (2003) is preferred for meta-analysis treatment since it is considered to better reflect risk in the target population than the two other studies. Another study (Charbotel et al., 2006) of similar exposure conditions of a different base population and of different case and control ascertainment methods as the Arnsberg region studies has become available since the Arnsberg studies. This study shows a statistically significant elevation of risk and high cumulative TCE exposure in addition to a positive trend with rank-order exposure levels. Charbotel et al. (2006) adds evidence to observations from earlier studies on high TCE exposures in Southern Germany and suggests that peak exposure may add to risk associated with cumulative TCE exposure.

4.4.2.3. *Examination of Possible Confounding Factors*

Examination of potential confounding factors is an important consideration in the evaluation of observations in the epidemiologic studies on TCE and kidney cancer. A known risk factor for kidney cancer is cigarette smoking. Obesity, diabetes, hypertension and antihypertensive medications, and analgesics are linked to kidney cancer, but causality has not

been established (Moore et al., 2005; McLaughlin et al., 2006). On the other hand, fruit and vegetable consumption is considered protective of kidney cancer risk (McLaughlin et al., 2006). Studies by Asal et al. (1988), Partanen et al. (1991), McCredie and Stewart (1993), Aupérin et al. (1994), Chow et al. (1994), Mellemgaard et al. (1994), Mandel et al. (1995), Vamvakas et al. (1998), Dosemeci et al. (1999), Pesch et al. (2000a), Brüning et al. (2003), and Charbotel et al. (2006) controlled for smoking and all studies except Pesch et al. (2000a) controlled for BMI. Vamvakas et al. (1998) and Dosemeci et al. (1999) controlled for hypertension and or diuretic intake in the statistical analysis. Because it is unlikely that exposure to trichloroethylene is associated with smoking, body mass index, hypertension, or diuretic intake, these possible confounders do not significantly affect the estimates of risk (NRC, 2006).

Direct examination of possible confounders is less common in cohort studies than in case-control studies where information is obtained from study subjects or their proxies. Use of internal controls, such as for Zhao et al. (2005), in general minimizes effects of potential confounding due to smoking or socioeconomic status since exposed and referent subjects are drawn from the same target population. Effect of smoking as a possible confounder may be assessed indirectly through (1) examination of risk ratios for other smoking-related sites and (2) examination of the expected contribution by these three factors to cancer risks. Lung cancer risk in Zhao et al. (2005) was not elevated compared to referent subjects and this observation suggests smoking patterns were similar between groups. Smoking was more prevalent in the Raaschou-Nielsen et al. (2003) cohort than the background population as suggested by the elevated risks for lung and other smoking-related sites; however, Raaschou-Nielsen et al. (2003) do not consider smoking to fully explain the 20 and 40% excesses in renal cell carcinoma risk in the cohort and subcohort. A high percentage of smokers in the cohort would be needed to account for the magnitude of renal cell carcinoma excess. Specifically, Raaschou-Nielsen et al. (2003) noted "a high smoking rate would be expected to generate a much higher excess risk of lung cancer than was observed in this study."

The magnitude of confounding bias related to cigarette smoking in occupationally employed populations to the observed lung, bladder and stomach cancer risk is minimal; less than 20% for lung cancer and less than 10% for bladder and stomach cancers (Siemiatycki et al., 1988; Leigh, 1996; Bang and Kim, 2001; Blair et al., 2007). Thus, in cohort studies lacking direct adjustment for smoking and use of external referents, difference in cigarette smoking between exposed and referent subjects is not sufficient to fully explain observed excess kidney cancer risks associated with TCE, particularly, high TCE exposure. Information on possible confounding due to BMI (obesity) and to diabetes is lacking in cohort studies; however, any

uncertainties are likely small given the generally healthy nature of an employed population and its favorable access to medical care.

Mineral oils such as cutting fluids or hydrazine common to some job titles with potential TCE exposures (such as machinists, metal workers, and test stand mechanics) were included as covariates in statistical analyses of Zhao et al. (2005), Boice et al. (2006) and Charbotel et al. (2006, 2009). A TCE effect on kidney cancer incidence was still evident although effect estimates were often imprecise due to lowered statistical power (Zhao et al., 2005; Charbotel et al., 2006, 2009). Observed associations were similar in analyses including chemical coexposures in both Zhao et al. (2005) and Charbotel et al. (2006, 2009) compared to chemical coexposure unadjusted risks. The association or OR between high TCE score and kidney cancer incidence in Zhao et al. (2005) was 7.71 (95% CI: 0.65, 91.4) after adjustment for other carcinogens including hydrazine and cutting oils, compared to analyses unadjusted for chemical coexposures (4.90, 95% CI: 1.23, 19.6).

In Charbotel et al. (2006), exposure to TCE was strongly associated with exposure to cutting fluids and petroleum oils (22 of the 37 TCE-exposed cases were exposed to both). Statistical modeling of all factors significant at 10% threshold showed the OR for cutting fluids to be almost equal to 1, whereas the OR for the highest level of TCE exposure was close to two (Charbotel et al., 2006). Moreover, when exposure to cutting oils was divided into three levels, a decrease in OR with level of exposure was found. In conditional logistic regression adjusted for cutting oil exposure, the relative risk (OR) was similar to relative risks from unadjusted for cutting fluid exposures (high cumulative TCE exposure: 1.96 [95% CI: 0.71−5.37] compared to 2.16 [95% CI: 1.02−4.60]; high cumulative and peak: 2.63 [95% CI: 0.79−8.83] compared to 2.73 [95% CI: 1.06−7.07] [Charbotel, 2006]). Charbotel et al. (2009) further examined TCE exposure defined as the highest TWA in any job held, inferior to cumulative exposure given its lack of consideration of TCE exposure potential in other jobs, either as exposure to TCE alone, cutting fluids alone, or to both after adjusting for smoking, body mass index, age, sex, and exposure to other oils (TCE alone: 1.62 [95% CI: 0.75, 3.44]); cutting fluids alone: 2.39 (95% CI: 0.52, 11.03); TCE >50-ppm TWA + cutting fluids: 2.70 (95% CI: 1.02, 7.17). There were few cases exposed to cutting fluids alone ($n = 3$) or to TCE alone ($n = 15$), all of whom had TCE exposure (in the highest exposed job held) of <35-ppm TWA, and the subgroup analyses were of limited statistical power. A finding of higher risk for both cutting oil and TCE exposure ≥50 ppm compared to cutting oil alone supports a TCE effect for kidney cancer. Adjustment for cutting oil exposures, furthermore, did not greatly affect the magnitude of TCE effect measures in the many analyses presented by Charbotel et al. (2006, 2009) suggesting cutting fluid exposure as not greatly confounding TCE effect measures.

Boice et al. (2006) was unable to directly examine hydrazine exposure on TCE effect measures because of a lack of model convergence in statistical analyses. Three of 7 TCE-exposed kidney cancer cases were identified with hydrazine exposure of 1.5 years or less and the absence of exposure to the other 4 cases suggested confounding related to hydrazine was unlikely to greatly modify observed association between TCE and kidney cancer.

4.4.2.4. *Susceptible Populations—Kidney Cancer and Trichloroethylene (TCE) Exposure*

Two studies of kidney cancer cases from the Arnsberg region in Germany have examined the influence of polymorphisms of the glutathione-S-transferase metabolic pathway on renal cell carcinoma risk and TCE exposure (Brüning et al., 1997b; Wiesenhütter et al., 2007). In their study of 45 TCE-exposed male and female renal cell carcinoma cases pending legal compensation and 48 unmatched male TCE-exposed controls, Brüning et al. (1997b) observed a higher prevalence of exposed cases homozygous and heterozygous for GST-M1 positive, 60%, than the prevalence for this genotype among exposed controls, 35%. The frequency of GST-M1 positive was lower among this control series than the frequency found in other European population studies, 50% (Brüning et al., 1997b). The prevalence of the GST-T1 positive genotype was 93% among exposed cases and 77% among exposed controls. The prevalence of GST-T1 positive genotype in the European population is 75% (Brüning et al., 1997b).

Wiesenhütter et al. (2007) compares the frequency of genetic polymorphism among subjects from the renal cancer case-control study of Brüning et al. (2003) and to the frequencies of genetic polymorphisms in the areas of Dormund and Lutherstadt Wittenberg, Germany. Wiesenhütter et al. (2007) identified the genetic frequencies of GST-M1 and GST-T1 phenotypes for 98 of the original 134 cases (73%) and 324 of the 401 controls (81%). The prevalence of GST-M1 positive genotype was 48% among all renal cell carcinoma cases, 40% among TCE-exposed cases, and 52% among all controls. The prevalence of GST-T1 positive genotypes was 81% among all cases and 81% among all controls. The prevalence of GST-T1 positive genotypes reported in this paper for all TCE-exposed cases was 20%. The numbers of exposed ($n = 4$) and unexposed ($n = 15$) GST-T1 positive cases does not sum to the 79 cases with the GST-T1 positive genotype identified in the table's first row; U.S. Environmental Protection Agency (U.S. EPA) staff has written Professor Bolt requesting clarification of the data in Table 1 of Wiesenhütter et al. (2007) (personal communication from Cheryl Siegel Scott to Professor Herman Bolt, email dated August 05, 2008) [no reply received as of January, 2009 to request]). Wiesenhütter et al. (2007) noted background frequencies in the German population in the expanded control group were 50% for GST-M1 positive and 81% for GST-T1 positive genotypes.

Observations in Brüning et al. (1997b) and Wiesenhütter et al. (2007) must be interpreted cautiously. Few details are provided in these studies on selection criteria and not all subjects from the Brüning et al. (2003) case-control study are included. For GST-M1 positive, the higher prevalence among exposed cases in Brüning et al. (1997b) compared Wiesenhütter et al. (2007) and the lower prevalence among controls compared to background frequency in the European population may reflect possible selection biases. On the other hand, the broader base population included in Brüning et al. (2003) may explain the observed lower frequency of GST-M1 positive cases in Wiesenhütter et al. (2007). Moreover, Wiesenhütter et al. (2007) does not report genotype frequencies for controls by exposure status and this information is essential to an examination of whether renal cell carcinoma risk and TCE exposure may be modified by polymorphism status.

Of the three larger (in terms of number of cases) studies that did provide results separately by sex, Dosemeci et al. (1999) suggest that there may be a sex difference for TCE exposure and renal cell carcinoma (OR: 1.04, [95% CI: 0.6, 1.7]) in males and 1.96 (95% CI: 1.0, 4.0 in females), while Raaschou-Nielsen (2003) report the same standardized incidence ration (SIR = 1.2) for both sexes and crude ORs calculated from data from the Pesch et al. (2000a) study (provided in a personal communication from Beate Pesch, Forschungsinstitut für Arbeitsmedizin, to Cheryl Scott, U.S. EPA, 21 February 2008) are 1.28 for males and 1.23 for females. Whether the Dosemeci et al. (1999) observations are due to susceptibility differences or to exposure differences between males and females cannot be evaluated. Blair et al. (1998) and Hansen et al. (2001) also present some results by sex, but these two studies have too few cases to be informative about a sex difference for kidney cancer.

4.4.2.5. *Meta-Analysis for Kidney Cancer*

Meta-analysis (detailed methodology in Appendix C) was adopted as a tool for examining the body of epidemiologic evidence on kidney cancer and TCE exposure and to identify possible sources of heterogeneity. The meta-analyses of the overall effect of TCE exposure on kidney cancer suggest a small, statistically significant increase in risk that was stronger in a meta-analysis of the highest exposure group. There was no observable heterogeneity across the studies for any of the meta-analyses and no indication of publication bias. Thus, these findings of increased risks of kidney cancer associated with TCE exposure are robust.

The meta-analysis of kidney cancer examines 14 cohort and case-control studies identified through a systematic review and evaluation of the epidemiologic literature on TCE exposure (Siemiatycki et al., 1991; Parent et al., 2000; Axelson et al., 1994; Anttila et al., 1995;

Blair et al., 1998; Morgan et al., 1998; Boice et al., 1999; Dosemeci et al., 1999; Greenland et al. 1994; Pesch et al., 2000a; Hansen et al., 2001; Brüning et al., 2003; Raaschou-Nielsen et al., 2003; Zhao et al., 2005; Charbotel et al., 2006). Details of the systematic review and meta-analysis of the TCE studies are fully discussed in Appendix B and C.

The pooled estimate from the primary random effects meta-analysis of the 14 studies was 1.25 (95% CI: 1.11, 1.41). The analysis was dominated by two (contributing almost 70% of the weight) or three (almost 80% of the weight) large studies (Dosemeci et al., 1999; Pesch et al., 2000a; Raaschou-Nielsen et al., 2003). Figure 4-1 arrays individual studies by their weight. No single study was overly influential; removal of individual studies resulted in pooled RR (RRp) estimates that were all statistically significant ($p < 0.005$) and that ranged from 1.22 (with the removal of Brüning et al. [2003]) to 1.27 (with the removal of Raaschou-Nielsen et al. [2003]). Similarly, the overall RRp estimate was not highly sensitive to alternate RR estimate selections nor was heterogeneity or publication bias apparent. Subgroup analyses were done examining the cohort and case-control studies separately with the random effects model; the resulting RRp estimates were 1.16 (95% CI: 0.96, 1.40) for the cohort studies and 1.41 (1.08, 1.83) for the case-control studies. There was heterogeneity in the case-control subgroup, but it was not statistically significant ($p = 0.17$).

Nine studies reported risks for higher exposure groups (Siemiatycki et al., 1991; Parent et al., 2000; Blair et al., 1998; Morgan et al., 1998; Boice et al., 1999; Dosemeci et al., 1999; Pesch et al., 2000a; Brüning et al., 2003; Raaschou-Nielsen et al., 2003; Zhao et al., 2005; Charbotel et al., 2006). Different exposure metrics were used in the various studies, and the purpose of combining results across the different highest exposure groups was not to estimate an RRp associated with some level of exposure. Instead, the focus on the highest exposure category was meant to result in an estimate less affected by exposure misclassification. In other words, it is more likely to represent a greater differential TCE exposure compared to people in the referent group than the exposure differential for the overall (typically any versus none) exposure comparison. Thus, if TCE exposure increases the risk of kidney cancer, the effects should be more apparent in the highest exposure groups.

The RRp estimate from the random effects meta-analysis of the studies with results presented for higher exposure groups was 1.59 (95% CI: 1.26, 2.01), higher than the RRp from the overall kidney cancer meta-analysis. As with the overall analyses, the meta-analyses of the highest-exposure groups were dominated by Pesch et al. (2000a) and Raaschou-Nielsen et al. (2003), which provided about 70% of the weight. Axelson et al. (1994), Anttila et al. (1995) and

TCE and Kidney Cancer

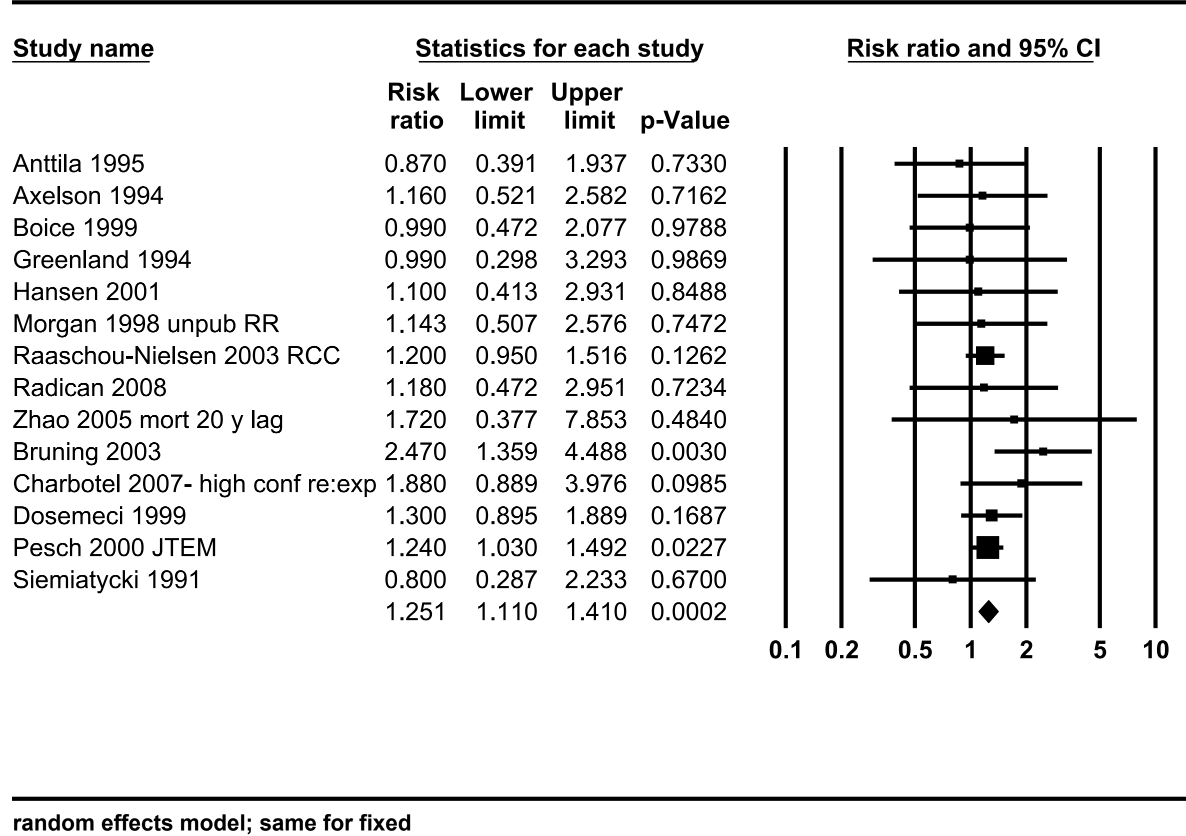

Figure 4-1. Meta-analysis of kidney cancer and overall TCE exposure (the pooled estimate is in the bottom row). Symbol sizes reflect relative weights of the studies. The horizontal midpoint of the bottom diamond represents the pooled RR estimate and the horizontal extremes depict the 95% CI limits.)

Hansen et al. (2001) do not report risk ratios for kidney cancer by higher exposure and a sensitivity analysis was carried out to address reporting bias. The RRp estimate from the primary random effects meta-analysis with null RR estimates (i.e., RR = 1.0) included for Axelson et al. (1994), Anttila et al. (1995) and Hansen et al. (2001) to address reporting bias associated with ever exposed was 1.53 (95% CI: 1.23, 1.91). Figure 4-2 arrays individual studies by their weight. The inclusion of these 3 additional studies contributed less than 8% of the total weight. No single study was overly influential; removal of individual studies resulted in RRp estimates that were all statistically significant ($p < 0.02$) and that ranged from 1.43 (with the removal of Raaschou-Nielsen et al. [2003]) to 1.58 (with the removal of Pesch et al. [2000a]). Similarly, the RRp estimate was not highly sensitive to alternate RR estimate selections and heterogeneity observed across the studies for any of the meta-analyses conducted with the highest-exposure groups (all have $p < 0.002$).

NRC (2006) deliberations on trichloroethylene commented on two prominent evaluations of the then-current TCE epidemiologic literature using meta-analysis techniques, Wartenberg et al. (2000) and Kelsh et al. (2005), submitted by Exponent-Health Sciences to NRC during their deliberations. Wartenberg et al. (2000) reported an RRp of 1.7 (95% CI: 1.1, 2.7) for kidney cancer incidence in the TCE subcohorts (Axelson et al., 1994; Anttila et al., 1995; Blair et al., 1998; Henschler et al., 1995). For kidney cancer mortality in TCE subcohorts (Henschler et al., 1995; Blair et al., 1998; Boice et al., 1999; Morgan et al., 1998; Ritz, 1999), Wartenberg et al. (2000) reported an RRp of 1.2 (95% CI: 0.8, 1.7). Kelsh et al. (2005) examined a slightly different grouping of cohort studies as did Wartenberg et al. (2000), presenting a pooled relative risk estimate for kidney cancer incidence and mortality combined. The RRp for kidney cancer in cohort studies (Axelson et al., 1994; Anttila et al., 1995; Blair et al., 1998; Morgan et al., 1998; Boice et al., 1999; Hansen et al., 2001; Raaschou-Nielsen et al., 2003) was 1.29 (95% CI: 1.06−1.57) with no evidence of heterogeneity. Kelsh et al. (2005), also, presented separately a pooled relative risk for renal cancer case-control studies and TCE. For case-control studies (Siemiatycki et al., 1991; Greenland et al., 1994; Vamvakas et al., 1998; Dosemeci et al., 1999; Pesch et al., 2000a; Brüning et al., 2003), the RRp for renal cell carcinoma was 1.7 (95% CI: 1.0, 2.7) (interpolated from Figure 26 of NRC presentation) with evidence of heterogeneity, and RRp of 1.2 (95% CI: 0.9, 1.4) (interpolated from Figure 26 of NRC presentation) and no evidence of heterogeneity in a sensitivity analysis removing Vamvakas et al. (1998) and Brüning et al. (2003), two studies Kelsh et al. (2005) considered as "outliers."

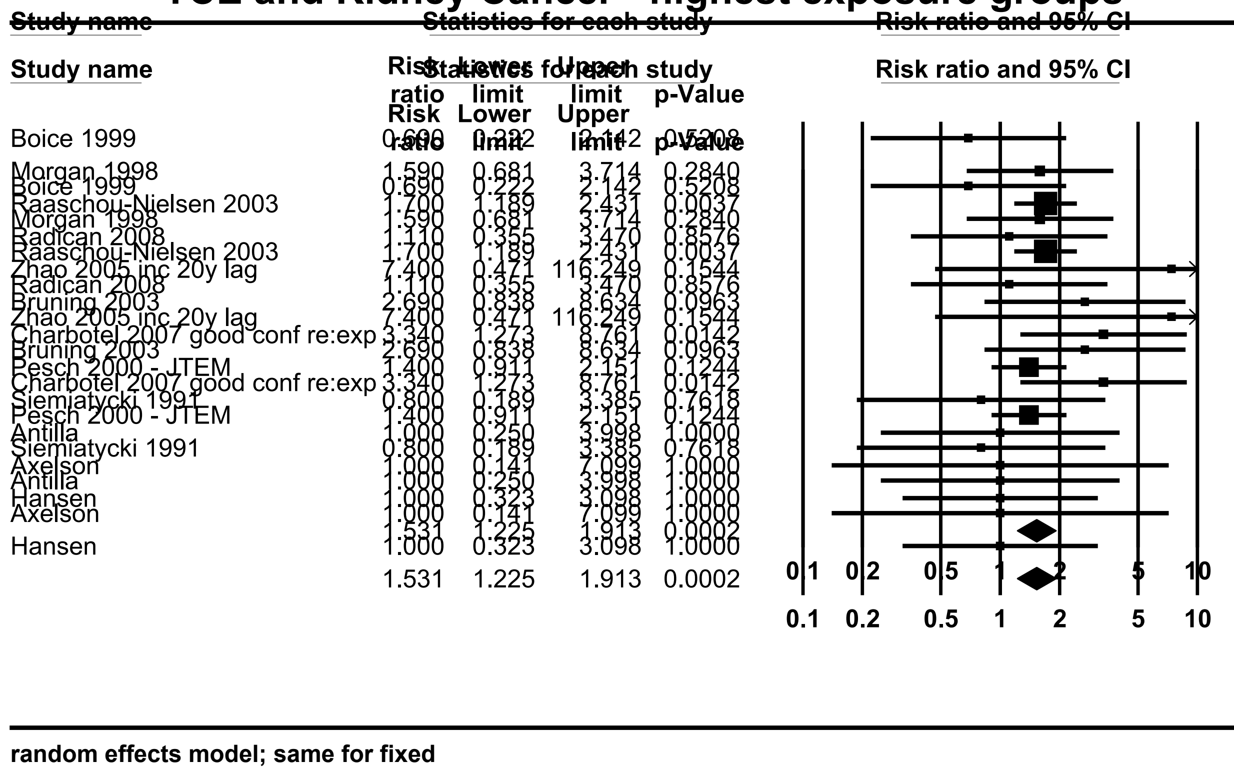

Figure 4-2. Meta-analysis of kidney cancer and TCE exposure—highest exposure groups. With assumed null RR estimates for Antilla, Axelson, and Hansen (see Appendix C text).

The present analysis was conducted according to NRC (2006) suggestions for transparency, systematic review criteria, and examination of both cohort and case-control studies. The present analysis includes the recently published study of Charbotel et al. (2006) and an analysis that examines both the TCE subcohort and case-control studies together. As discussed above, the pooled estimate from the primary random effects meta-analysis of the 14 studies was 1.25 (95% CI: 1.11, 1.41). Additionally, U.S. EPA examined kidney cancer risk for higher exposure group. The RRp estimate from the random effects meta-analysis of the studies with results presented for higher exposure groups was 1.59 (95% CI: 1.26, 2.01), higher than the RRp from the overall kidney cancer meta-analysis, and 1.53 (95% CI: 1.23, 1.91) in the meta-analysis with null RR estimates (i.e., RR = 1.0) to address possible reporting bias for three studies.

4.4.3. Human Studies of Somatic Mutation of von Hippel-Lindau (*VHL*) Gene

Studies have been conducted to identify mutations in the *VHL* gene in renal cell carcinoma patients, with and without TCE exposures (Wells et al., 2009; Charbotel et al., 2007; Schraml et al., 1999; Brauch et al., 1999, 2004; Toma et al., 2008; Furge et al., 2007; Kenck et al., 1996). Inactivation of the *VHL* gene through mutations, loss of heterozygosity (LOH) and imprinting has been observed in about 70% of sporadic renal clear cell carcinomas, the most common renal cell carcinoma subtype (Kenck et al., 1996). Other genes or pathways, including c-myc activation and VEGF, have also been examined as to their role in various renal cell carcinoma subtypes (Furge et al., 2007; Toma et al., 2008). Furge et al. (2007) reported that there are molecularly distinct forms of RCC and possibly molecular differences between clear-cell renal cell carcinoma subtypes. This study was performed using tissues obtained from paraffin blocks. These results are supported by a more recent study which examined the genetic abnormalities of clear cell renal cell carcinoma using frozen tissues from 22 cc-RCC patients and paired normal tissues (Toma et al., 2008). This study found that 20 (91%) of the 22 cases had LOH on chromosome 3p (harboring the *VHL* gene). Alterations in copy number were also found on chromosome 9 (32% of cases), chromosome arm 14q (36% of cases), chromosome arm 5q (45% of cases) and chromosome 7 (32% of cases), suggesting roles for multiple genetic changes in RCC, and is also supported by genomes-wide single-nucleotide polymorphism analysis (Toma et al., 2008).

Several papers link mutation of the *VHL* gene in renal cell carcinoma patients to TCE exposure. These reports are based on comparisons of *VHL* mutation frequencies in TCE exposed cases from renal cell carcinoma case-control studies or from comparison to background mutation rates among renal cell carcinoma case series (see Table 4-40). Brüning et al. (1997a) first

reported a high somatic mutation frequency (100%) in a series of 23 renal cell carcinomas cases with medium to high intensity TCE exposure as determined by an abnormal SSCP pattern, with most variations found in exon two. Only four samples were sequenced at the time of publication and showed mutations in exon one, two and three (see Table 4-40). Some of the cases in this study were from the case-control study of Vamvakas et al. (1998) (see Section 4.4.3 and Appendix C).

Brauch et al. (1999, 2004) analyzed renal cancer cell tissues for mutations of the *VHL* gene and reported increased occurrence of mutations in patients exposed to high concentrations of TCE. In the first study (Brauch et al., 1999), an employer's liability or worker's compensation registry was used to identify 44 renal cell carcinoma cases, 18 of whom were also included in Brüning et al. (1997a). Brauch et al. (1999) found multiple mutations in 42% of the exposed patients who experienced any mutation and 57% showed loss of heterozygosity. A hot spot mutation of cytosine to thymine at nucleotide 454 (C454T) was found in 39% of samples that had a *VHL* mutation and was not found in renal cell cancers from nonexposed patients or in lymphocyte DNA from either exposed or nonexposed cases or controls. As discussed above, little information was given on how subjects were selected and whether there was blinding of exposure status during the DNA analysis. In the second study, Brauch et al. (2004) investigated 21 of the 39 renal cell carcinoma patients identified as non-TCE exposed from Vamvakas et al. (1998) for which tissue specimens were available. The earlier studies of Brüning et al. (1997a) or Brauch et al. (1999) included *VHL* sequencing of tissue specimens from TCE-exposed cases from the renal cell carcinoma case-control study of Vamvakas et al. (1998). Brauch et al. (2004) compared age at diagnosis and histopathologic parameters of tumors as well as somatic mutation characteristics in the *VHL* tumor suppressor gene between the TCE-exposed and non-TCE exposed renal cell carcinoma patient groups (TCE-exposed from their previous 1999 publication to the non TCE-exposed cases newly sequenced in this study). Renal cell carcinoma did not differ with respect to histopathologic characteristics in either patient group. Comparing results from TCE-exposed and nonexposed patients revealed clear differences with respect to (1) frequency of somatic *VHL* mutations, (2) incidence of C454T transition, and (3) incidence of multiple mutations. The C454T hot spot mutation at codon 81 was exclusively detected in tumors from TCE-exposed patients, as were multiple mutations. Also, the incidence of *VHL* mutations in the TCE-exposed group was at least 2-fold higher than in the nonexposed group. Overall, these finding support the view that the effect of TCE is not limited to clonal expansion of cells mutated spontaneously or by some other agent.

Table 4-40. Summary of human studies on somatic mutations of the *VHL* gene[a]

TCE exposure status	Brüning et al., 1997a	Brauch et al., 1999		Schraml et al., 1999		Brauch et al., 2004		Charbotel et al., 2007	
	Exposed	Exposed	Unexposed	Exposed	Unexposed	Exposed	Unexposed	Exposed	Unexposed
Number of subjects/ Number with mutations (%)	23/23 (100%)	44/33 (75%)	73/42 (58%)	9/3 (33%)	113/38 (34%)	17/14 (82%)	21/2 (10%)	25/2 (9%)	23/2 (8%)
Renal cell carcinoma subtype	Unknown	Unknown		Clear cell 9 (75%) Papillary 2 (18%) Oncocytomas 1 (8%)	Unknown	Clear cell 37 (%) Oncocytic adenoma 1 (%) Bilateral metachronous 1 (%)		Clear cell 51 (75%) Papillary 10 (10−15%) Chromophobe 4 (5%) Oncocytomas 4 (5%)	
Tissue type analyzed	Paraffin	Paraffin, fresh (lymphocyte)		Paraffin		Paraffin		Paraffin, frozen tissues, Bouin's fixative	
Assay	SSCP,[b] sequencing[b]	SSCP, sequencing, restriction enzyme digestion		CGH, sequencing		Sequencing		Sequencing	
Number of mutations	23	50	42	4	50	24	2	2	2
Type of mutation									
Missense	1	27	NA	1	Unknown	17	2	1	1
Nonmissense[c]	3	23	NA	3	Unknown	7	0	1	1

[a]Adapted from NRC (2006) with addition of Schraml et al. (1999) and Charbotel et al. (2007).
[b]By single stand conformation polymorphism (SSCP). Four (4) sequences confirmed by comparative genomic hybridization.
[c]Includes insertions, frameshifts, and deletions.

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Brauch et al. (2004) were not able to analyze all RCCs from the Vamvakas study (Vamvakas et al., 1998), in part because samples were no longer available. Using the data described by Brauch et al. (2004) (*VHL* mutation found in 15 exposed and 2 nonexposed individuals, and *VHL* mutation not found in 2 exposed and 19 unexposed individuals), the calculated OR is 71.3. The lower bound of the OR including the excluded RCCs is derived from the assumption that all 20 cases that were excluded were exposed but did not have mutations in *VHL* (*VHL* mutations were found in 15 exposed and 2 unexposed individuals and *VHL* was not found in 22 exposed and 18 unexposed individuals), leading to an OR of 6.5 that remains statistically significant.

Charbotel et al. (2007) examines somatic mutations in the three *VHL* coding exons in RCC cases from their case-control study (Charbotel et al., 2006). Of the 87 RCCs in the case-control study, tissue specimens were available for 69 cases (79%) of which 48 were cc-RCC. *VHL* sequencing was carried out for only the cc-RCC cases, 66% of the 73 cc-RCC cases in Charbotel et al. (2006). Of the 48 cc-RCC cases available for *VHL* sequencing, 15 subjects were identified with TCE exposure (31%), an exposure prevalence lower than 43% observed in the case-control study. Partial to full sequencing of the *VHL* gene was carried out using polymerase chain reaction (PCR) amplification and *VHL* mutation pattern recognition software of Béroud et al. (1998). Full sequencing of the *VHL* gene was possible for only 26 RCC cases (36% of all RCC cases). Single point mutations were identified in 4 cases (8% prevalence): 2 unexposed cases, a G>C mutation in exon 2 splice site and a G>A in exon 1; one case identified with low/medium exposure, T>C mutation in exon 2, and, one case identified with high TCE exposure, T>C in exon 3. It should be noted that the two cases with T>C mutations were smokers unlike the cases with G>A or G>C mutations. The prevalence of somatic *VHL* mutation in this study is quite low compared to that observed in other RCC case series from this region; around 50% (Bailly et al., 1995; Gallou et al., 2001). To address possible bias from misclassification of TCE exposure, Charbotel et al. (2006) examined renal cancer risk for jobs associated with a high level of confidence for TCE exposure. As would be expected if bias was a result of misclassification, they observed a stronger association between higher confidence TCE exposure and RCC, suggesting that some degree of misclassification bias is associated with their broader exposure assessment approach. Charbotel et al. (2007) do not present findings on *VHL* mutations for those subjects with higher level of confidence TCE exposure assignment.

Schraml et al. (1999) did not observe statistically significant differences in DNA sequence or mutation type in a series of 12 renal cell carcinomas from subjects exposed to solvents including varying TCE intensity and a parallel series of 113 clear cell carcinomas from non-TCE exposed patients. Only 9 of the RCC were cc-RCC and were sequenced for mutations.

VHL mutations were observed in clear cell tumors only; four mutations in three TCE-exposed subjects compared to 50 mutations in tumors of 38 nonexposed cases. Details as to exposure conditions are limited to a statement that subjects had been exposed to high doses of solvents, potential for mixed solvent exposures, and that exposure included a range of TCE concentrations. Limitations of this study include having a wider range of TCE exposure intensities as compared to the studies described above (Brüning et al., 1997a; Brauch et al., 1999, 2004), which focused on patients exposed to higher levels of TCE, and the limited number of TCE-exposed subjects analyzed, being the smallest of all available studies on RCC, TCE and *VHL* mutation. For these reasons, Schraml et al. (1999) is quite limited for examining the question of *VHL* mutations and TCE exposure.

A number of additional methodological issues need to be considered in interpreting these studies. Isolation of DNA for mutation detection has been performed using various tissue preparations, including frozen tissues, formalin fixed tissues and tissue sections fixed in Bouin's solution. Ideally, studies would be performed using fresh or freshly frozen tissue samples to limit technical issues with the DNA extraction. When derived from other sources, the quality and quantity of the DNA isolated can vary, as the formic acid contained in the formalin solution, fixation time and period of storage of the tissue blocks often affect the quality of DNA. Picric acid contained in Bouin's solution is also known to degrade nucleic acids resulting in either low yield or poor quality of DNA. In addition, during collection of tumor tissues, contamination of neighboring normal tissue can easily occur if proper care is not exercised. This could lead to the 'dilution effect' of the results—i.e., because of the presence of some normal tissue, frequency of mutations detected in the tumor tissue can be lower than expected. These technical difficulties are discussed in these papers, and should be considered when interpreting the results. Additionally, selection bias is possible given tissue specimens were not available for all RCC cases in Vamvakas et al. (1998) or in Charbotel et al. (2006). Some uncertainty associated with misclassification bias is possible given the lack of TCE exposure information to individual subjects in Schraml et al. (1999) and in Charbotel et al. (2007) from their use of broader exposure assessment approach compared to that associated with the higher confident exposure assignment approach. A recent study by Nickerson et al. (2008) addresses many of these concerns by utilizing more sensitive methods to look at both the genetic and epigenetic issues related to *VHL* inactivation. This study was performed on DNA from frozen tissue samples and used a more sensitive technique for analysis for mutations (endonuclease scanning) as well as analyzing for methylation changes that may lead to inactivation of the *VHL* gene. This method of analysis was validated on tissue samples with known mutations. Of the 205 cc-RCC samples analyzed, 169 showed mutations in the *VHL* gene (82.4%). Of those 36 without mutation, 11

were hypermethylated in the promoter region, which will also lead to inactivation of the *VHL* gene. Therefore, this study showed inactivating alterations in the *VHL* gene (either by mutation or hypermethylation) in 91% tumor samples analyzed.

The limited animal studies examining the role of *VHL* mutation following exposure to chemicals including TCE are described below in Section 4.4.6.1.1. Conclusions as to the role of *VHL* mutation in TCE-induced kidney cancer, taking into account both human and experimental data, are presented below in Section 4.4.7.

4.4.4. Kidney Noncancer Toxicity in Laboratory Animals

Acute, subchronic, and chronic exposures to TCE cause toxicity to the renal tubules in rats and mice of both sexes. Nephrotoxicity from acute exposures to TCE has only been reported at relatively high doses, although histopathological changes have not been investigated in these experiments. Chakrabarty and Tuchweber (1988) found that TCE administered to male F344 rats by intraperitoneal injection (723−2,890 mg/kg) or by inhalation (1,000−2,000 ppm for 6 hours) produced elevated urinary NAG, γ-glutamyl transpeptidase (GGT), glucose excretion, blood urea nitrogen (BUN), and high molecular weight protein excretion, characteristic signs of proximal tubular, and possibly glomerular injury, as soon as 24 hours postexposure. In the intraperitoneal injection experiments, inflammation was observed, although some inflammation is expected due to the route of exposure, and nephrotoxicity effects were only statistically significantly elevated at the highest dose (2,890 mg/kg). In the inhalation experiments, the majority of the effects were statistically significant at both 1,000 and 2,000 ppm. Similarly, at these exposures, renal cortical slice uptake of *p*-aminohippurate was inhibited, indicating reduced proximal tubular function. Cojocel et al. (1989) found similar effects in mice administered TCE by intraperitoneal injection (120−1,000 mg/kg) at 6 hours postexposure, such as the dose-dependent increase in plasma BUN concentrations and decrease in *p*-aminohippurate accumulation in renal cortical slices. In addition, malondialdehyde (MDA) and ethane production were increased, indicating lipid peroxidation.

Kidney weight increases have been observed following inhalation exposure to TCE in both mice (Kjellstrand et al., 1983b) and rats (Woolhiser et al., 2006). Kjellstrand et al. (1983b) demonstrated an increase in kidney weights in both male (20% compared to control) and female (10% compared to control) mice following intermittent and continuous TCE whole-body inhalation exposure (up to 120 days). This increase was significant in males as low as 75 ppm exposure and in females starting at 150-ppm exposure. The latter study, an unpublished report by Woolhiser et al. (2006), was designed to examine immunotoxicity of TCE but also contains information regarding kidney weight increases in female Sprague Dawley (SD) rats exposed to

1 0-, 100-, 300-, and 1,000-ppm TCE for 6 hours/day, 5 days/week, for 4 weeks. Relative kidney
2 weights were significantly elevated (17.4% relative to controls) at 1,000-ppm TCE exposure.
3 However, the small number of animals and the variation in initial animal weight limit the ability
4 of this study to determine statistically significant increases.
5 Similarly, overt signs of subchronic nephrotoxicity, such as changes in blood or urinary
6 biomarkers, are also primarily a high dose phenomenon, although histopathological changes are
7 evident at lower exposures. Green et al. (1997b) reported administration of 2,000 mg/kg/d TCE
8 by corn oil gavage for 42 days in F344 rats caused increases of around 2-fold of control results in
9 urinary markers of nephrotoxicity such as urine volume and protein (both 1.8×), NAG (1.6×),
10 glucose (2.2×) and ALP (2.0×), similar to the results of the acute study of Chakrabarty and
11 Tuchweber (1988), above. At lower dose levels, Green et al. (1998b) reported that plasma and
12 urinary markers of nephrotoxicity were unchanged. In particular, after 1−28 day exposures to
13 250 or 500 ppm TCE for 6 hours/day, there were no statistically significant differences in plasma
14 levels of BUN or in urinary levels of creatinine, protein, ALP, NAG, or GGT. However,
15 increased urinary excretion of formic acid, accompanied by changes in urinary pH and increased
16 ammonia, was found at these exposures. Interestingly, at the same exposure level of 500 ppm
17 (6 hours/day, 5 days/week, for 6 months), Mensing et al. (2002) reported elevated excretion of
18 low molecular weight proteins and NAG, biomarkers of nephrotoxicity, but after the longer
19 exposure duration of 6 months.
20 Numerous studies have reported histological changes from TCE exposure for subchronic
21 and chronic durations (Maltoni et al., 1988, 1986; Mensing et al., 2002; NTP, 1990, 1988). As
22 summarized in Table 4-41, in 13-week studies in F344 rats and B6C3F1 mice, NTP (1990)
23 reported relatively mild cytomegaly and karyomegaly of the renal tubular epithelial cells at the
24 doses 1,000−6,000 mg/kg/d (at the other doses, tissues were not examined). The NTP report
25 noted that "these renal effects were so minimal that they were diagnosed only during a
26 reevaluation of the tissues ... prompted by the production of definite renal toxicity in the 2-year
27 study." In the 6 month, 500-ppm inhalation exposure experiments of Mensing et al. (2002),
28 some histological changes were noted in the glomeruli and tubuli of exposed rats, but they
29 provided no detailed descriptions beyond the statement that "perivascular, interstitial infections
30 and glomerulonephritis could well be detected in kidneys of exposed rats."

Table 4-41. Summary of renal toxicity and tumor findings in gavage studies of trichloroethylene by NTP (1990)

Sex	Dose (mg/kg)[a]	Cytomegaly and karyomegaly incidence (severity[b])	Adenoma (overall; terminal)	Adenocarcinoma (overall; terminal)
1/d, 5 d/wk, 13-wk study, F344/N rats				
Male	0, 125, 250, 500, 100	Tissues not evaluated	None reported	
	2,000	8/9 (Minimal/mild)		
Female	0, 62.5, 125, 250, 500	Tissues not evaluated		
	1,000	5/10 (Equivocal/minimal)		
1/d, 5 d/wk, 13-wk study, $B6C3F_1$ mice				
Male	0, 375, 750, 1,500	Tissues not evaluated	None reported	
	3,000	7/10[c] (Mild/moderate)		
	6,000	—[d]		
Female	0, 375, 750, 1,500	Tissues not evaluated		
	3,000	9/10 (Mild/moderate)		
	6,000	1/10 (Mild/moderate)		
1/d, 5 d/wk, 103-wk study, F344/N rats				
Male	0	0% (0)	0/48; 0/33	0/48; 0/33
	500	98% (2.8)	2/49; 0/20	0/49; 0/20
	1,000	98% (3.1)	0/49; 0/16	3/49; 3/16[e]
Female	0	0% (0)	0/50; 0/37	0/50; 0/37
	500	100% (1.9)	0/49; 0/33	0/49; 0/33
	1,000	100% (2.7)	0/48; 0/26	1/48; 1/26
1/d, 5 d/wk, 103-wk study, $B6C3F_1$ mice				
Male	0	0% (0)	1/49; 1/33	0/49; 0/33
	1,000	90% (1.5)	0/50; 0/16	1/50; 0/16
Female	0	0% (0)	0/48; 0/32	0/48; 0/32
	1,000	98% (1.8)	0/49; 0/23	0/49; 0/23

[a]Corn oil vehicle.
[b]Numerical scores reflect the average grade of the lesion in each group (1, slight; 2, moderate; 3, well marked; and 4, severe).
[c]Observed in four mice that died after 7−13 weeks and in three that survived the study.
[d]All mice died during the first week.
[e]$p = 0.028$.

1 After 1−2 years of chronic TCE exposure by gavage (NCI, 1976; NTP, 1990, 1988) or
2 inhalation (Maltoni et al., 1988) (see Tables 4-41−4-45), both the incidence and severity of these
3 effects increases, with mice and rats exhibiting lesions in the tubular epithelial cells of the inner
4 renal cortex that are characterized by cytomegaly, karyomegaly, and toxic nephrosis. As with
5 the studies at shorter duration, these chronic studies reported cytomegaly and karyomegaly of
6 tubular cells. NTP (1990) specified the area of damage as the pars recta, located in the
7 corticomedullary region. It is important to note that these effects are distinct from the chronic
8 nephropathy and inflammation observed in control mice and rats (Lash et al., 2000b; Maltoni et
9 al., 1988; NCI, 1976).
10 These effects of TCE on the kidney appear to be progressive. Maltoni et al. (1988) noted
11 that the incidence and degree of renal toxicity increased with increased exposure time and
12 increased time from the start of treatment. As mentioned above, signs of toxicity were present in
13 the 13 week study (NTP, 1988), and NTP (1990) noted cytomegaly at 26 weeks. NTP (1990)
14 noted that as "exposure time increased, affected tubular cells continued to enlarge and additional
15 tubules and tubular cells were affected," with toxicity extending to the cortical area as kidneys
16 became more extensively damaged. NTP (1988, 1990) noted additional lesions that increased in
17 frequency and severity with longer exposure, such as dilation of tubules and loss of tubular cells
18 lining the basement membrane ("stripped appearance" [NTP, 1988] or flattening of these cells
19 [NTP, 1990]). NTP (1990) also commented on the intratubular material and noted that the
20 tubules were empty or "contained wisps of eosinophilic material."
21 With gavage exposure, these lesions were present in both mice and rats of both sexes, but
22 were on average more severe in rats than in mice, and in male rats than in female rats (NTP,
23 1990). Thus, it appears that male rats are most sensitive to these effects, followed by female rats
24 and then mice. This is consistent with the experiments of Maltoni et al. (1988), which only
25 reported these effects in male rats. The limited response in female rats or mice of either sex in
26 these experiments may be related to dose or strain. The lowest chronic gavage doses in the
27 National Cancer Institute (NCI, 1976) and NTP (1988, 1990) F344 rat experiments was
28 500 mg/kg/d, and in all these cases at least 80% (and frequently 100%) of the animals showed
29 cytomegaly or related toxicity. By comparison, the highest gavage dose in the Maltoni et al.
30 (1988) experiments (250 mg/kg/d) showed lower incidences of renal cytomegaly and
31 karyomegaly in male Sprague-Dawley rats (47% and 67%, overall and corrected incidences) and
32 none in female rats. The B6C3F1 mouse strain was used in the NCI (1976), NTP (1990), and
33 Maltoni et al. (1988) studies (see Tables 4-41−4-45). While the two gavage studies (NCI, 1976;
34 NTP, 1990) were consistent, reporting at least 90% incidence of cytomegaly and karyomegaly at

1 **Table 4-42. Summary of renal toxicity and tumor findings in gavage studies**
2 **of trichloroethylene by NCI (1976)**
3

Sex	Dose (mg/kg)[a]	Toxic nephrosis (overall; terminal)	Adenoma or adenocarcinoma (overall; terminal)[b]
1/d, 5 d/wk, 2-yr study, Osborn-Mendel rats			
Males	0	0/20; 0/2	0/20; 0/2
	549	46/50; 7/7	1/50;[c] 0/7
	1,097	46/50; 3/3	0/50; 0/3
Females	0	0/20; 0/8	0/20; 0/8
	549	39/48; 12/12	0/48; 0/12
	1,097	48/50; 13/13	0/50; 0/13
1/d, 5 d/wk, 2-yr study, B6C3F1 mice			
Males	0	0/20; 0/8	0/20; 0/8
	1,169	48/50; 35/35	0/50; 0/35
	2,339	45/50; 20/20	1/50;[d] 1/20
Females	0	0/20; 0/17	0/20; 0/17
	869	46/50; 40/40	0/50; 0/40
	1,739	46/47;[e] 39/39	0/47; 0/39

4 [a]Treatment period was 48 weeks for rats, 66 weeks for mice. Doses were changed several times during the study
5 based on monitoring of body weight changes and survival. Dose listed here is the time-weighted average dose
6 over the days on which animals received a dose.
7 [b]A few malignant mixed tumors and hamartomas of the kidney were observed in control and low dose male rats, but
8 are not counted here.
9 [c]Tubular adenocarcinoma.
10 [d]Tubular adenoma.
11 [e]One mouse was reported with “nephrosis,” but not “nephrosis toxic,” and so was not counted here.
12
13 **Table 4-43. Summary of renal toxicity findings in gavage studies of**
14 **trichloroethylene by Maltoni et al. (1988)**
15

Sex	Dose (mg/kg)[a]	Megalonucleocytosis[b] (overall; corrected[c])
1/d, 4−5 d/wk, 52-wk exposure, observed for lifespan, Sprague-Dawley rats		
Males	0	0/20; 0/22
	50	0/30; 0/24
	250	14/30; 14/21
Females	0	0/30; 0/30
	50	0/30; 0/29
	250	0/30; 0/26

16
17 [a]Olive oil vehicle.
18 [b]Renal tubuli megalonucleocytosis is the same as cytomegaly and karyomegaly of renal tubuli cells (Maltoni et al.,
19 1988).
20 [c]Denominator for “corrected” incidences is the number of animals alive at the time of the first kidney lesion in this
21 experiment (39 weeks).

Table 4-44. Summary of renal toxicity and tumor incidence in gavage studies of trichloroethylene by NTP (1988)

Sex	Dose (mg/kg)*	Cytomegaly	Toxic Nephropathy	Adenoma (overall; terminal)	Adenocarcinoma (overall; terminal)
1/d, 5 d/wk, 2-yr study, ACI rats					
Male	0	0/50	0/50	0/50; 0/38	0/50; 0/38
	500	40/49	18/49	0/49; 0/19	1/49; 0/19
	1,000	48/49	18/49	0/49; 0/11	0/49; 0/11
Female	0	0/48	0/48	0/48; 0/34	0/48; 0/34
	500	43/47	21/47	2/47; 1/20	1/47; 1/20
	1,000	42/43	19/43	0/43; 0/19	1/43; 0/19
1/d, 5 d/wk, 2-yr study, August rats					
Male	0	0/50	0/50	0/50; 0/21	0/50; 0/21
	500	46/50	10/50	1/50; 0/13	1/50; 1/13
	1,000	46/49	31/49	1/49; 1/16	0/49; 0/16
Female	0	0/49	0/49	1/49; 1/23	0/49; 0/23
	500	46/48	8/48	2/48; 1/26	2/48; 2/26
	1,000	50/50	29/50	0/50; 0/25	0/50; 0/25
1/d, 5 d/wk, 2-yr study, Marshall rats					
Male	0	0/49	0/49	0/49; 0/26	0/49; 0/26
	500	48/50	18/50	1/50; 0/12	0/50; 0/12
	1,000	47/47	23/47	0/47; 0/6	1/47; 0/6
Female	0	0/50	0/50	1/50; 0/30	0/50; 0/30
	500	46/48	30/48	1/48; 1/12	1/48; 0/12
	1,000	43/44	30/44	0/44; 0/10	1/44; 1/10
1/d, 5 d/wk, 2-yr study, Osborne-Mendel rats					
Male	0	0/50	0/50	0/50; 0/22	0/50; 0/22
	500	48/50	39/50	6/50; 5/17	0/50; 0/17
	1,000	49/50	35/50	1/50; 1/15	1/50; 0/15
Female	0	0/50	0/50	0/50; 0/20	0/50; 0/20
	500	48/50	30/50	0/50; 0/11	0/50; 0/11
	1,000	49/49	39/49	1/49; 0/7	0/49; 0/7

*Corn oil vehicle.

1 **Table 4-45. Summary of renal toxicity and tumor findings in inhalation**
2 **studies of trichloroethylene by Maltoni et al. (1988)[a]**
3

Sex	Concentration (ppm)	Meganucleocytosis[b] (overall; corrected)	Adenoma (overall; corrected)	Adenocarcinoma (overall; corrected)
7 h/d, 5 d/wk, 2-yr exposure, observed for lifespan, Sprague-Dawley rats[c]				
Male	0	0/135; 0/122	0/135; 0/122	0/135; 0/122
	100	0/130; 0/121	1/130; 1/121	0/130; 0/121
	300	22/130; 22/116	0/130; 0/116	0/130; 0/116
	600	101/130; 101/124	1/130; 1/124	4/130; 4/124
Female	0	0/145; 0/141	0/145; 0/141	0/145; 0/141
	100	0/130; 0/128	1/130; 1/128	0/130; 0/128
	300	0/130; 0/127	0/130; 0/127	0/130; 0/127
	600	0/130; 0/127	0/130; 0/127	1/130; 1/127
7 h/d, 5 d/wk, 78-wk exposure, observed for lifespan, B6C3F1 mice[d]				
Male	0	0/90	0/90	0/90
	100	0/90	0/90	1/90
	300	0/90	0/90	0/90
	600	0/90	0/90	0/90
Female	0	0/90	0/90	1/90
	100	0/90	0/90	0/90
	300	0/90	0/90	0/90
	600	0/90	0/90	0/90

4
5 [a]Three inhalation experiments in this study found no renal megalonucleocytosis, adenomas, or adenocarcinomas:
6 BT302 (8-week exposure to 0, 100, 600 ppm in Sprague-Dawley rats); BT303 (8-week exposure to 0, 100, or 600
7 ppm in Swiss mice); and BT305 (78-week exposure to 0, 100, 300, or 600 ppm in Swiss mice).
8 [b]Renal tubuli meganucleocytosis is the same as cytomegaly and karyomegaly of renal tubuli cells (Maltoni et al.,
9 1988).
10 [c]Combined incidences from experiments BT304 and BT304bis. Corrected incidences reflect number of rats alive at
11 47 weeks, when the first renal tubular megalonucleocytosis in these experiments appeared.
12 [d]Female incidences are from experiment BT306, while male incidences are from experiment BT306bis, which was
13 added to the study because of high, early mortality due to aggressiveness and fighting in males in experiment
14 BT306. Corrected incidences not show, because only the renal adenocarcinomas appeared at 107 weeks in the
15 male and 136 in the female, when the most of the mice were already deceased.
16
17
18 all studied doses, whether dose accounts for the lack of kidney effects in Maltoni et al. (1988)
19 requires comparing inhalation and gavage dosing. Such comparisons depend substantially on the
20 internal dose metric, so conclusions as to whether dose can explain differences across studies
21 cannot be addressed without dose-response analysis using physiologically based

1 pharmacokinetic (PBPK) modeling. Some minor differences were found in the multistrain NTP
2 study (1988), but the high rate of response makes distinguishing among them difficult. Soffritti
3 (personal communication with JC Caldwell, February 14, 2006) did note that the colony from
4 which the rats in Maltoni et al. (1986, 1988) experiments were derived had historically low
5 incidences of chronic progressive nephropathy and renal cancer.
6

7 **4.4.5. Kidney Cancer in Laboratory Animals**

8 ***4.4.5.1. Inhalation Studies of Trichloroethylene (TCE)***

9 A limited number of inhalation studies examined the carcinogenicity of TCE, with no
10 statistically-significantly increases in kidney tumor incidence reported in mice or hamsters
11 (Fukuda et al., 1983; Henschler et al., 1980; Maltoni et al., 1988, 1986). The cancer bioassay by
12 (Maltoni et al., 1986, 1988) reported no statistically significant increase in kidney tumors in mice
13 or hamsters, but renal adenocarcinomas were found in male (4/130) and female (1/130) rats at
14 the high dose (600 ppm) after 2 years exposure and observation at natural death. In males, these
15 tumors seemed to have originated in the tubular cells, and were reported to have never been
16 observed in over 50,000 Sprague-Dawley rats (untreated, vehicle-treated, or treated with
17 different chemicals) examined in previous experiments in the same laboratory (Maltoni et al.,
18 1986). The renal adenocarcinoma in the female rat was cortical and reported to be similar to that
19 seen infrequently in historical controls. This study also demonstrated the appearance of
20 increased cytokaryomegaly or megalonucleocytosis, a lesion that was significantly and dose-
21 dependently increased in male rats only (see Table 4-45). Maltoni et al. (1986) noted that some
22 considerations supported either the hypothesis that these were precursor lesions of renal
23 adenocarcinomas cancer or the hypothesis that these are not precursors but rather the
24 morphological expression of TCE-induced regressive changes. The inhalation studies by Fukuda
25 et al. (1983) in Sprague-Dawley rats and female ICR mice, reported one clear cell carcinoma in
26 rats exposed to the highest concentration (450 ppm) but saw no increase in kidney tumors in
27 mice. This result was not statistically significant (see Table 4-46). One negative study
28 (Henschler et al., 1980) tested NMRI mice, Wistar rats, and Syrian hamsters of both sexes (60
29 animals per strain), and observed no significant increase in renal tubule tumors any of the species
30 tested. Benign adenomas were observed in male mice and rats, a single adenocarcinoma was
31 reported in male rats at the highest dose, and no renal adenocarcinomas reported in females of
32 either species (see Table 4-46). Renal cell carcinomas appear to be very rare in Wistar rats, with
33 historical control rates reported to be about 0.4% in males and 0.2% in females (Potericki and
34 Walsh, 1998), so these data are very limited in power to detect small increases in their incidence.
35

Table 4-46. Summary of renal tumor findings in inhalation studies of trichloroethylene by Henschler et al. (1980)[a] and Fukuda et al. (1983)[b]

Sex	Concentration (ppm)	Adenomas	Adenocarcinomas
6 h/d, 5 d/wk, 18-month exposure, 30-month observation, Han:NMRI mice (Henschler et al., 1980)			
Males	0	4/30	1/30
	100	1/29	0/30
	500	1/29	0/30
Females	0	0/29	0/29
	100	0/30	0/30
	500	0/28	0/28
6 h/d, 5 d/wk, 18-month exposure, 36-month observation, Han:WIST rats (Henschler et al., 1980)			
Males	0	2/29	0/29
	100	1/30	0/30
	500	2/30	1/30
Females	0	0/28	0/28
	100	0/30	0/30
	500	1/30	0/30
7 h/d, 5 d/wk, 2-yr study, Crj:CD (SD) rats (Fukuda et al., 1983)			
Females	0	0/50	0/50
	50	0/50	0/50
	150	0/47	0/47
	450	0/51	1/50

[a]Henschler et al. (1980) observed no renal tumors in control or exposed Syrian hamsters.
[b]Fukuda et al. (1983) observed no renal tumors in control or exposed Crj:CD-1 (ICR) mice.

4.4.5.2. *Gavage and Drinking Water Studies of Trichloroethylene (TCE)*

Several chronic gavage studies exposing multiple strains of rats and mice to 0−3,000 mg/kg TCE for at least 52 weeks have been conducted (see Tables 4-41−4-44, 4-47) (Henschler et al., 1984; Maltoni et al., 1986; NCI, 1976; NTP, 1988, 1990; Van Duuren et al., 1979). Van Duuren et al. (1979) examined TCE and 14 other halogenated compounds for carcinogenicity in both sexes of Swiss mice. While no excess tumors were observed, the dose rate (0.5 mg once per week, or an average dose rate of approximately 2.4 mg/kg/d for a 30 g mouse) is about 400-fold lower than that in the other gavage studies. Inadequate design and reporting of this study limit the ability to use the results as an indicator of TCE carcinogenicity. In the NCI (1976)

study, the results for Osborne-Mendel rats were considered by the authors to be inconclusive due to significant early mortality. In rats of both sexes, no increase was seen in primary tumor induction over that observed in controls. While both sexes of B6C3F1 mice showed a compound-related increase in nephropathy, no increase in tumors over controls was observed. The NCI study (1976) used technical grade TCE which contained two known carcinogenic compounds as stabilizers (epichlorohydrin and 1,2-epoxybutane). However, a subsequent study by Henschler et al. (1984) in mice reported no significant differences in systemic tumorigenesis between pure, industrial, and stabilized TCE, suggesting that concentrations of these stabilizers are too low to be the cause of tumors. A later gavage study by NTP (1988), using TCE stabilized with diisopropylamine, observed an increased incidence of renal tumors in all four strains of rats (ACI, August, Marshall, and Osborne-Mendel). All animals exposed for up to 2 years (rats and mice) had non-neoplastic kidney lesions, even if they did not later develop kidney cancer (see Table 4-44). This study was also considered inadequate by the authors because of chemically induced toxicity, reduced survival, and incomplete documentation of experimental data. The final NTP study (1990) in male and female F344 rats and B6C3F1 mice used epichlorohydrin-free TCE. Only in the highest-dose group (1,000 mg/kg) of male F344 rats was renal carcinoma statistically significant increased. The results for detecting a carcinogenic response in rats were considered by the authors to be equivocal because both groups receiving TCE showed significantly reduced survival compared to vehicle controls and because of a high rate (e.g., 20% of the animals in the high-dose group) of death by gavage error. However, historical control incidences at NTP of kidney tumors in F344 rats is very low,[2] lending biological significance to their occurrence in this study, despite the study's limitations. Cytomegaly and karyomegaly were also increased, particularly in male rats. The toxic nephropathy observed in both rats and mice and contributed to the poor survival rate (see Table 4-41). As discussed previously, this toxic nephropathy was clearly distinguishable from the spontaneous chronic progression nephropathy commonly observed in aged rats.

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[2] NTP (1990) reported a historical control incidence of 0.4% in males. The NTP web site reports historical control rates of renal carcinomas for rats dosed via corn oil gavage on the NIH-07 diet (used before 1995, when the TCE studies were conducted) to be 0.5% (2/400) for males and 0% (0/400) for females (http://ntp-server.niehs.nih.gov/ntp/research/database_searches/historical_controls/path/r_gavco.txt). In addition, the 2 occurences in males came from the same study, with all other studies reporting 0/50 carcinomas.

1 **Table 4-47. Summary of renal tumor findings in gavage studies of**
2 **trichloroethylene by Henschler et al. (1984)[a] and Van Duuren et al. (1979)[b]**
3

Sex (TCE dose)	Control or TCE Exposed (Stabilizers if present)	Adenomas	Adenocarcinomas
5 d/wk, 18-month exposure, 24-month observation, Swiss mice (Henschler et al., 1984)			
Males (2.4g/kg bw)	Control (none)	1/50	1/50
	TCE (triethanolamine)	1/50	1/50
	TCE (industrial)	0/50	0/50
	TCE (epichlorohydrin (0.8%))	0/50	0/50
	TCE (1,2-epoxybutane (0.8%))	2/50	2/50
	TCE (both epichlorohydrin (0.25%) and 1,2-epoxybutane (0.25%))	0/50	0/50
Females (1.8 g/kg bw)	Control (none)	0/50	1/50
	TCE (triethanolamine)	4/50	0/50
	TCE (industrial)	0/50	0/50
	TCE (epichlorohydrin (0.8%))	0/50	0/50
	TCE (1,2-epoxybutane (0.8%))	0/50	0/50
	TCE (both epichlorohydrin (0.25%) and 1,2-epoxybutane (0.25%))	0/50	0/50
1 d/wk, 89-week exposure, Swiss rats (Van Duuren et al., 1979)			
Males (0.5mg)	Control	0/30	0/30
	TCE (unknown)	0/30	0/30
Females (0.5mg)	Control	0/30	0/30
	TCE(unknown)	0/30	0/30

4
5 [a]Henschler et al. (1984) Due to poor condition of the animals resulting from the nonspecific toxicity of high doses of
6 TRI and/or the additives, gavage was stopped for all groups during weeks 35−40, 65 and 69−78, and all doses were
7 reduced by a factor of 2 from the 40th week on.
8 [b]Van Duuren et al. (1979) observed no renal tumors in control or exposed Swiss mice.
9
10
11 **4.4.5.3.** ***Conclusions: Kidney Cancer in Laboratory Animals***
12 Chronic TCE carcinogenicity bioassays have shown evidence of neoplastic lesions in the
13 kidney in rats (mainly in males, with less evidence in females), treated via inhalation and gavage.
14 As discussed above, individual studies have a number of limitations and have shown limited

increases in kidney tumors. However, given the rarity of these tumors as assessed by historical controls and the repeatability of this result, these are considered biologically significant.

4.4.6. Role of Metabolism in Trichloroethylene (TCE) Kidney Toxicity

It is generally thought that one or more TCE metabolites rather than the parent compound are the active moieties for TCE nephrotoxicity. As reviewed in Section 3.3, oxidation by CYPs, of which CYP2EI is thought to be the most active isoform, results in the production of chloral hydrate, trichloroacetic acid, dichloroacetic acid and trichloroethanol. The glutathione conjugation pathway produces metabolites such as DCVG, DCVC, dichlorovinylthiol, and NAcDCVC. Because several of the steps for generating these reactive metabolites occur in the kidney, the GSH conjugation pathway has been thought to be responsible for producing the active moiety or moieties of TCE nephrotoxicity. A comparison of TCE's nephrotoxic effects with the effects of TCE metabolites, both *in vivo* and *in vitro*, thus, provides a basis for assessing the relative roles of different metabolites. While most of the available data have been on metabolites from GSH conjugation, such as DCVC, limited information is also available on the major oxidative metabolites TCOH and TCA.

4.4.6.1. *In Vivo Studies of the Kidney Toxicity of Trichloroethylene (TCE) Metabolites*

4.4.6.1.1. ***Role of GSH conjugation metabolites of Trichloroethylene (TCE).*** In numerous studies, DCVC has been shown to be acutely nephrotoxic in rats and mice. Mice receiving a single dose of 1 mg/kg DCVC (the lowest dose tested in this species) exhibited karyolytic proximal tubular cells in the outer stripe of the outer medulla, with some sloughing of cells into the lumen and moderate desquamation of the tubular epithelium (Eyre et al., 1995b). Higher doses in mice were associated with more severe histological changes similar to those induced by TCE, such as desquamation and necrosis of the tubular epithelium (Darnerud et al., 1989; Terracini and Parker, 1965a; Vaidya et al., 2003a, b). In rats, no histological changes in the kidney were reported after single doses of 1, 5, and 10 mg/kg DCVC (Eyre et al., 1995a; Green et al., 1997a), but cellular debris in the tubular lumen was reported at 25 mg/kg (Eyre et al., 1995b) and slight degeneration and necrosis were seen at 50 mg/kg (Green et al., 1997). Green et al. (1997) reported no histological changes were noted in rats after 10 doses of 0.1−5.0 mg/kg DCVC (although increases in urinary protein and GGT were found), but some karyomegaly was noted in mice after 10 daily doses of 1 mg/kg. Therefore, mice appear more sensitive than rats to the nephrotoxic effects of acute exposure to DCVC, although the number of animals used at each dose in these studies was limited (10 or less). Although the data are not sufficient to assess the relatively sensitivity of other species, it is clear that multiple species, including rabbits, guinea

pigs, cats, and dogs, are responsive to DCVC's acute nephrotoxic effects (Jaffe et al., 1984; Krejci et al., 1991; Terracini and Parker, 1965b; Wolfgang et al., 1989b).

Very few studies are available at longer durations. Terracini and Parker (1965) gave DCVC in drinking water to rats at a concentration of 0.01% for 12 weeks (approximately 10 mg/kg/d), and reported consistent pathological and histological changes in the kidney. The progression of these effects was as follows: (1) during the first few days, completely necrotic tubules, with isolated pyknotic cells being shed into the lumen; (2) after 1 week, dilated tubules in the inner part of the cortex, lined with flat epithelial cells that showed thick basal membranes, some with big hyperchromatic nuclei; (3) in the following weeks, increased prominence of tubular cells exhibiting karyomegaly, seen in almost all animals, less pronounced tubular dilation, and cytomegaly in the same cells showing karyomegaly. In addition, increased mitotic activity was reported the first few days, but was not evident for the rest of the experiment. Terracini and Parker (1965) also reported the results of a small experiment (13 male and 5 female rats) given the same concentration of DCVC in drinking water for 46 weeks, and observed for 87 weeks. They noted renal tubular cells exhibiting karyomegaly and cytomegaly consistently throughout the experiment. Moreover, a further group of 8 female rats given DCVC in drinking water at a concentration of 0.001% (approximately 1 mg/kg/d) also exhibited similar, though less severe, changes in the renal tubules. In mice, Jaffe et al. (1984) gave DCVC in drinking water at concentrations of 0.001, 0.005, and 0.01% (estimated daily dose of 1−2, 7−13, and 17−22 mg/kg/d), and reported similar effects in all dose groups, including cytomegaly, nuclear hyperchromatism, and multiple nucleoli, particularly in the pars recta section of the kidney. Thus, effects were noted in both mice and rats under chronic exposures at doses as low as 1−2 mg/kg/d (the lowest dose tested). Therefore, while limited, the available data do not suggest differences between mice and rats to the nephrotoxic effects of DCVC under chronic exposure conditions, in contrast to the greater sensitivity of mice to acute and subchronic DCVC-induced nephrotoxicity.

Importantly, as summarized in Table 4-48, the histological changes and their location in these subchronic and chronic experiments with DCVC are quite similar to those reported in chronic studies of TCE, described above, particularly the prominence of karyomegaly and cytomegaly in the pars recta section of the kidney. Moreover, the morphological changes in the tubular cells, such as flattening and dilation, are quite similar. Similar pathology is not observed with the oxidative metabolites alone (see Section 4.4.6.1.2).

Table 4-48. Summary of histological changes in renal proximal tubular cells induced by chronic exposure to TCE, DCVC, and TCOH

Effects	TCE	DCVC	TCOH
Karyomegaly	Enlarged, hyperchromatic nuclei, irregular to oblong in shape. Vesicular nuclei containing prominent nucleoli.	Enlarged, hyperchromatic nuclei with and multiple nucleoli. Nuclear pyknosis and karyorrhexis.	None reported.
Cytomegaly	Epithelial cells were large, elongated and flattened.	Epithelial cells were large, elongated and flattened cells.	No report of enlarged cells.
Cell necrosis/ hyperplasia	Stratified epithelium that partially or completely filled the tubular lumens. Cells in mitosis were variable in number or absent. Cells had abundant eosinophilic or basophilic cytoplasm.	Thinning of tubular epithelium, frank tubular necrosis, re-epitheliation. Tubular atrophy, interstitial fibrosis and destruction of renal parenchyma. More basophilic and finely vacuolated.	No flattening or loss of epithelium reported. Increased tubular cell basophilia, followed by increased cellular eosinophilia, tubular cell vacuolation.
Morphology/ content of tubules	Some tubules enlarged/dilated to the extent that they were difficult to identify. Portions of basement membrane had a stripped appearance. Tubules were empty or contained "wisps of eosinophilic material."	Tubular dilation, denuded tubules. Thick basal membrane. Focal areas of dysplasia, intraluminal casts.	No tubular dilation reported. Intratubular cast formation.

Sources: NCI (1976); NTP (1988, 1990); Maltoni et al. (1988); Terracini and Parker (1965); Jaffe et al. (1985); Green et al. (2003).

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1 Additionally, it is important to consider whether sufficient DCVC may be formed from
2 TCE exposure to account for TCE nephrotoxicity. While direct pharmacokinetic measurements,
3 such as the excretion of NAcDCVC, have been used to argue that insufficient DCVC would be
4 formed to be the active moiety for nephrotoxicity (Green et al., 1997), as discussed in Chapter 3,
5 urinary NAcDCVC is a poor marker of the flux through the GSH conjugation pathway because
6 of the many other possible fates of metabolites in that pathway. In another approach, Eyre et al.
7 (1995b) using acid-labile adducts as a common internal dosimeter between TCE and DCVC, and
8 reported that a single TCE dose of 400 mg/kg in rats (similar to the lowest daily doses in the NCI
9 and NTP rat bioassays) and 1,000 mg/kg (similar to the lowest daily doses in the NCI and NTP
10 mouse bioassays) corresponded to a single equivalent DCVC dose of 6 and 1 mg/kg/d in rats and
11 mice, respectively. These equivalent doses of DCVC are greater or equal to those in which
12 nephrotoxicity has been reported in these species under chronic conditions. Therefore, assuming
13 that this dose correspondence is accurate under chronic conditions, sufficient DCVC would be
14 formed from TCE exposure to explain the observed histological changes in the renal tubules.
15 The Eker rat model (*Tsc-2*$^{+/-}$) is at increased risk for the development of spontaneous
16 renal cell carcinoma and as such has been used to understand the mechanisms of renal
17 carcinogenesis (Stemmer et al., 2007; Wolf et al., 2000). One study has demonstrated similar
18 pathway activation in Eker rats as that seen in humans with *VHL* mutations leading to renal cell
19 carcinoma, suggesting *Tsc-2* inactivation is analogous to inactivation of *VHL* in human renal cell
20 carcinoma (Liu et al., 2003). Although the Eker rat model is a useful tool for analyzing
21 progression of renal carcinogenesis, it has some limitations in analysis of specific genetic
22 changes, particularly given the potential for different genetic changes depending on type of
23 exposure and tumor. The results of short-term assays to genotoxic carcinogens in the Eker rat
24 model (Morton et al., 2002; Stemmer et al., 2007) reported limited preneoplastic and neoplastic
25 lesions which may be related to the increased background rate of renal carcinomas in this animal
26 model.
27 Recently, Mally et al. (2006) exposed male rats carrying the Eker mutation to TCE
28 (0−1,000 mg/kg BW) by corn oil gavage and demonstrated no increase in renal preneoplastic
29 lesions or tumors. Primary Eker rat kidney cells exposed to DCVC in this study did induce an
30 increase in transformants *in vitro* but no DCVC-induced *vhl* or *Tsc-2* mutations were observed.
31 *In vivo* exposure to TCE (5 days/week for 13 weeks), decreased body weight gain and increased
32 urinary excretion at the two highest TCE concentrations analyzed (500 and 1,000 mg/kg BW)
33 but did not change standard nephrotoxicity markers (GGT, creatinine and urinary protein).
34 Renal tubular epithelial cellular proliferation as measured by BrdU incorporation was
35 demonstrated at the three highest concentrations of TCE (250, 500 and 1,000 mg/kg/d). A

minority of these cells also showed karyomegaly at the two higher TCE concentrations. Although renal cortical tumors were demonstrated in all TCE exposed groups, these were not significantly different from controls (13 weeks). These studies were complemented with *in vitro* studies of DCVC (10−50 μM) in rat kidney epithelial (RKE) cells examining proliferation at 8, 24, and 72 hours and cellular transformation at 6−7 weeks. Treatment of RKE cells from susceptible rats with DCVC gave rise to morphologically transformed colonies consistently higher than background (Mally et al., 2006). Analyzing ten of the renal tumors from the TCE exposed rats and nine of the DCVC transformants from these studies for alterations to the *VHL* gene that might lead to inactivation found no alterations to *VHL* gene expression or mutations.

One paper has linked the *VHL* gene to chemical-induced carcinogenesis. Shiao et al. (1998) demonstrated *VHL* gene somatic mutations in *N*-nitrosodimethylamine-induced rat kidney cancers that were of the clear cell type. The clear cell phenotype is rare in rat kidney cancers, but it was only the clear cell cancers that showed *VHL* somatic mutation (three of eight tumors analyzed). This provided an additional link between *VHL* inactivation and clear cell kidney cancer. However, this study examined archived formalin fixed paraffin embedded tissues from previous experiments. As described previously (see Section 4.4.2), DNA extraction from this type of preparation creates some technical issues. Similarly, archived formalin-fixed paraffin embedded tissues from rats exposed to potassium bromide were analyzed in a later study by Shiao et al. (2002). This later study examined the *VHL* gene mutations following exposure to potassium bromide, a rat renal carcinogen known to induce clear cell renal tumors. Clear cell renal tumors are the most common form of human renal epithelial neoplasms, but are extremely rare in animals. Although F344 rats exposed to potassium bromide in this study did develop renal clear cell carcinomas, only two of nine carried the same C to T mutation at the core region of the Sp1 transcription-factor binding motif in the *VHL* promoter region, and one of four untreated animals had a C to T mutation outside the conserved core region. Mutation in the *VHL* coding region was only detected in one tumor, so although the tumors developed following exposure to potassium bromide were morphologically similar to those found in humans; no similarities were found in the genetic changes.

Elfarra et al. (1984) found that both DCVG and DCVC administered to male F344 rats by intraperitoneal injections in isotonic saline resulted in elevations in BUN and urinary glucose excretion. Furthermore, inhibition of renal GGT activity with acivicin protected rats from DCVG-induced nephrotoxicity. In addition, both the ß-lyase inhibitor AOAA and the renal organic anion transport inhibitor probenecid provided protection from DCVC, demonstrating a requirement for metabolism of DCVG to the cysteine conjugate by the action of renal GGT and dipeptidase, uptake into the renal cell by the organic anion transporter, and subsequent activation

by the ß-lyase. This conclusion was supported further by showing that the -methyl analog of DCVC, which cannot undergo a ß-elimination reaction due to the presence of the methyl group, was not nephrotoxic.

Korrapati et al. (2005) builds upon a series of investigations of hetero- (by $HgCl_2$) and homo-(by DCVC, 15 mg/kg) protection against a lethal dose of DCVC (75 mg/kg). Priming, or preconditioning, with pre-exposure to either $HgCl_2$ or DCVC of male Swiss-Webster mice was said to augment and sustain cell division and tissue repair, hence protecting against the subsequent lethal DCVC dose(Vaidya et al., 2003a, b, c). Korrapati et al. (2005) showed that a lethal dose of DCVC downregulates phosphorylation of endogenous retinoblastoma protein (pRb), which is considered critical in renal proximal tubular and mesangial cells for the passage of cells from G1 to S-phase, thereby leading to a block of renal tubule repair. Priming, in contrast, upregulated P-pRB which was sustained even after the administration of a lethal dose of DCVC, thereby stimulating S-phase DNA synthesis, which was concluded to result in tissue repair and recovery from acute renal failure and death. These studies are more informative about the mechanism of autoprotection than on the mechanism of initial injury caused by DCVC. In addition, the priming injury (not innocuous, as it caused 25−50% necrosis and elevated blood urea nitrogen) may have influenced the toxicokinetics of the second DCVC injection.

4.4.6.1.2. ***Role of oxidative metabolites of Trichloroethylene (TCE).*** Some investigators (Green et al., 1998, 2003; Dow and Green, 2000) have proposed that TCE nephrotoxicity is related to formic acid formation. They demonstrated that exposure to either trichloroethanol or trichloroacetic acid causes increased formation and urinary excretion of formic acid (Green et al., 1998). The formic acid does not come from trichloroethylene. Rather, trichloroethylene (or a metabolite) has been proposed to cause a functional depletion of vitamin B_{12}, which is required for the methionine salvage pathway of folate metabolism. Vitamin B_{12} depletion results in folate depletion. Folate is a cofactor in one-carbon metabolism and depletion of folate allows formic acid to accumulate, and then to be excreted in the urine (Dow and Green, 2000).

TCE (1 and 5 g/L), TCA (0.25, 0.5 and 1 g/L) and TCOH (0.5 and 1.0 g/L) exposure in male Fisher rats substantially increased excretion of formic acid in urine, an effect suggested as a possible explanation for TCE-induced renal toxicity in rats (Green et al., 1998a). Green et al. (2003a) reported tubular toxicity as a result of chronic (1 year) exposure to TCOH (0, 0.5, and 1.0 g/L). Although TCOH causes tubular degeneration in a similar region of the kidney as TCE, there are several dissimilarities between the characteristics of nephrotoxicity between the two compounds, as summarized in Table 4-48. In particular, Green et al. (1998) did not observe TCOH causing karyomegaly and cytomegaly. These effects were seen as early as 13 weeks after

1 the commencement of TCE exposure (NTP, 1990), with 300 ppm inhalation exposures to TCE
2 (Maltoni et al., 1988), as well as at very low chronic exposures to DCVC (Terracini and Parker,
3 1965; Jaffe et al., 1984). In addition, Green et al. (2003) reported neither flattening nor loss of
4 the tubular epithelium nor hyperplasia, but suggested that the increased early basophilia was due
5 to newly divided cells, and therefore, represented tubular regeneration in response to damage.
6 Furthermore, they noted that such changes were seen with the spontaneous damage that occurs in
7 aging rats. However, several of the chronic studies of TCE noted that the TCE-induced damage
8 observed was distinct from the spontaneous nephropathy observed in rats. A recent *in vitro*
9 study of rat hepatocytes and primary human renal proximal tubule cells from two donors
10 measured formic acid production following exposure to CH (0.3−3 mM, 3−10 days) (Lock et al.,
11 2007). This study observed increased formic acid production at day 10 in both human renal
12 proximal tubule cell strains, but a similar level of formic acid was measured when CH was added
13 to media alone. The results of this study are limited by the use of only two primary human cell
14 strains, but suggest exposure to CH does not lead to significant increases in formic acid
15 production *in vivo*.
16 Interestingly, it appears that the amount of formic acid excreted reaches a plateau at a
17 relatively low dose. Green et al. (2003) added folic acid to the drinking water of the group of
18 rats receiving the lower dose of TCOH (18.3 mg/kg/d) in order to modulate the excretion of
19 formic acid in that dose group, and retain the dose-response in formic acid excretion relative to
20 the higher-dose group (54.3 mg/kg/d). These doses of TCOH are much lower than what would
21 be expected to be formed *in vivo* at chronic gavage doses. For instance, after a single 500-mg/kg
22 dose of TCE (the lower daily dose in the NTP rat chronic bioassays), Green and Prout (1985)
23 reported excretion of about 41% of the TCE gavage dose in urine as TCOH or trichloroethanol-
24 glucuronide conjugate (TCOG) in 24 hours. Thus, using the measure of additional excretion
25 after 24 hours and the TCOH converted to TCA as a lower bound as to the amount of TCOH
26 formed by a single 500 mg/kg dose of TCE, the amount of TCOH would be about 205 mg/kg,
27 almost 4-fold greater than the high dose in the Green et al. (2003) study. By contrast, these
28 TCOH doses are somewhat smaller than those expected from the inhalation exposures of TCE.
29 For instance, after 6 hour exposure to 100 and 500 ppm TCE (similar to the daily inhalation
30 exposures in Maltoni et al., 1988), male rats excreted 1.5 and 4.4 mg of TCOH over 48 hours,
31 corresponding to 5 and 15 mg/kg for a rat weighing 0.3 kg (Kaneko et al., 1994). The higher
32 equivalent TCOH dose is similar to the lower TCOH dose used in Green et al. (2003), so it is
33 notable that while Maltoni et al. (1988) reported a substantial incidence of cytomegaly and
34 karyomegaly after TCE exposure (300 and 600 ppm), none was reported in Green et al. (2003).

TCOH alone does not appear sufficient to explain the range of renal effects observed after TCE exposure, particularly cytomegaly, karyomegaly, and flattening and dilation of the tubular epithelium. However, given the studies described above, it is reasonable to conclude that TCOH may contribute to the nephrotoxicity of TCE, possibly due to excess formic acid production, because (1) there are some similarities between the effects observed with TCE and TCOH and (2) the dose at which effects with TCOH are observed overlap with the approximate equivalent TCOH dose from TCE exposure in the chronic studies.

Dow and Green (2000) noted that TCA also induced formic acid accumulation in rats, and suggested that TCA may therefore, contribute to TCE-induced nephrotoxicity. However, TCA has not been reported to cause any similar histologic changes in the kidney. Mather et al. (1990) reported an increase of kidney-weight to body-weight ratio in rats after 90 days of exposure to trichloroacetic acid in drinking water at 5,000 ppm (5 g/L) but reported no histopathologic changes in the kidney. DeAngelo et al. (1997) reported no effects of trichloroacetic acid on kidney weight or histopathology in rats in a 2-year cancer bioassay. Dow and Green (2000) administered TCA at quite high doses (1 and 5 g/L in drinking water), greater than the subsequent experiments of Green et al. (2003) with TCOH (0.5 and 1 g/L in drinking water), and reported similar amounts of formic acid produced (about 20 mg/day for each compound). However, cytotoxicity or karyomegaly did not appear to be analyzed. Furthermore, much more TCOH is formed from TCE exposure than TCA. Therefore, if TCA contributes substantially to the nephrotoxicity of TCE, its contribution would be substantially less than that of TCOH. Lock et al. (2007) also measured formic acid production in human renal proximal tubule cells exposed to 0.3−3 mM CH for 10 days CH. This study measured metabolism of CH to TCOH and TCA as well as formic acid production and subsequent cytotoxicity. Increased formic acid was not observed in this study, and limited cytotoxicity was observed. However, this study was performed in human renal proximal tubular cells from only two donors, and there is potential for large interindividual variability in response, particularly with CYP enzymes.

In order to determine the ability of various chlorinated hydrocarbons to induce peroxisomal enzymes, Goldsworthy and Popp (1987) exposed male Fisher-344 rats and male B6C3F1 mice to TCE (1,000 mg/kg BW) and TCA (500 mg/kg BW) by corn oil gavage for 10 consecutive days. Peroxisomal activation was measured by palmitoyl CoA oxidase activity levels. TCE led to increased peroxisomal activation in the kidneys of both rats (300% of control) and mice (625% of control), while TCA led to an increase only in mice (280% of control). A study by Zanelli et al. (1996) exposed Sprague-Dawley rats to TCA for 4 days and measured both renal and hepatic peroxisomal and cytochrome P450 enzyme activities. TCA-treated rats

had increased activity in CYP 4A subfamily enzymes and peroxisomal palmitoyl-CoA oxidase. Both of these acute studies focused on enzyme activities and did not further analyze resulting histopathology.

4.4.6.2. *In Vitro Studies of Kidney Toxicity of Trichloroethylene (TCE) and Metabolites*

Generally, it is believed that TCE metabolites are responsible for the bulk of kidney toxicity observed following exposure. In particular, studies have demonstrated a role for DCVG and DCVC in kidney toxicity. The work by Lash and colleagues (Cummings et al., 2000a, b; Cummings and Lash, 2000; Lash et al., 2000a) examined the effect of trichloroethylene and its metabolites *in vitro*. Trichloroethylene and DCVC are toxic to primary cultures of rat proximal and distal tubular cells (Cummings et al., 2000b) while the TCE metabolites DCVG and DCVC have been demonstrated to be cytotoxic to rat and rabbit kidney cells *in vitro* (Groves et al., 1993; Hassall et al., 1983; Lash et al., 2000a, 2001; Wolfgang et al., 1989a). Glutathione-related enzyme activities were well maintained in the cells, whereas CYP activities were not. The enzyme activity response to DCVC was greater than the response to trichloroethylene; however, the proximal and distal tubule cells had similar responses even though the proximal tubule is the target *in vivo*. The authors attributed this to the fact that the proximal tubule is exposed before the distal tubule *in vivo* and to possible differences in uptake transporters. They did not address the extent to which transporters were maintained in the cultured cells.

In further studies, Lash et al. (2001) assessed the toxicity of trichloroethylene and its metabolites DCVC and DCVG using *in vitro* techniques (Lash et al., 2001) as compared to *in vivo* studies. Experiments using isolated cells were performed only with tissues from Fischer 344 rats, and lactate dehydrogenase release was used as the measure of cellular toxicity. The effects were greater in males. DCVC and trichloroethylene had similar effects, but DCVG exhibited increased efficacy compared with trichloroethylene and DCVC.

In vitro mitochondrial toxicity was assessed in renal cells from both Fischer 344 rats and B6C3F1 mice following exposure to both DCVC and DCVG (Lash et al., 2001). Renal mitochondria from male rats and mice responded similarly; a greater effect was seen in cells from the female mice. These studies show DCVC to be slightly more toxic than trichloroethylene and DCVG, but species differences are not consistent with the effects observed in long-term bioassays. This suggests that *in vitro* data be used with caution in risk assessment, being mindful that *in vitro* experiments do not account for *in vivo* pharmacokinetic and metabolic processes.

In LLC-PK1 cells, DCVC causes loss of mitochondrial membrane potential, mitochondrial swelling, release of cytochrome c, caspase activation, and apoptosis (Chen et al.,

2001). Thus, DCVC is toxic to mitochondria, resulting in either apoptosis or necrosis. DCVC-induced apoptosis also has been reported in primary cultures of human proximal tubule cells (Lash et al., 2001).

DCVC was further studied in human renal proximal tubule cells for alterations in gene expression patterns related to proposed modes of action in nephrotoxicity (Lock et al., 2006). In cells exposed to subtoxic levels of DCVC to better mimic workplace exposures, the expression of genes involved with apoptosis (caspase 8, FADD-like regulator) was increased at the higher dose (1 μM) but not at the lower dose (0.1 μM) of DCVC exposure. Genes related to oxidative stress response (SOD, NFkB, p53, c-Jun) were altered at both subtoxic doses, with genes generally upregulated at 0.1 μM DCVC being downregulated at 1 μM DCVC. The results of this study support the need for further study, and highlight the involvement of multiple pathways and variability of response based on different concentrations.

Lash et al. (2007) examined the effect of modulation of renal metabolism on toxicity of TCE in isolated rat cells and microsomes from kidney and liver. Following exposure to modulating chemicals, lactate dehydrogenase (LDH) was measured as a marker of cytotoxicity, and the presence of specific metabolites was documented (DCVG, TCA, TCOH, and CH). Inhibition of the CYP stimulated an increase of GSH conjugation of TCE and increased cytotoxicity in kidney cells. This modulation of CYP had a greater effect on TCE-induced cytotoxicity in liver cells than in kidney cells. Increases in GSH concentrations in the kidney cells led to increased cytotoxicity following exposure to TCE. Depletion of GSH in hepatocytes exposed to TCE, however, led to an increase in hepatic cytotoxicity. The results of this study highlight the role of different bioactivation pathways needed in both the kidney and the liver, with the kidney effects being more affected by the GSH conjugation pathways metabolic products.

In addition to the higher susceptibility of male rats to TCE-induced nephrocarcinogenicity and nephrotoxicity, isolated renal cortical cells from male F344 rats are more susceptible to acute cytotoxicity from TCE than cells from female rats. TCE caused a modest increase in LDH release from male rat kidney cells but had no significant effect on LDH release from female rat kidney cells. These results on male susceptibility to TCE agree with the *in vivo* data.

4.4.6.3. *Conclusions as to the Active Agents of Trichloroethylene (TCE)-Induced Nephrotoxicity*

In summary, the TCE metabolites DCVC, TCOH, and TCA have all been proposed as possible contributors to the nephrotoxicity of TCE. Both *in vivo* and *in vitro* data strongly

support the conclusion that DCVC and related GSH conjugation metabolites are the active agents of TCE-induced nephrotoxicity. Of these, DCVC induces effects in renal tissues, both *in vivo* and *in vitro*, that are most similar to those of TCE, and formed in sufficient amounts after TCE exposure to account for those effects. A role for formic acid due to TCOH or TCA formation from TCE cannot be ruled out, as it is known that substantial TCOH and TCA are formed from TCE exposure, that formic acid is produced from all three compounds, and that TCOH exposure leads to toxicity in the renal tubules. However, the characteristics of TCOH-induced nephrotoxicity do not account for the range of effects observed after TCE exposure while those of DCVC-induced nephrotoxicity do. Also, TCOH does not induce the same pathology as TCE or DCVC. TCA has also been demonstrated to induce peroxisomal proliferation in the kidney (Goldsworthy and Popp, 1987), but this has not been associated with kidney cancer. Therefore, although TCOH and possibly TCA may contribute to TCE-induced nephrotoxicity, their contribution is likely to be small compared to that of DCVC.

4.4.7. Mode(s) of Action for Kidney Carcinogenicity

This section will discuss the evidentiary support for several hypothesized modes of action for kidney carcinogenicity, including mutagenicity, cytotoxicity and regenerative proliferation, peroxisome proliferation, α2μ-related nephropathy and formic acid-related nephropathy, following the framework outlined in the *Cancer Guidelines* (U.S. EPA, 2005a, b).[3]

4.4.7.1. *Hypothesized Mode of Action: Mutagenicity*

One hypothesis is that TCE acts by a mutagenic mode of action in TCE-induced renal carcinogenesis. According to this hypothesis, the key event leading to TCE-induced kidney tumor formation constitute the following: TCE GSH conjugation metabolites (e.g., DCVG, DCVC, NAcDCVC, and/or other reactive metabolites derived from subsequent beta-lyase, flavin monooxygenases [FMO], or CYP metabolism) derived from the GSH-conjugation pathway, after being either produced *in situ* in or delivered systemically to the kidney, cause direct alterations to

[3] As recently reviewed (Guyton et al., 2008) the approach to evaluating mode of action information described in U.S. EPA's Cancer Guidelines (2005a, b) considers the issue of human relevance of a hypothesized mode of action in the context of hazard evaluation. This excludes, for example, consideration of toxicokinetic differences across species; specifically, the *Cancer Guidelines* state, "the toxicokinetic processes that lead to formation or distribution of the active agent to the target tissue are considered in estimating dose but are not part of the mode of action." In addition, information suggesting quantitative differences in the occurrence of a key event between test species and humans are noted for consideration in the dose-response assessment, but is not considered in human relevance determination. In keeping with these principles, a formal analysis of the dose-response of key events in the hypothesized modes of action is not presented unless it would aid in the overall weight of evidence analysis for carcinogenicity, as presented in Section 4.11.

DNA (e.g., mutation, DNA damage, and/or micronuclei induction). Mutagenicity is a well-established cause of carcinogenicity.

Experimental Support for the Hypothesized Mode of Action. Evidence for the hypothesized mode of action for TCE includes (1) the formation of GSH-conjugation pathway metabolites in the kidney demonstrated in TCE toxicokinetics studies; and (2) the genotoxicity of these GSH-conjugation pathway metabolites demonstrated in most existing *in vitro* and *in vivo* assays of gene mutations (i.e., Ames test) and in assays of unscheduled DNA synthesis, DNA strand breaks, and micronuclei using both "standard" systems and renal cells/tissues.[4] Additional relevant data come from analyses of *VHL* mutations in human kidney tumors and studies using the Eker rat model. These lines of evidence are elaborated below.

Toxicokinetic data are consistent with these genotoxic metabolites either being delivered to or produced in the kidney. As discussed in Chapter 3, following *in vivo* exposure to TCE, the metabolites DCVG, DCVC, and NAcDCVC have all been detected in the blood, kidney, or urine of rats, and DCVG in blood and NAcDCVC in urine have been detected in humans (Birner et al., 1993; Bernauer et al., 1996; Lash et al., 1999a, 2006). In addition, *in vitro* data have shown DCVG formation from TCE in cellular and subcellular fractions from the liver, from which it would be delivered to the kidney via systemic circulation, and from the kidney (see Tables 3-23−3-24, and references therein). Furthermore, *in vitro* data in both humans and rodents support the conclusion that DCVC is primarily formed from DCVG in the kidney itself, with subsequent *in situ* transformation to NAcDCVC by *N*-Acetyl transferase or to reactive metabolites by beta-lyase, FMO, or CYPs (see Sections 3.3.3.2.2−3.3.3.2.5). Therefore, it is highly likely that both human and rodent kidneys are exposed to these TCE metabolites. .

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[4] The U.S. EPA *Cancer Guidelines* (2005a ,b) note reliance on "evaluation of *in vivo* or *in vitro* short-term testing results for genetic endpoints" and evidence that "the carcinogen or a metabolite is DNA-reactive and/or has the ability to bind to DNA"as part of this weight of evidence supporting a mutagenic mode of action. While evidence from hypothesis-testing experiments that mutation is an early step in the carcinogenic process is considered if available, it is not required for determination of a mutagenic mode of action; rather, reliance on short-term genotoxicity tests is emphasized. Thus, such tests are the focus of this analysis, which also includes an analysis of other available data from humans and animals. In keeping with these principles, a formal analysis of the temporal concordance of key events in the hypothesized modes of action is not presented unless it would aid in the overall weight of evidence analysis for carcinogenicity, as presented in Section 4.11.

1 As discussed in Section 4.2.1.4.2, DCVG, DCVC, and NAcDCVC have been
2 demonstrated to be genotoxic in most available *in vitro* assays.[5] In particular, DCVC was
3 mutagenic in the Ames test in three of the tested strains of *S. typhimurium* (TA100, TA2638,
4 TA98) (Dekant et al., 1986; Vamvakas et al., 1988a), and caused dose-dependent increases in
5 unscheduled DNA synthesis in the two available assays: porcine kidney tubular epithelial cell
6 line (Vamvakas et al., 1996) and Syrian hamster embryo fibroblasts (Vamvakas et al., 1988b).
7 DCVC has also been shown to induce DNA strand breaks in both available studies (Jaffe et al.,
8 1985; Robbiano et al., 2004), and induce micronucleus formation in primary kidney cells from
9 rats and humans (Robbiano et al., 2004) but not in Syrian hamster embryo fibroblasts
10 (Vamvakas et al., 1988b). Only one study each is available for DCVG and *N*-AcDCVC, but
11 notably both were positive in the Ames test (Vamvakas et al., 1988a; Vamvakas et al., 1987).
12 Although the number of test systems was limited, these results are consistent.
13 These *in vitro* results are further supported by studies reporting kidney-specific
14 genotoxicity after *in vivo* administration of TCE or DCVC. In particular, Robbiano et al. (1998)
15 reported increased numbers of micronucleated cells in the rat kidney following oral TCE
16 exposure. Oral exposure to DCVC in both rabbits (Jaffe et al., 1985) and rats (Clay, 2008)
17 increased DNA strand breaks in the kidney. However, in one inhalation exposure study in rats,
18 TCE did not increase DNA breakage in the rat kidney, possibly due to study limitations (limited
19 exposure time [6 hours/day for only 5d] and small number of animals exposed [*n* = 5] [Clay,
20 2008]). One study of TCE exposure in the Eker rat, a rat model heterozygous for the tumor
21 suppressor gene *Tsc-2*, reported no significant increase in kidney tumors as compared to controls
22 (Mally et al., 2006). Inactivation of *Tsc-2* in this rat model is associated with spontaneous renal
23 cell carcinoma with activation of pathways similar to that of *VHL* inactivation in humans
24 (Liu et al., 2003). TCE exposure for 13-weeks (corn oil gavage) led to increased nephrotoxicity
25 but no significant increases in preneoplastic or neoplastic lesions as compared to controls
26 (Mally et al., 2006). This lack of increased incidence of neoplastic or preneoplastic lesions
27 reported by Mally et al. (2006) in the tumor-prone Eker rat is similar to lack of significant short-

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[5] Evaluation of genotoxicity data entails a weight of evidence approach that includes consideration of the various types of genetic damage that can occur. In acknowledging that genotoxicity tests are by design complementary evaluations of different mechanisms of genotoxicity, a recent IPCS publication (Eastmond et al., 2009) notes that "multiple negative eresults may not be sufficient to remove concern for mutagenicity raised by a clear positive result in a single mutagenicity assay.". These considerations inform the present approach. In addition, consistent with U.S. EPA's *Cancer Guidelines* (2005a, b), the approach does not address relative potency (e.g., among TCE metabolites, or of such metabolites with other known genotoxic carcinogens) *per se*, nor does it consider quantitative issues related to the probable production of these metabolites in vivo. Instead, the analysis of genetic toxicity data presented in Section 4.2 and summarized here focuses on the identification of a genotoxic hazard of these metabolites; a quantitative analysis of TCE metabolism to reactive intermediates, via PBPK modeling, is presented in Section 3.5.

term response exhibited by other genotoxic carcinogens in the Eker rat (Morton et al., 2002; Stemmer et al., 2007) and may be related to the increased background rate of renal carcinomas in this animal model. Mally et al. (2006) also exposed primary kidney epithelial cells from the Eker rat to DCVC *in vitro* and demonstrated increased transformation similar to that of other renal carcinogens (Horesovsky et al., 1994).

As discussed in Section 4.2.1.4.1, although Douglas et al. (1999) did not detect increased mutations in the kidney of *lacZ* transgenic mice exposed to TCE for 12 days, these results are not highly informative as to the role of mutagenicity in TCE-induced kidney tumors, given the uncertainties in the production in genotoxic GSH conjugation metabolites in mice and the low carcinogenic potency of TCE for kidney tumors in rodents relative to what is detectable in experimental bioassays. Limited, mostly *in vitro*, toxicokinetic data do not suggest mice have less GSH conjugation or subsequent renal metabolism/bioactivation (see Section 3.3.3.2.7), but quantitatively, the uncertainties in the flux through these pathways remain significant (see Section 3.5). In additional, similar to other genotoxic renal carcinogens analyzed by NTP, there is limited evidence of mouse kidney tumors following TCE exposure. However, given the already low incidences of kidney tumors observed in rats, a relatively small difference in potency in mice would be undetectable in available chronic bioassays. Notably, of seven chemicals categorized as direct-acting genotoxic carcinogens that induced rat renal tumors in NTP studies, only two also led to renal tumors in the mouse (tris[2,3-dibromopropyl]phosphate and ochratoxin A) (Reznik et al., 1979; Kanisawa and Suzuki, 1978), so the lack of detectable response in mouse bioassays does not preclude a genotoxic MOA.

VHL inactivation (via mechanisms such as deletion, silencing or mutation) observed in human renal clear cell carcinomas, is the basis of a hereditary syndrome of kidney cancer predisposition, and is hypothesized to be an early and causative event in this disease (e.g., Nickerson et al., 2008). Therefore, specific actions of TCE metabolites that produce or select for mutations of the *VHL* suppressor gene could lead to kidney tumorigenesis. Several studies have compared *VHL* mutation frequencies in cases with TCE exposures with those from control or background populations. Brüning et al. (1997a) and Brauch et al. (1999, 2004) reported differences between TCE-exposed and nonexposed renal cell carcinoma patients in the frequency of somatic *VHL* mutations, the incidence of a hot spot mutation of cytosine to thymine at nucleotide 454, and the incidence of multiple mutations. These data suggest that kidney tumor genotype data in the form of a specific mutation pattern may potentially serve to discriminate TCE-induced tumors from other types of kidney tumors in humans. If validated, this would also suggest that TCE-induced kidney tumors are dissimilar from those occurring in unexposed individuals. Thus, while not confirming a mutation MOA, these data suggest that TCE-induced

tumors may be distinct from those induced spontaneously in humans. However, it has not been examined whether a possible linkage exists between *VHL* loss or silencing and mutagenic TCE metabolites.

By contrast, Schraml et al. (1999) and Charbotel et al. (2007) reported that TCE-exposed renal cell carcinoma patients did not have significantly higher incidences of *VHL* mutations compared to nonexposed patients. However, details as to the exposure conditions were lacking in Schraml et al. (1999). In addition, the sample preparation methodology employed by Charbotel et al. (2007) and others (Brüning et al., 1997a; Brauch et al., 1999) often results in poor quality and/or low quantity DNA, leading to study limitations (less than 100% of samples were able to be analyzed). Therefore, further investigations are necessary to either confirm or contradict the validity of the genetic biomarkers for TCE-related renal tumors reported by Brüning et al. (1997a) and Brauch et al. (1999, 2004).

In addition, while exposure to mutagens is certainly associated with cancer induction (as discussed with respect to the liver in Appendix E, Sections E.3.1 and E.3.2), examination of end-stage tumor phenotype or genotype has limitations concerning determination of early key events. The mutations that are observed with the progression of neoplasia are associated with increased genetic instability and an increase in mutation rate. Further, inactivation of the *VHL* gene also occurs through other mechanisms in addition to point mutations, such as loss of heterozygosity or hypermethylation (Kenck et al., 1996; Nickerson et al., 2008) not addressed in these studies. Recent studies examining the role of other genes or pathways suggest roles for multiple genes in renal cell carcinoma development (Furge et al., 2007; Toma et al., 2008). Therefore, the inconsistent results with respect to *VHL* mutation status do not constitute negative evidence for a mutational MOA and the positive studies are suggestive of a TCE-induced kidney tumor genotype.

In sum, the predominance of positive genotoxicity data in the database of available studies of TCE metabolites derived from GSH conjugation (in particular the evidence of kidney-specific genotoxicity following *in vivo* exposure to TCE or DCVC), coupled with the toxicokinetic data consistent with the *in situ* formation of these GSH-conjugation metabolites of TCE in the kidney, is consistent with the hypothesis that a mutagenic MOA is operative in TCE-induced kidney tumors. Available data on the *VHL* gene in humans add biological plausibility to these conclusions.

4.4.7.2. *Hypothesized Mode of Action: Cytotoxicity and Regenerative Proliferation*

Another hypothesis is that TCE acts by a cytotoxicity mode of action in TCE-induced renal carcinogenesis. According to this hypothesis, the key events leading to TCE-induced

kidney tumor formation comprise the following: the TCE GSH-conjugation metabolite DCVC, after being either produced *in situ* in or delivered systemically to the kidney, causes cytotoxicity, leading to compensatory cellular proliferation and subsequently increased mutations and clonal expansion of initiated cells.

Experimental Support for the Hypothesized Mode of Action. Evidence for the hypothesized MOA consist primarily of (1) the demonstration of nephrotoxicity following TCE exposure at current occupational limits in human studies and chronic TCE exposure in animal studies; (2) the relatively high potential of the TCE metabolite DCVC to cause nephrotoxicity; and (3) toxicokinetic data demonstrating that DCVC is formed in the kidney following TCE exposure. Data on nephrotoxicity of TCE and DCVC are discussed in more detail below, while the toxicokinetic data were summarized previously in the discussion of mutagenicity. However, there is a lack of experimental support linking TCE nephrotoxicity and sustained cellular proliferation to TCE-induced nephrocarcinogenicity.

There is substantial evidence that TCE is nephrotoxic in humans and laboratory animals and that its metabolite DCVC is nephrotoxic in laboratory animals. Epidemiological studies have consistently demonstrated increased excretion of nephrotoxicity markers (NAG, protein, albumin) at occupational (Green et al., 2004) and higher (Bolt et al., 2004; Brüning et al., 1999a, b) levels of TCE exposure. However, direct evidence of tubular toxicity, particularly in renal cell carcinoma cases, is not available. These studies are supported by the results of multiple laboratory animal studies. Chronic bioassays have reported very high (nearly 100%) incidences of nephrotoxicity of the proximal tubule in rats (NTP, 1988, 1990) and mice (NCI, 1976; NTP, 1990) at the highest doses tested. *In vivo* studies examining the effect of TCE exposure on nephrotoxicity showed increased proximal tubule damage following intraperitoneal injection and inhalation of TCE in rats (Chakrabarty and Tuchweber, 1988) and intraperitoneal injection in mice (Cojocel et al., 1989). Studies examining DCVC exposure in rats (Terracini and Parker, 1965; Elfarra et al., 1986) and mice (Jaffe et al., 1984; Darnerud et al., 1989) have also shown increases in kidney toxicity. The greater potency for kidney cytotoxicity for DCVC compared to TCE was shown by *in vitro* studies (Lash et al., 1995, 1986; Stevens et al., 1986). These studies also further confirmed the higher susceptibility of male rats or mice to DCVC-induced cytotoxicity. Cytokaryomegaly (an effect specific to TCE and not part of the chronic progressive nephropathy or the pathology that occurs in aging rat kidneys) was observed in the majority of rodent studies and may or may not progress to carcinogenesis. Finally, as discussed extensively in Section 4.4.6.1, a detailed comparison of the histological changes in the

kidney caused by TCE and its metabolites supports the conclusion that DCVC is the predominant moiety responsible for TCE-induced nephrotoxicity.

Because it is known that not all cytotoxins are carcinogens (i.e., cytotoxicity is not a specific predictor of carcinogenicity), additional experimental support is required to link nephrotoxicity to carcinogenicity. Clearly, cytotoxicity occurs at doses below those causing carcinogenicity, as the incidence of nephrotoxicity in chronic bioassays is an order of magnitude higher than that of renal tumors. However, there are multiple mechanisms by which TCE has been hypothesized to induce cytotoxicity, including oxidative stress, disturbances in calcium ion homeostasis, mitochondrial dysfunction, and protein alkylation (Lash et al., 2000a). Some of these effects may therefore, have ancillary consequences related to tumor induction which are independent of cytotoxicity per se. Under the hypothesized MOA, cytotoxicity leads to the induction of repair processes and compensatory proliferation that could lead to an increased production or clonal expansion of cells previously initiated by mutations occurred spontaneously, from coexposures, or from TCE or its metabolites. Data on compensatory cellular proliferation and the subsequent hypothesized key events in the kidney are few, with no data from rat strains used in chronic bioassays. In rats carrying the Eker mutation, Mally et al. (2006) reported increased DNA synthesis as measured by BrdU incorporation in animals exposed to the high dose of TCE (1,000 mg/kg/d) for 13 weeks, but there was no evidence of clonal expansion or tumorigenesis in the form of increased preneoplastic or neoplastic lesions as compared to controls. While chronic nephrotoxicity was reported in the same bioassays showing increased kidney tumor incidences, the use of such data to inform MOA is indirect and associative. Moreover, chronic animal studies with reduced (in female rats) or absent (in mice of both sexes) carcinogenic response have also demonstrated cytotoxicity (NTP, 1990, NCI, 1976). Therefore, in both rodent and human studies of TCE, data demonstrating a causal link between tubular toxicity and the induction of kidney tumors are lacking.

4.4.7.3. *Additional Hypothesized Modes of Action with Limited Evidence or Inadequate Experimental Support*

Along with metabolites derived from GSH conjugation of TCE, oxidative metabolites are also present and could induce toxicity in the kidney. After TCE exposure, the oxidative metabolite and peroxisome proliferator TCA is present in the kidney and excreted in the urine as a biomarker of exposure. Hypotheses have also been generated regarding the roles of $\alpha 2\mu$-globulin or formic acid in nephrotoxicity induced by TCE oxidative metabolites TCA or TCOH. However, the available data are limited or inadequate for supporting these hypothesized MOAs.

4.4.7.3.1. *Peroxisome proliferation.* Although not as well studied as the effects of glutathione metabolites in the kidney, there is evidence that oxidative metabolites affect the kidney after TCE exposure. Both TCA and DCA are peroxisome proliferator activated receptor alpha (PPARα) agonists although most activity has been associated with TCA production after TCE exposure. Exposure to TCE has been found to induce peroxisome proliferation not only in the liver but also the kidney. Peroxisome proliferation in the kidney has been evaluated by only one study of TCE (Goldsworthy and Popp, 1987), using increases in cyanide-insensitive palmitoyl-CoA oxidation (PCO) activity as a marker. Increases in renal PCO activity were observed in rats (3.0-fold) and mice (3.6-fold) treated with TCE at 1,000 mg/kg/d for 10 days, with smaller increases in both species from TCA treatment at 500 mg/kg/d for 10 days. However, no significant increases in kidney/body weight ratios were observed in either species. There was no relationship between induction of renal peroxisome proliferation and renal tumors (i.e., a similar extent of peroxisome proliferation-associated enzyme activity occurred in species with and without TCE-induced renal tumors). However, the increased peroxisomal enzyme activities due to TCE exposure are indicative of oxidative metabolites being present and affecting the kidney. Such metabolites have been associated with other tumor types, especially liver, and whether coexposures to oxidative metabolites and glutathione metabolites contribute to kidney tumorigenicity has not been examined.

4.4.7.3.2. *α2μ-Globulin-related nephropathy.* Induction of α2μ-globulin nephropathy by TCE has been investigated by Goldsworthy et al. (1988), who reported that TCE did not induce increases in this urinary protein, nor did it stimulate cellular proliferation in rats. In addition, whereas kidney tumors associated with α2μ-globulin nephropathy are specific to the male rat, as discussed above, nephrotoxicity is observed in both rats and mice and kidney tumor incidence is elevated (though not always statistically significant) in both male and female rats. TCOH was recently reported to cause hyaline droplet accumulation and an increase in α2μ-globulin, but these levels were insufficient to account for the observed nephropathy as compared to other exposures (Green et al., 2003b). Therefore, it is unlikely that α2μ-globulin nephropathy contributes significantly to TCE-induced renal carcinogenesis.

4.4.7.3.3. *Formic acid-related nephrotoxicity.* Another MOA hypothesis proposes that TCE nephrotoxicity is mediated by increased formation and urinary excretion of formic acid mediated by the oxidative metabolites TCA or TCOH (Green et al., 1998, 2003; Dow and Green, 2000). The subsequent hypothesized key events are the same as those for DCVC-induced cytotoxicity, discussed above (see Section 4.4.7.2). As discussed extensively in Section 4.4.6.1.2, these

oxidative metabolites do not appear sufficient to explain the range of renal effects observed after TCE exposure, particularly cytomegaly, karyomegaly, and flattening and dilation of the tubular epithelium. Although TCOH and possibly TCA may contribute to the nephrotoxicity of TCE, perhaps due to excess formic acid production, these metabolites do not show the same range of cytotoxic effects observed following TCE exposure (see Table 4-48). Therefore, without specific evidence linking the specific nephrotoxic effects caused by TCOH or TCA to carcinogenesis, and in light of the substantial evidence that DCVC itself can adequately account for the nephrotoxic effects of TCE, the weight of evidence supports a conclusion that cytotoxicity mediated by increased formic acid production induced by oxidative metabolites TCOH and possibly TCA is not responsible for the majority of the TCE-induced cytotoxicity in the kidneys, and therefore, would not be the major contributor to the other hypothesized key events in this MOA, such as subsequent regenerative proliferation.

4.4.7.4. *Conclusions About the Hypothesized Modes of Action*

4.4.7.4.1. *1. Is the hypothesized mode of action sufficiently supported in the test animals?*

4.4.7.4.1.1. *Mutagenicity*. The predominance of positive genotoxicity data in the database of available studies of TCE metabolites derived from GSH conjugation (in particular the evidence of kidney-specific genotoxicity following *in vivo* exposure to TCE or DCVC), coupled with the toxicokinetic data consistent with the *in situ* formation of these GSH-conjugation metabolites of TCE in the kidney, supports the conclusion that a mutagenic MOA is operative in TCE-induced kidney tumors.

4.4.7.4.1.2. *Cytotoxicity*. As reviewed above, *in vivo* and *in vitro* studies have shown a consistent nephrotoxic response to TCE and its metabolites in proximal tubule cells from male rats. Therefore, it has been proposed that cytotoxicity seen in this region of the kidney is a precursor to carcinogenicity. However, it has not been determined whether tubular toxicity is a necessary precursor of carcinogenesis, and there is a lack of experimental support for causal links, such as compensatory cellular proliferation or clonal expansion of initiated cells, between nephrotoxicity and kidney tumors induced by TCE. Nephrotoxicity is observed in both mice and rats, in some cases with nearly 100% incidence in all dose groups, but kidney tumors are only observed at low incidences in rats at the highest tested doses. Therefore, nephrotoxicity alone appears to be insufficient, or at least not rate-limiting, for rodent renal carcinogenesis, since maximal levels of toxicity are reached before the onset of tumors.

4.4.7.4.1.3. *Additional hypotheses*. The kidney is also exposed to oxidative metabolites that have been shown to be carcinogenic in other target organs. TCA is excreted in kidney after its

metabolism from TCE and also can cause peroxisome proliferation in the kidney, but there are inadequate data to define a MOA for kidney tumor induction based on peroxisome proliferation. TCE induced little or no α2μ-globulin and hyaline droplet accumulation to account for the observed nephropathy, so available data do not support this hypothesized MOA. The production of formic acid following exposure to TCE and its oxidative metabolites TCOH and TCA may also contribute to nephrotoxicity; however, the available data indicate that TCOH and TCA are minor contributors to TCE-induced nephrotoxicity, and therefore, do not support this hypothesized MOA. Because these additional MOA hypotheses are either inadequately defined or are not supported by the available data, they are not considered further in the conclusions below.

4.4.7.4.2. ***2. Is the hypothesized mode of action relevant to humans?***

4.4.7.4.2.1. ***Mutagenicity.*** The evidence discussed above demonstrates that TCE GSH-conjugation metabolites are mutagens in microbial as well as test animal species. Therefore, the presumption that they would be mutagenic in humans. Available data on the *VHL* gene in humans add biological plausibility to this hypothesis. The few available data from human studies concerning the mutagenicity of TCE and its metabolites suggest consistency with this MOA, but are not sufficiently conclusive to provide direct supporting evidence for a mutagenic MOA. Therefore, this MOA is considered relevant to humans.

4.4.7.4.2.2. ***Cytotoxicity.*** Although data are inadequate to determine that the MOA is operative, none of the available data suggest that this MOA is biologically precluded in humans. Furthermore, both animal and human studies suggest that TCE causes nephrotoxicity at exposures that also induce renal cancer, constituting positive evidence of the human relevance of this hypothesized MOA.

4.4.7.4.3. ***3. Which populations or lifestages can be particularly susceptible to the hypothesized mode of action?***

4.4.7.4.3.1. ***Mutagenicity.*** The mutagenic MOA is considered relevant to all populations and lifestages. According to U.S. EPA's *Cancer Guidelines* (U.S. EPA, 2005a) and *Supplemental Guidance* (U.S. EPA, 2005b), there may be increased susceptibility to early-life exposures for carcinogens with a mutagenic mode of action. Therefore, because the weight of evidence supports a mutagenic mode of action for TCE carcinogenicity and in the absence of chemical-specific data to evaluate differences in susceptibility, early-life susceptibility should be assumed and the age-dependent adjustment factors (ADAFs) should be applied, in accordance with the *Supplemental Guidance*.

In addition, because the MOA begins with GSH-conjugation metabolites being delivered systemically or produced *in situ* in the kidney, toxicokinetic differences—i.e., increased production or bioactivation of these metabolites—may render some individuals more susceptible to this MOA. Toxicokinetic-based susceptibility is discussed further in Section 4.10.

In rat chronic bioassays, TCE-treated males have higher incidence of kidney tumors than similarly treated females. However, the basis for this sex-difference is unknown, and whether it is indicative of a sex difference in human susceptibility to TCE-induced kidney tumors is likewise unknown. The epidemiologic studies generally do not show sex differences in kidney cancer risk. Lacking exposure-response information, it is not known if the sex-difference in one renal cell carcinoma case-control study (Dosemeci et al., 1999) may reflect exposure differences or susceptibility differences.

4.4.7.4.3.2. *Cytotoxicity*. Populations which may be more susceptible based on the toxicokinetics of the production of GSH conjugation metabolites and the sex differences observed in rat chronic bioassays are the same as for a mutagenic MOA. No data are available as to whether other factors may lead to different populations or lifestages being more susceptible to a cytotoxic MOA for TCE-induced kidney tumors. For instance, it is not known how the hypothesized key events in this MOA interact with known risk factors for human renal cell carcinoma.

The weight of evidence sufficiently supports a mutagenic MOA for TCE in the kidney, based on supporting data that GSH-metabolites are genotoxic and produced in sufficient quantities in the kidney to lead to tumorigenesis. Cytotoxicity and regenerative proliferation were considered as an alternate MOA, however, there are inadequate data to support a causal association between cytotoxicity and kidney tumors. Further, hypothesized MOAs relating to peroxisomal proliferation, α2μ-globulin nephropathy and formic acid-related nephrotoxicity were considered and rejected due to limited evidence and/or inadequate experimental support.

4.4.8. Summary: Trichloroethylene (TCE) Kidney Toxicity, Carcinogenicity, and Mode-of-Action

Human studies have shown increased levels of proximal tubule damage in workers exposed to high levels of TCE (NRC, 2006). These studies analyzed workers exposed to TCE alone or in mixtures and reported increases in various urinary biomarkers of kidney toxicity (β2-microglublin, total protein, NAG, α1-microglobulin) (Nagaya et al., 1989; Seldén et al., 1993; Brüning et al. 1999a, b; Bolt et al., 2004; Green et al., 2004; Radican et al., 2006). Laboratory animal studies examining TCE exposure provide additional support, as multiple

studies by both gavage and inhalation exposure show that TCE causes renal toxicity in the form of cytomegaly and karyomegaly of the renal tubules in male and female rats and mice. By gavage, incidences of these effects under chronic bioassay conditions approach 100%, with male rats appearing to be more sensitive than either female rats or mice of either sex based on the severity of effects. Under chronic inhalation exposures, only male rats exhibited these effects. Further studies with TCE metabolites have demonstrated a potential role for DCVC, TCOH, and TCA in TCE-induced nephrotoxicity. Of these, DCVC induces the renal effects that are most like TCE, and it is formed in sufficient amounts following TCE exposure to account for these effects.

Kidney cancer risk from TCE exposure has been studied related to TCE exposure in cohort, case-control and geographical studies. These studies have examined TCE in mixed exposures as well as alone. Elevated risks are observed in many of the cohort and case-control studies examining kidney cancer incidence in industries or job titles with historical use of TCE (see Tables 4-38 and 4-39), particularly among subjects ever exposed to TCE (Dosemeci et al., 1999; Brüning et al., 2003; Raaschou-Nielsen et al., 2003) or subjects with TCE surrogate for high exposure (Brüning et al., 2003; Raaschou-Nielsen et al., 2003; Zhao et al., 2005; Charbotel et al., 2006). Although there are some controversies related to deficiencies of the epidemiological studies (Vamvakas et al., 1998; Henschler et al., 1995), many of these are overcome in later studies (Brüning et al., 2003; Charbotel et al., 2006). A meta-analysis of the overall effect of TCE exposure on kidney cancer, additionally, suggests a small, statistically significant increase in risk (pooled RR = 1.25 95% CI: 1.11, 1.41) with a pooled relative risk estimate in the higher exposure group of 1.53, (95% CI: 1.23, 1.91), robust in sensitivity to alternatives and lacking observed statistical heterogeneity among studies meeting explicitly-defined inclusion criteria.

In vivo laboratory animal studies to date suggest a small increase in renal tubule tumors in male rats and, to a lesser extent, in female rats, with no increases seen in mice or hamsters. These results are based on limited studies of both oral and inhalation routes, some of which were deemed insufficient to determine carcinogenicity based on various experimental issues. However, because of the rarity of kidney tumors in rodents, the repeatability of this finding across strains and studies supports their biological significance despite the limitations of individual studies and relatively small increases in reported tumor incidence.

Some but not all human studies have suggested a role for *VHL* mutations in TCE-induced kidney cancer (Brüning et al., 1997a; Brauch et al., 1999, 2004; Schraml et al., 1999; Charbotel et al., 2007). Certain aspects of these studies may explain some of these discrepant results. The majority of these studies have examined paraffinized tissue that may lead to technical difficulties

in analysis, as paraffin extractions yield small quantities of often low-quality DNA. The chemicals used in the extraction process itself may also interfere with enzymes required for further analysis (PCR, sequencing). Although these studies do not clearly show mutations in all TCE-exposed individuals, or in fact in all kidney tumors examined, this does not take into account other possible means of *VHL* inactivation, including silencing or loss, and other potential targets of TCE mutagenesis were not systematically examined. A recent study by Nickerson et al. (2008) analyzed both somatic mutation and promoter hypermethylation of the *VHL* gene in cc-RCC frozen tissue samples using more sensitive methods. The results of this study support the hypothesis that *VHL* alterations are an early event in clear cell RCC carcinogenesis, but these alterations may not be gene mutations. No experimental animal studies have been performed examining *vhl* inactivation following exposure to TCE, although one *in vitro* study examined *vhl* mutation status following exposure to the TCE-metabolite DCVC (Mally et al., 2006). This study found no mutations following DCVC exposure, although this does not rule out a role for DCVC in *vhl* inactivation by some other method or *vhl* alterations caused by other TCE metabolites.

Although not encompassing all of the actions of TCE and its metabolites that may be involved in the formation and progression of neoplasia, available evidence supports the conclusion that a mutagenic MOA mediated by the TCE GSH-conjugation metabolites (predominantly DCVC) is operative in TCE-induced kidney cancer. This conclusion is based on substantial evidence that these metabolites are genotoxic and are delivered to or produced in the kidney, including evidence of kidney-specific genotoxicity following *in vivo* exposure to TCE or DCVC. Cytotoxicity caused by DCVC leading to compensatory cellular proliferation is also a potential MOA in renal carcinogenesis, but available evidence is inadequate to conclude that this MOA is operative, either together with or independent of a mutagenic MOA. The additional MOA hypotheses of peroxisome proliferation, accumulation of α2μ-globulin, and cytotoxicity mediated by TCE-induced excess formic acid production are not supported by the available data.

4.5. LIVER TOXICITY AND CANCER

4.5.1. Liver Noncancer Toxicity in Humans

The complex of chronic liver disease is a spectrum of effects and comprises nonalcoholic fatty liver disease (nonalcoholic steatohepatitis) and cirrhosis, more rare anomalies ones such as autoimmune hepatitis, primary biliary cirrhosis, and primary sclerosing cholangitis, and hepatocellular and cholangiocarcinoma (intrahepatic bile duct cancer) (Juran and Lazaridis, 2006). Chronic liver disease and cirrhosis, excluding neoplasia, is the 12th leading cause of death

in the United States in 2005 with 27,530 deaths (Kung et al., 2008) with a morality rate of 9.0 per 100,000 (Jemal et al., 2008).

Eight studies reported on liver outcomes and TCE exposure and are identified in Table 4-49. Three studies are suggestive of effects on liver function tests in metal degreasers occupationally exposed to trichloroethylene (Nagaya et al., 1993; Rasmussen et al., 1993; Xu et al., 2009). Nagaya et al. (1993) in their study of 148 degreasers in metal parts factories, semiconductor factors, or other factories, observed total mean serum cholesterol concentration, mean serum high density lipoprotein-cholesterol (HDL-C) concentrations to increase with increasing TCE exposure, as defined by U-TTC), although a statistically significant linear trend was not found. Nagaya et al. (1993) estimated subjects in the low exposure group had TCE exposure to 1 ppm-, 6-ppm TCE in the moderate exposure group, and 210-ppm TCE in the high exposure group. No association was noted between serum liver function tests and U-TTC, a finding not surprising given individuals with a history of hepatobiliary disease were excluded from this study. Nagaya et al. (1993) follows 13 workers with higher U-TTC concentrations over a 2-year period; serum HDL-C and two hepatic function enzymes, GGT and aspartate aminotrasferase (AST) concentrations were highest during periods of high level exposure, as indicated from U-TTC concentrations. Similarly, in a study of 95 degreasers, 70 exposed to trichloroethylene exposure and 25 to CFC113 (Rasmussen et al., 1993), mean serum GGT concentration for subjects with the highest TCE exposure duration was above normal reference values and were about 3-fold higher compared to the lowest exposure group. Rasmussen et al. (1993) estimated mean urinary TCE concentration in the highest exposure group as 7.7 mg/L with past exposures estimated as equivalent to 40−60 mg/L. Multivariate regression analysis showed a small statistically nonsignificant association due to age and a larger effect due to alcohol abuse that reduced and changed direction of a TCE exposure affect. The inclusion of CFC113 exposed subjects introduces a downward bias since liver toxicity is not associated with CFC113 exposure (U.S. EPA, 2008) and would underestimate any possible TCE effect. Xu et al. (2009) reported symptoms and liver function tests of 21 metal degreasers with severe hypersensitivity dermatitis (see last paragraph in this section for discussion of other liver effects in hypersensitivity dermatitis cases). TCE concentration of agent used to clean metal parts ranged from 10.2 to 63.5% with workplace ambient monitoring time-weighted-average TCE concentrations of 18 to 683 mg/m^3 (3 to 127 ppm). Exposure was further documented by urinary TCA levels in 14 of 21 cases above the recommended occupation level of 50 mg/L. The prevalence of elevated liver enzymes among these subjects was 90% (19 cases) for alanine aminotrasferase, 86% (18 cases) for asparatate aminotrasferase, and 76% (16 cases) for total bilirubin (Xu et al., 2009).

1 **Table 4-49. Summary of human liver toxicity studies**
2

Subjects	Effect	Exposure	Reference
148 male metal degreasers in metal parts, semiconductor and other factories	Serum liver function enzyme (HDL-C, AST, and GGT) concentrations did not correlated with TCE exposure assesses in a prevalence study but did correlate with TCE concentration over a 2-yr follow-up period	U-TTC levels obtained from spot urine sample obtained during working hours used to assign exposure category included the following: High: 209 ± 99 mg/g Cr Medium: 35 ± 27 mg/g Cr Low: 5 ± 2 mg/g Cr Note: this study does not include an unexposed referent group	Nagaya et al., 1993
95 workers (70 TCE exposed, 25 CFC113 exposed) selected from a cohort of 240 workers at 72 factors engaged in metal degreasing with chlorinated solvents	Increased serum GGT concentration with increasing cumulative exposure	4 groups (cumulative number of years exposed over a working life): I: 0.6 (0−0.99) II: 1.9 (1−2.8) III: 4.4 (2.9−6.7) IV: 14.4 (6.8−35.6)	Rasmussen et al., 1993
21 metal degreasers with severe hypersensitivity dermatitis	High prevalence of serum liver function enzymes above normal levels: ALT, 19 or 21 cases; AST, 18 of 21 cases, and T-Bili, 16 of 21 cases	TWA mean ambient TCE concentration occupational setting of cases, 18 mg/m^3 to 683 mg/m^3 14 of 21 cases with U-TCE above recommended occupational level of 50 mg/L	Xu et al., 2009
5 healthy workers engaged in decreasing activities in steel industry and 5 healthy workers from clerical section of same company	Total serum bile acid concentration increased between pre- and postexposure (2-d period)	8-h TWA mean personal air: 8.9 ± 3.2 ppm postexposure	Neghab et al., 1997
22 workers at a factory manufacturing small appliances	Increased in several bile acids	Regular exposure to <5 ppm TCE; peak exposure for 2 workers to >250 00m	Driscoll et al., 1992
4,489 males and female residents from 15 Superfund site and identified from ATSDR Trichloroethylene Exposure Subregistry	Liver problems diagnosed with past year	Residency in community with Superfund site identified with TCE and other chemicals	Davis et al., 2006
Case reports from 8 countries of individuals with idiosyncratic generalized skin disorders	Hepatitis in 46 to 94% of cases; other liver effects includes hepatomegaly and elevated liver function enzymes; and in rare cases, acute liver failure	If reported, TCE, from <50 mg/m^3 to more than 4,000 mg/m^3. Symptoms developed within 2−5 wks of initial exposure, with some intervals up to 3 months	Kamijima et al., 2007
Deaths in California between 1979−1981 due to cirrhosis	SMR of 211 (95% CI: 136, 287) for white male sheet metal workers and SMR = 174 (95% CI: 150−197) for metal workers	Occupational title on death certificate	Leigh and Jiang, 1993

3
4 ALT = alanine aminotrasferase.

Two studies provide evidence of plasma or serum bile acids changes among TCE-exposed degreasers. Neghab et al. (1997) in a small prevalence study of 10 healthy workers (5 unexposed controls and 5 exposed) observed statistically significantly elevated total serum bile acids, particularly deoxycholic acid and the subtotal of free bile acids, among TCE subjects at postexposure compared to their pre-exposure concentrations and serum bile acid levels correlated well with TCE exposure ($r = 0.94$). Total serum bile acid concentration did not change in control subjects between pre- and postexposure, nor did enzyme markers of liver function in either unexposed or exposed subjects differ between pre and postexposure period. However, the statistical power of this study is quite limited and the prevalence design does not include subjects who may have left employment because of possible liver problems. The paper provides minimal details of subject selection and workplace exposure conditions, except that pre-exposure testing was carried out on the 1st work day of the week (pre-exposure), repeated sampling after 2 days (postexposure), and a postexposure 8-hour time-weighted-average TCE concentration of 9 ppm for exposed subjects; no exposure information is provided for control subjects. Driscoll et al. (1992) in a study of 22 subjects (6 unexposed and 16 exposed) employed at a factory manufacturing small appliances reported statistically significant group differences in logistic regression analyses controlling for age and alcohol consumption in mean fasting plasma bile acid concentrations. Other indicators of liver function such as plasma enzyme levels were statistically significant different between exposed and unexposed subjects. Laboratory samples were obtained at the start of subject's work shift. Exposure data are not available on the 22 subjects and assignment of exposed and unexposed was based on work duties. Limited personal monitoring from other nonparticipating workers at this facility indicated TCE exposure as low, less than 5 ppm, with occasional peaks over 250 ppm although details are lacking whether these data represent exposures of study subjects.

Davis et al. (2006) in their analysis of subjects from the TCE subregistry of ATSDR's National Exposure Registry examined the prevalence of subjects reporting liver problems (defined as seeking treatment for the problem from a physician within the past year) using rates for the equivalent health condition from the National Health Interview Survey (a nationwide multipurpose health survey conducted by the National Center for Health Statistics, Centers for Disease Control and Prevention). The TCE subregistry is a cohort of exposed persons from 15 sites in 5 states. The shortest time interval from inclusion in the exposure registry and last follow-up was 5 years for one site and 10 years for seven sites. Excess in past-year liver disorders relative to the general population persisted for much of the lifetime of follow-up. SMRs for liver problems were 3rd follow-up, SMR = 2.23 (99% CI: 1.13, 3.92); 4th follow-up, SMR = 3.25 (99% CI: 1.82, 5.32); and, 5th follow-up, SMR = 2.82 (99% CI: 1.46, 4.89).

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Examination by TCE exposure, duration or cumulative exposure to multiple organic solvents did not show exposure-response patterns. Overall, these observations are suggestive of liver disorders as associated with potential TCE exposure, but whether TCE caused these conditions is not possible to determine given the study's limitations. These limitations include a potential for misclassification bias, the direction of which could dampen observations in a negative direction, and lack of adjustment in statistical analyses for alcohol consumption, which could bias observations in a positive direction.

Evaluation in epidemiologic studies of risk factors for cirrhosis other than alcohol consumption and Hepatitis A, B, and C is quite limited. NRC (2006) cited a case report of cirrhosis developing in an individual exposed occupationally to TCE for 5 years from a hot-process degreaser and to 1,1,1-trichloroethane for 3 months thereafter (Thiele et al., 1982). One cohort study on cirrhosis deaths in California between 1979 and 1981 and occupational risk factors as assessed using job title observed elevated risks with occupational titles of sheet metal workers and metalworkers and cirrhosis among white males who comprised the majority of deaths (Leigh and Jiang, 1993). This analysis lacks information on alcohol patterns by occupational title in addition to specific chemical exposures. Few deaths attributable to cirrhosis are reported for nonwhite male and for both white and nonwhite female metalworkers with analyses examining these individuals limited by low statistical power. Some but not all trichloroethylene mortality studies report risk ratios for cirrhosis (see Table 4-50). A statistically significant deficit in cirrhosis mortality is observed in three studies (Morgan et al., 1998; Boice et al., 1999, 2006) and with risk ratios including a risk of 1.0 in the remaining studies (Garabrant et al., 1988; Blair et al., 1989, 1998; Ritz, 1999; ATSDR, 2004). These results do not rule out an effect of TCE on liver cirrhosis since disease misclassification may partly explain observations. Available studies are based on death certificates where a high degree of underreporting, up to 50%, is know to occur (Blake et al., 1988).

1 **Table 4-50. Selected results from epidemiologic studies of TCE exposure and**
2 **cirrhosis**
3

Study population	Exposure group	Relative risk (95% CI)	No. obs. events	Reference
Cohort and PMR-mortality				
Aerospace workers (Rocketdyne)				
	Any TCE (utility/eng flush)	0.39 (0.16, 0.80)	7	Boice et al., 2006
	Low cumulative TCE score	Not reported		Zhao et al., 2005
	Medium cumulative TCE score			
	High TCE score			
	p for trend			
View-master workers				
	Males	0.76 (0.16, 2.22)	3	ATSDR, 2003, 2004
	Females	1.51 (0.72, 2.78)	10	
Electronic workers (Taiwan)				
	Primary liver, males	Not reported		Chang et al., 2005, 2003
	Primary liver, females	Not reported		
Uranium-processing workers				
	Any TCE exposure	0.91 (0.63, 1.28)	33	Ritz, 1999
	Light TCE exposure, >2 yrs duration	Not reported		
	Mod TCE exposure, >2 yrs duration	Not reported		
Aerospace workers (Lockheed)				
	TCE routine exposure	0.61 (0.39, 0.91)	23	Boice et al., 1999
	TCE routine-intermittent	Not reported	13	
Aerospace workers (Hughes)				
	TCE subcohort	0.55 (0.30, 0.93)	14	Morgan et al., 1998, 2000
	Low intensity (<50 ppm)	0.95 (0.43, 1.80)	9	
	High intensity (>50 ppm)	0.32 (0.10, 0.74)	5	
Aircraft maintenance workers (Hill AFB, Utah)				
	TCE subcohort	1.1 (0.6, 1.9)[a]	44	Blair et al., 1998
	Males, cumulative exposure			
	0	1.0 [a]		
	<5 ppm-yr	0.6 (0.2, 1.3)	10	
	5−25 ppm-yr	0.8 (0.3, 1.9)	9	
	>25 ppm-yr	1.2 (0.6, 2.4)	17	

1

Table 4-50. Selected results from epidemiologic studies of TCE exposure and cirrhosis (continued)

Study population	Exposure group	Relative risk (95% CI)	No. obs. events	Reference
Aircraft maintenance workers (continued)	Females, cumulative exposure			
	0	1.0 [a]		
	<5 ppm-yr	2.4 (1.4, 13.7)	6	
	5−25 ppm-yr	1.8 (0.2, 15.0)	1	
	>25 ppm-yr	0.6 (0.1, 4.8)	1	
	TCE subcohort	1.04 (0.56, 1.93) [a,b]	37	Radican et al., 2008
	Males, cumulative exposure	0.87 (0.43, 1.73)	31	
	0	1.0 [a,b]		
	<5 ppm-yr	0.56 (0.23, 1.40)	8	
	5−25 ppm-yr	1.07 (0.45, 2.53)	10	
	>25 ppm-yr	1.06 (0.48, 2.38)	13	
	Females, cumulative exposure	1.79 (0.54, 5.93)	6	
	0	1.00 [a]		
	<5 ppm-yr	3.30 (0.88, 12.41)	4	
	5−25 ppm-yr	2.20 (0.26, 18.89)	1	
	>25 ppm-yr	0.59 (0.97, 5.10)	1	
Deaths reported to GE pension fund (Pittsfield, MA)		Not reported		Greenland et al., 1994
U. S. Coast Guard employees				Blair et al. (1989)
	Marine inspectors	1.36 (0.79, 2.17)	17	
	Noninspectors	0.53 (0.23, 1.05)	8	
Aircraft manufacturing plant employees (Italy)				Costa et al., 1989
	All subjects	Not reported		
Aircraft manufacturing plant employees (San Diego, CA)				Garabrant et al., 1988
	All subjects	0.86 (0.67, 1.11)	63	

2
3 [a]Referent group are subjects from the same plant or company, or internal referents.
4 [b]Numbers of cirrhosis deaths in Radican et al. (2009) are fewer than Blair et al. (1998) because Radican et al. (2008)
5 excluded cirrhosis deaths due to alcohol.
6
7
8 A number of case reports exist of liver toxicity including hepatitis accompanying
9 immune-related generalized skin diseases described as a variation of erythema multiforme,
10 Stevens-Johnson syndrome, toxic epiderma necrolysis patients, and hypersensitivity syndrome
11 (Section 4.6.1.2 describes these disorders and evidence on TCE) (Kamijima et al., 2007).
12 Kamijima et al. (2007) reported hepatitis was seen in 92−94% of cases presenting with an
13 immune-related generalized skin diseases of variation of erythema multiforme, Stevens-Johnson

syndrome, and toxic epiderma necrolysis patients, but the estimates within the hypersensitivity syndrome group were more variable (46−94%). Many cases developed with a short time after initial exposure and presented with jaundice, hepatomegaly or hepatosplenomegaly, in addition, to hepatitis. Hepatitis development was of a nonviral etiology, as antibody titers for Hepatitis A, B, and C viruses were not detectable, and not associated with alcohol consumption (Huang et al., 2002; Kamijima et al., 2007). Liver failure was moreover a leading cause of death among these subjects. Kamijima et al. (2007) note the similarities between specific skin manifestations and accompanying hepatic toxicity and case presentations of TCE-related generalized skin diseases and conditions that have been linked to specific medications (e.g., carbamezepine, allupurinol, antibacterial sulfonamides), possibly in conjunction with reactivation of specific latent viruses. However, neither cytomegalovirus or Epstein-Barr viruses are implicated in the few reports which did include examination of viral antibodies.

4.5.2. Liver Cancer in Humans

Primary hepatocellular carcinoma and cholangiocarcinoma (intrahepatic and extrahepatic bile ducts) are the most common primary hepatic neoplasms (El-Serag, 2007; Blehacz and Gores, 2008). Primary hepatocellular carcinoma is the 5th most common of cancer deaths in males and 9th in females (Jemal et al., 2008). Age-adjusted incidence rates of hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (ICC) are increasing, with a 2-fold increase in HCC over the past 20 years. This increase has not attributable to an expanded definition of liver cancer to include primary or secondary neoplasms since International Classification of Disease (ICD)-9, incorrect classification of hilar cholangiocarcinomas in ICD-O as ICC, or to improved detection methods (Welzel et al., 2006; El-Serag, 2007). It is estimated that 21,370 Americans will be diagnosed in 2008 with liver and intrahepatic bile cancer; age-adjusted incidence rates for liver and intrahepatic bile duct cancer for all races are 9.9 per 100,000 for males and 3.5 per 100,000 for females (Ries et al., 2008). Survival for liver and biliary tract cancers remains poor and age-adjusted mortality rates are just slightly lower than incidence rates. While hepatitis B and C viruses and heavy alcohol consumption are believed major risk factors for HCC and intrahepatic cholangiocarcinoma, these risk factors cannot fully account for roughly 10 and 20% of HCC cases (Kulkarni et al., 2004). Cirrhosis is considered a premalignant condition for HCC, however, cirrhosis is not a sufficient cause for HCC since 10 to 25% of HCC cases lack evidence of cirrhosis at time of detection (Chiesa et al., 2000; Fattovich et al., 2004; Kumar et al., 2007). Nonalcoholic steatohepatitis reflecting obesity and metabolic syndrome is recently suggested as contributing to liver cancer risk (El-Serag, 2007).

All cohort studies, except Zhao et al. (2005), present risk ratios (SIRs or SMRs) for liver and biliary tract cancer. More rarely reported in cohort studies are risk ratios for primary liver cancer (hepatocellular carcinoma or HCC) or for gallbladder and extrahepatic bile duct cancer. Four community studies also presented risk ratios for liver and biliary tract cancer including a case-control study of primary liver cancer of residents of Taiwanese community with solvent-contaminated drinking water wells (Vartiainen et al., 1993; Morgan and Cassidy, 2002; Lee et al., 2003; ATSDR, 2006). Several population case-control studies examine liver cancer and organic solvents or occupational job titles with possible TCE usage (Stemhagen et al., 1983; Hardell et al., 1984; Hernberg et al., 1984, 1988; Austin et al., 1987; Dossing et al., 1997; Heinemann et al., 2000; Porru et al., 2001; Weiderpass et al., 2003; Ji and Hemminki, 2005; Kvam et al., 2005; Lindbohm et al., 2009); however, the lack of detailed exposure assessment to TCE, specifically in the population case-control studies as well as in geographic-based studies, or, too few exposed cases and controls in those studies that do present some information limits their usefulness for evaluating hepatobiliary or gall bladder cancer and TCE exposure. Table 4-51 presents observations from cohort, case-control, and community studies on liver and biliary tract cancer, primary liver, and gallbladder and extrahepatic bile duct cancer and trichloroethylene.

Excess liver cancer incidence is observed in most high quality studies (Axelson et al., 1994; Anttila et al., 1995; Hansen et al., 2001; Raaschou-Nielsen et al., 2003) as is mortality in studies which assess TCE exposure by job exposure matrix approaches (Blair et al., 1998; Morgan et al., 1998; Ritz, 1999; ATSDR, 2004; Boice et al., 2006; Radican et al., 2008). Risks for primary liver cancer and for gallbladder and biliary tract cancers in females were statistically significantly elevated only in Raaschou-Nielsen et al. (2003), the study with the largest number of observed cases without suggestion of exposure duration-response patterns. Cohort studies with more uncertain exposure assessment approaches, e.g., studies of all subjects working at a factory (Garabrant et al., 1998; Blair et al., 1989; Costa et al., 1989; Chang et al., 2003, 2005), do not show association but are quite limited given their lacking attribution of who may have higher or lower exposure potentials. Ritz (1999), the exception, found evidence of an exposure-response relationship; mortality from hepatobiliary cancer was found to increase with degree and duration of exposure and time since first exposure with a statistically significant but imprecise (wide confidence intervals) liver cancer risk for those with the highest exposure and longest time since first exposure. This observation is consistent with association with TCE, but with uncertainty given one TCE exposed case in the highest exposure group and correlation between TCE, cutting fluids, and radiation exposures.

Table 4-51. Selected results from epidemiologic studies of TCE exposure and liver cancer

Study population	Exposure group	Liver and intrahepatic bile ducts		Primary liver		Gallbladder and extrahepatic bile ducts		
		Relative risk (95% CI)	No. obs. events	Relative risk (95% CI)	No. obs. events	Relative risk (95% CI)	No. obs. events	Reference
Cohort and PMR studies—incidence								
Aerospace workers (Rocketdyne)								
	Low cumulative TCE score	Not reported						Zhao et al., 2005
	Medium cumulative TCE score	Not reported						
	High TCE score	Not reported						
	p for trend							
Danish blue-collar workers with TCE exposure								Raaschou-Nielson et al., 2003
	Males + females	1.3 (1.0, 1.6)[a]	82					
	Males + females	1.4 (1.0, 1.8)[b]	57					
	Males, any exposure	1.1 (0.8, 1.5)[b]	41	1.1 (0.7, 1.6)	27	1.1 (0.6, 1.9)	14	
	<1 yr employment duration	1.2 (0.7, 2.1)[b]	13	1.3 (0.6, 2.5)	9	1.1 (0.3, 2.9)	4	
	1−4.9 yrs employment duration	0.9 (0.5, 1.6)[b]	13	1.0 (0.5, 1.9)	9	0.8 (0.2, 2.1)	4	
	≥5 yrs employment duration	1.1 (0.6, 1.7)[b]	15	1.1 (0.5, 2.1)	9	1.4 (0.5, 3.1)	6	
	Females, any exposure	2.8 (1.6, 4.6)[b]	16	2.8 (1.1, 5.8)	7	2.8 (1.3, 5.3)	9	
	<1 yr employment duration	2.5 (0.7, 6.5)[b]	4	2.8 (0.3, 10.0)	2	2.3 (0.3, 8.4)	2	
	1−4.9 yrs employment duration	4.5 (2.2, 8.3)[b]	10	4.1 (1.1, 10.5)	4	4.8 (1.7, 10.4)	6	
	≥5 yrs employment duration	1.1 (0.1, 3.8)[b]	2	1.3 (0.0, 7.1)	1	0.9 (0.0, 5.2)	1	
Biologically-monitored Danish workers								Hansen et al., 2001
	Males + females	2.1 (0.7, 5.0)[b]	5	1.7 (0.2, 6.0)	2	2.5 (0.5, 7.3)	3	
	Males	2.6 (0.8, 6.0)[b]	5	1.8 (0.2, 6.6)	2	3.3 (0.7, 9.7)	3	
	Females		0 (0.4 exp)		0 (0.1 exp)		0 (0.3 exp)	

Table 4-51. Selected results from epidemiologic studies of TCE exposure and liver cancer (continued)

Study population	Exposure group	Liver and intrahepatic bile ducts		Primary liver		Gallbladder and extrahepatic bile ducts		
		Relative risk (95% CI)	No. obs. events	Relative risk (95% CI)	No. obs. events	Relative risk (95% CI)	No. obs. events	Reference
	Cumulative exposure (Ikeda)	Not reported						
	<17 ppm-yr							
	≥17 ppm-yr							
	Mean concentration (Ikeda)	Not reported						
	<4 ppm							
	4+ ppm							
	Employment duration	Not reported						
	<6.25 yr							
	≥6.25							
Aircraft maintenance workers from Hill Air Force Base								Blair et al., 1998
	TCE subcohort	Not reported	9	Not reported				
	Males, cumulative exposure							
	0	1.0[c]		1.03				
	<5 ppm-yr	0.6 (0.1, 3.1)	3	1.2 (0.1, 2.1)	2			
	5−25 ppm-yr	0.6 (0.1, 3.8)	2	1.0 (0.1, 16.7)	1			
	>25 ppm-yr	1.1 (0.2, 4.8)	4	2.6 (0.3, 25.0)	3			
	Females, cumulative exposure		0		0			
Biologically-monitored Finnish workers								Anttila et al., 1995
	All subjects	1.89 (0.86, 3.59)[b]	9	2.27 (0.74, 5.29)	5	1.56 (0.43, 4.00)	4	
	Mean air-TCE (Ikeda extrapolation from U-TCA)							
	<6 ppm	Not reported		1.64 (0.20, 5.92)	2			
	6+ ppm			2.74 (0.33, 9.88)	2			

Table 4-51. Selected results from epidemiologic studies of TCE exposure and liver cancer (continued)

Study population	Exposure group	Liver and intrahepatic bile ducts		Primary liver		Gallbladder and extrahepatic bile ducts		Reference
		Relative risk (95% CI)	No. obs. events	Relative risk (95% CI)	No. obs. events	Relative risk (95% CI)	No. obs. events	
Biologically-monitored Swedish workers								Axelson et al., 1994
	Males	1.41 (0.38, 3.60)[b]	4					
	Females	Not reported						
Cohort and PMR-mortality								
Computer manufacturing workers (IBM), NY		Not reported	1					Clapp and Hoffman, 2008
Aerospace workers (Rocketdyne)								
	Any TCE (utility/eng flush)	1.28 (0.35, 3.27)	4					Boice et al., 2006
	Low cumulative TCE score	Not reported						Zhao et al., 2005
	Med cumulative TCE score							
	High TCE score							
	p for trend							
View-Master workers								
	Males	2.45 (0.50, 7.12)[d]	3	1.01 (0.03, 5.63)[d]	1	8.41 (1.01, 30.4)[d]	2	ATSDR, 2003, 2004
	Females		0 (2.61 exp)		0 (1.66 exp)		0 (0.95 exp)	
Electronic workers (Taiwan)								
	Primary liver, males	Not reported			0 (0.69 exp)			Chang et al., 2005, 2003
	Primary liver, females	Not reported			0 (0.57 exp)			

Table 4-51. Selected results from epidemiologic studies of TCE exposure and liver cancer (continued)

Study population	Exposure group	Liver and intrahepatic bile ducts		Primary liver		Gallbladder and extrahepatic bile ducts		
		Relative risk (95% CI)	No. obs. events	Relative risk (95% CI)	No. obs. events	Relative risk (95% CI)	No. obs. events	Reference
Uranium-processing workers								
	Any TCE exposure	Not reported						Ritz, 1999
	Light TCE exposure, >2 yrs ration	0.93 (0.19, 4.53)[e]	3					
	Mod TCE exposure, >2 yrs duration	4.97 (0.48, 51.1)[e]	1					
	Light TCE exposure, >5 yrs duration	2.86 (0.48, 17.3)[f]	3					
	Mod TCE exposure, >5 yrs duration	12.1 (1.03, 144)[f]	1					
Aerospace workers (Lockheed)								
	TCE routine exposure	0.54 (0.15, 1.38)	4					Boice et al., 1999
	TCE routine-intermittent							
	0 yrs	1.00[c]	22					
	Any exposure	Not reported	13					
	<1 yr	0.53 (0.18, 1.60)	4					
	1−4 yrs	0.52 (0.15, 1.79)	3					
	≥5 yrs	0.94 (0.36, 2.46)	6					
	p for trend	>0.20						
Aerospace workers (Hughes)								
	TCE subcohort	0.98 (0.36, 2.13)	6					Morgan et al., 1998, 2000
	Low intensity (<50 ppm)[e]	1.32 (0.27, 3.85)	3					
	High intensity (>50 ppm)[e]	0.78 (0.16, 2.28)	3					

Table 4-51. Selected results from epidemiologic studies of TCE exposure and liver cancer (continued)

Study population	Exposure group	Liver and intrahepatic bile ducts		Primary liver		Gallbladder and extrahepatic bile ducts		
		Relative risk (95% CI)	No. obs. events	Relative risk (95% CI)	No. obs. events	Relative risk (95% CI)	No. obs. events	Reference
	TCE subcohort (Cox analysis)							
	Never exposed	1.00[c]	14					
	Ever exposed	1.48 (0.56, 3.91)[g,h]	6					
	Cumulative							
	Low	2.12 (0.59, 7.66)[h]	3					
	High	1.19 (0.34, 4.16)[h]	3					
	Peak							
	No/low	1.00[c]	17					
	Medium/high	0.98 (0.29, 3.35)[h]	3					
Aircraft maintenance workers (Hill AFB, Utah)								Blair et al., 1998
	TCE subcohort	1.3 (0.5, 3.4)[c]	15	1.7 (0.2, 16.2)3	4			
	Males, cumulative exposure							
	0	1.0[c]						
	<5 ppm-yr	1.1 (0.3, 4.1)	6					
	5−25 ppm-yr	0.9 (0.2, 4.3)	3					
	>25 ppm-yr	0.7 (0.2, 3.2)	3					
	Females, cumulative exposure							
	0	1.0[c]						
	<5 ppm-yr	1.6 (0.2, 18.2)	1					
	5−25 ppm-yr		0					
	>25 ppm-yr	2.3 (0.3, 16.7)	2					
	TCE subcohort	1.12 (0.57, 2.19)[c,i]	31	1.25 (0.31, 4.97)[c,i]	8			Radican et al., 2008

Table 4-51. Selected results from epidemiologic studies of TCE exposure and liver cancer (continued)

Study population	Exposure group	Liver and intrahepatic bile ducts: Relative risk (95% CI)	Liver and intrahepatic bile ducts: No. obs. events	Primary liver: Relative risk (95% CI)	Primary liver: No. obs. events	Gallbladder and extrahepatic bile ducts: Relative risk (95% CI)	Gallbladder and extrahepatic bile ducts: No. obs. events	Reference
Aircraft maintenance workers (continued)	Males, cumulative exposure	1.36 (0.59, 3.11) [c]	28	2.72 (0.34, 21.88) [c]	8			
	0	1.0 [c]		1.03				
	<5 ppm-yr	1.17 (0.45, 3.09)	10	3.28 (0.37, 29,45)	4			
	5−25 ppm-yr	1.16 (0.39, 3.46)	6		0			
	>25 ppm-yr	1.72 (0.68, 4.38)	12	4.05 (0.45, 36.41)	4			
	Females, cumulative exposure	0.74 (0.18, 2.97) [c]	3		0			
	0	1.03						
	<5 ppm-yr	0.69 (0.08, 5.74)	1					
	5−25 ppm-yr		0					
	>25 ppm-yr	0.98 (0.20, 4.90)	2					
Deaths reported to GE pension fund (Pittsfield, MA)		0.54 (0.11, 2.63) [j]	9					Greenland et al., 1994
U. S. Coast Guard employees								Blair et al., 1989
	Marine inspectors	1.12 (0.23, 3.26)	3					
	Noninspectors	Not reported	0 (2 exp)					
Aircraft manufacturing plant employees (Italy)								Costa et al., 1989
	All subjects	0.70 (0.23, 1.64)	5					
Aircraft manufacturing plant employees (San Diego, CA)								Garabrant et al., 1988
	All subjects	0.94 (0.40, 1.86)	8					
Case-control studies								
Residents of community with contaminated drinking water (Taiwan)								Lee et al., 2003
	Village of residency, males							
	Upstream	1.00						
	Downstream	2.57 (1.21, 5.46)	26					

Table 4-51. Selected results from epidemiologic studies of TCE exposure and liver cancer (continued)

Study population	Exposure group	Liver and intrahepatic bile ducts		Primary liver		Gallbladder and extrahepatic bile ducts		
		Relative risk (95% CI)	No. obs. events	Relative risk (95% CI)	No. obs. events	Relative risk (95% CI)	No. obs. events	Reference
Geographic studies								
Residents in two study areas in Endicott, NY		0.71 (0.09, 2.56)	<6					ATSDR, 2006
Residents in 13 census tracts in Redlands, CA		1.29 (0.74, 2.05)[k]	28					Morgan and Cassidy, 2002
Finnish residents								Vartiainen et al., 1993
	Residents of Hausjarvi	0.7 6 (0.3, 1.4)	7					
	Residents of Huttula	0.6 (0.2, 1.3)	6					

[a]ICD-7, 155 and 156; Primary liver (155.0), gallbladder, and biliary passages (155.1), and liver secondary and unspecified (156).
[b]ICD-7, 155; Primary liver, gallbladder, and biliary passages.
[c]Internal referents, workers without TCE exposure.
[d]Proportional mortality ratio (PMR).
[e]Logistic regression analysis with a 0-year lag for TCE exposure.
[f]Logistic regression analysis with a 15-year lag for TCE exposure.
[g]Risk ratio from Cox Proportional Hazard Analysis, stratified by age, sex, and decade (Environmental Health Strategies, 1997).
[h]Morgen et al. (1998) do not identify if SIR is for liver and biliary passage or primary liver cancer; identified as primary liver in NRC (2006).
[i]Radican et al. (2008) provide results for TCE exposure for follow-up through 1990, comparing the Poisson model rate ratios as reported by Blair et al. (1998) with Cox model hazard ratios. Relative risk from Cox model adjusted for age and gender for liver and intrahepatic bile duct cancer was 1.2 (95% CI: 0.5, 3.4) and for primary liver cancer was 1.3 (95% CI: 0.1, 12.0).
[j]Odds ratio.
[k]99% confidence intervals.

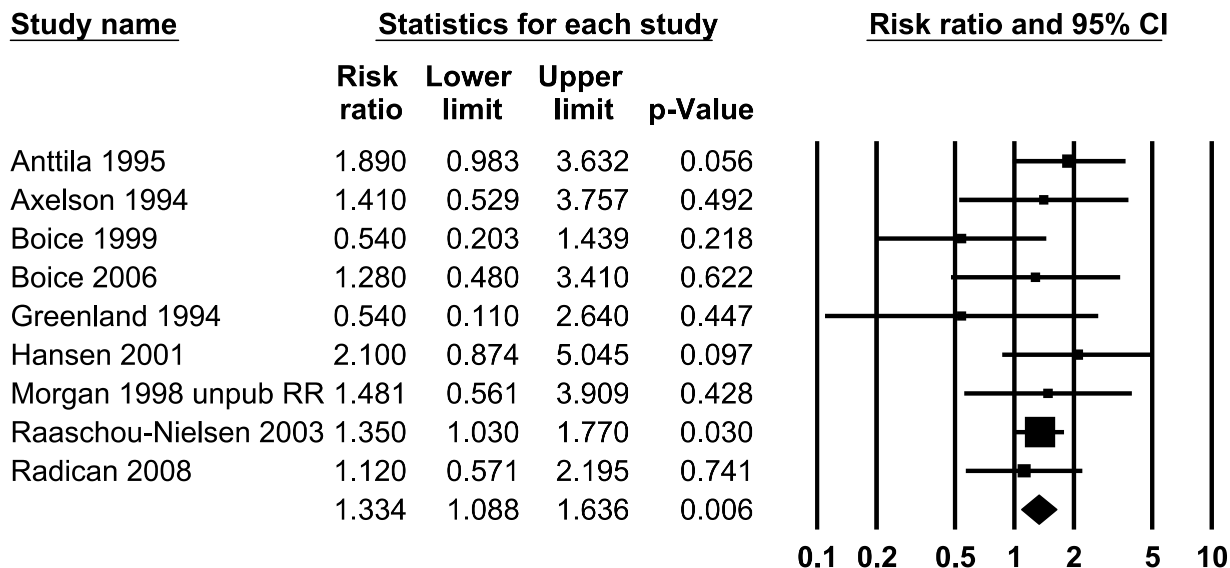
exp = exposures.

Observations in these studies provide some evidence of susceptibility of liver, gallbladder and biliary tract; observations consistent with pharmacokinetic processing of TCE and the extensive intra- and extrahepatic recirculation of metabolites. Magnitude of risk of gallbladder and biliary tract cancer is slightly higher than that for primary liver cancer in Raaschou-Nielsen et al. (2003), the study with the most cases. Observations in Blair et al. (1998), Hansen et al. (2001), and Radican et al. (2008), three smaller studies, suggest slightly larger risk ratios for primary liver cancer compared to gallbladder and biliary tract cancer. Overall, these studies are not highly informative for cross-organ comparison of relative magnitude of susceptibility.

The largest geographic studies (Morgan and Cassidy, 2002; Lee et al., 2003) are also suggestive of association with the risk ratio (mortality odds ratio) in Lee et al. (2003) as statistically significantly elevated. The geographic studies do not include a characterization of TCE exposure to individual subjects other than residency in a community with groundwater contamination by TCE with potential for exposure misclassification bias dampening observations; these studies lack characterization of TCE concentrations in drinking water and exposure characteristics such as individual consumption patterns. For this reason, observations in Morgan and Cassidy (2002) and Lee et al. (2003) are noteworthy, particularly if positive bias leading to false positive finding is considered minimal, and the lack of association with liver cancer in the two other community studies (Vartiainen et al., 1993; ATSDR, 2006) does not detract from Morgan and Cassidy (2002) or Lee et al. (2003). Lee et al. (2003), however, do not address possible confounding related to hepatitis viral infection status, a risk factor for liver cancer, or potential misclassification due to the inclusion of secondary liver cancer among the case series, factors which may amplify observed association.

Meta-analysis is adopted as a tool for examining the body of epidemiologic evidence on liver cancer and TCE exposure, to identify possible sources of heterogeneity and as an additional means to identify cancer hazard. The meta-analyses of the overall effect of TCE exposure on liver (and gall bladder/biliary passages) cancer suggest a small, statistically significant increase in risk. The pooled estimate from the primary random effects meta-analysis of the 9 (all cohort) studies is 1.33 (95% CI: 1.09, 1.64) (see Figure 4-3). The study of Raaschou-Nielsen et al. (2003) contributes about 57% of the weight; its removal from the analysis does not noticeably change the RRp estimate, but the estimate is no longer statistically significant (RRp = 1.31; 95% CI: 0.96, 1.79). The pooled estimate was not overly influenced by any other single study, nor was it overly sensitive to individual RR estimate selections. There is no evidence of publication bias in this data set, and no observable heterogeneity across the study results.

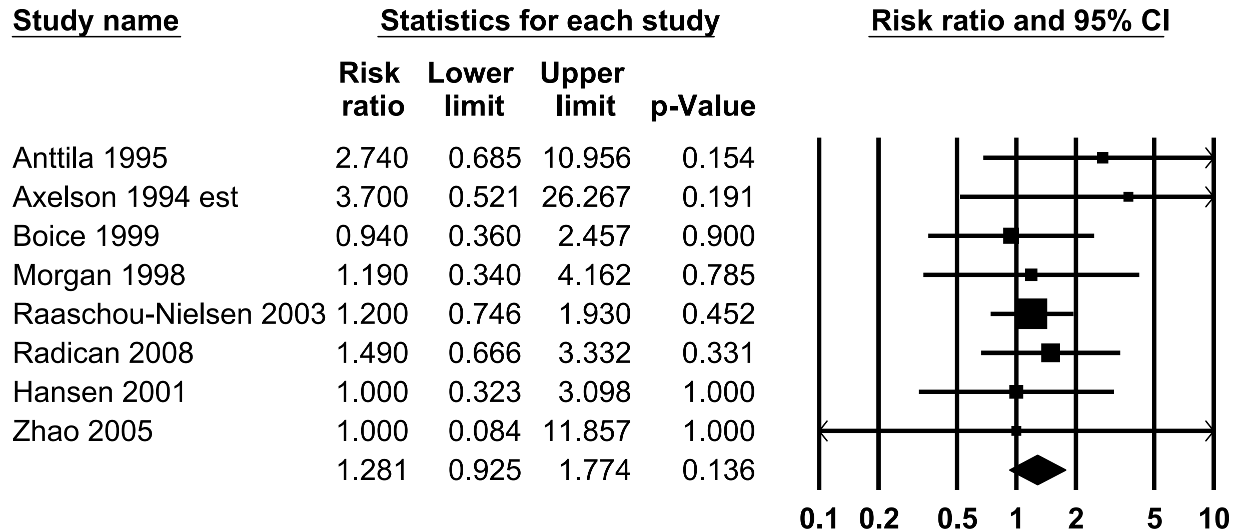
TCE and Liver Cancer



random effects model; same for fixed

Figure 4-3. Relative risk estimates of liver and biliary tract cancer and overall TCE exposure. The pooled estimate is in the bottom row. Symbol sizes reflect relative weights of the studies. The horizontal midpoint of the bottom diamond represents the pooled RR estimate and the horizontal extremes depict the 95% CI limits.

1 Examination of sites individually (i.e., primary liver and intrahepatic bile ducts separate
2 from the combined liver and gallbladder/biliary passage grouping) resulted in the RRp estimate
3 for liver cancer alone (for the 3 studies for which the data are available; for the other studies,
4 results for the combined grouping were used) slightly lower than the one based entirely on
5 results from the combined cancer categories (1.31; 95% CI: 1.02, 1.67). This result is driven by
6 the fact that the risk ratio estimate from the large Raaschou-Nielsen et al. (2003) study decreased
7 from 1.35 for liver and gall bladder/biliary passage cancers combined to 1.28 for liver cancer
8 alone.
9 The RRp estimate from the random effects meta-analysis of liver cancer in the highest
10 exposure groups in the 6 studies which provide risk estimates associated with highest exposure
11 primary liver cancer is 1.32 (95% CI: 0.93, 1.86), slightly lower than the RRp estimate for liver
12 and gallbladder/biliary cancer and any TCE exposure of 1.33 (95% CI: 1.09, 1.64), and not
13 statistically significant (see Figure 4-4). Again, the RRp estimate of the highest-exposure groups
14 is dominated by one study (Raaschou-Nielsen et al., 2003). Two studies lack reporting of liver
15 cancer risk associated with highest exposure, so consideration of reporting bias (considered the
16 primary analysis) let to a result of 1.28 (95% CI: 0.93, 1.77), similar to that estimated in the more
17 restricted set of studies presenting risk ratios association with highest exposure groups in
18 published papers.
19 Different exposure metrics are used in the various studies, and the purpose of combining
20 results across the different highest exposure groups is not to estimate an RRp associated with
21 some level of exposure, but rather to examine impacts of combining RR estimates that should be
22 less affected by exposure misclassification. In other words, the highest exposure category is
23 more likely to represent a greater differential TCE exposure compared to people in the referent
24 group than the exposure differential for the overall (typically any versus none) exposure
25 comparison. Thus, if TCE exposure increases the risk of liver and gallbladder/biliary cancer, the
26 effects should be more apparent in the highest exposure groups. The findings of a lower RRp
27 associated with highest exposure group reflects observations in Radican et al. (2008) and
28 Raaschou-Nielsen et al. (2003), the study contributing greatest weight to the meta-analysis, that
29 RR estimates for the highest-exposure groups, although greater than 1.0, are less than the RR
30 estimates with any TCE exposure.



Study name	Risk ratio	Lower limit	Upper limit	p-Value
Anttila 1995	2.740	0.685	10.956	0.154
Axelson 1994 est	3.700	0.521	26.267	0.191
Boice 1999	0.940	0.360	2.457	0.900
Morgan 1998	1.190	0.340	4.162	0.785
Raaschou-Nielsen 2003	1.200	0.746	1.930	0.452
Radican 2008	1.490	0.666	3.332	0.331
Hansen 2001	1.000	0.323	3.098	1.000
Zhao 2005	1.000	0.084	11.857	1.000
	1.281	0.925	1.774	0.136

Figure 4-4. Meta-analysis of liver cancer and TCE exposure—highest exposure groups. With assumed null RR estimates for Hansen and Zhao (see Appendix C text).

1 Thus, while the finding of an elevated and statistically significant RRp for liver and
2 gallbladder/biliary cancer and any TCE exposure provides evidence of association, the statistical
3 significance of the pooled estimates is dependent on one study, which provides the majority of
4 the weight in the meta-analyses. Furthermore, combining results from the highest-exposure
5 groups yields lower RRp estimates than for an overall effect. These results do not rule out an
6 effect of TCE on liver cancer, because the liver cancer results are relatively underpowered with
7 respect to numbers of studies and number of cases; overall, the meta-analysis provides only
8 minimal support for association between TCE exposure and liver and gallbladder/biliary cancer.
9 NRC (2006) deliberations on trichloroethylene commented on two prominent evaluations
10 of the then-current TCE epidemiologic literature using meta-analysis techniques, Wartenberg et
11 al. (2000) and Kelsh et al. (2005), submitted by Exponent-Health Sciences to NRC during their
12 deliberations and published afterwards in the open literature as Alexander et al. (2007) with the
13 substitution of the recently published study of Boice et al. (2006) for Ritz (1999) which Kelsh et
14 al. (2005) included in their NRC presentation. NRC (2006) found weaknesses in the techniques
15 used in Wartenberg et al. (2000) and the Exponent analyses. U.S. EPA staff conducted their
16 analysis according to NRC (2006) suggestions for transparency, systematic review criteria, and
17 examination of both cohort and case-control studies. The U.S. EPA analysis of liver cancer
18 considered a similar set of studies as Alexander et al. (2007) although treatment of these studies
19 differs between analyses. Alexander et al. (2007) in their Table 2, for example, present pooled
20 relative risk estimates, grouping of studies with differing exposure potentials, for example,
21 including the large cohort of Boice et al. (1999) of 77,965 subjects, 2.267 (3%) identified with
22 TCE exposure, with biomarker studies (Axelson et al., 1994; Anttila et al., 1995; Hansen et al.,
23 2001), whereas studies in the U.S. EPA analysis were identified using a systematic review and
24 objective criteria. Alexander et al. (2007) lacks a defined rationale for grouping studies with
25 subjects of different TCE exposure potentials, particularly studies with well-defined TCE
26 exposure assessment with large cohorts which include both TCE-exposed and non-TCE
27 exposure subjects. The inclusion of studies whose subjects have little to no TCE exposure over
28 background levels has the potential to introduce misclassification bias and dampen observed risk
29 ratios, a likely alternative explanation for observed inconsistency across occupational groups
30 reported by the authors. Additionally, Alexander et al. (2007) lacks quantitative examination of
31 liver cancer risk in the higher TCE exposure groups without explanation given their meta-
32 analysis of NHL did present such an examination (Mandel et al., 2006). A third difference
33 between the U.S. EPA and previous meta-analyses is their treatment of Ritz (1999), included in
34 Wartenberg et al. (2000), Kelsh et al. (2005), and Alexander et al. (2007), but not in this analysis.
35 In spite the weaknesses in past meta-analyses, pooled liver and gall bladder/biliary tract cancer

risk estimates for overall TCE exposure for TCE subcohorts is of a similar magnitude as that observed in U.S. EPA's updated and expanded analysis, Wartenberg et al. (2000), 1.1 (95% CI: 0.3, 4.8) for incidence and 1.1 (95% CI: 0.7, 1.7) for mortality, Kelsh et al. (2005), 1.32 (95% CI: 1.05, 1.66) and Alexander et al. (2007), 1.30 (95% CI: 1.09−1.55).

4.5.3. Experimental Studies of Trichloroethylene (TCE) in Rodents—Introduction

The previous sections have described available human data for TCE-induced noncancer effects (e.g., disturbances in bile production) and whether an increased risk of liver cancer in humans has been established from analysis of the epidemiological literature. A primary concern for effects on the liver comes from a large database in rodents indicating that, not only TCE, but a number of its metabolites are capable of inducing hepatocellular adenomas and carcinomas in rodent species. Thus, many of rodent bioassays have focused on the study of liver cancer for TCE and its metabolites and possible early effects specifically that may be related to tumor induction.

This section describes the hazard data for TCE effects in the rodent liver and inferences from studies of its metabolites. For more detailed descriptions of the issues providing context for these data in terms the state of the science of liver physiology (see Section E.1), cancer (see Section E.3), liver cancer (see Section E.3), and the MOA of liver cancer and other TCE-induced effects (see Section E.3.4), please see Appendix E. A more comprehensive review of individual studies of TCE-induced liver effects in laboratory animals is also provided in Section E.2 that includes detailed analyses of the strengths and the limitations of these studies. Issues have been raised regarding the relevance of mouse liver tumor data to human liver cancer risk that are addressed in Sections E.3.2 and E.3.3. Given that activation of the PPARα receptor has received great attention as a potential MOA for TCE induced liver tumors, the current status of that hypothesis is reviewed in Section E.3.4.1. Finally, comparative studies of TCE metabolites and the similarities and differences of such study results are described in summary sections of Appendix E (i.e., Section E.2.4) as well as discussions of proposed MOAs for TCE-induced liver cancer (i.e., Sections E.2.4 and E.3.4.2).

A number of acute and subchronic studies have been undertaken to describe the early changes in the rodent liver after TCE administration with the majority using the oral gavage route of administration. Several key issues affect the interpretation of these data. The few drinking water studies available for TCE have recorded significant loss of TCE through volatilization in drinking water solutions and thus, this route of administration is generally not used. Some short-term studies of TCE have included detailed examinations while others have reported primarily liver weight changes as a marker of TCE response. The matching and

1 recording of age, but especially initial and final body weight, for control and treatment groups is
2 of particular importance for studies using liver weight gain as a measure of TCE response as
3 differences in these parameters affect TCE-induced liver weight gain. Most data are for TCE
4 exposures of at least 10 days to 42 days. For many of the subchronic inhalation studies
5 (Kjellstrand et al., 1981, 1983a, b), issues associated with whole body exposures make
6 determination of dose levels more difficult. The focus of the long-term studies of TCE is
7 primarily detection and characterization of liver tumor formation.
8 For gavage experiments, death due to gavage errors and specifically from use of this
9 route of administration, especially at higher TCE exposure concentrations, has been a recurring
10 problem, especially in rats. Unlike inhalation exposures, the effects of vehicle can also be an
11 issue for background liver effects in gavage studies. Concerns regarding effects of oil vehicles,
12 especially corn oil, have been raised (Kim et al., 1990; Charbonneau et al., 1991). Several oral
13 studies in particular document that use of corn oil as the vehicle for TCE gavage dosing induces
14 a different pattern of toxicity, especially in male rodents (see Merrick et al., 1989;
15 Section E.2.2.1). Several studies also report the effects of corn oil on hepatocellular DNA
16 synthesis and indices of lipid peroxidation (Channel et al., 1998; Rusyn et al., 1999). For
17 example, Rusyn et al. (1999) report that a single dose of dietary corn oil increases hepatocyte
18 DNA synthesis 24 hours after treatment by ~3.5-fold of control, activates of NF-κB to a similar
19 extent ~2 hours after treatment almost exclusively in Kupffer cells, and induces an ~3−4-fold
20 increase of control NF-κB in hepatocytes after 8 hours and an increase in TNFα mRNA between
21 8 and 24 hours after a single dose in female rats.
22 In regard to studies that have used the i.p. route of administration, as noted by
23 Kawamoto et al. (1988), injection of TCE may result in paralytic ileus and peritonitis and that
24 subcutaneous treatment paradigm will result in TCE not immediately being metabolized but
25 retained in the fatty tissue. Wang and Stacey (1990) state that "intraperitoneal injection is not
26 particularly relevant to humans" and suggest that intestinal interactions require consideration in
27 responses such as increase serum bile acid.
28 While studies of TCE metabolites have been almost exclusively conducted via drinking
29 water, and thus, have avoided vehicle effects and gavage error, they have issues of palatability at
30 high doses and decreased drinking water consumption as a result that not only raises issues of the
31 resulting internal dose of the agent but also of effects of drinking water reduction.
32 Although there are data for both mice and rats for TCE exposure and studies of its
33 metabolites, the majority of the available information has been conducted in mice. This is
34 especially the case for long-term studies of DCA and TCA in rats. There is currently one study
35 each available for TCA and DCA in rats and both were conducted with such few numbers of

animals that the ability to detect and discern whether there was a treatment-related effect are very limited (DeAngelo et al., 1997, 1996; Richmond et al., 1995).

With regard to the sensitivity of studies used to detect a response, there are issues regarding not only the number of animals used but also the strain and weight of the animals. For some studies of TCE strains were used that have less background rate of liver tumor development and carcinogenic response. As for the B6C3F1 mouse, the strain most used in the bioassays of TCE metabolites, the susceptibility of the B6C3F1 to hepatocarcinogenicity has made the strain a sensitive biomarker for a variety of hepatocarcinogens. Moreover, Leakey et al. (2003b) demonstrated that increased body weight at 45 weeks of life is an accurate predictor of large background tumor rates. Unfortunately a 2-year study of chloral hydrate (George et al., 2000) and the only available 2-year study of TCA (DeAngelo et al., 2008), which used the same control animals, were both conducted in B6C3F1 mice that grew very large (~50 g) and prone to liver cancer (64% background incidence of hepatocellular adenomas and carcinomas) and premature mortality. Thus, these bioassays are of limited value for determination of the dose-response for carcinogenicity.

Finally, as discussed below, the administration of TCE to laboratory animals as well as environmental exposure of TCE in humans are effectively coexposure studies. TCE is metabolized to a number of hepatoactive as well as hepatocarcinogenic agents. A greater variability of response is expected than from exposure to a single agent making it particularly important to look at the TCE database in a holistic fashion rather than the results of a single study, especially for quantitative inferences. This approach is particularly useful given that the number of animals in treatment groups in a variety of TCE and TCE metabolite studies have been variable and small for control and treatment groups. Thus, their statistical power was not only limited for detection of statistically significant changes but also in many cases to be able to determine whether there is not a treatment related effect (i.e., Type II error for power calculation). Section E.2.4.2 provides detailed analyses of the database for liver weight induction by TCE and its metabolites in mice and the results of those analyses are described below. Specifically, the relationship of liver weight induction, but also other endpoints such as peroxisomal enzyme activation and increases in DNA synthesis to liver tumor responses are also addressed as well.

4.5.4. Trichloroethylene (TCE)-Induced Liver Noncancer Effects

A number of effects have been studied as indicators of TCE effects on the liver but also as proposed events whose sequellae could be associated with resultant liver tumors after chronic TCE exposure in rodents. Similar effects have been studied in rodents exposed to TCE

metabolites which may be useful for not only determining whether such effects are associated with liver tumors induced by these metabolites but also if they are similar to what has been observed for TCE.

4.5.4.1. *Liver Weight*

Increases in liver weight in mice, rats, and gerbils have been reported as a result of acute and short-term, and subchronic TCE treatment by inhalation and oral routes of exposure (Nunes et al., 2001; Tao et al., 2000; Tucker et al., 1982; Goldsworthy and Popp, 1987; Elcombe et al., 1985; Dees and Travis, 1993; Nakajima et al., 2000; Berman et al., 1995; Melnick et al., 1987; Laughter et al., 2004; Merrick et al., 1989; Goel et al., 1992; Kjellstrand et al., 1981, 1983a, b; Buben and O'Flaherty, 1985). The extent of TCE-induced liver weight gain is dependent on species, strain, gender, nutrition status, duration of exposure, route of administration, vehicle used in oral studies, and the concentration of TCE administered. Of great importance to the determination of the magnitude of response is whether the dose of TCE administered also affects whole body weight, and thus, liver weight and the percent liver/body weight ratio. Therefore, studies which employed high enough doses to induce whole body weight loss generally showed a corresponding decrease in percent liver/body weight at such doses and "flattening" of the dose-response curve, while studies which did not show systemic toxicity reported liver/body weight ratios generally proportional to dose. Chronic studies, carried out for longer durations, that examine liver weight are few and often confounded by the presence of preneoplastic foci or tumors that also affect liver weight after an extended period of TCE exposure. The number of studies that examine liver weight changes in the rat are much fewer than for mouse. Overall, the database for mice provides data for examination of the differences in TCE-induced effects from differing exposure levels, durations of exposure, vehicle, strain, and gender. One study provided a limited examination of TCE-induced liver weight changes in gerbils.

TCE-induced increases in liver weight have been reported to occur quickly. Kjellstrand et al. (1981) reported liver weight increases after 2 days inhalation exposure in NMRI mice, Laughter et al. (2004) reported increased liver weight in SV129 mice in their 3-days study (see below), and Tao et al. (2000) reported a increased in percent liver/body weight ratio in female B6C3F1mice for after 5 days. Elcombe et al. (1985) and Dees and Travis (1993) reported gavage results in mice and rats after 10 days exposure to TCE which showed TCE-induced increases in liver weight. Tucker et al. (1982) reported that 14 days of exposure to 24 mg/kg and 240 mg/kg TCE via gavage to induce a dose-related increase in liver weight in male CD-1 mice but did not show the data.

For mice, the inhalation studies of Kjellstrand et al provided the most information on the affect of duration of exposure, dose of exposure, strain tested, gender, initial weight, and variability in response between experiments on TCE-induced liver weight increases. These experiments also provided results that were independent of vehicle effect. Although the determination of the exact magnitude of response is limited by experimental design, Kjellstrand et al. (1981) reported that in NMRI mice, continuous TCE inhalation exposure induced increased percent liver/body weight by 2 days and that by 30 days (the last recorded data point) the highest percent liver/body weight ratio was reported (~1.75-fold over controls) in both male and female mice. Kjellstrand et al. (1983b) exposed seven different strains of mice (wild, C57BL, DBA, B6CBA, A/sn, NZB, NMRI) to 150-ppm TCE for 30 days and demonstrated that strain, gender, and toxicity, as reflected by changes in whole body weight, affected the percent liver/body weight ratios induced by 30 days of continuous TCE exposure. In general for the 7 strains of mice examined, female mice had the less variable increases in TCE-induced liver weight gain across duplicate experiments than male mice. For instance, in strains that did not exhibit changes in body weight (reflecting systemic toxicity) in either gender (wild-type and DBA), 150-ppm TCE exposure for 30 days induced 1.74- to 1.87-fold of control percent liver/body weight ratios in female mice and 1.45- to 2.00-fold of control percent liver/body weight ratios in male mice. The strain with the largest TCE-induced increase in percent liver/body weight increase was the NZB strain (~2.08-fold of control for females and 2.34- to 3.57-fold of control for males). Kjellstrand et al. (1983b) provided dose-response information for the NMRI strain of mice (A Swiss-derived strain) that indicated dose-related increases in percent liver/body weight ratios between 37- and 300-ppm TCE exposure for 30 days. The 150-ppm dose was reported to induce a 1.66- and 1.69-fold increases in percent liver/body weight ratios in male and female mice, respectively. Interestingly, they also reported similar liver weight increases among groups with the same cumulative exposure, but with different daily exposure durations (1 hour/day at 3,600 ppm to 24 hours/day at 150 ppm for 30 days).

Not only have most gavage experiments have been carried out in male mice, which Kjellstrand et al. (1983a) had demonstrated to have more variability in response than females, but also vehicle effects were noted to occur in experiments that examined them. Merrick et al. (1989) reported that corn oil induced a similar increase in percent liver/body weight ratios in female mice fed TCE in emulphor and corn oil for 4 weeks, male mice TCE administered in the corn oil vehicle induced a greater increase in liver weight than emulphor but less mortality at a high does.

Buben and O'Flaherty (1985) treated male, outbred Swiss-Cox mice for 6 weeks at doses ranging from 100 to 3,200 mg/kg/d, and reported increased liver/body-weight ratios at all tested

1 doses (1.12- to 1.75-fold of controls). Given the large strain differences observed by Kjellstrand
2 et al. (1983b), the use of predominantly male mice, and the effects of vehicle in gavage studies,
3 interstudy variability in dose-response relationships is not surprising.
4 Dependence of PPARα activation for TCE-liver weight gain has been investigated in
5 PPARα null mice by both Nakajima et al. (2000) and Laughter et al. (2004). Nakajima et al.
6 (2000) reported that after 2 weeks of 750 mg/kg TCE exposure to carefully matched SV129
7 wild-type or PPARα-null male and female mice ($n = 6$ group), there was a reported 1.50-fold
8 increase in wild-type and 1.26-fold of control percent liver/body weight ratio in PPARα-null
9 male mice. For female mice, there was ~1.25-fold of control percent liver/body weight ratios for
10 both wild-type and PPARα-null mice. Thus, TCE-induced liver weight gain was not dependent
11 on a functional PPARα receptor in female mice and some portion of it may have been in male
12 mice. Both wild-type male and female mice were reported to have similar increases in the
13 number of peroxisome in the pericentral area of the liver and TCE exposure and, although
14 increased 2-fold, were still only ~4% of cytoplasmic volume. Female wild-type mice were
15 reported to have less TCE-induced elevation of very long chain acyl-CoA synthetase, D-type
16 peroxisomal bifunctional protein, mitochondrial trifunctional protein α subunits α and β, and
17 cytochrome P450 4A1 than males mice, even though peroxisomal volume was similarly elevated
18 in male and female mice. The induction of PPARα protein by TCE treatment was also reported
19 to be slightly less in female than male wild-type mice (2.17- vs. 1.44-fold of control induction,
20 respectively). Thus, differences between genders in this study were for increased liver weight
21 were not associated with differences in peroxisomal volume in the hepatocytes but there was a
22 gender-related difference in induction of enzymes and proteins associated with PPARα.
23 The study of Laughter et al. (2004) used SV129 wild-type and PPARα-null male mice
24 treated with 3 daily doses of TCE in 0.1% methyl cellulose for either 3 days (1,500 mg/kg TCE)
25 or 3 weeks (0, 10, 50, 125, 500, 1,000, or 1,500 mg/kg TCE 5 days a week). However, the
26 paradigm is not strictly comparable to other gavage paradigms due to the different dose vehicle
27 and the documented impacts of vehicles such as corn oil on TCE-induced effects. In addition, no
28 initial or final body weights of the mice were reported and thus, the influence of differences in
29 initial body weight on percent liver/body weight determinations could not be ascertained. While
30 control wild-type and PPARα-null mice were reported to have similar percent liver/body weight
31 ratios (i.e., ~4.5%) at the end of the 3-day study, at the end of the 3-week experiment the percent
32 liver/body weight ratios were reported to be larger in the control PPARα-null male mice (5.1%).
33 TCE treatment for 3 days was reported for percent liver/body weight ratio to be 1.4-fold of
34 control in the wild-type mice and 1.07-fold of control in the null mice. After 3 weeks of TCE
35 exposure at varying concentrations, wild-type mice were reported to have percent liver/body

weight ratios that were within ~2% of control values with the exception of the 1,000 mg/kg and 1,500 mg/kg treatment groups (~1.18- and 1.30-fold of control, respectively). For the PPARα-null mice the variability in percent liver/body weight ratios were reported to be greater than that of the wild-type mice in most of the TCE groups and the baseline levels of percent liver/body weight ratio for control mice 1.16-fold of that of wild-type mice. TCE exposure was apparently more toxic in the PPARα-null mice. Decreased survival at the 1,500 mg/kg TCE exposure level resulted in the prevention of recording of percent liver/body weight ratios for this group. At 1,000 mg/kg TCE exposure level, there was a reported 1.10-fold of control percent liver/body weight ratio in the PPARα-null mice. None of the increases in percent liver/body weight in the null mice were reported to be statistically significant by Laughter et al. (2004). However, the power of the study was limited due to low numbers of animals and increased variability in the null mice groups. The percent liver/body weight ratio after TCE treatment reported in this study was actually greater in the PPARα-null mice than the wild-type male mice at the 1,000 mg/kg TCE exposure level (5.6 ± 0.4% vs. 5.2 ± 0.5%, for PPARα-null and wild-type mice, respectively) resulting in a 1.18-fold of wild-type and 1.10-fold of PPARα-null mice. Although the results reported in Laughter et al. (2004) for DCA and TCA were not conducted in experiments that used the same paradigm, the TCE-induced increase in percent liver/body weight more closely resembled the dose-response pattern for DCA than for DCA wild-type SV129 and PPARα-null mice.

No study examined strain differences among rats, and cross-study comparisons are confounded by heterogeneity in the age of animals, dosing regimen, and other design characteristics that may affect the degree of response. For rats, TCE-induced percent liver/body weight ratios were reported to range from 1.16- to 1.46-fold of control values depending on the study paradigm. The studies which employed the largest range of exposure concentrations (Melnick et al., 1987; Berman et al., 1995) examined 4 doses in the rat. In general, there was a dose-related increase in percent liver/body weight in the rat, especially at doses that did not cause concurrent decreased survival or significant body weight loss. For gerbils, Kjellstrand et al. (1981) reported a similar value of ~1.25-fold of control percent liver/body weight as for S-D rats exposed to 150 ppm TCE continuously for 30 days. Woolhiser et al. (2006) also reported inhalation TCE exposure to increase the percent liver/body weight ratios in female Sprague-Dawley rats although this strain appeared to be less responsive that others tested for induction of hepatomegaly from TCA exposure and to also be less prone to spontaneous liver cancer.

The size of the liver is under tight control and after cessation of a mitogenic stimulus or one inducing hepatomegaly, the liver will return to its preprogrammed size (see Appendix E). The increase in liver weight from TCE-exposure also appears to be reversible. Kjellstrand et al.

(1981) reported a reduction in liver weight gain increases after cessation of TCE exposure for 5 or 30 days in male and female mice. However, experimental design limitations precluded discernment of the magnitude of decrease. Kjellstrand et al. (1983b) reported that mice exposed to 150 ppm TCE for 30 days and then examined 120 days after the cessation of exposure, had liver weights were 1.09-fold of control for TCE-exposed female mice and the same as controls for TCE-exposed male mice. However, the livers were not the same as untreated liver in terms of histopathology. The authors reported that "after exposure to 150 ppm for 30 days, followed by 120 days of rehabilitation, the morphological picture was similar to that of the air-exposure controls except for changes in cellular and nuclear sizes." Qualitatively, the reduction in liver weight after treatment cessation is consistent with the report of Elcombe et al. (1985) in Alderly Park mice. The authors report that the reversibility of liver effects after the administration of TCE to Alderly Park mice for 10 consecutive days. Effects upon liver weight, DNA concentration, and tritiated thymidine incorporation 24 and 48 hours after the last dose of TCE were reported to still be apparent. However, 6 days following the last dose of TCE, all of these parameters were reported to return to control values with the authors not showing the data to support this assertion. Thus, cessation of TCE exposure would have resulted in a 75% reduction in liver weight by 4 days in mice exposed to the highest TCE concentration. Quantitative comparisons are not possible because Elcombe et al. (1985) did not report data for these results (e.g., how many animals, what treatment doses, and differences in baseline body weights) and such a large decrease in such a short period of time needs to be verified.

4.5.4.2. *Cytotoxicity*

Acute exposure to TCE appears to induce low cytotoxicity below subchronically lethal doses. Relatively high doses of TCE appear necessary to induce cytotoxicity after a single exposure with two available studies reported in rats. Okino et al. (1991) reported small increases in the incidence of hepatocellular necrosis in male Wistar rats exposed to 2,000 ppm (8 hours) and 8,000 ppm (2 hours), but not at lower exposures. In addition, "swollen" hepatocytes were noted at the higher exposure when rats were pretreated with ethanol or Phenobarbital. Serum transaminases increased only marginally at the 8,000-ppm exposure, with greater increases with pretreatments. Berman et al. (1995) reported hepatocellular necrosis, but not changes in serum markers of necrosis, after single gavage doses of 1,500 and 5,000 mg/kg TCE in female F344 rats. However, they did not report any indications of necrosis after 14 days of treatment at 50−1,500 mg/kg/d nor the extent of necrosis.

At acute and subchronic exposure periods to multiple doses, the induction of cytotoxicity, though usually mild, appears to differ depending on rodent species, strain, dosing vehicle and

duration of exposure, and the extent of reporting to vary between studies. For instance, Elcombe et al. (1985) and Dees and Travis(1993), which used the B6C3F1 mouse strain and corn oil vehicle, reported only slight or mild necrosis after 10 days of treatment with TCE at doses up to 1,500 mg/kg/d. Elcombe et al. (1985) also reported cell hypertrophy in the centrilobular region. Dees and Travis (1993) reported some loss of vacuolization in hepatocytes of mice treated at 1,000 mg/kg/d. Laughter et al. (2004) reported that "wild-type" SV129 mice exposed to 1,500 mg/kg TCE exposure for 3 weeks exhibited mild granuloma formation with calcification or mild hepatocyte degeneration but gave not other details or quantitative information as to the extent of the lesions or what parts of the liver lobule were affected. The authors noted that "wild-type mice administered 1,000 and 1,500 mg/kg exhibited centrilobular hypertrophy" and that "the mice in the other groups did not exhibit any gross pathological changes" after TCE exposure. Channel et al. (1998) reported no necrosis in B6C3F1 mice treated by 400−1,200 mg/kg/d TCE by corn oil gavage for 2 days to 8 weeks.

However, as stated above, Merrick et al. (1989) reported that corn oil resulted in more hepatocellular necrosis, as described by small focal areas of 3−5 hepatocytes, in male B6C3F1 mice than use of emulphor as a vehicle for 4-week TCE gavage exposures. Necrotic hepatocytes were described as surrounded by macrophages and polymorphonuclear cells. The authors reported that visible necrosis was observed in 30−40% of male mice administered TCE in corn oil but not that there did not appear to be a dose-response. For female mice, the extent of necrosis was reported to be 0 for all control and TCE treatment groups using either vehicle. Serum enzyme activities for alanine aminotransferase (ALT), AST, and LDH (markers of liver toxicity) showed that there was no difference between vehicle groups at comparable TCE exposure levels for male or female mice. Except for LDH levels in male mice exposed to TCE in corn oil there was not a correlation with the extent of necrosis and the patterns of increases in ALT and AST enzyme levels.

Ramdhan et al. (2008) assessed TCE-induced hepatotoxicity by measuring plasma ALT and AST activities and histopathology in Sv/129 mice treated by inhalation exposure, which are not confounded by vehicle effects. Despite high variability and only six animals per dose group, all three measures showed statistically significant increases at the high dose of 2,000 ppm (8 hours/day for 7 days), although a nonstatistically significant elevation is evident at the low dose of 1,000 ppm. Even at the highest dose, cytotoxicity was not severe, with ALT and AST measures increased 2-fold or less and an average histological score less than 2 (range 0−4).

Kjellstrand et al. (1983b) exposed male and female NRMI mice to 150 ppm for 30 to 120 days. Kjellstrand et al. (1983b) reported more detailed light microscopic findings from their study and stated that

> After 150 ppm exposure for 30 days, the normal trabecular arrangement of the liver cells remained. However, the liver cells were generally larger and often displayed a fine vacuolization of the cytoplasm. The nucleoli varied slightly to moderately in size and shape and had a finer, granular chromatin with a varying basophilic staining intensity. The Kupffer cells of the sinusoid were increased in cellular and nuclear size. The intralobular connective tissue was infiltrated by inflammatory cells. There was not sign of bile stasis. Exposure to TCE in higher or lower concentrations during the 30 days produced a similar morphologic picture. After intermittent exposure for 30 days to a time-weighted-average concentration of 150 ppm or continuous exposure for 120 days, the trabecular cellular arrangement was less well preserved. The cells had increased in size and the variations in size and shape of the cells were much greater. The nuclei also displayed a greater variation in basophilic staining intensity, and often had one or two enlarged nucleoli. Mitosis was also more frequent in the groups exposed for longer intervals. The vacuolization of the cytoplasm was also much more pronounced. Inflammatory cell infiltration in the interlobular connective tissue was more prominent. After exposure to 150 ppm for 30 days, followed by 120 days of rehabilitation, the morphological picture was similar to that of the air-exposure controls except for changes in cellular and nuclear sizes.

Although not reporting comparisons between male and female mice in the results section of the paper for TCE-induced histopathological changes, the authors stated in the discussion section that "However, liver mass increase and the changes in liver cell morphology were similar in TCE-exposed male and female mice." Kjellstrand et al. (1983b) did not present any quantitative data on the lesions they describe, especially in terms of dose-response. Most of the qualitative description presented was for the 150-ppm exposure level and the authors suggest that lower concentrations of TCE give a similar pathology as those at the 150-ppm level, but do not present data to support that conclusion. Although stating that Kupffer cells were reported to be increased in cellular and nuclear size, no differential staining was applied light microscopy sections to distinguish Kupffer from endothelial cells lining the hepatic sinusoid in this study. Without differential staining such a determination is difficult at the light microscopic level.

Indeed, Goel et al. (1992) describe proliferation of "sinusoidal endothelial cells" after 1,000 and 2,000 mg/kg/d TCE exposure for 28 days in male Swiss mice. They reported that histologically, "the liver exhibits swelling, vacuolization, widespread degeneration/necrosis of hepatocytes as well as marked proliferation of endothelial cells of hepatic sinusoids at 1,000 and 2,000 mg/kg TCE doses." Only one figure is given, at the light microscopic level, in which it is impossible to distinguish endothelial cells from Kupffer cells and no quantitative measures or proliferation were examined or reported to support the conclusion that endothelial cells are proliferating in response to TCE treatment. Similarly, no quantitative analysis regarding the extent or location of hepatocellular necrosis was given. The presence or absence of

inflammatory cells were not noted by the authors as well. In terms of white blood cell count, the authors note that it is slightly increased at 500 mg/kg/d but decreased at 1,000 and 2,000 mg/kg/d TCE, perhaps indicating macrophage recruitment from blood to liver and kidney, which was also noted to have pathology at these concentrations of TCE.

The inflammatory cell infiltrates described in the Kjellstrand et al. (1983b) study are consistent with invasion of macrophages and well as polymophorphonuclear cells into the liver, which could activate resident Kupffer cells. Although not specifically describing the changes as consistent with increased polyploidization of hepatocytes, the changes in cell size and especially the continued change in cell size and nuclear staining characteristics after 120 days of cessation of exposure are consistent with changes in polyploidization induced by TCE. Of note is that in the histological description provided by the authors, although vacuolization is reported and consistent with hepatotoxicity or lipid accumulation, which is lost during routine histological slide preparation, there is no mention of focal necrosis or apoptosis resulting from these exposures to TCE.

Buben and O'Flaherty (1985) reported liver degeneration "as swollen hepatocytes" and to be common with treatment of TCE to Male Swiss-Cox mice after 6 weeks. They reported that "Cells had indistinct borders; their cytoplasm was clumped and a vesicular pattern was apparent. The swelling was not simply due to edema, as wet weight/dry weight ratios did not increase." Karyorrhexis (the disintegration of the nucleus) was reported to be present in nearly all specimens and suggestive of impending cell death. No Karyorrhexis, necrosis, or polyploidy was reported in controls, but a low score Karyorrhexis was given for 400 mg/kg TCE and a slightly higher one given for 1,600 mg/kg TCE. Central lobular necrosis reported to be present only at the 1,600 mg/kg TCE exposure level and assigned a low score. Polyploidy was described as characteristic in the central lobular region but with low score for both 400 mg/kg and 1,600 mg/kg TCE exposures. The authors reported that "hepatic cells had two or more nuclei or had enlarged nuclei containing increased amounts of chromatin, suggesting that a regenerative process was ongoing" and that there were no fine lipid droplets in TCE exposed animals. The finding of "no polyploidy" in control mouse liver in the study of Buben and O'Flaherty (1985) is unexpected given that binucleate and polyploid hepatocytes are a common finding in the mature mouse liver. It is possible that the authors were referring to unusually high instances of "polyploidy" in comparison to what would be expected for the mature mouse. The score given by the authors for polyploidy did not indicate a difference between the two TCE exposure treatments and that it was of the lowest level of severity or occurrence. No score was given for centrolobular hypertrophy although the DNA content and liver weight changes suggested a dose-response. The "Karyrrhexis" described in this study could have been a sign of cell death

associated with increased liver cell number or dying of maturing hepatocytes associated with the increased ploidy, and suggests that TCE treatment was inducing polyploidization. Consistent with enzyme analyses, centrilobular necrosis was only seen at the highest dose and with the lowest qualitative score, indicating that even at the highest dose there was little toxicity.

At high doses, Kaneko et al. (2000) reported sporadic necrosis in male Mrl-lpr/lpr mice, which are "genetically liable to autoimmune disease," exposed to 500 to 2,000 ppm, 4 hours/day, 6 days/week, for 8 weeks (*n* = 5). Dose-dependent mild inflammation and associated changes were reported to be found in the liver. The effects on hepatocytes were reported to be minimal by the authors with 500-ppm TCE inducing sporadic necrosis in the hepatic lobule. Slight mobilization and activation of sinusoid lining cells were also noted. These pathological features were reported to increase with dose.

NTP (1990), which used the B6C3F1 mouse strain, reported centrilobular necrosis in 6/10 male and 1/10 female B6C3F1 mice treated at a dose of 6,000 mg/kg/d for up to 13 weeks (all the male mice and 8 of the 10 female mice died in the first week of treatment). At 3,000 mg/kg/d exposure level, although centrilobular necrosis was not observed, 2/10 males had multifocal areas of calcification in their livers, which the authors suggest is indicative of earlier hepatocellular necrosis. However, only 3/10 male mice at this dose survived to the end of the 13-week study.

For the NTP (1990) 2-year study, B6C3F1 mice were reported to have no treatment-related increase in necrosis in the liver. A slight increase in the incidence of focal necrosis was noted TCE-exposed male mice (8 vs. 2%) with a slight reduction in fatty metamorphosis in treated male mice (0 treated vs. 2 control animals) and in female mice a slight increase in focal inflammation (29 vs. 19% of animals) and no other changes. Therefore, this study did not show concurrent evidence of liver toxicity with TCE-induced neoplasia after 2 years of TCE exposure in mice.

For the more limited database in rats, there appears to be variability in reported TCE induced cytotoxicity and pathology. Nunes et al. (2001) reported no gross pathological changes in rats gavaged with corn oil or with corn oil plus 200 mg/kg TCE for 7 days. Goldsworthy and Popp (1987) gave no descriptions of liver histology given in this report for TCE-exposed animals or corn-oil controls. Kjellstrand et al. (1981) gave also did not give histological descriptions for livers of rats in their inhalation study.

Elcombe et al. (1985) provided a description of the histopathology at the light microscopy level in Osborne-Mendel rats, and Alderly Park rats exposed to TCE via gavage for 10 days. However, they did not provide a quantitative analysis or specific information regarding the variability of response between animals within group and there was no indication by the

authors regarding how many rats were examined by light microscopy. Hematoxylin and eosin sections from Osborne-Mendel rats were reported to show that

> Livers from control rats contained large quantities of glycogen and isolated inflammatory foci, but were otherwise normal. The majority of rats receiving 1,500 mg/kg body weight TCE showed slight changes in centrilobular hepatocytes. The hepatocytes were more eosinophilic and contained little glycogen. At lower doses these effects were less marked and were restricted to fewer animals. No evidence of treatment-related hepatotoxicity (as exemplified by single cell or focal necrosis) was seen in any rat receiving TCE. H&E [hematoxylin and eosin] sections from Alderly Park Rats showed no signs of treatment-related hepatotoxicity after administration of TCE. However, some signs of dose-related increase in centrilobular eosinophilia were noted.

Thus, both mice and rats were reported to exhibit pericentral hypertrophy and eosinophilia as noted from the histopathological examination in Elcombe et al. (1985).

Berman et al. (1995) reported that for female rats exposed to TCE for 14 days hepatocellular necrosis was noted to occur in the 1,500 and 5,000 mg/kg groups in 6/7 and 6/8 female rats, respectively but not to occur in lower doses. The extent of necrosis was not noted by the authors for the two groups exhibiting a response after 1 day of exposure. Serum enzyme levels, indicative of liver necrosis, were not presented and because only positive results were presented in the paper, presumed to be negative. Therefore, the extent of necrosis was not of a magnitude to affect serum enzyme markers of cellular leakage.

Melnick et al. (1987) reported that the only treatment-related lesion observed microscopically in rats from either dosed-feed or gavage groups was individual cell necrosis of the liver with the frequency and severity of this lesion similar at each dosage levels of TCE microencapsulated in the feed or administered in corn oil. The severity for necrosis was only mild at the 2.2 and 4.8 g/kg feed groups and for the 6 animals in the 2.8 g/kg group corn oil group. The individual cell necrosis was reported to be randomly distributed throughout the liver lobule with the change to not be accompanied by an inflammatory response. The authors also reported that there was no histologic evidence of cellular hypertrophy or edema in hepatic parenchymal cells. Thus, although there appeared to be TCE-treatment related increases in focal necrosis after 14 days of exposure, the extent was mild even at the highest doses and involved few hepatocytes.

For the 13-week NTP study (1990), only control and high dose F344/N rats were examined histologically. Pathological results were reported to reveal that 6/10 males and 6/10 female rats had pulmonary vasculitis at the highest concentration of TCE. This change was also reported to have occurred in 1/10 control male and female rats. Most of those animals were

also reported to have had mild interstitial pneumonitis. The authors report that viral titers were positive during this study for Sendai virus.

Kumar et al. (2001) reported that male Wistar rats exposed to 376 ppm, 4 hours/day, 5 days/week for 8−24 weeks showed evidence of hepatic toxicity. The authors stated that, "after 8 weeks of exposure enlarged hepatocytes, with uniform presence of fat vacuoles were found in all of the hepatocytes affecting the periportal, midzonal, and centriolobular areas, and fat vacuoles pushing the pyknosed nuclei to one side of hepatocytes. Moreover, congestion was not significant. After exposure of 12 and 24 weeks, the fatty changes became more progressive with marked necrosis, uniformly distributed in the entire organ." No other description of pathology was provided in this report. In regard to the description of fatty change, the authors only did conventional H&E staining of sections with no precautions to preserve or stain lipids in their sections. However, as noted below, the NCI study also reports long-term TCE exposure in rats to result in hepatocellular fatty metamorphosis. The authors provided a table with histological scoring of simply + or—for minimal, mild or moderate effects and do not define the criteria for that scoring. There is also no quantitative information given as to the extent, nature, or location of hepatocellular necrosis. The authors report "no change was observed in glutamic oxoacetate transaminase and glutamic pyruvated transaminase levels of liver in all the three groups. The GSH level was significantly decreased while "total sulphydryl" level was significantly increased during 8, 12, and 24 weeks of TCE exposure. The acid and alkaline phosphatases were significantly increased during 8, 12, and 24 weeks of TCE exposure." The authors present a series of figures that are poor in quality to demonstrate histopathological TCE-induced changes. No mortality was observed from TCE exposure in any group despite the presence of liver necrosis.

Thus, in this limited database that spans durations of exposure from days to 24 weeks and uses differing routes of administration, generally high doses for long durations of exposure are required to induce hepatotoxicity from TCE exposure in the rat. The focus of 2-year bioassays in rats has been the detection of a cancer response with little or no reporting of noncancer pathology in most studies. Henschler et al. (1984) and Fukuda et al. (1983) do not report noncancer histopathology, but do both report rare biliary cell derived tumors in rats in relatively insensitive assays. For male rats, noncancer pathology in the NCI (1976) study was reported to include increased fatty metamorphosis after TCE exposure and angiectasis or abnormally enlarged blood vessels. Angiectasis can be manifested by hyperproliferation of endothelial cells and dilatation of sinusoidal spaces. For the NTP (1990) study there was little reporting of non-neoplastic pathology or toxicity and no report of liver weight at termination of the study. In the NTP (1988) study, the 2 year study of TCE exposure reported no evidence of TCE-induced liver

toxicity described as non-neoplastic changes in ACI, August, Marshal, and Osborne-Mendel rats. Interestingly, for the control animals of these four strains there was, in general, a low background level of focal necrosis in the liver of both genders. Obviously, the negative results in this bioassay for cancer are confounded by the killing of a large portion of the animals accidently by experimental error but TCE-induced overt liver toxicity was not reported.

In sum, the cytotoxic effects in the liver of TCE treatment appear include little or no necrosis in the rodent liver, but rather, a number of histological changes such as mild focal hepatocyte degeneration at high doses, cellular "swelling" or hypertrophy, and enlarged nuclei. Histological changes consistent with increased polyploidization and specific descriptions of TCE-induced polyploidization have been noted in several experiments. Several studies note proliferation of nonparenchymal cells after TCE exposure as well. These results are more consistently reported in mice, but also have been reported in some studies at high doses in rats, for which fewer studies are available. In addition, the increase in cellular and nuclear sizes appeared to persist after cessation of TCE treatment. In neither rats nor mice is there evidence that TCE treatment results in marked necrosis leading to regenerative hyperplasia.

4.5.4.3. *Measures of DNA Synthesis, Cellular Proliferation, and Apoptosis*

The increased liver weight observed in rodents after TCE exposure may result from either increased numbers of cells in the liver, increased size of cells in the liver, or a combination of both. Studies of TCE in rodents have studied whole liver DNA content of TCE-treated animals to determine whether the concentration of DNA per gram of liver decreases as an indication of hepatocellular hypertrophy (Buben and O'Flaherty, 1985; Dees and Travis, 1993; Elcombe et al., 1985). While the slight decreases observed in some studies are consistent with hypertrophy, the large variability in controls and lack of dose-response limits the conclusions that can be drawn from these data. In addition, multiple factors beyond hypertrophy affect DNA concentration in whole-liver homogenates, including changes in ploidy and the number of hepatocytes and nonparenchymal cells.

The incorporation of tritiated thymidine or BrdU has also been analyzed in whole liver DNA and in individual hepatocytes as a measure of DNA synthesis. Such DNA synthesis can occur from either increased numbers of hepatocytes in the liver or by increased polyploidization. Section E.1.1 describes polyploidization in human and rodent liver and its impacts on liver function, while Sections E.3.1.2 and E.3.3.1 discuss issues of target cell identification for liver cancer and changes in ploidy as a key even in liver cancer using animals models, respectively. Along with changes in cell size (hypertrophy), cell number (cellular proliferation), and the DNA content per cell (cell ploidy), the rate of apoptosis has also been noted or specifically examined

in some studies of TCE and its metabolites. All of these phenomena have been identified in proposed hypotheses as key events possibly related to carcinogenicity. In particular, changes in cell proliferation and apoptosis have been postulated to be part of the MOA for PPARα-agonists by Klaunig et al. (2003) (see Section E.3.4).

In regard to early changes in DNA synthesis, the data for TCE are very limited Mirsalis et al. (1989) reported measurements of *in vivo-in vitro* hepatocyte DNA repair and S-phase DNA synthesis in primary hepatocytes from male Fischer-344 rats and male and female B6C3F1 mice administered single doses of TCE by gavage in corn oil. They reported negative results 2−12 hours after treatment from 50−1,000 mg/kg TCE in rats and mice (male and female) for unscheduled DNA synthesis and repair using 3 animals per group. After 24 and 48 hours of 200 or 1,000 mg/kg TCE in male mice ($n = 3$) and after 48 hours of 200 ($n = 3$) or 1,000 ($n = 4$) mg/kg TCE in female mice, similar values of 0.30 to 0.69% of hepatocytes were reported as undergoing DNA synthesis in primary culture. Only the 1,000 mg/kg TCE dose in male mice at 48 hours was reported to give a result considered to be positive (~2.2% of hepatocytes) but no statistical analyses were performed on these measurements. These results are limited by both the number of animals examined and the relevance of the paradigm.

As noted above, TCE treatment in rodents has been reported to result in hepatocellular hypertrophy and increased centrilobular eosinophilia. Elcombe et al. (1985) reported a small decrease in DNA content with TCE treatment (consistent with hepatocellular hypertrophy) that was not dose-related, increased tritiated thymidine incorporation in whole mouse liver DNA that was that was treatment but not dose-related (i.e., a 2-, 2-, and 5-fold of control in mice treated with 500, 1,000, and 1,500 mg/kg TCE), and slightly increased numbers of mitotic figures that were treatment but not dose-related and not correlated with DNA synthesis as measured by thymidine incorporation. Elcombe et al., reported no difference in response between 500 and 1,000 mg/kg TCE treatments for tritiated thymidine incorporation. Dees and Travis (1993) also reported that incorporation of tritiated thymidine in DNA from mouse liver was elevated after TCE treatment with the mean peak level of tritiated thymidine incorporation occurred at 250 mg/kg TCE treatment level and remaining constant for the 500 and 1,000 mg/kg treated groups. Dees and Travis (1993) specifically report that mitotic figures, although very rare, were more frequently observed after TCE treatment, found most often in the intermediate zone, and found in cells resembling mature hepatocytes. They reported that there was little tritiated thymidine incorporation in areas near the bile duct epithelia or close to the portal triad in liver sections from both male and female mice. Channel et al. (1998) reported proliferating cell nuclear antigen (PCNA) positive cells, a measure of cells that have undergone DNA synthesis, was elevated only on Day 10 (out of the 21 studied) and only in the 1,200 mg/kg/d TCE exposed

group with a mean of ~60 positive nuclei per 1,000 nuclei for 6 mice (~6%). Given that there was little difference in PCNA positive cells at the other TCE doses or time points studied, the small number of affected cells in the liver could not account for the increase in liver size reported in other experimental paradigms at these doses. The PCNA positive cells as well as "mitotic figures" were reported to be present in centrilobular, midzonal, and periportal regions with no observed predilection for a particular lobular distribution. No data were shown regarding any quantitative estimates of mitotic figures and whether they correlated with PCNA results. Thus, whether the DNA synthesis phases of the cell cycle indicated by PCNA staining were indentifying polyploidization or increased cell number cannot be determined.

For both rats and mice, the data from Elcombe et al. (1985) showed that tritiated thymidine incorporation in total liver DNA observed after TCE exposure did not correlate with mitotic index activity in hepatocytes. Both Elcombe et al. (1985) and Dees and Travis (1993) reported a small mitotic indexes and evidence of periportal hepatocellular hypertrophy from TCE exposure. Neither mitotic index or tritiated thymidine incorporation data support a correlation with TCE-induced liver weight increase in the mouse, but rather the increase to be most likely due to hepatocellular hypertrophy. If higher levels of hepatocyte replication had occurred earlier, such levels were not sustained by 10 days of TCE exposure. These data suggest that increased tritiated thymidine levels were targeted to mature hepatocytes and in areas of the liver where greater levels of polyploidization occur (see Section E.1.1). Both Elcombe et al. (1985) and Dees and Travis (1993) show that tritiated thymidine incorporation in the liver was ~2-fold greater than controls between 250−1,000 mg/kg TCE, a result consistent with a doubling of DNA. Thus, given the normally quiescent state of the liver, the magnitude of this increase over control levels, even if a result of proliferation rather than polyploidization, would be confined to a very small population of cells in the liver after 10 days of TCE exposure.

Laughter et al. (2004) reported that there was an increase in DNA synthesis after aqueous gavage exposure to 500 and 1,000 mg/kg TCE given as 3 boluses a day for 3 weeks with BrdU given for the last week of treatment. An examination of DNA synthesis in individual hepatocytes was reported to show that 1 and 4.5% of hepatocytes had undergone DNA synthesis in the last week of treatment for the 500 and 1,000 mg/kg doses, respectively. Again, this level of DNA synthesis is reported for a small percentage of the total hepatocytes in the liver and not reported to be a result of regenerative hyperplasia.

Finally, Dees and Travis (1993) and Channel et al. (1998) reported evaluating changes in apoptosis with TCE treatment. Dees and Travis (1993) enumerated identified by either hematoxylin and eosin or feulgen staining in male and female mice after 10 days of TCE treatment by. Only 0 or 1 apoptosis was observed per 100 high power (400×) fields in controls

and all dose groups except for those given 1,000 mg/kg/d, in which 8 or 9 apoptoses per 100 fields were reported. None of the apoptoses were in the intermediate zones where mitotic figures were observed, and all were located near the central veins. This is the same region where one would expect endogenous apoptoses as hepatocytes "stream" from the portal triad toward the central vein (Schwartz-Arad, 1989). In addition, this is the same region where Buben and O'Flaherty (1985) noted necrosis and polyploidy. By contrast Channel et al. (1998) reported no significant differences in apoptosis at any treatment dose (400 to 1,200 mg/kg/d) examined after any time from 2 days to 4 weeks.

4.5.4.4. *Peroxisomal Proliferation and Related Effects*

Numerous studies have reported that TCE administered to mice and rats by gavage leads to proliferation of peroxisomes in hepatocytes. Some studies have measured changes in the volume and number of peroxisomes as measures of peroxisome proliferation while others have measured peroxisomal enzyme activity such catalase and cyanide-insensitive PCO. Like liver weight, the determination of a baseline level of peroxisomal volume, number, or enzyme activity can be variable and have great effect on the ability to determine the magnitude of a treatment-related effect.

Elcombe et al. (1985) reported increases in the percent of the cytoplasm occupied by peroxisomes in B6C3F1 and Alderley Park mice treated for 10 days at 500 to 1,500 mg/kg/d. Although the increase over controls appeared larger in the B6C3F1 strain, this is largely due to the 2-fold smaller control levels in that strain, as the absolute percentage of peroxisomal volume was similar between strains after treatment. All these results showed high variability, as evidenced from the reported standard deviations. Channel et al. (1998) found a similar absolute percentage of peroxisomal volume after 10 days treatment in the B6C3F1 mouse at 1,200 mg/kg/d TCE but with the percentage in vehicle controls similar to the Alderley-Park mice in the Elcombe et al. (1985) study. Interestingly, Channel et al. (1998) found that the increase in peroxisomes peaked at 10 days, with lower values after 6 and 14 days of treatment. Furthermore, the vehicle control levels also varied almost 2-fold depending on the number of days of treatment. Nakajima et al. (2000), who treated male wild-type SV129 mice at 750 mg/kg/d for 14 days, found even higher baseline values for the percentage of peroxisomal volume, but with an absolute level after treatment similar to that reported by Channel et al. (1998) in B6C3F1 mice treated at 1,200 mg/kg/d TCE for 14 days. Nakajima et al. (2000) also noted that the treatment-related increases were smaller for female wild-type mice, and that there were no increases in peroxisomal volume in male or female PPARα-null mice, although vehicle control levels were slightly elevated (not statistically significant). Only Elcombe et al. (1985)

examined peroxisomal volume in rats, and reported smaller treatment-related increases in two strains (OM and AP), but higher baseline levels. In particular, at 1,000 mg/kg/d, after 10 days treatment, the percent peroxisomal volume was similar in OM and AP rats, with similar control levels as well. While the differences from treatment were not statistically significant, only five animals were used in each group, and variability, as can be seen by the standard deviations, was high, particularly in the treated animals.

The activities of a number of different hepatic enzymes have also been as markers for peroxisome proliferation and/or activation of PPARα. The most common of these are catalase and cyanide-insensitive PCO. In various strains of mice (B6C3F1, Swiss albino, SV129 wild-type) treated at doses of 500 to 2,000 mg/kg/d for 10 to 28 days, increases in catalase activity have tended to be more modest (1.3- to 1.6-fold of control) as compared to increases in PCO (1.4- to 7.9-fold of control) (Elcombe et al., 1985; Goel et al., 1992; Goldsworthy and Popp, 1987; Laughter et al., 2004; Nakajima et al., 2000; Watanabe and Fukui, 2000). In rats, Elcombe et al. (1985) reported no increases in catalase or PCO activity in Alderley-Park rats treated at 1,000 mg/kg/d TCE for 10 days. In F344 rats, Goldsworthy and Popp (1987) and Melnick et al. (1987) reported increases of up to 2-fold in catalase and 4.1-fold in PCO relative to controls treated at 600 to 4,800 mg/kg/d for 10 to 14 days. The changes in catalase were similar to those in mice at similar treatment levels, with 1.1- to 1.5-fold of control enzyme activities at doses of 1,000 to 1,300 mg/kg/d (Elcombe et al., 1985; Melnick et al., 1987). However, the changes in PCO were smaller, with 1.1- to 1.8-fold of control activity at these doses, as compared to 6.3- to 7.9-fold of control in mice (Goldsworthy and Popp, 1987; Melnick et al., 1987).

In SV129 mice, Nakajima et al. (2000) and Laughter et al. (2004) investigated the dependence of these changes on PPARα by using a null mouse. Nakajima et al. reported that neither male nor female wild-type or PPARα null mice had significant increases in catalase after 14 days of treatment at 750 mg/kg/d. However, given the small number of animals (4 per group) and the relatively small changes in catalase observed in other (wild-type) strains of mice, this study had limited power to detect such changes. Several other markers of peroxisome proliferation, including acyl-CoA oxidase and CYP4A1 (PCO was not investigated), were induced by TCE in male wild-type mice, but not in male null mice or female mice of either type. Unfortunately, none of these markers have been investigated using TCE in female mice of any other strain, so it is unclear whether the lack of response is characteristic of female mice in general, or just in this strain. Interestingly, as noted above, liver/body weight ratio increases were observed in both sexes of the null mice in this study. Laughter et al. (2004) only quantified activity of the peroxisome proliferation marker PCO in their study, and found in null mice a slight decrease (0.8-fold of control) at 500 mg/kg/d TCE and an increase (1.5-fold of control) at

1,500 mg/kg/d TCE after 3 weeks of treatment, with neither statistically significant (4−5 mice per group). However, baseline levels of PCO were almost 2-fold higher in the null mice, and the treated wild-type and null mice differed in PCO activity by only about 1.5-fold.

In sum, oral administration of TCE for up to 28 days causes proliferation of peroxisomes in hepatocytes along with associated increases in peroxisomal enzyme activities in both mice and rats. Male mice tend to be more sensitive in that at comparable doses, rats and female mice tend to exhibit smaller responses. For example, for peroxisomal volume and PCO, the fold-increase in rats appears to be lower by 3- to 6-fold than that in mice, but, for catalase, the changes were similar between mice in F344 rats. No inhalation or longer-term studies were located, and only one study examined these changes at more than one time-point. Therefore, little is known about the route-dependence, time course, and persistence of these changes. Finally, two studies in PPARα-null mice (Laughter et al., 2004; Nakajima et al., 2000) found diminished responses in terms of increased peroxisomal volume and peroxisomal enzyme activities as compared to wild-type mice, although there was some confounding due to baseline differences between null and wild-type control mice in several measures.

4.5.4.5. *Oxidative Stress*

Several studies have attempted to study the possible effects of "oxidative stress" and DNA damage resulting from TCE exposures. The effects of induction of metabolism by TCE, as well as through coexposure to ethanol, have been hypothesized to in itself increase levels of "oxidative stress" as a common effect for both exposures (see Sections E.3.4.2.3 and E.4.2.4). Oxidative stress has been hypothesized to be a key event or MOA for peroxisome proliferators as well, but has been found to neither be correlated with cell proliferation nor carcinogenic potency of peroxisome proliferators (see Section E.3.4.1.1). As a MOA, it is not defined or specific as the term "oxidative stress" is implicated as part of the pathophysiologic events in a multitude of disease processes and is part of the normal physiologic function of the cell and cell signaling.

In regard to measures of oxidative stress, Rusyn et al. (2006) noted that although an overwhelming number of studies draw a conclusion between chemical exposure, DNA damage, and cancer based on detection of 8OHdG, a highly mutagenic lesion, in DNA isolated from organs of *in vivo* treated animals, a concern exists as to whether increases in 8OHdG represent damage to genomic DNA, a confounding contamination with mitochondrial DNA, or an experimental artifact. As noted in Sections E.2.1.1 and E.2.2.11, studies of TCE which employ the i.p. route of administration can be affected by inflammatory reactions resulting from that routes of administration and subsequent toxicity that can involve oxygen radical formation from inflammatory cells. Finally, as described in Section E.2.2.8, the study by Channel et al. (1998)

demonstrated that corn oil as vehicle had significant effects on measures of "oxidative stress" such as TBARS.

The TBARS results presented by Channel et al. (1988) indicate suppression of TBARS with increasing time of exposure to corn oil alone with data presented in such a way for 8OHdG and total free radical changes that the pattern of corn oil administration was obscured. It was not apparent from that study that TCE exposure induced oxidative damage in the liver.

Toraason et al. (1999) measured 8OHdG and a "free radical-catalyzed isomer of arachidonic acid and marker of oxidative damage to cell membranes, 8-Epi-prostaglandin F2α (8-epiPGF)", excretion in the urine and TBARS (as an assessment of malondialdehyde and marker of lipid peroxidation) in the liver and kidney of male Fischer rats exposed to single i.p. injections in of TCE in Alkamuls vehicle. Using this paradigm, 500-mg/kg TCE was reported to induce Stage II anesthesia and a 1,000 mg/kg TCE to induce Level III or IV (absence of reflex response) anesthesia and burgundy colored urine with 2/6 rats at 24 hours comatose and hypothermic. The animals were sacrificed before they could die and the authors suggested that they would not have survived another 24 hours. Thus, using this paradigm there was significant toxicity and additional issues related to route of exposure. Urine volume declined significantly during the first 12 hours of treatment and while water consumption was not measured, it was suggested by the authors to be decreased due to the moribundity of the rats. Given that this study examined urinary markers of "oxidative stress" the effects on urine volume and water consumption, as well as the profound toxicity induced by this exposure paradigm, limit the interpretation of the study. The issues of bias in selection of the data for this analysis, as well as the issues stated above for this paradigm limit interpretation of these data while the authors suggest that evidence of oxidative damage was equivocal.

4.5.4.6. *Bile Production*

Effects of TCE exposure in humans and in experimental animals is presented in Section E.2.6. Serum bile acids (SBA) have been suggested as a sensitive indicator of hepatotoxicity to a variety of halogenated solvents with an advantage of increased sensitivity and specificity over conventional liver enzyme tests that primarily reflect the acute perturbation of hepatocyte membrane integrity and "cell leakage" rather than liver functional capacity (i.e., uptake, metabolism, storage, and excretion functions of the liver) (Bai et al., 1992b; Neghab et al., 1997). While some studies have reported negative results, a number of studies have reported elevated SBA in organic solvent-exposed workers in the absence of any alterations in normal liver function tests. These variations in results have been suggested to arise from failure of some methods to detect some of the more significantly elevated SBA and the short-lived and reversible

nature of the effect (Neghab et al., 1997). Neghab et al. (1997) have reported that occupational exposure to 1,1,2-trichloro-1,2,2-trifluoroethane and trichloroethylene has resulted in elevated SBA and that several studies have reported elevated SBA in experimental animals to chlorinated solvents such as carbon tetrachloride, chloroform, hexachlorobutadiene, tetrachloroethylene, 1,1,1-trichloroethane, and trichloroethylene at levels that do not induce hepatotoxicity (Bai et al., 1992a, b; Hamdan and Stacey, 1993; Wang and Stacey, 1990). Toluene, a nonhalogenated solvent, has also been reported to increase SBA in the absence of changes in other hepatobiliary functions (Neghab and Stacey, 1997). Thus, disturbance in SBA appears to be a generalized effect of exposure to chlorinated solvents and nonchlorinated solvents and not specific to TCE exposure.

Wang and Stacey (1990) administered TCE in corn oil via i.p. injection to male Sprague-Dawley rats with liver enzymes and SBA examined 4 hours after the last TCE treatment. The limitations of i.p injection experiments have already been discussed. While reporting no overt liver toxicity there was, generally, a reported dose-related increase in cholic acid, chenodeoxycholic acid, deoxycholic acid, taurocholic acid, tauroursodeoxycholic acid with cholic acid and taurochlolic acid increased at the lowest dose. The authors report that "examination of liver sections under light microscopy yielded no consistent effects that could be ascribed to trichloroethylene." In the same study a rats were also exposed to TCE via and using this paradigm, cholic acid and taurocholic acid were also significantly elevated but the large variability in responses between rats and the low number of rats tested in this paradigm limit its ability to determine quantitative differences between groups. Nevertheless, without the complications associated with i.p. exposure, inhalation exposure of TCE at relatively low exposure levels that were not associated with other measures of toxicity *were* associated with increased SBA level.

Hamdan et al. (1993) administered TCE in corn oil (1 mmol/kg) in male Sprague-Dawley rats and followed the time-course of SBA elevation, TCE concentration, and trichloroethanol in the blood up to 16 hours. Liver and blood concentration of TCE were reported to peak at 4 hours while those of trichloroethanol peaked at 8 hours after dosing. TCE levels were not detectable by 16 hours in either blood or liver while those of trichloroethanol were still elevated. Elevations of SBA were reported to parallel those of TCE with cholic acid and taurochloate acid reported to show the highest levels of bile acids. The authors state that liver injury parameters were checked and found unaffected by TCE exposure but did not show the data. Thus, it was TCE concentration and not that of its metabolite that was most closely related to changes in SBA and after a single exposure and the effect appeared to be reversible. In an *in vitro* study by Bai and Stacey (1993), TCE was studied in isolated rat hepatocytes with TCE reported to cause a

dose-related suppression of initial rates of cholic acid and taurocholic acid but with no significant effects on enzyme leakage and intracellular calcium contents, further supporting a role for the parent compound in this effect.

4.5.4.7. *Summary: Trichloroethylene (TCE)-Induced Noncancer Effects in Laboratory Animals*

In laboratory animals, TCE leads to a number of structural changes in the liver, including increased liver weight, small transient increases in DNA synthesis, cytomegaly in the form of "swollen" or enlarged hepatocytes, increased nuclear size probably reflecting polyploidization, and proliferation of peroxisomes. Liver weight increases proportional to TCE dose are consistently reported across numerous studies, and appear to be accompanied by periportal hepatocellular hypertrophy. There is also evidence of increased DNA synthesis in a small portion of hepatocytes at around 10 days *in vivo* exposure. The lack of correlation of hepatocellular mitotic figures with whole liver DNA synthesis or DNA synthesis observed in individual hepatocytes supports the conclusion that cellular proliferation is not the predominant cause of increased DNA synthesis. The lack of correlation of whole liver DNA synthesis and those reported for individual hepatocytes suggests that nonparenchymal cells also contribute to such synthesis. Indeed, nonparenchymal cell activation or proliferation has been noted in several studies. Moreover, the histological descriptions of TCE exposed liver are consistent with and in some cases specifically note increased polyploidy after TCE exposure. Interestingly, changes in TCE-induced hepatocellular ploidy, as indicated by histological changes in nuclei, have been noted to remain after the cessation of exposure. In regard to apoptosis, TCE has been reported to either not change apoptosis or to cause a slight increase at high doses. Some studies have also noted effects from dosing vehicle alone (such as corn oil in particular) not only on liver pathology, but also on DNA synthesis.

Available data also suggest that TCE does not induce substantial cytotoxicity, necrosis, or regenerative hyperplasia, as only isolated, focal necroses and mild to moderate changes in serum and liver enzyme toxicity markers having been reported. Data on peroxisome proliferation, along with increases in a number of associated biochemical markers, show effects in both mice and rats. These effects are consistently observed across rodent species and strains, although the degree of response at a given mg/kg/d dose appears to be highly variability across strains, with mice on average appearing to be more sensitive.

In addition, like humans, laboratory animals exposed to TCE have been observed to have increased serum bile acids, though the toxicologic importance of these effects is unclear.

4.5.5. Trichloroethylene (TCE)-Induced Liver Cancer in Laboratory Animals

For 2-year or lifetime studies of TCE exposure a consistent hepatocarcinogenic response has been observed using mice of differing strains and genders and from differing routes of exposure. However, some rat studies have been confounded by mortality from gavage error or the toxicity of the dose of TCE administered. In some studies, a relative insensitive strain of rat has been used. However, in general it appears that the mouse is more sensitive than the rat to TCE-induced liver cancer. Three studies give results the authors consider to be negative for TCE-induced liver cancer in mice, but have either design and/or reporting limitations, or are in strains and paradigms with apparent low ability for liver cancer induction or detection. Findings from these studies are shown in Tables 4-52 through 4-57, and discussed below.

4.5.5.1. *Negative or Inconclusive Studies of Mice and Rats*

Fukuda et al. (1983) reported a 104-week inhalation bioassay in female Crj:CD-1 (ICR) mice and female Crj:CD (SD) rats exposed to 0-, 50-, 150-, and 450-ppm TCE ($n = 50$). There were no reported incidences of mice or rats with liver tumors for controls indicative of relatively insensitive strains and gender used in the study for liver effects. While TCE was reported to induce a number of other tumors in mice and rats in this study, the incidence of liver tumors was less than 2% after TCE exposure. Of note is the report of cystic cholangioma reported in 1 group of rats.

Henschler et al. (1980) exposed NMRI mice and WIST random bred rats to 0-, 100-, and 500-ppm TCE for 18 months ($n = 30$). Control male mice were reported to have one hepatocellular carcinoma and 1 hepatocellular adenoma with the incidence rate unknown. In the 100-ppm TCE exposed group, 2 hepatocellular adenomas and 1 mesenchymal liver tumor were reported. No liver tumors were reported at any dose of TCE in female mice or controls. For male rats, only 1 hepatocellular adenomas at 100 ppm was reported. For female rats no liver tumors were reported in controls, but 1 adenoma and 1 cholangiocarcinoma was reported at 100-ppm TCE and at 500-ppm TCE, 2 cholangioadenomas, a relatively rare biliary tumor, was reported. The difference in survival in mice, did not affect the power to detect a response, as was the case for rats. However, the low number of animals studied, abbreviated exposure duration, low survival in rats, and absent background response (suggesting low intrinsic sensitivity to this endpoint) suggest a study of limited ability to detect a TCE carcinogenic liver response. Of note is that despite their limitations, both Fukuda et al. (1983) and Henschler et al. (1980) report rare biliary cell derived tumors in TCE-exposed rats.

Table 4-52. Summary of liver tumor findings in gavage studies of trichloroethylene by NTP (1990)[a]

Sex	Dose (mg/kg)[b]	Adenoma (overall; terminal[c])	Adenocarcinoma (overall; terminal[c])
1/d, 5 d/wk, 103-wk study, F344/N rats			
Male	0	NA[d]	0/49
	500	NA	0/49
	1,000	NA	1/49
Female	0	NA	0/50
	500	NA	1/48
	1,000	NA	1/48
1/d, 5 d/wk, 103-wk study, $B6C3F_1$ mice			
Male	0	7/48; 6/33	8/48; 6/33
	1,000	14/50; 6/16	31/50; 14/16[f]
Female	0	4/48; 4/32	2/48; 2/32
	1,000	16/49; 11/23[e]	13/49; 8/23[g]

[a]Liver tumors not examined in 13-week study, so data shown only for 103-week study.
[b]Corn oil vehicle.
[c]Terminal values not available for rats.
[d]Data not available.
[e]$p < 0.003$.
[f]$p < 0.001$.
[g]$p \leq 0.002$.

1 **Table 4-53. Summary of liver tumor findings in gavage studies of**
2 **trichloroethylene by NCI (1976)**
3

Sex	Dose (mg/kg)[a]	Hepatocarcinoma
1/d, 5 d/wk, 2-yr study, Osborn-Mendel rats		
Males	0	0/20
	549	0/50
	1,097	0/50
Females	0	0/20
	549	1/48
	1,097	0/50
1/d, 5 d/wk, 2-yr study, B6C3F1 mice		
Males	0	1/20
	1,169	26/50[b]
	2,339	31/48[b]
Females	0	0/20
	869	4/50
	1,739	11/47[b]

4
5 [a]Treatment period was 48 weeks for rats, 66 weeks for mice. Doses were changed several times during the
6 study based on monitoring of body weight changes and survival. Dose listed here is the time-weighted
7 average dose over the days on which animals received a dose.
8 [b]p <0.01.

Table 4-54. Summary of liver tumor incidence in gavage studies of trichloroethylene by NTP (1988)

Sex	Dose (mg/kg)*	Adenoma	Adenocarcinoma
1/d, 5 d/wk, 2-yr study, ACI rats			
Male	0	0/50	1/50
	500	0/49	1/49
	1,000	0/49	1/49
Female	0	0/49	2/49
	500	0/46	0/46
	1,000	0/39	0/39
1/d, 5 d/wk, 2-yr study, August rats			
Male	0	0/50	0/50
	500	0/50	1/50
	1,000	0/48	1/48
Female	0	0/48	2/48
	500	0/48	0/48
	1,000	0/50	0/50
1/d, 5 d/wk, 2-yr study, Marshall rats			
Male	0	1/49	1/49
	500	0/50	0/50
	1,000	0/47	1/47
Female	0	0/49	0/49
	500	0/48	0/48
	1,000	0/46	0/46
1/d, 5 d/wk, 2-yr study, Osborne-Mendel rats			
Male	0	1/50	1/50
	500	1/50	0/50
	1,000	1/49	2/49
Female	0	0/50	0/50
	500	0/48	2/48
	1,000	0/49	2/49

*Corn oil vehicle.

Table 4-55. Summary of liver tumor findings in inhalation studies of trichloroethylene by Maltoni et al. (1988)[a]

Sex	Concentration (ppm)	Hepatoma
7 h/d, 5 d/wk, 8-wk exposure, observed for lifespan, Swiss mice		
Male	0	1/100
	100	3/60
	600	4/72
Female	0	1/100
	100	1/60
	600	0/72
7 h/d, 5 d/wk, 78-wk exposure, observed for lifespan, Swiss mice		
Male	0	4/90
	100	2/90
	300	8/90
	600	13/90
Female	0	0/90
	100	0/90
	300	0/90
	600	1/90
7 h/d, 5 d/wk, 78-wk exposure, observed for lifespan, B6C3F1 mice[b]		
Male	0	1/90
	100	1/90
	300	3/90
	600	6/90
Female	0	3/90
	100	4/90
	300	4/90
	600	9/90

[a]Three inhalation experiments in this study found no hepatomas: BT302 (8-week exposure to 0, 100, 600 ppm in Sprague-Dawley rats); BT303 (8-week exposure to 0, 100, or 600 ppm in Swiss mice); and BT304 (78-week exposure to 0, 100, 300, or 600 ppm in Sprague-Dawley rats).
[b]Female incidences are from experiment BT306, while male incidences are from experiment BT306bis, which was added to the study because of high, early mortality due to aggressiveness and fighting in males in experiment BT306.

1 **Table 4-56. Summary of liver tumor findings in inhalation studies of**
2 **trichloroethylene by Henschler et al. (1980)[a] and Fukuda et al. (1983)**
3

Sex	Concentration (ppm)	Adenomas	Adenocarcinomas
6 h/d, 5 d/wk, 18-mo exposure, 30-mo observation, Han:NMRI mice (Henschler et al., 1980)			
Males	0	1/30 [b]	1/30
	100	2/29 [b]	0/30
	500	0/29	0/30
Females	0	0/29	0/29
	100	0/30	0/30
	500	0/28	0/28
6 h/d, 5 d/wk, 18-mo exposure, 36-mo observation, Han:WIST rats (Henschler et al., 1980)			
Males	0	1/29	0/29
	100	1/30	0/30
	500	0/30	0/30
Females	0	0/28	0/28
	100	1/30	1/30
	500	2/30	0/30
7 h/d, 5 d/wk, 2-yr study, Crj:CD (SD) rats (Fukuda et al., 1983)			
Females	0	0/50	0/50
	50	1/50	0/50
	150	0/47	0/47
	450	0/51	1/50
7 h/d, 5 d/wk, 2-yr study, Crj:CD (ICR) mice (Fukuda et al., 1983)			
Females	0	0/49	0/49
	50	0/50	0/50
	150	0/50	0/50
	450	1/46	0/46

4
5 [a]Henschler et al. (1980) observed no liver tumors in control or exposed Syrian hamsters.
6 [b]One additional hepatic tumor of undetermined class not included.

1 **Table 4-57. Summary of liver tumor findings in gavage studies of**
2 **trichloroethylene by Henschler et al. (1984)[a]**
3

Sex (TCE conc.)	TCE (Stabilizers if present)	Benign[b]	Malignant[c]
5 d/wk, 18-mo exposure, 24-mo observation, Swiss mice (Henschler et al., 1984)			
Males (2.4g/kg BW)	Control (none)	5/50	0/50
	TCE (triethanolamine)	7/50	0/50
	TCE (industrial)	9/50	0/50
	TCE (epichlorohydrin (0.8%))	3/50	1/50
	TCE (1,2-epoxybutane (0.8%))	4/50	0/50
	TCE (both epichlorohydrin (0.25%) and 1,2-epoxybutane (0.25%))	5/50	0/50
Females (1.8 g/kg BW)	Control (none)	1/50	0/50
	TCE (triethanolamine)	7/50	0/50
	TCE (industrial)	9/50	0/50
	TCE (epichlorohydrin (0.8%))	3/50	0/50
	TCE (1,2-epoxybutane (0.8%))	2/50	0/50
	TCE (both epichlorohydrin (0.25%) and 1,2-epoxybutane (0.25%))	4/50	1/50

4
5 [a]Henschler et al. (1984) Due to poor condition of the animals resulting from the nonspecific toxicity of high doses of
6 TRI and/or the additives, gavage was stopped for all groups during weeks 35-40, 65 and 69-78, and all doses were
7 reduced by a factor of 2 from the 40th week on.
8 [b]Includes hepatocellular adenomas, hemangioendothelioma, cholangiocellular adenoma.
9 [c]Includes hepatocellular carcinoma, malignant hemangiosarcoma, cholangiocellular carcinoma.
10
11 Conc. = concentration.
12
13
14 Van Duuren et al. (1979), exposed mice to 0.5 mg/mouse to TCE via gavage once a week
15 in 0.1 mL trioctanion (*n* = 30). Inadequate design and reporting of this study limit that ability to
16 use the results as an indicator of TCE carcinogenicity.
17 The NCI (1976) study of TCE was initiated in 1972 and involved the exposure of
18 Osborn-Mendel rats to varying concentrations of TCE. A low incidence of liver tumors was
19 reported for controls and carbon tetrachloride positive controls in rats from this study. The
20 authors concluded that due to mortality, "the test is inconclusive in rats." They note the
21 insensitivity of the rat strain used to the positive control of carbon tetrachloride exposure.

1 The NTP (1990) study of TCE exposure in male and female F344/N rats, and B6C3F1
2 mice (500 and 1,000 mg/kg for rats) is limited in the ability to demonstrate a dose-response for
3 hepatocarcinogenicity. For rats, the NTP (1990) study reported no treatment-related non-
4 neoplastic liver lesions in males and a decrease in basophilic cytological change reported from
5 TCE-exposure in female rats. The results for detecting a carcinogenic response in rats were
6 considered to be equivocal because both groups receiving TCE showed significantly reduced
7 survival compared to vehicle controls and because of a high rate (e.g., 20% of the animals in the
8 high-dose group) of death by gavage error.
9 The NTP (1988) study of TCE exposure in four strains of rats to "diisopropylamine-
10 stabilized TCE" was also considered inadequate for either comparing or assessing TCE-induced
11 liver carcinogenesis in these strains of rats because of chemically induced toxicity, reduced
12 survival, and incomplete documentation of experimental data. TCE gavage exposures of 0, 500,
13 or 1,000 mg/kg/d (5 days/week, for 103 weeks) male and female rats was also marked by a large
14 number of accidental deaths (e.g., for high-dose male Marshal rats 25 animals were accidentally
15 killed).
16 Maltoni et al. (1986) reported the results of several studies of TCE via inhalation and
17 gavage in mice and rats. A large number of animals were used in the treatment groups but the
18 focus of the study was detection of a neoplastic response with only a generalized description of
19 tumor pathology phenotype given and limited reporting of non-neoplastic changes in the liver.
20 Accidental death by gavage error was reported not to occur in this study. In regards to effects of
21 TCE exposure on rat survival, "a nonsignificant excess in mortality correlated to TCE treatment
22 was observed only in female rats (treated by ingestion with the compound)".
23 For rats, Maltoni et al. (1986) reported 4 liver angiosarcomas (1 in a control male rat,
24 1 both in a TCE-exposed male and female at 600 ppm TCE for 8 weeks, and 1 in a female rat
25 exposed to 600-ppm TCE for 104 weeks), but the specific results for incidences of hepatocellular
26 "hepatomas" in treated and control rats were not given. Although the Maltoni et al. (1986)
27 concluded that the small number was not treatment related, the findings were brought forward
28 because of the extreme rarity of this tumor in control Sprague-Dawley rats, untreated or treated
29 with vehicle materials. In rats treated for 104 weeks, there was no report of a TCE treatment-
30 related increase in liver cancer in rats. This study only presented data for positive findings so it
31 did not give the background or treatment-related findings in rats for liver tumors in this study.
32 Thus, the extent of background tumors and sensitivity for this endpoint cannot be determined.
33 Of note is that the Sprague-Dawley strain used in this study was also noted in the Fukuda et al.
34 (1983) study to be relatively insensitive for spontaneous liver cancer and to also be negative for
35 TCE-induced hepatocellular liver cancer induction in rats. However, like Fukuda et al. (1983)

1 and Henschler et al. (1980), that reported rare biliary tumors in insensitive strains of rat for
2 hepatocellular tumors, Maltoni et al. (1986) reported a relatively rare tumor type, angiosarcoma,
3 after TCE exposure in a relatively insensitive strain for "hepatomas." As noted above, many of
4 the rat studies were limited by premature mortality due to gavage error or premature mortality
5 (Henschler et al., 1980; NCI, 1976; NTP, 1990, 1988), which was reported not occur in
6 Maltoni et al. (1986).
7

8 **4.5.5.2. *Positive Trichloroethylene (TCE) Studies of Mice***

9 In the NCI (1976) study of TCE exposure in B6C3F1 mice, TCE was reported to increase
10 incidence of hepatocellular carcinomas in both doses and both genders of mice (~1,170 and
11 2,340 mg/kg for males and 870 and 1,740 mg/kg for female mice). Hepatocellular carcinoma
12 diagnosis was based on histologic appearance and metastasis to the lung. The tumors were
13 described in detail and to be heterogeneous "as described in the literature" and similar in
14 appearance to tumors generated by carbon tetrachloride. The description of liver tumors in this
15 study and tendency to metastasize to the lung are similar to descriptions provided by
16 Maltoni et al. (1986) for TCE-induced liver tumors in mice via inhalation exposure.
17 The NTP (1990) study of TCE exposure in male and female B6C3F1 mice (1,000 mg/kg
18 for mice) reported decreased latency of liver tumors, with animals first showing carcinomas at
19 57 weeks for TCE-exposed animals and 75 weeks for control male mice. The administration of
20 TCE was also associated with increased incidence of hepatocellular carcinoma (tumors with
21 markedly abnormal cytology and architecture) in male and female mice. Hepatocellular
22 adenomas were described as circumscribed areas of distinctive hepatic parenchymal cells with a
23 perimeter of normal appearing parenchyma in which there were areas that appeared to be
24 undergoing compression from expansion of the tumor. Mitotic figures were sparse or absent but
25 the tumors lacked typical lobular organization. Hepatocellular carcinomas had markedly
26 abnormal cytology and architecture with abnormalities in cytology cited as including increased
27 cell size, decreased cell size, cytoplasmic eosinophilia, cytoplasmic basophilia, cytoplasmic
28 vacuolization, cytoplasmic hyaline bodies, and variations in nuclear appearance. Furthermore, in
29 many instances several or all of the abnormalities were present in different areas of the tumor
30 and variations in architecture with some of the hepatocellular carcinomas having areas of
31 trabecular organization. Mitosis was variable in amount and location. Therefore, the phenotype
32 of tumors reported from TCE exposure was heterogeneous in appearance between and within
33 tumors. However, because it consisted of a single-dose group in addition to controls, this study
34 is limited of limited utility for analyzing the dose-response for hepatocarcinogenicity. There was

also little reporting of non-neoplastic pathology or toxicity and no report of liver weight at termination of the study.

Maltoni et al. (1986) reported the results of several studies of TCE in mice. A large number of animals were used in the treatment groups but the focus of the study was detection of a neoplastic response with only a generalized description of tumor pathology phenotype given and limited reporting of non-neoplastic changes in the liver. There was no accidental death by gavage error reported to occur in mice but, a "nonsignificant" excess in mortality correlated to TCE treatment was observed in male B6C3F1 mice. TCE-induced effects on body weight were reported to be absent in mice except for one experiment (BT 306 bis) in which a slight nondose correlated decrease was found in exposed animals. "Hepatoma" was the term used to describe all malignant tumors of hepatic cells, of different subhistotypes, and of various degrees of malignancy and were reported to be unique or multiple, and have different sizes (usually detected grossly at necropsy) from TCE exposure. In regard to phenotype tumors were described as usual type observed in Swiss and B6C3F1 mice, as well as in other mouse strains, either untreated or treated with hepatocarcinogens and to frequently have medullary (solid), trabecular, and pleomorphic (usually anaplastic) patterns. Swiss mice from this laboratory were reported to have a low incidence of hepatomas without treatment (1%). The relatively larger number of animals used in this bioassay (n = 90 to 100), in comparison to NTP standard assays, allows for a greater power to detect a response.

TCE exposure for 8 weeks via inhalation at 100 or 600 ppm may have been associated with a small increase in liver tumors in male mice in comparison to concurrent controls during the life span of the animals. In Swiss mice exposed to TCE via inhalation for 78 weeks, there a reported increase in hepatomas associated with TCE treatment that was dose-related in male but not female Swiss mice. In B6C3F1 mice exposed via inhalation to TCE for 78 weeks, increases in hepatomas were reported in both males and females. However, the experiment in males was repeated with B6C3F1 mice from a different source, since in the first experiment more than half of the mice died prematurely due to excessive fighting. Although the mice in the two experiments in males were of the same strain, the background level of liver cancer was significantly different between mice from the different sources (1/90 versus 19/90), though the early mortality may have led to some censoring. The finding of differences in response in animals of the same strain but from differing sources has also been reported in other studies for other endpoints. However, for both groups of male B6C3F1 mice the background rate of liver tumors over the lifetime of the mice was no greater than about 20%.

There were other reports of TCE carcinogenicity in mice from chronic exposures that were focused primarily on detection of liver tumors with limited reporting of tumor phenotype or

non-neoplastic pathology. Herren-Freund et al. (1987) reported that male B6C3 F1 mice given 40 mg/L TCE in drinking water had increased tumor response after 61 weeks of exposure. However, concentrations of TCE fell by about ½ at this dose of TCE during the twice a week change in drinking water solution so the actual dose of TCE the animals received was less than 40 mg/L. The percent liver/body weight was reported to be similar for control and TCE-exposed mice at the end of treatment. However, despite difficulties in establishing accurately the dose received, an increase in adenomas per animal and an increase in the number of animals with hepatocellular carcinomas were reported to be associated with TCE exposure after 61 weeks of exposure and without apparent hepatomegaly. Anna et al. (1994) reported tumor incidences for male B6C3F1 mice receiving 800 mg/kg/d TCE via gavage (5 days/week for 76 weeks). All TCE-treated mice were reported to be alive after 76 weeks of treatment. Although the control group contained a mixture of exposure durations (76−134 weeks) and concurrent controls had a very small number of animals, TCE-treatment appeared to increase the number of animals with adenomas, the mean number of adenomas and carcinomas, but with no concurrent TCE-induced cytotoxicity.

4.5.5.3. *Summary: Trichloroethylene (TCE)-Induced Cancer in Laboratory Animals*

Chronic TCE bioassays have consistently reported increased liver tumor incidences in both sexes of B6C3F1 mice treated by inhalation and gavage exposure in a number of bioassays. The only inhalation study of TCE in Swiss mice also showed an effect in males. Data in the rat, while not reporting statistically significantly increased risks, are not entirely adequate due to low numbers of animals, inadequate reporting, use of insensitive bioassays, increased systemic toxicity, and/or increased mortality. Notably, several studies in rats noted a few very rare types of liver or biliary tumors (cystic cholangioma, cholangiocarcinoma, or angiosarcomas) in treated animals.

4.5.6. Role of Metabolism in Liver Toxicity and Cancer

It is generally thought that TCE oxidation by CYPs is necessary for induction of hepatotoxicity and hepatocarcinogenicity (Bull, 2000). Direct evidence for this hypothesis is limited, e.g., the potentiation of hepatotoxicity by pretreatment with CYP inducers such as ethanol and phenobarbital (Nakajima et al., 1988; Okino et al., 1991). Rather the presumption that CYP-mediated oxidation is necessary for TCE hepatotoxicity and hepatocarcinogenicity is largely based on similar effects (e.g., increases in liver weight, peroxisome proliferation, and hepatocarcinogenicity) having been observed with TCE's oxidative metabolites. The discussion below focuses the similarities and differences between the major effects in the liver of TCE and

1 of the oxidative metabolites CH, TCA, and DCA. In addition, CH is largely converted to TCOH,
2 TCA, and possibly DCA.
3

4 **4.5.6.1.** ***Pharmacokinetics of Chloral Hydrate (CH), Trichloroacetic Acid (TCA), and***
5 ***Dichloroacetic Acid (DCA) From Trichloroethylene (TCE) Exposure***

6 As discussed in Chapter 3, *in vivo* data confirm that CH and TCA, are oxidative
7 metabolites of TCE. In addition, there are indirect data suggesting the formation of DCA.
8 However, direct *in vivo* evidence of the formation of DCA is confounded by its rapid clearance
9 at low concentrations, and analytical artifacts in its detection *in vivo* that have yet to be entirely
10 resolved. PBPK modeling (see Section 3.5) predicts that the proportions of TCE metabolized to
11 CH and TCA varies considerably in mice (ranging from 15−97 and 4−38%, respectively) and
12 rats (ranging 7−75 and 0.5−22%, respectively). Therefore, a range of smaller concentrations of
13 TCA or CH may be relevant for comparisons with TCE-induced liver effects. For example, for
14 1,000 mg/kg/d oral doses of TCE, the relevant comparisons would be approximately
15 0.25−1.5 g/L in drinking water for TCA and CH. For DCA a corresponding range is harder to
16 determine and has been suggested to be an upper limit of about 12% (Barton et al., 1999).
17

18 **4.5.6.2.** ***Comparisons Between Trichloroethylene (TCE) and Trichloroacetic Acid (TCA),***
19 ***Dichloroacetic Acid (DCA), and Chloral Hydrate (CH) Noncancer Effects***

20 **4.5.6.2.1.** ***Hepatomegaly—qualitative and quantitative comparisons.*** As discussed above,
21 TCE causes hepatomegaly in rats, mice, and gerbils under both acute and chronic dosing. Data
22 from a few available studies suggest that oxidative metabolism is important for mediating these
23 effects. Buben and O'Flaherty (1985) collected limited pharmacokinetic data in a sample of the
24 same animals for which liver weight changes were being assessed. While liver weight increases
25 had similarly strong correlations with applied dose and urinary metabolites for doses up to
26 1,600 mg/kg/d (R^2 of 0.97 for both), above that dose, the linear relationship was maintained with
27 urinary metabolites but not with applied dose. Ramdhan et al. (2008) conducted parallel
28 experiments at TCE 1,000 and 2,000 ppm (8 hours/day, 7 days) in wild-type and cyp2e1-null
29 mice, which did not exhibit increased liver/body weight ratios with TCE treatment and excreted
30 2-fold lower amounts of oxidative metabolites TCA and TCOH in urine as compared to wild-
31 type mice. However, among control mice, those with the null genotype had 1.32-fold higher
32 absolute liver weights and 1.18-fold higher liver/body weight ratios than wild-type mice,
33 reducing the sensitivity of the experiment, particularly with only 6 mice per dose group.
34 With respect to oxidative metabolites themselves, data from CH studies are not
35 informative—either because data were not shown (Sanders et al., 1982) or, because at the time

points measured, liver weight increases are substantially confounded by foci and carcinogenic lesions (Leakey et al., 2003a). TCA and DCA have both been found to cause hepatomegaly in mice and rats, with mice being more sensitive to this effect. DCA also increases liver/body weight ratios in dogs, but TCE and TCA have not been tested in this species (Cicmanec et al., 1991).

As noted above, TCE-induced changes in liver weight appear to be proportional to the exposure concentration across route of administration, gender and rodent species. As an indication of the potential contribution of TCE metabolites to this effect, a quantitative comparison of the shape of the dose-response curves for liver weight induction for TCE and its metabolites is informative. The analysis below was reported in Evans et al. (2009).

A number of short-term (<4 weeks) studies of TCA and DCA in drinking water have attempted to measure changes in liver weight induction, with the majority of these studies being performed in male B6C3F1 mice. Studies conducted from 14 to 30 days show a consistent increase in percent liver/body weight induction by TCA or DCA. However, as stated in many of the discussions of individual studies (see Appendix E), there is a limited ability to detect a statistically significant change in liver weight change in experiments that use a relatively small number of animals or do not match control and treatment groups for age and weight. The experiments of Buben and O'Flaherty used 12−14 mice per group giving it a greater ability to detect a TCE-induced dose response. However, many experiments have been conducted with 4−6 mice per dose group. For example, the data from DeAngelo et al. (2008) for TCA-induced percent liver/body weight ratio increases in male B6C3F1 mice were only derived from 5 animals per treatment group after 4 weeks of exposure. The 0.05 and 0.5 g/L exposure concentrations were reported to give a 1.09- and 1.16-fold of control percent liver/body weight ratios which were consistent with the increases noted in the cross-study database above. However, a power calculation shows that the Type II error (which should be >50% and thus, greater than the chances of "flipping a coin") was only a 6 and 7% and therefore, the designed experiment could accept a false null hypothesis. In addition, some experiments took greater care to age and weight match the control and treatment groups before the start of treatment.

Therefore, given these limitations and the fact that many studies used a limited range of doses, an examination of the combined data from multiple studies (Parrish et al., 1996; Sanchez and Bull, 1990; Carter et al., 1995; Kato-Weinstein et al., 2001; DeAngelo et al., 1989, 2008) can best inform/discern differences in DCA and TCA dose-response relationships for liver weight induction (described in more detail in Section E.2.4.2). The dose-response curves for similar concentrations of DCA and TCA are presented in Figure 4-5 for durations of exposure from 14−28 days in the male B6C3F1 mouse, which was the most common sex and strain used. As

noted in Appendix E, there appears to be a linear correlation between dose in drinking water and liver weight induction up to 2 g/L of DCA. However, the shape of the dose-response curve for TCA appears to be quite different. Lower concentrations of TCA induce larger increase that does DCA, but the TCE response reaches an apparent plateau while that of DCA continues to increase the response. TCA studies did not show significant duration-dependent difference in liver weight induction in this duration range. Short duration studies (10−42 days) were selected because (1) in chronic studies, liver weight increases are confounded by tumor burden, (2) multiple studies are available, and (3) TCA studies do not show significant duration-dependent differences in this duration range.

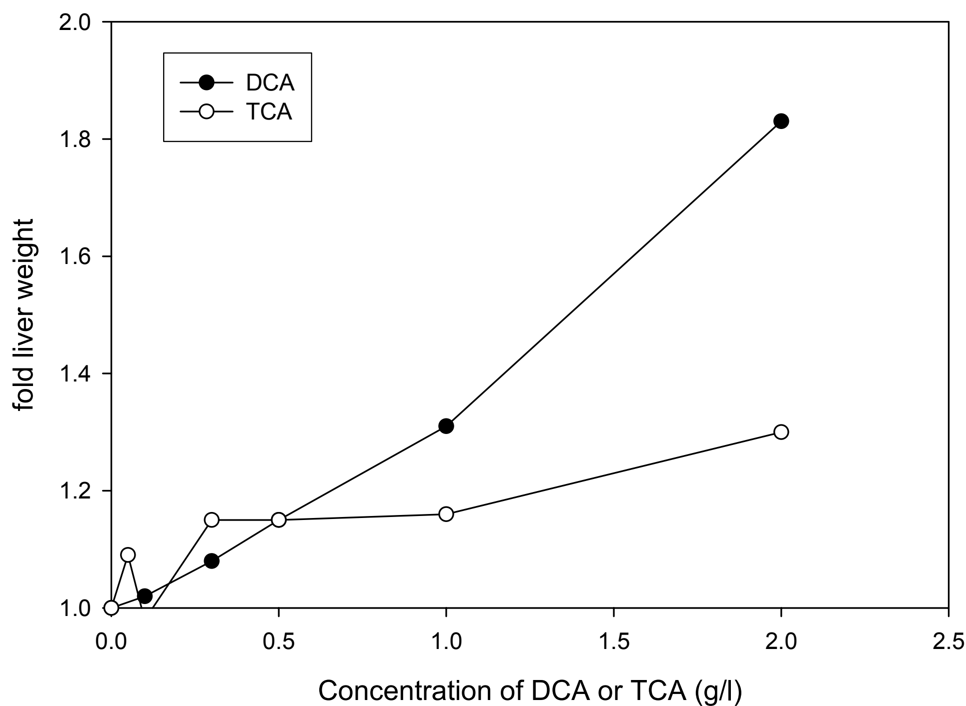
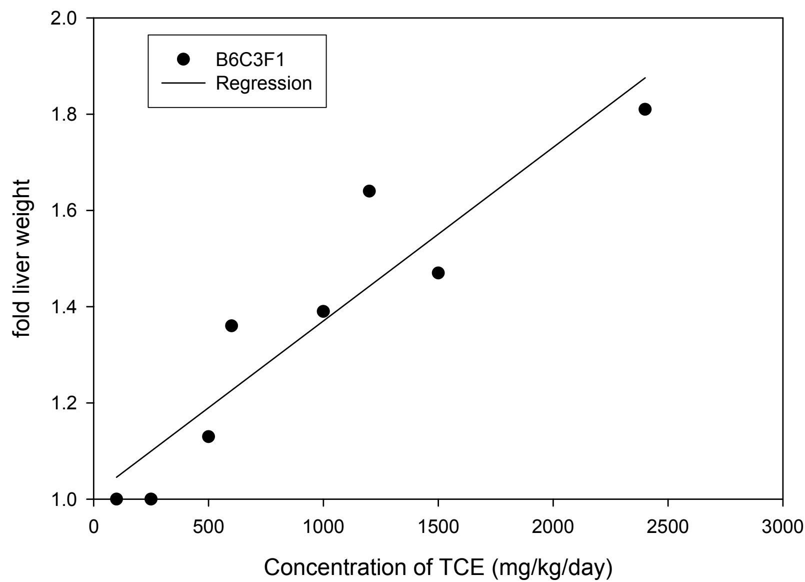


Figure 4-5. Comparison of average fold-changes in relative liver weight to control and exposure concentrations of 2 g/L or less in drinking water for TCA and DCA in male B6C3F1 mice for 14−30 days (Parrish et al.,1996; Sanchez and Bull, 1990; Carter et al., 1995; Kato-Weinstein et al., 2001; DeAngelo et al., 1989, 2008).

Of interest is the issue of how the dose-response curves for TCA and DCA compare to that of TCE in a similar model and dose range. Since TCA and DCA have strikingly different dose-response curves, which one if either best fits that of TCE and thus, can give insight as to which is causative agent for TCE's effects in the liver? The carcinogenicity of chronic TCE

1 exposure has been predominantly studies in two mouse strains, Swiss and B6C3F1, both of
2 which reportedly developed liver tumors. Rather than administered in drinking water, oral TCE
3 studies have been conducted via oral gavage and generally in corn oil for 5 days of exposure per
4 week. Factors adding to the increased difficulty in establishing the dose-response relationship
5 for TCE across studies and for comparisons to the DCA and TCA database include vehicle
6 effects, the difference between daily and weekly exposures, the dependence of TCE effects in the
7 liver on its metabolism to a variety of agents capable inducing effects in the liver, differences in
8 response between strains, and the inherent increased variability in use of the male mouse model.
9 Despite difference in exposure route, etc., a consistent pattern of dose-response emerges from
10 combining the available TCE data. The effects of oral exposure to TCE from 10−42 days on
11 liver weight induction is shown below in Figure 4-6 using the data of Elcombe et al. (1985),
12 Dees and Travis (1993), Goel et al. (1992), Merrick et al. (1987), Goldsworthy and Popp (1987),
13 and Buben and O'Flaherty (1985). Oral TCE administration in male B6C3F1 and Swiss mice
14 appeared to induce a dose-related increase in percent liver/body weight that was generally
15 proportional to the increase in magnitude of dose, though as expected, with more variability than
16 observed for a similar exercise for DCA or TCA in drinking water. Some of the variability is
17 due to the inclusion of the 10 day studies, since as discussed in Section E.2.4.2, there was a
18 greater increase in TCE-induced liver weight at 28−42 days of exposure Swiss mice than the
19 10-day data in B6C3F1 mice, and Kjellstrand et al. (1981) noted that TCE-induced liver weight
20 increases are still increasing at 10 days inhalation exposure. A strain difference is not evident
21 between the Swiss and B6C3F1 males, as both the combined TCE data and that for only B6C3F1
22 mice show similar correlation with the magnitude of dose and magnitude of percent liver/body
23 weight increase. The correlation coefficients for the linear regressions presented for the B6C3F1
24 data are $R^2 = 0.861$ and for the combined data sets is $R^2 = 0.712$. Comparisons of the slopes of
25 the dose-response curves suggest a greater consistency between TCE and DCA than between
26 TCE and TCA. There did not appear to be evidence of a plateau with higher TCE doses, and the
27 degree of fold-increase rises to higher levels with TCE than with TCA in the same strain of
28 mouse.

1



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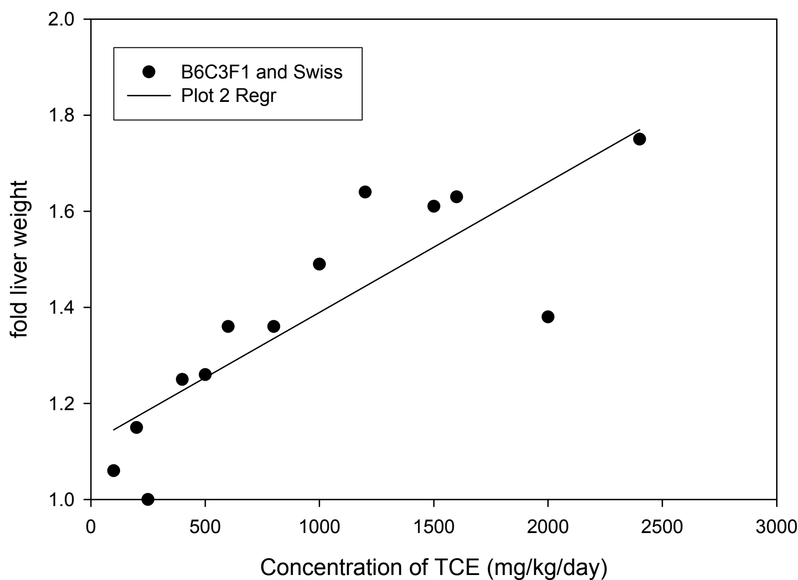

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4 **Figure 4-6. Comparisons of fold-changes in average relative liver weight and**
5 **gavage dose of (top panel) male B6C3F1 mice for 10−28 days of exposure**
6 **(Merrick et al., 1989; Elcombe et al., 1985; Goldsworthy and Popp, 1987;**
7 **Dees and Travis, 1993) and (bottom panel) in male B6C3F1 and Swiss mice.**

8

9

10 A more direct comparison would be on the basis of dose rather than drinking water
11 concentration. The estimations of internal dose of DCA or TCA from drinking water studies,
12 while varying considerably (DeAngelo et al., 1989, 2008), nonetheless suggest that the doses of

TCE used in the gavage experiments were much higher than those of DCA or TCA. However, only a fraction of ingested TCE is metabolized to DCA or TCA, as, in addition to oxidative metabolism, TCE is also cleared by GSH conjugation and by exhalation. While DCA dosimetry is highly uncertain (see Sections 3.3 and 3.5), the mouse PBPK model, described in Section 3.5 was calibrated using extensive *in vivo* data on TCA blood, plasma, liver, and urinary excretion data from inhalation and gavage TCE exposures, and makes robust predictions of the rate of TCA production. If TCA were predominantly responsible for TCE-induced liver weight increases, then replacing administered TCE dose (e.g., mg TCE/kg/day) by the rate of TCA produced from TCE (mg TCA/kg/day) should lead to dose-response curves for increased liver weight consistent with those from directly administered TCA. Figure 4-7 shows this comparison using the PBPK model-based estimates of TCA production for 4 TCE studies from 28−42 days in the male NMRI, Swiss, and B6C3F1 mice (Kjellstrand et al., 1983b; Buben and O’Flaherty, 1985; Merrick et al., 1989; Goel et al., 1992) and 4 oral TCA studies in B6C3F1 male mice at 2 g/L or lower drinking water exposure (DeAngelo et al., 1989, 2008; Parrish et al., 1996; Kato-Weinstein et al., 2001) from 14−28 days of exposure. The selection of the 28−42 day data for TCE was intended to address the decreased opportunity for full expression of response at 10 days. PBPK modeling predictions of daily internal doses of TCA in terms of mg/kg/d via produced via TCE metabolism would be are indeed lower than the TCE concentrations in terms of mg/kg/d given orally by gavage. The predicted internal dose of TCA from TCE exposure studies are of a comparable range to those predicted from TCA drinking water studies at exposure concentrations in which palpability has not been an issue for estimation of internal dose. Thus, although the TCE data are for higher exposure concentrations, they are predicted to produce comparable levels of TCA internal dose estimated from direct TCA administration in drinking water.

Figure 4-7 clearly shows that for a given amount of TCA produced from TCE, but going through intermediate metabolic pathways, the liver weight increases are substantially greater than, and highly inconsistent with, that expected based on direct TCA administration. In particular, the response from direct TCA administration appears to "saturate" with increasing TCA dose at a level of about 1.4-fold, while the response from TCE administration continues to increase with dose to 1.75-fold at the highest dose administered orally in Buben and O'Flaherty (1985) and over 2-fold in the inhalation study of Kjellstrand et al. (1983b). Because TCA liver concentrations are proportional to the dose TCA, and do not depend on whether it is administered in drinking water or internally produced in the liver, the results of the comparison using the TCA liver dose metric are identical.

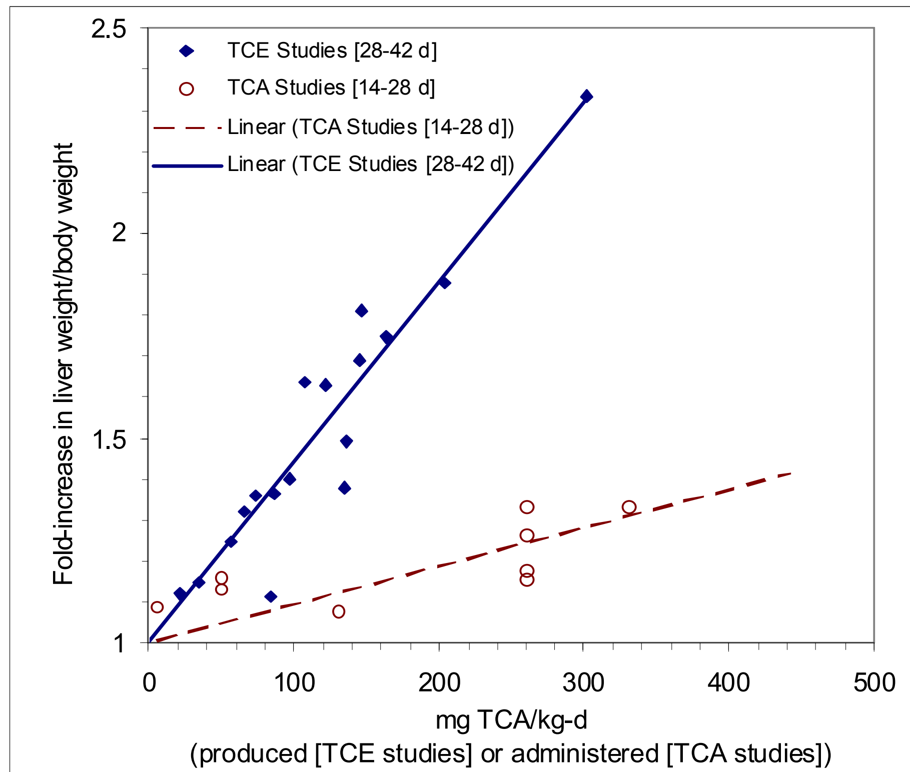
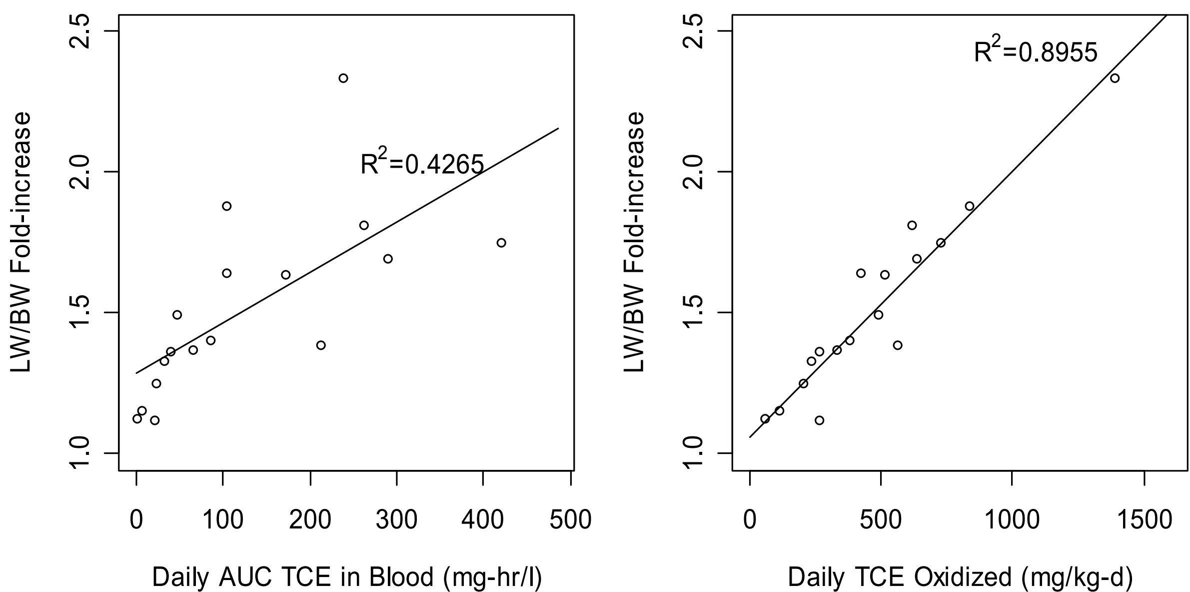


Figure 4-7. Comparison of fold-changes in relative liver weight for data sets in male B6C3F1, Swiss, and NRMI mice between TCE studies (Kjellstrand et al., 1983b; Buben and O'Flaherty, 1985; Merrick et al., 1989; Goel et al., 1992 [duration 28−42 days]) and studies of direct oral TCA administration to B6C3 F1 mice (DeAngelo et al., 1989; Parrish et al., 1996; Kato-Weinstein et al., 2001; DeAngelo et al., 2008 [duration 14−28 days]). Abscissa for TCE studies consists of the median estimates of the internal dose of TCA predicted from metabolism of TCE using the PBPK model described in Section 3.5 of the TCE risk assessment. Lines show linear regression with intercept fixed at unity. All data were reported fold-change in mean liver weight/body weight ratios, except for Kjellstrand et al. (1983b), with were the fold-change in the ratio of mean liver weight to mean body weight. In addition, in Kjellstrand et al. (1983b), some systemic toxicity as evidence by decreased total body weight was reported in the highest-dose group.

Furthermore, while as noted previously, oral studies appear to report a linear relationship between TCE exposure concentration and liver weight induction, the inclusion of inhalation studies on the basis of internal dose led to a highly consistent dose-response curve for among TCE study. Therefore, it is unlikely that differing routes of exposure can explain the inconsistencies in dose-response.

1 Additional analyses do, however, support a role for oxidative metabolism in TCE-
2 induced liver weight increases, and that the parent compound TCE is not the likely active moiety
3 (suggested previously by Buben and O'Flaherty [1985]). In particular, the same studies are
4 shown in Figure 4-8 using PBPK-model based predictions of the area-under-the-curve (AUC) of
5 TCE in blood and total oxidative metabolism, which produces chloral, TCOH, DCA, and other
6 metabolites in addition to TCA. The dose-response relationship between TCE blood levels and
7 liver weight increase, while still having a significant trend, shows substantial scatter and a low
8 R^2 of 0.43. On the other hand, using total oxidative metabolism as the dose metric leads to
9 substantially more consistency dose-response across studies, and a much tighter linear trend with
10 an R^2 of 0.90 (see Figure 4-8). A similar consistency is observed using liver-only oxidative
11 metabolism as the dose metric, with R^2 of 0.86 (not shown). Thus, while the slope is similar
12 between liver weight increase and TCE concentration in the blood and liver weight increase and
13 rate of total oxidative metabolism, the data are a much better fit for total oxidative metabolism.
14



15
16 **Figure 4-8. Fold-changes in relative liver weight for data sets in male**
17 **B6C3F1, Swiss, and NRMI mice reported by TCE studies of duration**
18 **28−42 days (Kjellstrand et al., 1983b; Buben and O'Flaherty, 1985; Merrick**
19 **et al., 1989; Goel et al., 1992) using internal dose metrics predicted by the**
20 **PBPK model described in Section 3.5: (A) dose metric is the median estimate**
21 **of the daily AUC of TCE in blood, (B) dose metric is the median estimate of**
22 **the total daily rate of TCE oxidation.** Lines show linear regression. Use of
23 liver oxidative metabolism as a dose metric gives results qualitatively similar to
24 (B), with $R^2 = 0.86$.

Although the qualitative similarity to the linear dose-response relationship between DCA and liver weight increases is suggestive of DCA being the predominant metabolite responsible for TCE liver weight increases, due to the highly uncertain dosimetry of DCA derived from TCE, this hypothesis cannot be tested on the basis of internal dose. Similarly, another TCE metabolite, chloral hydrate, has also been reported to induce liver tumors in mice, however, there are no adequate comparative data to assess the nature of liver weight increases induced by this TCE metabolite (see Section E.2.5 and Section 4.5.1.2.4 below). Whether its formation in the liver after TCE exposure correlates with TCE-induced liver weight changes cannot be determined.

4.5.6.2.2. ***Cytotoxicity.*** As discussed above, TCE has sometimes been reported to cause minimal/mild focal hepatocellular necrosis or other signs of hepatic injury, albeit of low frequency and mostly at doses ≥1,000 mg/kg/d (Dees and Travis, 1993; Elcombe et al., 1985) or at exposures ≥1,000 ppm in air (Ramdhan et al., 2008) from 7−10 days of exposure. Data from available studies are supportive of a role for oxidative metabolism in TCE-induced cytotoxicity in the liver, though they are not informative as to the actual active moiety(ies). Buben and O'Flaherty (1985) noted a strong correlation (R-squared of between glucose-6-phosphatase inhibition and total urinary oxidative metabolites). Ramdhan et al. (2008) conducted parallel experiments at TCE 1,000 and 2,000 ppm (8 hours/day, 7 days) in wild-type and cyp2e1-null mice, the latter of which did not exhibit hepatotoxicity (assessed by serum ALT, AST, and histopathology) and excreted 2-fold lower amounts of oxidative metabolites TCA and TCOH in urine as compared to wild-type mice. In addition, urinary TCA and TCOH excretion was correlated with serum ALT and AST measures, though the R-squared values (square of the reported correlation coefficients) were relatively low (0.54 and 0.67 for TCOH and TCA, respectively).

With respect to CH (166 mg/kg/d) and DCA (~90 mg/kg/d), Daniel et al. (1992) reported that after drinking water treatment, hepatocellular necrosis and chronic active inflammation were reported to be mildly increased in both prevalence and severity in all treated groups after 104 weeks of exposure. The histological findings, from interim sacrifices (n = 5), were considered by the authors to be unremarkable and were not reported. TCA has not been reported to induce necrosis in the liver under the conditions tested. Relatively high doses of DCA (≥1 g/L in drinking water) appear to result in mild focal necrosis with attendant reparative proliferation at lesion sites, but no such effects were reported at lower doses (≤0.5 g/L in drinking water) more relevant for comparison with TCE (DeAngelo et al., 1999; Sanchez and Bull, 1990; Stauber et

al., 1998). Enlarged nuclei and changes consistent with increased ploidy, are further discussed below in the context of DNA synthesis.

4.5.6.2.3. ***DNA synthesis and polyploidization.*** The effects on DNA synthesis and polyploidization observed with TCE treatment have similarly been observed with TCA and DCA. With respect to CH, George et al. (2000) reported that CH exposure did not alter DNA synthesis in rats and mice at any of the time periods monitored (all well past 2 weeks), with the exception of 0.58 g/L chloral hydrate at 26 weeks slightly increasing hepatocyte labeling (~2−3-fold of controls) in rats and mice but the percent labeling still representing 3% or less of hepatocytes.

In terms of whole liver or hepatocyte label incorporation, the most comparable exposure duration between TCE, TCA, and DCA studies is the 10- and 14-day period. Several studies have reported that in this time period, peak label incorporation into individual hepatocytes and whole liver for TCA and DCA have already passed (Styles et al., 1991; Sanchez and Bull, 1990; Pereira, 1996; Carter et al., 1995). A direct time-course comparison is difficult, since data at earlier times for TCE are more limited.

There are conflicting reports of DNA synthesis induction in individual hepatocytes for up to 14 days of DCA or TCA exposure. In particular, Sanchez and Bull (1990) reported tritiated thymidine incorporation in individual hepatocytes up to 2 g/L exposure to DCA or TCA induced little increase in DNA synthesis except in instances and in close proximity to areas of proliferation/necrosis for DCA treatment after 14 days of exposure in male mice. The largest percentage of hepatocytes undergoing DNA synthesis for any treatment group was less than 1% of hepatocytes. However, they reported treatment- and exposure duration-changes in hepatic DNA incorporation of tritiated thymidine for DCA and TCA. For TCA treatment, the largest increases over control levels for hepatic DNA incorporation (at the highest dose) was a 3-fold increase after 5 days of treatment and a 2-fold increase over controls after 14 days of treatment. For DCA whole-liver tritiated thymidine incorporation was only slightly elevated at necrogenic concentrations and decreased at the 0.3 g/L non-necrogenic level after 14 days of treatment. In contrast to Sanchez and Bull (1990), Stauber and Bull (1997) reported increased tritiated thymidine incorporation for individual hepatocytes after 14 days of treatment with 2 g/L DCA or TCA in male mice. They used a more extended period of tritiated thymidine exposure of 3−5 days and so these results represent aggregate DNA synthesis occurring over a more extended period of time. A "1-day labeling index" was reported as less than 1% for the highest level of increased incorporation. However, after 14 days, the labeling index was reported to be increased by ~3.5-fold for TCA and ~5.5-fold for DCA over control values. After 28 days, the labeling

index was reported to be decreased ~2.3-fold by DCA and increased ~2.5-fold after treatment with TCA. Pereira (1996) reported that for female B6C3F1 mice, 5-day incorporation of BrDU, as a measure of DNA synthesis, was increased at 0.86 g/L and 2.58 g/L DCA treatment for 5 days (~2-fold at the highest dose) but that by Day 12 and 33 levels had fallen to those of controls. For TCA exposures, 0.33 g/L, 1.10 g/L and 3.27 g/L TCA all gave a similar ~3-fold increase in BrdU incorporation by 5 days, but that by 12 and 33 days were not changed from controls. Nonetheless, what is consistent is that these data report that, similar to TCE-exposed mice at 10 days of exposure, cells undergoing DNA synthesis in DCA- or TCA-exposed mice for up to 14 days of exposure to be confined to a very small population of cells in the liver. Thus, these data are consistent with hypertrophy being primarily responsible for liver weight gains as opposed to increases in cell number in mice.

Interestingly, a lack of correlation between whole liver label incorporation and that in individual hepatocytes has been reported by several studies of DCA (Sanchez and Bull, 1990; Carter et al., 1995). For example, Carter et al. (1995) reported no increase in labeling of hepatocytes in comparison to controls for any DCA treatment group from 5 to 30 days of DCA exposure. Rather than increase hepatocyte labeling, DCA induced no change from days 5 though 15 but significantly decreased levels between days 20 and 30 for 0.5 g/L that were similar to those observed for the 5 g/L exposures. However, for whole liver DNA tritiated thymidine incorporation, Carter et al. (1995) reported 0.5g/L DCA treatments to show trends of initial inhibition of DNA tritiated thymidine incorporation followed by enhancement of labeling that was not statistically significant from 5 to 30 days of exposure. Examination of individual hepatocytes does not include the contribution of nonparenchymal cell DNA synthesis that would be detected in whole liver DNA. As noted above, proliferation of the nonparenchymal cell compartment of the liver has been noted in several studies of TCE in rodents, and thus, this is one possible reason for the reported discrepancy.

Another possible reason for this inconsistency with DCA treatment is polyploidization, as was suggested above for TCE. Although this was not examined for DCA or TCA exposure by Sanchez and Bull (1990), Carter et al. (1995) reported that hepatocytes from both 0.5 and 5 g/L DCA treatment groups had enlarged, presumably polyploidy nuclei, with some hepatocyte nuclei labeled in the mid-zonal area. There were statistically significant changes in cellularity, nuclear size, and multinucleated cells during 30 days exposure to DCA. The percentage of mononucleated cells hepatocytes was reported to be similar between control and DCA treatment groups at 5- and 10-day exposure. However, at 15 days and beyond DCA treatments were reported to induce increases in mononucleated hepatocytes with later time periods to also showing DCA-induced increases nuclear area, consistent with increased polyploidization without

mitosis. The consistent reporting of an increasing number of mononucleated cells between 15 and 30 days could be associated with clearance of mature hepatocytes as suggested by the report of DCA-induced loss of cell nuclei. The reported decrease in the numbers of binucleate cells in favor of mononucleate cells is not typical of any stage of normal liver growth (Brodsky and Uryvaeva, 1977). The pattern of consistent increase in percent liver/body weight induced by 0.5 g/L DCA treatment from days 5 though 30 was not consistent with the increased numbers of mononucleate cells and increase nuclear area reported from Day 20 onward. Specifically, the large differences in liver weight induction between the 0.5 g/L treatment group and the 5 g/L treatment groups at all times studied also did not correlate with changes in nuclear size and percent of mononucleate cells. Thus, increased liver weight was not a function of cellular proliferation, but probably included both aspects of hypertrophy associated with polyploidization and increased glycogen deposition (see below) induced by DCA. Carter et al. (1995) suggested that although there is evidence of DCA-induced cytotoxicity (e.g., loss of cell membranes and apparent apoptosis), the 0.5 g/L exposure concentration has been shown to increase hepatocellular lesions after 100 weeks of treatment without concurrent peroxisome proliferation or cytotoxicity (DeAngelo et al., 1999).

In sum, the observation of TCE-treatment related changes in DNA content, label incorporation, and mitotic figures are generally consistent with patterns observed for both TCA and DCA. In all cases, hepatocellular proliferation is confined to a very small fraction of hepatocytes, and hepatomegaly observed with all three treatments probably largely reflects cytomegaly rather than cell proliferation. Moreover, label incorporation likely largely reflects polyploidization rather than hepatocellular proliferation, with a possible contribution from nonparenchymal cell proliferation. As with TCE, histological changes in nuclear sizes and number also suggest a significant degree of treatment-related polyploidization, particularly for DCA.

4.5.6.2.4. ***Apoptosis.*** As for apoptosis, Both Elcombe et al. (1985) and Dees and Travis (1993) reported no changes in apoptosis other than increased apoptosis only at a treatment level of 1,000-mg/kg TCE. Dees and Travis (1993) reported that increased apoptoses from TCE exposure “did not appear to be in proportion to the applied TCE dose given to male or female mice.” Channel et al. (1998) reported that there was no significant difference in apoptosis between TCE treatment and control groups with data not shown. However, the extent of apoptosis in any of the treatment groups, or which groups and timepoints were studied for this effect cannot be determined. While these data are quite limited, it is notable that peroxisome

proliferators have been suggested inhibit, rather than increase, apoptosis as part of their carcinogenic MOA (Klaunig et al., 2003).

However, for TCE metabolites, DCA has been most studied, though it is clear that age and species affect background rates of apoptosis. Snyder et al. (1995), in their study of DCA, report that control mice were reported to exhibit apoptotic frequencies ranging from ~0.04 to 0.085%, that over the 30-day period of their study the frequency rate of apoptosis declined, and suggest that this pattern is consistent with reports of the livers of young animals undergoing rapid changes in cell death and proliferation. They reported rat liver to have a greater the estimated frequency of spontaneous apoptosis (~0.1%) and therefore, greater than that of the mouse. Carter et al. (1995) reported that after 25 days of 0.5 g/L DCA treatment apoptotic bodies were reported as well as fewer nuclei in the pericentral zone and larger nuclei in central and midzonal areas. This would indicate an increase in the apoptosis associated with potential increases in polyploidization and cell maturation. However, Snyder et al. (1995) report that mice treated with 0.5 g/L DCA over a 30-day period had a similar trend as control mice of decreasing apoptosis with age. The percentage of apoptotic hepatocytes decreased in DCA-treated mice at the earliest time point studied and remained statistically significantly decreased from controls from 5 to 30 days of exposure. Although the rate of apoptosis was very low in controls, treatment with 0.5g/L DCA reduced it further (~30−40% reduction) during the 30-day study period. The results of this study not only provide a baseline of apoptosis in the mouse liver, which is very low, but also to show the importance of taking into account the effects of age on such determinations. The significance of the DCA-induced reduction in apoptosis reported in this study, from a level that is already inherently low in the mouse, for the MOA for induction of DCA-induce liver cancer is difficult to discern.

4.5.6.2.5. ***Glycogen accumulation.*** As discussed in Sections E.3.2 and E.3.4.2.1, glycogen accumulation has been described to be present in foci in both humans and animals as a result from exposure to a wide variety of carcinogenic agents and predisposing conditions in animals and humans. The data from Elcombe et al. (1985) included reports of TCE-induced pericentral hypertrophy and eosinophilia for both rats and mice but with "fewer animals affected at lower doses." In terms of glycogen deposition, Elcombe report "somewhat" less glycogen pericentrally in the livers of rats treated with TCE at 1,500 mg/kg than controls with less marked changes at lower doses restricted to fewer animals. They do not comment on changes in glycogen in mice. Dees and Travis (1993) reported TCE-induced changes to "include an increase in eosinophilic cytoplasmic staining of hepatocytes located near central veins, accompanied by loss of cytoplasmic vacuolization." Since glycogen is removed using conventional tissue processing

and staining techniques, an increase in glycogen deposition would be expected to increase vacuolization and thus, the report from Dees and Travis is consistent with less not more glycogen deposition. Neither study produced a quantitative analysis of glycogen deposition changes from TCE exposure. Although not explicitly discussing liver glycogen content or examining it quantitatively in mice, these studies suggest that TCE-induced liver weight increases did not appear to be due to glycogen deposition after 10 days of exposure and any decreases in glycogen were not necessarily correlated with the magnitude of liver weight gain either.

For TCE and TCA 500 mg/kg treatments in mice for 10 days, changes in glycogen were not reported in the general descriptions of histopathological changes (Elcombe et al., 1985; Styles et al., 1991; Dees and Travis, 1993) or were specifically described by the authors as being similar to controls (Nelson et al., 1989). However, for DCA, glycogen deposition was specifically noted to be increased with treatment, although no quantitative analyses was presented that could give information as to the nature of the dose-response (Nelson et al., 1989).

In regard to cell size, although increased glycogen deposition with DCA exposure was noted by Sanchez and Bull (1990) to occur to a similar extent in B6C3F1 and Swiss Webster male mice despite differences in DCA-induced liver weight gain. Lack of quantitative analyses of that accumulation in this study precludes comparison with DCA-induced liver weight gain. Carter et al. (1995) reported that in control mice there was a large variation in apparent glycogen content and also did not perform a quantitative analysis of glycogen deposition. The variability of this parameter in untreated animals and the extraction of glycogen during normal tissue processing for light microscopy make quantitative analyses for dose-response difficult unless specific methodologies are employed to quantitatively assess liver glycogen levels as was done by Kato-Weinstein et al. (2001) and Pereira et al. (2004).

Bull et al. (1990) reported that glycogen deposition was uniformly increased from 2 g/L DCA exposure with photographs of TCA exposure showing slightly less glycogen staining than controls. However, the abstract and statements in the paper suggest that there was increased PAS positive material from TCA treatment that has caused confusion in the literature in this regard. Kato-Weinstein et al. (2001) reported that in male B6C3F1 mice exposed to DCA and TCA, the DCA treatment increased glycogen and TCA decreased glycogen content of the liver by using both chemical measurement of glycogen in liver homogenates and by using ethanol-fixed sections stained with PAS, a procedure designed to minimize glycogen loss.

Kato-Weinstein et al. (2001) reported that glycogen rich and poor cells were scattered without zonal distribution in male B6C3F1 mice exposed to 2 g/L DCA for 8 weeks. For TCA treatments, they reported centrilobular decreases in glycogen and ~25% decreases in whole liver by 3 g/L TCA. Kato-Weinstein et al. (2001) reported whole liver glycogen to be increased

~1.50-fold of control (90 vs. 60 mg glycogen/g liver) by 2 g/L DCA after 8 weeks exposure male B6C3F1 mice with a maximal level of glycogen accumulation occurring after 4 weeks of DCA exposure. Pereira et al. (2004) reported that after 8 weeks of exposure to 3.2 g/L DCA liver glycogen content was 2.20-fold of control levels (155.7 vs. 52.4 mg glycogen/g liver) in female B6C3F1 mice. Thus, the baseline level of glycogen content reported by (~60 mg/g) and the increase in glycogen after DCA exposure was consistent between Kato-Weinstein et al. (2001) and Pereira et al. (2004). However, the increase in liver weight reported by Kato-Weinstein et al. (2001) of 1.60-fold of control percent liver/body weight cannot be accounted for by the 1.50-fold of control glycogen content. Glycogen content only accounts for 5% of liver mass so that 50% increase in glycogen cannot account for the 60% increase liver mass induced by 2 g/L DCA exposure for 8 weeks reported by Kato-Weinstein (2001). Thus, DCA-induced increases in liver weight are occurring from other processes as well. Carter et al. (2003) and DeAngelo et al. (1999) reported increased glycogen after DCA treatment at much lower doses after longer periods of exposure (100 weeks). Carter reported increased glycogen at 0.5 g/L DCA and DeAngelo et al. (1999) at 0.03 g/L DCA in mice. However, there is no quantitation of that increase.

4.5.6.2.6. ***Peroxisome proliferation and related effects.*** TCA and DCA have both been reported to induce peroxisome proliferation or increase in related enzyme markers in rodent hepatocytes (DeAngelo et al., 1989, 1997; Mather et al., 1990; Parrish et al., 1996). Between TCA and DCA, both induce peroxisome proliferation in various strains of mice, but it clear that TCA and DCA are weak PPARα agonists and that DCA is weaker than TCA in this regard (Nelson et al., 1989) using a similar paradigm.

George et al. (2000) reported that CH exposure did not hepatic PCO activity in rats and mice at any of the time periods monitored. It is notable that the only time at which DNA synthesis index was (slightly) increased, at 26 weeks, there remained a lack of induction of PCO. A number of measures that may be related to peroxisome proliferation were investigated in Leakey et al. (2003a). Of the enzymes associated with PPARα agonism (total CYP, CYP2B isoform, CYP4A, or lauric acid β-hydroxylase activity), only CYP4A and lauric acid β-hydroxylase activity were significantly increased at 15 months of exposure in the dietary-restricted group administered the highest dose (100 mg/kg CH) with no other groups reported showing a statistically significant increased response (n = 12/group). There is an issue of interpretation of peroxisomal enzyme activities and other enzymes associated with PPARα receptor activation to be a relevant event in liver cancer induction at a time period in which tumors or foci are already present. Although not statistically significant, the 100 mg/kg CH

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exposure group of *ad libitum*-fed mice also had an increase in CH-induced increases of CYP4A and lauric acid β-hydroxylase activity. Seng et al. (2003) described CH toxicokinetics and peroxisome proliferation-associated enzymes in mice at doses up to 1,000 mg/kg/d for 2 weeks with dietary control or caloric restriction. Lauric acid β-hydroxylase and PCO activities were reported to be induced only at doses >100 mg/kg in all groups, with dietary-restricted mice showing the greatest induction. Differences in serum levels of TCA, the major metabolite remaining 24 hours after dosing, were reported not to correlate with hepatic lauric acid β-hydroxylase activities across groups.

Direct quantitative inferences regarding the magnitude of response in these studies in comparison to TCE, however, are limited by possible variability and confounding. In particular, many studies used cyanide-insensitive PCO as a surrogate for peroxisome proliferation, but the utility of this marker may be limited for a number of reasons. First, several studies have shown that this activity is not well correlated with the volume or number of peroxisomes that are increased as a result of exposure to TCE or it metabolites (Nakajima et al., 2000; Elcombe et al., 1985; Nelson et al., 1989). In addition, this activity appears to be highly variable both as a baseline measure and in response to chemical exposures. Laughter et al. (2004) presented data showing WY-14,643 induced increases in PCO activity that varied up to 6-fold between different experiments in wild-type mice. They also showed that, in some instances, PCO activity in untreated PPARα-null mice was up to 6-fold greater than that in wild-type mice. Parrish et al. (1996) noted that control values between experiments varied as much as a factor of 2-fold for PCO activity and thus, their data were presented as percent of concurrent controls. Furthermore, Melnick et al. (1987) reported that corn oil administration alone can elevate PCO (as well as catalase) activity, and corn oil has also been reported to potentiate the induction of PCO activity of TCA in male mice (DeAngelo et al., 1989). Thus, quantitative inferences regarding the magnitude of response in these studies are limited by a number of factors. For example, in the studies reported in DeAngelo et al. (2008) a small number of animals was studied for PCO activity at interim sacrifices ($n = 5$). PCO activity varied 2.7-fold as baseline controls. Although there was a 10-fold difference in TCA exposure concentration, the increase in PCO activity at 4 weeks was 1.3-, 2.4-, and 5.3-fold of control. More information on the relationship of PCO enzyme activity and its relationship to carcinogenicity is discussed in Section E.3.4 and below.

4.5.6.2.7. *Oxidative stress.* Very limited data are available as to oxidative stress and related markers induced by the oxidative metabolites of TCE. As discussed in Appendix E, above, there are limited data that do not indicate significant oxidative stress and associated DNA damage associated with acute and subacute TCE treatment. In regard to DCA and TCA, Larson and Bull

(1992) exposed male B6C3F1 mice or Fischer 344 rats to single doses TCA or DCA in distilled water by oral gavage ($n = 4$). In the first experiment, TBARS was measured from liver homogenates and assumed to be malondialdehyde. The authors stated that a preliminary experiment had shown that maximal TBARS was increased 6 hours after a dose of DCA and 9 hours after a dose of TCA in mice and that by 24 hours TBARS concentrations had declined to control values. Time-course information in rats was not presented. A dose of 100 mg/kg DCA (rats or mice) or TCA (mice) did not elevate TBARS concentrations over that of control liver with this concentration of TCA not examined in rats. For TCA, there was a slight dose-related increase in TBARS over control values starting at 300 mg/kg in mice with the increase in TBARS increasing at a rate that was lower than the magnitude of increase in dose. Of note, is the report that the induction of TBARS in mice is transient and has subsided within 24 hours of a single dose of DCA or TCA, that the response in mice appeared to be slightly greater with DCA than TCA at similar doses, and that for DCA, there was similar TBARS induction between rats and mice at similar dose levels.

Austin et al. (1996) appears to a follow-up publication of the preliminary experiment cited in Larson and Bull (1992). Male B6C3F1 mice were treated with single doses of DCA or TCA via gavage with liver examined for 8OHdG. The authors stated that in order to conserve animals, controls were not employed at each time point. There was a statistically significant increase over controls in 8OHdG for the 4- and 6-hour time points for DCA (~1.4- and 1.5-fold of control, respectively) but not at 8 hours in mice. For TCA, there was a statistically significant increase in 8OHdG at 8 and 10 hours for TCA (~1.4- and 1.3-fold of control, respectively).

Consistent results as to low, transient increases in markers of "oxidative stress" were also reported by Parrish et al. (1996), who in addition to examining oxidative stress alone, attempted to examine its possible relationship to PCO and liver weight in male B6C3F1 mice exposed to TCA or DCA for 3 or 10 weeks ($n = 6$). The dose-related increase in PCO activity at 21 days for TCA was reported to not be increased similarly for DCA. Only the 2.0 g/L dose of DCA was reported to induce a statistically significant increase at 21-days of exposure of PCO activity over control (~1.8-fold of control). After 71 days of treatment, TCA induced dose-related increases in PCO activities that were approximately twice the magnitude as that reported at 21 days. Treatments with DCA at the 0.1 and 0.5 g/L exposure levels produced statistically significant increase in PCO activity of ~1.5- and 2.5-fold of control, respectively. The administration of 1.25 g/L clofibric acid in drinking water, used as a positive control, gave ~6−7-fold of control PCO activity at 21 and 71 days exposure. Parrish et al. (1996) reported that laurate hydroxylase activity was reported to be elevated significantly only by TCA at 21 days and to approximately the same extent (~1.4- to 1.6-fold of control) increased at all doses tested and at 71 days both the

0.5 and 2.0 g/L TCA exposures to a statistically significant increase in laurate hydroxylase activity (i.e., 1.6- and 2.5-fold of control, respectively). No change was reported after DCA exposure. Laurate hydroxylase activity within the control values varying 1.7-fold between 21 and 71 days experiments. Levels of 8OHdG in isolated liver nuclei were reported to not be altered from 0.1, 0.5, or 2.0 g/L TCA or DCA after 21 days of exposure and this negative result was reported to remain even when treatments were extended to 71 days of treatment. The authors noted that the level of 8OHdG increased in control mice with age (i.e., ~2-fold increase between 71-day and 21-day control mice). Thus, the increases in PCO activity noted for DCA and TCA were not associated with 8OHdG levels (which were unchanged) and also not with changes laurate hydrolase activity observed after either DCA or TCA exposure. Of note, is that the authors report taking steps to minimize artifactual responses for their 8OHdG determinations. The authors concluded that their data suggest that peroxisome proliferative properties of TCA were not linked to oxidative stress or carcinogenic response.

4.5.6.3. *Comparisons of Trichloroethylene (TCE)-Induced Carcinogenic Responses With Trichloroacetic Acid (TCA), Dichloroacetic Acid (DCA), and Chloral Hydrate (CH) Studies*

4.5.6.3.1. ***Studies in rats.*** As discussed above, data on TCE carcinogenicity in rats, while not reporting statistically significantly increased risks, are not entirely adequate due to low numbers of animals, increased systemic toxicity, and/or increased treatment-related or accidental mortality. Notably, several studies in rats noted a few very rare types of liver or biliary tumors (cystic cholangioma, cholangiocarcinoma, or angiosarcomas) in treated animals. For TCA, DCA and CH, there are even fewer studies in rats, so there is a very limited ability to assess the consistency or lack thereof in rat carcinogenicity among these compounds.

For TCA, the only available study in rats (DeAngelo et al., 1997) has been frequently cited in the literature to indicate a lack of response in this species for TCA-induced liver tumors. However, this study does report an apparent dose-related increase in multiplicity of adenomas and an increase in carcinomas over control at the highest dose. The use by DeAngelo et al. (1997) of a relatively low number of animals per treatment group ($n = 20-24$) limits this study's ability to determine a statistically significant increase in tumor response. Its ability to determine an absence of treatment-related effect is similarly limited. In particular, a power calculation of the study shows that for most endpoints (incidence and multiplicity of all tumors at all exposure DCA concentrations), the Type II error, which should be >50%, was less than 8%. The only exception was for the incidence of adenomas and adenomas and carcinomas for the 0.5 g/L treatment group (58%), at which, notably, there was a reported increase in reported adenomas or adenomas and carcinomas combined over control (15 vs. 4%). Therefore, the likelihood of a

false null hypothesis was not negligible. Thus, while suggesting a lower response than for mice for liver tumor induction, this study is inconclusive for determining of whether TCA induces a carcinogenic response in the liver of rats.

For DCA, there are two reported long-term studies in rats (DeAngelo et al, 1996; Richmond et al., 1995) that appear to have reported the majority of their results from the same data set and which consequently were subject to similar design limitations and DCA-induced neurotoxicity in this species. DeAngelo et al. (1996) reported increased hepatocellular adenomas and carcinomas in male F344 rats exposed to DCA for 2 years. However, the data from exposure concentrations at a 5 g/L dose had to be discarded and the 2.5 g/L DCA dose had to be continuously lowered during the study due to neurotoxicity. There was a DCA-induced increased in adenomas and carcinomas combined reported for the 0.5 g/L DCA (24.1 vs. 4.4% adenomas and carcinomas combined in treated vs. controls) and an increase at a variable dose started at 2.5 g/L DCA and continuously lowered (28.6 vs. 3.0% adenomas and carcinomas combined in treated vs. controls). Only combined incidences of adenomas and carcinomas for the 0.5 g/L DCA exposure group was reported to be statistically significant by the authors although the incidence of adenomas was 17.2 vs. 4% in treated vs. control rats. Hepatocellular tumor multiplicity was reported to be increased in the 0.5 g/L DCA group (0.31 adenomas and carcinomas/animal in treated vs. 0.04 in control rats) but was reported by the authors to not be statistically significant. At the starting dose of 2.5 g/L that was continuously lowered due to neurotoxicity, the increased multiplicity of hepatocellular carcinomas was reported by the authors to be to be statistically significant (0.25 carcinomas/animals vs. 0.03 in control) as well as the multiplicity of combined adenomas and carcinomas (0.36 adenomas and carcinomas/animals vs. 0.03 in control rats). Issues that affect the ability to determine the nature of the dose-response for this study include (1) the use of a small number of animals ($n = 23$, $n = 21$, and $n = 23$ at final sacrifice for the 2.0 g/L NaCl control, 0.05 g/L and 0.5 g/L treatment groups) that limit the power of the study to both determine statistically significant responses and to determine that there are not treatment-related effects (i.e., power) (2) apparent addition of animals for tumor analysis not present at final sacrifice (i.e., 0.05 and 0.5 g/L treatment groups), and (3) most of all, the lack of a consistent dose for the 2.5 g/L DCA exposed animals.

Similar issues are present for the study of Richmond et al. (1995) which was conducted by the same authors as DeAngelo et al. (1996) and appeared to be the same data set. There was a small difference in reports of the results between the two studies for the same data for the 0.5 g/L DCA group in which Richmond et al. (1995) reported a 21% incidence of adenomas and DeAngelo et al. (1996) reported a 17.2% incidence. The authors did not report any of the results of DCA-induced increases of adenomas and carcinomas to be statistically significant. The same

issues discussed above for DeAngelo et al. (1996) apply to this study. Similar to the DeAngelo et al. (1997) study of TCA in rats, the use in these DCA studies (DeAngelo et al., 1996; Richmond et al., 1995) of relatively small numbers of rats limits the detection of treatment-related effects and the ability to determine whether there was no treatment related effects (Type II error), especially at the low concentrations of DCA exposure.

For CH, George et al. (2000) exposed male F344/N rats to CH in drinking water for 2 years. Groups of animals were sacrificed at 13, 26, 52, and 78 weeks following the initiation of dosing, with terminal sacrifices at Week 104. Only a few animals received a complete pathological examination. The number of animals surviving >78 weeks and the number examined for hepatocellular proliferative appeared to differ (42−44 animals examined but 32−35 surviving till the end of the experiment). Only the lowest treatment group had increased liver tumors which were marginally significantly increased.

Leuschner and Beuscher (1998) examined the carcinogenic effects of CH in male and female Sprague-Dawley rats (69−79 g, 25−29 days old at initiation of the experiment) administered 0, 15, 45, and 135 mg/kg CH in unbuffered drinking water 7 days/week (n = 50/group) for 124 weeks in males and 128 weeks in females. Two control groups were noted in the methods section without explanation as to why they were conducted as two groups. The authors report no substance-related influence on organ weights and no macroscopic evidence of tumors or lesions in male or female rats treated with CH for 124 or 128 weeks. However, no data are presented on the incidence of tumors in either treatment or control groups. The authors did report a statistically significant increase in the incidence of hepatocellular hypertrophy in male rats at the 135 mg/kg dose (14/50 animals vs. 4/50 and 7/50 in Controls I and II). For female rats, the incidence of hepatocellular hypertrophy was reported to be 10/50 rats (Control I) and 16/50 (Control II) rats with 18/50, 13/50 and 12/50 female rats having hepatocellular hypertrophy after 15, 45, and 135 mg/kg CH, respectively. The lack of reporting in regard to final body weights, histology, and especially background and treatment group data for tumor incidences, limit the interpretation of this study. Whether this paradigm was sensitive for induction of liver cancer cannot be determined.

Therefore, given the limitations in the available studies, a comparison of rat liver carcinogenicity induced by TCE, TCA, DCA, and CH reveals no strong inconsistencies, but nor does it provide much insight into the relative importance of different TCE metabolites in liver tumor induction.

4.5.6.3.2. ***Studies in mice.*** Similar to TCE, the bioassay data in mice for DCA, TCA, and CH are much more extensive and have shown that all three compounds induce liver tumors in mice.

Several 2-year bioassays have been reported for CH (Daniel et al., 1992; George et al., 2000; Leakey et al., 2003a). For many of the DCA and TCA studies, the focus was not carcinogenic dose-response but rather investigation of the nature of the tumors and potential MOAs in relation to TCE. As a result, studies often employed relatively high concentrations of DCA or TCA and/or were conducted for a year or less. As shown previously in Section 4.5.4.2.1, the dose-response curves for increased liver weight for TCE administration in male mice are more similar to those for DCA administration and TCE oxidative metabolism than for direct TCA administration (inadequate data were available for CH). An analogous comparison for DCA-, TCA-, and CH-induced tumors would be informative, ideally using data from 2-year studies.

4.5.6.3.2.1. *Trichloroethylene (TCE) carcinogenicity dose-response data*. Unfortunately, the database for TCE, while consistently showing an induction of liver tumors in mice, is very limited for making inferences regarding the shape of the dose-response curve. For many of these experiments multiplicity was not given only liver tumor incidence. NTP (1990), Bull et al. (2002), Anna et al. (1994) conducted gavage experiments in which they only tested one dose of ~1,000 mg/kg/d TCE. NCI (1976) tested two doses that were adjusted during exposure to an average of 1,169 and 2,339 mg/kg/d in male mice with only 2-fold dose spacing in only 2 doses tested. Maltoni et al. (1986) conducted inhalation experiments in two sets of B6C3F1 mice and one set of Swiss mice at 3 exposure concentrations that were 3-fold apart in magnitude between the low and mid-dose and 2-fold apart in magnitude between the mid- and high-dose. However, for one experiment in male B6C3F1 mice (BT306), the mice fought and suffered premature mortality and for two the experiments in B6C3F1 mice, although using the same strain, the mice were obtained from differing sources with very different background liver tumor levels. For the Maltoni et al. (1988) study a general descriptor of "hepatoma" was used for liver neoplasia rather than describing hepatocellular adenomas and carcinomas so that comparison of that data with those from other experiments is difficult. More importantly, while the number of adenomas and carcinomas may be the same between treatments or durations of exposure, the number of adenomas may decrease as the number of carcinomas increase during the course of tumor progression. Such information is lost by using only a hepatoma descriptor.

Given the limited database, it would be useful if different studies could be combined to yield a more comprehensive dose-response curve, as was done for liver weight, above. However, this is probably not appropriate for several reasons. First, only NTP (1990) was performed with dosing duration and time of sacrifice both being the "standard" 104 weeks. NCI (1976), Maltoni et al. (1986), Anna et al. (1994), and Bull et al. (2002) all had shorter dosing periods and either longer (Maltoni et al., 1986) or shorter (the other three studies) observation times. Therefore,

because of potential dose-rate effects and differences in the degree of expression of TCE-induced tumors, it is difficult to even come up with a comparable administered dose metric across studies. Moreover, the background tumor incidences are substantially different across experiments, even controlling for mouse strain and sex. For example, across gavage studies in male B6C3F1 mice, the incidence of hepatocellular carcinomas ranged from 1.2 to 16.7% (NCI, 1976; Anna et al., 1994; NTP, 1990) and the incidence of adenomas ranged from 1.2 to 14.6% (Anna et al., 1994; NTP, 1990) in control B6C3F1 mice. After ~1,000 mg/kg/d TCE treatment, the incidence of carcinomas ranged from 19.4 to 62% (Bull et al., 2002; NCI, 1976; Anna et al., 1994; NTP, 1990), with three of the studies (NCI, 1976; Anna et al., 1994; NTP, 1990) reporting a range of incidences between 42.8 to 62.0%). The incidence of adenomas ranged from 28 to 66.7% (Bull et al., 2002; Anna et al., 1994; NTP, 1990). In the Maltoni et al. (1986) inhalation study as well, male B6C3F1 mice from two different sources had very different control incidences of hepatomas (~2% versus about ~20%).

Therefore, only data from the same experiment in which more than a single exposed dose group was used provide reliable data on the dose-response relationship for TCE hepatocarcinogenicity, and incidences from these experiments are shown in Figures 4-9 and 4-10. Except for one of the two Maltoni et al. (1986) inhalation experiments in male B6C3F1 mice, all of these data sets show relatively proportional increases with dose, albeit with somewhat different slopes as may be expected across strains and sexes. Direct comparison is difficult, since the "hepatomas" reported by Maltoni et al. (1986) are much more heterogeneous, including neoplastic nodules, adenomas, and carcinomas, than the carcinomas reported by NCI (1976). Nonetheless, although the data limitations preclude a conclusive statement, these data are generally consistent with the linear relationship observed with TCE-induced liver weight changes.

4.5.6.3.2.2. ***Dichloroacetic acid (DCA) carcinogenicity dose-response data*****.** With respect to DCA, Pereira (1996) reported that for 82 week exposure to DCA in female B6C3F1 mice, DCA exposure concentrations of 0, 2, 6.67, and 20 mmol/L (0, 0.26, 0.86, and 2.6 g/L) led to close proportionally increasing adenoma prevalences of 2.2, 6, 25, and 84.2%, though adenoma multiplicity increased more than linearly between the highest two doses. Unfortunately, too few carcinomas were observed at these doses and duration to meaningfully inform the shape of the dose-response relationship. More useful is DeAngelo et al. (1999), which reported on a study of DCA hepatocarcinogenicity in male B6C3F1 mice over a lifetime exposure. DeAngelo et al. (1999) used 0.05 g/L, 0.5 g/L, 1.0 g/L, 2.0 g/L and 3.5 g/L exposure concentrations of DCA in their 100-week dirking water study. The number of animals at final sacrifice was generally low

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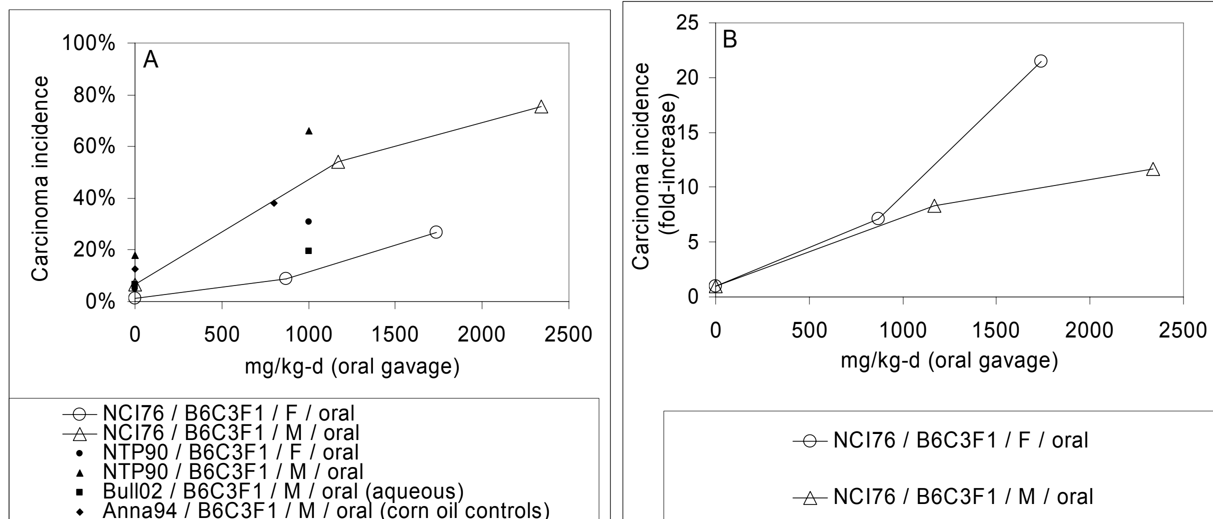
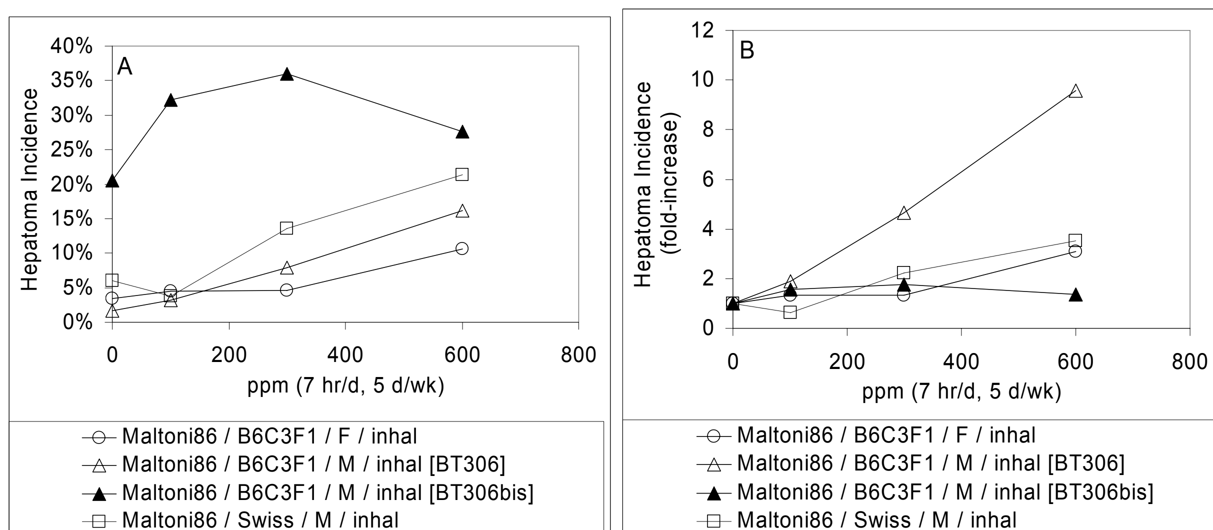


2 **Figure 4-9. Dose-response relationship, expressed as (A) percent incidence**
3 **and (B) fold-increase over controls, for TCE hepatocarcinogenicity in NCI**
4 **(1976).** For comparison, incidences of carcinomas for NTP (1990), Anna et al.
5 (1994), and Bull et al. (2002) are included, but without connecting lines since they
6 are not appropriate for assessing the shape of the dose-response relationship.
7
8



9
10 **Figure 4-10. Dose-response relationship, expressed as (A) incidence and (B)**
11 **fold-increase over controls, for TCE hepatocarcinogenicity in Maltoni et al.**
12 **(1986).** Note that the BT306 experiment reported excessive mortality due to
13 fighting, and so the paradigm was repeated in experiment BT306bis using mice
14 from a different source.

in the DCA treatment groups and variable. The multiplicity or number of hepatocellular carcinomas/animals was reported to be significantly increased over controls in a dose-related manner at all DCA treatments including 0.05 g/L DCA, and a no-observed-effect level (NOEL) reported not to be observed by the authors. Between the 0.5 g/L and 3.5 g/L exposure concentrations of DCA the magnitude of increase in multiplicity was similar to the increases in magnitude in dose. The incidence of hepatocellular carcinomas were reported to be increased at all doses as well but not reported to be statistically significant at the 0.05 g/L exposure concentration. However, given that the number of mice examined for this response (n = 33), the power of the experiment at this dose was only 16.9% to be able to determine that there was not a treatment related effect. Indeed, Figure 4-11 replots the data from DeAngelo et al. (1999) with an abscissa drawn to scale (unlike the figure in the original paper, which was not to scale), suggests even a slightly greater than linear effect at the lowest dose (0.05 g/L, or 8 mg/kg/d) as compared to the next lowest dose (0.5 g/L, or 84 mg/kg/d), though of course the power of such a determination is limited. The authors did not report the incidence or multiplicity of adenomas for the 0.05 g/L exposure group in the study or the incidence or multiplicity of adenomas and carcinomas in combination. For the animals surviving from 79 to 100 weeks of exposure, the incidence and multiplicity of adenomas peaked at 1 g/L while hepatocellular carcinomas continued to increase at the higher doses. This would be expected where some portion of the adenomas would either regress or progress to carcinomas at the higher doses.

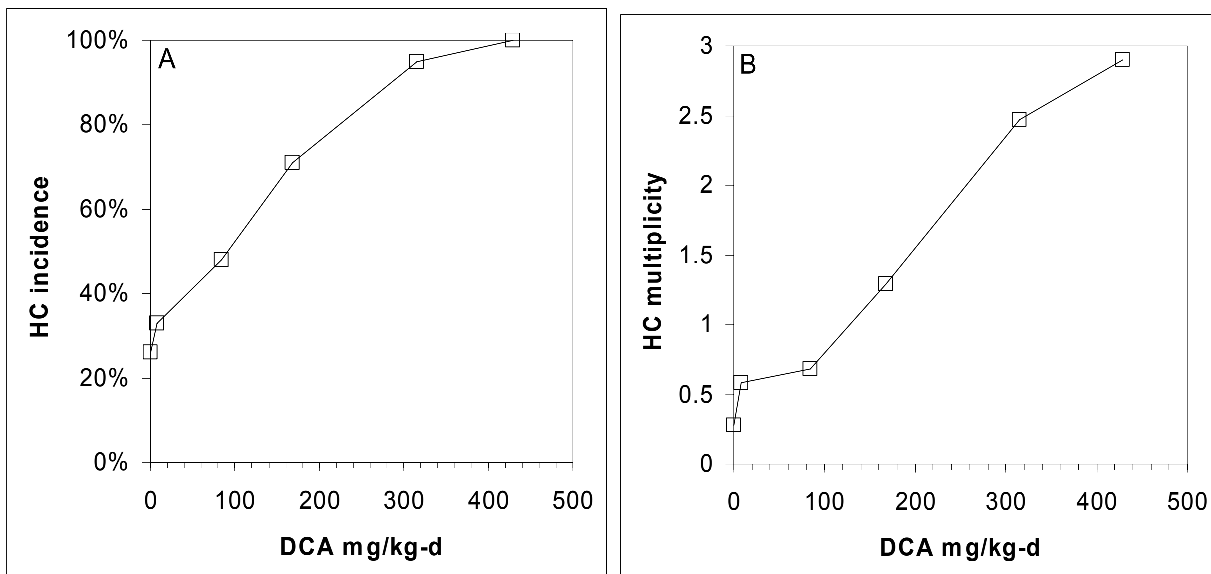


Figure 4-11. Dose-response data for hepatocellular carcinomas (HC) (A) incidence and (B) multiplicity, induced by DCA from DeAngelo et al. (1999). Drinking water concentrations were 0, 0.05, 0.5, 1, 2, and 3.5 g/L, from which daily average doses were calculated using observed water consumption in the study.

Associations of DCA carcinogenicity with various noncancer, possibly precursor, effects was also investigated. Importantly, the doses that induced tumors in DeAngelo et al. (1999) were reported to not induce widespread cytotoxicity. An attempt was also made to relate differing exposure levels to subchronic changes and peroxisomal enzyme induction. Interestingly, DeAngelo et al. (1999) reported that peroxisome proliferation was significantly increased at 3.5 g/L DCA only at 26 weeks, not correlated with tumor response, and to not be increased at either 0.05 g/L or 0.5 g/L treatments. The authors concluded that DCA-induced carcinogenesis was not dependent on peroxisome proliferation or chemically sustained proliferation, as measured by DNA synthesis. Slight hepatomegaly was present by 26 weeks in the 0.5 g/L group and decreased with time. By contrast, increases in both percent liver/body weight and the multiplicity of hepatocellular carcinomas increased proportionally with DCA exposure concentration after 79−100 weeks of exposure. DeAngelo et al. (1999) presented a figure comparing the number of hepatocellular carcinomas/animal at 100 weeks compared with the percent liver/body weight at 26 weeks that showed a linear correlation (r^2 = 0.9977) while peroxisome proliferation and DNA synthesis did not correlate with tumor induction profiles. The proportional increase in liver weight with DCA exposure was also reported for shorter durations of exposure as noted previously. Therefore, for DCA, both tumor incidence and liver weight appear to increase proportionally with dose.

4.5.6.3.2.3. ***Trichloroacetic acid (TCA) carcinogenicity dose-response data*.** With respect to TCA, Pereira (1996) reported that for 82 week exposure to TCA in female B6C3F1 mice, TCA exposure concentrations of 0, 2, 6.67, and 20 mmol/L (0, 0.33, 1.1, and 3.3 g/L) led to increasing incidences and multiplicity of adenomas and of carcinomas (Figure 4-12). DeAngelo et al. (2008) reported the results of three experiments exposing male B6C3F1 mice to neutralized TCA in drinking water (incidences also in Figure 4-12). Rather than using 5 exposure levels that were generally 2-fold apart, as was done in DeAngelo et al. (1999) for DCA, DeAngelo et al. (2008) studied only 3 doses of TCA that were an order of magnitude apart which limits the elucidation of the shape of the dose-response curve. In addition, the 104-week data, DeAngelo et al. (2008) contained 2 studies, each conducted in a separate laboratories—the two lower doses were studied in one study and the highest dose in another. The first 104-week study was conducted using 2 g/L NaCl, or 0.05, 0.5, or 5 g/L TCA in drinking water for 60 weeks (Study #1) while the other two were conducted for a period of 104 weeks (Study #2 with 2.5 g/L neutralized acetic acid or 4.5 g/L TCA exposure groups and Study #3 with deionized water, 0.05 g/L TCA and 0.5 g/L TCA exposure groups). In addition, a relatively small number of animals were used for the determination of a tumor response (n ~ 30 at final necropsy).

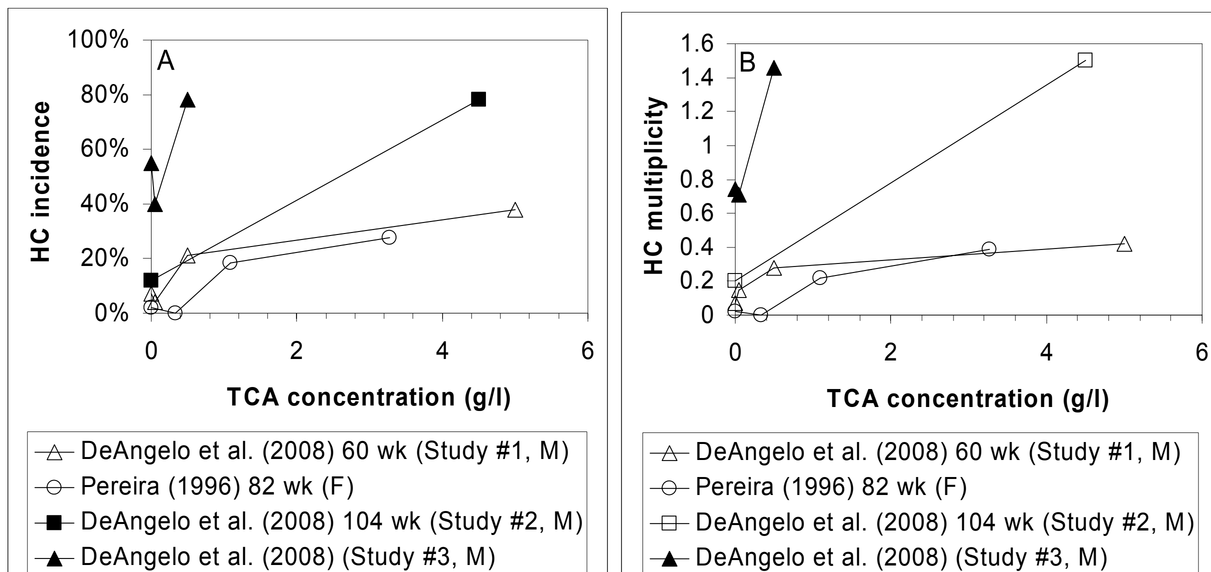


Figure 4-12. Reported incidences of hepatocellular carcinomas (HC) and adenomas plus carcinomas (HA+HC) in various studies in B6C3F1 mice (Pereira, 1996; DeAngelo et al., 2008). Combined HA + HC were not reported in (Pereira, 1996).

In Study #1, the incidence data for adenomas observed at 60 weeks at 0.05 g/L, 0.5 g/L and 5.0 g/L TCA were 2.1-, 3.0- and 5.4-fold of control values, with similar fold increases in multiplicity. As shown by Pereira (1996), 60 weeks does not allow for full tumor expression, so whether the dose-response relationship is the same at 104 weeks is not certain. For instance, Pereira (1996) examined the tumor induction in female B6C3F1 mice and demonstrated that foci, adenoma, and carcinoma development in mice are dependent on duration of exposure (period of observation in controls). In control female mice a 360- vs. 576-day observation period showed that at 360 days no foci or carcinomas and only 2.5% of animals had adenomas whereas by 576 days of observation, 11% had foci, 2% adenomas, and 2% had carcinomas. For DCA and TCA treatments, foci, adenomas, and carcinoma incidence and multiplicity did not reach full expression until 82 weeks at the 3 doses employed. Although the numbers of animals were relatively low and variable at the two highest doses (18−28 mice) there were 50−53 mice studied at the lowest dose level and 90 animals studied in the control group.

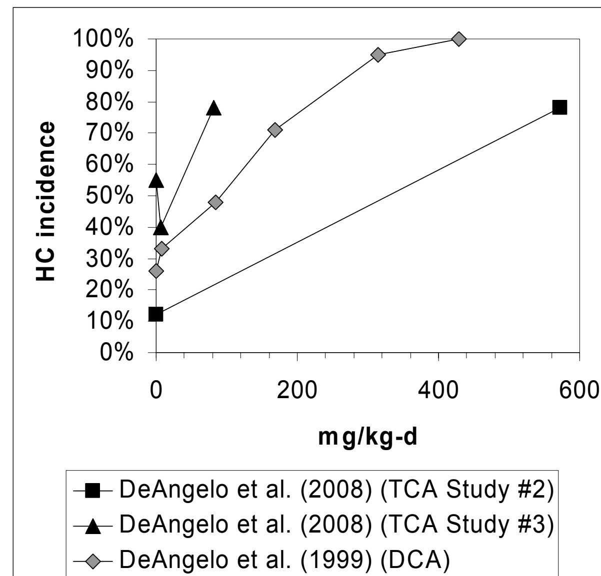
Therefore, the 104-week DeAngelo et al. (2008) data from Studies #2 and #3 would generally be preferred for elucidating the TCA dose-response relationship. However, Study #2 was only conducted at one dose, and although Study #3 used lower doses, it exhibited extraordinarily high control incidences of liver tumors. In particular, while the incidence of

adenomas and carcinomas was 12% in Study #2, it was reported to be 64% in Study #3. The mice in Study #3 were of very large size (weighing ~50 g at 45 weeks) as compared to Study #1, Study #2, or most other bioassays in general, and the large background rate of tumors reported is consistent with the body-weight-dependence observed by Leakey et al. (2003b).

To put into context the 64% incidence data for carcinomas and adenomas reported in DeAngelo et al. (2008) for the control group of Study #3, other studies cited in this review for male B6C3F1 mice show a much lower incidence in liver tumors with (1) NCI (1976) study of TCE reporting a colony control level of 6.5% for vehicle and 7.1% incidence of hepatocellular carcinomas for untreated male B6C3F1 mice (n = 70−77) at 78 weeks, (2) Herren-Freund et al. (1987) reporting a 9% incidence of adenomas in control male B6C3F1 mice with a multiplicity of 0.09 ± 0.06 and no carcinomas (n = 22) at 61 weeks, (3) NTP (1990) reporting an incidence of 14.6% adenomas and 16.6% carcinomas in male B6C3F1 mice after 103 weeks (n = 48), and (4) Maltoni et al. (1986) reporting that B6C3F1 male mice from the "NCI source" had a 1.1% incidence of "hepatoma" (carcinomas and adenomas) and those from "Charles River Co." had a 18.9% incidence of "hepatoma" during the entire lifetime of the mice (n = 90 per group). The importance of examining an adequate number of control or treated animals before confidence can be placed in those results in illustrated by Anna et al. (1994) in which at 76 weeks 3/10 control male B6C3F1 mice that were untreated and 2/10 control animals given corn oil were reported to have adenomas but from 76 to 134 weeks, 4/32 mice were reported to have adenomas (multiplicity of 0.13 ± 0.06) and 4/32 mice were reported to have carcinomas (multiplicity of 0.12 ± 0.06). Thus, the reported combined incidence of carcinomas and adenomas of 64% reported by DeAngelo et al. (2008) for the control mice of Study # 3, not only is inconsistent and much higher than those reported in Studies #1 and #2, but also much higher than reported in a number of other studies of TCE.

Therefore, this large background rate and the increased mortality for these mice limit their use for determining the nature of the dose-response for TCA liver carcinogenicity. At the two lowest doses of 0.05 g/L and 0.5 g/L TCA from Study #3, the differences in the incidences and multiplicities for all tumors were 2-fold at 104 weeks. However, there was no difference in any of the tumor results (i.e., adenoma, carcinoma, and combinations of adenoma and carcinoma incidence and multiplicity) between the 4.5 g/L dose group in Study #2 and the 0.5 g/L dose group in Study #3 at 104 weeks. By contrast, at 60 weeks of exposure, but within the same study (Study #1), there was a 2-fold increase in multiplicity for adenomas, and for adenomas and carcinomas combined between the 0.5 and 5.0 g/L TCA exposure groups. These results are consistent with the two highest exposure levels reaching a plateau of response after a long enough duration of exposure for full expression of the tumors (i.e., ~90% of animals having liver

1 tumors at the 0.5 g/L and 5 g/L exposures). However, whether such a plateau would have been
2 observed in mice with a more "normal" body weight, and hence a lower background tumor
3 burden cannot be determined.
4 Because of the limitations of different studies, it is difficult to discern whether the liver
5 tumor dose-response curves of TCA and DCA are different in a way analogous to that for liver
6 weight (see Figure 4-13). Certainly, it is clear that at the same concentration in drinking water or
7 estimated applied dose, DCA is more potent than TCA, as DCA induces nearly 100% incidence
8 of carcinomas at a lower dose than TCA. Therefore, like with liver weight gains, DCA has a
9 steeper dose-response function than TCA. However, the evidence for a "plateau" in tumor
10 response at high doses with TCA, as was observed for liver weight, is equivocal, as it is
11 confounded by the highly varying background tumor rates and the limitations of the available
12 study paradigms.



13
14 **Figure 4-13. Reported incidence of hepatocellular carcinomas induced by**
15 **DCA and TCA in 104-week studies (DeAngelo et al., 1999, 2008).** Only
16 carcinomas were reported in DeAngelo et al. (1999), so combined adenomas and
17 carcinomas could not be compared.
18
19
20 DeAngelo et al. (2008) attempt to identify a NOEL for tumorigenicity using tumor
21 multiplicity data and estimated TCA dose. However, it is not an appropriate descriptor for these
22 data, especially given that "statistical significance" of the tumor response is the determinant used
23 by the authors to support the conclusions regarding a dose in which there is no TCA-induced
24 effect. Due to issues related to the appropriateness of use of the concurrent control in Study #3,

only the 60-week experiment (i.e., Study # 1) is useful for the determination of tumor dose-response. Not only is there not allowance for full expression of a tumor response at the 60-week time point but a power calculation of the 60-week study shows that the Type II error, which should be >50% and thus, greater than the chances of "flipping a coin," was 41 and 71% for incidence and 7 and 15% for multiplicity of adenomas for the 0.05 and 0.5 g/L TCA exposure groups. For the combination of adenomas and carcinomas, the power calculation was 8 and 92% for incidence and 6 and 56% for multiplicity at 0.05 and 0.5 g/L TCA exposure. Therefore, the designed experiment could accept a false null hypothesis, especially in terms of tumor multiplicity, at the lower exposure doses and erroneously conclude that there is no response due to TCA treatment.

In terms of correlations with other noncancer, possibly precursor effects, DeAngelo et al. (2008) also reported that PCO activity, which varied 2.7-fold as baseline controls, was 1.3-, 2.4-, and 5.3-fold of control for the 0.05, 0.5, and 5 g/L TCA exposure groups in Study #1 at 4 weeks was for adenomas incidence 2.1-, 3.0-, and 5.4-fold of control and not similar at the lowest dose level at 60 weeks. However, it is not clear whether the similarly between PCO and carcinogenicity at 60 weeks would persist for tumor incidence at 104 weeks. DeAngelo et al. (2008) report a regression analyses that compare "percent of hepatocellular neoplasia," indicated by tumor multiplicity, with TCA dose, represented by estimations of the TCA dose in mg/kg/d, and with PCO activity for the 60-week and 104-week data. Whether adenomas and carcinomas combined or individual tumor type were used in these analysis was not reported by the authors. However, it would be preferable to compare "precursor" levels of PCO at earlier time points, rather than at a time when there was already a significant tumor response. In addition, linear regression analyses of these data are difficult to interpret because of the wide dose spacing of these experiments. In such a situation, for a linear regression, control and 5 g/L exposure levels will basically determine the shape of the dose-response curve since the 0.05 g/L and 0.5 g/L exposure levels are so close to the control (0) value. Thus, dose response appears to be linear between control and the 5.0 g/L value with the two lowest doses not affectively changing the slope of the line (i.e., "leveraging" the regression). Moreover, at the 5 g/L dose level, there is potential for effects due to palatability, as reported in one study in which drinking water consumption declined at this concentration (DeAngelo et al., 2008). Thus, the value of these analyses is limited by (1) use of data from Study # 3 in a tumor prone mouse that is not comparable to those used in Studies #1 and #2, (2) the appropriateness of using PCO values from later time points and the variability in PCO control values, (3) the uncertainty of the effects of palatability on the 5 g/L TCA results which were reported in one study to reduce drinking water consumption, and (4) the dose-spacing of the experiment.

4.5.6.3.2.4. ***Chloral hydrate (CH) carcinogenic dose-response.*** Although a much more limited database in rodents than for TCA or DCA, there is evidence that chloral hydrate is also a rodent liver hepatocarcinogen (see also Section E.2.5 and Caldwell and Keshava [2006]).

Daniel et al. (1992) exposed adult male B6C3F1 28-day-old mice to 1 g/L CH in drinking water for 30 and 60 weeks (*n* = 5 for interim sacrifice) and for 104 weeks (*n* = 40). The concentration of CH was 1 g/L and estimated to provide a 166-mg/kg/d dose. It is not clear from the report what control group better matched the CH group, as the mean initial body weights of the groups as well as the number of animals varied considerably in each group (i.e., ~40% difference in mean body weights at the beginning of the study). Liver tumors were increased by CH treatment. The percent incidence of liver carcinomas and adenomas in the surviving animals was 15% in control and 71% in CH-treated mice and the incidence of hepatocellular carcinoma reported to be 46% in the CH-treated group. The number of tumors/animals was also significantly increased with CH treatment. However, because this was a single dose study, a comparison with the dose-response relationship with TCE, TCA, or DCA is not feasible.

George et al. (2000) exposed male B6C3F1 mice to CH in drinking water for 2 years. Groups of animals were sacrificed at 26, 52, and 78 weeks following the initiation of dosing, with terminal sacrifices at Week 104. Only a few animals received a complete pathological examination. Preneoplastic foci and adenomas were reported to be increased in the livers of all CH treatment groups at 104 weeks. The percent incidence of hepatocellular adenomas was reported to be 21.4, 43.5, 51.3, and 50% in control, 13.5, 65.0 and 146.6 mg/kg/d CH treatment groups, respectively. The percent incidence of hepatocellular carcinomas was reported to be 54.8, 54.3, 59.0 and 84.4% in these same groups. The resulting percent incidence of hepatocellular adenomas and carcinomas was reported to be 64.3, 78.3, 79.5 and 90.6%. Of concern is the reporting of a 64% incidence of hepatocellular carcinomas and adenomas in the control group of mice for this experiment, which is the same as that for another study published by this same laboratory (DeAngelo et al., 2008). DeAngelo et al. (2008) did not identify them as being contemporaneous studies or sharing controls, but a comparison of the control data published by DeAngelo et al. (2008) for TCA and that published by George et al. (2000) for the CH studies shows them to be the same data set. Therefore, as discussed above, this data set was derived from B6C3F1 mice that were large (~50 g) and resultantly tumor prone, making determinations of the dose-response of CH from this experiment difficult. Therefore, for the purposes of comparison of dose-response relationships, this study has the same limitations as the DeAngelo et al. (2008) study, discussed above.

Leakey et al. (2003a) studied the effects of CH exposure (0, 25, 50, and 100 mg/kg/d, 5 days/week, 104−105 weeks via gavage) in male B6C3F1 mice with dietary control used to manipulate body growth (*n* = 48 for 2-year study and *n* = 12 for the 15-month interim study). Dietary control was reported to decrease background liver tumor rates (decreased by15−20%) and was reported to be associated with decreased variation in liver-to-body weight ratios, thereby potentially increasing assay sensitivity. In dietary-controlled groups and groups fed *ad libitum*, liver adenomas and carcinomas (combined) were reported to be increased with CH treatment. With dietary restriction there was a more discernable CH tumor-response with overall tumor incidence reduced, and time-to-tumor increased by dietary control in comparison to *ad libitum* fed mice. Incidences of hepatocellular adenoma and carcinoma overall rates were reported to be 33, 52, 49, and 46% for control, 25, 50, and 100 mg/kg *ad libitum*-fed mice, respectively. For dietary controlled mice the incidence rates were reported to be 22.9, 22.9, 29.2, and 37.5% for controls, 25, 50, and 100 mg/kg CH, respectively. Body weights were matched and carefully controlled in this study. These data are shown in Figure 4-14, relative to control incidences. It is evident from these data that dietary control significantly changes the apparent shape of the dose-response curve, presumably by reducing variability between animals. While the *ad libitum* dose groups had an apparent "saturation" of response, this was not evident with the dietary controlled group. Of note all the other bioassays for TCE, TCA, DCA, and CH were in *ad libitum* fed mice. Therefore, it is difficult to compare the dose-response curves for CH-treated mice on dietary restriction to those fed *ad libitum*. However, the rationale for dietary restriction in the B6C3F1 mouse is to prevent the types of weight gain and corresponding high background tumor levels observed in DeAngelo et al. (2008) and George et al. (2000). As stated previously, most other studies of TCA, DCA, and TCE had background levels that, while varied, were lower than the *ad libitum* fed mice studied in Leakey et al. (2003a).

Of note is that incidences of adenomas and carcinomas combined do not show differences in tumor progression as carcinomas may increase and adenomas may regress. Liver weight increases at 15-months did not correlate with 2-year tumor incidences in the *ad libitum* group, but a consistent dose-response shape between these two measures is evident in the dietary controlled group. However, of note is the reporting of liver weight at 15 months is for a time period in which foci and liver tumors have been reported to have already occurred in other studies, so hepatomegaly in the absence of these changes is hard to detect.

In terms of other noncancer effects that may be associated with tumor induction, it is notable that while dietary restriction reduced the overall level of CH-mediated tumor induction, it led to greater CH-mediated induction of peroxisome proliferation-associated enzymes. Moreover, between control groups, dietary restricted mice appeared to have higher levels of

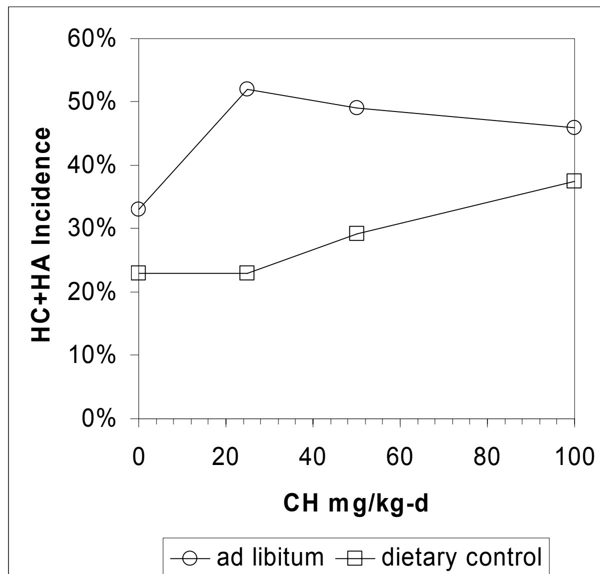


Figure 4-14. Effects of dietary control on the dose-response curves for changes in liver tumor incidences induced by CH in diet (Leakey et al., 2003a).

lauric acid ω-hydrolase activity than *ad libitum*-fed mice. Seng et al. (2003) report that lauric acid β-hydroxylase and PCO were induced only at exposure levels >100 mg/kg CH, again with dietary restricted groups showing the greatest induction. Such data argue against the role of peroxisome proliferation in CH-liver tumor induction in mice.

Leakey et al. (2003a) gave no descriptions of liver pathology were given other than incidence of mice with fatty liver changes. Hepatic malondialdehyde concentration in *ad libitum* fed and dietary controlled mice did not change with CH exposure at 15 months but the dietary controlled groups were all approximately half that of the *ad libitum*-fed mice. Thus, while overall increased tumors observed in the *ad libitum* diet correlated with increased malondialdehyde concentration, there was no association between CH dose and malondialdehyde induction for either diet.

Overall, from the CH studies in mice, there is an apparent increase in liver adenomas and carcinomas induced by CH treatment by either drinking water or gavage with all available studies performed in male B6C3F1 mice. However, the background levels of hepatocellular adenomas and carcinomas in these mice in George et al. (2000) and body-weight data from this study are high, consistent with the association between large body weight and background tumor susceptibility shown with dietary control (Leakey et al., 2003a). With dietary control, Leakey et

al. (2003a) report a dose-response relationship between exposure and tumor incidence that is proportional to dose.

4.5.6.3.2.5. ***Degree of concordance among trichloroethylene (TCE), trichloroacetic acid (TCA), dichloroacetic acid (DCA), and chloral hydrate (CH) dose-response relationships*.** Comparison of the dose-response for TCE hepatocarcinogenicity with that for TCA and DCA is weakly suggestive a better concordance in dose-response shape between TCE and DCA or TCE and CH than between TCE and TCA. However, differences across the databases of these compounds, especially with respect to the comparability of study durations and control tumor incidences, preclude a definitive conclusion from these data.

4.5.6.3.3. ***Inferences from liver tumor phenotype and genotype.*** A number of studies have investigation tumor phenotypes, such as c-Jun staining, tincture, and dysplacity, or genotypes, such as H-ras mutations, to inform both the identification of the active agents of TCE liver tumor induction as well as what MOA(s) may be involved.

4.5.6.3.3.1. ***Tumor phenotype—staining and appearance*.** The descriptions of tumors in mice reported by the NCI, NTP, and Maltoni et al studies are also consistent with phenotypic heterogeneity as well as spontaneous tumor morphology (see Section E.3.4.1.5). As noted in Section E.3.1, hepatocellular carcinomas observed in humans are also heterogeneous. For mice, Maltoni et al. (1986) described malignant tumors of hepatic cells to be of different subhistotypes, and of various degrees of malignancy and were reported to be unique or multiple, and have different sizes (usually detected grossly at necropsy) from TCE exposure. In regard to phenotype, tumors were described as usual type observed in Swiss and B6C3F1 mice, as well as in other mouse strains, either untreated or treated with hepatocarcinogens and to frequently have medullary (solid), trabecular, and pleomorphic (usually anaplastic) patterns. For the NC I (1976) study, the mouse liver tumors were described in detail and to be heterogeneous "as described in the literature" and similar in appearance to tumors generated by carbon tetrachloride. The description of liver tumors in this study and tendency to metastasize to the lung are similar to descriptions provided by Maltoni et al. (1986) for TCE-induced liver tumors in mice via inhalation exposure. The NTP (1990) study reported TCE exposure to be associated with increased incidence of hepatocellular carcinoma (tumors with markedly abnormal cytology and architecture) in male and female mice. Hepatocellular adenomas were described as circumscribed areas of distinctive hepatic parenchymal cells with a perimeter of normal appearing parenchyma in which there were areas that appeared to be undergoing compression

from expansion of the tumor. Mitotic figures were sparse or absent but the tumors lacked typical lobular organization. Hepatocellular carcinomas were reported to have markedly abnormal cytology and architecture with abnormalities in cytology cited as including increased cell size, decreased cell size, cytoplasmic eosinophilia, cytoplasmic basophilia, cytoplasmic vacuolization, cytoplasmic hyaline bodies and variations in nuclear appearance. Furthermore, in many instance several or all of the abnormalities were reported to be present in different areas of the tumor and variations in architecture with some of the hepatocellular carcinomas having areas of trabecular organization. Mitosis was variable in amount and location. Therefore, the phenotype of tumors reported from TCE exposure was heterogeneous in appearance between and within tumors from all 3 of these studies.

Caldwell and Keshava (2006) report "that Bannasch (2001) and Bannasch et al. (2001) describe the early phenotypes of preneoplastic foci induced by many oncogenic agents (DNA-reactive chemicals, radiation, viruses, transgenic oncogenes and local hyperinsulinism) as insulinomimetic. These foci and tumors have been described by tincture as eosinophilic and basophilic and to be heterogeneous. The tumors derived from them after TCE exposure are consistent with the description for the main tumor lines of development described by Bannasch et al. (2001) (see Section 3.4.1.5). Thus, the response of liver to DCA (glycogenesis with emergence of glycogen poor tumors) is similar to the progression of preneoplastic foci to tumors induced from a variety of agents and conditions associated with increased cancer risk." Furthermore Caldwell and Keshava (2006) note that Bull et al. (2002) report expression of insulin receptor to be elevated in tumors of control mice or mice treated with TCE, TCA and DCA but not in nontumor areas suggesting that this effect is not specific to DCA.

There is a body of literature that has focused on the effects of TCE and its metabolites after rats or mice have been exposed to "mutagenic" agents to "initiate" hepatocarcinogenesis and this is discussed in Section E.4.2. TCE and its metabolites were reported to affect tumor incidence, multiplicity, and phenotype when given to mice as a coexposure with a variety of "initiating" agents and with other carcinogens. Pereira and Phelps (1996) reported that methylnitrosourea (MNU) alone induced basophilic foci and adenomas. MNU and low concentrations of DCA or TCA in female mice were reported to induce heterogeneous for foci and tumor with a higher concentration of DCA inducing more eosinophilic and a higher concentration of TCA inducing more tumors that were basophilic. Pereira et al. (2001) reported that not only dose, but gender also affected phenotype in mice that had already been exposed to MNU and were then exposed to DCA. As for other phenotypic markers, Lantendresse and Pereira (1997) reported that exposure to MNU and TCA or DCA induced tumors that had some

1 commonalities, were heterogeneous, but for female mice were overall different between DCA
2 and TCA as coexposures with MNU.
3 With regard to the phenotype of TCA and DCA-induced tumors, Stauber and Bull (1997)
4 reported the for male B6C3F1 mice, DCA-induced "lesions" contained a number of smaller
5 lesions that were heterogeneous and more eosinophilic with larger "lesions" tending to less
6 numerous and more basophilic. For TCA results using this paradigm, the "lesions" were
7 reported to be less numerous, more basophilic, and larger than those induced by DCA. Carter et
8 al. (2003) used tissues from the DeAngelo et al. (1999) and examined the heterogeneity of the
9 DCA-induced lesions and the type and phenotype of preneoplastic and neoplastic lesions pooled
10 across all time points. Carter et al. (2003) examined the phenotype of liver tumors induced by
11 DCA in male B6C3 F1 mice and the shape of the dose-response curve for insight into its MOA.
12 They reported a dose-response of histopathologic changes (all classes of premalignant lesions
13 and carcinomas) occurring in the livers of mice from 0.05−3.5 g/L DCA for 26−100 weeks and
14 suggest foci and adenomas demonstrated neoplastic progression with time at lower doses than
15 observed DCA genotoxicity. Preneoplastic lesions were identified as eosinophilic, basophilic
16 and/or clear cell (grouped with clear cell and mixed cell) and dysplastic. Altered foci were
17 50% eosinophilic with about 30% basophilic. As foci became larger and evolved into
18 carcinomas they became increasingly basophilic. The pattern held true through out the exposure
19 range. There was also a dose and length of exposure related increase in atypical nuclei in
20 "noninvolved" liver. Glycogen deposition was also reported to be dose-dependent with
21 periportal accumulation at the 0.5 g/L exposure level. Carter et al. (2003) suggested that size and
22 evolution into a more malignant state are associated with increasing basophilia, a conclusion
23 consistent with those of Bannasch (1996) and that there a greater periportal location of lesions
24 suggestive as the location from which they arose. Consistent with the results of DeAngelo et al.
25 (1999), Carter et al. (2003) reported that DCA (0.05−3.5 g/L) increased the number of lesions
26 per animal relative to animals receiving distilled water, shortened the time to development of all
27 classes of hepatic lesions, and that the phenotype of the lesions were similar to those
28 spontaneously arising in controls. Along with basophilic and eosinophilic lesions or foci,
29 Carter et al. (2003) concluded that DCA-induced tumors also arose from isolated, highly
30 dysplastic hepatocytes in male B6C3F1 mice chronically exposed to DCA suggesting another
31 direct neoplastic conversion pathway other than through eosinophilic or basophilic foci.
32 Rather than male B6C3F1 mice, Pereira (1996) studied the dose-response relationship for
33 the carcinogenic activity of DCA and TCA and characterized their lesions (foci, adenomas and
34 carcinomas) by tincture in females (the generally less sensitive gender). Like the studies of TCE
35 by Maltoni et al. (1986), female mice were also reported to have increased liver tumors after

TCA and DCA exposures. Pereira (1996) pool lesions were pooled for phenotype analysis so the affect of duration of exposure could not be determined nor adenomas separated from carcinomas for "tumors." However, as the concentration of DCA was decreased the number of foci was reported by Pereira (1996) to be decreased but the phenotype of the foci to go from primarily eosinophilic foci (i.e., ~95% eosinophilic at 2.58 g/L DCA) to basophilic foci (~57% eosinophilic at 0.26 g/L). For TCA the number of foci was reported to ~40 basophilic and ~60 eosinophilic regardless of dose. Spontaneously occurring foci were more basophilic by a ratio of 7/3. Pereira (1996) described the foci of altered hepatocytes and tumors induced by DCA in female B6C3F1 mice to be eosinophilic at higher exposure levels but at lower or intermittent exposures to be half eosinophilic and half basophilic. Regardless of exposure level, half of the TCA-induced foci were reported to be half eosinophilic and half basophilic with tumors 75% basophilic. In control female mice, the limited numbers of lesions were mostly basophilic, with most of the rest being eosinophilic with the exception of a few mixed tumors. The limitations of descriptions tincture and especially for inferences regarding peroxisome proliferator from the description of "basophilia" is discussed in Section E.3.4.1.5.

Thus, the results appear to differ between male and female B6C3F1 mice in regard to tincture for DCA and TCA at differing doses. What is apparent is that the tincture of the lesions is dependent on the stage of tumor progression, agent (DCA or TCA), gender, and dose. Also what is apparent from these studies is the both DCA and TCA are heterogeneous in their tinctoral characteristics.

Overall, tumors induced by TCA, DCA, CH, and TCE are all heterogeneous in their physical and tinctural characteristics in a manner this not markedly distinguishable from spontaneous lesions or those induced by a wide variety of chemical carcinogens. For instance, Daniel et al. (1992), which studies DCA and CH carcinogenicity (discussed above) noted that morphologically, there did not appear to be any discernable differences in the visual appearance of the DCA- and CH-induced tumors. Therefore, these data do not provide strong insights into elucidating the active agent(s) for TCE hepatocarcinogenicity or their MOA(s).

4.5.6.3.3.2. ***C-Jun staining*****.** Stauber and Bull (1997) reported that in male B6C3F1 mice, the oncoproteins c-Jun and c-Fos were expressed in liver tumors induced by DCA but not those induced by TCA. Although Bull et al. (2004) have suggested that the negative expression of c-Jun in TCA-induced tumors may be consistent with a characteristic phenotype shown in general by peroxisome proliferators as a class, as pointed out by Caldwell and Keshava (2006), there is no supporting evidence of this. Nonetheless, the observation that TCA and DCA have

different levels of oncogene expression led to a number of follow-up studies by this group. No data on oncoprotein immunostaining are available for CH.

Stauber et al. (1998) studied induction of "transformed" hepatocytes by DCA and TCE treatment *in vitro*, including an examination of c-Jun staining. Stauber et al. (1998) isolated primary hepatocytes from 5−8 week old male B6C3F1 mice (n = 3) and subsequently cultured them in the presence of DCA or TCA. In a separate experiment 0.5 g/L DCA was given to mice as pretreatment for 2 weeks prior to isolation. The authors assumed that the anchorage-independent growth of these hepatocytes was an indication of an "initiated cell." After 10 days in culture with DCA or TCA (0, 0.2, 0.5 and 2.0 mM), concentrations of 0.5 mM or more DCA and TCA both induced an increase in the number of colonies that was statistically significant, with DCA showing dose-dependence as well as slightly greater overall increases than TCA. In a time course experiment the number of colonies from DCA treatment *in vitro* peaked by 10 days and did not change through Days 15−25 at the highest dose and, at lower concentrations of DCA, increased time in culture induced similar peak levels of colony formation by Days 20−25 as that reached by 10 days at the higher dose. Therefore, the number of colonies formed was independent of dose if the cells were treated long enough *in vitro*. However, not only did treatment with DCA or TCA induce anchorage independent growth but untreated hepatocytes also formed larger numbers of colonies with time, although at a lower rate than those treated with DCA. The level reached by untreated cells in tissue culture at 20 days was similar to the level induced by 10 days of exposure to 0.5 mM DCA. The time course of TCA exposure was not tested to see if it had a similar effect with time as did DCA. The colonies observed at 10 days were tested for c-Jun expression with the authors noting that "colonies promoted by DCA were primarily c-Jun positive in contrast to TCA promoted colonies that were predominantly c-Jun negative." Of the colonies that arose spontaneously from tissue culture conditions, 10/13 (76.9%) were reported to be c-Jun +, those treated with DCA 28/34 (82.3%) were c-Jun +, and those treated with TCA 5/22 (22.7%) were c-Jun +. Thus, these data show heterogeneity in cell in colonies but with more that were c-Jun + colonies occurring by tissue culture conditions alone than in the presence of DCA, rather than in the presence of TCA.

Bull et al. (2002) administered TCE, TCA, DCA, and combinations of TCA and DCA to male B6C3F1 mice by daily gavage (TCE) or drinking water (TCA, DCA, and TCA+DCA) for 52−79 weeks, in order to compare a number of tumor characteristics, including c-Jun expression, across these different exposures. Bull et al. (2002) reported lesion reactivity to c-Jun antibody to be dependent on the proportion of the DCA and TCA administered after 52 weeks of exposure. Given alone, DCA was reported to produce lesions in mouse liver for which approximately half displayed a diffuse immunoreactivity to a c-Jun antibody, half did not, and none exhibited a

mixture of the two. After TCA exposure alone, no lesions were reported to be stained with this antibody. When given in various combinations, DCA and TCA coexposure induced a few lesions that were only c-Jun+, many that were only c-Jun-, and a number with a mixed phenotype whose frequency increased with the dose of DCA. For TCE exposure of 79 weeks, TCE-induced lesions were reported to also have a mixture of phenotypes (42% c-Jun+, 34% c-Jun-, and 24% mixed) and to be most consistent with those resulting from DCA and TCA coexposure but not either metabolite alone.

A number of the limitations of the experiment are discussed in Caldwell et al. (2008) Specifically, for the DCA and TCA exposed animals, the experiment was limited by low statistical power, a relatively short duration of exposure, and uncertainty in reports of lesion prevalence and multiplicity due to inappropriate lesions grouping (i.e., grouping of hyperplastic nodules, adenomas, and carcinomas together as "tumors"), and incomplete histopathology determinations (i.e., random selection of gross lesions for histopathology examination). For determinations of immunoreactivity to c-Jun, Bull et al. (2002) combined hyperplastic nodules, adenomas, and carcinomas in most of their treatment groups, so differences in c-Jun expression across differing types of lesions were not discernable.

Nonetheless, these data collectively strongly suggest that TCA is not the sole agent of TCE-induced mouse liver tumors. In particular, TCE-induced tumors that were, in order of frequency, c-Jun+, c-Jun–, and of mixed phenotype, while c-Jun+ tumors have never been observed with TCA treatment. Nor do these data support DCA as the sole contributor, since mixed phenotypes were not observed with DCA treatment.

4.5.6.3.3.3. ***Tumor genotype: H-ras mutation frequency and spectrum*****.** An approach to determine the potential MOAs of DCA and TCA through examination of the types of tumors each "induced" or "selected" was to examine H-ras activation (Ferreira-Gonzalez et al., 1995; Anna et al., 1994; Bull et al., 2002; Nelson et al., 1990). No data of this type were available for CH. This approach has also been used to try to establish an H-ras activation pattern for "genotoxic" and "nongenotoxic" liver carcinogens compounds and to make inferences concerning peroxisome proliferator-induced liver tumors. However, as noted by Stanley et al. (1994), the genetic background of the mice used and the dose of carcinogen may affect the number of activated H-ras containing tumors which develop. In addition, the stage of progression of "lesions" (i.e., foci vs. adenomas vs. carcinomas) also has been linked the observance of H-ras mutations. Fox et al. (1990) note that tumors induced by phenobarbital (0.05% drinking water [H_2O], 1 year), chloroform (200 mg/kg corn oil gavage, 2 times weekly for 1 year) or ciprofibrate (0.0125% diet, 2 years) had a much lower frequency of H-ras gene

activation than those that arose spontaneously (2-year bioassays of control animals) or induced with the "genotoxic" carcinogen benzidine-2 hydrochloric acid (HCl) (120 ppm, drinking H_2O, 1 year) in mice. In that study, the term "tumor" was not specifically defined but a correlation between the incidence of H-ras gene activation and development of either a hepatocellular adenoma or hepatocellular carcinoma was reported to be made with no statistically significant difference between the frequency of H-ras gene activation in the hepatocellular adenomas and carcinomas. Histopathological examination of the spontaneous tumors, tumors induced with benzidine-2 HCl, Phenobarbital, and chloroform was not reported to reveal any significant changes in morphology or staining characteristics. Spontaneous tumors were reported to have 64% point mutation in codon 61 (n = 50 tumors examined) with a similar response for Benzidine of 59% (n = 22 tumors examined), whereas for Phenobarbital the mutation rate was 7% (n = 15 tumors examined), chloroform 21% (n = 24 tumors examined) and ciprofibrate 21% (n = 39 tumors examined). The ciprofibrate-induced tumors were reported to be more eosinophilic as were the surrounding normal hepatocytes.

Hegi et al. (1993) tested ciprofibrate-induced tumors in the NIH3T3 cotransfection-nude mouse tumorigenicity assay, which the authors state is capable of detecting a variety of activated protooncogenes. The tumors examined (ciprofibrate-induced or spontaneously arising) were taken from the Fox et al. study (1990), screened previously, and found to be negative for H-ras activation. With the limited number of samples examined, Hegi et al concluded that ras protooncogene activation or activation of other protooncogenes using the nude mouse assay were not frequent events in ciprofibrate-induced tumors and that spontaneous tumors were not promoted with it. Using the more sensitive methods, the H-ras activation rate was reported to be raised from 21 to 31% for ciprofibrate-induced tumors and from 64 to 66% for spontaneous tumors. Stanley et al. (1994) studied the effect of methylclofenapate (MCP) (25 mg/kg for up to 2 years), a peroxisome proliferator, in B6C3F1 (relatively sensitive) and C57BL/10J (relatively resistant) mice for H-ras codon 61 point mutations in MCP-induced liver tumors (hepatocellular adenomas and carcinomas). In the B6C3F1 mice the number of tumors with codon 61 mutations was 11/46 and for C57BL/10J mice 4/31. Unlike the findings of Fox et al. (1990), Stanley et al. (1994) reported an increase in the frequency of mutation in carcinomas, which was reported to be twice that of adenomas in both strains of mice, indicating that stage of progression was related to the number of mutations in those tumors, although most tumors induced by MCP did not have this mutation.

Anna et al. (1994) reported that the H-ras codon 61 mutation frequency was not statistically different in liver tumors from DCA and TCE-treated mice from a highly variable number of tumors examined. From their concurrent controls, they reported that H-ras codon 61

mutations in 17% (*n* = 6) of adenomas and 100% (*n* = 5) of carcinomas. For historical controls (published and unpublished), they reported mutations in 73% (*n* = 33) of adenomas and mutations in 70% (*n* = 30) of carcinomas. For tumors from TCE-treated animals, they reported mutations in 35% (*n* = 40) of adenomas and 69% (*n* = 36) of carcinomas, while for DCA-treated animals, they reported mutations in 54% (*n* = 24) of adenomas and in 68% (*n* = 40) of carcinomas. Anna et al. (1994) reported more mutations in TCE-induced carcinomas than adenomas. In regard to mutation spectra in H-ras oncogenes in control or spontaneous tumors, the patterns were slightly different but those from TCE treatment were mostly similar to that of DCA-induced tumors (0.5% in drinking water).

The study of Ferreira-Gonzalez (1995) in male B6C3 F1 mice has the advantage of comparison of tumor phenotype at the same stage of progression (hepatocellular carcinoma), for allowance of the full expression of a tumor response (i.e., 104 weeks), and an adequate number of spontaneous control lesions for comparison with DCA or TCA treatments. However, tumor phenotype at an end stage of tumor progression may not be indicative of earlier stages of the disease process. In spontaneous liver carcinomas, 58% were reported to show mutations in H-61 as compared with 50% of tumor from 3.5 g/L DCA-treated mice and 45% of tumors from 4.5 g/L TCA-treated mice. A number of peroxisome proliferators have been reported to have a much smaller mutation frequency that spontaneous tumors (e.g., 13−24% H-ras codon 61 mutations after methylclofenopate depending on mouser strain, Stanely et al. [1994]: 21 to 31% for ciprofibrate-induced tumors and from 64 to 66% for spontaneous tumors, Fox et al. [1990] and Hegi et al [1993]). Thus, there was a heterogeneous response for this phenotypic marker for the spontaneous, DCA-, and TCA- treatment induced hepatocellular carcinomas had similar patterns H-ras mutations that differed from the reduced H-ras mutation frequencies reported for a number of peroxisome proliferators.

In his review, Bull (2000) suggested "the report by Anna et al. (1994) indicated that TCE-induced tumors possessed a different mutation spectra in codon 61 of the H-ras oncogene than those observed in spontaneous tumors of control mice." Bull (2000) stated that "results of this type have been interpreted as suggesting that a chemical is acting by a mutagenic mechanism" but went on to suggest that it is not possible to *a priori* rule out a role for selection in this process and that differences in mutation frequency and spectra in this gene provide some insight into the relative contribution of different metabolites to TCE-induced liver tumors. Bull (2000) noted that data from Anna et al. (1994), Ferreira-Gonzalez et al. (1995), and Maronpot et al. (1995) indicated that mutation frequency in DCA-induced tumors did not differ significantly from that observed in spontaneous tumors. Bull (2000) also noted that the mutation spectra

found in DCA-induced tumors has a striking similarity to that observed in TCE-induced tumors, and DCA-induced tumors were significantly different than that of TCA-induced liver tumors.

Bull et al. (2002) reported that mutation frequency spectra for the H-ras codon 61 in mouse liver "tumors" induced by TCE (*n* = 37 tumors examined) were reported to be significantly different than that for TCA (*n* = 41 tumors examined), with DCA-treated mice tumors giving an intermediate result (*n* = 64 tumors examined). In this experiment, TCA-induced "tumors" were reported to have more mutations in codon 61 (44%) than those from TCE (21%) and DCA (33%). This frequency of mutation in the H-ras codon 61 for TCA is the opposite pattern as that observed for a number of peroxisome proliferators in which the number of mutations at H-ras codon 61 in tumors has been reported to be much lower than spontaneously arising tumors (see above). Bull et al. (2002) noted that the mutation frequency for all TCE, TCA or DCA tumors was lower in this experiment than for spontaneous tumors reported in other studies (they had too few spontaneous tumors to analyze in this study), but that this study utilized lower doses and was of shorter duration than that of Ferreira-Gonzalez (1995). Furthermore, the disparities from previous studies may also be impacted by lesion grouping, mentioned above, in which lower stages of progression are grouped with more advanced stages.

Overall, in terms of H-ras mutation, TCE-induced tumors appears to be more like DCA-induced tumors (which are consistent with spontaneous tumors), or those resulting from a coexposure to both DCA and TCA (Bull et al., 2002), than from those induced by TCA. As noted above, Bull et al. (2002) reported the mutation frequency spectra for the H-ras codon 61 in mouse liver tumors induced by TCE to be significantly different than that for TCA, with DCA-treated mice tumors giving an intermediate result and for TCA-induced tumors to have a H-ras profile that is the opposite than those of a number of other peroxisome proliferators. More importantly, however, these data, along with the measures discussed above, show that mouse liver tumors induced by TCE are heterogeneous in phenotype and genotype in a manner similar to that observed in spontaneous tumors.

4.5.6.3.4. ***"Stop" experiments.*** Several stop experiments, in which treatment is terminated early in some dose groups, have attempted to ascertain the whether progression differences exist between TCA and DCA. After 37 weeks of treatment and then a cessation of exposure for 15 weeks, Bull et al. (1990) reported that after combined 52 week period, liver weight and percent liver/body weight were reported to still be statistically significantly elevated after DCA or TCA treatment. The authors partially attribute the remaining increases in liver weight to the continued presence of hyperplastic nodules in the liver. In terms of liver tumor induction, the authors stated that "statistical analysis of tumor incidence employed a general linear model

ANOVA with contrasts for linearity and deviations from linearity to determine if results from groups in which treatments were discontinued after 37 weeks were lower than would have been predicted by the total dose consumed." The multiplicity of tumors (incidence was not used) observed in male mice exposed to DCA or TCA at 37 weeks and then sacrificed at 52 weeks were compared with those exposed for a full 52 weeks. The response in animals that received the shorter duration of DCA exposure was very close to that which would be predicted from the total dose consumed by these animals. By contrast, the response to TCA exposure for the shorter duration was reported by the authors to deviate significantly ($p = 0.022$) from the linear model predicted by the total dose consumed. However, in the prediction of "dose-response," foci, adenomas, and carcinomas were combined into one measure. Therefore, foci, a certain percentage of which have been commonly shown to spontaneously regress with time, were included in the calculation of total "lesions." Moreover, only a sample of lesions were selected for histological examination, and as is evident in the sample, some lesions appeared "normal" upon microscopic examination (see below). Therefore, while suggesting that cessation of exposure diminished the number of "lesions," methodological limitations temper any conclusions regarding the identity and progression of lesion with continuous vs. noncontinuous DCA and TCA treatment.

Additionally, Bull et al. (1990) noted that after stopping treatment, DCA lesions appeared to arrest their progression in contrast to TCA lesions, which appeared to progress. In particular, among those in the stop treatment group (at 2 g/L) with 0/19 lesions examined histologically were carcinomas, while in the continuous treatment groups, a significant fraction of lesions examined were carcinomas at the higher exposure (6/23 at 2 g/L). By contrast, at terminal sacrifice, TCA lesions a larger fraction of the lesions examined were carcinomas in the stop treatment group (3/5 at 2 g/L) than in the continuous treatment group (2/7 and 4/16 at 1 g/L and 2 g/L, respectively).

However, as mentioned above, these inferences are based on examination of only a subset of lesions. Specifically, for TCA treatment the number of animals examined for determination of which "lesions" were foci, adenomas, and carcinomas was 11 out of the 19 mice with "lesions" at 52 weeks while all 4 mice with lesions after 37 weeks of exposure and 15 weeks of cessation were examined. For DCA treatment the number of animals examined was only 10 out of 23 mice with "lesions" at 52 weeks while all 7 mice with lesions after 37 weeks of exposure and 15 weeks of cessation were examined. Most importantly, when lesions were examined microscopically, some did not all turn out to be preneoplastic or neoplastic—for example, two lesions appeared "to be histologically normal" and one necrotic.

While limited, the conclusions of Bull et al. (1990) are consistent with later experiments performed by Pereira and Phelps (1996). They noted that in MNU-treated mice that were then treated with DCA, the yield of altered hepatocytes decreases as the tumor yields increase between 31 and 51 weeks of exposure suggesting progression of foci to adenomas, but that adenomas did not appear to progress to carcinomas. For TCA, Pereira and Phelps (1996) reported that “MNU-initiated” adenomas promoted with TCA continued to progress. However, the use of MNU initiation complicates direct comparisons with treatment with TCA or DCA alone.

No similar data comparing stop and continued treatment of TCE are available to assess the consistency or lack-thereof with TCA or DCA. Moreover, the informative of such a comparison would be limited by designs of the available TCA and DCA studies, which have used higher concentrations in conjunction with the much lower durations of exposure. While higher doses allow for responses to be more easily detected, it introduces uncertainty as to the effects of the higher doses alone. In addition, because the overall duration of the experiments is also generally much less than 104 weeks, it is not possible to discern whether the differences in results between those animals in which treatment was suspended in comparison to those in which had not had been conducted would persist with longer durations.

4.5.6.4. *Conclusions Regarding the Role of Trichloroacetic Acid (TCA), Dichloroacetic Acid (DCA), and Chloral Hydrate (CH) in Trichloroethylene (TCE)-Induced Effects in the Liver*

In summary, it is likely that oxidative metabolism is necessary for TCE-induced effects in the liver. However, the specific metabolite or metabolites responsible for both noncancer and cancer effects is less clear. TCE, TCA, and DCA exposures have all been associated with induction of peroxisomal enzymes but are all weak PPARα agonists. The available data strongly support TCA ***not*** being the sole or predominant active moiety for TCE-induced liver effects. With respect to hepatomegaly, TCE and TCA dose-response relationships are quantitatively inconsistent, for TCE leads to greater increases in liver/body weight ratios that expected from predicted rates of TCA production. In fact, above a certain dose of TCE, liver/body weight ratios are greater than that observed under any conditions studied so far for TCA. Histological changes and effects on DNA synthesis are generally consistent with contributions from either TCA or DCA, with a degree of polyploidization, rather than cell proliferation, likely to be significant for TCE, TCA, and DCA. With respect to liver tumor induction, TCE leads to a heterogeneous population of tumors, not unlike those that occur spontaneously or that are observed following TCA-, DCA-, or CH-treatment. Moreover, some liver phenotype experiments, particularly those utilizing immunostaining for c-Jun, support a role for both DCA

1 and TCA in TCE-induced tumors, with strong evidence that TCA cannot solely account for the
2 characteristics of TCE-induced tumors. In addition, H-ras mutation frequency and spectrum of
3 TCE-induced tumors more closely resembles that of spontaneous tumors or of those induced by
4 DCA, and were less similar in comparison to that of TCA-induced tumors. The heterogeneity of
5 TCE-induced tumors is similar to that observed to be induced by a broad category of
6 carcinogens, and to that observed in human liver cancer. Overall, then, it is likely that multiple
7 TCE metabolites, and therefore, multiple pathways, contribute to TCE-induced liver tumors.
8
9 **4.5.7. Mode of Action (MOA) for Trichloroethylene (TCE) Liver Carcinogenicity**

10 This section will discuss the evidentiary support for several hypothesized modes of action
11 for liver carcinogenicity (including mutagenicity and peroxisome proliferation, as well as several
12 additional proposed hypotheses and key events with limited evidence or inadequate experimental
13 support), following the framework outlined in the *Cancer Guidelines* (U.S. EPA, 2005a, b).[6]
14
15 **4.5.7.1. *Mutagenicity***

16 The hypothesis is that TCE acts by a mutagenic mode of action in TCE-induced
17 hepatocarcinogenesis. According to this hypothesis, the key events leading to TCE-induced liver
18 tumor formation constitute the following: TCE oxidative metabolite CH, after being produced in
19 the liver, cause direct alterations to DNA (e.g., mutation, DNA damage, and/or micronuclei
20 induction). Mutagenicity is a well established cause of carcinogenicity.
21
22 ***Experimental support for the hypothesized mode of action.*** The genotoxicity, as described by
23 the ability of TCE, CH, TCA, and DCA to induce mutations, was discussed previously in
24 Section 4.2. The strongest data for mutagenic potential are for CH, thought to be a relatively
25 short-lived intermediate in the metabolism of TCE that is rapidly converted to TCA and TCOH
26 in the liver (see Section 3.3). CH causes a variety of genotoxic effects in available *in vitro* and *in*
27 *vivo* assays, with particularly strong data as to its ability to induce aneuploidy. It has been
28 argued that CH mutagenicity is unlikely to be the cause of TCE carcinogenicity because the
29 concentrations required to elicit these responses are generally quite high, several orders of

[6] As recently reviewed (Guyton et al., 2008) the approach to evaluating mode of action information described in US EPA's *Cancer Guidelines* (2005a, b) considers the issue of human relevance of a hypothesized mode of action in the context of hazard evaluation. This excludes, for example, consideration of toxicokinetic differences across species; specifically, the Cancer Guidelines state, "the toxicokinetic processes that lead to formation or distribution of the active agent to the target tissue are considered in estimating dose but are not part of the mode of action." In addition, information suggesting quantitative differences in the occurrence of a key event between test species and humans are noted for consideration in the dose-response assessment, but is not considered in human relevance determination.

magnitude higher that achieved *in vivo* (Moore and Harrington-Brock, 2000). For example, peak concentrations of CH in the liver of around 2−3 mg/kg have been reported after TCE administration at doses that are hepatocarcinogenic in chronic bioassays (Abbas and Fisher, 1997; Greenberg et al., 1999). Assuming a liver density of about 1 kg/L, these concentrations are orders of magnitude less than the minimum concentrations reported to elicit genotoxic responses in the Ames test and various *in vitro* measures of micronucleus, aneuploidy, and chromosome aberrations, which are in the 100−1,000 mg/L range. However, it is not clear how much of a correspondence is to be expected from concentrations in genotoxicity assays *in vitro* and concentrations *in vivo*, as reported *in vivo* CH concentrations are in whole-liver homogenate while *in vitro* concentrations are in culture media. In addition, a few *in vitro* studies have reported positive results at concentrations as low as 1 or 10 mg/L, including Furnus et al. (1990) for aneuploidy in Chinese hamster CHED cells (10 mg/L), Eichenlaub-Ritter et al. (1996) for bivalent chromosomes in meiosis I in MF1 mouse oocytes (10 mg/L), and Gibson et al. (1995) for cell transformation in Syrian hamster embryo cells after 7 day treatment. Moreover, some *in vivo* genotoxicity assays of CH reported positive results at doses similar to those eliciting a carcinogenic response in chronic bioassays. For example, Nelson and Bull (1988) reported increased DNA single strand breaks at 100 CH mg/kg (oral) in male B6C3F1 mice, although the result was not replicated by Chang et al. (1992). In another example, four of six *in vivo* mouse genotoxicity studies reported that CH induced micronuclei in mouse bone-marrow erythrocytes, with the lowest effective doses in positive studies ranging from 83 to 500 mg/kg (positive: Russo and Levis [1992], Russo et al. [1992], Marrazini et al. [1994], Beland et al. [1999]; negative: Leuschner and Leuschner [1991], Leopardi et al. [1993]). However, the use of i.p. administration in these and many other *in vivo* genotoxicity assays complicates the comparison with carcinogenicity data. Also, it is difficult with the available data to assess the contributions from the genotoxic effects of CH along with those from the genotoxic and nongenotoxic effects of other oxidative metabolites (discussed below in Sections 4.5.5.2 and 4.5.5.3).

Furthermore, altered DNA methylation, another heritable mechanism by which gene expression may be altered, is discussed below in the in Section 4.5.1.3.2.6. As discussed previously, the differential patterns of H-ras mutations observed in liver tumors induced by TCE, TCA, and DCA may be more indicative of tumor selection and tumor progression resulting from exposure to these agents rather than a particular mechanism of tumor induction. The state of the science of cancer and the role of epigenetic changes, in addition to genetic changes, in the initiation and progression of cancer and specifically liver cancer, are discussed in Section E.3.1.

1 Therefore, while data are insufficient to conclude that a mutagenic MOA mediated by CH
2 is operant, a mutagenic MOA, mediated either by CH or by some other oxidative metabolite of
3 TCE, cannot be ruled out.
4

5 **4.5.7.2. *Peroxisome Proliferator Activated Receptor Alpha (PPARα) Receptor Activation***

6 The hypothesis is that TCE acts by a PPARα agonism MOA in TCE-induced
7 hepatocarcinogenesis. According to this hypothesis, the key events leading to TCE-induced liver
8 tumor formation constitute the following: the TCE oxidative metabolite TCA, after being
9 produced in the liver, activates the PPARα receptor, which then causes alterations in cell
10 proliferation and apoptosis and clonal expansion of initiated cells. This MOA is assumed to
11 apply only to the liver.
12

13 ***Experimental support for the hypothesized mode of action.*** Proliferation of peroxisomes and
14 increased activity of a number of related marker enzymes has been observed in rodents treated
15 with TCE, TCA, and DCA. The peroxisome-related effects of TCE are most likely mediated
16 primarily through TCA based on TCE metabolism producing more TCA than DCA and the
17 lower doses of TCA required to elicit a response relative to DCA. However, Bull (2004) and
18 Bull et al. (2004) have recently suggested that peroxisome proliferation occurs at higher
19 exposure levels than those that induce liver tumors for TCE and its metabolites. They report that
20 a direct comparison in the no-effect level or low-effect level for induction of liver tumors in the
21 mouse and several other endpoints shows that, for TCA, liver tumors occur at lower
22 concentrations than peroxisome proliferation *in vivo* but that PPARα activation occurs at a lower
23 dose than either tumor formation or peroxisome proliferation. A similar comparison for DCA
24 shows that liver tumor formation occurs at a much lower exposure level than peroxisome
25 proliferation or PPARα activation. *In vitro* transactivation studies have shown that human and
26 murine versions of PPARα are activated by TCA and DCA, while TCE itself is relatively
27 inactive in the *in vitro* system, at least with mouse PPARα (Maloney and Waxman, 1999; Zhou
28 and Waxman, 1998). In addition, Laughter et al. (2004) reported that the responses of ACO,
29 PCO, and CYP4A induction by TCE, TCA, and DCA were substantially diminished in
30 PPARα-null mice. Therefore, evidence suggests that TCE, through its metabolites TCA and
31 DCA, activate PPARα, and that at doses relevant to TCE-induced hepatocarcinogenesis, the role
32 of TCA in PPARα agonism is likely to predominate.
33 It has been suggested that PPARα receptor activation is both the MOA for TCA liver
34 tumor induction as well as the MOA for TCE liver tumor induction, as a result of the metabolism
35 of TCE to TCA (NRC, 2006; Corton, 2008). Section E.3.4 addressed the status of the PPARα

MOA hypothesis for liver tumor induction and provides a more detailed discussion. However, as discussed previously and in Section E.2.1.10, TCE-induced increases in liver weight have been reported in male and female mice that do not have a functional PPARα receptor (Nakajima et al., 2000). The dose-response for TCE-induced liver weight increases differs from that of TCA (see Section E.2.4.2). The phenotype of the tumors induced by TCE have been described to differ from those by TCA and to be more like those occurring spontaneously in mice, those induced by DCA, or those resulting from a combination of exposures to both DCA and TCA (see Section E.2.4.4). As to whether TCA induces tumors through activation of the PPARα receptor, the tumor phenotype of TCA-induced mouse liver tumors has been reported to have a different pattern of H-ras mutation frequency from other peroxisome proliferators (see Section E.2.4.4; Bull et al., 2002; Stanely et al., 1994; Fox et al., 1990; Hegi et al., 1993). While TCE, DCA, and TCA are weak peroxisome proliferators, liver weight induction from exposure to these agents has not correlated with increases in peroxisomal enzyme activity (e.g., PCO activity) or changes in peroxisomal number or volume. By contrast, as discussed above, liver weight induction from subchronic exposures appears to be a more accurate predictor of carcinogenic response for DCA, TCA and TCE in mice (see also Section E.2.4.4). The database for cancer induction in rats is much more limited than that of mice for determination of a carcinogenic response to these chemicals in the liver and the nature of such a response.

While many compounds known to cause rodent liver tumors with long-term treatment also activate the nuclear receptor PPARα, the mechanisms by which PPARα activation contributes to tumorigenesis are not completely known (Klaunig et al., 2003; NRC, 2006; Yang et al., 2007). As reviewed by Keshava and Caldwell (2006), PPARα activation leads to a highly pleiotropic response and may play a role in toxicity in multiple organs as well as in multiple chronic conditions besides cancer (obesity, atherosclerosis, diabetes, inflammation). Klaunig et al. (2003) and NRC (2006) proposed that the key causal events for PPARα agonist-induced liver carcinogenesis, after PPARα activation, are perturbation of cell proliferation and/or apoptosis, mediated by gene expression changes, and selective clonal expansion. It has also been proposed that sufficient evidence for this MOA consists of evidence of PPARα agonism (i.e., in a receptor assay) in combination with either light- or electron-microscopic evidence for peroxisome proliferation or both increased liver weight and one more of the *in vivo* markers of peroxisome proliferation (Klaunig et al., 2003). However, it should be noted that peroxisome proliferation and *in vivo* markers such as PCO are not considered causal events (Klaunig et al., 2003; NRC, 2006), and that their correlation with carcinogenic potency is poor (Marsman et al., 1988). Therefore, for the purposes of this discussion, peroxisome proliferation and its markers

are considered indicators of PPARα activation, as it is well established that these highly specific effects are mediated through PPARα (Klaunig et al., 2003; Peters et al., 1997).

As recently reviewed by Guyton et al. (2009), recent data suggest that PPARα activation along with these hypothesized causal events may not be sufficient for carcinogenesis. In particular, Yang et al. (2007) reported comparisons between mice treated with Wy-14643 and transgenic mice in which PPARα was constitutively activated in hepatocytes without the presence of ligand. Yang et al. (2007) reported that, in contrast to Wy-14643-treatment, the transgene did not induce liver tumors at 11 months, despite inducing PPARα-mediated effects of a similar type and magnitude seen in response to tumorigenic doses of Wy-14643 in wild-type mice (decreased serum fatty acids, induction of PPARα target genes, altered expression of cell-cycle control genes, and a sustained increase in cellular proliferation). Nonetheless, it is important to discuss the extent to which PPARα activation mediates the effects proposed by Klaunig et al. (2003) and NRC (2006), even if the hypothesized sequence of key events may not be sufficient for carcinogenesis. Investigation continues into additional events that may also contribute, such as nonparenchymal cell activation and micro-RNA-based regulation of protooncogenes (Yang et al., 2007; Shah et al., 2007). Specifically addressed below are gene expression changes, proliferation, clonal expansion, and mutation frequency or spectrum.

With respect to gene expression changes due to TCE, Laughter et al. (2004) evaluated transcript profiles induced by TCE in wild-type and PPARα-null mice. As noted in Sections E.3.4.1.3 and E.3.1.2, there are limitations to the interpretation of such studies, some of which are discussed below. Also noted in Appendix E are discussions of how studies of peroxisome proliferators, indicate of the need for phenotypic anchoring, especially since gene expression is highly variable between studies and within studies using the same experimental paradigm. Section E.3.4 in also provides detailed discussions of the status of the PPARα hypothesis. Of note, all null mice at the highest TCE dose (1,500 mg/kg/d) were moribund prior to the end of the planned 3-week experiment(Laughter et al., 2004), and it was proposed that this may reflect a greater sensitivity in PPARα-null mice to hepatotoxins due to defects in tissue repair abilities. Laughter et al. (2004) also noted that four genes known to be regulated by other peroxisome proliferators also had altered expression with TCE treatment in wild-type, but not null mice. However, in a comparative analysis, Bartosiewicz et al. (2001) concluded that TCE induced a different pattern of transcription than two other peroxisome proliferators, di(2-ethylhexyl) phthalate (DEHP) and clofibrate. In addition, Keshava and Caldwell (2006) compared gene expression data from Wy-14643, dibutyl phthalate (DBP), GEM, and DEHP, and noted a lack of consistent results across PPARα agonists. Thus, available data are insufficient to conclude that TCE gene expression changes are similar to other PPAR agonists, or even that

there are consistent changes (beyond the *in vivo* markers of peroxisome proliferation, such as ACO, PCO, CYP4A, etc.) among different agonists. It should also be noted that Laughter et al. (2004) did not compare baseline (i.e., control levels of) gene expression between null and wild-type control mice, hindering interpretation of these results (Keshava and Caldwell, 2006). The possible relationship between PPARα activation and hypomethylation are discussed below in Section 4.5.7.1.9.

In terms of proliferation, mitosis itself has not been examined in PPARα-null mice, but BrdU incorporation, a measure of DNA synthesis that may reflect cell division, polyploidization, or DNA repair, was observed to be diminished in null mice as compared to wild-type mice at 500 and 1,000 mg/kg/d TCE (Laughter et al., 2004). However, BrdU incorporation in null mice was still about 3-fold higher than controls, although it was not statistically significantly different due to the small number of animals, high variability, and the 2- to 3-fold higher baseline levels of BrdU incorporation in control null mice as compared to control wild-type mice. Therefore, while PPARα appears to contribute to the short-term increase in DNA synthesis observed with TCE treatment, these results cannot rule out other contributing mechanisms. However, since it is likely that both cellular proliferation and increased ploidy contribute to the observed TCE-induced increases in DNA synthesis, it is not clear to whether the observed decrease in BrdU incorporation is due to reduced proliferation, reduced polyploidization, or both.

With respect to clonal expansion, it has been suggested that tumor characteristics such as tincture (i.e., the staining characteristics light microscopy sections of tumor using H&E stains) and oncogene mutation status can be used to associate chemical carcinogens with a particular MOA such as PPARα agonism (Klaunig et al., 2003; NRC, 2006). This approach is problematic primarily because of the lack of specificity of these measures. For example, with respect to tincture, it has been suggested that TCA-induced foci and tumors resemble those of other peroxisome proliferators in basophilia and lack of expression of GGT and GST-pi. However, as discussed in Caldwell and Keshava (2006), the term "basophilic" in describing foci and tumors can be misleading, because, for example, multiple lineages of foci and tumors exhibit basophilia, including those not associated with peroxisome proliferators (Bannasch, 1996; Bannasch et al., 2001; Carter et al., 2003). Moreover, a number of studies indicate that foci and tumors induced by other "classic" peroxisome proliferators may have different phenotypic characteristics from that attributed to the class through studies of WY-14643, including DEHP (Voss et al., 2005) and clofibric acid (Michel et al., 2007). Furthermore, even the combination of GGT and GST-pi negative, basophilic foci are nonspecific to peroxisome proliferators, as they have been observed in rats treated with AfB1 and AfB1 plus PB, none of which are peroxisome proliferators (Kraupp-Grasl et al., 1998; Grasl-Kraupp et al., 1993). Finally, while Bull et al. (2004)

suggested that negative expression of *c-jun* in TCA-induced tumors may be consistent with a characteristic phenotype of peroxisome proliferators, no data could be located to support this statement. Therefore, of phenotypic information does not appear to be reliable for associating a chemical with a PPARα agonism MOA.

Mutation frequency or spectrum in oncogenes has also been suggested to be an indicator of a PPARα agonism MOA being active (NRC, 2006), with the idea being that specific genotypes are being promoted by PPARα agonists. Although not a highly specific marker, H-*ras* codon 61 mutation frequency and spectra data do not support a similarity between mutations in TCE-induced, TCA-, or DCA- tumors and those due to other peroxisome proliferators. For example, while ciprofibrate and methylclofenopate had lower mutation frequencies than historical controls (Hegi et al., 1993; Stanley et al., 1994), TCA-induced tumors had mutation frequencies similar to or higher than historical controls (Ferreira-Gonzalez et al., 1995; Bull et al., 2002). Anna et al. (1994) and Ferreira-Gonzalez et al. (1995) also reported TCE and DCA-induced tumors to have mutation frequencies similar to historical controls, although Bull et al. (2002) reported lower frequencies for these chemicals. However, the data reported by Bull et al. (2002) consist of mixed lesions at different stages of progression, and such differing stages, in addition to differences in genetic background and dose, can influence the frequency of H-*ras* mutations (Stanley et al., 1994). In addition, a greater frequency of mutations was reported in carcinomas than adenomas, and Bull et al. (2002) stated that this suggested that H-*ras* mutations were a late event. Moreover, Fox et al. (1990) noted that tumors induced by phenobarbital, chloroform, and ciprofibrate all had a much lower frequency of H-*ras* gene activation than those that arose spontaneously, so this marker does not have good specificity. Mutation spectrum is similarly of low utility for supporting a PPARα agonism MOA. First, because many peroxisome proliferators been reported to have low frequency of mutations, the comparison of mutation spectrum would be limited to a small fraction tumors. In addition to the low power due to small numbers, the mutation spectrum is relatively nonspecific, as Fox et al. (1990) reported that of the tumors with mutations, the spectra of the peroxisome proliferator ciprofibrate, historical controls, and the genotoxic carcinogen benzidine-2 HCl were similar.

In summary, TCE clearly activates PPARα, and some of the effects contributing to tumorigenesis that Klaunig et al. (2003) and NRC (2006) propose to be the result of PPARα agonism are observed with TCE, TCA, or DCA treatment. While this consistency is supportive a role for PPARα, all of the proposed key causal effects with the exception of PPARα agonism itself are nonspecific, and may be caused by multiple mechanisms. There is more direct evidence that several of these effects, including alterations in gene expression and changes in DNA synthesis, are mediated by multiple mechanisms in the case of TCE, and a causal linkage

1 to PPARα specifically is lacking. Therefore, because, as discussed further in the MOA
2 discussion below, there are multiple lines of evidence supporting the role of multiple pathways
3 of TCE-induced tumorigenesis, the hypothesis that PPARα agonism and the key causal events
4 proposed by Klaunig et al. (2003) and NRC (2006) constitute the sole or predominant MOA for
5 TCE-induced carcinogenesis is considered unlikely.
6 Furthermore, as reviewed by Guyton et al. (2009), recent data strongly suggest that
7 PPARα and key events hypothesized by Klaunig et al. (2003) are not sufficient for
8 carcinogenesis induced by the purported prototypical agonist Wy-14643. Therefore, the
9 proposed PPARα MOA is likely "incomplete" in the sense that the sequence of key events[7]
10 necessary for cancer induction has not been identified. A recent 2-year bioassay of the
11 peroxisome proliferator DEHP showed that it can induce a liver tumor response in mice lacking
12 PPARα similar to that in wild-type mice (Ito et al., 2007). Klaunig et al. (2003) previously
13 concluded that PPARα agonism was the sole MOA for DEHP-induced liver tumorigenesis based
14 on the lack of tumors in PPARα-null mice after 11 months treatment with Wy-14643 (Peters et
15 al., 1997). They also assumed that due to the lack of markers of PPARα agonism in PPARα-null
16 mice after short-term treatment with DEHP (Ward et al., 1998), a long-term study of DEHP in
17 PPARα-null mice would yield the same results as for Wy-14643. However, due the finding by
18 Ito et al. (2007) that PPARα-null mice exposed to DEHP do develop liver tumors, they
19 concluded that DEHP can induce liver tumors by multiple mechanisms (Ito et al., 2007;
20 Takashima et al., 2008). Hence, since there is no 2-year bioassay in PPARα-null mice exposed
21 to TCE or its metabolites, it is not justifiable to use a similar argument based on Peters et al.
22 (1997) and short-term experiments to suggest that the PPARα MOA is operative. Therefore, the
23 conclusion is supported that the hypothesized PPARα MOA is inadequately specified because
24 the data do not adequately show the proposed key events individually being required for
25 hepatocarcinogenesis, nor do they show the sequence of key events collectively to be sufficient
26 for hepatocarcinogenesis.
27

[7] As defined by the U.S. EPA *Cancer Guidelines* (2005a, b) a "key event" is "an empirically observable precursor step that is itself a necessary element of the mode of action or is a biologically based marker for such an element," and the term "mode of action" (MOA) is defined as "a sequence of key events and processes, starting with interaction of an agent with a cell, proceeding through operational and anatomical changes, and resulting in cancer formation." Therefore, a single key event alone is necessary, but not necessarily sufficient for carcinogenesis; however, the *sequence* of key events constituting a MOA needs to be sufficient for carcinogenesis.

1 **4.5.7.3. *Additional Proposed Hypotheses and Key Events with Limited Evidence or***
2 ***Inadequate Experimental Support***

3 Several effects that been hypothesized to be associated with liver cancer induction are
4 discussed in more detail below, including increased liver weight, DNA hypomethylation, and
5 pathways involved in glycogen accumulation such as insulin signaling proteins. As discussed
6 above, TCE and its metabolites reportedly increase nuclear size and ploidy in hepatocytes, and
7 these effects likely account for much of the increases in labeling index and DNA synthesis
8 caused by TCE. Importantly, these changes appear to persist with cessation of treatment, with
9 liver weights, but not nuclear sizes, returning to control levels(Kjellstrand et al., 1983a). In
10 addition, glycogen deposition, DNA synthesis, increases in mitosis, or peroxisomal enzyme
11 activity do not appear correlated with TCE-induced liver weight changes.
12
13 **4.5.7.3.1. *Increased liver weight.*** Increased liver weight or liver/body weight ratios
14 (hepatomegaly) is associated with increased risk of liver tumors in rodents, but it is relatively
15 nonspecific (Allen et al., 2004). The evidence presented above for TCE and its metabolites
16 suggest a similarity in dose-response between liver weight increases at short-term durations of
17 exposure and liver tumor induction observed from chronic exposure. Liver weight increases may
18 results from several concurrent processes that have been associated with increase cancer risk
19 (e.g., hyperplasia, increased ploidy, and glycogen accumulation) and when observed after
20 chronic exposure may result from the increased presence of foci and tumors themselves.
21 Therefore, there are inadequate data to adequately define a MOA hypothesis for
22 hepatocarcinogenesis based on liver weight increases.
23
24 **4.5.7.3.2. *"Negative selection."*** As discussed above, TCE, TCA, and DCA all cause transient
25 increases in DNA synthesis. This DNA synthesis has been assumed to result from proliferation
26 of hepatocytes. However, the dose-related TCA- and DCA-induced increases in liver weight not
27 correlate with patterns of DNA synthesis; moreover, there have been reports that DNA synthesis
28 in individual hepatocytes does not correlate with whole liver DNA synthesis measures
29 (Sanchez and Bull, 1990; Carter et al., 1995). With continued treatment, decreases in DNA
30 synthesis have been reported for DCA (Carter et al., 1995). More importantly, several studies
31 show that transient DNA synthesis is confined to a very small population of cells in the liver in
32 mice exposed to TCE for 10 days or to DCA or TCA for up to 14 days of exposure. Therefore,
33 generalized mitogenic stimulation is not likely to play a role in TCE-induced liver
34 carcinogenesis.

Bull has proposed that the TCE metabolites TCA and DCA may contribute to liver tumor induction through so-called "negative selection" by way of several possible processes (Bull, 2000). First, it is hypothesized that the mitogenic stimulation by continued TCA and DCA exposure is down-regulated in normal hepatocytes, conferring a growth advantage to initiated cells that either do not exhibit the down-regulation of response or are resistant to the down-regulating signals. This is implausible as both the normal rates of cell division in the liver and the TCE-stimulated increases are very low. Polyploidization has been reported to decrease the normal rates of cell division even further. That the transient and relatively low level of DNA synthesis reported for TCE, DCA, and TCA is reflective of proliferation rather than polyploidization is not supported by data on mitosis. A mechanism for such "down-regulation" has not been identified experimentally.

A second proposed contributor to "negative-selection" is direct enhancement by TCA and DCA in the growth of certain populations of initiated cells. While differences in phenotype of end stage tumors have been reported between DCA and TCA, the role of selection and emergence of potentially different foci has not been elucidated. Neither have pathway perturbations been identified that are common to liver cancer in human and rodent for TCE, DCA, and TCA. The selective growth of clones of hepatocytes that may progress fully to cancer is a general feature of cancer and not specific to at TCE, TCA, or DCA MOA.

A third proposed mechanism by which TCE may enhance liver carcinogenesis within this "negative selection" paradigm is through changing apoptosis. However, as stated above, TCE has been reported to either not change apoptosis or to cause a slight increase at high doses. Rather than increases in apoptosis, peroxisome proliferators have been suggested to inhibit apoptosis as part of their carcinogenic MOA. However, the age and species studied appear to greatly affect background rates of apoptosis (Snyder et al., 1995) with the rat having a greater rate of apoptosis than the mouse. DCA has been reported to induce decreases in apoptosis in the mouse (Carter et al., 1995; Snyder et al., 1995). However, the significance of the DCA-induced reduction in apoptosis, from a level that is already inherently low in the mouse, for the MOA for induction of DCA-induce liver cancer is difficult to discern.

Therefore, for a MOA for hepatocarcinogenesis based on "negative selection," there are inadequate data to adequately define the MOA hypothesis, or the available data do not support such a MOA being operative.

4.5.7.3.3. *Polyploidization.* Polyploidization may be an important key event in tumor induction. For example, in addition to TCE, partial hepatectomy, nafenopin, methylclofenopate, DEHP, diethylnitrosamine, *N*-nitrosomorpholine, and various other exposures that contribute to

liver tumor induction also shift the hepatocyte ploidy distribution to be increasingly diploid or polypoid (Hasmal and Roberts, 2000; Styles et al., 1988; Melchiorri et al., 1993; Miller et al., 1996; Vickers et al., 1996). As discussed by Gupta (2000), "[w]orking models indicate that extensive polyploidy could lead to organ failure, as well as to oncogenesis with activation of precancerous cell clones." However, the mechanism(s) by which increased polypoidy enhances carcinogenesis is not currently understood. Due to increased DNA content, polypoid cells will generally have increased gene expression. However, polyploid cells are considered more highly differentiated and generally divide more slowly and are more likely to undergo apoptosis, perhaps thereby indirectly conferring a growth advantage to initiated cells (see Section E.1). Of note is that changes in ploidy have been observed in transgenic mouse models that are also prone to develop liver cancer (see Section E.3.3.1). It is likely that polyploidization occurs with TCE exposure and it is biologically plausible that polyploidization can contribute to liver carcinogenesis, although the mechanism(s) is (are) not known. However, whether polyploidization is necessary for TCE-induced carcinogenesis is not known, as no experiment in which polyploidization specifically is blocked or diminished has been performed and the extent of polyploidization has not been quantified. Therefore, there are inadequate data to adequately define a MOA hypothesis for hepatocarcinogenesis based on polyploidization.

4.5.7.3.4. *Glycogen storage.* As discussed above, several studies have reported that DCA causes accumulation of glycogen in mouse hepatocytes. Such glycogen accumulation has been suggested to be pathogenic, as it is resistant to mobilization by fasting (Kato-Weinstein et al., 1998). In humans, glycogenesis due to glycogen storage disease or poorly controlled diabetes has been associated with increased risk of liver cancer (LaVecchia et al., 1994; Adami et al., 1996; Wideroff et al., 1997; Rake et al., 2002). Glycogen accumulation has also been reported to occur in rats exposed to DCA.

For TCE exposure in mice or rats, glycogen content of hepatocytes has been reported to be somewhat less than or the same as controls, or not remarked upon in the studies. TCA exposure has been reported to decrease glycogen content in rodent hepatocytes while DCA has been reported to increase it (Kato-Weinstein et al., 2001). There is also evidence that DCA-induced increases in glycogen accumulation are not proportional to liver weight increases and only account for a relatively small portion of increases in liver mass. DCA-induced increases in liver weight are not a function of cellular proliferation but probably include hypertrophy associated with polyploidization, increased glycogen deposition and other factors.

While not accounting for increases in liver weight, excess glycogen can still be not only be pathogenic but a predisposing condition for hepatocarcinogenesis. Some hypotheses

1 regarding the possible relationship between glycogenesis and carcinogenesis have been posed
2 that lend them biological plausibility. Evert et al. (2003), using an animal model of hepatocyte
3 exposure to a local hyperinsulinemia from transplanted islets of Langerhans with remaining
4 tissue is hypoinsulinemic, reported that insulin induces alterations resembling preneoplastic foci
5 of altered hepatocytes that develop into hepatocellular tumors in later stages of carcinogenesis.
6 Lingohr et al. (2001) suggest that normal hepatocytes down-regulate insulin-signaling proteins in
7 response to the accumulation of liver glycogen caused by DCA and that the initiated cell
8 population, which does not accumulate glycogen and is promoted by DCA treatment, responds
9 differently from normal hepatocytes to the insulin-like effects of DCA. Bull et al. (Bull et al.,
10 2002) reported increased insulin receptor protein expression in tumor tissues regardless of
11 whether they were induced by TCE, TCA, or DCA. Given the greater activity of DCA relative
12 to TCA on carbohydrate metabolism, it is unclear whether changes in these pathways are causes
13 or simply reflect the effects of tumor progression. Therefore, it is biologically plausible that
14 changes in glycogen status may occur from the opposing actions of TCE metabolites, but
15 changes in glycogen content due to TCE exposure has not been quantitatively studied. The
16 possible contribution of these effects to TCE-induced hepatocarcinogenesis is unclear.
17 Therefore, there are inadequate data to adequately define a MOA hypothesis for TCE-induced
18 hepatocarcinogenesis based on changes in glycogen storage or even data to support increased
19 glycogen storage to result from TCE exposure.
20
21 **4.5.7.3.5.** ***Inactivation of GST-zeta.*** DCA has been shown to inhibit its own metabolism in that
22 pretreatment in rodents prior to a subsequent challenge dose leads to a longer biological half-life
23 (Schultz et al., 2002). This self-inhibition is hypothesized to occur through inactivation of
24 GST-zeta (Schultz et al., 2002). In addition, TCE has been shown to cause the same
25 prolongation of DCA half-life in rodents, suggesting that TCE inhibits GST-zeta, probably
26 through the formation of DCA (Schultz et al., 2002). DCA-induced inhibition of GST-zeta has
27 also been reported in humans, with GST-zeta polymorphisms reported to influence the degree of
28 inactivation (Blackburn et al., 2000; Blackburn et al., 2001; Tzeng et al., 2000). Board et al.
29 (2001) report one variant to have significantly higher activity with DCA as a substrate than other
30 GST-zeta isoforms, which could affect DCA susceptibility.
31 GST-zeta, which is identical to maleylacetoacetate isomerase, is part of the tyrosine
32 catabolism pathway which is disrupted in Type 1 hereditary tyrosinemia, a disease associated
33 with the development of hepatocellular carcinoma at a young age (Tanguay et al., 1996). In
34 particular, GST-zeta metabolizes maleylacetoacetate (MAA) to fumarylacetoacetate (FAA) and
35 maleylacetone (MA) to fumarylacetone(Cornett et al., 1999; Tanguay et al., 1996). It has been

suggested that the increased cancer risk with this disease, as well as through DCA exposure, results from accumulation of MAA and MA, both alkylating agents, or FAA, which displays apoptogenic, mutagenic, aneugenic, and mitogenic activities (Bergeron et al., 2003; Cornett et al., 1999; Jorquera and Tanguay, 2001; Kim et al., 2000; Tanguay et al., 1996). However, the possible effects of DCA through this pathway will depend on whether MAA, MA, or FAA is the greater risk factor, since inhibition of GST-zeta will lead to greater concentrations of MAA and MA and lower concentrations of FAA. Therefore, if MAA is the more active agent, DCA may increase carcinogenic risk, while if FAA is the more active, DCA may decrease carcinogenic risk. Tzeng et al. (2000) propose the later based on the greater genotoxicity of FAA, and in fact suggest that DCA may "merit consideration for trial in the clinical management of hereditary tyrosinemia type 1."

Therefore, TCE-induced inactivation GST-zeta, probably through formation of DCA, may play a role in TCE-induced hepatocarcinogenesis. However, this mode of action is not sufficiently delineated at this point for further evaluation, as even the question of whether its actions through this pathway may increase or decrease cancer risk has yet to be experimentally tested.

4.5.7.3.6. ***Oxidative stress.*** Several studies have attempted to study the possible effects of "oxidative stress" and DNA damage resulting from TCE exposures. The effects of induction of metabolism by TCE, as well as through coexposure to ethanol, have been hypothesized to in itself increase levels of "oxidative stress" as a common effect for both exposures (see Section E.4.2.4). In terms of contributing to a carcinogenic MOA, the term "oxidative stress" is a somewhat nonspecific term, as it is implicated as part of the pathophysiologic events in a multitude of disease processes and is part of the normal physiologic function of the cell and cell signaling. Commonly, it appears to refer to the formation of reactive oxygen species leading to cellular or DNA damage. As discussed above, however, measures of oxidative stress induced by TCE, TCA, and DCA appear to be either not apparent, or at the very most transient and nonpersistent with continued treatment (Larson and Bull, 1992; Channel et al., 1998; Toraason et al., 1999; Parrish et al., 1996). Therefore, while the available data are limited, there is insufficient evidence to support a role for such effects in TCE-induced liver carcinogenesis.

Oxidative stress has been hypothesized to be part of the MOA for peroxisome proliferators, but has been found to neither be correlated with cell proliferation nor carcinogenic potency of peroxisome proliferators (see Section E.3.4.1.1). For instance, Parrish et al. (1996) reported that increases in PCO activity noted for DCA and TCA were not associated with 8OHdG levels (which were unchanged) and also not with changes laurate hydrolase activity

1 observed after either DCA or TCA exposure. The authors concluded that their data do not
2 support an increase in steady state oxidative damage to be associated with TCA initiation of
3 cancer and that extension of treatment to time periods sufficient to insure peroxisome
4 proliferation failed to elevate 8OHdG in hepatic DNA. The authors thus, suggested that
5 peroxisome proliferative properties of TCA were not linked to oxidative stress or carcinogenic
6 response.
7
8 **4.5.7.3.7.** ***Changes in gene expression (e.g., hypomethylation).*** Studies of gene expression as
9 well as considerations for interpretation of studies of using the emerging technologies of DNA,
10 siRNA, and miRNA microarrays for MOA analyses are included in Sections E.3.1.2 and
11 E.3.4.2.2. Caldwell and Keshava (2006) and Keshava and Caldwell (2006) report on both
12 genetic expression studies and studies of changes in methylation status induced by TCE and its
13 metabolites as well as differences and difficulties in the patterns of gene expression between
14 differing PPARα agonists. In particular are concerns for the interpretation of studies which
15 employ pooling of data as well as interpretation of "snapshots in time of multiple gene changes."
16 For instance, in the Laughter et al. (2004) study, it is not clear whether transcription arrays were
17 performed on pooled data as well as the issue of phenotypic anchoring as data on percent
18 liver/body weight indicates significant variability within TCE treatment groups, especially in
19 PPARα-null mice. For studies of gene expression using microarrays Bartosiewicz et al. (2001)
20 used a screening analysis of 148 genes for xenobiotic-metabolizing enzymes, DNA repair
21 enzymes, heat shock proteins, cytokines, and housekeeping gene expression patterns in the liver
22 in response TCE. The TCE-induced gene induction was reported to be highly selective; only
23 Hsp 25 and 86 and Cyp2a were up-regulated at the highest dose tested. Collier et al. (2003)
24 reported differentially expressed mRNA transcripts in embryonic hearts from Sprague-Dawley
25 rats exposed to TCE with sequences down-regulated with TCE exposure appearing to be those
26 associated with cellular housekeeping, cell adhesion, and developmental processes. TCE was
27 reported to induce up-regulated expression of numerous stress-response and homeostatic genes.
28 For the Laughter et al. (2004) study, transcription profiles using macroarrays containing
29 approximately 1,200 genes were reported in response to TCE exposure with 43 genes reported to
30 be significantly altered in the TCE-treated wild-type mice and 67 genes significantly altered in
31 the TCE-treated PPARα knockout mice. However, the interpretation of this information is
32 difficult because in general, PPARα knockout mice have been reported to be more sensitive to a
33 number of hepatotoxins partly because of defects in the ability to effectively repair tissue damage
34 in the liver (Shankar et al., 2003; Mehendale, 2000) and because a comparison of gene
35 expression profiles between controls (wild-type and PPARα knockout) were not reported. As

reported by Voss et al. (2006), dose-, time course-, species-, and strain-related differences should be considered in interpreting gene array data. The comparison of differing PPARα agonists presented in Keshava and Caldwell (2006) illustrate the pleiotropic and varying liver responses of the PPARα receptor to various agonists, but did not imply that these responses were responsible for carcinogenesis.

As discussed above in Section E.3.3.5, Aberrant DNA methylation is a common hallmark of all types of cancers, with hypermethylation of the promoter region of specific tumor suppressor genes and DNA repair genes leading to their silencing (an effect similar to their mutation) and genome-wide hypomethylation (Ballestar and Esteller, 2002; Berger and Daxenbichler, 2002; Herman et al., 1998; Pereira et al., 2004; Rhee et al., 2002). Whether DNA methylation is a consequence or cause of cancer is a long-standing issue (Ballestar and Esteller, 2002). Fraga et al. (2004, 2005) reported global loss of monoacetylation and trimethylation of histone H4 as a common hallmark of human tumor cells; they suggested, however, that genomewide loss of 5-methylcytosine (associated with the acquisition of a transformed phenotype) exists not as a static predefined value throughout the process of carcinogenesis but rather as a dynamic parameter (i.e., decreases are seen early and become more marked in later stages).

DNA methylation is a naturally occurring epigenetic mechanism for modulating gene expression, and disruption of this mechanism is known to be relevant to human carcinogenesis. As reviewed by Calvisi et al. (2007),

> [a]berrant DNA methylation occurs commonly in human cancers in the forms of genome-wide hypomethylation and regional hypermethylation. Global DNA hypomethylation (also known as demethylation) is associated with activation of protooncogenes, such as c-Jun, c-Myc, and c-HA-Ras, and generation of genomic instability. Hypermethylation on CpG islands located in the promoter regions of tumor suppressor genes results in transcriptional silencing and genomic instability.

While clearly associated with cancer, it has not been conclusively established whether these epigenetic changes play a causative role or are merely a consequence of transformation (Tryndyak et al., 2006). However, as Calvisi et al. (2007) note, “Current evidence suggests that hypomethylation might promote malignant transformation via multiple mechanisms, including chromosome instability, activation of protooncogenes, reactivation of transposable elements, and loss of imprinting.”

Although little is known about how it occurs, a hypothesis has also been proposed that that the toxicity of TCE and its metabolites may arise from its effects on DNA methylation status. In regard to methylation studies, many are coexposure studies as they have been

conducted in initiated animals with some studies being very limited in their reporting and conduct. Caldwell and Keshava (2006) review the body of work regarding TCE, DCA, and TCA. Methionine status has been noted to affect the emergence of liver tumors (Counts et al., 1996). Tao et al. (2000) and Pereira et al. (2004) have studied the effects of excess methionine in the diet to see if it has the opposite effects as a deficiency (i.e., and reduction in a carcinogenic response rather than enhancement). However, Tao et al. (2000) report that the administration of excess methionine in the diet is not without effect and can result in percent liver/body weight ratios. Pereira et al. (2004) report that methionine treatment alone at the 8 g/kg level was reported to increase liver weight, decrease lauryl-CoA activity and to increase DNA methylation.

Pereira et al. (2004) reported that very high level of methionine supplementation to an AIN-760A diet, affected the number of foci and adenomas after 44 weeks of coexposure to 3.2 g/L DCA. However, while the highest concentration of methionine (8.0 g/kg) was reported to decrease both the number of DCA-induce foci and adenomas, the lower level of methionine coexposure (4.0 g/kg) increased the incidence of foci. Coexposure of methionine (4.0 or 8.0 g/kg) with 3.2 g/L DCA was reported to decrease by ~25% DCA-induced glycogen accumulation, increase mortality, but not to have much of an effect on peroxisome enzyme activity (which was not elevated by more than 33% over control for DCA exposure alone). The authors suggested that their data indicate that methioninine treatment slowed the progression of foci to tumors. Given that increasing hypomethylation is associated with tumor progression, decreased hypomethylation from large doses of methionine are consistent with a slowing of progression. Whether, these results would be similar for lower concentrations of DCA and lower concentrations of methionine that were administered to mice for longer durations of exposure, cannot be ascertained from these data. It is possible that in a longer-term study, the number of tumors would be similar. Finally, a decrease in tumor progression by methionine supplementation is not shown to be a specific event for the MOA for DCA-induced liver carcinogenicity.

Tao et al. (2000) reported that 7 days of gavage dosing of TCE (1,000 mg/kg in corn oil), TCA (500 mg/kg, neutralized aqueous solution), and DCA (500 mg/kg, neutralized aqueous solution) in 8-week old female B6C3F1 mice resulted in not only increased liver weight but also increased hypomethylation of the promoter regions of c-*jun* and c-*myc* genes in whole liver DNA. However, data were shown for 1−2 mice per treatment. Treatment with methionine was reported to abrogate this response only at a 300 mg/kg i.p dose with 0−100 mg/kg doses of methionine having no effect. Ge et al. (2001) reported DCA- and TCA-induced DNA hypomethylation and cell proliferation in the liver of female mice at 500 mg/kg and decreased methylation of the c-*myc* promoter region in liver, kidney and urinary bladder. However,

increased cell proliferation preceded hypomethylation. Ge et al. (2002) also reported hypomethylation of the c-*myc* gene in the liver after exposure to the peroxisome proliferators 2,4-dichlorophenoxyacetic acid (1,680 ppm), DBP (20,000 ppm), gemfibrozil (8,000 ppm), and Wy-14,643 (50−500 ppm, with no effect at 5 or 10 ppm) after 6 days in the diet. Caldwell and Keshava (2006) concluded that hypomethylation did not appear to be a chemical-specific effect at these concentrations. As noted Section E.3.3.5, chemical exposure to a number of differing carcinogens have been reported to lead to progressive loss of DNA methylation..

After initiation by *N*-methyl-*N*-nitrosourea (25 mg/kg) and exposure to 20 mmol/L DCA or TCA (46 weeks), Tao et al. (2004) report similar hypomethylation of total mouse liver DNA by DCA and TCA with tumor DNA showing greater hypomethylation. A similar effect was noted for the differentially methylated region-2 of the insulin-like growth factor-II (IGF-II) gene. The authors suggest that hypomethylation of total liver DNA and the IGF-II gene found in nontumorous liver tissue would appear to be the result of a more prolonged activity and not cell proliferation, while hypomethylation of tumors could be an intrinsic property of the tumors. As pointed out by Caldwell and Keshava (2006) over expression of IGF-II gene in liver tumors and preneoplastic foci has been shown in both animal models of hepatocarcinogenesis and humans, and may enhance tumor growth, acting via the over-expressed IGF-I receptor (Scharf et al., 2001; Werner and Le Roith, 2000).

Diminished hypomethylation was observed in Wy-14643-treated PPARα-null mice as compared to wild-type mice, suggestive of involvement of PPARα in mediating hypomethylation (Pogribny et al., 2007), but it is unclear how relevant these results are to TCE and its metabolites. First, the doses of Wy-14643 administered are associated with substantial liver necrosis and mortality with long-term treatment (Woods et al., 2007), adding confounding factors the interpretation of their results. Hypomethylation by Wy-14643 progressively increased with time up to 5 months (Pogribny et al., 2007), consistent with the sustained DNA synthesis caused by Wy-14643 and a role for proliferation in causing hypomethylation. Regardless, as discussed above, it is unlikely that PPARα is the mediator of the observed transient increase in DNA synthesis by DCA, so even if it is important for hypomethylation by TCA, there may be more than one pathway for this effect.

To summarize, aberrant DNA methylation status, including hypomethylation, is clearly associated with both human and rodent carcinogenesis. Hypomethylation itself appears to be sufficient for carcinogenesis, as diets deficient in choline and methionine that induce hypomethylation have been shown to cause liver tumors in both rats and mice (Ghoshal and Farber, 1984; Mikol et al., 1983; Henning and Swendseid, 1996; Wainfan and Poirier, 1992). However, it is not known to what extent hypomethylation is necessary for TCE-induced

carcinogenesis. However, as noted by Bull (2004) and Bull et al. (2004), the doses of TCA and DCA that have been tested for induction of hypomethylation are quite high compared to doses at which tumor induction occurs—at least 500 mg/kg/d. Whether these effects are still manifest at lower doses relevant to TCE carcinogenicity, particularly with respect to DCA, has not been investigated. Finally, the role of PPARα in modulating hypomethylation, possibly through increased DNA synthesis as suggested by experiments with Wy-14643, are unknown for TCE and its metabolites.

4.5.7.3.8. ***Cytotoxicity.*** Cytotoxicity and subsequent induction of reparative hyperplasia have been proposed as key events for a number of chlorinated solvents, such as chloroform and carbon tetrachloride.. However, as discussed above and discussed by Bull (2004) and Bull et al. (2004), TCE treatment at doses relevant to liver carcinogenicity results in relatively low cytotoxicity. While a number of histological changes with TCE exposure are observed, in most cases necrosis is minimal or mild, associated with vehicle effects, and with relatively low prevalence. This is consistent with the low prevalence of necrosis observed with TCA and DCA treatment at doses relevant to TCE exposure. Therefore, it is unlikely that cytotoxicity and reparative hyperplasia play a significant role in TCE carcinogenicity

4.5.7.4. *Mode of Action (MOA) Conclusions*

Overall, although a role for many of the proposed key events discussed above cannot be ruled out, there are inadequate data to support the conclusion that any of the particular MOA hypotheses reviewed above are operant. Thus, the MOA of liver tumors induced by TCE is considered unknown at this time, and the answer to the first key question "***1. Is the hypothesized mode of action sufficiently supported in the test animals?"*** is "no" at this time. Consequently, the other key questions of "***2. Is the hypothesized mode of action relevant to humans?***" and "***3. Which populations or lifestages can be particularly susceptible to the hypothesized mode of action?***" will not be discussed in a MOA-specific manner. Rather, they are discussed below in more general terms, first qualitatively and then quantitatively, using available relevant data.

4.5.7.4.1. ***Qualitative human relevance and susceptibility.*** No data exist that suggests that TCE-induced liver tumorigenesis is caused by processes that irrelevant in humans. In addition, as discussed above, several of the other effects such as polyploidization, changes in glycogen storage, and inhibition of GST-zeta—are either clearly related to human carcinogenesis or areas of active research as to their potential roles. For example, the effects of DCA on glycogen storage parallel the observation that individuals with conditions that lead to glycogenesis appear

to be at an increased risk of liver cancer (LaVecchia et al., 1994; Adami et al., 1996; Wideroff et al., 1997; Rake et al., 2002). In addition, there may be some relationship between the effects of DCA and the mechanism of increased liver tumor risk in childhood in those with Type 1 hereditary tyrosinemia, though the hypotheses needs to be tested experimentally. Similarly, with respect to PPARα activation and downstream events hypothesized to be causally related to liver carcinogenesis, it is generally acknowledged that "a point in the rat/mouse key events cascade where the pathway is biologically precluded in humans cannot be identified, in principle" (Klaunig et al, 2003; NRC, 2006).

In terms of human relevance and susceptibility, it is also useful to briefly review what is known about human HCC. A number of risk factors have been identified for human hepatocellular carcinoma, including ethanol consumption, hepatitis B and C virus infection, aflatoxin B1 exposure, and, more recently, diabetes and perhaps obesity (El-Serag and Rudolph, 2007). However, it is also estimated that a substantial minority of HCC patients, perhaps 15 to 50%, have no established risk factors (El-Serag and Rudolph, 2007). In addition, cirrhosis is present in a large proportion of HCC patients, but the prevalence of HCC without underlying cirrhosis, while not precisely known, is still significant, with estimates based on relatively small samples ranging from 7 to 54% (Fattovisch, 2004).

However, despite the identification of numerous factors that appear to play a role in the human risk of HCC, the mechanisms are still largely unclear (Yeh et al., 2007). Interestingly, the observation by Leakey et al. (2003a, b) that body weight significantly and strongly impacts background liver tumor rates in B6C3F1 mice parallels the observed epidemiologic associations between liver cancer and obesity (review in El-Serag and Rudolph [2007]). This concordance suggests that similar pathways may be involved in spontaneous liver tumor induction between mice and humans. The extent to which TCE exposure may interact with known risk factors for HCC cannot be determined at this point, but several hypotheses can be posed based on existing data. If TCE affects some of the same pathways involved in human HCC, as suggested in the discussion of several TCE-induced effects above, then TCE exposure may lead a risk that is additive to background.

As discussed above, there are several parallels between the possible key events in TCE-induced liver tumors in mice and what is known about mechanisms of human HCC, though none have been experimentally tested. Altered ploidy distribution and DNA hypomethylation are commonly observed in human HCC (Zeppa et al., 1998; Lin et al., 2003; Calvisi et al., 2007). Interestingly, El-Serag and Rudolph (2007) have been suggested that the risk of HCC increases with cirrhosis in part because the liver parenchymal cells have decreased proliferative capacity, resulting in an altered milieu that promotes tumor cell proliferation. This description suggests a

similarity in mode of action, though via different mechanisms, with the "negative selection" hypothesis proposed by Bull (2000) for TCE and its metabolites although for TCE changes in apoptosis and cell proliferation have not been noted or examined to such an extent to provide evidence of a similar environment. Increased ploidy decreases proliferative capacity, so that may be another mechanism through which the effects of TCE mimic the conditions thought to facilitate the induction of human HCC.

In sum, from the perspective of hazard characterization, the available data support the conclusion that the mode of action for TCE-induced mouse liver tumors is relevant to humans. No data suggest that any of the key events are biologically precluded in humans, and a number of qualitative parallels exist between hypotheses for the mode of action in mice and what is known about the etiology and induction of human HCC. A number of risk factors have been identified that appear to modulate the risk of human HCC, and these may also modulate the susceptibility to the effects from TCE exposure. As noted in Section E.4, TCE exposure in the human population is accompanied not only by external exposures to its metabolites, but brominated analogues of those metabolites that are also rodent carcinogens, a number of chlorinate solvents that are hepatocarcinogenic and alcohol consumption. The types of tumors and the heterogeneity of tumors induced by TCE in rodents parallel those observed in humans (see Section E.3.1.8). The pathways identified for induction of cancer in humans for cancer are similar to those for the induction of liver cancer (see Section E.3.2.1). However, while risk factors have been identified for human liver cancer that have similarities to TCE-induced effects and those of its metabolites, both the mechanism for human liver cancer induction and that for TCE-induced liver carcinogenesis in rodents are not known.

4.5.7.4.2. ***Quantitative species differences.*** As a precursor to the discussion of quantitative differences between humans and rodents and among humans, it should be noted that an adequate explanation for the difference in response for TCE-liver cancer induction between rats and mice has yet to be established or for that difference to be adequately described given the limitations in the rat database. For TCA, there is only one available long-term study in rats that, while suggestive that TCA is less potent in rats than mice, is insufficient to determine if there was a TCA-induced effect or what its magnitude may be. While some have proposed that the lower rate of TCA formation in rats relative to mice would explain the species difference, PBPK modeling suggests that the differences (3−5-fold) may be inadequate to fully explain the differences in carcinogenic potency. Moreover, inferences from comparing the effects of TCE and TCA on liver weight, using PBPK model-based estimates of TCA internal dose metrics as a result of TCE or TCA administration, indicate that TCA is not likely to play a predominant role

in hepatomegaly. Combined with the qualitative correlation between rodent hepatomegaly and hepatocarcinogenesis observed across many chemicals, this suggests that TCA similarly is not a predominant factor in TCE-induced hepatocarcinogenesis. Indeed, there are multiple lines of evidence that TCA is insufficient to account for TCE-induced tumors, including data on tumor phenotype (e.g., c-Jun immunostaining) and genotype (e.g., H-ras mutation frequency and spectrum). For DCA, only a single experiment in rats is available (reported in two publications), and although it suggests lower hepatocarcinogenic potency in rats relative to mice, its relatively low power limits the inferences that can be made as to species differences.

As TCA induces peroxisome proliferation in the mouse and the rat, some have suggested that difference in peroxisomal enzyme induction is responsible for the difference in susceptibility to TCA liver carcinogenesis. The study of DeAngelo et al. (1989) has been cited in the literature as providing evidence of differences between rats and mice for peroxisomal response to TCA. However, data from the most resistant strain of rat (Sprague-Dawley) have been cited in comparisons of peroxisomal enzyme effects but the Osborne-Mendel and F344 rat were not refractory and showed increased PCO activity so it is not correct to state that the rat is refractory to TCA-induction of peroxisome activity (see Section E.2.3.1.5). In addition, as discussed above, inferences based on PCO activity are limited by its high variability, even in control animals, as well as its not necessarily being predictive of the peroxisome number or cytoplasmic volume.

The same assumption of lower species sensitivity by measuring peroxisome proliferation has been applied to humans, as peroxisome proliferation caused by therapeutic PPARα agonists such as fibrates in humans is generally lower (<2-fold induction) than that observed in rodents (20- to 50-fold induction). However, as mentioned above, it is known that peroxisome proliferation is not a good predictor of potency (Marsman et al., 1988).

Limited data exist on the relative sensitivity of the occurrence of key events for liver tumor induction between mice and humans and among humans. Pharmacokinetic differences are addressed with PBPK modeling to the extent that data allow, so the discussion here will concentrate on pharmacodynamic differences. Most striking is the difference in "background" rates of liver tumors. Data from NTP indicates that control B6C3F1 mice in 2-year bioassays have a background incidence of hepatocellular carcinomas of 26% in males and 10% in females, with higher incidences for combined hepatocellular adenomas and carcinomas (Maronpot, 2007). However, as discussed above, Leakey et al. (2003a, b) report that the background incidence rates are very dependent on the weight of the mice. By contrast, the estimated lifetime risk of liver and biliary tract cancer in the United States (about 75% of which are hepatocellular carcinomas) is 0.97% for men and 0.43% for women (Ries et al., 2008). However, regions of the world

where additional risk factors (hepatitis infection, alflatoxin exposure) have high prevalence have liver cancer incidences up to more than 6-fold greater than the United States (Ferlay et al., 2004). Therefore, one possible quantitative difference that can be flagged for use in dose-response assessment is the background rate of liver tumors between species. Biologically-based dose-response modeling by Chen (2000) suggested that the data were consistent with a purely promotional model in which potency would be proportional to background tumor incidence. However, it is notable that male Swiss mice, which have lower background liver tumor rates than the B6C3F1 strain, were also positive in one long-term bioassay (Maltoni et al., 1986).

Similarly, in terms of intraspecies susceptibility, to the extent that TCE may independently promote pre-existing initiated cells, it can be hypothesized that those with greater risk for developing HCC due to one more of the known risk factors would have a proportional increase in the any contributions from TCE exposure. In addition, in both humans and mice, males appear to be at increased risk of liver cancer, possibly due to sexually dimorphism in inflammatory responses (Lawrence et al., 2007; Naugler et al., 2007; Rakoff-Nahoun and Medzhitov, 2007), suggesting that men may also be more susceptible to TCE-induced liver tumorigenesis than women. It has been observed that human HCC is highly heterogeneous histologically, but within patients and between patients, studies are only beginning to distinguish the different pathways that may be responsible for this heterogeneity (Feitelson et al., 2002; Chen et al., 2002; Yeh et al., 2007).

Appropriate quantitative data are generally lacking on interspecies differences in the occurrence of most other proposed key events, although many have argued that there are significant quantitative differences between rodents and humans related to PPARα activation (Klaunig et al., 2003; NRC, 2006). For instance, it has been suggested that lower levels of PPARα receptor in human hepatocytes relative to rodent hepatocytes contributes to lower human sensitivity (Tugwood et al., 1996; Palmer et al., 1998; Klaunig et al., 2003). However, out of a small sample of human livers ($n = 6$) show similar protein levels to mice (Walgren et al., 2000a). Another proposed species difference has been ligand affinity, but while transactivation assays showed greater affinity of Wy-14643 and perfluorooctanoic acid for rodent relative to human PPARα, they showed TCA and DCA had a similar affinities between species (Maloney and Waxman, 1999). Furthermore, it is not clear that receptor-ligand kinetics (capacity and affinity) are rate-limiting for eliciting hepatocarcinogenic effects, as it is known that maximal receptor occupation is not necessary for a maximal receptor mediated response (Stephenson, 1956, see also review by Danhof et al., 2007).

There is also limited *in vivo* and *in vitro* data suggesting that increases in cell proliferation mediated by PPARα agonists are diminished in humans and other primates relative

to rodents (Klaunig et al., 2003; NRC, 2006; Hoivik et al., 2004). However, Walgren et al. (2000b) reported that TCA and DCA were not mitogenic in either human or rodent hepatocytes *in vitro*. Furthermore, TCE, TCA, and DCA all induce only transient increases in cell proliferation, so the relevance to TCE of interspecies differences from PPARα agonists that to produce sustained proliferation, such as Wy-14643, is not clear. In addition, comparisons between primate and rodent models should take into account the differences in the ability to respond to any mitogenic stimulation (see Section E.3.2). Primate and human liver respond differently (and much more slowly) to a stimulus such as partial hepatectomy.

Recent studies in "humanized" mice (PPARα-null mice in which a human PPARα gene was subsequently inserted and expressed in the liver) reported that treatment with a PPARα agonist lead to greatly lower incidence of liver tumors as compared to wild-type mice (Morimura et al., 2006). However, these experiments were performed with WY-14643 at a dose causing systemic toxicity (reduced growth and survival), had a duration of less than 1 year, and involved a limited number of animals. In addition, because liver tumors in mice at less than 1 year are extremely rare, the finding a one adenoma in WY-14643-treated humanized mice suggests carcinogenic potential that could be further realized with continued treatment (Keshava and Caldwell, 2006). In addition, Yang et al. (2007) recently noted that let-7C, a microRNA involved in cell growth and thought to be a regulatory target of PPARα (Shah, 2008), was inhibited by Wy-14643 in wild-type mice, but not in "humanized mice" in which had human PPARα was expressed throughout the body on a PPARα-null background. However, these humanized mice had about a 20-fold higher baseline expression of let-7C, as reported in control mice, potentially masking any treatment effects. More generally, it is not known to what extent PPARα-related events are rate-limiting in TCE-induced liver tumorigenesis, for which multiple pathways appear to be operative. So even if quantitative differences mediated by PPARα were well estimated, they would not be directly usable for dose-response assessment in the absence of way to integrate the contributions from the different pathways.

In sum, the only quantitative data and inter- and intraspecies susceptibility suitable for consideration in dose-response assessment are differences background liver tumor risk. These may modulate the effects of TCE if relative risk, rather than additional risk, is the appropriate common inter- and intraspecies metric. However, the extent to which relative risk would provide a more accurate estimate of human risk is unknown.