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DRINKING WATER CRITERIA DOCUMENT FOR
POLYCYCLIC AROMATIC HYDROCARBONS (PAHS)

Prepared for

OFFICE OF WATER

Prepared by

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This document provides the health effects basis to be considered in establishing the MCLG. To achieve this objective, data on pharmacokinetics, human exposure, acute and chronic toxicity to animals and humans, epidemiology and mechanisms of toxicity are evaluated. Specific emphasis is placed on literature data providing dose-response information. Thus, while the literature search and evaluation performed in support of this document has been comprehensive, only the reports considered most pertinent in the derivation of the MCLG are cited in the document. The comprehensive literature data base in support of this document includes information published up to 1985; however, more recent data may have been added during the review process.

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-Anthracene
-Benz[a]anthracene
-Benzo[a]pyrene
-Benzo[b] fluoranthene

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FOREWORD

Section 1412 (b)(3)(A) of the Safe Drinking Water Act, as amended in 1986, requires the Administrator of the Environmental Protection Agency to publish maximum contaminant level goals (MCLGs) and promulgate National Primary Drinking Water Regulations for each contaminant, which, in the judgment of the Administrator, may have an adverse effect on public health and which is known or anticipated to occur in public water systems. The MCLG is nonenforceable and is set at a level at which no known or anticipated adverse health effects in humans occur and which allows for an adequate margin of safety. Factors considered in setting the MCLG include health effects data and sources of exposure other than drinking water.

This document provides the health effects basis to be considered in establishing the MCLG. To achieve this objective, data on pharmacokinetics, human exposure, acute and chronic toxicity to animals and humans, epidemiology and mechanisms of toxicity are evaluated. Specific emphasis is placed on literature data providing dose-response information. Thus, while the literature search and evaluation performed in support of this document has been comprehensive, only the reports considered most pertinent in the derivation of the MCLG are cited in the document. The comprehensive literature data base in support of this document includes information published up to 1985; however, more recent data may have been added during the review process.

When adequate health effects data exist, Health Advisory values for less than lifetime exposures (1-day, 10-day and longer-term, ~10% of an individual's lifetime) are included in this document. These values are not used in setting the MCLG, but serve as informal guidance to municipalities and other organizations when emergency spills or contamination situations occur.

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LIST OF ABBREVIATIONS

AHH	Aryl hydrocarbon hydroxylase
B[a]P	Benzo[a]pyrene
BUN	Blood urea nitrogen
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic acid
DWEL	Drinking water equivalent level
GI	Gastrointestinal
GLC	Gas liquid chromatography
GSH	Glutathione
HA	Health advisory
HDL	High density lipoprotein
HPLC	High performance liquid chromatography
i.m.	Intramuscular
i.p.	Intraperitoneal
i.v.	Intravenous
LDL	Low density lipoprotein
LOAEL	Lowest-observed-adverse-effect level
LOEL	Lowest-observed-effect level
MFO	Mixed function oxidase
NOAEL	No-observed-adverse-effect level
NOEL	No-observed-effect level
PAH	Polycyclic aromatic hydrocarbon
PEG	Polyethylene glycol
q_1^*	Potency slope for carcinogenic risk estimate
RfD	Reference dose
RNA	Ribonucleic acid
s.c.	Subcutaneous
SGOT	Serum glutamic oxaloacetic transaminase
SGPT	Serum glutamic pyruvic transaminase
TPA	12-o-Tetradecanoyl phorbol-13-acetate
UV	Ultraviolet

I. SUMMARY

This document includes information on 15 polycyclic aromatic hydrocarbons (PAHs) included among those compounds identified as priority (water) pollutants by the U.S. EPA.

PAHs are a class of diverse compounds that are formed during the incomplete combustion of organic material. They are ubiquitous and enter the environment from many sources. Generally PAHs are not very soluble in water. Solubility decreases greatly with increasing molecular weight. The log of the octanol/water partition coefficient (P) also increases rapidly with increasing molecular weight. This increases adsorption to particulate matter and exposure of the compounds to microbial degradation. However, it has been found that PAHs with more than four aromatic rings are less amenable to microbial action. The larger P values also increase the probability of bioaccumulation. Volatilization does not appear to be a significant route of exit from aquatic systems due to the low vapor pressures of PAHs.

As they are highly lipid soluble, PAHs readily pass through cellular membranes. However, the rate of absorption is increased when the PAHs are present in an oil carrier vehicle. This is particularly true for oral and dermal routes of exposure.

The highly lipid soluble nature of PAH compounds results in their distribution throughout the body in fatty tissues. The primary sites of storage in the body have been found to be similar in a variety of mammalian species. These include the kidneys, liver and fat with some accumulation occurring in the spleen, adrenals and ovaries.

PAH compounds are metabolized by the MFO system of enzymes associated with cytochrome P-450. The liver has the highest activity for this system although MFO activity is found in other organs such as kidneys, lungs and skin. PAH coumpounds have been found to induce increased levels of activity as well as synthesis of isozymes of cytochrome P-450-associated enzymes.

The major routes of excretion for PAH compounds are hepatobiliary and urinary. Some evidence for mammary gland excretion also exists. Although PAHs present in the body tend to be present in fatty tissues, available evidence does not indicate extensive bioaccumulation of PAHs in these or other tissues.

The primary focus of research on biologic effects of PAHs has been on their carcinogenicity. There is generally a lack of research data on the noncarcinogenic toxic effects of oral exposures to PAH compounds. What little research has been conducted has centered on three or four compounds in this large class. Target organs are diverse, probably due to the wide distribution of PAH compounds throughout the body. Toxicity centers primarily on hematopoietic and lymphoid systems. Immunosuppression measured in various experimental systems has been observed following exposure to a number of PAHs. Nonoral exposure to PAHs has also been observed to effect changes in lymphoid and hematopoietic systems.

There is an extensive data base on the carcinogenicity of selected PAHs. Most studies, however, employed dermal, inhalation or subcutaneous rather than oral exposure. Overall there is a great deal of variation in carcinogenic potential among this class of compounds. PAHs may produce

tumors in the vicinity where they are introduced; that is, oral exposure produces stomach tumors and inhalation produces lung and upper stomach tumors. Distant site tumors may form dependent on systemic distribution of metabolites and/or metabolism at target tissues removed from site of introduction. Most PAHs have been observed to produce genotoxic effects in one or more test systems. There are data indicating that some PAHs can serve as promoters or cocarcinogens.

Reproductive and teratogenic effects have not been well studied in these compounds. There is evidence, however, that ingestion of benzo[a]pyrene by pregnant mice results in reproductive deficits in the F_1 generation and that this compound produces oōtoxicity.

While very little information exists on the effects of specific PAHs on humans, there are numerous reports linking exposure to environmental and occupationally generated PAH-containing mixtures to human health effects. These mixtures include coal tar, soots, coke oven emissions and cigarette smoke.

The ability of a PAH to induce carcinogenic responses depends on its distribution to target organs, the presence of potentially reactive areas in its structure and its potential for transformation to reactive electrophiles. This last factor is species and tissue dependent, and also, to some extent, a function of inducibility of the cytochrome P-450-associated enzymes.

Target tissues for PAH toxicity other than carcinogenesis are generally those engaged in active DNA synthesis. Mechanisms involving PAH-mediated DNA-damage or suppression of DNA synthesis have been proposed for some target organs. Naphthalene exposure is associated with anemias and cataract formation; this latter effect is not seen with other PAHs.

There were no data suitable for calculation of 1- or 10-day health advisories for any PAH described in this document. For all PAHs in this document classified as B2, probable human carcinogen, data were insufficient for calculation of DWELs. Subchronic studies (90-day gavage exposure in CD-1 mice) were used as the bases for the following DWELs: anthracene, 10.5 mg/ℓ; fluoranthene, 1.4 mg/ℓ; fluorene, 1.4 mg/ℓ; pyrene, 1.0 mg/ℓ. Although there is a 90-day study on acenaphthylene, only frank effects were reported; data were, thus, not appropriate for derivation of criteria. Several studies have been evaluated as the basis for a DWEL for naphthalene; consensus, however, has not been reached as to the critical study.

Evaluation of carcinogenicity data prompted the following classification of these PAHs: Group D, not classifiable as to human carcinogenicity -- acenaphthylene, anthracene, benzo[g,h,i]perylene, fluoranthene, fluorene, naphthalene, phenanthrene and pyrene; Group B2, probable human carcinogen -- benz[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene; benzo[a]-pyrene, chrysene, dibenz[a,h]anthracene and indeno[1,2,3-cd]pyrene.

Dose-response data for benzo[a]pyrene carcinogenicity were used to derive an upper bound estimate of the slope of the dose-response curve at low doses. This evaluation, based on the linearized multistage procedure,

resulted in an upper-bound slope factor of 11.5 mg/kg/day. This use of the multistage model was inadequate because the experimental high-dose data were excluded from the analysis and a single exposure time was assumed. Other experimental data and modeling techniques were used. Nine risk estimates were calculated from three different studies in two species of outbred rodents. Several different models and data sets were selected. All nine slope factors spanned less than one order of magnitude; four estimates were selected from these nine. The data, from which these estimates are derived, are considered to be less-than-optimal, but acceptable. These selected studies have several commonalities, including mode of administration, tumor sites, tumor types and the presumed mechanisms of action. The data sets could not be combined prior to modeling (the preferred approach) because they employed significantly dissimilar protocols. The range of these four estimates is 4.5-9.0. Each estimate is based on a low-dose extrapolation procedure and entails the use of multiple assumptions and default procedures. The geometric mean from four slope factors, each considered to be of equal merit, was used to calculate a single oral slope factor of 5.8 per (mg/kg/day). Using standard assumptions for human body weight and water consumption, a drinking water unit risk of 1.7E-4 per mg/L was derived. Concentrations of benzo[a]pyrene corresponding to lifetime risks of 10^{-5}, 10^{-6} and 10^{-7} were determined to be $6x10^{-2}$, $6x10^{-3}$ and $6x10^{-4}$ µg/L, respectively.

An analysis of the relative carcinogenic potency of 12 PAH compounds, using benzo[a]pyrene as the basis of comparison, was made using the two-stage model applied to a recent compilation of animal carcinogenesis data in which each experiment tested both benzo[a]pyrene and one or more of the other compounds. A peer review panel suggested that different criteria

be used to select data for a final relative potency determination and that additional studies be analyzed in order to extend the list of PAH compounds for which potencies are evaluated. Until this is done, no recommendations can be made concerning the quantitative cancer risk of the other PAH compounds.

II. PHYSICAL AND CHEMICAL PROPERTIES

Polycyclic aromatic hydrocarbons (PAHs) are a class of diverse compounds that are formed during the incomplete combustion of organic material. A large number of PAHs have been identified in the environment, most of which derive from anthropogenic sources (Santodonato et al., 1980). As a group, PAHs are ubiquitous in the atmosphere, water and soil. They enter aquatic systems from wastewater, urban stormwater runoff, leaching from soil, and wet and dry deposition from the atmosphere (Kveseth et al., 1982; Andren and Strand, 1981). Some PAHs have been found in surface and groundwater as well as finished drinking water. This document focuses on those PAHs identified as priority (water) pollutants by the U.S. EPA (U.S. EPA, 1980a-d). Table II-1 lists these PAHs; some of their physical and chemical characteristics are given in Table II-2. The structural formulas are shown schematically in Figures II-1 and II-2.

The persistence of PAHs in the aquatic environment is a direct function of their physical and chemical properties. These govern the susceptibility of the compounds to various degradation processes, including photolysis, volatilization, and sedimentation and microbial degradation (Callahan et al., 1979). To begin with, many of the PAHs have low vapor pressures and, thus, low volatility. Consequently, volatilization may not be important in the removal of PAHs from water. Second, the solubility of PAHs in water tends to be low, which increases their susceptibility to adsorption onto aquatic particulate matter. This, in turn, suggests that sedimentation and ultimately, microbial degradation, constitutes the primary removal process.

TABLE II-1

Priority Pollutant Polycyclic Aromatic Hydrocarbons
Found in the Environment*

Acenaphthylene	Chrysene
Anthracene	Dibenz[a,h]anthracene
Benz[a]anthracene	Fluoranthene
Benzo[a]pyrene	Fluorene
Benzo[b]fluoranthene	Indeno[1,2,3-cd]pyrene
Benzo[k]fluoranthene	Naphthalene
Benzo[g,h,i]perylene	Phenanthrene
	Pyrene

*Source: U.S. EPA, 1980d

TABLE II-2

Selected Physical and Chemical Characteristics of Important PAHs[a]

Compound	CAS Number	Physical and Chemical Characteristics						
		Molecular Weight	Melting Point (°C)	Boiling Point (°C)	Vapor Pressure (torr)	Solubility in H_2O at 25°C (mg/L)	Density	Log Octanol/ Water Partition Coefficient
Acenaphthylene	208-96-8	152.20	92	265-275	$1x10^{-3}$ to $1x10^{-2}$	3.93	0.899	4.07
Anthracene	120-12-7	178.23	218	342	$1.95x10^{-4}$	0.045, 0.073	1.25	4.45
Benz[a]anthracene	56-55-3	228.28	167	435	$5.0x10^{-9}$	0.014, 0.009	1.274	5.61
22nzo[a]pyrene	50-32-8	252.32	178	310-312[b]	$5.0x10^{-9}$	0.0038	1.35	6.04
Benzo[b]fluoranthene	205-99-2	252.32	168		$1x10^{-11}$ $1x10^{-6}$	Insoluble		6.57
Benzo[k]fluoranthene	207-08-9	252.32	215.7	480	$9.59x10^{-11}$	Insoluble		6.84
Benzo[g,h,i]perylene	191-24-2	276.34	278	550	$1x10^{-10}$[c]	0.00026		7.23
Chrysene	218-01-9	228.28	255-256	448	$1x10^{-11}$ $1x10^{-8}$	0.002	1.274	5.61
Dibenz[a,h]anthracene	53-70-3	278.36	266.6		$1x10^{-10}$	0.0005	1.282	5.97
Fluoranthene	206-44-0	202.3	111	375	$1x10^{-2}$[c]	0.265		5.20
Fluorene	86-73-7	166.21	116-117	295	$1x10^{-3}$ $1x10^{-2}$	1.68-1.98	1.203[d]	4.18
Indeno[1,2,3-cd]pyrene	193-39-5	276.34	162.5-164		~$1x10^{-10}$			7.66
Naphthalene	91-20-3	128.16	80.2	218	$4.9x10^{-2}$[e]	30-40	1.145	3.37

TABLE II-2 (cont.)

Compound	CAS Number	Physical and Chemical Characteristics						
		Molecular Weight	Melting Point (°C)	Boiling Point (°C)	Vapor Pressure (torr)	Solubility in H_2O at 25°C (mg/ℓ)	Density	Log Octanol/ Water Partition Coefficient
Phenanthrene	85-01-8	178.23	100	340	6.8×10^{-4}	1.00, 1.29	0.98[f]	4.46
Pyrene	129-00-0	202.26	156	385	6.85×10^{-7}	0.14, 0.132	1.271	5.32

[a]Source: Smith et al., 1978; Weast, 1983; Cleland and Kingsbury, 1977; Davis et al., 1942; May et al., 1978; IARC, 1973, 1983; U.S. EPA, 1980c; Hansch and Leo, 1985

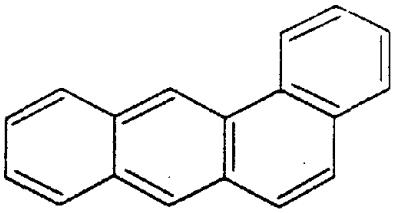
[b]Determined at 10 mm Hg

[c]Determined at 25°C

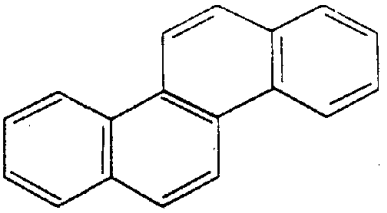
[d]Determined at 0°C

[e]Determined at 19.8°c

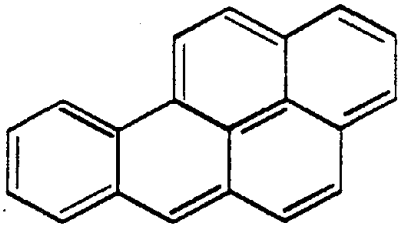
[f]Determined at 4°C



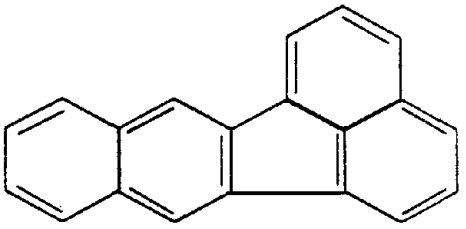
Benz[a]anthracene



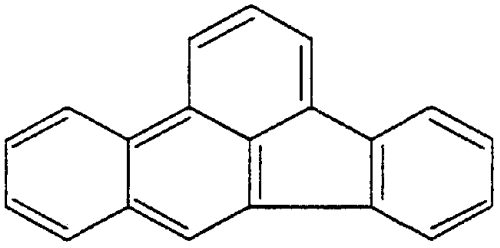
Chrysene



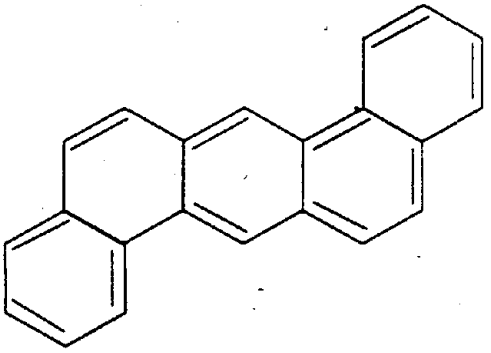
Benzo[a]pyrene



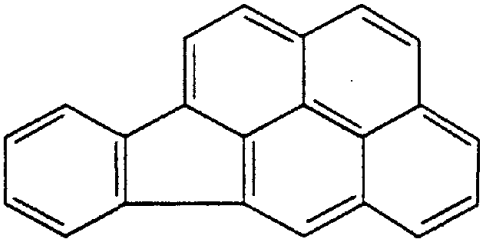
Benzo[k]fluoranthene



Benzo[b]fluoranthene



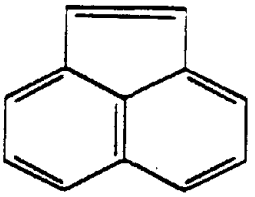
Dibenz[a,h]anthracene



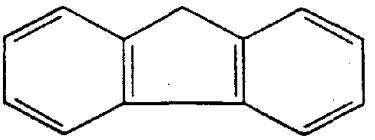
Indeno[1,2,3-cd]pyrene

FIGURE II-1

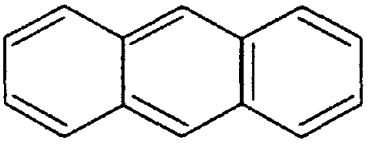
Chemical Structure of Selected PAHs



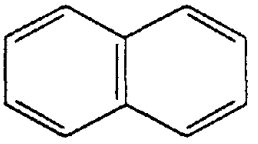
Acenaphthylene



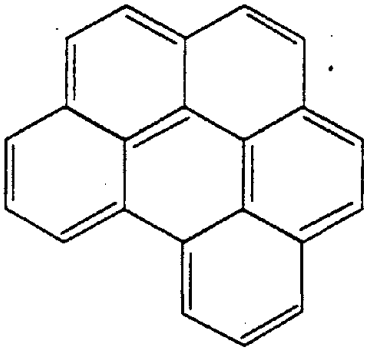
Fluorene



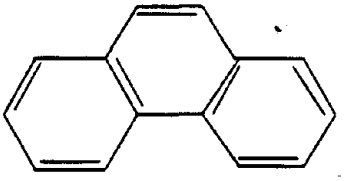
Anthracene



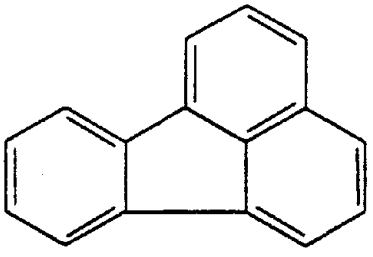
Naphthalene



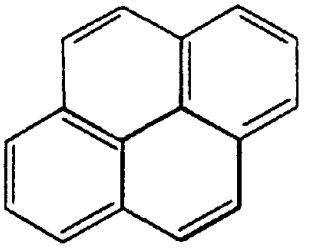
Benzo[g,h,i]perylene



Phenanthrene



Fluoranthene



Pyrene

FIGURE II-1 (cont.)

Chemical Structure of Selected PAHs

Those PAHs composed of four or more aromatic rings (therefore, having high molecular weight) are less amenable to microbial degradation; they are environmentally more stable compounds. Third, the log octanol/water partition coefficients of the PAHs also tend to be high, and there is a positive correlation between these coefficients and bioconcentration potential. Therefore, if PAH concentrations increase quickly enough, toxicity could occur in aquatic life. PAHs, however, do tend to be rapidly metabolized and excreted. Fourth, the previous characteristics seem to be most representative of the so-called carcinogenic PAHs (see Chapter VIII), which implies that such PAHs are more likely to persist in the aquatic environment, yielding longer-term exposures in comparison with less potent PAHs. The production, use and occurrence in water for each of the 15 PAHs described in this document follows.

Acenaphthylene

Pertinent data regarding the production, use and occurrence of acenaphthylene could not be located in the available literature.

Anthracene

Production and Use. In 1981, U.S. imports of anthracene totalled 21,000 kg, down sharply from 510,000 kg imported in 1979. Separate data on U.S. exports were not available (IARC, 1973). Anthracene has been used as an intermediate in dye production. It has also been used in smoke screens, scintillation counter crystals and organic semiconductor research.

Occurrence. Anthracene occurs ubiquitously as a product of incomplete combustion; it also occurs in fossil fuels. It has been identified in

surface water, tap water (1.1-59.7 ng/ℓ), wastewater (1.6-7.0 µg/ℓ) and dried sediment of lakes (30-650 µg/kg) (IARC, 1973).

Benz[a]anthracene

Production and Use. There is no commercial production or known use of this compound.

Occurrence. Benz[a]anthracene occurs ubiquitously in products of incomplete combustion; it is also found in fossil fuels. It has been identified in surface water, tap water (0.4-10.7 ng/ℓ), rainfall (3.2-12.3 ng/ℓ), subterranean water (0-1.3 ng/ℓ), wastewater (0.5-4.9 µg/ℓ), sludge (230-1760 µg/kg) and freeze-dried sewage sludge (0.62-19 mg/kg) (IARC, 1973).

Benzo[a]pyrene

Production and Use. There is no commercial production or known use of this compound.

Occurrence. Benzo[a]pyrene occurs ubiquitously in products of incomplete combustion; it also occurs in fossil fuels. It has been identified in surface water (0.2-13,000 ng/ℓ), tap water (0.2-1000 ng/ℓ), rain water (2.2-7.3 ng/ℓ), subterranean water (0.4-7 ng/ℓ), wastewater (0.001-6000 µg/ℓ), sludge (3-1330 µg/kg) and freeze-dried sewage sludge (540-13,300 µg/kg) (IARC, 1973, 1983).

Benzo[b]fluoranthene

Production and Use. There is no commercial production or known use of this compound.

Occurrence. Benzo[b]fluoranthene occurs ubiquitously in products of incomplete combustion; it also occurs in fossil fuels. It has been identified in surface water (0.6-1.1 ng/ℓ), tap water (0.4-5.4 ng/ℓ), rain water (4.4-14.6 ng/ℓ), subterranean water (0.6-9.0 ng/ℓ), wastewater (0.04-23.7 µg/ℓ) and sludge (510-2160 µg/kg) (IARC, 1973).

Benzo[k]fluoranthene

Production and Use. There is no commercial production or known use of this compound.

Occurrence. Benzo[k]fluoranthene occurs ubiquitously as a product of incomplete combustion; it also occurs in fossil fuels. It has been identified in surface water (0.2-0.8 ng/ℓ), tap water (1-3.4 ng/ℓ), rain water (1.6-10.1 ng/ℓ), subterranean water (1-3.5 ng/ℓ), effluent discharge (0.01-8 µg/ℓ) and sludge (150-1270 µg/kg) (IARC, 1973).

Benzo[g,h,i]perylene

Production and Use. There is no commercial production or known use of this compound.

Occurrence. Benzo[g,h,i]perylene occurs ubiquitously in products of incomplete combustion; it also occurs in considerable amounts in coal tar and is an important component of gasoline engine exhaust. It has been identified in surface water (0.3-28.5 ng/ℓ), tap water (0.8-7.1 ng/ℓ), rain water (2.3-10.8 ng/ℓ), subterranean water (0.7-6.4 ng/ℓ), wastewater (0.4-2.8 µg/ℓ), sludge (200-1220 µg/kg), freeze-dried sewage sludge samples (400-8700 µg/kg) and dried sediments from lakes (1-1930 µg/kg) (IARC, 1973).

Chrysene

Production and Use. There is no commercial production or known use of this compound.

Occurrence. Chrysene occurs ubiquitously and in approximately the same concentration as benzo[a]pyrene in products of incomplete combustion. In addition, chrysene and, preferentially, related structures (methyl-substituted and partially hydrogenated chrysenes) occur in higher concentrations than most of the PAHs in fossil fuels such as crude oil and lignite. Chrysene has been identified in surface water (7.9-62.0 ng/ℓ), wastewater (0.732-6.44 ng/ℓ), freeze-dried sewage sludge (780-23,700 µg/kg) and sediments (40-240 µg/kg) (IARC, 1983).

Dibenz[a,h]anthracene

Production and Use. There is no commercial production or known use of this compound.

Occurrence. Dibenz[a,h]anthracene occurs ubiquitously as a product of incomplete combustion; it also occurs in fossil fuels. It has been identified in wastewater (IARC, 1983).

Fluoranthene

Production and Use. There is no commercial production or known use of this compound.

Occurrence. Fluoranthene occurs ubiquitously in products of incomplete combustion; it also occurs in fossil fuels. It has been

identified in surface water (4.7-6.5 ng/ℓ), tap water (2.6-132.6 ng/ℓ), rain water (5.6-1460 ng/ℓ), subterranean water (9.9-100.0 ng/ℓ), wastewater (0.1-45 µg/ℓ), sludge (580-4090 µg/kg), freeze-dried sewage sludge (610-5160 µg/kg) and dried sediment of lakes (13-5870 µg/kg) (IARC, 1983).

Fluorene

Production and Use. There is no commercial production or known use of this compound.

Occurrence. Fluorene occurs ubiquitously in products of incomplete combustion; it also occurs in fossil fuels. It has been identified in surface water (4.1-102.1 ng/ℓ), tap water (4-16 ng/ℓ) and sewage sludge (0.61-51.60 mg/kg) (IARC, 1973).

Indeno[1,2,3-cd]pyrene

Production and Use. There is no commercial production or known use of this compound.

Occurrence. Indeno[1,2,3-cd]pyrene occurs ubiquitously in products of incomplete combustion; it also occurs in fossil fuels. It has been identified in surface water (0.2-0.5 ng/ℓ), tap water (0.3-4.8 ng/ℓ), rainfall (0.2-8.7 ng/ℓ), subterranean water (0.2-5.0 ng/ℓ), wastewater (0.01-15 µg/ℓ), sludge (470-1200 µg/kg), freeze-dried sewage sludge samples (300-7400 µg/kg) and dried sediment from lakes (1-2070 µg/kg) (IARC, 1973).

Naphthalene

Production and Use. Domestic production capacity of naphthalene was estimated to be 660 million pounds annually as of January 1, 1984. Total domestic consumption of naphthalene for 1985 has been estimated to be 540 million pounds; this amount includes imports of 8 million pounds. Exportation volumes are believed to be in the range of 5 million pounds annually. Major applications of naphthalene include use as an intermediate in the production of phthalic anhydride (55% of consumption), the insecticide carbaryl (20%), β-naphthol (8%), synthetic tanning agents (6%), surfactants (5%), miscellaneous organic intermediates (2%), and use as a moth repellant (2%) (Chemical Economics Handbook, 1981).

Occurrence. Naphthalene is the most abundant single constituent of coal tar (Schmeltz et al., 1978). It is released in the environment via industrial gaseous and particulate emissions, aqueous waste streams, and through consumer uses.

Naphthalene has been detected in industrial effluents (up to 32 mg/ℓ), municipal wastewater treatment plant effluents (22 µg/ℓ), ambient river water (2.0 µg/ℓ), seawater, drinking water (1.4 µg/ℓ), well water, and groundwater (U.S. EPA, 1980c; Shackelford and Keith, 1976; Eganhouse and Kaplan, 1982; Desideri et al., 1984). Stuermer et al. (1982) detected naphthalene in groundwater samples collected near underground coal gasification sites at concentrations of 380-1800 µg/ℓ 15 months after gasification activity had ended. Pankow et al. (1984) reported mean dissolved naphthalene concentrations of 11 and 72 ng/ℓ in rainwater samples collected in semirural and residential locations, respectively, in Oregon.

Phenanthrene

Production and Use. There is no commercial production or known use of this compound. Its derivative, cyclopentenaphenanthrene, has been used as a starting material for synthesizing bile acids, cholesterol and other steroids.

Occurrence. Phenanthrene is present in products of incomplete combustion; it also occurs in fossil fuels. It has been identified in surface water (0-1300 ng/ℓ), tap water (3.1-90 µg/ℓ), wastewater (70 µg/ℓ) and dried sediment in lakes (140-274 µg/kg) (IARC, 1973).

Pyrene

Production and Use. There is no commercial production or known use of this compound. Pyrene from coal-tar has been used as the starting material for the synthesis of benzo(a)pyrene.

Occurrence. Pyrene occurs ubiquitously in products of incomplete combustion; it also occurs in fossil fuels. It is found in relatively high quantities in coal-tar. Pyrene has been identified in surface water (2.0-3.7 ng/ℓ), tap water (1.1 ng/ℓ), rain water (5.8-27.8 ng/ℓ), subterranean water (1.6-2.5 ng/ℓ), wastewater (0.00023-11.8 µg/ℓ), sludge (900-47,200 µg/kg) and dried sediment from lakes (7-3940 µg/kg) (IARC, 1973).

Summary

PAHs are a class of diverse compounds resulting from incomplete combustion. They are ubiquitous pollutants and are found in ambient air, soil and aquatic systems.

PAHs are characterized by low volatility, very slight water solubility and a propensity for adsorption to particulates in aquatic systems. PAHs of four or more rings (characteristic of the carcinogenic PAHs) are less amenable to microbial degradation than are smaller compounds, making them environmentally stable and, therefore, increasing the potential for longer-term exposure. The high log octanol/water partition coefficients of PAHs indicate a propensity for bioaccumulation. However, animal data indicate that PAHs tend to be rapidly metabolized and excreted.

With the exceptions of anthracene and naphthalene there are no reported commercial productions of any PAH described in this document.

III. TOXICOKINETICS

Essentially no human pharmacokinetic data are available from which to develop a coherent picture of PAH absorption, distribution and elimination. The experimental data base consists almost entirely of studies in rodents (mice, rats and hamsters) with occasional studies in larger mammals. While there are certainly quantitative differences among these species (in the relative importance of bile and urine as routes of excretion, for example) the qualitative picture that emerges appears to be much the same across species. PAH kinetics are characterized by rapid, capacity-limited metabolism to both biologically active and inactive metabolites. Both increased levels of enzymes responsible for the metabolism of PAHs and isozymes of these metabolic enzymes are inducible by chemical inducers of the 3-methylcholanthrene class. Excretion of chemically stable metabolites into urine and bile is rapid. Differences in disposition among different PAHs are associated at least in part with differences in their lipophilicities.

Absorption

Oral. Fractional absorption of PAHs from the GI tract is dependent on the specific hydrocarbon ingested. In general, the more lipophilic members of this class must be solubilized in water before they can be absorbed. PAHs with some intrinsic hydrophilic character may be absorbed to some degree even in the absence of emulsifiers such as bile salts. Absorption of all PAHs, however, is expected to be influenced by the properties of other chemicals coadministered or coabsorbed. These include, of course, the constituents of the diet (Chang, 1943; Modica et al., 1983).

Chang's early (1943) work illustrates the dependence of fractional absorption on the compound ingested. While absorption of naphthalene by the rat was complete, and that of acenaphthene and phenanthrene nearly so, anthracene, chrysene, dibenz[a,h]anthracene, benzo[a]pyrene and 3-methylcholanthrene were only 5-50% absorbed either from the diet or from a suspension in starch solution. Absorption of benzo[a]pyrene was ~60% from a stock diet containing 1% benzo[a]pyrene, and ~40% when the benzo[a]pyrene was given as the starch suspension. Modica et al. (1983) similarly observed that chrysene was much less efficiently absorbed than either benzanthracene or triphenylene by young fasted rats given the compounds orally as emulsions.

The dependence of PAH absorption on the vehicle in which it is administered has been well documented. A group of coordinated studies conducted in the early 1950s (Setälä and Ekwall, 1950; Ermala et al., 1951; Ekwall et al., 1951; Setälä, 1954) examined the effects of single solvents. Benzo[a]pyrene was administered to mice and cats by stomach tube, in solution or in a suspension of natural fats (olive oil, arachidis oil); natural fats emulsified in bile; bile alone; long-chain fatty acids and alcohols (oleyl alcohol, oleic acid); polyethylene glycols; and synthetic emulsifiers such as the Triton ethers, among other vehicles. Animals were fasted for at least 12 hours prior to benzo[a]pyrene administration and throughout the experiment, so that the effect of the solvents could be studied in the absence of food. Benzo[a]pyrene was absorbed well in the forestomach irrespective of the vehicle. Benzo[a]pyrene in natural fats or other lipophilic solvents did not penetrate the stomach wall, which is not surprising since

fat itself is not absorbed in the stomach under normal conditions. Hydrophobic solvents, as well as the hydrophobic PAHs, are unable to penetrate the protective mucous layer lining the stomach.

Oleic acid and oleyl alcohol; predominantly lipophilic but with hydrophilic groups, effected a slight penetration of benzo[a]pyrene into the glandular stomach wall. Greater penetration was associated with administration of benzo[a]pyrene in vehicles with both hydrophilic and lipophilic properties; penetration was greatest when the hydrophilic character of the solvent was dominant (Ekwall et al., 1951). Thus, a solvent capable of solubilizing benzo[a]pyrene in aqueous solution is required for its absorption in the stomach.

The effective solubilizers studied by this group of investigators are not present naturally in foods to any significant extent, and it is unlikely that much PAH absorption occurs in the stomach at all under normal conditions. However, once the PAHs have entered the small intestine, they are solubilized by the bile salts in concert with fatty acid anions and monoglycerides (Laher and Barrowman, 1983), and are absorbed in both the small and large intestines (Ermala et al., 1951).

Working with a group of PAHs of increasing ring number and correlated decreasing aqueous solubility, Rahman et al. (1986) showed that the presence of bile salts in the rat intestine is essential for optimum absorption of anthracene, 7,12-dimethylbenzanthracene, and benzo[a]pyrene but not for absorption of 2,6-dimethylnaphthalene or phenanthrene, whose aqueous solubilities are 2-3 orders of magnitude greater than those of the other three hydrocarbons. When the bile was diverted from the intestine by

bile duct cannulation, benzo[a]pyrene was absorbed only 23% as well as it was in the presence of normal amounts of bile. In addition, fractional absorption of the 4- and 5-ringed compounds 7,12-dimethylbenzanthracene and benzo[a]pyrene was not as great as that of the smaller hydrocarbons, particularly in the absence of bile. Thus, coadministration of benzo[a]-pyrene with food is expected to enhance its absorption as a result of activation of the natural mechanisms for absorption of dietary lipids.

There is no convincing evidence that the absorption of PAHs occurs by mechanisms other than passive uptake. Rees et al. (1971) proposed the possibility that uptake might increase exponentially as the amount of benzo[a]pyrene in the GI tract increases. This suggestion was based on studies of the entry of benzo[a]pyrene into everted sacs of rat small intestine. Transfer of benzo[a]pyrene from sac tissue to the enclosed medium was proportional to the concentration in the sac tissue. Uptake by the sac tissue was not affected by metabolic inhibitors, indicating that uptake was not an energy-requiring process. However, the amount bound to the sac tissue increased exponentially with increasing incubation medium concentration >200 μM, suggesting multilayer adsorption to the tissue surface at high concentrations. That such an adsorption/absorption process might be relevant to absorption *in vivo* was suggested by the observation that the amount of benzo[a]pyrene found in retroperitoneal fat and mammary tissue of young female rats given benzo[a]pyrene intragastrically in sesame oil 18 hours earlier appeared to be exponentially related to the dose. The *in vitro* conditions employed by Rees et al. (1971), however, were grossly unphysiologic, and "multilayer adsorption" cannot be a significant component of benzo[a]pyrene absorption *in vivo*. Furthermore, the *in vivo* observation of exponentially increasing tissue concentrations is not

substantiated by the results of other studies. Bock and Dao (1961) found that the concentration of 3-methylcholanthrene in mammary tissue and mammary fat of female rats was proportional to the intubation dose, while Modica et al. (1983) calculated that the area under the tissue concentration curve for chrysene was less than proportional to the dose in blood, liver, brain and parametrial adipose tissue, and roughly proportional to the dose in mammary tissue. Thus, there is no evidence for greater than proportional absorption with dose.

Uptake of PAHs is partly into thoracic duct lymph but principally into portal venous blood (Laher et al., 1984). According to Rees et al. (1971), 10-20% of a 10 mg intragastric dose of benzo[a]pyrene entered the thoracic duct lymph of rats. Daniel et al. (1967) found 5% of the radiolabel from an intragastric dose of 8.4 µg of ^{14}C-labeled dibenz[a,h]anthracene in thoracic lymph during the first 24 hours after administration to rats. Reid (1977) recovered 18% of an absorbed dose of benzo[a]pyrene in olive oil from thoracic lymph of rats given benzo[a]pyrene intraduodenally.

Gastrointestinal absorption of PAHs is rapid, as would be anticipated for compounds utilizing absorption mechanisms designed for uptake of nutrients. The concentration of benzo[a]pyrene peaked in the liver ~1 hour after dosing in female rats intubated with 22.8 mg of benzo[a]pyrene, while the blood concentration peaked at 1.5-2 hours, the lag reflecting passage from liver to blood (Modica et al., 1983). Excretion of metabolites in bile also follows rapidly, the rate generally peaking by 2 hours irrespective of the PAH administered (Rahman et al., 1986). It is interesting to note that in general the fraction of total metabolites excreted in bile rather than in urine increases as the PAHs increase in size and decrease in solubility.

Radiolabel from ^{14}C-dibenz[a,h]anthracene given by intubation continued to appear in thoracic lymph of two rats for at least 24 hours, peaking at around 3-4 hours (Daniel et al., 1967). That some of this radiolabel was probably associated with metabolite reabsorption is suggested by the observations that radiolabel did not peak in the blood plasma of these two rats until ~7 hours after dibenzanthracene administration, and that much of the radiolabeled material extracted from plasma, urine, and bile was metabolized dibenz[a,h]anthracene. Rees et al. (1971) reported that benzo[a]pyrene peaked in the thoracic lymph of rats 3-4 hours after administration of 10 mg by intubation, but the fluorescence-based analytical technique used by these investigators would not have distinguished between benzo[a]pyrene and its metabolites.

Inhalation

The absorption of PAHs from the lung has been investigated in both inhalation studies and in experiments in which the PAHs were given by intratracheal administration. Both pure PAHs and PAHs adsorbed on particles of various sizes and chemical composition have been used. Adsorption of a PAH onto ultrafine particles (<1 μm) from which it can be released into biologic fluids has been shown to increase both the lung retention time of the PAH and its carcinogenicity. The nature of the adsorbent particle is critical: adsorbents that do not readily release adsorbed chemicals are not as likely to enhance tumor yield and other biologic effects in experimental animals as are adsorbents with lower affinity for the carcinogen (Bevan and Worrell, 1985). In addition to adsorption onto particulates, factors such as particle or aerosol size and size distribution, the test species' airway anatomy, metabolism and defense mechanisms, as well as

properties of the PAH itself, will influence the penetration, deposition and retention of an inhaled PAH and the probability and routes of its subsequent absorption. Other chemicals, including those adsorbed to particulates, may also influence the disposition and biologic activity of PAHs taken into the lung.

Kotin et al. (1959) reported a vehicle effect on intratracheally instilled benzo[a]pyrene. Radiolabeled-^{14}C-benzo[a]pyrene (25 µg/0.3 mℓ vehicle) was administered to Wistar male rats either as an aqueous suspension with distilled water or in a solution with triethylene glycol. The esophagus was ligated in each to prevent benzo[a]pyrene from entering the GI tract by mucociliary escalator clearance and swallowing. At 24 hours, 38% of the administered aqueous suspension dose persisted in the lung, whereas only 17% could be recovered at 4 hours in the lungs when administered in triethylene glycol. The rapid mobilization was attributed to the greater speed of solubilization of the fine particles from triethylene glycol vs. the slower speed of solubilization of the larger crystals that form in an aqueous suspension.

The role of particles as carriers of PAHs and enhancers of their pulmonary activity has been the subject of a number of investigations. Coadministration of PAHs with fine adsorbent particles was shown nearly 30 years ago to result in a higher incidence of lung tumors in rats than administration of the PAH alone (Pylev et al., 1969; Saffiotti et al., 1965, 1968). Coadministration by intratracheal instillation of a suspension of ^{14}C-benzo[a]pyrene with carbon black or asbestos (Pylev et al., 1969) or by intratracheal instillation of particles of ferric oxide, aluminum oxide,

or carbon coated with benzo[a]pyrene (Henry and Kaufman, 1973) resulted in prolongation of radiolabel retention in the hamster lung. However, an increase in benzo[a]pyrene retention was not necessarily correlated with an increase in tumorigenicity. Farrell and Davis (1974) showed that carbon black was a more effective tumor promoter than ferric oxide in the golden hamster lung; aluminum oxide was only minimally active as a promoter in this system. In general, the probability of tumor occurrence was shown to increase as particle size range decreased when either carbon black or ferric oxide was used as the adsorbent (Farrell and Davis, 1974; Henry et al., 1974). In the absence of carrier particles, large (77% <42 μm, 0% <10 μm) benzo[a]pyrene particles were cleared less rapidly from the lung and were more tumorigenic than small (77% <5.2 μm, 3% <1.3 μm) particles (Feron et al., 1980). Henry et al. (1975) showed that benzo[a]pyrene had to be physically adsorbed on the ferric oxide to be most effective as a promoter.

Henry and Kaufman (1973) suggested that the ability of the hydrocarbon to be eluted from its particulate adsorption sites might be an important determinant of its biologic activity. Creasia et al. (1976) demonstrated that the rate of elution of benzo[a]pyrene from its adsorption sites on carbon particles in the mouse lung was greater for small (0.5-1.0 μm) particles than for large (15-30 μm) particles. Benzo[a]pyrene adsorbed to the larger particles was cleared with the particles themselves, with a half-time for elimination of 4-5 days. However, while the smaller particles themselves were cleared more slowly ($t_{1/2}$=7 days), the benzo[a]pyrene adsorbed to these particles was cleared more quickly ($t_{1/2}$=36 hours).

This indicates that the benzo[a]pyrene was being eluted from the smaller carbon particles at a rate of 15% per day. Thus, for at least 4 days, 15% of the initial dose of benzo[a]pyrene was free to interact with the respiratory tissue. In the absence of carbon particles, 50% of a benzo[a]pyrene dose was cleared from the lung within 1.5 hours and >95% was cleared within 24 hours. These results established that a combination of prolonged retention time and biologic availability of the adsorbate are involved in the cocarcinogenic effect observed for particulate and benzo[a]pyrene in the lung.

Based on their studies with model phospholipid bilayer membranes (Lakowicz and Hylden, 1978; Lakowicz et al., 1980) and with microsomal preparations (Lakowicz and Bevan, 1979), the authors suggested that cocarcinogenic particles facilitate the uptake of adsorbed chemical carcinogens by cell membranes. Later work by the same group of investigators (Bevan et al., 1981; Bevan and Worrell, 1985) supports this mechanistic hypothesis. Chang and Hart (1983), who demonstrated that the chrysotile form of asbestos enhanced uptake of benzo[a]pyrene into human dermal fibroblasts in vitro, proposed that cocarcinogenicity is mediated in part by enhanced cellular proliferation. It should be noted that binding to cellular DNA was also enhanced by the presence of asbestos in this study, so that the proposed mechanisms of enhanced uptake and enhanced cellular proliferation are fully compatible and not mutually exclusive. Other in vitro studies (Eastman et al., 1983), using hamster tracheal epithelial cells incubated with benzo[a]-pyrene and asbestos, showed enhanced benzo[a]pyrene uptake and DNA alkylation 4 days post-treatment, which was attributed to the presence of asbestos.

Absorption and distribution of benzo[a]pyrene following inhalation exposure of rats to the compound alone or after adsorption onto particles has been studied by investigators at the Inhalation Toxicology Research Institute (Mitchell and Tu, 1979; Mitchell, 1982, 1983; Sun et al., 1983, 1984). Mitchell (1982, 1983) reported the disposition of an aerosol of 500 μg ^{3}H-benzo[a]pyrene/ℓ, 1-2 μm mass median diameter, inhaled by rats for ≤1 hour. Clearance of radiolabel from the respiratory tract was biphasic. Fifty percent of the radiolabel was cleared within 2-3 hours; radiolabel remaining after this time was cleared much more slowly, the half-life being 25-50 hours, depending on location in the lung. The stomach and small intestine contained higher concentrations of benzo[a]pyrene 0.5 hour after termination of exposure than any other tissue, and feces contained ~10 times the amount of radiolabel found in urine during the first day following exposure. The observation that the amount of radiolabel in the stomach and small intestine was at its maximum immediately following termination of exposure suggests mucociliary clearance and swallowing of inhaled material. The presence of radioactivity in other soft tissues (e.g., kidney and liver) 0.5 hour after exposure indicates rapid absorption and distribution of the benzo[a]pyrene from its initial site of deposition.

The concentrations of organic-soluble radiolabel, water-soluble radiolabel and covalently-bound radiolabel were determined in the lung at 0.5, 6 and 24 hours postexposure (Mitchell, 1982, 1983). The 2-3-hours clearance half-life appeared to represent loss of organic-soluble radiolabel; after 24 hours, 80% of total lung radiolabel was covalently bound to macromolecules, inviting the speculation that it is this bound radiolabel that persists in the lung with a half-life of 25-50 hours.

Similar inhalation studies demonstrated that pyrene was also cleared from the rat lung rapidly, although not as rapidly as benzo[a]pyrene (Mitchell and Tu, 1979). The pyrene aerosol had a mass median diameter of 0.45 μm. The amount of pyrene left in the lung 24 hours after exposure was 69% of that remaining 0.5 hour after exposure; at 2 days, it was 5%. Covalent binding was not measured.

Sun et al. (1983) examined the deposition, retention and excretion patterns of the radiolabel associated with ^{3}H-benzo[a]pyrene coated on ultrafine (0.1 μm median diameter) particles of gallium oxide, Ga_2O_3. The purpose of this study was to determine whether association with ultrafine particles affects the disposition of organic air pollutants. Rats were exposed by inhalation to the aerosols (3500 ng/ℓ) for 30 minutes, and tissues and excreta were collected for 16 days after the exposures. Studies with pure aerosols (600 ng/ℓ) of ^{3}H-benzo[a]pyrene of the same median diameter were also conducted for comparison, with the exposure period extended to 50 minutes in order to insure that total deposition was similar to that observed in the particle inhalation experiments. About 28% of each aerosol had been deposited in the respiratory tract at the end of the exposure period.

Initial clearance from the lung was rapid, with half-lives of <1 hour for both aerosols. During the subsequent slow excretion phase, lung levels of radiolabel resulting from exposure to benzo[a]pyrene-coated particles were 4-10 times higher than those resulting from exposure to pure benzo[a]-pyrene aerosols, although the half-lives, had they been calculated, would probably not have been greatly different. Lung clearance of the pure

benzo[a]pyrene aerosol was also very similar to that reported by Mitchell (1982) for aerosol concentrations 800 times higher. Of particular interest is that levels of radiolabel in the stomach increased with time after exposure to coated particles. Thirty-five percent of the benzo[a]pyrene initially deposited in the lung was recovered from the stomach 2 hours after exposure to benzo[a]pyrene-coated particles, while no more than ~0.5% of the amount of pure aerosolized benzo[a]pyrene (initially) deposited in the lung was found in the stomach at any time following exposure. Thus, exposure to a benzo[a]pyrene-coated particle rather than to a pure benzo[a]pyrene aerosol resulted in a very marked shift in the pattern of lung clearance, from direct absorption into blood to clearance by mucociliary action followed by ingestion. Consequently, of the total radiolabel excreted in urine and feces through day 16, ~15% was recovered from the urine of animals exposed to the pure aerosols and only ~8% from the urine of animals exposed to the coated aerosols.

It should be noted that pure aerosols of ^{3}H-nitropyrene, as well as gallium oxide aerosols coated with ^{3}H-nitropyrene, were studied in a parallel set of experiments, with results that were different from the benzo[a]pyrene results. Thus, it is not advisable to generalize retention and absorption data for a particular PAH to predict the quantitative behavior of other PAHs or nitropyrenes under similar conditions.

A possible effect of diesel exhaust particles on retention of PAHs by the lung is currently of some concern (Tyrer et al., 1981). A study reported by Sun et al. (1984) showed that the retention and excretion patterns of ^{3}H-benzo[a]pyrene-coated diesel engine exhaust particles with

a mass median diameter of ~0.14 μm (4-6 μg/ℓ) were qualitatively similar to those recorded for the gallium oxide study. However, the amounts of radiolabel retained in the lung during the second, slow phase of clearance were much higher in the diesel exhaust study than in the gallium oxide study. Most of this radiolabel was present as unchanged compound.

Dermal. PAHs can be absorbed percutaneously. The rate and extent of absorption are strongly dependent on the size and configuration of the hydrocarbon molecule, and are also dependent on the concentration of the hydrocarbon applied to the skin.

Heidelberger and Weiss (1951) showed that ^{14}C-benzo[a]pyrene dissolved in benzene was absorbed from a single application site on the shaved back skin of mice and excreted principally in the feces. Fifty percent of the radiolabel from a 63.7 μg/cm² dose of benzo[a]pyrene was lost from the site of application by 1 hour; only 6% remained after 7 days. Dibenz[a,h]-anthracene was only very slowly lost from the application site and was not detected in the feces. By contrast, Bock and Burnham (1961) showed that 7,12-dimethylbenzanthracene and benzo[a]pyrene in a 99:1 mixture of benzene and mineral oil reached similar concentrations in shaved back skin of mice 2 hours after cutaneous application of ~400 μg/cm².

Sanders et al. (1984) confirmed that benzo[a]pyrene and 7,12-dimethyl-benzanthracene are rapidly absorbed across the skin of mice. Six percent of a dose of 125 μg benzo[a]pyrene/cm² in acetone had disappeared from the application site by 1 hour and 40% by 24 hours, with 7% remaining at the site after 7 days. Eighty percent of a 5.4 μg 7,12-dimethylbenzanthra-

cene/cm² dose had disappeared by 24 hours, and 96% by 1 week. Absorption was dose-dependent; increasing the 7,12-dimethylbenzanthracene dose from 5.4 to 56 and to 515 μg/cm² reduced absorption during the first 24 hours from 82% to 71% and to 33%, respectively; decreasing the benzo[a]pyrene dose from 125 to 12.5 and to 1.25 μg/cm² increased absorption during the first 24 hours from 41% to 83% and to 82%, respectively. Use of muzzles to prevent grooming of the treated area reduced the fraction of the dose found in the stomach and its contents to one-third of the amount found when muzzles were not used; this fraction, 0.5-1.5% of the administered dose, was so small that it had no significant impact on measurement of either absorption or excretion.

Molecular size (number of rings) and structure affected the concentrations of 12 different hydrocarbons, including phenanthrene, anthracene, benz[a]anthracene and 3-methylcholanthrene, studied in mouse skin under the conditions used by Bock and Burnham (1961). While this measurement reflects both rate of uptake by the skin and rate of transfer into the systemic circulation, it does indicate that skin penetration occurs and that the rate of the overall dermal absorption process is dependent on molecular size and configuration. It was also determined that the nature of the solvent makes a difference in the absorption of PAHs. Small amounts (≤5%) of mineral oil added to benzene resulted in greater dermal absorption. However, the absorption was not as great when a mixture of 50% mineral oil was used as the solvent.

Fifty percent of a single application of 9.3 μg anthracene/cm² (dissolved in 71 μl of a 1:7 mixture of hexane:acetone) to the shaved

back skin of female Sprague-Dawley rats was absorbed in 5 days, as measured by the appearance in urine, feces and other tissues (Yang et al., 1986).

Distribution

Elimination of benzo[a]pyrene and of 7,12-dimethylbenzanthracene from blood is biphasic, the first phase being extremely rapid. About 90% of either chemical was lost in the first 10-30 minutes following administration of an intravenous dose to mice or rats (Heidelberger and Weiss, 1951; Kotin et al., 1959; Lo, 1964; Iqbal et al., 1979). The remainder was lost more slowly.

Clearance of metabolites accounts for the second, slower phase of decline of radiolabel in the blood. Excretion of metabolites in bile and urine is preceded by a period during which the liver and kidney contain relatively large fractions of the dose. This period is brief. By 90 minutes following intravenous injection of ^{14}C-benzo[a]pyrene in mice, the liver contained only 1.36% of the radiolabel, and 72% had already entered the bile (Heidelberger and Weiss, 1951). Dibenz[a,h]anthracene was less rapidly metabolized; 90 minutes after an intravenous dose was given to mice, 89% of the radiolabel was still in the liver (Heidelberger and Weiss, 1951).

Selective accumulation does not occur in target tissues of PAH carcinogenic action. Depending on the lipophilicity of the PAH, significant accumulation in body fat is likely to occur; early authors attributed the induction of mammary tumors by PAHs to the fact that the mammary gland is in intimate association with fat (Bock and Dao, 1961; Daniel et al., 1967). Bock and Dao (1961) found little phenanthrene in the perirenal and mammary

fat of rats given the hydrocarbon by stomach tube 24 hours earlier. Concentrations of 7,12-dimethylbenzanthracene and 3-methylcholanthrene were somewhat higher, and benzo[a]pyrene was present in the grestest concentration. These concentrations correlate with the relative lipophilicity of phenanthrene, 7,12-dimethylbenzanthracene, and benzo[a]pyrene (Rahman et al., 1986).

Oral. When radiolabeled PAHs are administered into the GI tract by gastric intubation or by feeding, first-pass metabolism destroys the sharp initial drop in radiolabel characteristic of intravenous administration. Instead, radiolabel peaks after several hours; this radiolabel is associated with metabolites. The timing of the peak depends on the particular PAH, and is expected to depend on the dose as well. It has been seen to occur at ~7 hours for dibenz[a,h]anthracene and 15 hours for 7,12-dimethylbenzanthracene in rats (Daniel et al., 1967).

When a specific analytical technique (cyclohexane extraction of adipose tissue followed by gas liquid chromatography) is used for the determination of unchanged PAH, the peak time occurs earlier: at ~1.5 hour for benz[a]-anthracene in rats (Modica et al., 1983), and at ~1 hour for benz[a]anthracene and chrysene and 2.5-3 hours for triphenylene in rats (Bartosek et al., 1984). The concentrations of these PAHs in the liver peak shortly before their peaks in the blood (Modica et al., 1983; Bartosek et al., 1984). For this reason, the rate of appearance in the bile also tends to peak early (1-2 hours) for most PAHs (Kotin et al., 1959; Rahman et al., 1986). As far as can be determined from published data, distribution from the blood into peripheral tissues follows established principles: well-perfused tissues

establish a dynamic steady state with the blood early, while adipose tissue exchanges more slowly. Peak adipose tissue concentrations occurred at 2.5 hours for chrysene, ~4 hours for benz[a]anthracene and 8 hours for triphenylene (Bartosek et al., 1984), and at 3 hours for benz[a]anthracene in another study (Modica et al., 1983).

No kinetic evidence for this peripheral distribution is found in the concentrations of PAHs in the blood after a single dose, which can be fit by a biexponential equation with one term representing uptake and the other loss (Modica et al., 1983; Bartosek et al., 1984). Half-lives in rat tissues appear to be about the same for benz[a]anthracene and chrysene: 1 hour in blood, 0.8 hour in liver, 2.5 hours in brain, 5 hours in mammary tissue, and 14 hours in adipose tissue (Modica et al., 1983; Bartosek et al., 1984).

Inhalation. As has been discussed earlier (see Absorption Section), the pattern of distribution following a pulmonary exposure could resemble the pattern associated with intravenous administration or the pattern associated with oral exposure, or could be intermediate between the two. Kotin et al. (1959) instilled ^{14}C-benzo[a]pyrene intratracheally in triethylene glycol into rats whose esophagi had been ligated to prevent entry of benzo[a]pyrene into the GI tract. Except for the high initial pulmonary concentration, the pattern of tissue radiolabel was similar to that seen after subcutaneous or intravenous administration. One hour after instillation, 37% of the radiolabel was in the intestine, 1.3% in the liver, and 43.2% remained in the lung.

The study of Kotin et al. (1959) demonstrates that pulmonary absorption is rapid. Mitchell (1982) found radiolabel in soft tissues by 0.5 hour after a 1-hour inhalation exposure of rats to ^{3}H-benzo[a]pyrene. Radiolabel in testis, brain and kidney peaked at ~6 hours and was generally cleared from these tissues in ~1 day. At the 0.5-hour time point, the radiolabel in liver and kidney was largely polar metabolites of benzo[a]pyrene (Mitchell, 1983). In every salient point, this distribution pattern and timing resemble the pattern and timing of distribution of an intravenous dose. It can be concluded that an inhaled aerosol of pure benzo[a]pyrene is distributed essentially like a comparable intravenous dose save for the initial high pulmonary concentration. Inhaled pyrene aerosol also behaved much like an intravenous dose in rats, although a substantial amount of pyrene was found in the stomach 0.5 hour after exposure (Mitchell and Tu, 1979).

Clearance from the lung into the GI tract is not as rapid a process as absorption into blood. Only when PAHs are inhaled adsorbed to particulates does their residence time in the lung allow significant transfer to the GI tract. Sun et al. (1983) showed that during the first 2 hours after an inhalation exposure to ^{3}H-benzo[a]pyrene adsorbed onto gallium oxide particles, radioactivity in the stomach increased. In addition, radiolabel in the livers and kidneys of these rats was 5-6 times higher at 0.5 and 24 hours than radiolabel in the liver and kidneys of the rats inhaling pure benzo[a]pyrene aerosol. Although other tissue measurements were not made, these observations strongly suggest that the distribution pattern of an inhaled PAH adsorbed onto particulate material will have some of the characteristics of distribution associated with oral exposure. Inhalation

studies in rats exposed to benzo[a]pyrene adsorbed onto diesel engine exhaust particles (Sun et al., 1984) led to the same conclusion.

Tyrer et al. (1981) examined the effect of diesel exhaust exposure on the disposition of ^{14}C-benzo[a]pyrene administered intratracheally in gelatin solution (20 µℓ of 0.2% gelatin and 0.5 µg/µℓ of benzo[a]-pyrene) to A/J mice. Mice were exposed to raw diesel exhaust (6 mg/m^3 of particles) 8 hours/day, 7 days/week for 9 months prior to intratracheal instillation of benzo[a]pyrene. Autoradiography sections were processed from mice killed at 2.24 and 48 hours post-instillation. Qualitatively there were no obvious differences between mice exposed to both diesel fumes and benzo[a]pyrene or to benzo[a]pyrene only, possibly because of the large interindividual variance in expectorate ingestion. Within 2 hours after placement in the lungs, benzo[a]pyrene was found circulating in the blood-stream and may have entered the esophagus and stomach by swallowing of material cleared by the mucociliary escalator. ^{14}C-benzo[a]pyrene was cleared from the blood by the kidneys and then excreted. By 24 hours, ^{3}H-radioactivity accumulated in the stomach, lower GI tract, kidneys, bladder and slightly in the lung.

Dermal. Mitchell and Tu (1979) found trace amounts of pyrene-equivalent fluorescence in kidney, liver and trachea of rats 24 hours after a single application of pyrene to the unshaved back. No pyrene or metabolites were detectable in the lung. The GI tract contained 1.4% of the dose.

Six days after application of anthracene to the shaved back skin of rats, Yang et al. (1986) recovered a total of 1.3% of the applied dose from

15 (unspecified) selected tissues. At this time, 29.1% of the applied dose had been recovered in the urine and 21.9% in the feces.

To summarize, a number of studies with distribution data indicate that 1) detectable levels of PAHs can be observed in most internal organs from minutes to hours after various routes of administration; 2) adipose and mammary tissues are significant distribution sites where PAHs may be accumulated, stored and slowly released; and 3) after inhalation exposure, the GI tract contains relatively high levels of PAH or metabolites as the result of swallowing unmetabolized PAH from mucociliary clearance, or derived from hepatobiliary excretion of metabolites.

Metabolism

In the past, the relative lack of chemical reactivity for tumorigenic PAHs has been puzzling in light of their dramatic biologic effects. Early attempts to explain the carcinogenicity of various PAHs utilized physicochemical calculations (Pullman and Pullman, 1955). These early hypotheses were based on the assumption that those regions of the molecule favoring substitution or addition reactions would preferentially react with critical cellular target sites to initiate a carcinogenic transformation. This concept, however, did not prove successful for PAHs.

More recently it has been shown that PAHs are metabolized by enzyme-mediated oxidative mechanisms to form reactive electrophiles (reviewed in Conney, 1982; Gelboin, 1980; IARC, 1983; Pelkonen and Nebert, 1982; Santodonato et al., 1981; Zedeck, 1980). For many of the PAHs, certain "bioactivated" metabolites are formed having the capability for covalent

interaction with cellular constituents (i.e., RNA, DNA, proteins) and ultimately leading to tumor formation.

The obligatory involvement of metabolic activation for the expression of PAH-induced carcinogenesis has prompted the investigation of PAH metabolism in numerous animal models and human tissues. From these studies has emerged an understanding of the general mechanisms involved in PAH biotransformation. It is now known that PAHs are metabolized by the cytochrome P-450-dependent microsomal mixed-function oxidase (MFO) system, often designated aryl hydrocarbon hydroxylase. The activity of this enzyme system is readily inducible by exposure to PAH and is found in most mammalian tissues, although predominantly in the liver. The MFO system is involved in the metabolism of endogenous substrates (e.g., steroids) and the detoxification of many xenobiotics (Nebert et al., 1981). Paradoxically, however, the MFO system also catalyzes the formation of reactive epoxide metabolites from certain PAHs, possibly leading to carcinogenesis in experimental mammals.

The route by which PAHs and other xenobiotics enter the body may determine their fate and organ specificity. A compound absorbed from the lungs may bypass the "first pass" effect in the liver and reach the peripheral tissues in high concentrations. The unique enzymes in these extra-hepatic tissues may differ in terms of activity and specificity, from those in the liver. Thus, extra-hepatic metabolism may be extremely important in the bioavailability of a chemical to different parts of the body and in target tissue variability. Enzymes capable of metabolizing PAHs are found in the liver, lung, kidney, adrenals, testes, thyroid, skin, small intestine and sebaceous gland in a variety of species including human, baboon, monkey,

rat, mouse, hamster, guinea pig, rabbit and dog. Embryonic tissue of rat, mouse, hamster and chick also possess activity (Zedeck, 1980).

Benzo[a]pyrene as a Model of PAH Metabolism. A discussion of the metabolism of PAHs in mammalian species, including humans, may be approached by examining in detail the chemical fate of the most representative and well-studied compound in the alternant PAH class, namely benzo[a]pyrene. The metabolism of benzo[a]pyrene, subject of voluminous research, is shown schematically in Figure III-1. Only the most important pathways will be presented in this discussion that correlates with Figure III-2. This is a summary and the reader is referred to Gelboin (1980), Pelkonen and Nebert (1982), Yang et al. (1978), Zedeck (1980) and Thakker et al., (1985, 1988) for primary sources.

A monooxygenase first introduces an oxygen atom into any of several positions of the molecule to produce oxides or primary "simple" epoxides. This initial oxygenation is catalyzed by one of a number of different forms of P-450 (MFO). The epoxides then undergo spontaneous rearrangement to phenols. Another pathway for the epoxides is the reduction back to parent benzo[a]pyrene. The formation of quinones through the 6-phenol and 6-oxo radical is less well characterized and is discussed briefly in the Other Toxification Pathways Section. Quinones can also be produced nonenzymatically by lipid peroxidation and aerobic oxidation. The enzyme epoxide hydrolase can further metabolize epoxides to dihydrodiols. The glutathione transferases catalyze conjugation of epoxides with glutathione.

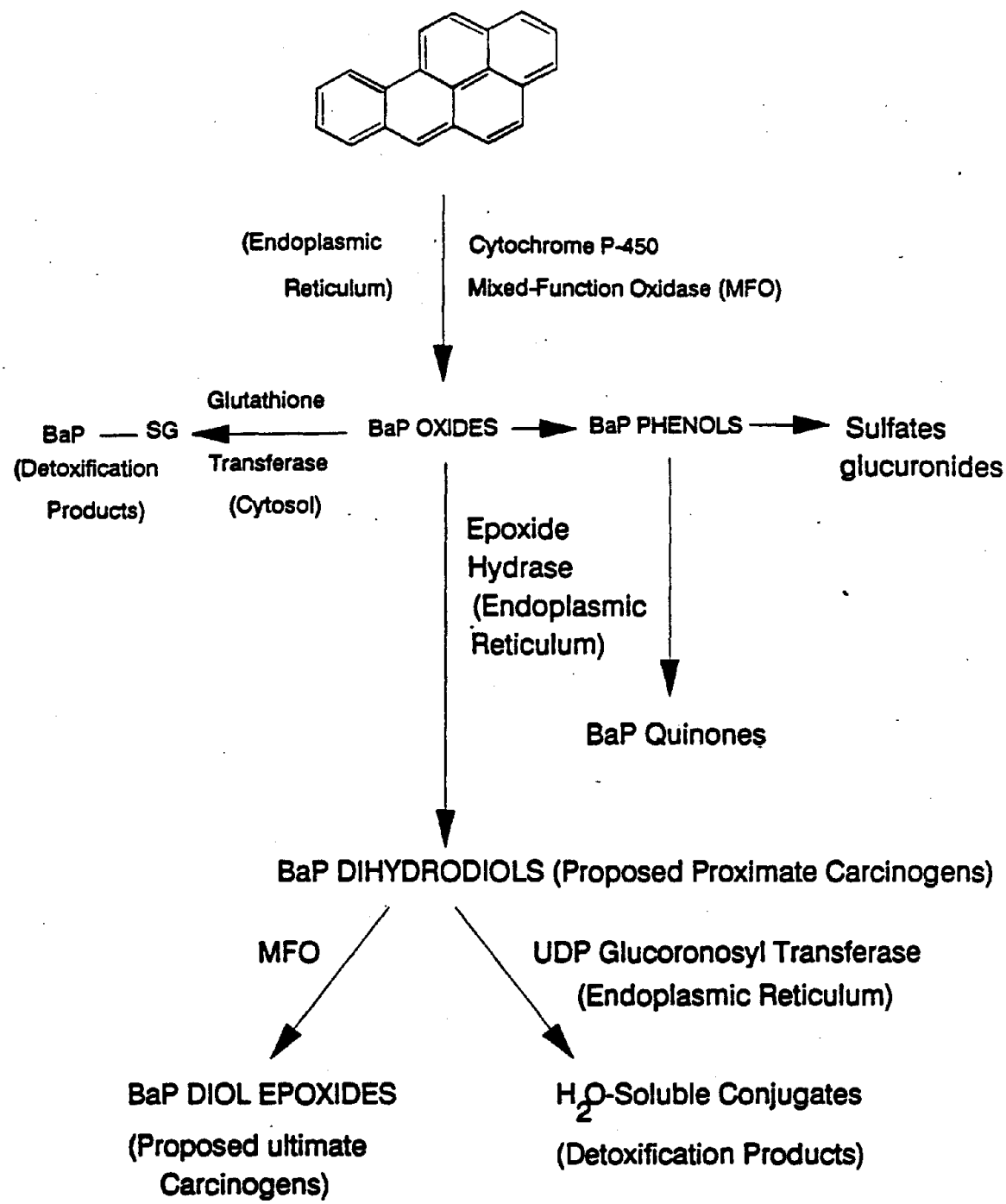


FIGURE III-1

Metabolism Schematic for PAHs

Source: U.S. EPA, 1980d

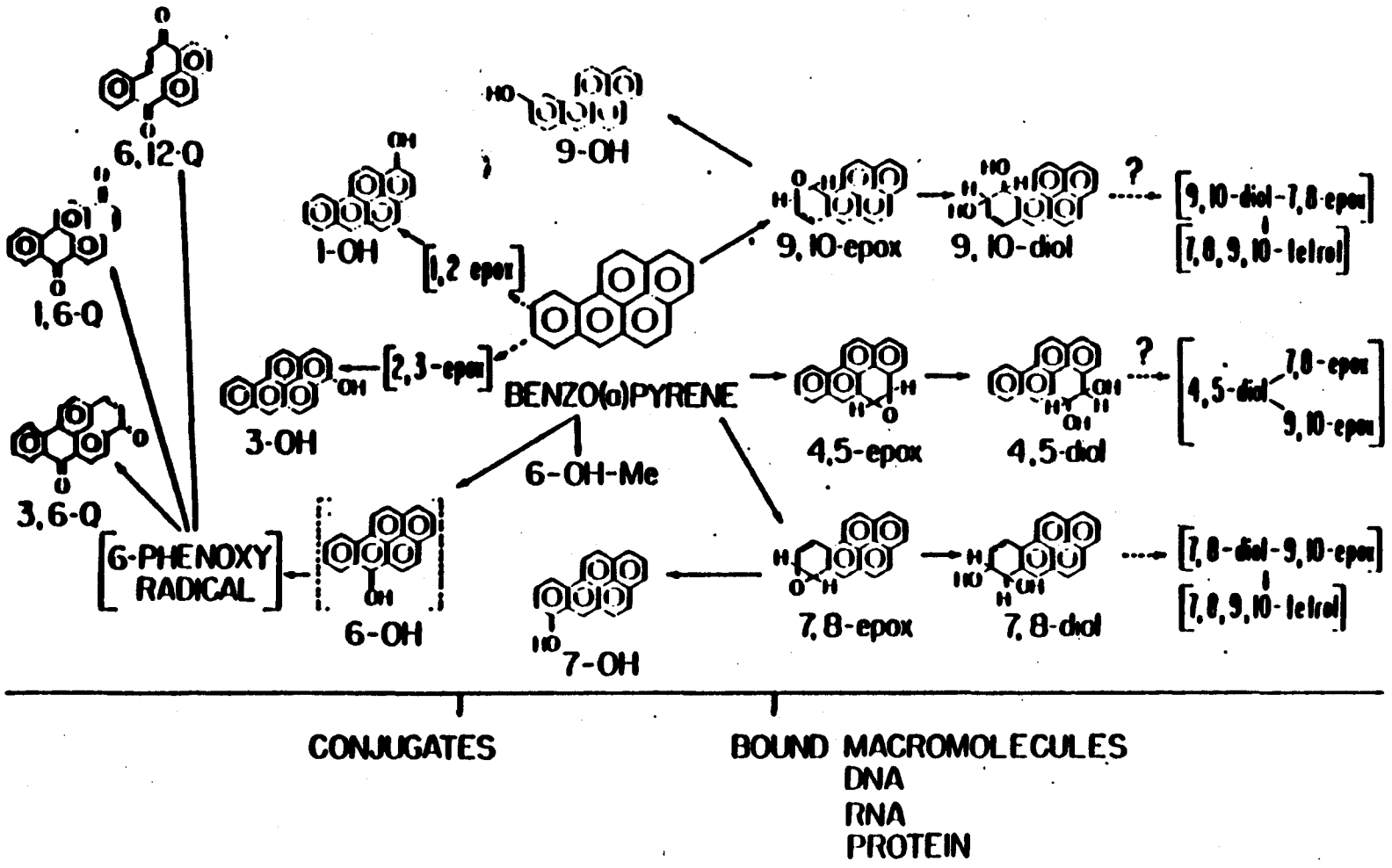


FIGURE III-2

Metabolism of Benzo[a]pyrene

Source: U.S. EPA, 1980d

Epoxides, dihydrodiols, phenols and quinones are generally regarded as "primary" metabolites of benzo[a]pyrene, which undergo further metabolism. Dihydrodiols and phenols can be substrates for the MFO, and another position of the molecule can become epoxidated (see following discussion of pathway). Dihydrodiols, phenols and quinones can also be conjugated with glucuronic acid in reactions catalyzed by UDP-glucuronosyltransferase or with sulfate in reactions catalzyed by sulfotransferase. Dihydrodiols can be dehydrogenated to catechols by a soluble dehydrogenase.

The metabolites of benzo[a]pyrene and other PAHs (both alternant and nonalternant) are often categorized on the basis of solubility in various laboratory extraction protocols (U.S. EPA, 1980d). Those metabolites that can be extracted from an aqueous incubation mixture using an organic solvent include the phenols, dihydrodiols, quinones and hydroxymethyl derivatives of PAHs having aliphatic side chains. Epoxides are included in this group although these are rather labile. The conjugation products, including those of glutathione, glucuronides and sulfates, remain in the water phase after extraction. Identification and quantification of metabolites in these fractions provide researchers with insight on the predominant pathways in the metabolism of a particular PAH.

The pathways leading to the 7,8-diol-9,10-epoxides of benzo[a]pyrene have been a focus of research since it was suggested that these are the predominant DNA-binding species in cell culture (Sims and Grover, 1974). For example the major adduct identified in human, hamster, rat and mouse endometrial DNA, exposed <u>in vitro</u> to benzo(a)pyrene, was anti-benzo(a)-pyrene-7,8-diol-9,10-epoxide (Kulkarni et al., 1986). The amount of this

adduct formed in hamster and mouse DNA was similar; human levels were 3-4 times higher and rat levels ~5-7 times lower. The quantity of B[a]P bound in humans was 7 times the amount bound in hamsters, while rats and mice were 4 and 2 times higher than hamsters, respectively. This study also showed that differences exist in the proportions and types of other adducts formed by these species.

Although the 4,5-oxide is the most mutagenic metabolite in bacterial systems relative to the 7,8-and 9,10-oxides, other data from assays such as DNA binding studies, cell transformation assays and carcinogenicity bioassays support the 7,8-diol-9,10-epoxides as ultimate carcinogenic forms of benzo[a]pyrene (Pelkonen and Nebert, 1982; IARC, 1983; Conney, 1982; Kulkarni et al., 1986; Thakker et al., 1988). The metabolic activation scheme for the formation of the 7,8-diol-9,10-epoxide is shown in Figure III-3. The 7,8-diol formed from benzo[a]pyrene is the trans isomer and there are four possible stereoisomeric 9,10-epoxides derived from this. The structure and nomenclature of these diol-epoxides are also shown in Figure III-3. The two isomers (anti and syn), also referred to as diol-epoxides I and II, are racemic and have been synthesized in order to study their stereochemistry. Diol-epoxide II (syn-isomer) has been found to be more chemically reactive than the diol-epoxide I (Gelboin, 1980). Both are unstable in aqueous media and are hydrolyzed nonenzymatically to tetrols. In the presence of NADPH or NADH they are nonenzymatically reduced to triols.

The idea that 7,8-diol-9,10-epoxide metabolite is the most probable ultimate carcinogen gave rise to the "bay-region" hypothesis (Jerina et al., 1978) for the metabolic toxification of PAHs. The theory was based on the

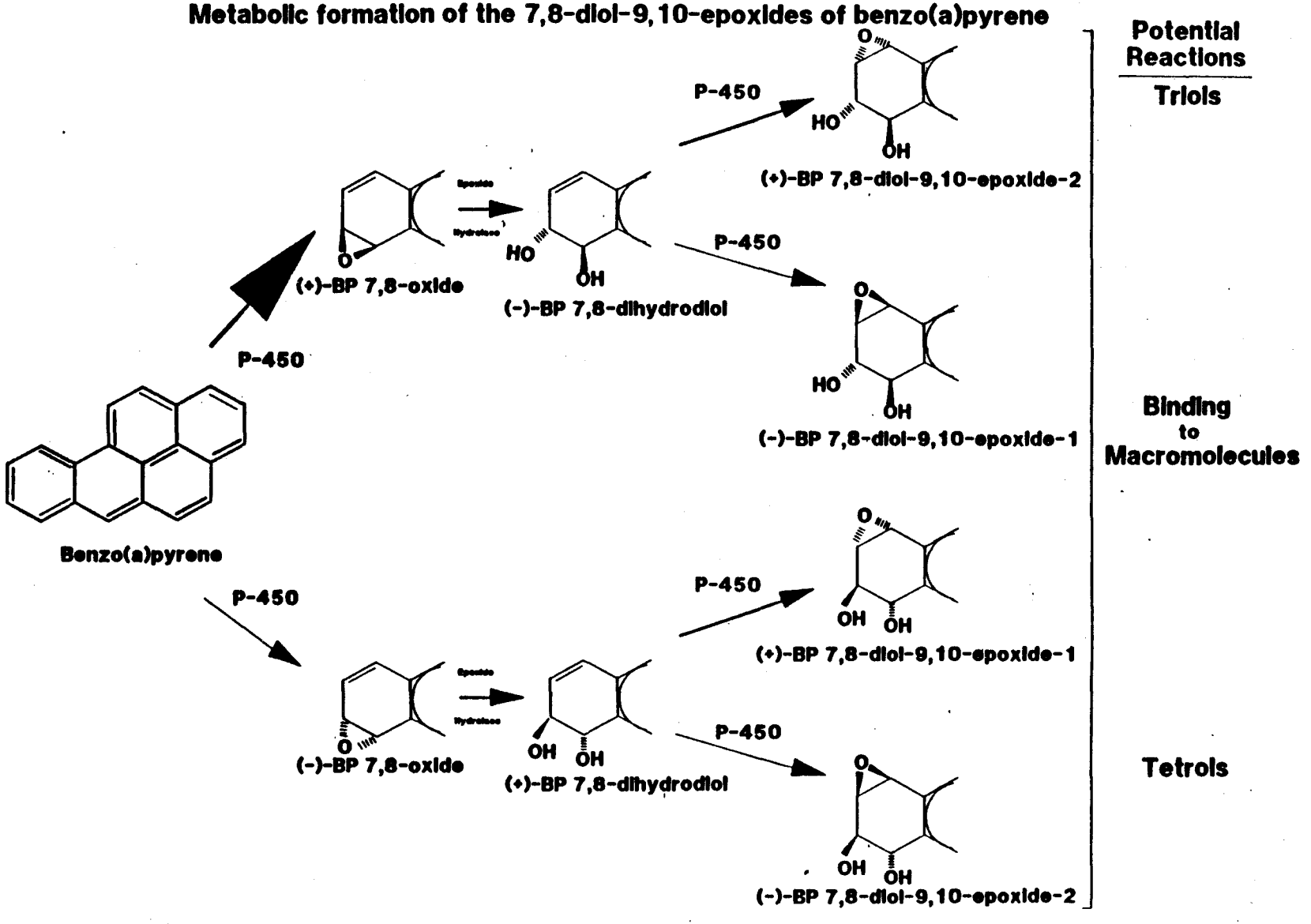


FIGURE III-3

Mechanisms of Enzymatic Activation of Benzo[a]pyrene to 7,8-Diol-9,10-Epoxides

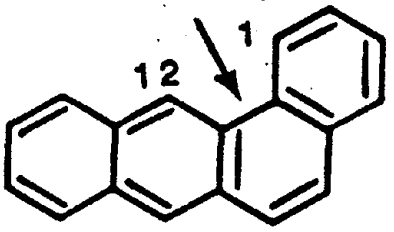
Source: Adapted from Yang et al., 1978

assumption that the unusually high chemical reactivity of such diol-epoxides and their high susceptibility to attack by nucleophiles can be attributed to their electronic properties. The bay-regions of four PAHs, benz[a]anthracene, benzo[a]pyrene, chrysene and phenanthrene, are shown in Figure III-4. The implications of the bay-region hypothesis for the mechanisms whereby PAHs may produce cancer are discussed in Chapter VII. Table III-1 lists examples of the enzymes involved in the toxification of PAHs from the production of diols, phenols and quinones, and diol-epoxides.

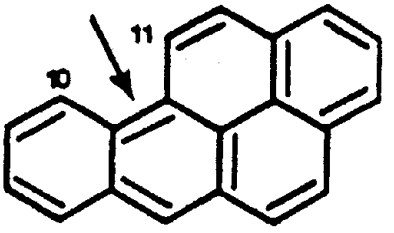
Metabolism of Nonalternant PAHs. The carcinogenicity of the alternant PAHs may be related to the intrinsic carcinogenicity of the bay-region diol-epoxides and the extent to which the parent compounds are metabolically converted to the bay-region diol epoxides (Thakker et al., 1988). However, it should be noted that there is increasing evidence that the bay-region theory of activation does not appear to be the principal route of activation for nonalternate PAHs such as benzo[b]fluoranthene and benzo[k]-fluoranthene and that other mechanisms of activation are involved for those PAHs that are devoid of a bay-region, such as indeno[1,2,3-cd]pyrene and benzo[k]fluoranthene (Amin et al., 1982; Geddie et al., 1982; Hecht et al., 1980; LaVoie et al., 1980; Rice et al., 1985a, 1986, 1987a,b).

Other Toxification Pathways. In additon to the MFO mediated formation of diol-epoxides and other oxygenated products, other routes of metabolism have been shown for PAHs.

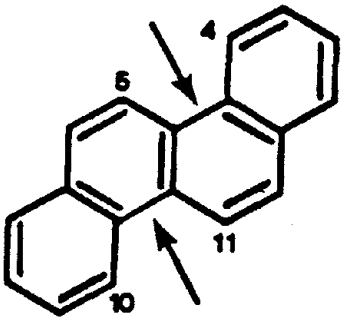
One-electron oxidation of PAHs can also form electrophilic metabolites that can bind to macromolecules (Cavalieri and Rogan, 1983). The inter-



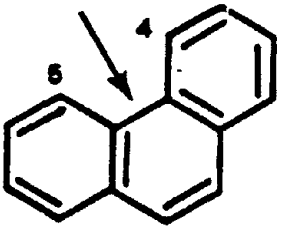
BENZ [a] ANTHRACENE



BENZO[a]PYRENE



CHRYSENE



PHENANTHRENE

FIGURE III-4

The Positions of Bay-Regions

Source: Jerina et al., 1978

TABLE III-1

Examples of Metabolism of PAHs to Biologically Active Forms by Various Enzyme Systems*

PAH	Toxification Enzymes	Biologically Active Intermediate
Benzo[a]pyrene	monooxygenase	4,5-oxide
	monooxygenase, epoxide hydrolase	7,8-diol-9,10-epoxide
	monooxygenase	6-oxoradical
Benz[a]anthracene	monooxygenase, epoxide hydrolase	3,4-diol-1,2-epoxide 8,9-diol-10,11-epoxide
Chrysene	monooxygenase, epoxide hydrolase	1,2-diol-3,4-epoxide
Dibenz[a,h]anthracene	monooxygenase, epoxide hydrolase	3,4-diol-1,2-epoxide
	monooxygenase, epoxide hydrolase	[10,11]-diol-[12,13]-epoxide

*Source: Adapted from Pelkonen and Nebert, 1982

mediates in these pathways are radical cations. Benzo[a]pyrene toxification by free-radical metabolism (see Figure III-2) occurs at position 6. The 6-hydroxybenzo[a]pyrene is unstable in solution and rapidly undergoes radical formation to the 6-oxobenzo[a]pyrene. This radical may interact with macromolecules or be converted to quinones (Jeftic and Adams, 1970). PAH quinones have been proposed to be biologically active because of activity in oxidation/reduction cycles involving quinone, hydroxyquinone and molecular oxygen (Lesko et al., 1978). The peroxides and oxygen radicals formed during these cycles may be responsible for the ultimate cellular injury. This oxidation/reduction cycle is shown in Figure III-5. PAHs, being photoreactive, can absorb visible light and become photooxidized. This photooxidation can result in the formation of quinones, dihydrodiols and phenols (see Chapter 7).

Marnett et al. (1978) and Marnett and Reed (1979) demonstrated oxygenation of benzo[a]pyrene catalzyed by prostaglandin H synthesis-dependent oxidation of arachidonic acid metabolism pathway. Benzo[a]pyrene thus activated has been shown to form covalent bonds with DNA and is mutagenic. Arachidonic acid has been shown to be released upon membrane pertubation. This metabolic pathway is particularly intriguing for instances such as occur in the lung with co-exposure to PAHs and irritants or particulates. These irritant chemicals may affect membrane physiology such that the metabolism of the PAH by prostaglandin synthetase could be enhanced locally in the extrahepatic tissue. For example, Warshawsky et al. (1984) examined the effects of Fe_2O_3 on the rate of metabolism of benzo[a]pyrene in the lungs of male white New Zealand rabbits. Benzo[a]pyrene was administered intratracheally to an isolated perfused lung preparation with and without

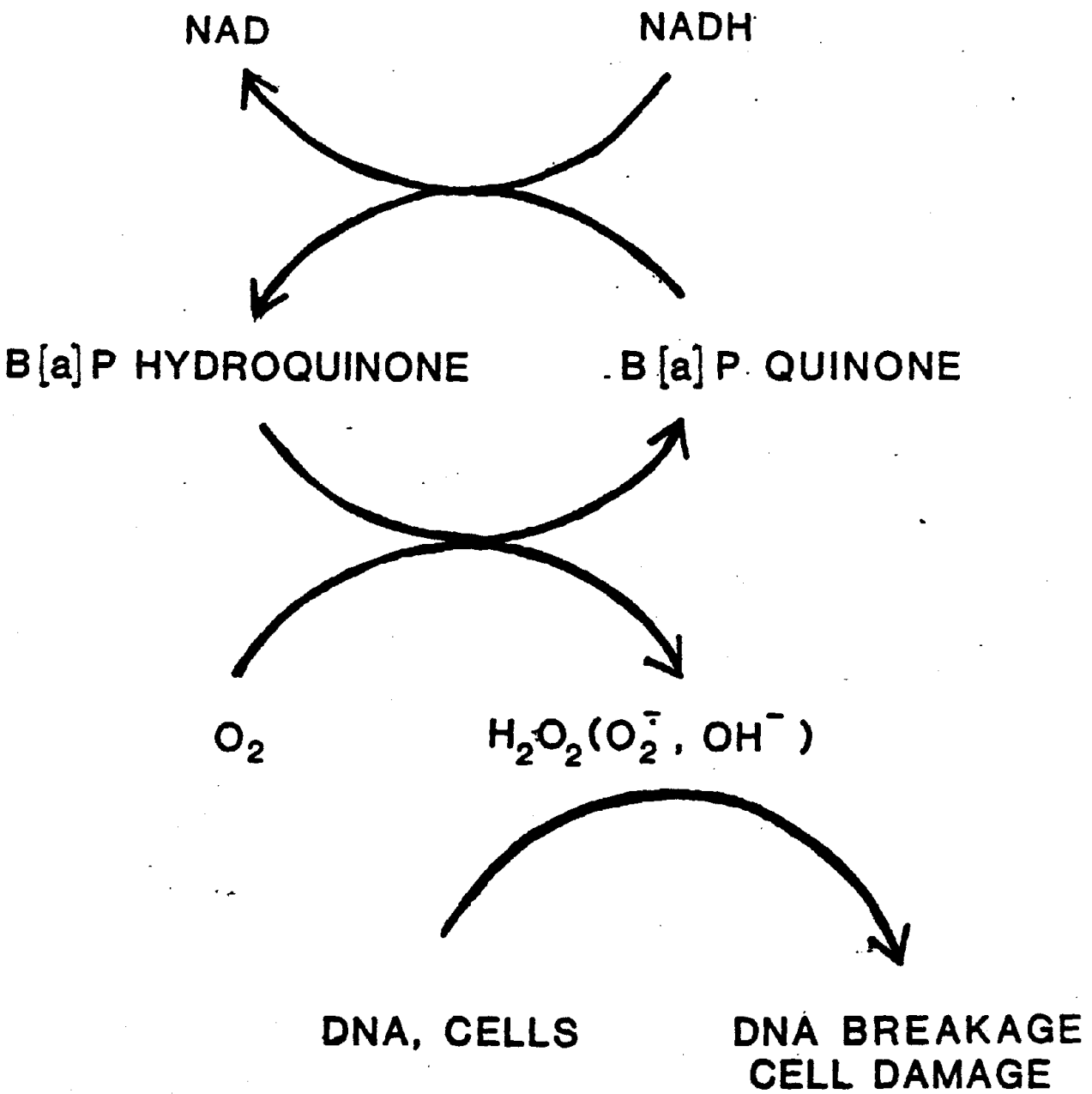


FIGURE III-5

Cyclic Scheme of Benzo[a]pyrene Dione/Diol Involvement in Redox Coupling

Source: Lesko et al., 1978

Fe_2O_3. After 180 minutes the Fe_2O_3 pretreated rabbit lungs showed an increase of benzo[a]pyrene metabolism over the benzo[a]pyrene-only treated rabbit lungs. The benzo[a]pyrene metabolites, namely dihydrodiols, were particularly enhanced by pretreatment with Fe_2O_3.

Hydroperoxide-dependent epoxidation of the 7,8-diol has been characterized in several other systems, such as microsomal lipid peroxidation (Dix and Marnett, 1983), hematin catalyzed decomposition of fatty acid hydroepoxides (Dix et al., 1985) and lipoxygenase catalyzed epoxidation (Hughes et al., 1989).

The toxification of benzo[a]pyrene by hydroxymethylation catalyzed by "hydroxymethyl synthetase" has also been suggested, but the presence of this pathway has not been confirmed (Rogan et al., 1980).

Both γ-radiation and UV-light convert benzo[a]pyrene to reactive forms, which bind covalently to macromolecules and are mutagenic, but the significance of these mechanisms in mammalian species is not known (Pelkonen and Nebert, 1982). Metabolites could also disturb DNA structure without binding covalently by slipping in between the planes of the helix (intercalation). PAH tetraols have been shown to do this in vitro (Geacintov et al., 1980).

Comparative Metabolism. One factor that affects the delicate balance of toxification/detoxification is the tissue site that catalyzes the formation of chemically reactive metabolites. The degree of induction or inhibition of enzyme systems by exogenous chemicals is known to vary for

specific organs; for example, liver is generally more inducible than is lung. The stability of reactive metabolites is also an important factor. If the biologic half-life is sufficiently long, the metabolite may be transported to other tissues for action or further metabolism. Several reactive metabolites in one tissue may compete for sites. Differences in site of administration will also affect metabolism.

Freudenthal et al. (1978) examined the metabolism of benzo[a]pyrene by lung microsomes isolated from humans, rhesus monkeys and Sprague-Dawley rats. Human samples were normal lung tissues of subjects with pulmonary tumors. The metabolites of ^{14}C-benzo[a]pyrene were analyzed by HPLC. Large individual variation in amounts of metabolites produced among monkey and human samples were seen, probably due to the genetic heterogenicity of the test subjects. Human subjects also had a wide range of age, were of both sexes and had dissimilar environmental contacts. Qualitative differences between the types of metabolites produced by humans as compared with those produced by the other animal species were less dramatic. This study demonstrated that the validity of comparison across species may not be compromised if caution is paid to the considerations of age, gender, exposure to inducing agents and other factors (U.S. EPA, 1980d).

That species differences in metabolism must be considered in carcinogenic potency, however, is demonstrated for chrysene by the work of Weston et al. (1985). They used short-term cultures of rat skin (in which chrysene is not a strong carcinogen), mouse skin (in which chrysene does cause a carcinogenic response) and human skin. For each skin type a major pathway leading to DNA adduct formation involved formation of chrysene bay-region

diol-epoxides (chrysene-1,2-diol-3,4-epoxide). Mouse skin, however, released larger quantitites of free dihydrodiols to the medium than either rats or humans. Moreover, there was greater chrysene adduct formation in mouse skin.

Excretion

Hepatobiliary excretion and elimination in the feces is the principal route by which metabolites of PAHs are excreted. Early work by Peacock (1936), Chalmers (1940), and Chalmers and Kirby (1940) established that the fluorescent material appearing in the bile of rabbits, guinea pigs, rats and fowl following intravenous injection of either colloidal benzo[a]pyrene or colloidal anthracene was not the administered hydrocarbon but a derivative. Excretion in the bile was well established within 15 minutes of injection, and only a small amount of fluorescence appeared in the urine of rabbits and rats. Almost none of the excreted material was unchanged benzo[a]pyrene. Chalmers and Kirby (1940) and Berenblum and Schoental (1942) found that only 1% of a subcutaneous dose of 60 mg benzo[a]pyrene was eliminated unchanged in the feces of rats or mice. Kotin et al. (1959) confirmed this fraction, and noted in addition that all elimination of unchanged benzo[a]pyrene occurred within the first 10-15 minutes following an intravenous dose.

Metabolism is the rate-determining step for excretion into the bile. Induction of metabolism with 3-methylcholanthrene enhanced the rate and extent of excretion of metabolites of benzo[a]pyrene in rat bile (Iqbal et al., 1979). Pre-treatment of rats with either 3-methylcholanthrene or benzo[a]pyrene itself also increased the rate of excretion into the bile of metabolites of subsequently administered benzo[a]pyrene. There was,

however, no such pretreatment effect on excretion when the metabolites themselves were given intravenously, demonstrating that the effect of the inducers is on metabolite formation and not on metabolite transfer to bile (Schlede et al., 1970).

Enterohepatic circulation of metabolites occurs, and a portion of urinary excretion of metabolites can be attributed to this source. Kotin et al. (1959) observed that in rats with biliary fistulas, urinary excretion of benzo[a]pyrene metabolites was reduced from 7-14% to 2-4% of the dose. The existence of an enterohepatic circulation suggested by this observation was confirmed by Chipman et al. (1982), who collected biliary metabolites of benzo[a]pyrene and introduced them into the duodena of bile-cannulated rats, following which the metabolites appeared in both bile and urine.

A very small fraction is excreted in pancreatic juice. Iqbal et al. (1979) observed that during the initial 2-hour period following intravenous administration of benzo[a]pyrene to rats, 0.03% of the dose was excreted in pancreatic juice while 39% was excreted in the bile. In view of the low metabolic potential of pancreatic microsomes in vitro, the authors suggested that metabolites of benzo[a]pyrene in the pancreas may have originated in the liver and been transferred to the pancreas by the systemic circulation.

Systemically absorbed benzo[a]pyrene or its metabolites (Heidelberger and Weiss, 1951; Kotin et al., 1959; Sanders et al., 1984) and systemically absorbed 7,12-dimethylbenzanthracene or its metabolites are also not eliminated in expired air (Lo, 1964; Sanders et al., 1984).

The fractions of ^{14}C-benzo[a]pyrene and ^{14}C-7,12-dimethylbenzanthracene-associated radiolabel excreted by rats in urine and bile have been quantitated. Thirty-eight percent of an intravenous dose of benzo[a]pyrene of unspecified size was excreted in bile by 2 hours; urine was not monitored in this study (Iqbal et al., 1979). Kotin et al. (1959) found 39% of intravenous doses $\leq$134 µg in the bile at 3 hours, and as much as 96% in the bile by 14 hours. In rats with biliary fistulas, a maximum of only 3-4% of radioactivity was recovered in the urine after a 24-hour period. Intact rats had a urinary excretion of 7-14% of the administered dose, suggesting that enterohepatic circulation serves as a secondary source of radioactivity that is excreted in the urine. The amount in the bile was not proportional to dose at doses of $\geq$150 µg; only 32% of a 400 µg dose was recovered in the bile at 24 hours. This amount (0.32x400 µg = 128 µg) is equal to that found in the bile 14 hours after the 134 µg dose (0.96x134 µg = 129 µg), illustrating the limited capacity of the rat to metabolize benzo[a]pyrene.

Lo (1964) administered 3 mg doses of ^{14}C-7,12-dimethylbenzanthracene intravenously to three rats, and reported that 10-16% of the radiolabel had been recovered in the urine and 18-44% in the feces by 17 days. Sanders et al. (1984) found 38% of recovered radiolabel from an intravenous dose of 1.04 µg in rat feces by 7 days, and 26% in urine. These observations indicate that the urine is a more important route of excretion for metabolites of 7,12-dimethylbenzanthracene than for metabolites of benzo[a]pyrene.

Additional data on relative utilization of urine and feces as routes of excretion are summarized in the following Dermal Section.

Oral. Because most of an absorbed oral dose of a PAH reaches the liver directly via the portal circulation before reaching the systemic circulation, hepatic metabolism and biliary excretion may determine the relationship between administered dose and systemic dose. It is to be expected that at reasonable doses a greater fraction of an oral dose than of an intravenous dose would be excreted in the feces. In fact, low doses of orally administered PAHs could, in principle, be fully metabolized and excreted in the bile without reaching the systemic circulation at all. Twenty-four hours after administration of 50 μg pyrene to rats, 24 μg was found in the GI tract and none in the liver, kidney, lung or trachea (Mitchell and Tu, 1979). However, controlled studies of elimination following oral doses of PAHs have generally utilized large amounts of the hydrocarbons, so that substantial fractions are not metabolized in the first pass. Interpretation of biliary excretion data following an oral dose is further complicated by enterohepatic circulation.

Rahman et al. (1986) administered five radiolabeled PAHs including benzo[a]pyrene and 7,12-dimethylbenzanthracene in 1.0 mg doses to rats intraduodenally, and cumulative excretion of radiolabel in bile and urine after 24 hours was measured and expressed as a fraction of administered radiolabel. For benzo[a]pyrene these figures were 6% for urine and 25% for bile, for 7,12-dimethylbenzanthracene they were 3% for urine and 25% for bile. These data for benzo[a]pyrene are consistent with the corresponding values obtained following intravenous administration of high doses (see preceding discussion), suggesting that hepatic capacity to metabolize the absorbed PAHs on the first pass had been greatly exceeded.

Modica et al. (1983) gave emulsions of benz[a]anthracene, chrysene and triphenylene to rats by stomach tube. Seventy-two hours later, cumulative fecal elimination was 6% of the benzanthracene dose of 22.8 mg, 3% of the triphenylene dose of 22.8 mg, 38% of the chrysene dose of 22.8 mg, and 41% of the chrysene dose of 11.4 mg, indicating that even these relatively massive doses are absorbed surprisingly well.

Eisele (1985) examined the uptake and distribution of tracer levels of ^{14}C-naphthalene in laying pullets, swine and dairy cattle. Each group of animals received either a single oral dose or daily oral doses of naphthalene for 31 days. The distribution of naphthalene and/or its metabolites in various tissues was measured 24 hours after the last dose. Considerable species differences in the distribution of naphthalene and/or its metabolites were observed. However, in each of the exposed animals naphthalene and/or its metabolites reached the systemic system and distributed to all tissues examined.

Inhalation. Elimination of an inhaled dose of a PAH follows its lung clearance pattern, that is, the fraction that is systemically absorbed from the lung is excreted in bile and urine, while the fraction that is cleared from the lung by mucociliary action and swallowed is subject to GI absorption. In this case, however, the amounts reaching the duodenum may be too low to approach the capacity of the liver to metabolize them on the first pass. In support of this supposition, Pylev et al. (1969) observed that excretion of radiolabel in feces and urine of hamsters given intratracheal instillations of ^{3}H-benzo[a]pyrene alone, or ^{3}H-benzo[a]pyrene-coated carbon or asbestos particles, followed the same general time course as loss

from the lung, and that for a period of 36 days elimination via urine remained at ~80% of that in feces.

The most detailed and precise studies in which excretion was measured following inhalation exposure are those from the Inhalation Toxicology Research Institute, in which rats were exposed by inhalation to pure ^{3}H-benzo[a]pyrene aerosols and to ^{3}H-benzo[a]pyrene adsorbed on gallium oxide particles, and excretion of radiolabel was followed for time periods $\leq$16 days. Using pure aerosols of ^{3}H-benzo[a]pyrene (500 µg/ℓ) with a mass median diameter of 1-2 µm, Mitchell (1982) observed that radiolabel was excreted both in the feces and in the urine, with radioactivity in the feces ~10 times that in the urine during the first 24 hours after exposure. Subsequently (Sun et al., 1983), this experiment was repeated with a lower concentration of ^{3}H-benzo[a]pyrene, 600 ng/ℓ, and was extended by exposures to gallium oxide particles coated with ^{3}H-benzo[a]pyrene. Both aerosols had the same mass median diameter, ~0.1 µm as in the previous study. The pure aerosol was, as before, eliminated in urine and feces, with the total amount of radiolabel eliminated in feces through the sixteenth day after exposure about 6 times the amount eliminated in the urine. Since the aerosol concentration in this study was 1/800 that used in the previous study, it must be concluded that the elimination pattern is independent of aerosol concentration at least up to ~500 µg/ℓ. That is, these aerosol concentrations do not stress hepatobiliary metabolism and excretion mechanisms. Based on a comparison with the work of Kotin et al. (1959), this is a reasonable conclusion. Kotin et al. (1959) found that oral doses $\leq$134 µg/rat did not exceed the maximum metabolic capacity of the animals.

When benzo[a]pyrene adsorbed to gallium oxide particles (3500 ng/ℓ) was inhaled, the total amount of radiolabel eliminated in the feces through the sixteenth day following exposure was ~12 times the amount eliminated in urine, demonstrating the shift in excretion pattern caused by the presence of the particles.

When benzo[a]pyrene was presented to rats as an aerosol coated on diesel exhaust particles (Sun et al., 1984), the amount excreted in feces by the twenty-sixth day following exposure was ~5 times the amount excreted in the urine.

Dermal. Since only an insignificant amount of radiolabel appears in the GI tract as a result of grooming by rats whose back skin was painted with PAHs (Mitchell and Tu, 1979; Sanders et al., 1984), the cumulative fractions of an absorbed cutaneous dose eliminated in urine and in feces should be comparable with the cumulative fractions found after intravenous administration. Excretion of radiolabel from ^{14}C-benzo[a]pyrene, ^{14}C-7,12-dimethylbenzanthracene and ^{14}C-anthracene, absorbed percutaneously, has been examined. The results are similar to the results of the intravenous studies discussed above.

Thirty-five percent of the radiolabel associated with a cutaneous dose of 12.5 μg ^{14}C-benzo[a]pyrene/cm^2 (22.5 μg total dose) was found in the feces at 24 hours and 80% at 7 days, with ~10% in the urine at 7 days, by which time nearly all the benzo[a]pyrene had been absorbed (Sanders et al., 1984). 7,12-dimethylbenzanthracene metabolites were excreted to a greater extent in the urine than benzo[a]pyrene metabolites. After 7 days,

30% of the radiolabel associated with a cutaneous dose of 5.4 μg ^{14}C-7,12-dimethylbenzanthracene/cm^2 (1.1 μg total dose) was recovered in the urine and only 62% in the feces (Sanders et al., 1984). Yang et al. (1986) found that of the 52% of the absorbed radiolabel from a cutaneous dose of 9.3 μg ^{14}C-anthracene/cm^2, 29% had been excreted in the urine and 21% in the feces after 6 days.

Interestingly, these fractions appear to be dose-dependent. Increasing the benzo[a]pyrene dose from 1.25 to 12.5 to 125 μg/cm^3 resulted in a shift in the excretion pattern at 24 hours in favor of the feces. In contrast, increasing the 7,12-dimethylbenzanthracene dose from 5.4 to 56 to 515 μg/cm^2 decreased the fraction of the dose excreted in both feces and urine, although fecal excretion was less markedly affected than urinary excretion (Sanders et al., 1984). These shifts were greater than could be accounted for by the reduction in percentage absorbed at the higher doses, encouraging the speculation that differential capacity limitations of the enzyme systems responsible for the formation of different metabolites might result in a relative decrease in production at high doses of metabolites for which the urine is the favored route of excretion.

Summary

The PAHs are a lipophilic class of xenobiotics that are readily absorbed across cellular membranes. Major routes of environmental exposure to PAHs are the following: the GI tract by contaminated food or water; the lungs by inhalation of aerosols or by hydrocarbon-adsorbed particles; and the skin by direct contact. Once absorbed, the PAHs are rapidly and widely distributed. The differing availability of the PAHs may depend on the

chemical form in which exposure occurs (for example, hydrophilic or lipophilic solution), varying intestinal absorption, metabolic transformation or removal rates. PAHs can be observed in most internal organs from minutes to hours after administration by various routes. Adipose and mammary tissues are significant distribution sites in which PAHs may be stored and slowly released.

PAH metabolism is affected by the route of administration. PAHs administered through the lungs or through i.p. or i.v. injection may avoid first pass metabolism in the liver. The blood will distribute the PAHs into peripheral tissues. This distribution will rapidly result in the establishment of a steady-state concentration between the blood and well-perfused tissues. Adipose tissues, however, will exchange the deposited PAHs more slowly and will accumulate and slowly release PAHs. On the other hand, oral administration of PAHs results in metabolism by the liver before systematic distribution can occur via the bloodstream; consequently, PAH metabolites will appear in the bloodstream several hours after administration.

PAHs can be metabolized by enzyme-mediated oxidative mechanisms to form reactive electrophiles. The cytochrome P-450 dependent microsomal mixed function oxidase (MFO) system (arylhydrocarbon hydrolase) is inducible by PAH exposure. These enzymes are found in many mammalian tissues; MFO enzymes of the liver are the best studied and have been found to be the most abundant. Similar types of metabolites are produced by mammalian tissues; however, the proportions of the phenols, diols and epoxides produced may differ among the various tissues. The mechanism of the diolepoxide

metabolic pathway for PAH toxification and the bay-region hypothesis are accepted. Reactive epoxides, formed by MFO mediated PAH metabolism, have the ability to interact with and thereby alter DNA, RNA and proteins. PAH metabolites are most commonly conjugated with glucuronic acid, glutathione or sulfate. They are excreted by the GI tract after hepatobiliary excretion or the swallowing of mucus material cleared from the respiratory tract. Nonenzymatic mechanisms of biologic PAH alteration also occur.

IV. HUMAN EXPOSURE

Text to be provided by the Office of Drinking Water.

V. HEALTH EFFECTS IN ANIMALS

The general and specific health effects associated with PAH ingestion, and to a lesser degree, inhalation, peritoneal and dermal exposures, are examined in this chapter. Much of the research on the health effects of selected PAHs has focused on their potential as skin carcinogens, genotoxic agents and on their inhalation effects (IARC, 1983). Studies of individual PAHs administered by the oral route are generally lacking.

Acute Oral Toxicity

Acenaphthylene. An English abstract of a paper by Knoblock et al. (1969) reported that acenaphthylene administration "to the stomach" resulted in an LD_{50} of 3 g/kg for rats and 2.2 g/kg for mice.

Anthracene. The abstract of a Russian study indicated that single oral doses of 1.47 or 2.44 g/kg of commercial grade anthracene or 17 g/kg of pure anthracene were not lethal to mice (Nagornyi, 1969). Toxic effects reportedly included fatigability; adynamia; histologic hyperemia in the kidney, liver, heart and lungs; "lipid dystrophy" in the liver; and leukocytosis with neutrophilia.

Systemically administered anthracene (50 mg/mℓ corn oil by gavage) followed by UV irradiation of the skin for 1 hour, 2 hours after dosing, produced keratitis of the exposed skin in mice (Dayhaw-Barker et al., 1985). This effect reportedly was less pronounced in mice exposed to UV light only and was not evident in vehicle controls.

Daily intragastric administration to five male Wistar rats of 100 mg/kg anthracene in olive oil for 4 days produced a nonsignificant (3-fold) increase in mean liver cytosolic aldehyde dehydrogenase activity (Törrönen et al., 1981). Neither treatment-related effects on aldehyde dehydrogenase activity in the liver microsomes or postmitochondrial fractions of small intestinal mucosa nor effects on liver/body weight ratios were observed.

Benz[a]anthracene. Pertinent data regarding the acute oral toxicity of benz[a]anthracene could not be located in the available literature.

Benzo[a]pyrene. Pertinent data regarding the acute oral toxicity of benzo[a]pyrene could not be located in the available literature.

Benzo[b]fluoranthene. Pertinent data regarding the acute oral toxicity of benzo[b]fluoranthene could not be located in the available literature.

Benzo[k]fluoranthene. Pertinent data regarding the acute oral toxicity of benzo[k]fluoranthene could not be located in the available literature.

Benzo[g,h,i]perylene. Pertinent data regarding the acute oral toxicity of benzo[g,h,i]perylene could not be located in the available literature.

Chrysene. Pertinent data regarding the acute oral toxicity of chrysene could not be located in the available literature.

Dibenz[a,h]anthracene. Pertinent data regarding the acute oral toxicity of dibenz[a,h]anthracene could not be located in the available literature.

Fluoranthene. The oral LD_{50} for male Carworth-Wistar rats exposed to fluoranthene is ~2000 mg/kg (Smyth et al., 1962).

Fluorene. Kizer et al. (1985), in an effort to identify enzyme changes as early indicators of hepatocarcinogenesis, examined the effect of several xenobiotics, including fluorene, on both the activity and amount of hepatic microsomal epoxide hydrolase. As part of this study, male Holtzman rats were fed a diet containing 0.06% (10.5 mg/kg/day) fluorene for 3 weeks. No hepatotoxicity, as measured by SGOT activity, was observed. Furthermore, fluorene exposure failed to result in an appreciable elevation of the activity of microsomal epoxide hydrolase. However, a 4-fold increase over controls of epoxide hydrolase antigen was detected by immunoassay. After comparing the activity and amount of hepatic microsomal epoxide hydrolase induced by feeding the various xenobiotics with their reported carcinogenic potential, the authors concluded that induction of microsomal epoxide hydrolase is not a "key change" leading to malignancy.

Indeno[1,2,3-cd]pyrene. Pertinent data regarding the acute oral toxicity of indeno[1,2,3-cd]pyrene could not be located in the available literature.

Naphthalene. The LD_{50} for oral exposure to naphthalene has been determined for several species. The LD_{50} values for naphthalene dissolved

in peanut oil for male and female Sherman rats were 2200 and 2400 mg/kg, respectively (Gaines, 1969). In two other studies, the LD_{50} values for rats were 1780 (Toxicology Data Bank, n.d.) and 9430 mg/kg (U.S. EPA, 1980c); the strain and sex of the animals was not specified. For male and female CD-1 mice, the acute oral LD_{50} values of naphthalene in corn oil were 533 and 710 mg/kg, respectively (Shopp et al., 1984). In a recent study (unpublished) conducted by Mallory et al. (1985a), the acute LD_{50} values of naphthalene in corn oil for male and female Sprague-Dawley rats were reported to be 2009 and 3310 mg/kg, respectively. An LD_{50} of 353 mg/kg was determined for CD-1 mice in a study in which Plasterer et al. (1985) treated mice at doses ranging from 125-2000 mg/kg/day for 8 days.

Although cataract formation in rats following oral administration of naphthalene has been known for many years (Fitzhugh and Buschke, 1949), recent studies have shown that ocular changes can result from a single dose of naphthalene. van Heyningen and Pirie (1967) found that lens changes developed in the eyes of rabbits after a single gavage dose of 1000 mg/kg. In CD-1 mice, oral doses of $\geq$400 mg/kg for males and $\geq$600 mg/kg for females resulted in ptosis with clear, red secretions around the eyes within 1 hour of dosing (Shopp et al., 1984).

Ikemoto and Iwata (1978) reported that oral administration of naphthalene (1 g/kg) to male and female albino rabbits for 2 consecutive days resulted in cataract formation. Occurrence of cataracts was accompanied by a decrease in sufhydryl content in both soluble and insoluble lens protein.

Rao and Pandya (1981) orally administered male BHD and AR albino rats 1000 mg/kg naphthalene each day for 10 days. Significant increases in the relative liver weight ($p<0.01$), and in liver aniline hydroxylase activity and lipid peroxidation ($p<0.001$) were observed in the treated animals. A moderate (nonsignificant) increase in lipid peroxidation was also observed in the eyes. No treatment-related changes in sulfhydryl content or alkaline phosphatase activity were observed in the liver, kidneys or eyes.

Yamauchi et al. (1986) reported that a single oral dose of napthalene (1000 mg/kg) to male Wistar rats resulted in a significant increase in serum lipid peroxide levels ($p<0.05$) beginning on the 4th day after administration and continuing through the 20-day observation period. This increase was paralleled by a significant ($p<0.05$) decrease in GSH content in the lens of the exposed animals. Zuelzer and Apt (1949) reported a hemolytic effect when naphthalene was administered to dogs in their diets.

Phenanthrene. Pertinent data regarding the acute oral toxicity of phenanthrene could not be located in the available literature.

Pyrene. Pertinent data regarding the acute oral toxicity of pyrene could not be located in the available literature.

Acute Toxicity By Other Routes

Acenaphthylene. In a study published in Russian (Rotenberg and Mashbits, 1965), acenaphthylene was administered to white rats intratracheally in a sunflower oil solution or by blowing acenaphthylene powder into the trachea. The dosing schedule used was not provided. The pulmonary tracts of animals sacrificed 1 month after the experiment began showed signs

of tracheobronchitis and hyperemia, edema and necrosis of the epithelium in the trachea and bronchi with the formation of ulcers. No further details of this study were available.

In a corn oil gavage study acenaphthylene was administered daily to 10 CD-1 mice/sex/group at 0, 125, 250, 500, 750 and 1000 mg/kg/day. In the 1000 mg/kg/day group the mean body weights were significantly reduced in both sexes at the end of the first week. Dose-related clinical symptomology was noted in groups receiving 500, 750 and 1000 mg/kg/day; these symptoms included languid behavior, prostration, decrease in body temperature (cold to touch) and toxic effects to the eyes. These eye effects included enlargement, drying out and eventual crusting over. In the 1000 mg/kg/day group eyes were adversely affected in 40% of the males and 50% of the females. The survival rate in the 750 mg/kg/day group was 90% among the males and 80% among the females; in the 1000 mg/kg/day group survival was significantly reduced in both sexes at 40%. Gross pathology from the 750 and 1000 mg/kg/day groups indicated that the liver, stomach, eyes and subcutaneous tissues were affected by the treatment (Hazelton Laboratories America Inc., 1989a).

Anthracene. Mice treated i.p. with anthracene were found to have an LD_{50} of >430 mg/kg bw (Salamone, 1981). In a study by Gerarde (1960), mice were given i.p. injections of 500 mg/kg bw/day for 7 days. Of the 10 mice treated, 9 survived. The ID_{50} (skin irritant dose) for the mouse was found to be 6.6×10^{-4} mmol/ear (Brune et al., 1978).

In a corn oil gavage study anthracene was administered daily for 14 days to 10 CD-1 mice/sex/group at 0, 125, 250, 500, 750 and 1000 mg/kg/day. No

treatment-related differences were observed between the groups in body weight gain, mortality and gross pathologic examination (Hazelton Laboratories America, Inc., 1989b).

Benz[a]anthracene. Pertinent data regarding the acute nonoral toxicity of benz[a]anthracene could not be located in the available literature.

Benzo[a]pyrene. The mouse LD_{50} (i.p.) for benzo[a]pyrene is ~250 mg/kg (Salamone, 1981). The ID_{50} for skin irritation in mice is 5.6×10^{-5} mmol/ear (Brune et al., 1978).

A single i.p. dose of 10 mg benzo[a]pyrene produced a reduction in the growth rate of immature rats (Haddow et al., 1937). Hellman et al. (1984) studied the acute toxicity of injections of benzo[a]pyrene in C57Bl male mice. Groups of 7-10 animals were injected i.p. with 1.12×10^{-4} mol/kg benzo[a]pyrene in corn oil. Two hours before sacrifice animals were injected with ^{3}H-thymidine to determine the rate of DNA turnover. A significant decrease (40%) in ^{3}H-thymidine incorporation into the thymus, spleen, small intestine and testis occurred 48 hours after benzo[a]pyrene treatment, which was indicative of decreased DNA synthesis in those organs. There was a stimulatory effect on thymidine incorporation in the liver 48 hours after the injection of benzo[a]pyrene.

Robinson et al. (1975) showed that "responsive" mice (those capable of producing increased levels of cytochrome P-450 mediated enzymes as a consequence of PAH exposure) had reduced survival time following a single i.p. dose of 500 mg/kg bw benzo[a]pyrene.

Wojdani et al. (1984) reported that target cell killing by lymphocytes from two mouse strains was decreased following a single i.p. injection of 5 or 50 mg benzo[a]pyrene/kg. This study is described in the Target Organ Toxicity Section.

Subcutaneous injections of benzo[a]pyrene in corn oil at doses of 5, 20 or 40 mg/kg in female B6C3F1 mice produced a dose-related suppression of antibody production to both T-cell independent and T-cell dependent antigens (White and Holsapple, 1984).

Benzo[b]fluoranthene. Pertinent data regarding the acute nonoral toxicity of benzo[b]fluoranthene could not be located in the available literature.

Benzo[k]fluoranthene. Pertinent data regarding the acute nonoral toxicity of benzo[k]fluoranthene could not be located in the available literature.

Benzo[g,h,i[perylene. Pertinent data regarding the acute nonoral toxicity of benzo[g,h,i]perylene could not be located in the available literature.

Chrysene. The LD_{50} (i.p.) for chrysene was found to be >320 mg/kg bw in mice (Simmon et al., 1979). A single i.p. injection of 7.5 mg chrysene in sesame oil produced no toxic effects in AKR/J or C57Bl/6J mice during a 20-day observation period or upon necropsy.

Dibenz[a,h]anthracene. One or two intraperitoneal administrations of 3-90 mg/kg bw dibenz[a,h]anthracene in sesame oil produced a reduction in the growth rate of young Lister strain hooded rats. This persisted for at least 15 weeks (Haddow et al., 1937).

Fluoranthene. The 24-hour LD_{50} value after dermal exposure to fluoranthene was found to be 3180 mg/kg in rabbits (Smyth et al., 1962). No information was reported concerning target organs or specific cause of death. As part of the same study, Smyth et al. (1962) observed no mortality in six male and six female albino rats exposed to concentrated vapors of fluoranthene for 8 hours.

Haddow et al. (1937) examined the effect of various PAHs, including fluoranthene, on body growth in hooded rats of the Lister strain. A single i.p. injection of 10 mg/kg fluoranthene dissolved in sesame oil had no adverse effect on body weight gain over a 24-day observation period.

Asokan et al. (1986) examined the induction of cutaneous and hepatic monoxygenase activities by fluoranthene. Twenty-four hours after a single topical application of 10 mg/kg fluoranthene to the backs of Sprague-Dawley rats, significant increases in aryl hydrocarbon hydroxylase, 7-ethoxyresorufin O-deethylase and 7-ethoxycoumarin O-deethylase activities were observed in the skin and liver.

Fluorene. Pertinent data regarding the acute nonoral toxicity of fluorene could not be located in the available literature.

Indeno[1,2,3-cd]pyrene. Pertinent data regarding the acute nonoral toxicity of indeno[1,2,3-cd]pyrene could not be located in the available literature.

Naphthalene. Irie et al. (1973) studied the effects of naphthalene in mice (strain not specified) following single subcutaneous injections at doses of 650-1348 mg/kg. Vigorous tremors were noted in the mice for 3-4 days following dosing. The LD_{50} value was calculated to be 969 (891-1053) mg/kg.

The 24-hour LD_{50} value of naphthalene in Swiss-Webster mice was 380 (350-413) mg/kg following i.p. injection (Warren et al., 1982; Shank et al., 1980). Those mice that died did so within 24 hours; survivors were observed for an additional 6 days. The target organ was identified by Warren et al. (1982) as the lungs, in which naphthalene caused a dose-dependent increase in bronchiolar epithelial necrosis at doses $\geq$200 mg/kg. The pulmonary damage and lethality resulting from naphthalene administration were markedly inhibited by prior treatment with piperonyl butoxide and enhanced by prior treatment with diethyl maleate. This supports the view that P-450-dependent metabolism of naphthalene is responsible for the observed toxicity and that glutathione plays an important role in the detoxification of the lung-damaging metabolite(s).

Reid et al. (1973) gave napthalene dissolved in sesame oil to C57Bl/6J mice by the intraperitoneal route and found coagulative necrosis of the bronchiolar and bronchial epithelium at a dose of 600 mg/kg. Controls received sesame oil alone, and no adverse effects were reported for this group. The size of the treatment groups was not stated.

Mahvi et al. (1977) administered naphthalene in corn oil intraperitoneally to C57Bl/6J mice. Two groups of 63 mice received corn oil alone or remained untreated. Groups of 21 mice each were given 67.4, 128 or 256 mg/kg. Three animals from each dosage group were sacrificed at 10 minutes, 1 hour, 6 hours, 12 hours, 24 hours, 48 hours, and 7 days following treatment. Lung tissue was rapidly fixed and examined by light, scanning electron, and transmission electron microscopy. No changes were noted in either control group. Minor bronchiolar epithelial changes were noted in the group receiving 67.4 mg/kg. Mice in the higher dose groups developed necrosis of secretory nonciliated bronchiolar cells and the adjacent ciliated cells. Epithelial structure returned to normal within 7 days in all cases.

Tong et al. (1982) found histologic changes in the lungs of C57Bl/6J mice treated i.p. with 225 mg/kg naphthalene. One day after dosing, the Clara cells in the terminal bronchioles were pyknotic, and hypereosinophilic nuclei appeared to be detaching from the bronchiolar wall. Three days after dosing, some surfaces appeared to be completely denuded of Clara cells, whereas other surfaces appeared to have immature Clara cells scattered circumferentially. Five days after treatment, there was still evidence of incomplete recovery; by 8 days, most of the terminal bronchioles were reepithelialized; and by 15 days, mature Clara cells were common, but recovery was evidently not complete.

O'Brien et al. (1985) investigated differences in naphthalene-induced toxicity subsequent to i.p. treatment of male Swiss T.O. mice and male Wistar-derived rats. In mice doses $\geq$200 mg/kg resulted in damage to the

nonciliated bronchiolar epithelial cells, and doses ≥400 mg/kg resulted in damage to cells in the proximal tubules of the kidney. Rats were more resistant to the cytotoxic effects of naphthalene, as doses of naphthalene as high as 1600 mg/kg caused no detectable pulmonary or renal damage. This species difference in toxicity was reflected by a larger depletion of nonprotein sulfhydryls in the lung and kidney of the mouse than in those organs in the rat.

It previously had been reported that the 8-hour inhalation LC_{50} value for naphthalene was 100 ppm (Union Carbide, 1968). However, Buckpitt (1985) suggested that this value may be too low. He estimated that in 8 hours the body burden would be <30 mg/rat, or ~150-200 mg/kg. This concentration is less than the oral or i.p. LD_{50} values for rats. Fait and Nachreiner (1985) reported that exposure of male and female Wistar rats to 78 ppm naphthalene for 4 hours resulted in no mortalities, nor any lung, liver, kidney, or nasal passage abnormalities. In an unpublished inhalation study with male Swiss-Wesbster mice, no deaths were noted following nose-only exposures to 90 ppm for 4 hours. Lung lesions however, were reported (Buckpitt, 1985).

van Heyningen and Pirie (1967) treated one rabbit intravenously with 300 mg of a dihydrodiol metabolite of naphthalene in divided doses over 3 days and noted retinal lesions. They also noted lens changes in four rabbits dosed externally with eye drops of the same compound (1% dissolved in water) over a period of 2-5 days for a total of 40-70 mg per rabbit.

Acute ocular irritation was noted in 2/6 New Zealand white rabbits receiving no postdose eye rinse after 24 and 48 hours of exposure to 0.1 mg

naphthalene (Mallory et al., 1985c). This response included slight iritis, moderate redness and slight swelling and discharge. All animals appeared normal by 72 hours postdosing. No positive response was noted in rabbits (three) receiving a postdose rinse.

No deaths occurred when 2500 mg/kg of naphthalene was applied to the skin of male and female Sherman rats (Gaines, 1969). The application of 2000 mg naphthalene/kg (dissolved in acetone) to the skin of New Zealand white rabbits did not cause mortality; the LD_{50} was, thus, >2000 mg/kg (Mallory et al., 1985b). These studies suggest that naphthalene may not be as readily absorbed through the skin as it is through the intestinal mucosa.

Naphthalene (moistened with 2 mℓ of acetone) was found to be slightly to moderately irritating to the skin of male and female New Zealand white rabbits 30-60 minutes postdosing (Mallory et al., 1985d). Dermal irritation was still evident up to 5 days after test material application. Fissuring of the skin was also noted.

Naphthalene (100%) did not cause delayed hypersensitivity in Hartley guinea pigs (Mallory et al., 1985e).

Phenanthrene. The LD_{50} for mice (i.p.) is 700 mg/kg bw (Simmon et al., 1979).

Yoshikawa et al. (1985) investigated effects of i.p. exposure to phenanthrene, pyrene and some of their oxidized products in Sprague-Dawley rats. As part of this experiment, groups of three males each were injected with

either 3.0 mL/kg saline, 3.0 mL/kg DMSO or 150 mg/kg phenanthrene in DMSO. An additional set of animals was similarly treated on a second occasion. Blood was obtained at 24 or 72 hours post-treatment by cardiac puncture and the following measurements taken: aspartate aminotransferase (AST), alanine aminotransferase, γ-glutamyl transpeptidase (GGTP), lactic dehydrogenase, glucose, bilirubin, BUN and creatinine. Gross observation was made of organs. Phenanthrene treatment resulted in a significant elevation of serum AST and GGTP by comparison with both controls. GGTP returned to control levels by 72 hours. Livers of animals killed at either 24 or 72 hours were described as congested with a distinct lobular pattern. Kidneys were reported to be somewhat smaller in size and congested.

Pyrene. The intraperitoneal $LD_{50(7)}$ (dose lethal to half the animals in 7 days) for B6C3F1 mice was found to be 514 mg/kg pyrene and the intraperitoneal $LD_{50(4)}$ (dose lethal to half the animals in 4 days) was 678 mg/kg bw (Salamone, 1981). The growth rate of young (46-48 days old) Lister rats was not affected by intraperitoneal administration of 10 mg of pyrene in sesame oil (Haddow et al., 1937).

Yoshikawa et al. (1985) administered 150 mg/kg pyrene to male Sprague-Dawley rats as described in the preceding section. This resulted in a small but significant elevation in both serum AST and bilirubin. At both 24 and 72 hours sacrificed rats were observed to have minimal congestion and swelling of livers.

Yoshikawa, T., W. Flory, L.P. Ruhr et al. (1987) treated male Sprague-Dawley rats with a single i.p. injection of 150 mg/kg pyrene in DMSO

and evaluated serum chemistry at 24 and 72 hours for indications of hepatotoxicity. No statistically significant differences were found for AST, alanine aminotransferase, sorbitol dehydrogenase, GGTP, lactate dehydrogenase, glucose, BUN or creatine between control rats receiving DMSO and rats administered pyrene. Furthermore, no histopathologic alterations were noted in animals necropsied 72 hours after treatment with pyrene.

Subchronic and Chronic Oral Toxicity

Bioassays for carcinogenicity are reported in this section only when they refer to health endpoints other than tumor incidence.

Acenaphthylene. Knobloch et al. (1969) reported on the effects associated with ingestion of both acenaphthylene and acenaphthene. One group of seven rats was given an oral dose of 0.6 g/kg bw of acenaphthylene in olive oil for 40 days. A second group of seven rats was given an oral dose of 2.0 g/kg bw of acenaphthene in olive oil for 32 days. Both PAHs yielded the following physiologic effects: "considerable" body weight loss, changes in the peripheral blood pattern, changes in renal function, and increased serum aminotransferase activities. Additional effects associated with acenaphthene only included mild morphologic damage to the liver and kidney, lung changes consisting of mild bronchitis, and localized inflammation of peribronchial tissue. Since the source of this information is an abstract, more precise quantification of the health effects is not possible.

In a Russian study (Rotenberg and Mashbits, 1965), acenaphthylene in oil was administered orally to white mice at a dose 1/10 the LD_{50} [LD_{50}=1760 (range of 1100-2800) mg/kg] every other day for 2 months. Treated mice

showed a significant lag in weight gain as compared with controls. Histopathologic examination of organs showed signs of stasis in the parenchymatous organs and albuminoid degeneration of the liver. The most severe changes were observed in the lungs, which showed hemorrhage with destruction of the interalveolar septa and focal bronchial pneumonia. Purulent foci were observed in isolated cases, and bronchogenic lung cancer was diagnosed in one mouse. Further details of this study were not provided.

In a study conducted by Hazelton Laboratories America, Inc. (1989C) for U.S. EPA acenaphthylene was administered to CD-1 mice (20/sex/group) by gavage at dosage levels of 0, 100, 200, or 400 mg/kg/day for at least 90 days. Effects examined included mortality, clinical signs, body weights, food consumption, opthalmology, hematology, clinical chemistry, organ weights, and gross and histopathology. There was no increase in mortality among males tested; however, the incidences of treatment-releated deaths in females were 15, 25 and 40% for low-, mid- and high-dose groups, respectively, compared with no deaths in female controls. No significant changes in mean body weights, body weight gains, or food consumption were found. Statistically significant ($p \leq 0.05$) treatment-related hematologic effects included decreased erythrocyte count in all male dose groups and high-dose females; decreased hemoglobin and hematocrit in mid- and high-dose males and high-dose females; increased platelet counts in mid- and high-dose males; and increased leucocyte and segmented neutrophil counts in high-dose females. Treatment-related clinical findings included significantly increased cholesterol and albumin in high-dose males and all groups of treated females, and increased total protein in mid- and high-dose males and all three female treatment groups. Mean absolute and relative liver weights

were significantly increased in all treatment groups of both sexes, accompanied by a dose-related increase in gross pathologic findings, including enlarged, dark, mottled, prominent reticular pattern or pale areas.

An increase in the incidence and severity of centrilobular hepatocellular hypertrophy was seen in all female treatment groups and in high-dose males. Individual cell necrosis was observed in high-dose females. No significant changes in mean absolute or relative kidney weights were found, but gross examination revealed a treatment-related increase in the occurrence of granular, pitted, rough, mottled, or small appearance. Nephropathy, increased incidence and severity of renal tubular dilatation, epithelial hyperplasia of the collecting ducts, slight hyperplasia of the transitional epithelium in the renal pelvis, and renal tubule microconcretions were observed in the kidneys of all treated females. An increase in the incidence of renal tubule regeneration was seen in high-dose males. Mean absolute and relative ovary weight decreases in mid- and high-dose females were accompanied by a slight increase in incidence and degree of inactivity, and fewer and smaller corpora lutea in the high-dose group. A small increase in the number of grossly observed ocular opacities was observed, but opthalmoscopic examination revealed no treatment-related ocular lesions. Based on liver and kidney changes and deaths in females the LOAEL was determined to be 100 mg/kg/day; no NOAEL could be determined since 100 mg/kg/day was the lowest dose given.

Anthracene. In a chronic bioassay for carcinogenic effects (Schmähl, 1955), a group of 28 BDI and BDIII rats received anthracene in the diet, starting when the rats were ~100 days old. The daily dosage was 5-15

mg/rat, and the experiment was terminated when a total dose of 4.5 g/rat was achieved on the 550th experimental day. The rats were observed until they died, with some living more than 1000 days. No treatment-related effects on lifespan or gross and histologic appearance of tissues were observed. Body weights were not mentioned, and hematologic parameters were not measured. No chronic LOAEL could be determined from this study.

In a 90-day subchronic toxicity study, the U.S. EPA (1989a) administered anthracene to groups of 20 male and female CD-1 (ICR)BR mice by gavage. Dose levels were 0, 250, 500 and 1000 mg/kg/day. Criteria evaluated for compound-related effects were mortality, clinical signs, body weights, food consumption, opthalmology, hematology, clinical chemistry, organ weights, organ-to-body weight ratios, gross pathology and histopathology. No treatment-related effects were noted; therefore, the NOAEL determined from this study is 1000 mg/kg/day.

Benz[a]anthracene. Pertinent data regarding the subchronic and chronic oral toxicity of benz[a]anthracene could not be located in the available literature.

Benzo[a]pyrene. Aplastic anemia, and ultimately death, have been linked to subchronic oral exposures to benzo[a]pyrene (Robinson et al., 1975). Strains of mice used in the experiment had been classified as either "responsive" or "nonresponsive", based on the strain's susceptibility to induction of cytochrome P-450 and associated enzymes by PAHs. Treatment groups, consisting of 30 animals/strain, were fed a laboratory diet ad libitum that had been soaked in corn oil containing benzo[a]pyrene; the

estimated oral dose was ~120 mg/kg/day. Responsive and nonresponsive control groups, each consisting of 30 animals, were fed the same diet that had been soaked in unadulterated corn oil. In three responsive strains (C57Bl/6, C3H/HeN, BALB/cAnN) fed benzo[a]pyrene, the following numbers of mice/group died over a 180-day period: 2/30, 3/30 and 1/30, respectively. This was by comparison with one mouse in the responsive strain (C57Bl/6) control group. Among the nonresponsive strains (AKR/N, DBA/2), all of the mice in the treatment groups died with at least half the deaths occurring within 15 days. Only two mice died in the nonresponsive (DBA/2) control group over the same period of time. The high mortality among the treated nonresponsive mice was attributed to pancytopenia, which led to death from hemorrhaging or overwhelming infection. The nonresponsive benzo[a]pyrene-treated mice also experienced a significant increase in relative liver-to-body weight ratios.

Benzo[b]fluoranthene. Pertinent data regarding the subchronic and chronic oral toxicity of benzo[b]fluoranthene could not be located in the available literature.

Benzo[k]fluoranthene. Pertinent data regarding the subchronic and chronic oral toxicity of benzo[k]fluoranthene could not be located in the available literature.

Benzo[g,h,i]perylene. Pertinent data regarding the subchronic and chronic oral toxicity of benzo[g,h,i]perylene could not be located in the available literature.

Chrysene. Pertinent data regarding the subchronic and chronic oral toxicity of chrysene could not be located in the available literature.

Dibenz[a,h]anthracene. Pertinent data regarding the subchronic and chronic oral toxicity of dibenz[a,h]anthracene could not be located in the available literature.

Fluoranthene. The U.S. EPA (1988) evaluated the oral toxicity of fluoranthene in a subchronic bioassay. For 13 weeks male and female CD-1 mice (20/sex/group) received either 0, 125, 250 or 500 mg/kg/day of fluoranthene dissolved in corn oil. Baseline blood evaluations were determined during the first week of study from an additional 30 animals/sex/group. Body weights, food consumption and clinical signs of toxicity were monitored at regular intervals during the experimental period. At the end of the study period the animals were sacrificed, submitted for autopsy, and hematologic and serum chemistry evaluations were performed.

All treatment groups exhibited increased salivation (never exceeding 10% of the population of any treatment group). There were dose-dependent increases in pigment accumulation in the liver and mild nephropathy characterized by the presence of multiple foci of tubular regeneration. A small, but statistically significant ($p \leq 0.01$), increase in relative liver weight was observed in mice receiving 125 mg/kg/day of fluoranthene. Statistically significant ($p \leq 0.05$) changes in mice receiving 250 mg/kg/day included increases in SGPT and absolute and relative liver weights, as well as decreases in packed cell volume and red blood cell numbers (females only) and albumin/globulin ratios. Statistically significant ($p \leq 0.05$) observa-

tions in mice receiving 500 mg/kg/day included increases in SGPT, serum globulin and absolute and relative liver weights, as well as decreases in packed cell volume (females only) and albumin/globulin ratios. Since there was no dose-related increase in clinical signs (i.e., salivation) and the changes in kidney and liver histopathology observed at 125 mg/kg/day were not considered adverse by the U.S. EPA (1991a), this dose level is considered the NOAEL. Based on hematologic alterations, increased SGPT levels, and changes in kidney and liver histopathology in animals receiving 250 mg/kg/day, the U.S. EPA (1991a) considered this dose level as the LOAEL.

Fluorene. Wilson et al. (1947) provided anecdotal reports on gross and histologic appearance of organs of rats exposed to fluorene in the diet as part of an oncology study. They observed significant decreases in the rate of growth among albino rats consuming 0.5 and 1.0% fluorene in the diet for 105 days. Furthermore, liver weights were increased in rats receiving $\geq$0.25% fluorene, spleen weights were decreased in all treated animals and testes weights were decreased in the high-dose rats. Neither numbers of animals nor any organ weights were published.

The U.S. EPA (1989a) conducted a subchronic toxicity study in which CD-1 mice (25/sex/group) were exposed for 13 weeks via gavage to 0, 125, 250 or 500 mg/kg/day fluorene suspended in corn oil. Parameters used to assess toxicity included food intake, body weight, clinical observations, hematology and serum chemistry and gross and histopathologic examinations. Increased salivation, hypoactivity and urine-wet abdomens in males were observed in all treated animals. The percentage of mice exhibiting hypoactivity was dose-related. In mice exposed at 500 mg/kg/day, labored

respiration, ptosis (drooping eyelids) and unkempt appearance were also observed. A significant decrease in erythrocyte count and hematocrit was observed in females treated with 250 mg/kg/day fluorene and in males and females exposed to 500 mg/kg/day. Decreased hemoglobin concentration and increased total serum bilirubin levels were also observed in the 500 mg/kg/day group. Decreases in erythrocyte count, hematocrit and hemoglobin concentration were all observed at 125 mg/kg. These effects, although apparently dose dependent, were not statistically significant at 125 mg/kg by comparison with controls. A significant decreasing trend in BUN and a significant increasing trend in total serum bilirubin were observed for high-dosed males and females. A dose-related increase in relative liver weight was observed in treated mice; a significant increase in absolute liver weight was also observed in the mice treated with $\geq$250 mg/kg/day fluorene. A significant increase in absolute and relative spleen and kidney weight was observed in males and females exposed to 500 mg/kg/day and males at 500 mg/kg/day, respectively. Increases in the absolute and relative liver and spleen weights in the high-dose males and females were accompanied by increased amounts of hemosiderin in the spleen and increased numbers of Kupffer cells of the liver. No other histopathologic lesions were observed. Using the data from U.S. EPA (1989a), the U.S. EPA (1991a) identified a LOAEL of 250 mg/kg/day for hematologic effects; the corresponding NOAEL is 125 mg/kg/day.

Indeno[1,2,3-cd]pyrene. Pertinent data regarding the subchronic and chronic oral toxicity of indeno[1,2,3-cd]pyrene could not be located in the available literature.

Naphthalene. Shopp et al. (1984) conducted a 14-day and a 90-day study on groups of male and female CD-1 mice administered naphthalene in corn oil by gavage. In the 14-day study, six groups of male and female mice (40-112/group) were given doses of 0, 27, 53 or 267 mg/kg/day; the highest dose was one-half the LD_{50} for male mice. Male mice demonstrated lower survival rates than females, apparently due to the aggressive behavior of group-housed male mice; however, the mortality in the high-dose groups of both male and female mice was 5-10% higher than in the control groups. There was a significant decrease (7-13%) in body weight in male and female mice receiving the high dose. The high-dose males exhibited a 30% decrease in thymus weight, while females exhibited a decrease in spleen weight and an increase in lung weight. Gross pathology but not histopathology was performed. No biologically relevant changes were noted in treated animals for the following measures: hematology, clinical chemistry, hexabarbital sleeping time, or immune function (humoral immune response, lymphocyte responsiveness, popliteal lymph node response, and bone marrow function).

For the 90-day study, five groups of 112 male and 112 female mice were given doses of 0, 5.3, 53 or 133 mg/kg/day. A high mortality was seen among all groups of male mice, but appeared to be due to the aggressive behavior of group-housed male mice.

No significant effects on body weight were noted for males or females. A significant decrease in the absolute weight of the brain, spleen and liver was noted for females receiving 133 mg/kg; however, organ-to-body weight ratios were significantly different only for the spleen. Of the changes noted in the clinical chemistry data, the increase in blood protein content in males and females receiving 53 or 133 mg/kg, the decrease in BUN in all

treated female groups, and the decrease in calcium ion concentrations in males receiving 53 or 133 mg/kg were considered to be treatment related. No significant changes were noted in hematology, in MFO activity, or in immune function for either sex. Histopathology data were not presented and it is not known if naphthalene caused bronchiolar lesions.

In a subchronic oral toxicity study performed for the NTP (1980b), naphthalene in corn oil was administered by gavage to male and female F344 rats (10/sex/dose) at dose levels of 0, 25, 50, 100, 200 or 400 mg/kg/day, 5 days/week for 13 weeks. At 400 mg/kg, two males died during the first week; this treatment dose caused diarrhea, lethargy, hunched posture and roughened haircoats in rats of both sexes. A significant (i.e., >10%) decrease in body weight gain was observed among males and females at 200 and 400 mg/kg and in females at 100 mg/kg. Food consumption was not affected.

All the rats in the study were necropsied and comprehensive histopathologic examinations were performed on rats from the 0 and 400 mg/kg groups. Histopathologic examinations of the kidneys and thymus were performed on rats from the 200 mg/kg group (according to the histopathology tables; the 100 mg/kg group according to the text). The authors stated that lesions of the kidney in males and thymus in females of the 400 mg/kg group may have been compound-induced, and that no eye lesions were found. The incidences of lesions of kidney and thymus were, however, very low. The renal lesions, which did not occur in females, were observed at incidences of 0/10 in controls, 2/10 in the 200 mg/kg group and 1/10 in the 400 mg/kg group. These renal lesions consisted of focal cortical lymphocytic infiltration or focal tubular regeneration in the two 200 mg/kg males and diffuse tubular degeneration in the one 400 mg/kg male. Lymphoid depletion of the thymus

occurred in 2/10 females of the 400 mg/kg group, in none of the control or 200 mg/kg females, and in none of the males of these groups. Hematologic analyses revealed marginal decreases in hemoglobin and hematocrit in males and females of the 400 mg/kg group, and a moderate increase in the number of mature neutrophils and a decrease in the number of lymphocytes in males of the 400 mg/kg group, relative to controls. No hematologic changes were observed at the lower dosages.

In a similar study, naphthalene was administered in corn oil by gavage at 0, 12.5, 25, 50, 100 or 200 mg/kg/day, 5 days/week, to B6C3F1 mice (10/sex/dose) for 13 weeks (NTP, 1980a). Seven mice (three males and two females of the 200 mg/kg group, one female of the 25 mg/kg group and one control male) died during the second, third and fourth weeks of the study from gavage trauma or accident. Transient signs of toxicity (lethargy, rough haircoats and decreased food consumption) occurred at weeks 3-5 in the 200 mg/kg groups. All treated groups of male mice gained somewhat more weight than did control males. Dose-related decreases in body weight gain were seen in females, but were not statistically significant. All the mice were necropsied and comprehensive histopathologic examinations were performed on the mice from the 0 and 200 mg/kg groups. No compound-related lesions were observed in any organs, including kidneys, thymus, eyes and lungs. Hematologic analyses, performed on all groups, revealed no significant, compound-related changes.

Fitzhugh and Buschke (1949) noted the formation of cataracts, within 3 weeks of treatment, in rats fed diets containing 2% naphthalene or one of several naphthalene derivatives. The effects of pigmentation on cataract

formation in rats and rabbits have been summarized by van Heyningen (1979): Albino rats do not readily convert naphthalene to 1,2-dihydroxynaphthalene, which auto-oxidizes to form 1,2-naphthoquinone (the naphthalene metabolite known to bind to the lens of the eye), possibly because polyphenol oxidase is found only in pigmented tissues. Secondary effects occur in the retinas of both albino and pigmented rats. For rabbits, pigmentation is not as important as a modifier of toxicity. Rather, the depletion of antioxidants is a critical step in rabbits since the reserve of ascorbic acid and other antioxidants is considerably less than in the rat.

The critical nature of the depletion of antioxidants in rabbits, as compared with the importance of pigmentation, was demonstrated by van Heyningen and Pirie (1967) in a gavage study in which naphthalene (1 mg/kg/day) was administered to Dutch and two strains of albino rabbits. In more than half of the treated rabbits, lens opacities and degeneration of the retina were observed. This occurred concomitantly with a depletion of ascorbic acid in the aqueous and vitreous humours. Some of those rabbits that received 10 or more doses of naphthalene showed a general yellowing of the eye fluids and yellow or brown cortical areas in the cataractous lens, suggesting the presence of 1,2-naphthaquinone and 1,2-dihydroxynaphthalene, respectively. Considerable variation exists among rabbits in their response to naphthalene. Cataract formation in rabbits was not noted following topical application of a 10% solution of naphthalene in oil or i.p. injection of 500 mg/day for 60 days (Ghetti and Mariani, 1956).

The effect of pigmentation on the development of cataracts in rats exposed to naphthalene was substantiated by Koch et al. (1976) using five

strains of Rattus norvegicus of different pigmentation. Groups of six animals of each strain were gavaged with either 0 or 1000 mg/kg naphthalene, dissolved in liquid paraffine, every second day for 75 days. No opacities or lens abnormalities were observed in any of the control groups. All animals of the pigmented strains (E3, BDE, DA) developed zonular cataracts between 16 and 28 days. Among the albino strains (Wistar, Sprague-Dawley) only some of the animals developed changes in the eyes. These changes were less pronounced in the albino strains and occurred after longer latencies of 32-61 days.

Cataract formation in C57Bl/6J and DBA/2N mice was evaluated by Shichi et al. (1980) and Shichi and Nebert (1982). The C57Bl/6J mice are "responsive" to the induction of AHH activity while the DBA/2N mice are "nonresponsive" to the induction of AHH activity (primarily P_1-450 enzyme activity that is believed to be involved in the toxification of PAHs). Groups of 15 mice were fed laboratory chow ad libitum that had been soaked for at least 24 hours in corn oil containing 5 or 10 mg/mℓ naphthalene. The feeding regimen was continued for 60 days. Daily ingestion was not calculated by the authors. A concomitant dose of β-naphthoflavone was given twice weekly as an inducer of AHH activity. A 6.7% incidence in cataract formation was observed in C57Bl/6J mice at each dose. In addition to cataract formation, tisssue degeneration in the choroid, ciliary body, and iris occurred. In support of the theory that the mechanism of naphthalene-induced cataract formation involves its metabolism by P_1-450 enzymes to toxic intermediates with subsequent binding to lens tissue, no cataracts were observed in DBA/2N mice.

Phenanthrene. Pertinent data regarding the subchronic and chronic oral toxicity of phenanthrene could not be located in the available literature.

Pyrene. Pyrene was fed at a concentration of 2000 mg/kg diet to young male rats for 100 days. An inhibition of growth was observed, which was reversible upon addition of cystine or methionine. The authors noted that livers of treated animals (including rats consuming benzo[a]pyrene or 3-methylcholanthrene in the diet) were enlarged and had a fatty appearance (White and White, 1939).

The U.S. EPA (1989b) conducted a 90-day subchronic toxicity study in which groups of male and female CD-1 mice (20/sex/group) were gavaged with either 0, 75, 125 or 250 mg/kg/day pyrene in corn oil for 13 weeks. Parameters used to assess toxicity included body and organ weights, food consumption, mortality, hematology and serum chemistry and gross and histopathology. Statistically significant ($p \leq 0.01$) decreases in absolute kidney weights were observed in males of all treatment groups and in females receiving 250 mg/kg/day. Statistically significant ($p \leq 0.01$) decreases in relative liver weights were observed in males receiving either 125 or 250 mg/kg/day and in females receiving 250 mg/kg/day. Nephropathy, characterized by the presence of multiple foci of renal tubular regeneration, often accompanied by interstitial lymphocytic infiltrates and/or foci of interstitial fibrosis, was observed in 4, 1, 1 and 9 male mice in the control, low, medium and high dosage groups, respectively. Similiar lesions were seen in 2, 3, 7 and 10 female mice in the 0, 75, 125 and 250 mg/kg/day treatment groups, respectively. The kidney lesions were described as minimal or mild

in all instances. Statistically significant ($p<0.01$) decreases in erythrocyte numbers, hematocrits and hemoglobin levels were observed in male mice receiving 250 mg/kg/day. Statistically significant ($p<0.01$) increases in absolute liver weights were observed in females receiving 250 mg/kg/day and in relative liver weights of females receiving either 125 or 250 mg/kg/day and in males receiving 250 mg/kg/day. Based on nephropathy, accompanied by changes in absolute and relative kidney weights, the U.S. EPA (1991a) identi- fied 125 mg/kg/day as the LOAEL; the corresponding NOAEL is 75 mg/kg/day.

Subchronic and Chronic Toxicity By Other Routes

Acenaphthylene. In a study reported by Rotenberg and Mashbits (1965), white rats were exposed to acenaphthylene dust at 0.5-1.25 mg/m³ for 4 hours/day for 4 months. After 3 weeks of exposure, a delay in weight gain and a tendency toward decreased blood pressure were observed. Histopathologic examination revealed various degrees of malignancy in the lungs of almost all treated rats. Focal bronchitis and peribronchitis with bronchiolization of the alveolar and metaplasia of the bronchial epithelium were observed in the mildest cases. Advanced cases showed desquamation of the bronchial and alveolar epithelium, papillar growths in the epithelium and, in three rats, isolated regions of carcinoma in the form of strands of epithelial cells. Further details of this study were not provided.

In a study by Reshetyuk et al. (1970) published in Russian, ~100 white male rats were exposed to vapors of acenaphthylene at a concentration of 18 ± 2.5 mg/m³, 4 hours/day, 6 exposures/week for 5 months. In exposed rats, reflexes of the upper airways were altered and an increase in the concentration of nucleic acids in the liver was observed. Histopathologic

examination of the lungs revealed aspecific pneumonia as the major pathology of inhalation exposure to acenaphthylene. Changes observed in the lungs included desquamation of the cells in the alveolar epithelium and focal bronchitis accompanied by hyperplasia and metaplasia of the bronchial epithelium. No signs of malignant growth were observed in this study. No further details of this study were available.

Anthracene. Pertinent data regarding the subchronic and chronic non-oral toxicity of anthracene could not be located in the available literature.

Benz[a]anthracene. An early study (Hoch-Ligeti, 1941) demonstrated lymphoid system effects as a consequence of benz[a]anthracene injection. Mice received a total dose of 10 mg in weekly s.c. treatments for 40 weeks. Lymph glands removed at weekly intervals showed treatment-related increases in reticulum cells and iron accumulation.

Benzo[a]pyrene. Male white carneau pigeons were given weekly injections in the pectoral muscle of 0.1, 10 or 100 mg/kg benzo[a]pyrene in corn oil (Revis et al., 1984). Controls were either not treated or were injected with corn oil. Four pigeons/group were cannulated at 3 months and at 6 months for recording of blood pressure and sampling of blood for determination of plasma cholesterol and lipoproteins. Atherosclerotic plaques were counted and sizes measured. By 6 months pigeons treated with 10 or 100 mg/kg/week benzo[a]pyrene were observed to have increased LDL protein and cholesterol ($p<0.01$), HDL protein and cholesterol ($p<0.01$) and plasma cholesterol ($p<0.05$). This was by comparison with corn oil-treated controls. Benzo[a]pyrene treatment did not produce changes in various

cardiovascular parameters including systolic and diastolic blood pressure, arterial pulse, left or right ventricular or central venous pressure, heart rate or relative heart weight.

Among pigeons treated with benzo[a]pyrene coronary artery plaques ranging in size from 0.6-0.85 mm were observed. Incidence of coronary artery plaques was 5/24 for benzo[a]pyrene-treated birds by comparison with 1/24 for combined corn oil and untreated controls. It was noted in this study that benzo[e]pyrene administered in the same fashion as was benzo[a]pyrene did not induce plaque formation.

Penn and Snyder (1988) tested benzo[a]pyrene, anthracene and dibenz-[a,h]anthracene to determine whether artherosclerotic plaque formation is related to mutagenicity or carcinogenic potency in chickens. In their study male White Leghorn chickens received weekly intramuscular injections with one of the test compounds for 16 weeks. The dosage and number of each group varied as follows: benzo[a]pyrene at 40 mg/kg with 6 chickens, anthracene at 20 mg/kg with 6 chickens, and dibenz[a,h]anthracene at 20 mg/kg with 5 chickens. At the end of the injection period, the cockerels were killed and the aortas isolated. The number of plaque-containing aorta segments/chicken was 6.8, 4.3 and 6.2 for the benzo[a]pyrene-, anthracene- and dibenz[a,h]anthracene-treated groups, respectively. The largest plaque volumes were noted in the animals treated with dibenz[a,h]anthracene.

Benzo[b]fluoranthene. Pertinent data regarding the subchronic and chronic nonoral toxicity of benzo[b]fluoranthene could not be located in the available literature.

Benzo[k]fluoranthene. Pertinent data regarding the subchronic and chronic nonoral toxicity of benzo[k]fluoranthene could not be located in the available literature.

Benzo[g,h,i]perylene. Pertinent data regarding the subchronic and chronic nonoral toxicity of benzo[g,h,i]perylene could not be located in the available literature.

Chrysene. Pertinent data regarding the subchronic and chronic nonoral toxicity of chrysene could not be located in the available literature.

Dibenz[a,h]anthracene. Chronic exposure to dibenz[a,h]anthracene has been associated with gross changes in the lymphoid system (Hoch-Ligeti, 1941). Weekly s.c. injections of dibenz[a,h]anthracene (0.5 mℓ of a 0.05% colloidal solution in 1% gelatine) were given to a total of 40 albino male and female mice for 40 weeks. The lymph glands were removed for examination from 2 mice/week over the study period. Splenectomies were done during weeks 21-30. Among the effects noted were an increase in reticulum (stem) cells, accumulation of iron, reduced lymphoid cells and dilated lymph sinuses. Reduction of lymphoid cells was more pronounced among dibenz[a,h]-anthracene-treated mice by comparison with anthracene and benz[a]anthracene treated animals. Moreover, the weight of the spleens in the treated mice were significantly lower than the spleen weights in the controls.

Histologic examination of spleens from dibenz[a,h]anthracene-treated animals showed diminishment of lymphoid and reticular elements. Livers were pale and soft and showed evidence of fatty degeneration and deposition of

iron in Kupfer cells. Adrenals were marked by iron deposition in the cortical zone reticularis, and kidneys showed signs of degeneration of tubules and Malphigian bodies. There was some degeneration of spermatogenic cells and two female mice had "many big corpora lutea in the ovaries" (Hoch-Ligeti, 1941).

Lasnitzki and Woodhouse (1944) conducted a more detailed study of the effects on the lymphatic system of long-term exposure to dibenz[a,h]anthracene. Subcutaneous injections (0.278 mg/injection) were given to male rats 5 times weekly for several weeks. The lymph nodes in the treated rats underwent hemolymphatic changes, including the appearance of extravascular red blood cells in the lymph spaces and large pigmented cells.

Malmgren et al. (1952) reported that 2- to 6-month-old homozygous strain C mice (3-4 mice/group) receiving three subcutaneous injections of either 50, 100 or 400 mg/kg/injection of dibenz[a,h]anthracene spread evenly over a 12-day period, which included the 5 days following antigen challenge, had reduced serum antibody levels.

Fluoranthene. Limited data are available concerning the toxic effects of fluoranthene produced by repeated administration. These consist of reports of mortality produced in mice by repeated dermal application or subcutaneous injection.

Shear (1938) administered four doses, each consisting of 10 mg of fluoranthene in glycerol, by subcutaneous injection to strain A mice. Six of 14 mice survived for 18 months.

Barry et al. (1935) applied a 0.3% solution of fluoranthene in benzene twice weekly to the interscapular region of mice. Mortality ranged from 60-70% after 6 months and 70-90% after 1 year.

Hoffman et al. (1972) applied 50 µl of a 1% fluoranthene solution in acetone 3 times weekly for 12 months to the backs of Swiss albino mice. No mortality had resulted after 15 months.

Fluorene. Pertinent data regarding the subchronic and chronic nonoral toxicity of fluorene could not be located in the available literature.

Indeno[1,2,3-cd]pyrene. Pertinent data regarding the subchronic and chronic nonoral toxicity of indeno[1,2,3-cd]pyrene could not be located in the available literature.

Naphthalene. A chronic inhalation study of naphthalene in mice has recently been conducted by the National Toxicology Program (NTP, 1991). The exposure phase of the 2-year inhalation study has been completed, but the histopathology data and the final report are not yet available. No other chronic toxicity data were found.

Phenanthrene. Pertinent data regarding the subchronic and chronic nonoral toxicity of phenanthrene could not be located in the available literature.

Pyrene. Pertinent data regarding the subchronic and chronic nonoral toxicity of pyrene could not be located in the available literature.

Target Organ Toxicity

The target, or preferred, organs for the toxic action of PAHs tend to be diverse. This is a consequence of two propensities of the PAHs. First, there is the preferential association for normally proliferating tissues such as bone marrow, lymphoid organs, gonads and intestinal epithelium (U.S. EPA, 1980d). Second, PAHs are distributed extensively throughout the body in general.

The toxicity of various PAHs seems to center on the hematopoietic and lymphoid systems of different animal species. Robinson et al. (1975) observed hemopoietic and lymphoid effects of benzo[a]pyrene in mice (of certain strains), and Lasnitzki and Woodhouse (1944) found dibenz-[a,h]anthracene to affect the lymph nodes of rats. While the hematopoietic and lymphoid systems were also shown to be affected in dogs treated with naphthalene (Zuelzer and Apt, 1949), the two major target organs for this PAH are nonciliated bronchiolar epithelial cells (Clara cells) and eye tissue. Cataracts have been shown to develop in several species of laboratory animals following exposure to naphthalene by routes other than inhalation (Fitzhugh and Buschke, 1949; Shichi et al., 1980; Shichi and Nebert, 1982).

Immunotoxic effects as a consequence of PAH exposure have been studied by a number of researchers. Malmgren et al. (1952) first reported reduced hemolysin titres to sheep red blood cells (SRBC) in mice exposed to dibenz-[a,h]anthracene and benz[a]anthracene. Subsequently, it was reported that exposure to carcinogenic levels of benzo[a]pyrene resulted in a depressed immune response to SRBC that persisted for 90 days (Stjernsward, 1966,

1969). Both humoral and cell mediated immunity have been shown to be depressed in C3H/Anf(H-$2^{k/k}$) mice injected with 150 µg benzo[a]pyrene/kg bw during days 11-17 of gestation. Production of plaque-forming colonies against SRBC (as measured by the Jerne plaque assay) was depressed severely from 1-4 weeks of age and persisted from 5-18 months of age. An in vitro graft vs. host assay also showed immunodepressive effects. Lymphocytes from immature mice exposed to benzo[a]pyrene (1-4 weeks) were severely limited in their ability to show mixed lymphocyte responses (Urso and Gengozian, 1984).

B6C3F1 mice were treated s.c. daily for 14 days with 5, 20 or 40 mg/kg benzo[a]pyrene (Blanton et al., 1986). Polyclonal antibody responses to lipopolysaccharide (LPS) and purified protein derivative (PPD) measured by Jerne plaque assay were decreased 50-66% after 7 days exposure. Exposure of B6C3F1 mice to s.c. injections of 40 mg/kg for 7 or 14 days resulted in a 73 and 98% suppression of the T-cell-dependent antibody response, respectively, as measured in a similar fashion.

Similar results were reported by Dean et al. (1983) for female B6C3F1 mice injected s.c. for 14 days with 5, 20 or 40 mg/kg bw. Exposure to benzo[a]pyrene resulted in decreased numbers of IgM and IgG plaque-forming cells in response to SRBC antigen and reduction in IgM plaque-forming cells in response to LPS. In this assay cell-mediated immunity (as measured by delayed cutaneous hypersensitivity to keyhole limpet hemocyanin), allograft rejection and susceptibility to Listeria monocytogenes was unaffected in benzo[a]pyrene treated mice.

A comparative study of the ability of PAHs to depress antibody response was undertaken by White et al. (1985). PAHs were administered s.c. in either single or multiple doses to B6C3F1 and DBA/2 mice. IgM response to SRBC was measured in a hemolytic plaque assay. Single exposures of B6C3F1 mice to 1 mmol/kg dibenz[a,h]anthracene and benzo[a]pyrene resulted in depression of antibody response. Fourteen days of exposure to a daily dose of 160 μmol/kg of the following PAHs also resulted in suppression (~60%) of the IgM response: benz[a]anthracene, dibenz[a,h]anthracene and benzo[a]pyrene. Neither anthracene nor chrysene exposure significantly affected response. Immunosuppression by benzo[a]pyrene was observed to a greater extent in DBA/2 mice. The B6C3F1 mouse strain is highly inducible for aryl hydrocarbon hydroxylase; the DBA/2 mouse strain is not.

The immunocytotoxic effects of benzo[a]pyrene were studied by Wojdani et al. (1984). Eight-week-old, inbred C3H/FCUM and C57B1/6CUM mice in groups of six animals were injected i.p. with P-815 tumor target cells. This was followed 10 days later by i.p. injection with either 0, 0.5, 5 or 50 mg/kg bw of benzo[a]pyrene in corn oil. After 24 hours splenic lymphocytes and peritoneal exudate lymphocytes were collected and assayed for target cell binding and target cell killing effects. A consistently decreasing relationship was noted between the dose of benzo[a]pyrene and both the binding and killing of target cells for splenic and peritoneal lymphocytes. At the two highest dosages of benzo[a]pyrene (5 and 50 mg/kg bw), significant decreases in the percent of lymphocytes binding to target cells or killing target cells existed as compared with the controls. This study also compared lymphocyte function of animals treated with 3-methylcholanthrene

and the weak carcinogen benzo[e]pyrene. Lymphocytes from benzo[a]pyrene treated animals were essentially similar to those from control animals regarding target cell binding and cytotoxic effects. The authors indicated that lymphocyte mediated immunity may be inhibited by PAHs and that this immunosuppressent effect can contribute to their carcinogenicity.

The effect of PAH exposure on interferon production in vivo was studied by Griffin et al. (1986). Female C3H mice were treated i.p. with 0.046, 0.46 or 4.6 mg benzo[a]pyrene in corn oil. At 12, 24, 48, 72 or 120 hours animals were injected with Sendai virus to induce interferon production. Eight hours after the virus was introduced mice were bled by cardiac puncture and serum was titred for interferon by a cytopathic effect inhibition assay in mouse L929 cells. Mice receiving 4.6 mg (180 mg/kg bw) were significantly depressed in their ability to produce interferon at 12, 48 and 120 hours after benzo[a]pyrene treatment. At the 48-hour challenge mice receiving the lower 0.46 mg dose were also significantly impaired with respect to their interferon production.

Carcinogenicity, Oral

Acenaphthylene. Pertinent data regarding the oral carcinogenicity of acenaphthylene could not be located in the available literature. In an abstract Knobloch et al. (1969) reported on the effects associated with ingestion of acenaphthylene. An oral dose of 0.6 g/kg bw of acenaphthylene in olive oil administered for 40 days to a group of seven rats yielded the following physiologic effects: "considerable" body weight loss, changes in the peripheral blood pattern, changes in renal function, and increased serum aminotransferase activities. No carcinogenic effects were reported; the mice were dosed for a short period of time.

Anthracene. Studies of orally administered anthracene have been negative for carcinogenic effects. Druckrey and Schmähl (1955) administered a diet containing anthracene in oil, 6 days/week to 28 BDI or BDIII rats of unspecified sex for a period of 78 weeks. The total dose was 4.5 g anthracene/rat. No tumors as a consequence of treatment were found to have developed. Similarly, in an unpublished report, Schmähl and Reiter (n.d.) administered a total dose of 4.4 g of anthracene orally to 31 rats during a 33-month study. Again, no tumors were reported to have occurred.

Benz[a]anthracene. Klein (1963) in a study of male B6AF1/J mice provides evidence of the carcinogenic potential of ingested benz[a]anthracene. A 3% solution of the compound in Methocel-Aerosol O.T. was administered by gavage at the rate of 3 doses/week for 5 weeks. Control animals received oral doses of Methocel-Aerosol O.T. alone. After 340-440 days and at 547-600 days, the animals were assessed for tumor development. The incidence of pulmonary adenomas and hepatomas was increased at both assessments; the hepatoma incidences at 547 days were higher than at 437 days. No statistical treatment of the data was reported. The findings are summarized in Table V-1.

The Klein (1963) paper cites an earlier study by White and Eschenbrenner (1945) wherein 2/6 rats receiving benz[a]anthracene in the diet developed multiple hepatomas.

A single gavage dose of 0.5 mg benz[a]anthracene in mineral oil produced no tumors in 13 mice after 16 months. Multiple gavage administration, 8 or 16 treatments at 3-7 day intervals resulted in forestomach papillomas in

TABLE V-1

Oral Carcinogenicity Testing of Benz[a]anthracene Administered by Gavage to Male B6AF1 Mice[a]

Administered Dose[b]	Duration of Study (days)	Target Organ	Tumor Type	Tumor Incidence (%)
0.5 mℓ of vehicle	444	lung	adenoma	10/38 (26)
		liver	hepatoma	0/38 (0)
0.5 mℓ 3% solution	437	lung	adenoma	37/39 (95)
		liver	hepatoma	18/39 (46)
0.5 mℓ of vehicle	547	lung	adenoma	7/20 (35)
		liver	hepatoma	2/20 (10)
0.5 mℓ 3% solution	547	lung	adenoma	19/20 (95)
		liver	hepatoma	20/20 (100)

[a]Source: Adapted from Klein, 1963

[b]Benz[a]anthracene in Methocel-Aerosol or vehicle only. All animals were treated 15 times (3 treatments/week for 5 weeks).

2/27 treated mice compared with 0/16 mineral oil-treated controls (Bock and King, 1959).

Benzo[a]pyrene. A relationship between the ingestion of benzo[a]-pyrene and the development of tumors has been documented in several studies in mice. Both benign and malignant tumors of the forestomach as a consequence of oral benzo[a]pyrene treatment have been reported by a number of authors (Hartwell, 1951; Shubik and Hartwell, 1957, 1969; Thompson and Co., 1971; Tracor/Jitco, 1973a,b; Wattenberg, 1972, 1974)..

Berenblum and Haran (1955) examined tumor induction in the forestomach of male C3H and Swiss mice using a number of carcinogenic substances, including benzo[a]pyrene. Mice were starved for 18 hours prior to treatment, which consisted of 0.3 mℓ of a 0.5% benzo[a]pyrene solution in PEG-400 administered by stomach tube on a weekly basis. No concurrent controls were reported. In one part of this study, treatment was for 30 weeks to C3H mice, resulting in the formation of one papilloma and 16 carcinomas of the forestomach. In a second experiment, Swiss mice were fed only milk and water for 3 days prior to the customary 18-hour fasting period to eliminate the effects of undigested food in the stomach at the time of dosing. Under these conditions, 85% (17/20) of the animals had tumors of the forestomach. In neither experiment was any tumor of the glandular mucosa of the stomach found.

As part of an experiment investigating tumor promotion by citrus oils, albino mice (sex not stated) were administered a single oral gavage of either 0, 12.5, 50 or 200 μg benzo(a)pyrene in polyethylene glycol 400

after food had been withheld overnight. The intestinal tracts of one-half of the surviving mice in each group were examined 280 days post-treatment. The incidences of benign forestomach tumors were 0/9, 2/10, 0/9 and 5/11 in the control, low-, mid- and high-dose groups, respectively (Pierce, 1961). The remaining mice were examined upon natural death or 569 days after treatment. The incidences of benign forestomach tumors were 0/17, 3/17, 0/19 and 8/17 in the control, low-, mid- and high-dose groups, respectively. The total number of tumors found in each group was 0, 3, 0 and 27, respectively. No carcinomas were observed and no tumors at other sites were noted by the authors (Field and Roe, 1965).

As part of a study on the effect of hormonal state on PAH tumor induction, virgin female BALB/c/Ch/Se mice were gavaged twice weekly with almond oil solutions of benzo[a]pyrene (Biancifiori et al., 1967). Treatment was for 15 weeks resulting in a total dose of 15 mg. Treatment groups consisted of ~25 each intact and ovariectomized animals receiving benzo[a]pyrene alone or in conjunction with 500-1000 µg/ℓ esterone in the drinking water. No concurrent untreated controls were reported. Among intact animals receiving benzo[a]pyrene alone forestomach tumors (5/25), mammary tumors (2/25) and lung tumors (no incidence reported) were observed.

Fedorenko and Yansheva (1967) administered benzo[a]pyrene in triethylene glycol by gavage to the "antium of the stomach" of CC_{57} mice (sex not specified). The authors used this solvent based on its purported ability to enhance the carcinogenic effects of hydrocarbons. The experimental period was stated to be 19 months. As it was also stated that the mice were kept until natural death, there is some confusion as to the length of exposure

and observation periods. Gavage doses of 100, 0.01, 0.1, 1 and 10 mg/animal were given 10 times. Incidence data were not reported for solvent controls. Tumors were found in the liver, mesentery, peritoneum and stomach (Table V-2).

Roe et al. (1970) investigated the use of sodium cyclamide saccharin and sucrose on the carcinogenic activity of benzo[a]pyrene in female Swiss mice. A single gavage dose of 50 μg benzo[a]pyrene in polyethylene glycol (PEG) was given and the animals were killed after 18 months. Tumor incidence is given in Table V-3.

As part of a study on the effects of caffeine in tumor induction in rats, Brune et al. (1981) administered 0.15 mg/kg/treatment of benzo[a]pyrene to Sprague-Dawley rats for 2 years. Benzo[a]pyrene was administered to three groups by 1.5% caffeine gavage and to two other groups through diet. Untreated and caffeine gavage (5 times/week) groups were used as controls (32 rats/sex/group). Three different gavage treatments were administered to 32 rats/sex/group; in the first treatment rats were gavaged with benzo[a]pyrene 5 times/week for a total annual dose of 39 mg/kg benzo[a]pyrene, in the second rats were gavaged every third day for a total annual dose of 18 mg/kg benzo[a]pyrene and in the final every ninth day for a total annual dose of 6 mg/kg benzo[a]pyrene. Rats that died spontaneously or were killed when moribund underwent histologic examination of various organs. The combined incidences of benign and malignant tumors were significantly different from controls ($p<0.05$, test) for the 5 times/week and the every third day gavage groups and at $p<0.10$ for the every ninth day gavage group. (For other results see Table V-4.)

TABLE V-2

Tumor Incidence in "Atrium of Stomach" Following Gavage Administration of Benzo[a]pyrene*

Total Benzo[a]pyrene Dose (mg)	Tumor Incidence	
	Carcinoma	Papilloma
0.01	0/16	0/16
0.10	0/26	2/26
1.00	0/24	5/24
10.00	11/30	12/30
100.00	16/27	7/27

*Adapted from Fedorenko and Yansheva, 1967

TABLE V-3

Incidence of Tumors Observed After a Single Gavage Treatment of Mice with Benzo[a]pyrene in Polyethylene Glycol*

Treatment	Tumor Incidence				
	Forestomach		Lung	Liver	Malignant Lymphoma
	Papilloma	Carcinoma			
PEG	2/65	0/65	15/65	5/65	3/65
PEG + 0.25 mg benzo[a] pyrene	20/61	1/61	18/61	9/61	0/61

*Adapted from Roe et al., 1970

TABLE V-4

Carcinogenicity of Oral Benzo[a]pyrene in Sprague-Dawley Rats[a]

Mode of Application	Total Dose B[a]P (mg/kg)	Forestomach Tumor Incidence[b]		Total Tumors
		Benign	Malignant	
Untreated control	0	2/64	0/64	3/64
Gavage caffeine control	0	3/64	0/64	6/64
Gavage[c] (5 times/week)	39	14/64	0/64	14/64
Gavage[c] (every 3rd day)	18	25/64	1/64	26/64
Gavage[c] (every 9th day)	6	11/64	1/64	13/64
Diet (5 days/week)	39	9/64	0/64	10/64
Diet (every 9th day)	6	1/64	0/64	3/64

[a]Source: Adapted from Brune et al., 1981

[b]Combined incidence for males and females. Effective number of animals was assumed to be 64.

[c]1.5% aqueous caffeine plus benzo[a]pyrene

Two other groups of 32 rats/sex were fed benzo[a]pyrene either 5 times/week [total annual dose 39 mg/kg benzo[a]pyrene or every ninth day [total annual dose of 6 mg/kg benzo[a]pyrene. Rats that died spontaneously or were killed when moribund underwent histologic examination of various organs. The combined incidences of benign and malignant tumors were significantly different only in the group fed benzo[a]pyrene 5 times/week ($p<0.05$) (see Table V-4).

Deml et al. (1983) dosed five groups of four female Sprague-Dawley rats with various combinations of benzo[a]pyrene and polychlorinated biphenyls (PCBs) dissolved in olive oil by means of gastric intubation for 12 weeks. After 12 weeks, the livers were examined to determine the number and area of enzyme-altered islands. Rats treated with PCBs before intubation with benzo[a]pyrene and promoted afterwards with PCBs showed a 3-fold increase in the number of enzyme-altered islands present. The total area of these islands was also 3 times larger than those found in the other test groups. PCB or benzo[a]pyrene alone appeared to produce no significant changes, but the pretreatment with PCB along with the administration of benzo[a]pyrene followed by PCB caused an alteration in benzo[a]pyrene metabolism that led to the formation of enzyme-altered foci.

As part of a study of inhibition of tumor formation by organoselenium compounds, CD-1 mice (25/group) were gavaged with benzo[a]pyrene. Corn oil solutions delivering 1 mg benzo[a]pyrene/0.2 mℓ were administered twice weekly for 4 weeks. Controls were treated with corn oil only. Mice began treatment at 9 weeks of age and were killed at 28 weeks. At this time no control animals had developed forestomach papillomas, whereas 85% (17/20) of

benzo[a]pyrene treated animals developed an average of 3.3±2.9 tumors/mouse (El-Bayoumy, 1985).

Robinson et al. (1987) administered benzo[a]pyrene to A/J mice by gavage as a positive control on a study of coal tar paints. Forty female mice were gavaged with 0.25 mg benzo[a]pyrene in 2.2 mℓ 2% emulphor twice weekly for 8 weeks (total dose 6 mg). Animals were killed at 8 weeks and were examined for lung adenomas and forestomach tumors. Benzo[a]pyrene treatment produced lung adenomas in 61% (22/36) of mice with an average of 1.42±0.40 tumors/mouse that was significantly increased by comparision with controls (29% or 11/38 incidence, 0.32±0.09 tumors/mouse). No forestomach tumors were observed in controls. Benzo[a]pyrene treatment resulted in 92% incidence; 67% of animals had papillomas and 61% carcinomas.

As part of a dietary carcinogenesis study, benzo[a]pyrene (1.5 mg, twice weekly for 4 weeks) was administered by gastric intubation to 25-30 female ICR mice/group (Benjamin et al., 1988). Three groups intubated with benzo[a]pyrene had different feeding regimens. The first two groups were administered a basal diet supplemented with 20% soy sauce and ±0.05% nitrite. The third group received only the basal diet and water. The feeding regimens were started 1 week before the initial gastric intubation and were continued throughout the experiment. After 21 weeks on study all mice were sacrificed. The tumor incidences of the mice in the three groups were similar (91%); however, mice receiving the soy supplemented diet and nitrite supplemented water had a significantly lower number of neoplasms per mouse (3.4) than the other two groups (5.2 and 4.0 for the group receiving no supplementation and the group receiving only the soy supplemented diet, respectively). In a follow-up experiment, a fourth group was added; this

group was fed the basal diet and received nitrite supplemented water. After the 4-week benzo[a]- pyrene dosing, the feed and water of some groups were changed to allow further evaluation of the effects. This experiment indicated that the inhibition of neoplasia by soy sauce and nitrite probably occurred during tumor promotion. In a subsequent study soy sauce at a dietary concentration of 20% was found to produce a significant reduction in forestomach neoplasms in benzo[a]pyrene-dosed female ICR mice (Benjamin et al., 1991; see also Benjamin et al., 1989).

Dietary benzo[a]pyrene at various doses was administered to mice (Neal and Rigdon, 1967; Rigdon and Neal, 1966, 1969). These findings are summarized in Tables V-5 and V-6.

Using male and female CFW-Swiss mice, 17-180 days old, Neal and Rigdon (1967) found that a dose-response relationship existed between the incidence of stomach tumors (papillomas and carcinomas) and long-term, oral exposure to benzo[a]pyrene in the diet. Animals were fed a diet containing 0-250 ppm of benzo[a]pyrene for $\leq$197 days. No tumors were found in the control group of 289 animals or in the groups treated with 1, 10 or 30 ppm benzo[a]pyrene. The incidence of tumors, however, increased between the 40 ppm and 250 ppm benzo[a]pyrene dosages (see Table V-5). In a second experiment by Neal and Rigdon (1967), mice were fed 100 or 250 ppm benzo[a]pyrene for 1 day with no gastric tumors developing within 105 days. However, 50% of mice fed 5000 ppm benzo[a]pyrene for 1 day did have gastric tumors upon examination ~113 days later. Groups of mice were also given food containing 250 ppm benzo-[a]pyrene for 1, 2, 4 or 30 days. Upon sacrifice 77-104 days later, the tumor incidences were 0/10 (1 day), 1/9 (2 days), 1/10 (4 days) and 26/26 (30 days). It was concluded that the development of gastric tumors in these

TABLE V-5

Incidence of Forestomach Papillomas and Carcinomas in Male and Female CFW Mice Administered Benzo[a]pyrene in the Diet*

Dose in Diet (ppm)	Total Consumed (mg)	Duration of Treatment (days)	Duration of Study (days)	Tumor Incidence (%)
0			70-300	0/289 (0)
1	0.48	110	140	0/25 (0)
10	4.48	110	140	0/24 (0)
20	8.88	110	226	1/23 (5)
30	13.32	110	143-177	0/37 (0)
40	17.76	110	143-211	1/40 (3)
45	19.8	110	141-183	4/40 (10)
50	21.4-29.4	107-197	124-219	24/34 (71)
100	39.2-48.8	98-122	118-146	19/23 (83)
250	70-165	70-165	88-185	66/73 (90)

*Source: Adapted from Neal and Rigdon, 1967

TABLE V-6

Carcinogenicity of Benzo[a]pyrene Administered in the Diet to Male and Female Swiss Mice[a]

Dose (ppm)	Duration of Treatment[b] (days)	Target Organ	Tumor Type	Tumor Incidence (%)
0*†	38-210+	stomach	papilloma/carcinoma	2/175 (1)
		lung	adenoma	33/151 (19)
		hematopoietic system	leukemia	0/175 (0)
250†	80-140	stomach	papilloma/carcinoma	69/108 (64)
		lung	adenoma	52/108 (48)
		hematopoietic system	leukemia	40/108 (37)
250*	72-99	stomach	papilloma/carcinoma	12/52 (23)
		lung	adenoma	26/52 (50)
250*	147-196	stomach	papillomas/carcinoma	9/13 (69)
		lung	adenoma	10/13 (77)
1000*	73-83	stomach	papilloma/carcinoma	5/9 (56)
		lung	adenoma	7/9 (78)
1000*	127-187	stomach	papilloma/carcinoma	13/13 (100)
		lung	adenoma	3/13 (23)

[a]Source: Adapted from Rigdon and Neal, 1966*, 1969†

[b]The duration of the treatment is equal to the duration of the study.

mice is influenced both by the amount of benzo[a]pyrene consumed per day and the number of days it is fed.

In another set of studies, Rigdon and Neal (1966, 1969) also found evidence of an association between chronic dietary exposure to benzo[a]-pyrene and tumor incidence in the stomach and lung, as well as the development of leukemia in mice (see Table V-6). Rigdon and Neal (1966) fed male and female Swiss mice dietary concentrations of benzo[a]pyrene of 0, 250 and 1000 ppm for different periods of time. The incidence of stomach tumors (papillomas and carcinomas) was related to both dose and length of exposure. All the mice in the 1000 ppm group were found to have gastric tumors after 86 days of benzo[a]pyrene consumption. A similar set of relationships between tumor incidence, dose and time held fairly well for lung adenomas as well. Rigdon and Neal (1969) also found that the occurrence of leukemia was related to ingestion of benzo[a]pyrene. Of mice fed 250 ppm benzo[a]pyrene in their diet over an 80-140 day period, 69/108 (63.9%) developed papillomas or carcinomas of the stomach. Similarly, lung adenomas occurred in 52/108 (48.1%) mice fed diets with 250 ppm benzo[a]pyrene. Finally, 40/108 (37%) of the treated mice developed leukemias. The ability of dietary benzo[a]-pyrene to produce mouse lung adenomas was also confirmed by the work of Wattenberg and Leong (1970) and Wattenberg (1974).

Previously, a single oral dose of 100 mg benzo[a]pyrene in the diet was shown to produce mammary tumors in 8/9 female Sprague-Dawley rats (Huggins and Yang, 1962). Sprague-Dawley rats of both sexes were treated daily with 2.5 mg benzo[a]pyrene. Forestomach and esophageal papillomas developed in 3/40 rats (Gibel, 1964).

Chouroulinkov et al. (1967) administered diets containing benzo[a]pyrene dissolved in olive oil to two groups of ~80 male albino mice for a period of 14 months. Based on an assumed food consumption of 5 g/day, the authors calculated a total dietary dose to be 8 mg. One group of treated animals was permitted water ad libitum while the second drank a 3% ethanol solution. Controls consisted of 40 mice given the standard diet supplemented with olive oil and a group of 81 fed the untreated diet only. Forestomach tumors (papillomas) were found in 5/81 surviving animals receiving benzo[a]pyrene and water and in 8/81 treated mice given the 3% ethanol solution. No gastric tumors were observed among controls.

As part of a study of the effects of phenolic antioxidants and ethoxyquin on PAH carcinogenicity, benzo[a]pyrene in sesame oil was administered in the diet to female Ha/ICR and A/HeJ mice (Wattenberg, 1972). The Ha/ICR mice received 0.4 mg/day for 28 days before being returned to a normal diet for 27 weeks or 1.26 mg/day for 28 days before being returned to a normal diet for 14 weeks. Forestomach tumors were observed in 11/20 low-dose and 13/19 high-dose animals. Data on untreated control Ha/ICR mice were not reported. The A/HeJ mice consumed 0 or 4.8 mg benzo[a]pyrene/day for 2 weeks, beginning at age 9 weeks, and were killed 10 weeks after the last treatment. All treated (12/12) but no control (0/12) animals developed forestomach tumors.

In a similar study of sulfur-containing compounds, female Ha/ICR and A/HeJ mice consumed diets containing 300 ppm benzo[a]pyrene in sesame oil (Wattenberg, 1974). The Ha/ICR mice were maintained on the diet from age 9 weeks to 15 weeks and observed until 29 weeks at which time they were killed and examined for forestomach tumors. These were observed in 8/20 animals;

no untreated controls were reported. A/HeJ mice started on treatment at 9 weeks of age and remained on the diet for 25 days. On days 7 and 21 of treatment animals were gavaged with 3 mg benzo[a]pyrene in 0.5 mℓ sesame oil. At 31 weeks the mice were killed and evaluated for pulmonary adenomas. These were present in 100% (12/12) of the animals with a mean number of 7.8 tumors/mouse. No untreated controls were reported.

Triolo et al. (1977) studied production of forestomach tumors in mice as related to inducibility of aryl hydrocarbon hydroxylase. Female Ha/ICR mice 9 weeks of age were fed diets containing 5% corn oil or 5% corn oil with benzo[a]pyrene to constitute 0.2 or 0.3 mg/g diet. Treatment was continued for 12 weeks at which point the animals were killed and stomachs only were examined histologically for tumors. Results are summarized in Table V-7. Tumors were of the squamous papilloma type. Gross observation of glandular stomach, lung and liver revealed no tumors in either control or treated animals.

Further evidence of carcinogenicity associated with ingestion of benzo[a]pyrene is found in the work of McCormick et al. (1981). A group of 20 inbred virgin female LEW/Mai rats received a single intragastric dose of 50 mg benzo[a]pyrene in sesame oil, while a second group received the same total dose in 8 weekly fractions of 6.25 mg. Mammary carcinoma incidence after 90 weeks was 77% in the 50 mg benzo[a]pyrene single dose group and 67% in the fractionated dose group. Mammary tumors were observed in 30% of untreated rats.

Adriaenssens et al. (1983) investigated the effect of dietary butylated hydroxyanisole, a phenolic antioxidant, on the formation of benzo[a]pyrene

TABLE V-7

Induction of Forestomach Tumors in Ha/ICR Mice Fed Dietary Benzo[a]pyrene[a]

Dose (mg/g diet)	Tumor Incidence	Tumors/Mouse	Carcinogenic Index[b]
0	0/9	0.0	0.0
0.2	6/9	1.8	121.9
0.3	9/9	4.0	400.0

[a]Source: Triolo et al., 1977

[b]Percentage of tumor-bearing mice x mean number of tumors/mouse

metabolite-DNA and -protein adducts in the lung, liver and forestomach of female A/HeJ mice. The mice were fed benzo[a]pyrene (from 2-1351 μmol/kg) in identical diets to which butylated hydroxyanisole (5 mg/g diet, ~1 g/kg/day) was or was not added. Adduct formation, thought by the authors to be a necessary, but not a sufficient, step in the development of benzo[a]pyrene induced tumors, was examined 48 hours after ingestion of benzo[a]pyrene. The major DNA adduct identified in each tissue at each dose was the (+)-7,8-dihydroxy-9,10-epoxy 7,8,9,10-tetrahydrobenzoa)pyrene: deoxyguanosine; other adducts, including 7,8-dihydroxy-9,10-epoxy 7,8,9,10-tetrahydrobenzo(a)pyrene:deoxyguanosine and (-)-7,8- dihydroxy-9,10-epoxy 7,8,9,10-tetrahydrobenzo(a)pyrene:deoxyguanosine, were identified.

Formation kinetics of the major adduct in lung and liver DNA from animals on the control diet showed a sigmoidal curve; forestomach adduct-DNA complexes exhibited no saturation over the levels tested. As the benzo[a]-pyrene dose approached 0, the dose-response curves became linear; however, in the three organs examined, no threshold dose appeared to exist below which benzo[a]pyrene metabolite adducts were not observed.

Dietary butylated hydroxyanisole treatment inhibited the formation benzo[a]pyrene metabolite adducts to forestomach, lung and liver DNA over a wide dietary benzo[a]pyrene range. Adduct formation in DNA from the fore-stomach of butylated hydroxyanisole-treated animals was 45% lower than the control group, but demonstrated the same binding kinetics as the animals in the control diet. The maximum inhibition of lung and liver DNA adduct formation in butylated hydroxyanisole-treated animals was 68 and 82% lower than the control group, respectively. As the benzo[a]pyrene dose approached

these values declined to 40 and 55%, respectively. Butylated hydroxyanisole treatment also diminished the curvilinear nature of the dose-response curve.

The level of benzo[a]pyrene metabolites binding to DNA was ~7% of the amount of binding to proteins in the three organs examined. The dose-response curves for benzo[a]pyrene metabolites binding to lung and liver proteins was parallel for both the control and butylated hydroxyanisole-treated groups. Inhibition of metabolite binding was not dose dependant in these organs. No consistent effects of butylated hydroxyanisole were noted in benzo[a]pyrene metabolite-forestomach protein binding kinetics.

Hamsters have also been observed to develop papillomas and carcinomas of the alimentary tract in response to gavage or dietary exposure to benzo[a]-pyrene (Dontenwill and Mohr, 1962; Chu and Malmgren, 1965). Chu and Malmgren (1965) fed male Syrian hamsters diets containing 500 mg benzo[a]-pyrene/kg food or 500 mg benzo[a]pyrene plus 5 g vitamin A palmitate or 5 g vitamin A palmitate only. This diet was provided 4 days/week and standard diet the remaining 3 days. Animals consumed ~5 g food/day, thus receiving ~10 mg benzo[a]pyrene and/or 100 mg vitamin A palmitate/week. All 10 animals fed vitamin A free diet died 1-4 weeks after the beginning of the assay, whereas those consuming vitamin A survived $\leq$5 months. Hamsters consuming benzo[a]pyrene with vitamin A lived longer (that is, from 6-14 months) than animals fed benzo[a]pyrene and no vitamin A. Nine of 13 treated animals developed tumors described by the authors as "cancer" in the forestomach (9) and intestine (2). Vitamin A in the diet eliminated intestinal tumors and decreased the severity of the forestomach lesions to

lesions described as papillomas and dyskeratoses. Tumor incidence in the group receiving only vitamin A was 0/27 for esophageal lesions; the incidence of forestomach tumors was not described.

Benzo[b]fluoranthene. Pertinent data regarding the oral carcinogenicity of benzo[b]fluoranthene could not be located in the available literature.

Benzo[k]fluoranthene. Pertinent data regarding the oral carcinogenicity of benzo[k]fluoranthene could not be located in the available literature.

Benzo[g,h,i]perylene. Pertinent data regarding the oral carcinogenicity of benzo[g,h,i]perylene could not be located in the available literature.

Chrysene. Pertinent data regarding the oral carcinogenicity of chrysene could not be located in the available literature.

Dibenz[a,h]anthracene. Mice (strain not specified) were fed a diet with added dibenz[a,h]anthracene. After 5-7 months exposure the total received doses of 9-19 mg resulted in the induction of forestomach tumors in 7/22 survivors at 1 year (Larinov and Soboleva, 1938).

Lorenz and Stewart (1948) administered strain A mice 0.4 mg dibenz[a,h]-anthracene/day in an aqueous mineral oil suspension given in place of drinking water. At 406 days exposure, 2 squamous cell carcinomas and 11 papillomas of the forestomach were observed (Lorenz and Stewart, 1948). The authors also conducted experiments in which C57B1, C3H, DBA/2 and A strain

mice were exposed to dibenz[a,h]anthracene in an olive oil water emulsion provided to the animals in lieu of drinking water (Lorenz and Stewart, 1947). The DBA/2 mice developed tumors including pulmonary adenomatosis, that Snell and Stewart (1962a) concluded were similar to adenomatous lesions found in humans. Snell and Stewart (1962a, 1962b) were thus prompted to undertake a similar experiment in mice. Groups of 21 male and female mice of the DBA/2 strain were given 0.2 mg/mℓ dibenz[a,h]anthracene in an aqueous olive oil emulsion ad libitum in place of drinking water. Twenty-five male and 10 female control animals received the olive oil emulsion in lieu of drinking water. Neither treated nor control mice tolerated the olive oil vehicle. Animals lost weight after a few weeks exposure, eventually becoming emaciated and dehydrated. Males were estimated to receive a daily dose of 0.85 mg/day while females received 0.76 mg/day of dibenz[a,h]anthracene. Duration of the experiment was 279 and 237 days for male and female mice in the experimental group and 351 and 226 days for controls. Treated mice developed pulmonary adenomatosis, alveologenic carcinomas, mammary tumors (females only), precancerous growths of the small intestine and hemangioendotheliomas of the pancreas, mesentery and abdominal lymph nodes. A pulmonary adenomatosis in a male mouse was the only lesion observed in either male or female control animals. Tumor incidences from the Snell and Stewart (1962a) study are compared with those of the Lorenz and Stewart (1947) study in Table V-8.

A series of assays was done wherein mice of several strains were gavaged twice weekly with preparations of 0.5% dibenz[a,h]anthracene in almond oil. After a 15-week treatment period, the total dose was 15 mg/animal. Mammary carcinomas were observed in 1/20 BALB/c females and in 13/24 pseudo-pregnant

TABLE V-8

Oral Carcinogenicity of Dibenz[a,h]anthracene in DBA/2 Mice[a]

Sex	Duration Feeding (days)	Duration Study (days)	Dose[b] (mg/day)	Tumor Type	Tumor Incidence[c]	Reference
M	177	281	0.56	Pulmonary adenomatosis	4/10	Lorenz and Stewart, 1947
				Alveologenic carcinoma	8/10	
				Carcinoma, small intestine	3/10	
				Precancer, small intestine	6/10	
				Hemangioendothelioma	4/10	
F	158	265	0.48	Pulmonary adenomatosis	3/9	Lorenz and Snell, 1947
				Alveologenic carcinoma	7/9	
				Carcinoma, small intestine	0/9	
				Precancer, small intestine	5/9	
				Hemangioendothelioma	2/9	
				Mammary carcinoma[d]	3/9	
M	279	279	0.85	Pulmonary adenomatosis	14/14	Snell and Stewart, 1962a
				Alveologenic carcinoma	14/14	
				Carcinoma, small intestine	0/14	
				Precancer, small intestine	2/14	
				Hemangioendothelioma	10/14	
F	237	237	0.76	Pulmonary adenomatosis	13/13	Snell and Stewart, 1962a
				Alveologenic carcinoma	10/13	
				Carcinoma, small intestine	0/13	
				Precancer, small intestine	4/13	
				Hemangioendothelioma	6/13	
				Mammary carcinoma[d]	12/13	

[a]Source: Adapted from Snell and Stewart, 1962a
[b]Compound was administered as an aqueous olive oil emulsion provided in lieu of drinking water.
[c]No tumors were observed in 10 female control mice in the Lorenz and Stewart (1947) study. One pulmonary adenomatosis was the only tumor observed in 25 male and 10 female control mice used in the Snell and Stewart (1962a) study.
[d]No mammary carcinomas were observed in the male mice.

BALB/c females (Biancifiori et al., 1976). A single 1.5 mg dose of dibenz[a,h]anthracene in PEG-400 produced forestomach papillomas in 2/42 male Swiss mice after 30 weeks (Berenblum and Haran, 1955).

Fluoranthene. Pertinent data regarding the oral carcinogenicity of fluoranthene could not be located in the available literature.

Fluorene. The carcinogenic potential of fluorene was studied by Wilson et al. (1947) and Morris et al. (1960). In the Morris et al. (1960) study, female buffalo rats were administered 0.05% fluorene in their diets containing either 3% added corn oil or propylene glycol. This resulted in the consumption of either 4.3 mg/day of fluorene for ~6 months or 4.6 mg/day for ~18 months. The incidence of tumors in the treatment and control groups was essentially the same (Table V-9). The authors of the study described fluorene as "slightly carcinogenic."

Wilson et al. (1947) studied the effect on tumor development in albino rats of exposure to various concentrations of fluorene in the diet over various periods of time. One set of rats was exposed to several concentrations (number not specified) ranging from 0.062-1.0% fluorene in the diet for 104 days while a second set received either 0.125, 0.25 or 0.5% fluorene in the diet for 453 days. Animals of the short-term group maintained on diets with fluorene concentrations of 0.5 and 1.0% experienced significant decreases in their rate of growth. In other aspects they appeared normal. The internal organs of rats exposed for 104 days were essentially normal in appearance and histology. Livers of rats consuming the 0.25% and higher dose diets in the longer study were significantly heavier than normal.

TABLE V-9

Carcinogenicity Testing of Fluorene Administered in the Diet to Female Buffalo Rats[a]

Dose or Exposure	Duration of Treatment	Duration of Study	Vehicle or Physical State	Target Organ	Tumor Type	Tumor Incidence (%)
0 mg/day	NA	15.5 months (range, 9.4-19.9)	diet with 3% added corn oil	uterus	adenocarcinoma	1/18 (6)
				uterus	fibroepithelial polyp	2/18 (11)
				adrenals	cortical adenoma	5/18 (28)
				pituitary	adenomas	6/18 (34)
				inguinal region	fibroma	1/18 (6)
4.6 mg/day[b]	18.1 months (range, 4.1-19.2)	19.0 months (range, 5.1-20.1)	diet, above plus fluorene in corn oil	uterus	fibrosarcoma	1/18 (6)
				uterus	carcinosarcoma	1/18 (6)
				R-E system	granulocytic leukemia	1/18 (6)
				pituitary	chromophobe adenoma	4/18 (22)
0 mg/day	NA	13.9 months (range, 7.8-18.2)	diet with 3% added propylene glycol	kidney	adenoma	1/18 (6)
				pituitary	chromophobe adenoma	2/18 (11)
				R-E system	granulocytic leukemia	1/18 (6)
4.3 mg/day[b]	6.1 months (range, 5.0-6.2)	10.2 months (range, 8.2-10.7)	diet, above plus fluorene added in corn oil	kidney	squamous-cell carcinoma	1/11 (6)
				ureter	squamous-cell carcinoma	1/11 (6)

[a]Source: Morris et al., 1960

[b]Reported daily intake of 0.05% dietary fluorene

NA = Not applicable

Spleens of all treated animals weighed less than normal as did testes of the highest dosed rats. In the longer-term exposure group, squamous metaplasia of the bronchial epithelium was noted in three rats while one rat exposed to 0.125% fluorene in the diet had developed a small benign kidney tubular adenoma. Total number of animals treated was not indicated, nor was a control group described.

Indeno[1,2,3-cd]pyrene. Pertinent data regarding the oral carcinogenicity of indeno[1,2,3-cd]pyrene could not be located in the available literature.

Naphthalene. There is only very limited information available on the carcinogenic potential of naphthalene following oral administration to laboratory animals.

Schmähl (1955) reported that naphthalene administered in food was not carcinogenic in rats (in-house strains BDI and BDIII). Naphthalene was dissolved in oil and given 6 times/week in food. The daily dose was between 10 and 20 mg. After reaching a total dose of 10 g/rat (food intake was not reported), treatment was stopped and animals observed until death, between 700 and 800 days of age.

Tsuda et al. (1980) administered a single gavage dose of 100 mg/kg naphthalene in corn oil to a group of 10 young adult F344 rats (sex not specified) at 12 hours after partial hepatectomy. A vehicle control group of 10 rats was included. At 2 weeks after surgery, 2-acetylaminofluorene was added to the diet at 200 ppm to inhibit proliferation of "nonresistant"

hepatocytes. After 1 week of dietary 2-acetylaminofluorene, a single 2.0 mℓ/kg dose of carbon tetrachloride was given to necrotize "nonresistant" hepatocytes and permit proliferation of "resistant" hepatocytes. Feeding of 2-acetylaminofluorene continued for 1 week, followed by a basal diet for 1 week. The rats were then sacrificed and livers were sectioned and histochemically examined for the number and size of γ-glutamyl transpeptidase (GGT)positive foci. These foci contain cells that are "resistant" to the necrotizing effects of carbon tetrachloride and to the proliferation-inhibiting effects of 2-acetylaminofluorene and are considered to represent an early stage in the process of neoplastic transformation. Neither the number nor the size of GGT foci appeared to be increased in naphthalene-treated rats compared with vehicle controls. The use of GGT as a biochemical marker of preneoplastic foci is generally accepted (Hendrich and Pitot, 1987).

Phenanthrene. A single oral dose of 200 mg phenanthrene dissolved in sesame oil was administered to ten, 50-day-old, female Sprague-Dawley rats. No mammary tumors were produced within 60 days. In a positive control group of 700 animals given 20 mg of 7,12-dimethylbenz[a]anthracene administered under the same conditions, mammary tumor incidence was 100% (Huggins and Yang, 1962).

Pyrene. Pertinent data regarding the oral carcinogenicity of pyrene could not be located in the available literature.

Carcinogenicity, Other Routes

Acenaphthylene. Hartwell (1951) cited a study wherein 20 mice (strain not specified) were skin painted with 0.25% acenaphthylene in benzene. At 6

months 13 animals were alive; 7 were alive at 1 year. No tumors were observed.

Rotenberg and Mashbits (1965) reported "various degrees of malignancy" in the lungs of almost all of an unspecified number of rats exposed to acenaphthylene dust at 0.5-1.25 mg/m^3 for 4 hours/day for 4 months. Further details were not provided. In another study (published in Russian) with male white rats, histopathologic lesions including hyperplasia and metaplasia of the bronchial epithelium, but no signs of malignancy, were reported following inhalation of acenaphthylene vapors at 18 mg/m^3, 4 hours/day, 6 days/week for 5 months (Reshetyuk et al., 1970).

Anthracene. The carcinogenic potential of anthracene has been tested by skin application with and without UV radiation in mice, in skin initiation-promotion assays with mice, by s.c. or i.p. routes in rats, by implantation into the lungs of rats, and by implantation into the brain or eyes in rabbits. These studies are summarized in Table V-10. The results of the skin painting bioassays for both complete carcinogenicity and for initiating activity do not provide evidence of carcinogenicity, but contradictory results were obtained when anthracene was applied to skin together with exposure to UV radiation. The injection and implantation studies do not provide evidence of carcinogenicity, but these results cannot be regarded as conclusive due to inadequacies in experimental design (e.g., small number of animals, limited number of exposures, inadequate controls).

Benz[a]anthracene. Shimkin and Stoner (1975) demonstrated negative results in the strain A mouse lung adenoma assay after a single i.v. injection of 0.25 mg benz[a]anthracene and an observation period of 6 months.

TABLE V-10

Dermal, Injection and Implantation Carcinogenicity Assays of Anthracene

Route	Species/	No.*/Sex	Purity	Treatment	Duration	Effects/Comments	Reference
Skin	mouse/NR	100/NR	NR	dose and number of applications not specified; 40% solution in lanolin	NR	No skin tumors; 45 and 6 mice survived >6 months and >160 days, respectively	Kennaway, 1924a,b
Skin	mouse/NR	41/NR	NR	dose and number of applications not specified; unspecified solutions in water, benzene or sesame oil	life (133 days average)	No skin tumors; dibenz[a,h]anthracene was tumorigenic in same study	Pollia, 1939
Skin	mouse/NR	50/NR	NR	dose not specified; 0.3% solution in benzene twice weekly	732 days	No skin tumors; 34 and 16 alive at 6 months and 1 year, respectively	Bachmann et al., 1937
Skin	mouse/Swiss	5/F	NR	dose not specified; 10% solution in acetone 3 times/week on the back	life (10-20 months)	No skin tumors; benzo[a]pyrene induced high incidences of skin papillomas and carcinomas under the same conditions	Wynder and Hoffmann, 1959a
Skin	mouse/NR	44/NR	NR	dose not specified; 5% solution in petroleum jelly-olive oil 3 times/week on the ear	life (11 months)	No skin tumors; 1/44 alive after 11 months; primary report not available	Miescher, 1942
Skin	mouse/NR	44/NR	NR	treatment as above but with ultraviolet radiation (>320 nm) for 40 or 60 minutes, 2 hours after skin application	life (10 months)	No skin tumors; 5/44 alive after 7 months	Miescher, 1942
Skin	mouse/NR	100/NR	NR	treatment as above but mice received ultraviolet radiation for 90 minutes	life (10 months)	No skin tumors; 7/100 alive after 7 months	Miescher, 1942
Skin	mouse/NR	NR/NR	NR	dose and number of applications not specified; 10% solution in petroleum jelly-olive oil followed by unspecified exposure to ultraviolet (320-405 nm) alone or with visible light	NR	"High incidence" of skin tumors, including many carcinomas, was observed after 5-8 weeks; no tumors in controls treated with anthracene, ultraviolet or ultraviolet with visible light; primary report not available; unusually short latency and inadequate histopathology reporting noted by IARC (1983)	Heller, 1950
Skin	mouse/Skh: hairless-1	24/mixed	NR	0 or 4 µg in methanol once daily, followed by ultraviolet (>290 nm) for 2 hours, 5 days/week	38 weeks	Incidence of skin tumors not significantly increased in treated group; survival was 20/24 and 19/24 in controls and treated groups	Forbes et al., 1976

TABLE V-10 (cont.)

Route	Species/	No.*/Sex	Purity	Treatment	Duration	Effects/Comments	Reference
Skin	mouse/S	20/NR	NR	two applications (0.3 mℓ of 0.5% solution in acetone per application) with a 30-minute interval 3 times/week for a total of 20 applications (30 mg/animal total); 18 weekly applications of croton oil in acetone (0.3 mℓ) consisting of 1/0.17%, 2/0.085% and 15/0.17% croton oil, beginning 25 days after the first anthracene application; controls received the same treatment with croton oil only.	25 weeks	Incidence of skin tumors not significantly increased in treated groups; survival was 19/20 and 17/20 in the control and treated groups, respectively	Salaman and Roe, 1956
Skin	mouse/CD-1	30/F	chromatographically purified	single application of 10 μmol (1782 μg) in benzene followed 1 week later by 5 μmol TPA twice weekly for 34 weeks; controls received the same treatment with TPA only	35 weeks	Incidence of skin papillomas was 4/28 (14%) in treated and 1/30 (3%) in controls	Scribner, 1973
Skin	mouse/CR1: CD/1(ICR)BR	20/F	NR	ten applications (0.1 mg in 0.1 mℓ acetone per application) applied every other day. Ten days later 0.25 μg TPA in 0.1 mℓ acetone was applied 3 times/week for 20 weeks	25 weeks	No significant increase in skin tumor incidence in treated groups	LaVoie et al., 1985
s.c.	rat/NR	10/NR	NR	weekly injections of 2 mℓ of 0.05% suspension in water for life (103 mg/animal maximum total dose)	>18 months	No subcutaneous sarcomas; survival was 7/10 after 12 months and 8/10 after 18 months; dibenz[a,h]-anthracene was tumorigenic under the same conditions	Boyland and Burrows, 1935
s.c.	rat/Wistar	5/NR	NR	5 mg in sesame oil, once weekly for 6 or 7 weeks	10 months	No tumors; examinations apparently included viscera; dibenz[a,h]-anthracene induced subcutaneous tumors in 2/5 similarly treated rats	Pollia, 1941
s.c.	rat/BDI and BDIII	10/NR	highly purified	20 mg in unspecified oil once weekly for 33 weeks	life	Injection site tumors (fibromas with sarcomatous areas) in 5/9 rats, mean latency, 26 months; no controls, but rats treated similarly with naphthalene in oil did not develop local tumors	Schmähl, 1955

TABLE V-10 (cont.)

Route	Species/	No.*/Sex	Purity	Treatment	Duration	Effects/Comments	Reference
i.p.	rat/BDI or BDIII	10/NR	highly purified	20 mg in unspecified oil once weekly for 33 weeks	life (mean ~2 years)	Tumor in one rat (spindle-cell sarcoma in abdominal cavity); no control group	Schmähl, 1955
Cerebral cortex implant	rabbit/NR	5/NR	NR	10 mg (1 rabbit) or 20 mg (4 rabbits) pellets	4.5 years	No tumors; survival was 4/5 after 4 years and 2/5 at 4.5 years	Russell, 1947
Cerebellar implant	rabbit/NR	2/NR	NR	12 mg pellets	4.5 years	No tumors; survival was 2/2 at 4.3 years	Russell, 1947
Optic implant	rabbit/NR	2/NR	NR	4 or 5 mg pellets	4.5 years	No tumors; survival was 2/2 at 4.5 years	Russell, 1947
Lung implant	rat/Osborne-Mendel	60/F	refined recrystallized	0.5 mg in 0.05 mℓ warm soft 1:1 beesway:tricaprylin	55 weeks	No tumors in treated or control animals	Stanton et al., 1972

*Numbers in treatment and control (if used) group(s) unless specified otherwise.

NR = Not reported; s.c. = subcutaneous; i.p. = intraperitoneal

As part of a large study of PAHs and their nitrated derivatives, newborn CD-1 mice were treated i.p. with a total of 2800 μmol benz[a]anthracene in DMSO. Liver tumors were not observed in treated female mice, but 31/39 males developed liver tumors of which 25/39 were carcinomas. This was a significant increase compared with controls. By contrast, female mice were observed to have a significantly increased incidence of lung tumors (6/32 compared with 0/31) while the males did not (6/39 compared with 1/28) (Wislocki et al., 1986).

Benz[a]anthracene is a well-documented complete skin carcinogen. Both the parent compound and the 3,4-dihydrodiol and the 3,4-diol-1,2-epoxide have produced tumors on mouse skin (IARC, 1973; Santodonato et al., 1981). By contrast neither Donryu rats nor Syrian golden hamsters developed tumors after topical benz[a]anthracene application (Tawfic, 1965; Shubik et al., 1960).

Subcutaneous administration of benz[a]anthracene in tricaprylin to mice resulted in injection site sarcomas. Incidences of sarcomas in C57Bl mice observed 9 months after being given graded doses of benz[a]anthracene were as follows: 0.05 mg, 5/43; 0.2 mg, 11/43; 1.0 mg, 15/31; 5.0 mg, 49/145; 10 mg, 5/16 (Steiner and Falk, 1951; Steiner and Edgecomb, 1952). Klein (1952) showed that intramuscular injection of albino mice derived from strain A with benz[a]anthracene in a 1 or 3% combination with croton oil produced injection site fibrosarcomas and hemangioendotheliomas.

Benzo[a]pyrene. Benzo[a]pyrene is known to produce tumors when administered by inhalation or intratracheal instillation. It is generally more

effective as a lung carcinogen when accompanied by a respirable particulate or a cocarcinogenic gas. Laskin et al. (1970) exposed 21 rats to 10 mg/m³ benzo[a]pyrene for 1 hour/day for 1 year. Two animals developed squamous cell carcinomas in this group. When rats were exposed to 10 ppm SO_2 for an additional 6 hours/day, carcinoma incidence was 5/21. No animals receiving SO_2 only developed tumors. Intratracheal instillation of benzo[a]pyrene accompanied by India ink resulted in a dose-dependent increase in lung tumors in rats (Yanisheva, 1971). Rats that received ^{14}C-benzo(a)pyrene along with carbon black or asbestos through intratracheal instillation had a higher incidence of lung tumors than those receiving only this PAH (Pylev et al., 1969).

Intratracheal instillation studies of particulate and benzo[a]pyrene in hamsters have also shown increased incidences of respiratory tumors. An increased incidence of respiratory tract tumors was reported in Syrian golden hamsters that had been administered benzo[a]pyrene along with ferric oxide particles (Saffiotti et al., 1965, 1968). In a similar experiment, Farrell and Davis (1974) showed that carbon black and ferric oxide were more effective tumor promoters than aluminum oxide when these three types of particles were bound to benzo[a]pyrene. The percentage of respiratory tract tumor-bearing hamsters in each group was 49, 49 and 22% for the carbon black, ferric oxide and aluminum oxide groups, respectively. Intratracheal instillation of radiolabeled benzo[a]pyrene-coated carbon, aluminum oxide and ferric oxide resulted in an increase in the retention time of the radiolabel in the hamster lung when compared with administration of only the radiolabeled benzo[a]pyrene (Henry and Kaufman, 1973).

Hamsters have provided a useful model for the study of benzo[a]pyrene lung carcinogenicity. Concurrent exposure with sesame oil resulted in tracheal papillomas and carcinomas (Mohr, 1971). Saffiotti et al. (1972) conducted several assays wherein a saline suspension of benzo[a]pyrene coated Fe_2O_3 particles were repeatedly instilled in hamster treacheas. In one assay the lowest dose employed, 0.25 mg benzo[a]pyrene/week, induced respiratory tumors in 10/88 hamsters. At the high-dose, 3 mg/week, the incidence was 34/57 with multiple tumors observed in some animals (Saffiotti et al., 1972). Using the same model, Sellakumar and Shubik (1972) reported a 30% incidence of respiratory tract tumors in animals receiving 20 weekly treatments of 0.5 mg benzo[a]pyrene.

In research by Thyssen et al. (1981), groups of 24 hamsters inhaled benzo[a]pyrene at concentrations of 2.2, 9.5 or 46.5 mg/m^3, 4.5 hours/day, 7 days/week for 10 weeks followed by an exposure for 3 hours/day, 7 days/week for a maximum of 675 days. Neither control animals nor animals receiving the lowest dose developed respiratory or upper digestive tract tumors. Above 2.2 mg/m^3 benzo[a]pyrene, the incidence of respiratory and upper digestive system tumors increased with dose. Table V-11 summarizes the findings.

A later report indicated that concurrent exposure of Syrian golden hamsters to SO_2 and benzo[a]pyrene-coated sodium chloride resulted in an enhancement of tumor response and a decreased latency period (Pauluhn et al., 1985).

Feron and Kruysse (1978) and Ketkar et al. (1978) also studied the incidence of respiratory tract neoplasms in male and female hamsters

TABLE V-11

Carcinogenicity of Benzo[a]pyrene to
Male Syrian Golden Hamsters By Inhalation[a,b]

Dose (mg/m^3)	mg/Hamster	Duration of Treatment[c] (weeks)	Target Organ	Tumor[d] Incidence (%)
0.0	0	96.4	respiratory tract	0/27 (0)
			upper digestive tract	0/27 (0)
2.2	29	95.2	respiratory tract	0/27 (0)
			upper digestive tract	0/27 (0)
9.5	127	96.4	respiratory tract	9/26[e] (35)
			upper digestive tract	7/26[e] (27)
46.5	383	59.5	respiratory tract	13/25[f] (52)
			upper digestive tract	14/25[f] (56)

[a]Source: Thyssen et al., 1981

[b]Exposure was for 4.5 hours/day for the first 10 weeks, 3 hours/day thereafter for 7 days/week as NaCl vapor (>99% of the particles had diameters between 0.2 and 0.5 μM) in air.

[c]The duration of the study is equal to the duration of the treatment

[d]Tumors were papillomas, papillary polyps, and squamous cell carcinomas

[e]3 nasal cavity, 8 laryngeal, 1 tracheal, 6 pharyngeal and 1 forestomach tumor

[f]1 nasal cavity, 13 laryngeal, 3 tracheal, 14 pharyngeal, 2 esophageal and 1 forestomach tumor

associated with intratracheal administration of benzo[a]pyrene. Ketkar et al. (1978) found the mean survival time of both male and female hamsters was positively related to the dose of benzo[a]pyrene administered as weekly instillations in bovine serum albumin (Table V-12). For male hamsters, the incidence of respiratory tract tumors tended to increase with dose except at the highest level where the tumor incidence rate declined slightly. For the female hamsters, tumor incidence was also increased by benzo[a]pyrene, but an inverse relationship held between dosage and tumor incidence. Why this inverse relationship was observed is not readily apparent.

Kobayashi (1975) treated 32 male and 28 female Syrian golden hamsters with intratracheal instillations of 1 mg of benzo[a]pyrene in saline weekly for 30 weeks. The control group (20 mice/sex) was administered an equivalent dose of saline. After 60 weeks the mice were sacrificed and complete necropsies performed. Survival rates appeared to be equivalent in the benzo[a]pyrene-treated and control groups (both sexes combined). The incidences of respiratory tract tumors were 11/26 in treated males and 14/26 in treated females. The majority of these tumors were found in the peripheral areas of the lung. No incidences of respiratory tract tumors were found in the control mice.

Stenback and Rowland (1978) studied the role of talc and benzo[a]pyrene in respiratory tumor formation. Two groups of 24 Syrian golden hamsters/sex received either 18 intratracheal instillations of 3 mg of talc in saline or 3 mg benzo[a]pyrene and 3 mg talc in saline weekly. Control groups received saline or remained untreated. Hamsters were autopsied upon spontaneous death. Animals treated with talc and benzo[a]pyrene had a shorter lifespan

TABLE V-12

Carcinogenicity of Benzo[a]pyrene Administered by Intratracheal Instillation to Syrian Hamsters[a]

Dose[b] (mg/week)	Duration of Treatment[c] (weeks)	Incidence of Respiratory Tract Neoplasms[d] (%)	
		Males	Females
0	41	0/29 (0)	0/30 (0)
0.10	40	5/26 (19)	12/30 (40)
0.33	24	7/29 (24)	10/28 (36)
1.0	10	6/27 (22)	6/20 (30)

[a]Source: Ketkar et al., 1978

[b]Benzo[a]pyrene (97% pure) was delivered in bovine serum albumin. Controls received vehicle only.

[c]Mean survival time. Survival time was also equal to study duration.

[d]Carcinomas, adenomas, adenocarcinomas and papillomas were reported.

(52 weeks) than the untreated controls (55 weeks), and these deaths were primarily attributed to pulmonary dysfunctions caused by either interstitial fibrosis or neoplastic involvement of the respiratory system. Only the hamsters treated with the talc and benzo[a]pyrene mixture displayed papillomas, squamous cell carcinomas, and undifferentiated tumors of the lung, larynx and trachea (incidence 33/48). Talc appeared to have no carcinogenic activity alone, but increased the carcinogenicity of benzo[a]-pyrene.

In an intratracheal instillation study, male Syrian golden hamsters were administered benzo[a]pyrene, arsenic (as arsenic trioxide) or arsenic and benzo[a]pyrene together. They were introduced with 3 mg of charcoal carbon dust carrier. The control group received only the carrier dust. The animals were dosed weekly. The benzo[a]pyrene group (50 hamsters) received 0.44 mg/exposure (~6 mg/kg), and the arsenic group (67 hamsters) received 0.25 mg/exposure (~3 mg/kg). The group receiving both chemicals (90 hamsters) received 0.45 mg and 0.23 mg of benzo[a]pyrene and arsenic, respectively. The 50-week survival rates of the control, benzo[a]pyrene, arsenic and combined groups were 40, 54, 52 and 41%, respectively. The 100-week survival rates of the control, benzo[a]pyrene, arsenic, and arsenic and benzo[a]pyrene combined groups were 13, 14, 25 and 13%, respectively. The incidences of carcinomas of the larynx, trachea, bronchii or lungs were 0/53, 3/47, 17/40 and 25/54 in the control, arsenic, benzo[a]pyrene, and arsenic and benzo[a]pyrene combined groups, respectively (Pershagen et al., 1984).

Male Sprague-Dawley rats were exposed to either [^{3}H]-benzo[a]pyrene microcrystals, [^{3}H]-benzo[a]pyrene microcrystals in iron oxide or

chrysotile asbestos, or [^{3}H]-benzo[a]pyrene absorbed to iron oxide or chrysotile asbestos particulates by intratracheal instillation. Rats were sacrificed at times ranging from 2 minutes to 72 hours after administration and the distribution of the radionuclide examined. Absorption of benzo[a]pyrene administered as microcrystals was rapid. Addition of asbestos decreased the absorption rate of the microcrystals, and addition of iron oxide had little or no effect on absorption. Particulates did not appear to increase the carcinogenicity of benzo[a]pyrene by particle enhanced uptake or increased retention time in the lungs (Bevan et al., 1988).

Two studies by Feron and co-workers showed benzo[a]pyrene instilled in saline suspension produced respiratory tumors in hamsters. Feron et al. (1973) reported that male Syrian golden hamsters receiving a total administered dose of 0, 3.25, 6.5, 13, 26 or 52 mg benzo[a]pyrene had the following incidences of respiratory tract tumors: 0/29, 3/30, 4/30, 9/30, 25/29 and 26/28, respectively. Feron and Kruysse (1978) found a positive dose-response relationship for tumor incidence in male and female hamsters given a total dose of 0, 18.2 or 36.4 mg of benzo[a]pyrene in saline intratracheally for 52 weeks. The incidence of respiratory tract tumors corresponding to these doses was 0, 13.8 and 63.3% in males, while in females the corresponding tumor incidences were 0, 11.1 and 29.2%. Papillomas and carcinomas of the trachea and pulmonary adenomas were most often observed.

Benzo[a]pyrene, 1 mg/mL in phosphate buffer solution, was administered intratracheally once a week for 15 weeks to 8-week-old female Syrian golden

hamsters. Treated animals and controls survived 443 and 614 days, respectively, after the initial instillation. No control animals were observed to develop lung or tracheal tumors whereas 6/13 treated animals had tracheal tumors (2 malignant, 4 benign) (Yamamoto et al., 1985).

Benzo[a]pyrene has been shown to produce tumors at various sites by a number of modes of administration. Intraperitoneal injection of newborn BLU:Ha(ICR) mice with benzo[a]pyrene produced lung adenomas. A dose of 280 µg/mouse resulted in a 94% tumor incidence (Busby et al., 1984). Individual Swiss mouse fetuses were treated with a single i.p. injection of benzo-[a]pyrene or derivatives in 1 µl trioctanoin and acetone. Doses of 0, 0.4, 4.0, 9.9 or 19.8 µmol/fetus were given on day 15 of intrauterine growth and animals were carried to term, weaned and kept until 12 weeks of age. At this time they were killed and examined for lung adenomas. Incidence of adenomas in the control group was 4/37 and in the treated groups at the stated dosages were 1/39, 10/42, 10/38 and 12/31. A racemic mixture of benzo[a]pyrene 7,8-diol-9,10-epoxides was more tumorigenic producing an incidence of 27/37 at a dose of 4.0 µmole/fetus (Rossi et al., 1983).

Male Wistar rats were given an 80 mg/kg bw intraperitoneal injection of benzo[a]pyrene 18 hours after partial hepatectomy (Dock et al., 1988). The rats were then placed on a specific diet schedule that consisted of 2 weeks of a basal diet, followed by 2 weeks of a diet supplemented with 0.02% 2-acetylaminofluorene (2-AAF). A single injection of carbon tetrachloride (2 ml/kg bw) was administered midway through the 2 weeks. The rats were then administered a basal diet for 2 weeks. The benzo[a]pyrene and 2-AAF

produced a significant number of enzyme-altered foci in the regenerating liver. There was a 50% reduction in the metabolism of benzo[a]pyrene in the proliferating liver.

Benzo[a]pyrene is carcinogenic when administered s.c. to mice, rats, hamsters, guinea pigs and some primates (IARC, 1983). Bryan and Shimkin (1943) attempted to establish dose-response curves for induction of tumors following s.c. administration in mice. Tricaprylin solutions (0.25 or 0.5 mℓ) were injected once only into the right axilla of male C3H mice, and animals were observed throughout their lifetime. No controls were reported, but several no effect doses were observed (Table V-13). In addition to injection site sarcomas, newborn mice administered benzo[a]pyrene s.c. developed hepatomas or lung adenomas (Pietra et al., 1961; Roe and Waters, 1967, Toth and Shubik, 1967; Grant et al., 1968).

Peraino et al. (1984) treated newborn (day 1) Sprague-Dawley rats by a single i.p. injection of 0.59 μmole benzo[a]pyrene/kg bw. At day 21 the animals were weaned and placed on a 30% casein diet containing 0.05% phenobarbital, a known promoter of hepatic neoplasms. Animals were killed at intervals up to ~500 days, and their livers were examined for histochemically detectable foci of altered hepatocytes as well as for hepatic tumors. Hepatic tumor incidence in females was ~57% and ~37% in males. Focus incidence had reached 100% in both males and females by day 200.

Benzo[a]pyrene is among the most potent and best documented of skin carcinogens. It is routinely used as a positive control in skin painting bioassays of other materials. Skin tumors have been produced by benzo[a]pyrene

TABLE V-13

Sarcomagenic Activity of Benzo[a]pyrene in Male C3H Mice Following a Single Subcutaneous Injection*

Dose (mg)	Tumor Incidence	
	Number Tumor-Bearing Animals/Number Effective Animals	Percentage
0.00195	2/81	2
0.0078	0/40	0
0.0156	0/19	0
0.031	0/16	0
0.062	4/20	20
0.125	15/19	79
0.25	14/21	67
0.5	19/19	100
1.0	18/20	90
2.0	19/19	100
4.0	16/19	84
8.0	20/21	95

*Source: Bryan and Shimkin, 1943

in mice, rats, rabbits and to a lesser extent in guinea pigs. In mice the tumorigenic dose is dependent upon the solvent used for delivery and on the strain of mice (IARC, 1973). Benzo[a]pyrene is both an initiator and a complete carcinogen. For example, Nesnow et al. (1986) studied the effects of dermal treatment with benzo[a]pyrene on 7- to 9-week-old SENCAR mice. The benzo[a]pyrene was applied as a single topical treatment in 0.2 mℓ of acetone and as five daily doses of 2 mg each. One week after treatment, 2.0 µg of the tumor promoter TPA was administered topically twice weekly. The results are presented in Table V-14.

Pereira et al. (1979) applied [3H]benzo[a]pyrene to shaved female HA/ICR mice; the mice were sacrificed 7 hours later. [^{3}H]Benzo[a]pyrene metabolite conjugated epidermal DNA, isolated from these treated mice, was found to contain two major benzo[a]pyrene-DNA adducts. The maximum concentrations of both aducts occurred 7 hours after a single dermal application. Benzo[a]pyrene-DNA adduct formation occurred in proportion to dose at doses several orders of magnitude below doses that normally yield a carcinogenic response.

In an analysis of two separate skin-painting experiments using benzo[a]-pyrene in acetone, Lee and O'Neill (1971) showed that the incidence rate of both tumors and infiltrating carcinomas could be described by the equation $d^2 (t-w)^k$ where t is time from first application, w and k are dose independent constants and d is the applied dose. In the first experiment 75 female albino mice/group (16 total groups) received either 6, 12, 24 or 48 µg of benzo[a]pyrene/week in either 2 applications/week, 4 applications/ week and 2 groups that were administered 3 applications/week. One group was

TABLE V-14

SENCAR Mouse Skin Tumorigenesis, Benzo[a]pyrene - Tumor Initiation[a]

Dose (Mg/mouse)	Sex	No. Mice Surviving	Mice with Papillomas[b] (%)	Papillomas/ Mouse[b]	Mice with Carcinomas (%)	Carcinomas per Mouse[c]
0	M	37	8	0.08	5	0.05
0	F	39	5	0.05	0	0
2.52	M	40	45	0.50	5	0.07
2.52	F	39	31	0.44	5	0.05
12.6	M	40	73	1.8	20	0.20
12.6	F	37	57	1.1	23	0.23
50.5	M	39	100	5.8	25	0.25
50.5	F	40	75	2.8	20	0.20
101	M	38	95	10.2	30	0.33
101	F	38	97	7.9	25	0.25

[a]Source: Nesnow et al., 1986

[b]Score at 6 months

[c]Cumulative score after 1 year

dosed on MWF and the second on TWF. In the second experiment, four groups of 40 albino female mice were treated with either 1, 3, 9 or 27 µg of benzo[a]pyrene every fourth day.

Several electrophilic metabolites of benzo[a]pyrene are skin carcinogens. These include dihydrodiols, diol-epoxides and some phenolic derivatives (Gelboin, 1980).

Benzo[a]pyrene has also been reported to be carcinogenic when administered by the following routes: i.v.; transplacentally; implantation in the stomach wall, renal parenchyma, and brain; injection into the renal pelvis; and vaginal painting (IARC, 1983).

Groups of partially hepatectomized male Sprague-Dawley rats were given either a single 30 mg benzo[a]pyrene i.p. injection 24 hours after the partial hepatectomy (10 rats), repeated intragastric injections of 4 mg benzo[a]pyrene for 6 days (15 rats) or a single olive oil injection 24 hours after the partial hepatectomy (10 rats in the control group) (Kitagawa et al., 1980). All groups were fed a diet containing 0.05% phenobarbital beginning 2 weeks after the partial hepatectomy. The animals were sacrificed at 52 weeks and were examined for hepatic tumors. In both groups receiving benzo[a]pyrene, many rats died of abdominal wall sarcomas prior to 52 weeks. In fact 0/10 rats injected with benzo[a]pyrene survived until week 52. Of the rats that received intragastric injections, 6/7 had hepatic tumors and 0/9 in the control group had hepatic tumors. It was reported that there were many enzyme-altered foci in the livers of rats receiving intragastric benzo[a]pyrene injections.

active as the parent compound, whereas the 9,10-diol of benzo[j]fluoranthene was less active than its parent compound (see Table V-15). The 8,9-diol of benzo[k]fluoranthene was not tumorigenic. The 9,10-diol of benzo[b]fluoranthene is a potential precursor to a bay region dihydrodiolepoxide. The benzo[j]fluoranthene 9,10-diol could be metabolized to diol-epoxide with the epoxide ring in a four-sided "pseudo bay region." Taken together, these results suggest that the formation of bay region dihydrodiol epoxides may not be the major activation mechanism in benzofluoranthene tumorigenesis.

This work is corroborated by the reports of Amin et al. (1985a,b). Initiation/promotion protocols using benzo[b]fluoranthene and benzo[k]-fluoranthene were carried out in Crl:CD-1(ICR)BR albino female mice. Each initiating compound was applied every other day in 10 doses as acetone solutions to the shaved backs of 20 mice/treatment group. Ten days after completion of the initiating treatment promotion was begun using thrice weekly applications of 2.5 µg of TPA (dissolved in 0.1 mL acetone). This was continued for 30 weeks. Results summarized in Table V-18 indicate that both benzo[b]fluoranthene and benzo[k]fluoranthene can serve as initiators of carcinogenesis. Benzo[b]fluoranthene, however, is more potent producing the same incidence of tumors with 1/100 the dose necessary to initiate tumors with benzo[k]fluoranthene.

Injection site sarcomas were observed in 18/24 survivors of a total group of 16 male and 14 female strain XVIInc/Z mice given three s.c. injections of 2.6 mg benzo[b]fluoranthene over a period of 2 months (Lacassagne et al., 1963).

TABLE V-15

Carcinogenicity of Benzofluoranthenes by Implantation in Rat Lungs[a]

Treatment	Dose (mg) Total	Median Survival Time (weeks)	Epidermoid Carcinomas	Sarcomas	Tumor Incidence
Untreated	--	118	0/35	0/35	0/35
Pellet[b]	--	104	0/35	0/35	0/35
Benzo[b]-fluoranthene	0.1	110	0/35	1/35	1/35
	0.3	113	1/35	2/35	3/35
	1.0	112	9/35	4/35	13/35
Benzo[k]-fluoranthene	0.16	114	0/35	0/35	0/35
	0.83	95	3/31	0/31	3/31
	4.15	98	12/27	0/27	12/27
Benzo[g,h,i,]-perylene	0.16	109	0/35	0/35	0/35
	0.83	114	1/35	0/35	1/35
	4.15	106	4/34	0/34	4/34

[a]Source: Deutsch-Wenzel et al., 1983

[b]Refers to beeswax and trioctanoin implanatation medium

TABLE V-16

Carcinogenicity Assay of PAH in Newborn Mice[a]

Compound	Total Dose (μmol)	Sex	Tumor Incidence[b]			
			Hepatic Tumors			Lung Adenomas
			Adenomas	Hepatomas	Total	
DMSO	--	M	0/17	1/17	1/17	0/17
		F	0/18	0/18	0/18	0/18
Benzo[a]pyrene	1.1	M	9/17	4/17	13/17[c]	14/17[c]
		F	0/14	0/14	0/14	9/14
Benzo[b]fluoranthene	0.5	M	6/15	2/15	8/15[c]	2/15
		F	0/17	0/17	0/17	3/17
Benzo[j]fluoranthene	1.1	M	8/21	3/21	11/21[c]	11/21[c]
		F	0/18	0/18	0/18	4/18[d]
Benzo[k]fluoranthene	2.1	M	2/16	1/16	3/16	1/16
		F	0/18	0/18	0/18	3/18
Indeno[1,2,3-cd]pyrene	2.1	M	0/11	0/11	0/11	1/11
		F	0/9	0/9	0/9	0/9

[a]Source: LaVoie et al., 1987

[b]Denominator denotes animals surviving until at least 35 weeks.

[c]$p<0.005$

[d]$p<0.05$

controls were run (Wynder and Hoffman, 1959b). As part of the same study, 20 Swiss mice were treated with benzo[k]fluoranthene. No tumors developed in animals painted with the 0.1% solution, but skin papillomas were observed in 2/20 mice treated with 0.5% benzo[k]fluoranthene. By contrast to this study, no significant increase in tumor incidence was observed when 40 female NMRI mice were given skin applications of 3.4, 5.6 or 9.2 μg benzo[k]fluoranthene 2 times/week for their lifetime. No effect on mortality was noted as a consequence of this treatment (Habs et al., 1980).

A single application of 11 mg benzo[k]fluoranthene did not induce tumors in 20 Swiss mice in a 63 week study. When this initiating dose was followed by promoting treatments with croton resin, 18/20 animals developed papillomas and 5/20 carcinomas (Van Düüren et al., 1966).

The tumor initiating activities of benzo[b]fluoranthene, benzo[j]fluoranthene and benzo[k]fluoranthene and three of their dihydrodiols (9,10-dihydro-9,10-dihydroxybenzo[b]fluoranthene, 9,10-dihydro-9,10-dihydroxybenzo-[j]fluoranthene and 8,9-dihydro-8,9-dihydroxybenzo[k]fluoranthene) were evaluated after application to the shaved backs of Crl:CD-1 mice (LaVoie et al., 1982a). Each compound was applied in acetone solution (0.1 mℓ) to the backs of 20 animals/group. Controls received acetone alone. Three initiating dose levels, 10, 30 and 100 μg for benzo[b]fluoranthene and 30, 100 and 1000 μg for the other two compounds (10 doses, every other day), were used followed by 2.5 μg TPA (3 times weekly for 20 weeks) (Table V-17). This study demonstrated that of the compounds tested, benzo[b]-fluoranthene was the most potent tumor initiator followed by benzo[j]fluor-anthene. Benzo[k]fluoranthene also showed tumor initiating activity but was not a complete carcinogen. The 9,10-diol of benzo[b]fluoranthene was as

TABLE V-17

Tumor Initiating Activity of Benzofluoranthenes in Crl:CD-1 Mice[a]

Compound	Total Initiating Dose (µg)	Percent Skin Tumor-Bearing Animals[b]	Skin Tumors/ Animal	Other Tumors
Benzo[b]fluoranthene	100	80	7.1	0
	30	60	2.3	1[c]
	10	45	0.9	1[d]
B[b]F-9,10-diol	100	95	8.8	0
	30	63	3.8	0
	10	26	1.0	1[d]
Benzo[j]fluoranthene	1000	95	7.2	1[c]
	100	55	1.9	0
	30	30	0.6	0
B[j]F-9,10-diol	1000	84	4.5	0
	100	20	0.3	1[e]
	30	5	0.1	0
Benzo[k]fluoranthene	1000	75	2.8	1[d]
	100	25	0.4	0
	30	5	0.1	0
B[k]F-8,9-diol	1000	10	0.4	0
	100	10	0.1	0
	30	15	0.1	0
Acetone	-	0	0	0

[a]Source: LaVoie et al., 1982a. Promoting treatment was 2.5 µg TPA, 3 times/week for 20 weeks.

[b]Skin tumors were predominantly squamous cell papillomas

[c]Malignant lymphoma

[d]Endometrial carcinoma of uterus

[e]Lung adenoma

active as the parent compound, whereas the 9,10-diol of benzo[j]fluoranthene was less active than its parent compound (see Table V-17). The 8,9-diol of benzo[k]fluoranthene was not tumorigenic. The 9,10-diol of benzo[b]fluoranthene is a potential precursor to a bay region dihydrodiolepoxide. The benzo[j]fluoranthene 9,10-diol could be metabolized to diol-epoxide with the epoxide ring in a four-sided "pseudo bay region." Taken together, these results suggest that the formation of bay region dihydrodiol epoxides may not be the major activation mechanism in benzofluoranthene tumorigenesis.

In another skin painting assay benzo[b]fluoranthene in acetone was applied to the skins of 20 female CD-1 mice/group for a total dose of either 0, 1.0 or 4.0 μmol (a total of 10 subdoses were applied every other day) (Weyand et al., 1990). Ten days after the final dose, 2.5 μg of tetradecanoylphorbol acetate was applied 3 times/week for 20 weeks. In the acetone control group, 2/20 mice developed skin tumors; the average number of skin tumors/mouse was 0.1. In both benzo[b]fluoranthene groups 20/20 mice developed skin tumors; the average number of skin tumors/mouse was 8.5 and 11.0 in the low- and high-dose groups, respectively.

This work is corroborated by the reports of Amin et al. (1985a,b). Initiation/promotion protocols using benzo[b]fluoranthene and benzo[k]-fluoranthene were carried out in Crl:CD-1(ICR)BR albino female mice. Each initiating compound was applied every other day in 10 doses as acetone solutions to the shaved backs of 20 mice/treatment group. Ten days after completion of the initiating treatment promotion was begun using thrice weekly applications of 2.5 μg of TPA (dissolved in 0.1 mℓ acetone). This was continued for 30 weeks. Results summarized in Table V-18 indicate

TABLE V-18

Tumor Initiating Activity of Benzofluoranthenes in Crl:CD-1(ICR)BR Mice[a]

Compound[b]	Total Initiating Dose (μmol)	Percent Animals with Tumors	Skin Tumors/ Animal
Acetone	--	10	0.2
Benzo[b]fluoranthene	40	45	0.9
	100	95	3.3
Acetone	--	5	0.1
Benzo[b]fluoranthene	40	42	0.5
	100	53	0.9
Acetone	--	0	0
Benzo[k]fluoranthene	4000	37	0.7

[a]Source: Amin et al., 1985a,b

[b]Each treatment is presented with its concurrent control. Promoting treatment consisted of thrice weekly applications of 2.5 μg TPA for 30 weeks.

that both benzo[b]fluoranthene and benzo[k]fluoranthene can serve as initiators of carcinogenesis. Benzo[b]fluoranthene, however, is more potent producing the same incidence of tumors with 1/100 the dose necessary to initiate tumors with benzo[k]fluoranthene.

Injection site sarcomas were observed in 18/24 survivors of a total group of 16 male and 14 female strain XVIInc/Z mice given three s.c. injections of 2.6 mg benzo[b]fluoranthene over a period of 2 months (Lacassagne et al., 1963).

Benzo[g,h,i,]perylene. Benzo[g,h,i]perylene did not produce a significant increase in tumor incidence (see Table V-15) when implanted as beeswax trioctanoin pellets in lungs of female OM rats (Deutsch-Wenzel et al., 1983). Although a few pulmonary tumors were observed in rats after intrapulmonary injection, the data were considered inadequate for evaluation (IARC, 1983).

No increased incidence of tumors was reported from two skin-painting bioassays of benzo[g,h,i]perylene conducted in female Swiss or Ha/ICR/mil Swiss mice (Hoffman and Wynder, 1966; Wynder and Hoffman, 1959b). Three initiation promotion assays for skin tumorigenesis in mice were likewise negative. Benzo[g,h,i]perylene did not produce injection site tumors in either of two studies wherein mice were exposed subcutaneously (IARC, 1983).

Van DQQren et al. (1973) reported some evidence that benzo[g,h,i]-perylene served as a cocarcinogen with benzo[a]pyrene when both were applied simultaneously to the skin of ICR/Ha Swiss mice.

Chrysene. As part of a study of nitrated PAHs, chrysene in DMSO was administered i.p. to CD-1 mice on days 1, 8 and 15 of age. Tumor incidences were recorded after 1 year. A total dose of 2800 nmol/mouse resulted in hepatic and lung tumors in 41 and 21% of the males, respectively. A total dose of 700 nmol induced liver tumors in 29% of the males; females were unaffected (Wislocki et al., 1986).

A series of early skin painting assays conducted in mice produced variable results. It was noted that in these bioassays the chrysene was likely to be contaminated with methylchrysene or other materials not detectable by the technology then available (IARC, 1983).

As part of a study of tobacco constituents, female Swiss mice were treated by brushing a 1% acetone solution of chrysene on the skin 3 times/week. No solvent control was reported, but 45% of the treated animals developed papillomas and 40% were observed to have carcinomas (Wynder and Hoffman, 1959a).

"Specially purified" chrysene was applied topically to 20 male C3H mice as either a decahydronaphthalene solution or as a 50:50 mix with n-dodecane. Applied alone, chrysene induced a papilloma in 1/12 mice at 76 weeks. In combination with n-dodecane 5/19 mice were observed to have papillomas and 12/19 bore carcinomas at 49 weeks (Horton and Christian, 1974).

Chrysene was assayed in a mouse skin initiation/promotion assay using ICR/Ha Swiss mice. Papillomas were induced in 5/20 animals treated with croton resin only and in 16/20 mice receiving croton oil preceded by a single application of 1 mg chrysene in acetone (Van Düüren et al., 1966). A

second study using pure TPA as the promoter yielded similar results. Female CD-1 mice received a single topical application of 1 mg of chrysene in acetone, followed by 25 µg TPA (in 0.1 mℓ acetone) 2 times/week. At 30 weeks, 3% of the promoter-only mice were observed to have papillomas, whereas 73% of the chrysene-treated animals developed these growths (Scribner, 1973).

Further evidence of chrysene's potential as an initiator of skin carcinogenesis comes from studies by Hecht et al. (1974) and Levin et al. (1978). In the first study, 20 female Swiss mice (Ha/ICR/M11) were given 10 daily treatments with 100 µg chrysene in acetone. After a 10-day resting period, 2.5 µg TPA in acetone was applied 3 times/week for 20 weeks. A group receiving chrysene only was observed at 72 weeks to have a carcinoma incidence of 4/11 as compared with an incidence of 11/18 (carcinomas and papillomas) in the chrysene plus TPA group (Hecht et al., 1974).

Levin et al. (1978) used female CD-1 mice, 30/group, in an assay wherein a single topical application of a 0.4, 1.25 or 4.0 µmol solution of chrysene in tetrahydrofuran:DMSO (95:5) was followed by twice weekly applications of TPA (200 µℓ of a 16 µmol solution in acetone). A second group receiving TPA alone had a papilloma incidence of 7% (0.07 tumors/mouse). Tumor incidence for the low, medium and high chrysene dose groups receiving TPA were the following: 25, 43 and 52% with 0.32, 0.97 and 1.45 tumors/mouse, respectively.

Three initiation promotion studies in female CD-1 mice also were positive for chrysene initiating activity. Wood et al. (1979) reported an incidence of 21/30 for observation of papillomas in mice given a single

application of 200 µℓ of a 2 µmol chrysene solution in acetone:DMSO: ammonium hydroxide (1000:100:1), followed by twice weekly applications TPA (200 µℓ of 16 µmol in acetone). Equivalent doses of the bay region 3,4-epoxy-1,2,3,4-tetrahydrochrysene and its precursor metabolite 1,2-dihydrochrysene produced essentially the same papilloma incidence. Control animals treated with acetone followed by TPA had a tumor incidence of 1/30. These data support the bay-region theory of carcinogenesis that suggests epoxides on saturated angular benzo rings which form part of a bay-region are particularly susceptible to undergoing ring opening to an electrophilic carbonium ion.

A second experiment by Wood et al. (1980) employed chrysene of higher purity (98%). Thirty mice were treated with 200 µℓ of an acetone solution of this chrysene preparation and subsequently with TPA (200 µℓ of 16 µmol solution) 2 times/week for 25 weeks. The acetone plus TPA controls were observed to have a tumor incidence of 4% (0.04 papillomas/mouse); the chrysene plus TPA group incidence was 80% (2.16 tumors/mouse).

In a third study, Rice et al. (1985b) initiated 25 CD-1 mice by application of 100 µg chrysene in acetone every other day for a total of 10 times (1.0 mg total dose). This was followed by thrice weekly applications of TPA beginning 10 days after completion of the initiation phase and continuing for 20 weeks. All animals thus treated survived, and 92% were observed to bear tumors.

Sencar mice were likewise sensitive to chrysene initiation. Papillomas were observed in 21/29 mice treated once with 2 µmol chrysene followed by

twice weekly TPA treatments (2 µg/treatment). TPA only animals had a papilloma incidence of 3/30 (Slaga et al., 1980).

Early studies with chrysene reported no injection site tumors as a consequence of i.m. or s.c. administration (Bottomly and Twort, 1934; Barry and Cook, 1934; Shear and Leiter, 1941). Small numbers of sarcomas at the site of chrysene injection were reported for C57Bl male and female mice when tricaprylin or arachis oil was used as the vehicle (Steiner and Falk, 1951; Steiner, 1955; Boyland and Sims, 1967). Pollia (1941) reported no tumors to be induced in rats receiving repeated injections of chrysene in water or sesame oil, while Barry and Cook (1934) reported four sarcomas in 10 rats repeatedly injected with 2-6 mg chrysene compared with two sarcomas in solvent control rats.

Perinatal exposure of mice to chrysene has resulted in tumor induction. Male and female Swiss mice were injected s.c. with 100 µg chrysene in PEG on the day of birth and the next 2 days. After 70 weeks tumor incidences were the following: one injection of PEG caused liver tumors in 5/20 males and 3/21 females and lung tumors in 2/20 males and 3/21 females; three injections of PEG caused liver tumors in 10/30 males and 0/15 females and lung tumors in 4/30 males and 1/15 females; chrysene caused liver tumors in 13/27 males and 0/21 females and lung tumors in 2/27 males and 1/21 females (Grover et al., 1975).

In a similar experiment, Swiss Webster BLU/Ha(ICR) mice received i.p. injections of a DMSO solution of chrysene on days 1, 8 and 15 of life. Total administered dose was 1.4 µmol. By 38-40 weeks treated animals had

developed lung tumors (5/24 males, 2/11 females) and hepatic tumors (6/24 males), and one lymphosarcoma was observed in a male mouse. DMSO-treated animals developed only lung tumors (2/21 males, 7/38 females) (Buening et al., 1979a). The same protocol was applied in a graded dose bioassay wherein the three i.p. injections were of 0.2, 0.4 and 0.8 μmol repurified chrysene. There was no increase in pulmonary tumors, but 22% of the treated male mice developed hepatic tumors compared with no hepatic tumors observed in the DMSO controls (Chang et al, 1983).

Dibenz[a,h]anthracene. Pulmonary administration of dibenz[a,h]anthracene has induced lung adenomas in mice (Andervont, 1937; Rask-Nielson, 1950, Kuschner et al., 1956). Intratracheal instillation of a dibenz[a,h]anthracene suspension in protein blood substitute with powdered India ink resulted in the development of squamous cell carcinomas (Yanishiva and Balenko, 1966). Single i.v. injections of a colloidal dispersion of this material were also tumorigenic in a dose-dependent fashion in strain A mice (Heston and Schneiderman, 1953).

Kennaway (1930) was the first of many researchers to report induction of skin tumors by dibenz[a,h]anthracene. In another study, a 0.2% solution of dibenz[a,h]anthracene in acetone/benzene was painted twice weekly on the skin of Swiss mice (38 μg/dose). Skin tumors were observed in 16/20 animals (Lijinsky et al., 1965). Van Düüren et al. (1967) topically exposed ICR/Ha Swiss mice 3 times/week to acetone dibenz[a,h]anthracene solutions at the following concentrations: 0.001, 0.01 and 0.1%. Tumor incidences were 1/30 (1 carcinoma), 43/50 (39 carcinomas) and 39/40 (32 carcinomas), respectively. The authors noted that latency was also dose related.

Dibenz[a,h]anthracene was compared with benzo[a]pyrene as to potency in inducing skin tumors in Swiss mice. Repeated skin exposure to a 0.001% acetone solution of benzo[a]pyrene induced papillomas in 43% of the mice and carcinomas in 3%. The same dibenz[a,h]anthracene exposure resulted in a papilloma incidence of 30% and a carcinoma incidence of 30%. Exposure of mice to a 0.01% solution of either compound resulted in papilloma and carcinoma development in >90% of the animals. Latent periods for induction were similar for both carcinogens (Wynder and Hoffman, 1959a).

Dibenz[a,h]anthracene has been reported to initiate skin tumor development in mice at doses as low as 0.02 μg given once (Klein, 1960). Other tumors such as papillomas and pulmonary tumors may also arise as a consequence of skin exposure to dibenz[a,h]anthracene (Buening et al., 1979b). Virgin C3H (Jax) mice were painted twice weekly with 0.25% dibenz[a,h]-anthracene in benzene (thiophene-free) for their lifetime. A 50% incidence of mammary tumors was observed in the control animals as compared with 10/11 in the treated mice (Ranadive and Karande, 1963).

In one study hamsters appeared to be somewhat more resistant to the skin tumorigenic properties of dibenz[a,h]anthracene. A group of 10 Syrian golden hamsters received 20 applications of 0.2% dibenz[a,h]anthracene in mineral oil over a 10 week period. At 50 weeks, 5 animals survived, but no tumors were observed (Shubik et al., 1960).

Several assays have been reported wherein dibenz[a,h]anthracene has been administered s.c. or i.m. Tumors at injection sites have been reported in rats, guinea pigs (low incidence), pigeons and unspecified fowl (Roussy et al., 1942; Shabad, 1938; Shabad and Urinson, 1938; Prichard et al., 1964;

Peacock, 1935). Mice are the animals most studied in this regard. Reports included those by Hartwell (1951), Shubik and Hartwell (1957, 1969) and Thompson and Co. (1971).

The effective dose for s.c. induction of tumors was established by Bryan and Shimkin (1943). Tricaprylin solutions of dibenz[a,h]anthracene were administered once to groups of ≥19 C3H mice so as to deliver the following doses: 0.0019, 0.0078, 0.016, 0.03, 0.06, 0.12, 0.25, 0.5, 1, 2, 4 or 8 mg. Incidences of injection site sarcomas were as follows: 2/79, 6/40, 6/19, 16/21, 20/20, 21/23, 19/21, 20/21, 22/22, 19/19, 17/20 and 16/21, respectively. It was noted that the lowest effective dose for dibenz[a,h]-anthracene was 0.0019 mg while that for 3-methylcholanthrene was >0.0039 mg. The authors also observed that the average latent period for dibenz[a,h]-anthracene sarcoma development was 3.7 months as compared with 2-5 months for 3-methylcholanthrene and 3 months for benzo[a]pyrene. Similar results were reported by Dobrovolskaïa-Zavadskaïa (1938) and Lettinga (1937).

In an abstract, Platt et al. (1983) reported that dibenz[a,h]anthracene showed stronger carcinogenic responses when administered s.c. to NMRI mice than did the following metabolites: 3,4-diol, 5-phenol, 5,6-oxide. The 1,2-diol and 5,6-diols of dibenz[a,h]anthracene were reported to be non-carcinogenic by this treatment regimen.

Newborn mice (general purpose/NIH) were exposed by s.c. injections of dibenz[a,h]anthracene doses between 0.003 and 6.7 µg/mouse. A dose-related increase in sarcoma incidence was noted for doses of ≥0.08 µg, and an increase in lung adenomas was observed for doses ≥0.2 µg (O'Gara et al., 1965).

Female NMRI mice were observed to develop injection site sarcomas after one s.c. treatment of as little as 2.35 μg dibenzo[a,h]anthracene in tricaprylin (Pfeiffer, 1977). No vehicle controls were reported but a group of "non-carcinogenic" PAHs tested concurrently were observed to have sarcoma incidences of 4-13%. Table V-19 compares sarcoma induction for dibenzo-[a,h]anthracene and benzo[a]pyrene.

Lubet et al. (1983a) found that s.c. injections of dibenz[a,h]anthracene were associated with fibrosarcoma development in mice, but only for some strains. Four strains of mice used included two, C3H/HeJ and C57Bl/6J, that respond to 3-methylcholanthrene treatment by increased levels and types of hepatic enzymes including AHH. Two strains, AKR/J and DBA/2J, were non-responders. Groups of 30 animals were injected with a single dose of 150 μg dibenz[a,h]anthracene in 0.05 mℓ trioctanoin and observed for 9 months. A control group for each strain, consisting of 10 animals each, received a s.c. injection of 0.05 mℓ trioctanoin alone. A summary of the findings is given in Table V-20. The tumor incidence in the treated animals varied between 0 and 80%, depending on the strain. Tumor incidences were higher in the C3H and C57Bl mice, which also were readily inducible for AHH. Likewise, the average latency period (in days) for fibrosarcoma development varied with the strain and tended to be inversely correlated with the tumor incidence rate. The authors concluded that, as for benzo[a]pyrene, the Ah receptor was involved in the process of tumor induction by s.c. injection for dibenz[a,h]anthracene.

Fluoranthene. Fluoranthene was first tested for carcinogenic activity more than 5 decades ago (Barry et al., 1935). The results from that

TABLE V-19

Sarcomagenic Activity of Subcutaneously Injected Dibenzo[a,h]anthracene and Benzo[a]pyrene in Female NMRI Mice*

Compound	Dose (μg)	Tumor Incidence
Dibenzo[a,h]anthracene	2.35	37/100
	4.7	39/100
	9.3	44/100
	18.7	56/100
	37.5	65/100
	75.0	69/100
Benzo[a]pyrene	3.12	9/100
	6.25	35/100
	12.5	51/100
	25.0	57/100
	50.0	77/100
	100.0	83/100

*Source: Pfeiffer, 1977

TABLE V-20

Incidence of Fibrosarcomas in Mice Associated with Subcutaneous Injections of Dibenz[a,h]anthracene[a]

Strain	Treatment[b]	Tumor Incidence	%	Average Latency (days)
C3H/HeJ	Dibenz[a,h]anthracene	24/30	80	165
	Control	0/10	0	0
C57Bl/6J	Dibenz[a,h]anthracene	16/30	33	242
	Control	0/10	0	0
AKR/J	Dibenz[a,h]anthracene	0/30	0	0
	Control	0/10	0	0
DBA/2J	Dibenz[a,h]anthracene	1/30	3	230
	Control	0/10	0	0

[a]Source: Lubet et al., 1983a

[b]Animals in the dibenz[a,h]anthracene group received a single injection of 150 µg of dibenz[a,h]anthracene in 0.05 mℓ of trioctanoin. Control animals received an injection of 0.05 mℓ trioctanoin alone.

investigation, and from several studies conducted since that time, indicated that fluoranthene had virtually no activity as a complete carcinogen.

Suntzeff et al. (1957) administered a 10% solution of fluoranthene in acetone 3 times weekly by topical application to CAF, Jackson, Swiss and Millerton mice. No tumors were found by 13 months.

Wynder and Hoffmann (1959a) administered a 0.1% solution of fluoranthene in acetone to the backs of 20 female Swiss (Millerton) mice 3 times/week for life. No tumors were found.

Hoffmann et al. (1972) administered 50 µl of a 1% fluoranthene solution to the backs of 20 female Swiss-Albino Ha/ICR/Mill mice 3 times/week for 12 months. All treated mice survived, and no tumors were observed. As part of the same study, 30 mice received 0.1 mg fluoranthene in 50 µl acetone every second day for a total of 10 doses; 10 days later promotion with 2.5% croton oil in acetone was started and continued for 20 weeks. A single papilloma was noted in 29 surviving mice.

Horton and Christian (1974) administered 50 mg fluoranthene in decalin or in decalin:n-dodecane (50:50) to the backs of 15 male C3H mice. The mice were treated 2 times/week for 82 weeks. No skin tumors were observed.

Van Düüren and Goldschmidt (1976) administered 40 µg fluoranthene in acetone 3 times weekly for 440 days to female ICR/Ha Swiss mice. No skin tumors were observed.

Barry et al. (1935) administered four doses of 10 mg fluoranthene in glycerol by subcutaneous injection to strain A mice. Six out of 14 mice survived for 18 months; no tumors were found by 19 months.

Shear (1938) administered four doses of 10 mg fluoranthene in glycerol by subcutaneous injection to strain A mice. Six out of 14 mice survived for 18 months; no tumors were found by 19 months.

Fluoranthene was tested by Busby et al. (1984) in a mouse lung adenoma assay. Newborn male and female mice of Swiss-Webster BLU:Ha strain were injected intraperitoneally with a total dose of 700 μg (163 mg/kg) or 3.5 mg (815 mg/kg) fluoranthene in three installments on days 1, 8 and 15 (1/7, 2/7 and 4/7 of the dose, respectively). Histopathologic examination of the lungs of the mice sacrificed at 24 weeks showed a significant increase in the high-dose group in the total incidence and number/mouse (28/48 or 58%, 1.08 tumors/mouse) of lung tumors compared with vehicle controls (5/55 or 9%, 0.09 tumors/mouse). Tumor response in the low-dose group (10/51 or 20%, 0.24 tumors/mouse) was not statistically significant. A positive response in a lung adenoma assay in the absence of corroborating studies is generally considered insufficient evidence of carcinogenicity.

Fluorene. Studies of fluorene as a complete mouse skin carcinogen and as a cocarcinogen with 3-methylcholanthrene were negative or inconclusive (Kennaway, 1924a; Riegel et al., 1951). IARC (1983) considered both reports inadequate for evaluation.

Fluorene was also found to be inactive both as a tumor initiator (with TPA as a promoter) and a complete carcinogen in skin-painting bioassays performed in female Ha/ICR mice by LaVoie et al. (1979, 1981b). No injection site tumors were produced in 10 strain A mice after seven s.c. injections of 10 mg fluorene in glycol. The study was terminated at 18 months (Shear, 1938).

Indeno[1,2,3-cd]pyrene. Indeno[1,2,3-cd]pyrene was administered to female OM rats by implantation in the lung of beeswax-trioctanoin pellets containing 0.16, 0.83 or 4.15 mg of the compound. Results summarized in Table V-21 indicate a dose-dependent increase in keratinized epidermoid carcinomas capable of invading the extrapulmonary chest wall. There was no increase in pleiomorphic sarcomas. A calculated potency from this assay relative to that of benzo[a]pyrene (=1.00) was 0.08 (Deutsch-Wenzel et al., 1983).

By contrast to these results, indeno[1,2,3-cd]pyrene was not tumorigenic in newborn CD-1 mice treated i.p. on days 1, 8 and 15 of life (total dose 2.1 μmol, see Table V-16) (LaVoie et al., 1987).

Groups of 20 female Swiss albino Ha/ICR/Mil mice were given topical applications of indeno[1,2,3-cd]pyrene prepared as dioxane or acetone solutions. Dioxane preparations of indeno[1,2,3-cd]pyrene at concentrations of 0.05 or 0.1% did not induce skin tumors whereas benzo[a]pyrene at the same concentration produced tumors in 90% of the treated mice in 7 months. By contrast, acetone solutions of indeno[1,2,3-cd]pyrene produced skin tumors in a dose-related fashion. No tumors were observed in animals painted with

TABLE V-21

Carcinogenicity of Indeno[1,2,3-cd]pyrene upon Implantation in Rat Lungs[a]

Treatment	Dose (mg)	Median Survival Time (Weeks)	Tumor Incidence		
			Epidermoid Carcinomas	Sarcomas	Total
Untreated	--	118	0/35	0/35	0/35
Pellet[b]	--	104	0/35	0/35	0/35
Indeno[1,2,3-cd]-pyrene	0.16	116	3/35	1/35	4/35
	0.83	109	8/35	0/35	8/35
	4.15	92	21/35	0/35	21/35

[a]Source: Deutsch-Wenzel et al., 1983

[b]Refers to beeswax and trioctanoin implantation medium

the 0.01 and 0.05% solutions. The 0.1% treatment induced six papillomas and three carcinomas beginning at 9 months; the 0.5% concentration of indeno-[1,2,3-cd]pyrene resulted in seven papillomas and five carcinomas with the first tumor appearing at 3 months. This paper also reported that a total dose of 250 µg indeno[1,2,3-cd]pyrene delivered in 10 applications was a sufficient initiating dose when followed by croton oil promoting treatment (Hoffman and Wynder, 1966).

Indeno [1,2,3-cd]pyrene was not shown to be a complete carcinogen when applied to the skin of female NMRI mice twice a week for their lifetime. Total doses applied in acetone were 0, 3.4, 5.6 or 9.2 mg/animal (Habs et al., 1980).

Solutions of 4.0 µmol indeno[1,2,3-cd]pyrene and two fluoridated metabolites, 2-fluoroindeno[1,2,3-cd]pyrene and 8,9-difluoroindeno-[1,2,3-cd]pyrene, were applied to the shaved backs of 30 female CD-1 mice/group every other day for a total of 10 doses. Mice treated with benzo[a]pyrene and acetone served as positive and negative controls. Ten days after the last initiating dose 12-O-tetradecanoylphorbol-13-acetate (TPA) was applied to the backs of the mice as a promoter 3 times/week for 20 weeks. At the end of the promotion period indeno[1,2,3-cd]pyrene and TPA treatments induced tumors in 72% of the mice (2.1 tumors/mouse), the 2-fluoroindeno[1,2,3-cd]pyrene and TPA treatments induced tumors in 76% of the mice (3.9 tumors/mouse) and the 8,9-difluoroindeno[1,2,3-cd]pyrene and TPA treatments induced tumors in 40% of the treated mice (0.6 tumors/mouse). At the end of the initiation period, DNA from the skins of 5 mice/group was isolated. [32]P-postlabeling analysis of the hydrolyzed DNA showed that

indeno[1,2,3-cd]pyrene and 2-fluoroindeno[1,2,3-cd]pyrene each form a single major DNA adduct when analyzed by thin-layer chromatography. These adducts have different retention behaviors. 8,9-Difluoroindeno[1,2,3-cd]pyrene does not have this adduct; the authors speculated that this could be one factor in the variance of the tumor incidences of the three compounds (Rice et al., 1990).

As part of a study of initiating capability of its major metabolites in mouse skin, indeno[1,2,3-cd]pyrene was applied to the shaved backs of 20 Crl:CD-1(ICR)BR female mice. Acetone solutions were applied every other day for 10 days for a total initiating dose of 1 mg. This was followed 10 days later by thrice weekly TPA applications (0.0025% in 100 µl acetone) for 20 weeks. Tumor incidence was essentially 100%. Indeno[1,2,3-cd]pyrene-1,2-diol and -1,2-oxide treatment both resulted in 80% tumor incidence by comparison with 8-hydroxy (~25%) and the acetone-treated controls (~5%) (Rice et al., 1986).

An earlier initiation-promotion bioassay performed by Rice et al. (1985c) showed a pronounced dose-response relationship. Following the same protocol described above, there was an 80% tumor incidence in mice receiving a total initiating dose of 1 mg indeno[1,2,3-cd]pyrene with an average of about four tumors/mouse after 22 weeks of promotion. However, when the total initiating dose was decreased to 100 or 300 µg, the number of tumor-bearing mice was below statistical significance.

Injection site sarcomas were reported in 10/14 male and 1/14 female XVIInc/Z mice administered three injections at 1 month intervals of 0.6 mg indeno[1,2,3-cd]pyrene in olive oil (Lacassagne et al., 1963).

Naphthalene. In an inhalation study, naphthalene (scintillation grade, purity >99%) vapor was administered at 0 and 10 ppm to 75 B6C3F1 mice/sex/dose for 6 hours/day, 5 days/week for 103 weeks (NTP, 1991b). Two groups of 75 B6C3F1 mice/sex were administered 30 ppm naphthalene for the same amount of time as the 0 and 10 ppm groups. In each group 25 mice/sex were designated for hematology evaluation; 5 mice/sex were to be sacrificed after 14 days and 3, 6, 12 and 18 months. Only the 14-day hematology mice were sacrificed in the control male mice because of high mortality. When compared with survival in exposed male groups, survival was decreased in the male control group; the decrease was the result of wound trauma from fighting among animals caged together. Survival was equivalent in all female groups. Final body weights were equivalent in all groups and no clinical observations could be attributed directly to naphthalene.

There was a significant increase in the incidence of alveolar or bronchiolar adenomas in the high-dose females by pairwise comparison with controls. The incidences were 5/68 (7%), 2/64 (3%) and 28/134 (21%) in the control, low- and high-dose groups, respectively. The recent historical control incidence for females in this laboratory is 91/1166 (7.8%, range 0-16%) and the recent historical control incidence for females specifically in inhalation studies is 39/466 (8.4%, range 0-12%). The first incidence of alveolar or bronchiolar adenomas was reported on experiment day 714, 736 and 656 in the control, low- and high-dose groups, respectively. In the high-dose females there was a single incidence of alveolar or bronchiolar carcinoma; this was not observed in the other groups. There was also a statistically significant increase in the incidence of respiratory epithelial cell hyperplasia in the naphthalene-treated groups. Because the

incidence of alveolar or bronchiolar adenomas was statistically significant and above historical control ranges, NTP judged the increased adenoma incidence to be directly related to naphthalene exposure.

There was a statistically significant increase in the incidence of respiratory epithelial cell hyperplasia in the naphthalene-treated male groups. There was no significant increase in the incidence of alveolar or bronchiolar adenomas or carcinomas (either separately or combined). The incidences of alveolar or bronchiolar adenomas or carcinomas (combined) were 7/69 (10%), 17/69 (25%) and 31/135 (23%) in the control, low- and high-dose groups, respectively. The male control incidences were 94/478 (19.7%, range 10-30%) in recent inhalation studies and 229/1172 (19.5%, range 6-42%) in all carcinogen studies conducted recently in this laboratory. As of this writing the U.S. EPA has not evaluated this study.

Adkins et al. (1986) exposed groups of 30 six- to eight-week-old female A/J strain mice by inhalation to naphthalene at concentrations of 0, 10 or 30 ppm, 6 hours/day, 5 days/week for 6 months. After the 6-month exposure period, excised lungs were examined for tumors. Naphthalene exposure did not significantly increase tumor incidence, but did cause a statistically significant increase ($p<0.05$) in the number of adenomas per tumor-bearing mouse lung. There was no dose-response.

Schmähl (1955) administered naphthalene by i.p. injection to a group of 10 rats (in-house strains BDI and BDIII) with another group of 10 rats serving as controls. The daily dose was 20 mg/rat and injections were given weekly for 40 weeks. Animals were observed until spontaneous death. Histologic examination offered no evidence of carcinogenic effects.

Boyland et al. (1964) implanted naphthalene into the bladder of stock Chester Beatty mice (23) in an effort to determine the suitability of naphthalene as a potential vehicle for carcinogenicity testing. The mice were observed for 30 weeks. Tumor incidence was as low as when paraffin wax was used and lower than with cholesterol. Naphthalene was judged to be inert but to have no advantage over cholesterol as a base for implantation pellets.

Coal tar-derived naphthalene that contained ~10% unidentified impurities was tested for carcinogenicity by Knake (1956). White rats (40, sex unspecified) were given seven subcutaneous injections of 500 mg/kg naphthalene in sesame oil at 2-week intervals. Lymphosarcomas were found in 5/34 surviving rats at 18 months (14.7%), whereas vehicle controls had a 2% incidence of these tumors. Mice (25, inbred black) were painted with 0.5% naphthalene in benzene 5 days/week for life. Four treated mice developed leukemias in contrast to 0/21 vehicle controls; the untreated control incidence was 0.4%. The value of these studies for assessing carcinogenicity is very limited due to the presence of potentially carcinogenic impurities. Moreover, the vehicle in the mouse study has been shown to cause leukemias, and the site of injection in the rats study was painted, prior to injection, with carbofuchsin, a known carcinogen.

Kennaway (1930) reported that naphthalene was not carcinogenic in skin painting studies in mice. The concentration, purity, dosing regimen, and other details were not provided. The reaction product of naphthalene and aluminum trichloride was reported to be carcinogenic, but the product was not identified.

Schmeltz et al. (1978) tested the carcinogenic activity of benzo[a]-pyrene and naphthalene in female ICR/HA (Sprague-Dawley) mice. A 100 µl solution containing 0.25% naphthalene and 0.003% benzo[a]pyrene was painted on the shaved backs of 30 mice 3 times/week for 78 weeks. Naphthalene inhibited benzo[a]pyrene-induced tumors; ~42% of the mice treated with benzo[a]pyrene alone had skin tumors whereas ~20% of mice had skin tumors when naphthalene and benzo[a]pyrene were administered together.

Phenanthrene. Data on phenanthrene's potential as a skin carcinogen have been summarized by IARC (1983). Phenanthrene did not serve as a complete carcinogen in two studies in mice that were incompletely reported (Kennaway, 1924b; Roe and Grant, 1964). Phenanthrene was reported to be active as an initiator in female CD-1 mice in one study (Scribner, 1973). Thirty-five weeks after initiation with 10 µmol phenanthrene and twice weekly treatments with 5 µmol TPA, 12/30 mice developed papillomas as compared with 0/30 TPA-treated controls. Phenanthrene, however, was ineffective as an initiator of skin tumorigenesis in Swiss Ha/ICR, albino, S and CD-1 mice (LaVoie et al., 1981a; Roe, 1962; Salaman and Roe, 1956; Wood et al., 1979) and inactive as a promoter in mice of unspecified strain (Roe and Grant, 1964). It was noted by Wood et al. (1979) that 30 female CD-1 mice given one topical application of 10 µmol phenanthrene or the 1,2- or 3,4-dihydrodiol followed by twice weekly applications of 16 µmol TPA (in 200 µl acetone) were observed to have papilloma incidences 2-4 times that of background. These incidences were not significantly increased by comparison with controls because of the small number of animals tested and the spontaneous tumor incidence.

Subcutaneous administration of phenanthrene to mice did not result in treatment-related increases in tumor incidence (Steiner, 1955; Roe, 1962). Likewise, neither s.c. nor i.p. treatment of neonatal mice resulted in significant tumor induction (Grant and Roe, 1963; Buening et al., 1979a). While the 1,2-diol-3,4-epoxides of phenanthrene are mutagenic to Salmonella typhimurium and to mammalian (V79) cells, they did not induce pulmonary tumors in newborn mice (Wood et al., 1979; Buening et al., 1979a).

Pyrene. Intratracheal instillation of pyrene with Fe_2O_3 particles did not result in increased numbers of respiratory tumors in male Syrian golden hamsters (Sellakumar and Shubik, 1974).

As part of a study of nitrated PAHs (Wislocki et al., 1986), a dimethyl sulfoxide solution of pyrene was administered i.p. to newborn CD-1 mice on days 1, 8 and 15 of age. Total administered doses were 200, 700 or 2800 nmole. Animals were weaned, separated by sex and observed without further treatment to 1 year. A small but significant increase in liver carcinomas was observed in the mid-dose male mice only (Table V-22). The incidences of total liver tumors (adenomas and carcinomas), lung tumors or malignant lymphomas were not significantly elevated in treated animals.

Tests of pyrene as a complete skin carcinogen and as an initiator of carcinogenicity in mice have been negative or inconclusive (Badger et al., 1940; Roe and Grant, 1964; Horton and Christian, 1974; Van Düüren and Goldschmidt, 1976; Salaman and Roe, 1956; Scribner, 1973). Co-administration of pyrene and benzo[a]pyrene to the backs of ICR/Ha mice resulted in an enhancement of the benzo[a]pyrene tumorigenicity (Van Düüren and Goldschmidt, 1976; Goldschmidt et al., 1973).

TABLE V-22

Assay of Pyrene in Newborn Mice[a]

Sex	Total Dose (nmol)	Tumor Incidence			
		Liver Adenomas	Liver Carcinomas	Lung Adenomas	Lung Carcinomas
M	0	7/73	0/73	3/73	2/73
F	0	0/65	0/65	1/65	1/65
M	200	0/29	0/29	1/29	0/29
F	200	0/31	0/31	1/31	0/31
M	700	0/25	3/25[b]	2/25	0/25
F	700	0/49	0/49	5/49	0/49
M	2800	2/14	1/14	1/14	0/14
F	2800	0/18	0/18	1/18	0/18

[a]Source: Wislocki et al., 1986

[b]Significantly different from controls ($p<0.05$), Fisher exact test

Pyrene did not produce tumors in Jackson A mice injected s.c. and observed 18 months (Shear and Leiter, 1941).

Reproductive/Teratogenic Effects

Evidence of deleterious reproductive effects, documented for PAHs in general and benzo[a]pyrene in particular, has been equivocal. This evidence is considered here.

Anthracene. A total of 8 mg anthracene/mouse was administered as daily s.c. doses or in a single oral dose during the last week of gestation to dams of the following strains: BALB/C, C3H/A and C57B1 X CBA F1. Fetuses were removed and the kidney cells established in culture. These cells exhibited enhanced plating efficiency as well as some hyperplastic changes by comparison with fetal cells obtained from untreated animals indicating the ability of anthracene to pass through the placenta (Shabad et al., 1972).

Benz[a]anthracene. Wolfe and Bryan (1939) reported fetal death and resorption in two pregnant rats as a consequence of s.c. injection of 5 mg benz[a]-anthracene beginning with day 1 of gestation.

Benzo[a]pyrene. Rigdon and Rennels (1964) conducted two series of experiments in rats (strain not specified) to ascertain possible reproductive consequences of dietary benzo[a]pyrene. In series one, eight females and an unspecified number of males were fed laboratory chow to which 1 mg/kg benzo[a]pyrene had been added, and a control group of six females and an unspecified number of males were fed a standard diet. Treated females were

mated with control males, and control females were mated to benzo[a]pyrene-treated males. Vaginal smears were taken during a 28-day period beginning with the first day of benzo[a]pyrene feeding. The authors observed no treatment-related effects on the estrus cycle. Three normal pregnancies were reported for the control females, and five benzo[a]pyrene-treated females also became pregnant. Of the treated females, only one delivered a total of four pups at day 23. Two of the four pups were stillborn, one of which was grossly malformed. One pup left with the dam died 3 days postpartum, presumably of starvation. Of the dams not delivering, one was found upon autopsy to carry four dead fetuses.

In the second series 6 control male and female rats were mated as were males and 7 females fed benzo[a]pyrene as described above. The pregnant control rats had apparently normal pregnancies. Two benzo[a]pyrene-fed females of seven mated became pregnant. On autopsy, it was found that one dam carried four dead fetuses and that fetal resorption had occurred in the other. While these data suggest a reproductive effect of benzo[a]pyrene, the report was unclear as to specifics of experimental design and length and timing of the feeding period for treated males and females. In this study the 1 mg/kg dose appears to be the LOAEL with no NOAEL for reproductive effects in rats.

Rigdon and Neal (1965) conducted a series of four experiments on reproductive effects in Swiss mice fed diets containing benzo[a]pyrene at concentrations of 0, 250, 500 or 1000 ppm. The animals were given the dietary benzo[a]pyrene for various time spans during mating, gestation and postpartum. Blue fluorescence, indicative of the presence of benzo[a]pyrene or

its metabolites, was demonstrated in the internal organs of treated mice. No teratogenic, embryotoxic, fetotoxic or other reproductive effects were observed in the treated animals. Fertility of male mice was apparently unaffected. Males fed the 500 ppm diet for 30 days were shown to have sperm present in the lumen of testicular tubules.

In a study of CD-1 strain pregnant mice, however, MacKenzie and Angevine (1981) found adverse reproductive effects in the offspring of animals dosed with benzo[a]pyrene. Benzo[a]pyrene in corn oil at doses of 0, 10, 40 or 160 mg/kg bw was administered by gavage on days 7-16 of gestation to groups of 30 or 60 dams. At these doses benzo[a]pyrene was not toxic to dams or fetuses. Pregnancies were carried to term and pups permitted to nurse until weaning. Number of pups and gross abnormalities were scored on day 1 postpartum, and on day 4 pups were weighed and sexed. At this time litters were culled to 8 pups for breeding studies. At 6 weeks of age 10 F_1 males and 10 F_1 females were sacrificed for histologic examination of reproductive organs. At 7 or 8 weeks the remaining F_1 mice were bred with untreated animals. Females, both those treated in utero and those mated with F_1 males, were sacrificed on days 14-19 of gestation, and numbers of implants, fetuses and resorptions were recorded. Gross abnormalities were also noted.

Reduced fertility and reproductive capacity were also observed in the F_1 mice. The F_1 males exposed to 10 mg/kg bw in utero showed a marked reduction in gonadal weight, although no effect on body weight was found. All control males (45/45) were fertile (sired at least one litter) and as a group impregnated 80% of the females to which they were exposed. Fertility

rates were 20/25, 3/45 and 0/20 for the 10, 40 and 160 mg/kg male dose groups, respectively, and the percent of impregnated females was 52, 7 and 0%, respectively.

In the control females the pregnancy rate was 35/35; the pregnancy rate for the female groups exposed in utero was 23/35, 0/55 and 0/20 for the 10, 0 and 160 mg/kg dose groups, respectively. In this study a 10 mg/kg dose is the LOAEL with no NOAEL for a reproductive effects in mice.

To examine embryotoxicity, fetal Swiss mice were microinjected in utero on day 10, 12 or 14 of gestation with benzo[a]pyrene, benzo[a]pyrene-4,5-oxide, a racemic mixture of 7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene (compounds thought to be carcinogenic metabolites of benzo[a]pyrene) or 6-methylbenzo[a]pyrene (Barbieri et al., 1986). The vehicle was 1:1 trioctanonin:acetone and the mice were administered the test compounds at doses of 0.4-16 nmol/embryo. An additional group was treated transplacentally with 47.5 nmole benzo[a]pyrene on day 10, 12 or 14. On day 18, the dams were sacrificed and inspected for implantation sites, as well as the number of dead and live fetuses; live fetuses were further examined for gross malformations. Results, given in Table V-23, show that intra-embryonal administration of benzo[a]pyrene and benzo[a]pyrene-4,5-oxide did not significantly increase the incidence of fetal malformations; however, the 7,8-diol-9,10-epoxide caused significant increases in the incidence of fetal malformations. Specific malformations included exencephaly, thoracoschisis, gastroschisis, phocomelia and edema. 6-Methylbenzo[a]pyrene also increased the incidence of terata; transplacental administration of benzo[a]pyrene, however, did not.

TABLE V-23

Embryotoxicity and Malformations in Swiss Mice Exposed by Intraembryonal Injection of Benzo[a]pyrene and Derivatives[a]

Treatment			Viability	Malformations
Compound	Day	Dose nmole/Embryo	No. Live/ No. Treated	No. Malformed/ Total No. Alive
Control[b]	10	0.5 (µl)	53/69	11/53
	12	1 (µl)	30/36	2/30
	14	2 (µl)	59/82	4/59
Benzo[a]pyrene	10	4	18/41[c]	6/18
	12	8	42/78[c]	6/42
	14	16	28/45	4/28
Benzo[a]pyrene-4,5-oxide	10	4	25/28	8/25
	12	8	21/43[c]	4/21
	14	16	51/66 [sic]	5/51
Benzo[a]pyrene-7,8-diol-9,10-epoxide	10	0.4	7/46[d]	7/7[d]
	12	2	59/111[c]	36/59[d]
	14	4	37/58	10/37[e]
6-Methylbenzo[a]pyrene	10	4	20/36[e]	10/20[e]
	12	8	13/27[c]	6/13[c]
	14	16	39/65	12/39[c]

[a]Source: Barbieri et al., 1986

[b]Acetone and trioctanoin (1:1)

[c]$p<0.01$

[d]$p<0.001$

[e]$p<0.05$

LeGraverand et al. (1984) found the extent of embryotoxicity and teratogenicity of benzo[a]pyrene to be related both to the route of administration and to the affinity of a receptor (AHH) for agents that induce hepatic cytochrome P-450 response. Benzo[a]pyrene (120 mg/kg/day) was orally administered to pregnant mice from day 2 to day 10 of gestation. The mice were either heterozygous or homozygous for the low-affinity receptor for inducers of cytochrome P-450 (the high-affinity receptor is dominant). Pharmacokinetic studies indicated that embryos developing in dams homozygous for the low-affininty receptors were exposed to higher levels of benzo[a]pyrene. Within this group of embryos, receptor homozygotes had an increased incidence of intrauterine toxicity and malformation when compared with receptor heterozygotes. Heterozygous dams exhibit an enhanced ability to metabolize benzo[a]pyrene in their intestines and liver; therefore, the developing embryos receive less benzo[a]pyrene. Consequently, less intrauterine toxicity and fewer malformations were seen. Intraperitonial administration of benzo[a]pyrene caused the exact opposite effects, indicating the importance of both the administration route and genotype of the exposed animal in the potential for a toxic response.

Swartz and Mattison (1985) showed that acute exposure to benzo[a]pyrene had an adverse, albeit transient, effect on oocyte follicle growth, ovulation and formation of corpora lutea. Five female C57Bl/6N mice/group were treated with a single i.p. injection of 0, 1, 5, 10, 50, 100 or 500 mg/kg bw benzo[a]pyrene in corn oil. One week post-treatment numbers of corpora lutea were significantly reduced in mice treated with 5-500 mg/kg benzo[a]-pyrene. By week 4 this depression was evident only in the mice in the two

highest treatment groups. Ovarian toxicity in the highest dose group was indicated not only by absences of corpora lutea but also by lack of signs of folliculogenesis.

Takizawa et al. (1984) established ED_{50} doses for small oocyte destruction by benzo[a]pyrene in several mouse strains. Treatment was by intraovarian injection of 0, 0.1, 1.0, 10 or 30 µg/ovary. Two weeks post-treatment mice were killed and ovarian tissue examined histologically. The ED_{50}s for various strains were the following: C57Bl/6N, ED_{50} = 3.38 µg/ovary; DBA/2N = 36.14; C57Bl/6J x DBA/2JF1 = 8.27.

Pregnant C3H/Anf mice were treated i.p. at either 11-13 or 16-18 days of gestation with 100 or 150 mg benzo[a]pyrene/kg bw. Body weights of progeny were comparable between control and treated groups. Offspring of treated animals, however, were observed to have a severe supression of immune function as measured by the ability to elicit an antibody response to sheep red blood cells. This supression of response persisted into adulthood indicating permanent immunosuppression when prenatal exposures occur (Urso and Gengozian, 1980).

Benzo[a]pyrene has been shown to be a transplacental carcinogen producing liver tumors in HA/ICR, strain A and C57Bl mice and lung tumors in the Ha/ICR mice (Bulay, 1970; Bulay and Wattenberg 1971; Nikonova, 1977).

Female A strain mice were subcutaneously administered 150 mg/bw benzo-[a]pyrene in sunflower oil on the 18th and 19th day of pregnancy (Turusov et al., 1990). The offspring were mated in a brother-sister system to create

the F2-F5 generations. Controls receiving only sunflower oil were maintained. The offspring were sacrificed at 1 year of age. When compared with controls, there was a statistically significant increase in the lung adenoma incidence in both the males and females of the first generation. The lung tumor incidences in F1 females were 42/78 and 12/78 in the exposed and control mice, respectively, and in F1 males the incidences were 52/67 and 6/75 in the exposed and control mice, respectively. The F2 females exhibited a statistically significant increase in lung tumor incidences also; these were 20/91 and 10/95 in the female F2 descendants of the treated dams and in the controls, respectively. This increased incidence of lung tumors was not seen in the F2 males or in later generations, but a statistically significant increase in tumor multiplicity was seen in the F2-F5 generations.

Rabbit progeny have also been shown to develop tumors after transplacental exposure to benzo[a]pyrene during the last third of preganancy (Beniashvili, 1978).

Chrysene. Mallard duck eggs were painted with 10 µl of a 0.1% solution of chrysene in an unspecified petroleum hydrocarbon reported to be of "relatively low embryotoxicity" (IARC, 1983). Embryotoxicity and teratogenic effects among ducklings were observed (Hoffman and Gay, 1981).

Fluoranthene. Irvin and Martin (1987) reported, in an abstract, a developmental study in which a single i.p. injection of fluoranthene (dose unspecified) was given to pregnant C57/B6 mice on gestational days 6, 7, 8 or 9. A gestation-dependent increased rate of embryo resorption was observed.

Naphthalene. In what appears to be two reports of the same study (Plasterer et al., 1985; Booth et al., 1983), single oral doses (300 mg/kg) of naphthalene were administered daily for 8 consecutive days to 50 pregnant CD-1 mice beginning on day 7 of gestation. This dose was estimated to be at or just below the maximum tolerated dose for acute lethality. A significant increase in maternal lethality ($p<0.05$) and a decrease in mean maternal body weights as well as the number of live pups per litter ($p<0.05$) on postpartum day 1 were noted when compared with the controls. There was not a concomitant increase in dead pups. There were no effects on pup survival and mean body weights. No gross congenital abnormalities were detected in the pups although the method used to examine the pups was not reported.

Hardin et al. (1981) administered naphthalene i.p. (395 mg/kg) in corn oil to pregnant Sprague-Dawley rats on day 1 of gestation. Daily injections continued through day 15. No treatment-related effects on maternal toxicity, fetal toxicity, or teratogenesis were reported.

In a pilot range-finding study, 20 artificially inseminated New Zealand white rabbits (at least 24 weeks of age and weighing 4-5 kg) were orally dosed with naphthalene (in 1% methylcellulose vehicle) at 50-1000 mg/kg from gestational days 6-18. Maternal lethality and/or abortion were increased at doses of $\geq$630 mg/kg, but no data were collected. No differences in reproductive parameters were noted, and no malformations or fetal death occurred at the lower dose levels (Naismith and Matthews, 1985).

In the main study by Naismith and Matthews (1986), 18 artificially inseminated New Zealand white rabbits per group were orally dosed with naphthalene (1% methylcellulose vehicle) at 0, 40, 200 or 400 mg/kg/day from

gestation days 6-18. Dams were at least 24 weeks of age (exact ages were not specified). Maternal body weights and body weight gains were comparable among all test groups and controls. Food consumption of high-dose (400 mg/kg) animals was significantly greater ($p \leq 0.05$) than controls during gestation days 7-15, 23-25 and 27-29. Pharmacotoxic signs observed during the study included decreased activity, dyspnea, weight loss, cyanosis, salivation, and loose stools or diarrhea; these signs occurred in an apparent dose-related manner. Gross examination of dams and controls indicated no differences in the following reproductive measures: number of corpora lutea, total number of implantations, viable or nonviable fetuses, pre- or postimplantation loss, fetal body weights, and fetal sex distribution. The malformations and variations were equally distributed among groups, and no dose-related trends were apparent. These malformations included 2 incidences of fused sternebrae and an incidence of umbilical herniation from 3 different litters in the control group, an incidence of viceral malformations in the mid-dose group and 3 incidences of fused sternebrae from 3 different litters in the high-dose group. The study authors concluded that oral administration of naphthalene to pregnant rabbits did not evoke a teratogenic effect. The U.S. EPA (1987a) concluded that the teratogenic potential could not be adequately assessed. The data were considered incomplete because of lack of information on the methods of fetal sacrifice and of visceral and skeletal examinations.

Matorova (1982) reported that naphthalene administered by gavage (0.015, 0.15 and 1.5 mg/kg) on a chronic basis (duration not reported) to pregnant female albino rats was associated with adverse effects on reproductive function and development of progeny. These effects included slow fetal development, an increase in number of hemorrhages and bleeding of internal

fetal organs, and a reduced viability. The reported threshold for effects was 0.075 mg/kg. A lack of information on protocol design, tests for significance, and experimental data renders these results difficult to interpret.

Mutagenicity/Genotoxicity

The PAHs have long been recognized as having mutagenic and other genotoxic effects. The literature contains many reports of short-term in vitro and in vivo tests of the genotoxicity of these compounds and of their metabolites. Because of the extensive scope of these reports, their results are presented in tabular form (Table V-24).

Synergism and/or Antagonism

Because PAHs rarely occur in isolation from each other in the environment, it is important to understand the potential health effects associated with mixtures of PAHs. Much research has focused on the promoting or inhibitory effect of noncarcinogenic PAHs, such as pyrene, on the carcinogenic potential of known carcinogens, often benzo[a]pyrene. The route of administration is typically nonoral (dermal, subcutaneous injection or inhalation). Some of the reports on cocarcinogenic activity are considered in this section.

Falk et al. (1964) conducted a series of experiments with C57B1 male mice (3-4 months old) to assess the potential inhibitory effects of phenanthrene and other PAHs considered noncarcinogenic on the tumorigenicity of dibenz[a,h]anthracene. Groups of 30 animals received single s.c. injections of various dosages of dibenz[a,h]anthracene alone and in combination with

TABLE V-24

Results of Short-Term Tests of Polycyclic Aromatic Hydrocarbons

Test	Organism (Assay)	Exogenous Activation* System	Results Reported	Comments	Reference
BACTERIA			ACENAPHTHYLENE		
Mutation	Salmonella typhimurium (reverse mutation, his)	Rat liver S9 (Aroclor)	Negative	Tested up to 250 µg/mL in strains TA1537 and TA1538	Gatehouse, 1980
		Rat liver S9 (3-methylcholanthrene)	Negative	Tested up to 3 µmol/plate in strains TA98 and TA100	Florin et al., 1980
	S. typhimurium (reverse mutation, his) (taped-plate assay)	Rat liver S9 (Aroclor)	Negative	Tested up to 50 µg/plate in strains TA98 and TA100	Bos et al., 1988
	S. typhimurium (forward mutation, 8AG^R)	Rat liver S9 (Aroclor or phenobarbital)	Positive	Strain TM677 tested at 1 µmol/mL	Kaden et al., 1979
MAMMALIAN CELLS					
Cell transformation	Syrian hamster embryo cells (morphologic changes)	None	Negative	1-50 µg/mL	Tu et al., 1986
BACTERIA			ANTHRACENE		
DNA damage	Escherichia coli (polA)	Rat liver S9 (none)	Negative	Tested up to 250 µg/mL	Rosenkrantz and Poirier, 1979
	E. coli (uvrA, recA, lexA, polA)	Rat liver S9 (Aroclor)	Negative. Potency (Δ minimal inhibitory concentration/ nmole) <0.0009	Minimal inhibitory concentration >100 µg/well in all strains with and without S9	Deflora et al., 1984
	Bacillus subtilis (rec)	Rat liver S9 (Aroclor)	Negative	Tested at 62 µg/well	McCarroll et al., 1981
Mutation	S. typhimurium (reverse mutation, his)	Rat liver S9 (Aroclor or 3-methylcholanthrene)	Negative	Tested up to 1000 µg/plate in strains TA1535, TA1537, TA1538, TA98 and TA100	McCann et al., 1975; Simmon, 1979b; La Voie et al., 1979; Salamone et al., 1979; Ho et al., 1981; Florin et al., 1980

TABLE V-24 (cont.)

Test	Organism (Assay)	Exogenous Activation[a] System	Results Reported	Comments	Reference
Mutation (cont.)	S. typhimurium (reverse mutation, his)	Hamster or rat liver S9 (Aroclor)	Positive	In strain TA100 from 50-400 µg/plate	Carver et al., 1986
		^{60}Co gamma irradiation of anthracene	Negative	In strains TA98, TA1535, TA1537 and TA1538	Gibson et al., 1978
		Rat liver S9 (Aroclor)	Negative	In strain TA1537 at 0.22 mM	Seixas et al., 1982
		Rat liver S9 (Aroclor)	Negative	Tested in TA1535, TA1537, TA1538, TA95 and TA100	Deflora et al., 1984
		Rat liver S9 (Aroclor)	Positive	TA97 at 5 and 10 µg/plate	Sakai et al., 1985
		Rat liver S9 (Aroclor)	Negative	Tested in TA98 and TA100 at up to 200 µg/plate	La Voie et al., 1985
	S. typhimurium (reverse mutation, his) (taped-plate assay)	Rat liver S9 (Aroclor)	Negative	Tested up to 50 µg/plate in strains TA98 and TA100	Bos et al., 1988
	S. typhimurium (forward mutation, 8AG^R)	Rat liver S9 (Aroclor or phenobarbitol)	Negative	Tested up to 225 nmol/mL	Kaden et al., 1979
	S. typhimurium (forward mutation, ARA^R)	Rat liver S9 (Aroclor)	Positive	In strain BA9 and 0.5-3 µmol	Dorado and Pueyo, 1988
FUNGI					
Recombination	Saccharomyces cerevisiae (mitotic recombination, D3)	Rat liver S9 (Aroclor)	Negative	None	Simmon, 1979a
MAMMALIAN CELLS					
DNA damage	Primary rat hepatocytes (unscheduled DNA synthesis)	None	Negative	Tested up to 1 µg/mL	Williams, 1977; Probst et al., 1981
	HeLa cells (unscheduled DNA synthesis)	Rat liver S9 (phenobarbital or 3-methylcholanthrene)	Negative	Tested up to 100 µg/mL	Martin et al., 1978; Martin and McDermid, 1981

TABLE V-24 (cont.)

Test	Organism (Assay)	Exogenous Activation* System	Results Reported	Comments	Reference
DNA damage (cont.)	F344 rat tracea epithelial organ culture (unscheduled DNA synthesis)	None	Negative	Tested from 0.1×10^{-4} to 10×10^{-4} M	Ide et al., 1981
	Chinese hamster ovary cells (DNA synthesis)	None	Negative	Tested 1000 µg/mL	Garrett and Lewtas, 1983
	Primary rat hepatocytes (unscheduled DNA synthesis)	None	Negative	Tested at 1×10^{-3} M	Williams et al., 1989
Mutation	Chinese hamster V79 cells (forward mutation, $6TG^R$)	Rat liver S9 (?)	Negative	Tested up to 125 µg/mL	Knapp et al., 1981
	Mouse lymphoma L5178Y cells (forward mutation, TFT^R)	Rat liver S9 (Aroclor)	Negative	Tested up to 100 nmol/mL	Amacher and Turner, 1980; Amacher et al., 1980
	Human lymphoblastoid 1K6 cells (forward mutation, TFT^R)	Rat liver S9 (Aroclor)	Negative	Tested up to 200 nmol/mL	Barfknecht et al., 1981
	Rat liver epithelial cell line ARL18 (forward mutation $6TG^R$)	None	Negative	Tested at 10^{-3} and 10^{-4}	Ved Brat et al., 1983
	Chinese hamster V79 cells (forward mutations; OUA^R, $6TG^R$)	Liver and lung cell mediated systems for OUA^R, kidney and bladder cell mediated systems for $6TG^R$	Negative	Tested at 3 µg/mL	Langenbach et al., 1983
Chromosome effects	Mouse lymphoma L5178Y cells (forward mutation, TK^-)	None	Negative	Up to 500 µg/mL	Mitchell et al., 1988
		Rat liver S9 (Aroclor)	Positive	Tested at 6-15 µg/mL	
	Chinese hamster ovary cells (forward mutation, HGPRT)	Rat liver S9 (Aroclor)	Negative	Tested at 3.5 µg/mL	Oshiro et al., 1988
	Chinese hamster D6 cells (sister-chromatid exchange; breaks)	None	Negative	Tested up to 1 µmol/mL	Abe and Sasaki, 1977
	Rat liver epithelial ARL-18 cells (sister-chromatid exchange)	None	Negative	Tested up to 1 µmol/mL	Tong et al., 1981b

TABLE V-24 (cont.)

Test	Organism (Assay)	Exogenous Activation* System	Results Reported	Comments	Reference
Chromosome effects (cont.)	Rat liver epithelial cell line ARL18 (sister-chromatid exchange)	None	Negative	Tested at 10^{-3} and 10^{-4} M	Ved Brat et al., 1983
	Chinese hamster bone marrow cells (*in vivo* sister-chromatid exchange)	None	Negative	450 mg/kg was injected 48 and 24 hours prior to test	Roszinsky-Köcher et al., 1979
	Rat hepatocytes (DNA single strand breaks)	None	Negative	Tested at 0.003, 0.03 and 0.3 mM	Sina et al., 1983
	Mouse lymphoma L5178Y/TK+/- cells (DNA strand unwinding assay)	Rat liver S9 (Aroclor)	Positive	Tested at 4.74×10^{-5} M to 9.48×10^{-5} M	Garberg et al., 1988
	Human peripheral lymphocytes (sister-chromatid exchange)	Intact rat hepatocytes	Negative	Tested at 10^{-4} to 10^{-5} M	Lindahl-Kiessling et al., 1989
		None	Negative	Tested at 10^{-4} to 10^{-6} M	
Cell transformation	Mouse BALB/3T3 cells (morphologic changes)	None	Negative	Tested at 10 µg/mL	DiPaolo et al., 1972
	Guinea pig fetal cells (morphologic changes)	None	Negative	Tested at 0.5 µg/mL	Evans and DiPaolo, 1975
	Syrian hamster embryo cells (morphologic changes)	None	Negative	Tested up to 50 µg/mL	Pienta et al., 1977
	C3H/10T1/2 cells mouse embryo fibroblast (morphologic changes)	None	Negative	Tested at 4 µM	Peterson et al., 1981
	Mouse embryo C3H10T1/2 cells (morphologic changes)	None	Negative	Tested up to 30 µg/mL	Lubet et al., 1983b
BACTERIA			BENZ[a]ANTHRACENE		
DNA damage	*E. coli* (*polA*)	Rat liver S9 (none)	Negative	Tested up to 250 µg/mL	Rosenkrantz and Poirier, 1979
	E. coli (*uvrA*, *recA*, *lexA*, *polA*)	Rat liver S9 (Aroclor)	Negative. Potency (Δ minimal inhibitory concentration/ nmol) >0.0001	Minimal inhibitory concentration >1000 µg/well with and without S9 in all strains	DeFlora et al., 1984

TABLE V-24 (cont.)

Test	Organism (Assay)	Exogenous Activation* System	Results Reported	Comments	Reference
Mutation	E. coli (expression of lac Z gene under control of SOS gene, sf1A)	Rat liver S9 (Aroclor)	Positive	In strain PQ37 up to 100 mM	von der Hude et al., 1988
	S. typhimurium (expression of lac Z gene under control of umu gene)	Rat liver S9 (Aroclor)	Weakly positive	Tested at 2.4 µg/ml	Nakamura et al., 1987
	S. typhimurium (reverse mutation, his)	Rat liver S9 (Aroclor)	Positive	20 µg/plate in strain TA100	McCann et al., 1975; Coombs et al., 1976; Simmon, 1979b; Salamone et al., 1979
			Negative	Strain TA1535	
		^{60}Co gamma irradiation of benz(a)anthracene	Positive	In strains TA98, TA1537 and TA1538 at 250 µg/plate	Gibson et al., 1978
			Negative	Tested in strain TA1535 up to 250 µg/plate	
		Rat liver S9 (methylcholanthrene)	Positive	In strains TA98 and TA100 up to 3 µmoles/plate	Florin et al., 1980
			Positive	Strains TA97, TA98, TA100, TA1537, TA1538. Potency (revertants/nmole)=12	Deflora et al., 1984
		Rat liver S9 (fluoranthene; benzo[e]pyrene; phenanthrene; pyrene; benzo[k]-fluoranthene; benzo[g,h,i]-perylene; anthanthrene; coronene; chrysene; benzo-[j]fluoranthene; benzo[b]-fluoranthene; indeno-[1,2,3-cd]pyrene; dibenz-[a,h]anthracene; clophen A50; benzo(c)phenanthrene, benzo[b]naphthal[2,3-d]-thiophene; benzo[b]-naphthol[1,2-d]thiophene; triphenylene; benzo[b]naph-thol[2,1-d]theophene)	Positive when rats were treated with benzo[j]fluor-anthene, clophen A50, benzo[b]fluor-anthene, indeno-[1,2,3-cd]pyrene, dibenz[a,h]-anthracene	In the presence of various liver S9 preparations induced by different PAHs, the weak carcinogen benz[a]anthracene exhibited mutagenic activities comparable to those of benzo-[a]pyrene in strain TA100	Norpoth et al., 1984

TABLE V-24 (cont.)

Test	Organism (Assay)	Exogenous Activation* System	Results Reported	Comments	Reference
Mutation (cont.)	S. typhimurium (reverse mutation, his)	Rat liver S9 (3-methylcholanthrene)	Positive	0.07 µmol/plate in strain TA100	Bartsch et al., 1980
		Rat hepatocytes intact and homogenized (Aroclor)	Positive	Suspension incubation TA100 weaker response with intact cells	Utesch et al., 1987
		Rat liver S9 (Aroclor)	Positive	In strains TA98 and TA100 at 50 µg/plate	Bos et al., 1988
		Rat liver S9 (polychlorinated biphenyl or phenobarbital)	Positive	In strains TA98 and TA100 at 50 µg/plate	Ito et al., 1988
		Hamster liver S9 (Aroclor)	Positive	In strain TA100 at 10-50 µg/plate	Phillpson and Ionnides, 1989
		Mouse, rat or pig S9 (Aroclor)	Negative	In strain TA100 at 10-50 µg/plate	
	S. typhimurium (reverse mutation, his) (taped-plate assay)	Rat liver S9 (Aroclor)	Negative	In strains TA98 and TA100 at 50 µg/plate	Bos et al., 1988
	S. typhimurium (forward mutation, BAG^R)	Rat liver S9 (Aroclor or phenobarbital)	Positive	65 nmol/ml in strain TM677	Kaden et al., 1979
FUNGI					
Recombination	S. cerevisiae (mitotic recombination, D3)	Rat liver S9 (Aroclor)	Negative	None	Simmon, 1979a
INSECTS					
Mutation	Drosophila melanogaster (lethals, visibles, bobbed mutants)	None	Positive	Administered by microinjection	Fahmy and Fahmy, 1973
MAMMALIAN CELLS					
DNA damage	Chinese hamster ovary cells (DNA synthesis)	None	Negative	Tested 1000 µg/ml	Garrett and Lewtas, 1983
	Primary rat hepatocytes (unscheduled DNA synthesis)	None	Positive	100 nmol/ml	Probst et al., 1981

TABLE V-24 (cont.)

Test	Organism (Assay)	Exogenous Activation* System	Results Reported	Comments	Reference
DNA damage (cont.)	HeLa cells (unscheduled DNA synthesis)	Rat liver S9 (3-methylcholanthrene)	Positive	Up to 100 pmol/mL	Martin et al., 1978
	Primary F344 rat tracheal epithelial organ culture (unscheduled DNA synthesis)	None	Negative	Tested at 0.1×10^{-4} to 10×10^{-4} M	Ide et al., 1981
	Primary F344 rat hepatocytes (unscheduled DNA synthesis)	None	Positive	Tested at 1×10^{-4} M	Williams et al., 1989
Mutation	Chinese hamster V79 cells (forward mutation, OUA^R)	Syrian hamster embryo cell feeder layer	Positive	44 nmol/mL	Slaga et al., 1978
	Chinese hamster V79 cells (forward mutation, $6TG^R$)	Rat liver S9 (3-methylcholanthrene)	Positive	46 nmol/mL	Krahn and Heidelberger, 1977
	Mouse lymphoma L5178Y cells (forward mutation, TFT^R)	Various	Positive	Small increase at 40 nmol/mL	Amacher et al. 1980; Amacher and Turner, 1980
	Rat liver epithelial cells (forward mutation, $6TG^R$)	None	Negative	Up to 100 nmol/mL	Tong et al., 1981a
Chromosomal effects	Chinese hamster ovary cells (sister-chromatid exchange)	None	Positive	2 µg/mL	Pal, 1981
	Simian virus transformed Chinese hamster ovary cells (selective DNA amplification)	None	Negative	Tested at 0.14 µmole	Pool et al., 1989
	Bone marrow cells of Long-Evans rats (chromosomal aberrations)	None	Negative	In vivo, rats received 25, 50 or 100 mg/kg	Ito et al., 1988
Cell transformation	Syrian hamster embryo cells (morphologic changes)	None	Positive	0.1 µg/mL 10 µg/mL	Pienta et al., 1977; DiPaolo et al., 1969, 1971
	Mouse prostate C3HG23 cells (morphologic changes)	None	Positive	1 µg/mL	Marquardt and Heidelberger, 1972

TABLE V-24 (cont.)

Test	Organism (Assay)	Exogenous Activation* System	Results Reported	Comments	Reference
Cell transformation (cont.)	Mouse C3H/10T1/2 cells (morphologic changes)	None	Negative	Tested up to 100 nmol/ml	Nesnow and Heidelberger, 1976
BACTERIA			BENZO[a]PYRENE		
DNA damage	E. coli (uvrA, recA, lexA, polA)	Rat liver S9 (Aroclor)	Negative. Potency (a minimal inhibition concentration/ nmole) <0.0002	Minimal inhibitory concentration >800 µg/well in all strains with and without S9	Deflora et al., 1984
	E. coli (expression of lac Z gene under control of SOS gene, sfiA)	Rat liver S9 (Aroclor)	Positive	In strain PQ37 up to 100 mM	von der Hude, et al., 1988
	S. typhimurium (expression of lac Z gene under control of umu C gene)	Rat liver S9 (Aroclor)	Positive	Tested at 1 µg/ml	Nakamura et al., 1987
Mutation	S. typhimurium (reverse mutation, his)	Rat liver S9 (Aroclor)	Positive	In strain TA100 up to 1000 µg/plate	Andrews et al., 1978
		^{60}Co gamma irradiation of benzo(a)pyrene	Positive	In strains TA98, TA1537 and TA1538 from 50-100 µg/plate	Gibson et al., 1978
		^{60}Co gamma irradiation of benzo(a)pyrene	Negative	In strain TA1535 up to 100 µg/plate	
		Rat liver S9 (Aroclor)	Negative Positive	Strain TA1535 Strain TA97, TA98, TA100, TA1537, TA1538. Potency (revertants/nmole) = 185	Deflora et al., 1984
		Rat liver S9 (Aroclor)	Positive	Tested at 2.5 µg/plate in strains TA98 and TA100. Positive only with metabolic activation	Lofroth et al., 1984
		Rat or hamster liver S9 (Aroclor)	Positive	Tested at 8 µM in strain TA100. Selenium at nontoxic concentrations inhibited the mutagenicity of benzo[a]pyrene in both the rat liver and hamster liver systems	Teel, 1984

TABLE V-24 (cont.)

Test	Organism (Assay)	Exogenous Activation* System	Results Reported	Comments	Reference
Mutation (cont.)	S. typhimurium (reverse mutation, his)	Rat liver S9 (Aroclor)	Positive	Strains TA97, TA98 and TA100 positive at 1 µg/plate	Sakai et al., 1985
		Rat hepatocytes intact and homogenized (Aroclor)	Positive	Suspension incubation; strain TA100; weaker response with intact cells	Utesch et al., 1987
	S. typhimurium (forward mutation, 8AG^R)	Rat liver S9 (Aroclor)	Positive	In strain TM677 at 4 µM	Kaden et al., 1979
	S. typhimurium (forward mutation, ARA^R)	Rat liver S9 (Aroclor)	Positive	In strain BA13 at a minimum dose of 0.77 nMol	Dorado and Pueyo, 1988
FUNGI					
Recombination	S. cerevisiae (mitotic recombination, D3)	Liver microsomes (Aroclor)	Negative	None	Simmon, 1979a
INSECTS					
Chromosomal effects	D. melanogaster (whole chromosome gain)	None	Negative	Larvae and adult flies exposed in feed. Oocytes and oogonia examined for chromosome gain	Fabian and Matoltsy, 1946
Mutagenicity	D. melanogaster (sex linked recessive lethal mutation)	None	Negative	Fed at 2500 and 50,000 ppm	Valencia et al., 1989
PLANTS					
Chromosome damage	Tradescantions clone 4430 (tetrad analysis)	None	Positive	Minimum effect level 12.6 ppm	Sandhu et al., 1989
MAMMALIAN CELLS					
DNA damage	F344 rat trachea epithelial organ culture (unscheduled DNA synthesis)	None	Positive	Tested at concentrations that ranged from 0.1×10^{-6} to 10×10^{-6} M	Ide et al., 1981
	Primary BALB/C mouse epidermal cells benzo[a]-pyrene-DNA adduct formation)	None	Positive	Increase in benzo[a]pyrene-DNA adduct formation with PAH concentrations in range of 50-250 nmol	Nakayama et al., 1984

TABLE V-24 (cont.)

Test	Organism (Assay)	Exogenous Activation[a] System	Results Reported	Comments	Reference
DNA damage (cont.)	Chinese hamster ovary cells (DNA single strand breaks)	Adult rat hepatocytes	Positive	Dose and exposure time dependent increases noted at $1x10^{-4}$ and $5x10^{-5}$ M	Yang et al., 1984
	Permeabilized human diploid fibroblasts (to which E. coli DNA polymerase was added) (DNA strand breaks)	None	Negative	Tested at 1 μM for 30 minutes	Snyder and Matheson, 1985
	Primary rat hepatocytes (unscheduled DNA synthesis)	None	Positive	Tested at $1x10^{-4}$ M	Williams et al., 1989
	Mouse lymphoma L5178/TK+/- cells (DNA strand-unwinding assay)	Rat liver S9 (Aroclor)	Positive	Tested at $5x10^{-4}$ to $50x10^{-4}$ M	Garberg et al., 1988
Mutation	Chinese hamster K_1-BH_4 cells (forward mutation, $6TG^R$)	Rat liver S9 (Aroclor)	Positive	Tested up to 6 μg/mL	Recio and Hsie, 1984
	Chinese hamster ovary cells (forward mutation, HGPRT)	Rat liver S9 (Aroclor)	Positive	Tested at 0.5-4.0 μg/mL	Oshiro et al., 1988
	Mouse lymphoma L5178Y (forward mutation, TK^-)	Rat liver S9 (Aroclor)	Positive	Tested at $5x10^{-4}$ to $10x10^{-4}$ mol/L	Wangenheim and Bolcsfoldi, 1988
	Mouse lymphoma L5178 cells (forward mutation, TK^-)	Rat liver S9 (Aroclor)	Positive	Tested from 3.2-10 μg/mL	Clay and Cross, 1990
Chromosomal effects	Mouse embryo cells C3H/10T1/2 (DNA single strand breaks)	None	Negative	Tested up to 10 μg/mL	Tubet et al., 1983b
	Rat liver epithelial cells RL-12 (sister-chromatid exchange)	None	Positive	Tested at $1.6x10^{-9}$ M to $1.6x10^{-7}$ M. Cell line can activate procarcinogen to genotoxic form	Murison et al., 1984
	Human peripheral lymphocytes (sister-chromatid exchange)	Intact rat hepatocytes	Positive	Tested at 10^{-4} to 10^{-5} M	Lindahl-Kiessling et al., 1989
		None	Positive	Tested at 10^{-4} to 10^{-6} M	
	Chinese hamster epithelial liver cells (sister-chromatid exchange)	None	Positive	Tested at 0.11-1 μg/mL	DeSalvia et al., 1988

TABLE V 24 (cont.)

Test	Organism (Assay)	Exogenous Activation[a] System	Results Reported	Comments	Reference
Chromosomal effects (cont.)	Simian virus transformed Chinese hamster ovary cells (selective DNA amplification)	None	Positive	Tested at 0.01 µmol	Pool et al., 1989
Cell transformation	Mouse BalbC/3T3 (morphologic changes)	None	Positive	At 10 and 20 µg/mL	DiPaolo et al., 1972
	C3H/10T1/2 mouse embryo fibroblast cells (morphologic changes)	None	Positive	Tested at 4 µM	Peterson et al., 1981
	Syrian hamster embryo cells (morphologic changes)	None	Positive	Tested at 1-5 µg/mL	Tu et al., 1986
BACTERIA			**BENZO[b]FLUORANTHENE**		
Mutation	S. typhimurium (reverse mutation, his)	Rat liver S9 (Aroclor)	Negative	Tested up to 100 µg/mL in strain TA100	Mossanda et al., 1979
		Rat liver S9 (Aroclor)	Positive	At 100 µg/plate in strain TA100. 7 nmol/plate in strain TA98, 0.5 µmol in TA100	LaVoie et al., 1979; Hermann, 1981; Amin et al., 1985b
MAMMALIAN CELLS					
Mutation	DPI-3 epithelial cell line (6-TG)	No data.	Negative	Tested up to 4 µM	Dooley et al., 1981
BACTERIA			**BENZO[k]FLUORANTHENE**		
Mutation	S. typhimurium (reverse mutation, his)	Rat liver S9 (Aroclor)	Positive	Tested up to 100 µg/plate in strain TA100	LaVoie et al., 1980
		Rat liver S9 (Aroclor)	Positive	5 µg/plate in strain TA98	Hermann et al., 1980
		Rat liver S9 (Aroclor)	Positive	From 10-50 µg/plate in strain TA100	Weyand et al., 1988
BACTERIA			**BENZO[g,h,i]PERYLENE**		
Mutation	S. typhimurium (reverse mutation, his)	Rat liver S9 (Aroclor)	Positive	At 20 µg/plate in strain TA100	Andrews et al., 1978; Mossanda et al., 1979

TABLE V-24 (cont.)

Test	Organism (Assay)	Exogenous Activation* System	Results Reported	Comments	Reference
Mutation (cont.)	S. typhimurium (reverse mutation, his)	Rat liver S9 (Aroclor)	Positive	In range 0.1-1000 µg/plate in strain TA98	Salamone et al., 1979
		^{60}Co gamma irradiation of benzo(g,h,i)pyrene	Positive	In strains TA98, TA1537 and TA1538 at 400 µg/plate	Gibson et al., 1978
		None	Negative	In strain TA1535 at concentrations up to 800 µg/plate	
		Rat liver S9 (Aroclor)	Positive	At 10 µg/plate in TA97 and TA98	Sakai et al., 1985
	S. typhimurium (forward mutation, BAG^R)	Rat liver S9 (Aroclor)	Positive	At 72 nmol/ml in strain TM677	Kaden et al., 1979
MAMMALIAN CELLS					
DNA damage	Chinese hamster ovary cells (DNA synthesis)	None noted	Positive	At 1000 µg/ml DNA synthesis at 3% of controls	Garrett and Lewtas, 1983
BACTERIA			CHRYSENE		
DNA damage	E. coli (polA)	Rat liver S9 (none)	Negative	Tested up to 250 µg/ml	Rosenkrantz and Poirier, 1979
			Negative	None	Leifer et al., 1981
	S. typhimurium (expression of lac Z gene under control of umu C gene)	Rat liver S9 (Aroclor)	Positive	Tested at 15 µg/ml	Nakamura et al., 1987
Mutation	S. typhimurium (reverse mutation, his)	Rat liver S9 (Aroclor)	Positive	At 10 µg/plate in strain TA100	McCann et al., 1975; LaVoie et al., 1979
		Rat liver microsomes (Aroclor)	Positive	At 125 nmol/plate in strain TA100; dose-response seen with increasing concentrations of microsomes	Wood et al., 1977
			Positive	None	Dunkel and Simmon, 1980
			Positive	None	Ishikwa et al., 1977

TABLE V-24 (cont.)

Test	Organism (Assay)	Exogenous Activation* System	Results Reported	Comments	Reference
Mutation (cont.)	S. typhimurium (reverse mutation, his)	Rat liver S9 (3-methylcholanthrene)	Positive	At 1.5 µmol/plate in strain TA100	Florin et al., 1980
			Negative	At 1.5 µmol/plate in strain TA98	
		Rat liver S9 (Aroclor)	Positive	Identified in highly mutagenic fraction of raw gas condensate. Mutagenicity assessed in strain TA98	Benson et al., 1984
		Rat liver S9 (Aroclor)	Positive	At 5 µg/plate, strains TA97, TA98 and TA100	Sakai et al., 1985
		Hamster and rat S9 (Aroclor)	Positive	In strain TA100 at 5 µg/plate	Carver et al., 1986
		None	Negative	In strains TA98 and TA100	Glatt et al., 1986
		Rat liver S9 (Aroclor)	Positive	In strain TA100 at 100 µg/plate	Glatt et al., 1986
			Negative	In strain TA98 at 100 µg/plate	
		Rat liver S9 (Aroclor)	Positive	In strains TA98 and TA100 at 50 µg/plate	Bos et al., 1988
		^{60}Co gamma irradiation in air	Positive	In strains TA98, TA1537 and TA1538 at 1000 µg/plate	Gibson et al., 1978
			Negative	In strain TA1535 up to 1000 µg/plate	
	S. typhimurium (reverse mutation, his) (taped-plate assay)	Rat liver S9 (Aroclor)	Negative	In strains TA98 and TA100 at 50 µg/plate	Bos et al., 1988
	S. typhimurium (forward mutation, 8AG^R)	Rat liver S9 (Aroclor)	Positive	At 45 nmol/mℓ in strain TM677	Kaden et al., 1979
FUNGI					
Recombination	S. cerevisiae (mitotic recombination, D3)	Rat liver S9 (Aroclor)	Negative	None	Simmon, 1979a

TABLE V-24 (cont.)

Test	Organism (Assay)	Exogenous Activation* System	Results Reported	Comments	Reference
MAMMALIAN CELLS					
DNA damage	Primary rat hepatocytes (unscheduled DNA synthesis)	None	Negative	Tested up to 100 nmol/mℓ	Tong et al., 1981a
	F344 rat trachea epithelial organ culture (unscheduled DNA synthesis)	None	Negative	Tested from 0.1×10^{-6} to 10×10^{-6} M	[illegible] et al.,
	Primary rat hepatocytes (unscheduled DNA synthesis)	None	Negative	Tested at concentrations up to 1×10^{-4} M	Williams et al., 1989
Mutation	Chinese hamster V79 cells (forward mutation OUA^R, $8AG^R$)	Syrian hamster embryo cell feeder layer	Negative	Tested up to 10 µg/mℓ	Huberman and Sachs, 1976
	V-79 Chinese hamster cells (forward mutation, $6\text{-}TG^R$)	Rat liver S9	Negative	Tested from 2.5-80 µg/mℓ	Glatt et al., 1986
		None	Negative	Tested from 2.5-80 µg/mℓ	
Chromosomal effects	Chinese hamster cells (sister chromatid exchange)	in vivo (gavage)	Positive	900 mg/kg	Roszinsky-Kocher et al., 1979
	Mouse oocytes (chromosome aberrations)	in vivo (gavage) (phenobarbital)	Positive	Weak positive at 450 mg/kg	Basler et al., 1977
	Hamster spermatogonia (chromosome aberrations)	in vivo (gavage) (phenobarbital)	Positive	Increase not significant at total dose of 900 mg/kg	
	Hamster bone marrow cells	in vivo (gavage) (phenobarbital)	Negative		
Cell transformation	Syrian hamster embryo cells (morphologic changes)	None	Positive	Tested at 10µg/mℓ	Pienta et al., 1977
	Mouse prostate C3HG23 cells (morphologic changes)	None	Negative	Tested up to 10 µg/mℓ	Marquardt and Heidelberger, 1972
	Mouse prostate M2 cells (morphologic changes)	Tested both with and without rat liver	Negative	Tested from 10-40 µg/mℓ	Glatt et al., 1986

TABLE V-24 (cont.)

Test	Organism (Assay)	Exogenous Activation* System	Results Reported	Comments	Reference
BACTERIA		DIBENZ[a,h]ANTHRACENE			
DNA damage	E. coli (recA)	Rat liver S9 (Aroclor)	Positive	At 25 µg/well	Ichinotsubo et al., 1977
	B. subtilis (recA)	Rat liver S9 (Aroclor)	Positive	Minimal inhibitory concentration for rec$^+$ 50 µg/well; for rec$^-$ 12 µg/well	McCarroll et al., 1981
Mutation	S. typhimurium (reverse mutation, his)	Rat or guinea pig liver (Aroclor) or (3-methylcholanthrene) rat liver S9	Positive	At 5 µg/plate in strain TA100	McCann et al., 1975; Andrews et al., 1978
		Rat liver S9 (Aroclor)	Positive	At 12 nmol/plate in strain TA98	Baker et al., 1980; Hermann, 1981
		Rat liver S9 (Aroclor)	Positive	In strain TA100 up to 1000 µg/plate	Andrews et al., 1978
		^{60}Co gamma irradiation of dibenz[a,h]anthracene	Negative	In strains TA98, TA1535 and TA1537	Gibson and Smith, 1979
		Rat, hamster, pig or human S9 (Aroclor)	Positive	In strain TA100 at 10-50 µg/plate	Phillipson and Ionnides, 1989
	S. typhimurium (forward mutation, 8AG^R)	Rat liver S9 (Aroclor)	Positive	At 75 nmol/mL in strain TM677	Kaden et al., 1979
PLANTS					
Chromosome damage	Tradescantia clone 4430 (tetrad analysis)	None	Positive	Minimum effective concentration was 12.5 ppm	Sandhu et al., 1989
MAMMALIAN CELLS					
DNA damage	Human foreskin epithelial cells (unscheduled DNA synthesis)	None	Positive	In range 1-100 µg/mL	Lake et al., 1978
	HeLa cells (unscheduled DNA synthesis)	3-methylcholanthrene	Positive	At 100 pmol/mL	Martin et al., 1978
	Syrian hamster embryo cells (unscheduled DNA synthesis)	None	Negative	Tested up to 20 µg/mL	Casto, 1979

TABLE V-24 (cont.)

Test	Organism (Assay)	Exogenous Activation* System	Results Reported	Comments	Reference
DNA damage (cont.)	Primary rat hepatocytes (unscheduled DNA synthesis)	None	Negative	Tested up to 100 nmol/ml	Probst et al., 1981
	F344 rat trachea epithelial organ culture (unscheduled DNA synthesis)	None	Negative	Tested from 0.1×10^{-6} to 10×10^{-6} M	Ide et al., 1981
Mutation	Chinese hamster V79 cells (forward mutation, OUA^R, BAG^R)	Syrian hamster embryo cell feeder layer	Positive	At 1 µg/ml	Huberman and Sachs, 1976; Huberman, 1978
	Chinese hamster V79 cells (forward mutation, $6TG^R$)	Rat liver S9 (3-methylcholanthrene)	Positive	At 56 nmol/ml	Krahn and Heidelberger, 1977
Cell transformation	Syrian hamster embryo cells (morphologic changes)	None	Positive	At 0.5-10 µg/ml	DiPaolo et al., 1969; Pienta et al., 1977; Casto et al., 1977; Casto, 1979
	Mouse C3H10T1/2 cells (morphologic changes)	None	Positive	At 20 µg/ml	Reznikoff et al., 1973
		None	Weakly positive	At 10 and 30 µg/ml	Lubet et al., 1983b
	Mouse prostate C3H cells (morphologic changes)	None	Positive	At 10 µg/ml	Chen and Heidelberger, 1969
	Mouse prostate C3HG23 cells (morphologic changes)	None	Negative	Tested up to 10 µg/ml	Marquardt and Heidelberger, 1972
BACTERIA			FLUORANTHENE		
DNA damage	B. subtilis (rec A)	With or without rat liver S9 (polychlorinated biphenyl)	Negative	At 10 mg/well	Kinae et al., 1981
	S. typhimurium (expression of lac Z gene under control of umu C gene)	Rat liver S9 (Aroclor)	Negative	Tested up to 167 µg/ml	Nakamura et al., 1987

TABLE V-24 (cont.)

Test	Organism (Assay)	Exogenous Activation* System	Results Reported	Comments	Reference
Mutation	S. typhimurium (reverse mutation, his)	Rat liver S9 (Aroclor)	Negative	Unknown concentration in airborne particulate materials (strain TA98)	Tokiwa et al., 1977
		Rat liver S9 (Aroclor)	Negative	Strains TA98 and TA100	LaVoie et al., 1979
		Rat liver S9 (Aroclor)	Weakly positive	Unknown concentration in strain TA98	Epler et al., 1978
		Rat liver S9 (Aroclor)	Negative	Tested up to 1000 µg/plate in strains TA98, TA100, TA1535, TA1537 and TA1538	Salamone et al., 1979
		Rat liver S9 (Aroclor)	Positive	At 5 µg/plate in strain TA98	Hermann et al., 1980
		With or without rat liver S9 (polychlorinated biphenyl)	Negative	Strains TA98 and TA1537 at 100 µg/plate	Kinae et al., 1981
	S. typhimurium (reverse mutation, his)	With or without rat liver S9 (polychlorinated biphenyl)	Positive	Strain TA100 at 100 µg/plate	Kinae et al., 1981
		Rat liver S9 (Aroclor)	Positive	Tested up to 200 µg/plate in strain TA100	LaVoie et al., 1982b
		Rat liver S9 (3-methylcholanthrene)	Negative	Tested in strains TA98 and TA100 at 3 µmol/plate	Florin et al., 1980
	S. typhimurium (reverse mutation, his) (taped-plate assay)	Rat liver S9 (Aroclor)	Positive	Tested up to 500 nmol/ml in strain TA97	Hera and Pueyo, 1988
		Rat liver S9 (Aroclor)	Positive	Tested up to 100 µg/bottom plate in strains TA98 and TA100. Results negative without the addition of S9 mix.	Bos et al., 1988
	S. typhimurium (forward mutation, 8AG^R)	Rat liver S9 (Aroclor)	Positive	Lowest concentration to show a significant mutation rate was 5 µM. Not mutagenic at up to 50 µM without the addition of S9 mix. Strain TM677	Rastetter et al., 1982

TABLE V-24 (cont.)

Test	Organism (Assay)	Exogenous Activation* System	Results Reported	Comments	Reference
Mutation (cont.)	S. typhimurium (forward mutation, $8AG^R$)	Rat liver S9 (Aroclor)	Positive	Strain TM677 at 1 µg/ml	Kaden et al., 1979
		Rat liver S9 (Aroclor)	Positive	Tested up to 10 µg/ml in strain TM677	Babson et al., 1986
	S. typhimurium (forward mutation, Ara^R)	Rat liver S9 (Aroclor)	Positive	Tested up to 500 nmol/ml in strain BA9	Hera and Pueyo, 1988
MAMMALIAN CELLS					
Mutation	Human lymphoblast cell line AHH-1 (forward mutation to $6GT^R$)	MFO induced by treatment with β-naphthaflavone	Negative	Tested at 10-100 µM, 48-hour exposure	Crespi and Thilly, 1984
	Chinese hamster ovary cells (differential cytotoxicity of repair-deficient strains)	Rat liver S9 (Aroclor)	Positive	Strains UV4 and UV5 deficient in nucleotide excision repair	Hoy et al., 1984
		Rat liver S9 (Aroclor)	Negative	Strain EM9 defective in DNA-strand-break rejoining	
	Chinese hamster ovary cell (forward mutation $6GI^R$)	Rat liver S9 (Aroclor)	Weakly positive	Tested at 20 µg/ml	Li, 1984
Chromosomal effects	Chinese hamster ovary cells (sister chromatid exchange)	Rat liver S9 (Aroclor)	Positive	Tested at 9, 18 and 36 µg/ml	Palitti et al., 1986
	Chinese hamster epithelial cell strain	None (using metabolically competent liver cells)	Negative	Tested at 25, 35 and 45 µg/ml	DeSalvia et al., 1988
BACTERIA			FLUORENE		
DNA damage	S. typhimurium (expression of lac Z gene under control of umu C gene)	Rat liver S9 (Aroclor)	Negative	Tested up to 16.7 µg/ml	Nakamura et al., 1987
Mutation	S. typhimurium (reverse mutation, his)	^{60}Co gamma irradiation of fluorene	Positive	In strains TA98 and TA1538 up to 1000 µg/plate	Gibson et al., 1978
			Negative	In strains TA1535 and TA1537 up to 1000 µg/plate	

TABLE V-24 (cont.)

Test	Organism (Assay)	Exogenous Activation[a] System	Results Reported	Comments	Reference
Mutation (cont.)	S. typhimurium (reverse mutation, his)	Rat liver S9 (Aroclor)	Negative	Tested up to 1000 µg/plate in strains TA1535, TA1537, TA98 and TA100	McCann et al., 1975; La Voie et al., 1979, 1981b
		Rat liver S9 (Aroclor)	Negative	Tested at up to 250 µg/plate strains TA97, TA98 and TA100	Sakai et al., 1985
		Rat liver S9 (3-MC or TCDD)	Negative	Tested up to 1504 nmol/plate in strain TA100	Pahlman and Pelkonen, 1987
		Rat liver S9 (Aroclor)	Negative	In strains TA98 and TA100 up to 50 µg/plate	Bos et al., 1988
	S. typhimurium (reverse mutation, his) (taped-plate assay)	Rat liver S9 (Aroclor)	Negative	In strains TA98 and TA100 up to 50 µg/plate	
	S. typhimurium (forward mutation, 8AG^R)	Rat liver S9 (Aroclor or phenobarbital)	Negative	Tested up to 300 nmol/mL in strain TM677	Kaden et al., 1979
MAMMALIAN CELLS					
DNA damage	Primary rat hepatocytes (unscheduled DNA synthesis)	None	Negative	Tested up to 10 nmol/mL	Probst et al., 1981
	Primary mouse or hamster hepatocytes (unscheduled DNA synthesis)	None	Negative	Tested up to 1 µM	McQueen et al., 1983
	Permeabilized human diploid fibroblasts and E. coli DNA polymerase (DNA strand breaks)	None	Negative	Tested at 3 mM for 2 hours	Snyder and Matheson, 1985
	Primary rat hepatocytes (unscheduled DNA synthesis)	None	Negative	Tested at $1x10^{-4}$ M	Williams et al., 1989
Mutation	Mouse L5178Y TK+/- lymphoma cells (forward mutation, TK$^-$)	Rat liver S9 (Aroclor)	Negative	Tested up to 30 µg/mL	Oberly et al., 1984
		None	Negative	Tested up to 60 µg/mL	
		Rat liver S9 (Aroclor)	Positive	Tested at $5.8x10^{-4}$ to $7.78x10^{-4}$ mol/L	Wangenheim and Bolcsfoldi, 1988
	Chinese hamster ovary cells (forward mutation, HGPRT)	Rat liver S9 (Aroclor)	Negative	Tested at 1-20 µg/mL	Oshiro et al., 1988

TABLE V-24 (cont.)

Test	Organism (Assay)	Exogenous Activation[a] System	Results Reported	Comments	Reference
CHROMOSOME EFFECTS	Mouse lymphoma cells (DNA strand breaks)	Rat liver S9 (Aroclor)	Positive	At 0.15 µM both with and without metabolic activation.	Garberg et al., 1988
	Chinese hamster cell line (chromosomal aberrations)	Liver S9 mix source unknown	Positive	25 µg/mℓ	Matsuoka et al., 1987
BACTERIA			INDENO[1,2,3-cd]PYRENE		
Mutation	S. typhimurium (reverse mutation, his)	Rat liver S9 (Aroclor)	Positive	At 20 µg/plate for TA100 and 2 µg/plate for TA98	LaVoie et al., 1979; Hermann et al., 1980
		Rat liver S9 (Aroclor)	Positive	At ~3 µg/plate TA100	Rice et al., 1985a
BACTERIA			NAPHTHALENE		
DNA damage	S. typhimurium (expression of lac Z gene under control of umu C gene)	Rat liver S9 (Aroclor)	Negative	Tested at 8.3 µg/mℓ	Nakamura et al., 1987
Mutation	S. typhimurium (reverse mutation, his)	With or without rat liver S9 (Aroclor)	Negative	Tested up to 1000 µg/plate in strains TA98, TA100, TA1535 and TA1537	McCann et al., 1975
		With or without rat liver S9	Negative	Tested up to 300 µg/plate in strains TA1535, TA1537, TA1538, TA98, TA100	Godek et al., 1985
		Rat liver S9 (3-methylcholanthrene)	Negative	Tested at 3 µmol/plate in strains TA98 and TA100	Florin et al., 1980
		Rat liver S9 (Aroclor)	Negative	Tested up to 1.6 mM in strain TA1537	Seixas et al., 1982
		With or without rat liver S9 (Aroclor)	Negative	Tested in two DNA-repair deficient strains, TA100 and TA98, and two strains which have full DNA repair capacity: UTH8414 and UTH8413	Connor et al., 1985
		Rat or hamster S9 (Aroclor)	Negative	Tested up to 10 mg/plate in strain TA100	Mortelmans et al., 1986
		Rat liver S9 (20-100 µℓ/plate) (Aroclor)	Negative	Tested up to 1000 µg/plate in TA98	Narbonne et al., 1987

TABLE V-24 (cont.)

Test	Organism (Assay)	Exogenous Activation* System	Results Reported	Comments	Reference
Mutation (cont.)	S. typhimurium (reverse mutation, his)	Rat liver S9 (20-100 µl/plate) (Aroclor)	Positive	Tested up to 1000 µg/plate in TA1535. Naphthalene gave >2 times the control value of revertants at 100 µg/plate with 20 µl S9/plate and at ≤50 µg/plate with 50 µl S9/plate	Narbonne et al., 1987
		With or without rat liver S9 (polychlorinated biphenyls)	Negative	Tested up to 250 µg/plate in strains TA97, TA98 and TA100	Sakai et al., 1985
		Rat liver S9 (Aroclor)	Weakly positive	Tested at up to 100 µg/bottom plate in strains TA97, TA102 and TA1537. Negative results without S9	Bos et al., 1987
		With or without rat liver S9 (Aroclor)	Negative	Tested at up to 100 µg/bottom plate in strain TA1535	Bos et al., 1987
		Rat liver S9 (Aroclor)	Negative	Tested up to 50 µg/plate in strains TA98 and TA100	Bos et al., 1988
		Rat liver S9 (Aroclor)	Negative	Tested in strains TA98, TA100, TA1535 and TA1537 with and without S9 at up to 100 µg/plate	NTP, 1991b
	S. typhimurium (reverse mutation, his) (taped-plate assay)	Rat liver S9 (Aroclor)	Positive	Tested at up to 100 µg/bottom plate in strains TA98 and TA100. Negative results without S9	Bos et al., 1987
		Rat liver S9 (Aroclor)	Negative	Tested up to 50 µg/plate in strains TA98 and TA100	Bos et al., 1988
MAMMALIAN CELLS					
DNA damage	Rat hepatocytes (unscheduled DNA synthesis)	None	Negative	Tested up to 16 µg/ml (0.32 µg). Concentrations >16 µg/ml were extremely cytotoxic.	Barfknecht et al., 1985
	Bone marrow of CD-1 mice (micronuclei induction)	None	Negative	Tested at 250 mg/kg (the MTD) in corn oil	Sorg et al., 1985
Chromosomal effects	Chinese hamster ovary cells (sister chromatid exchange)	Rat liver S9	Positive	Positive with and without rat liver S9 when tested at 15 and 27 µg/ml	NTP, 1991b

TABLE V-24 (cont.)

Test	Organism (Assay)	Exogenous Activation[a] System	Results Reported	Comments	Reference
Chromosomal effects (cont.)	Chinese hamster ovary cells (chromosomal abberations)	Rat liver S9	Positive	Tested at 45 and 67.5 µg/mL	NTP, 1991b
Cell transformation	Fischer rat embryo cell line (morphologic changes)	None	Negative	Tested up to 100 µg/mL	Freeman et al., 1973
	AKR leukemia, virus-infected Swiss mouse embryo cell line (morphologic changes)	None	Negative	Tested at 5 µg/mL	Rhim et al., 1974
BACTERIA			PHENANTHRENE		
DNA damage	B. subtilis (rec)	Rat liver S9 (Aroclor)	Negative	Tested at 125 µg/well	McCarroll et al., 1981
	E. coli (polA+)	Rat liver S9 (none)	Negative	Tested up to 250 µg/mL	Rosenkrantz and Poirier, 1979
	S. typhimurium (expression of lac Z gene under control of umu C gene)	Rat liver S9 (Aroclor)	Negative	Tested up to 8.3 µg/mL	Nakamura et al., 1987
	S. typhimurium (reverse mutation, his)	Rat liver S9 (Aroclor)	Positive	At 12 µg/plate in strain TA100	Oesch et al., 1981
		Rat liver microsomes (Aroclor)	Negative	Tested up to 50 nmol/plate in strain TA100	Wood et al., 1979
		Rat liver S9 (Aroclor)	Negative	Tested up to 50 µg/plate in strains TA1535, TA1537, TA98 and TA100	McCann et al., 1975
		Rat liver S9 (Aroclor)	Negative	Tested up to 200 µg/plate in strain TA100	La Voie et al., 1981a
		Rat liver S9 (Aroclor)	Negative	Tested at 0.28 mM in strain TA1537	Seixas et al., 1982
		Rat liver S9 (Aroclor)	Positive	Positively identified in a highly mutagenic fraction of raw gas condensate	Benson et al., 1984
		Rat liver S9 (Aroclor)	Positive	At 5 µg/plate TA97	Sakai et al., 1985
		Rat or hamster S9 (Aroclor)	Positive	Tested from 50-200 µg/plate in strain TA100	Carver et al., 1986

TABLE V-24 (cont.)

Test	Organism (Assay)	Exogenous Activation* System	Results Reported	Comments	Reference
DNA damage (cont.)	S. typhimurium (forward mutation, 8AG^R)	Rat liver S9 (Aroclor or phenobarbital)	Negative	Tested up to 300 nmol/mL in strain TM677	Kaden et al., 1979
FUNGI					
Recombination	S. cerevisiae (mitotic recombination, D3)	Rat liver S9 (Aroclor)	Negative	None	Simmon, 1979a
MAMMALIAN CELLS					
DNA damage	Human foreskin epithelial cells (unscheduled DNA synthesis)	None	Negative	Tested up to 400 µg/mL	Lake et al., 1978
	Primary rat hepatocytes (unscheduled DNA synthesis)	None	Negative	Tested up to 100 nmol/mL	Probst et al., 1981
	F344 rat trachea epithelial organ culture (unscheduled DNA synthesis)	None	Negative	Tested at 0.1×10^{-6} to 10×10^{-6} M	Ide et al., 1981
	Mouse skin (effect of co-application with benzo[a]-pyrene on formation of DNA adducts)	None	Negative	Average 17% decrease in formation of benzo[a]pyrene-DNA adducts upon co-application with benzo[a]pyrene	Rice et al., 1984
Mutation	Chinese hamster V79 cells (forward mutation, 8AG^R, OUA^R)	Syrian hamster embryo cell feeder layer	Negative	Tested at 1 µg/mL	Huberman and Sachs, 1976
	Human lymphoblastoid TK6 cells (forward mutation, TFT^R)	Rat liver S9 (Aroclor)	Positive	Tested at 50 nmol	Barfknecht et al., 1981
Chromosomal effects	Chinese hamster V79-4 cells (sister-chromatid exchange, aberrations)	Syrian hamster embryo cell feeder layer	Negative	Tested up to 10 µg/mL	Popescu et al., 1977
Cell transformation	Mouse prostate C3HG23 cells (morphologic changes)	None	Negative	Tested up to 10 µg/mL	Marquardt and Heidelberger, 1972
	Syrian hamster embryo cells (morphologic changes)	None	Negative	Tested up to 40 µg/mL	Pienta et al., 1977
	Mouse BALB/3T3 cells (morphologic changes)	None	Negative	Tested up to 50 µg/mL	Kakunaga, 1973

Test	Organism (Assay)	Exogenous Activation[a] System	Results Reported	Comments	Reference
Cell transformation (cont.)	Guinea pig fetal cells (morphologic changes)	None	Negative	Tested at 5 µg/mL	Evans and DiPaolo, 1975
	Mouse embryo C3H/10T1/2 fibroblast cells	None	Negative	Tested at 4 µM	Peterson et al., 1981
BACTERIA			PYRENE		
DNA damage	E. coli (rec, polA+, polA, uvrA)	Various	Negative	International collaborative program (consensus view of participants)	Ashby and Kilby, 1981
	B. subtilis (rec-)	Various	Negative	International collaborative program (consensus view of participants)	Ashby and Kilby, 1981
		None	Negative	No difference in inhibition at 100 µg/plate	Kinae et al., 1981
	S. typhimurium (expression of lac Z gene under control of umu C gene)	Rat liver S9 (Aroclor)	Negative	Tested up to 167 µg/mL	Nakamura et al., 1987
Mutation	S. typhimurium (reverse mutation, his)	^{60}Co gamma irradiation of pyrene	Positive	Tested at 160 µg/plate in strains TA98, TA1537 and TA1538	Gibson et al., 1978
		Rat liver S9 (polychlorinated biphenyl)	Positive	At 100 µg/plate with activation in strain TA1537	Kinae et al., 1981
			Negative	At 100 µg/plate with and without activation in strains TA98 and TA100	
		Rat liver S9 (Aroclor)	Negative	Tested up to 1000 µg/plate in strains TA1535, TA1537, TA98 and TA100	McCann et al., 1975; La Voie et al., 1979; Florin et al., 1980; Ho et al., 1981
		Various	Positive	At 25 µg/plate in strain TA1537; consensus view of participants in international collaborative program	Bridges et al., 1981
		Rat liver S9 (Aroclor)	Positive	Positive at 2 µg/plate, TA1537 TA97; higher doses TA100	Matljasevic and Zeiger, 1985

TABLE V 24 (cont.)

Test	Organism (Assay)	Exogenous Activation* System	Results Reported	Comments	Reference
Mutation (cont.)	S. typhimurium (reverse mutation, his)	Rat liver S9 (Aroclor)	Positive	At 2 µg/plate TA97, 4 µg/plate TA98	Sakai et al., 1985
		Rat liver S9 (Aroclor)	Negative	Tested up to 50 µg/plate in strain TA98	Bos et al., 1988
	S. typhimurium (reverse mutation, his) (taped-plate assay)	Rat liver S9 (Aroclor)	Negative	Tested up to 50 µg/plate in strain TA98	
	S. typhimurium (forward mutation, 8AG^R)	Rat liver S9 (Aroclor)	Positive	At 140 nmol/ml in strain TM677	Kaden et al., 1979
	S. typhimurium (forward mutation, ARA^R)	Rat liver S9 (Aroclor)	Positive	The minimum positive dose in strain B13 was 0.25 µmol	Dorado and Pueyo, 1988
FUNGI					
Mutation	S. cerevisiae and Schizosaccharomyces pombe (different genetic endpoints)	Various	Negative	Consensus view of participants in international collabrative program	de Serres and Hoffman, 1981
INSECTS					
Mutation	D. melanogaster (sex-linked recessive lethals)	None	Negative	800 mg/kg fed in diet for up to 72 hours	Valencia and Houtchens, 1981
MAMMALIAN CELLS					
DNA damage	Human foreskin epithelial cells (unscheduled DNA synthesis)	None	Negative	Tested up to 400 µg/ml	Lake et al., 1978
	Primary rat hepatocytes (unscheduled DNA synthesis)	None	Negative	Tested up to 500 mol/ml	Probst et al., 1981
	HeLa cells (unscheduled DNA synthesis)	Rat liver S9 (3-methylcholanthrene	Negative	None	Martin et al., 1978
	Human fibroblast cell line W138 (unscheduled DNA synthesis)	Rat liver S9 (Aroclor)	Positive	At 7.2 µg/ml	Robinson and Mitchell, 1981
	F344 rat trachea epithelial organ culture (unscheduled DNA synthesis)	None	Negative	Tested from 0.1×10^{-6} to 10×10^{-6} M	Ide et al., 1981

TABLE V-24 (cont.)

Test	Organism (Assay)	Exogenous Activation[a] System	Results Reported	Comments	Reference
DNA damage (cont.)	Mouse skin (effect of co-application with benzo[a]-pyrene on DNA adduct formation)	None	Positive	Co-application with benzo[a]-pyrene resulted in average increase in level of DNA adducts of 56%	Rice et al., 1984
	Primary F344 rat hepatocytes (unscheduled DNA synthesis)	None	Negative	Tested up to 1E-4 M	Williams et al., 1989
Mutation	Mouse lymphoma L5178Y cells (forward mutation, TFT^R)	Rat liver S9 (Aroclor)	Positive	At 10 µg/mL	Jotz and Mitchell, 1981
	Mouse lymphoma L5178Y cells	None	Negative	Tested up to 128 µg/mL	Mitchell et al., 1988
	Mouse lymphoma L5178Y cells (forward mutation, TK^-)	Rat liver S9 (Aroclor)	Positive	Tested from 5.9-9 µg/mL	Mitchell et al., 1988
	Mouse lymphoma L5178Y cells (forward mutation, TK^-)	Rat liver S9 (Aroclor)	Positive	Tested at $1.5x10^{-3}$ to $2.02x10^{-3}$ M	Wangenheim and Bolcsfoldi, 1988
	Chinese hamster ovary cells (forward mutation, HGPRT)	Rat liver S9 (Aroclor)	Negative	Tested from 2.5-15 µg/mL	Oshiro et al., 1988
Chromosomal effects	Rat liver epithelial ARL18 cells (sister-chromatid exchange)	None	Negative	Tested up to 1 µmol/mL	Tong et al., 1981b
	Chinese hamster ovary cells (sister-chromatid exchange)	Rat liver S9 (Aroclor)	Positive	Between 19 and 300 µg/mL	Evans and Mitchell, 1981
			Negative	Tested up to 100 µg/mL	Perry and Thomson, 1981
	Chinese hamster V79 cells (sister-chromatid exchange)	Syrian hamster embryo cell feeder layer	Positive	Tested at 10 µg/mL	Popescu et al. 1977
	Rat liver RL_1 cells (aberrations)	None	Negative	Tested up to 100 µg/mL	Dean, 1981
	Chinese hamster liver epithelial cells (sister-chromatid exchange)	None	Negative	Tested at 5-45 µg/mL	DeSalvia et al., 1988
	Human peripheral lymphocytes (sister-chromatid exchange)	None	Negative	Tested at 10^{-4} to 10^{-3} M	Lindahl Kiessling et al., 1989
		Intact rat hepatocytes	Positive	Tested at 10^{-4} M	

TABLE V-24 (cont.)

Test	Organism (Assay)	Exogenous Activation* System	Results Reported	Comments	Reference
Chromosomal effects (cont.)	Mouse lymphoma cells (DNA strand breaks)	Rat liver S9 (Aroclor)	Positive	Tested at 0.5×10^{-4} and 5×10^{-4} M	Garberg et al., 1988
Cell transformation	Syrian hamster embryo cells (morphologic changes)	None	Negative	Tested up to 20 µg/ml	DiPaolo et al., 1969; Pienta et al., 1977, Casto, 1979
	Mouse prostate C3H cells (morphologic changes)	Mouse embryo fibroblast feeder layer	Negative	Tested at 1 µg/ml	Chen and Heidelberger, 1969
	Mouse BALB/C-3T3 cells (morphologic changes)	None	Negative	Tested at 20 µg/ml Tested at 50 µg/ml	DiPaolo et al., 1972; Kakunaga, 1973
	Guinea pig fetal cells (morphologic changes)	None	Negative	Tested at 10 µg/ml	Evans and DiPaolo, 1975
	Syrian hamster embryo cells (morphologic changes)	None	Negative	Tested at 1-100 µg/ml	Tu et al., 1986

*Compound in parentheses refers to type of inducing treatment administered to animal from which the exogenous activation system was prepared.

other PAHs in an ethylene glycol, tricaprylin or ethyl laurate vehicle. The mice were then observed for 18 months to determine the incidence of sarcomas. Survival was 90-100% during the critical period of tumor formation, the 4th to 11th month. The authors found that both dose and interval between administration of the carcinogen and noncarcinogen affected the degree of observed inhibitory response. Phenanthrene had substantial inhibitory effects on the production of sarcomas by dibenz[a,h]anthracene, particularly at lower dosages. At 275 µg of the carcinogen, concomitant administration of a 24:1 molar ratio of phenanthrene to dibenz[a,h]-anthracene (in ethyl laurate) yielded ~50% reduction in the percentage of tumor-bearing animals as compared with administration of dibenz[a,h]-anthracene alone. When the dosage was decreased to 60 µg of the carcinogen, a similar molar ratio of phenanthrene to dibenz[a,h]anthracene was shown to yield a comparable reduction in tumor development. It was found that either concomitant administration of carcinogenic and noncarcinogenic PAHs or administration of the noncarcinogen either 2 days prior to or after the carcinogen administration resulted in maximum reduction in the percentage of tumor-bearing animals. When the vehicle was changed to triethylene glycol, phenanthrene in combination with dibenz[a,h]anthracene had a substantial promoting effect, approximately doubling the percentage of tumor-bearing animals.

In the same study benzo[a]pyrene was also tested for carcinogenic effects in combination with other noncarcinogenic, weakly carcinogenic and potent carcinogenic hydrocarbons in a tricaprylin vehicle (Falk et al., 1964). The chosen ratios of putative anticarcinogen to carcinogen approximated those that occur in the environment. PAHs, including benzo[a]-fluorene, chrysene, benzo[k]fluoranthene, perylene, and a mixture of

anthracene, phenanthrene and pyrene, had substantial inhibiting effects on the ability of benzo[a]pyrene to produce injection site sarcomas. Other PAHs, including acenaphthylene, fluorene, anthracene, benzo[g,h,i]perylene, indeno[1,2,3-cd]pyrene and coronene, had no such inhibiting effects.

By contrast, Pfeiffer (1973, 1977) found no inhibitory effects of 10 noncarcinogenic PAHs on benzo[a]pyrene or dibenz[a,h]anthracene carcinogenicity. Groups of 100 NRMI female mice were given single subcutaneous injections of various dosages of benzo[a]pyrene and dibenz[a,h]anthracene, and 10 noncarcinogenic PAHs, separately and in combination, in 0.5 mℓ tricaprylin. The PAHs, which the authors considered noncarcinogenic, included benzo[a]anthracene, phenanthrene, anthracene, pyrene, fluoranthene, chrysene, benzo[g,h,i]perylene, perylene, benzo[e]pyrene and coronene. The animals were examined weekly for the development of sarcomas for 114 weeks. Relationships were found to exist between increasing dose and tumor development for benzo[a]pyrene, dibenz[a,h]anthracene, benzo[a]pyrene plus dibenz[a,h]anthracene, and all 12 PAHs administered as mixtures. No treatment-related increases in sarcoma development were observed for any of the 10 noncarcinogenic PAHs when administered separately. Interestingly, the noted dose-response curves for combinations of PAHs most closely resembled the curve for dibenz[a,h]anthracene alone. Upon further statistical analysis to determine the relative effectiveness of the treatments in producing tumors, it was found that the mixture or benzo[a]pyrene and dibenz[a,h]anthracene alone, or in combination with the 10 noncarcinogenic PAHs, was slightly less potent in inducing tumors than was dibenz[a,h]anthracene alone.

Pott et al. (1977) conducted comparable experiments on the carcinogenic effects of automobile exhaust condensates (AEC) in mice. Female NRMI mice in groups of ~88 animals were administered single subcutaneous injections of various dosages of AEC and benzo[a]pyrene, separately and in combination, which had been dissolved or suspended in tricaprylin. AEC contained both PAHs and non-PAH substances. When AEC was present with benzo[a]pyrene, the incidence of tumors (most of which were sarcomas) was less than when benzo-[a]pyrene was administered alone. This inhibitory effect of AEC was particularly obvious at the higher dosages of benzo[a]pyrene, but a decreasing relationship between tumor incidence and level of AEC was observed at all dosages of benzo[a]pyrene. In a second series of experiments, Pott et al. (1977) sought to assess the effects of a PAH fraction and non-PAH substances prepared from the AEC. These were injected separately and in combination with benzo[a]pyrene. Mice injected in this set of experiments were observed for a year. The PAH-containing fraction of AEC was tumorigenic. When assayed in combination with other AEC fractions, tumorigenicity was reduced.

The relative proportions of PAHs found in automobile exhaust gas condensates were determined in relation to benzo[a]pyrene. The PAHs were divided into two groups; the first group was composed of benzo[a]pyrene, dibenz[a,h]anthracene, benz[a]anthracene and benzo[b]fluoranthene and the second group phenanthrene, anthracene, fluoranthene, pyrene, chrysene, benzo[e]pyrene and benzo[g,h,i]perylene. The concentrations used in each dose group were determined by the relative concentration of benzo[a]pyrene. The benzo[a]pyrene concentrations selected for the first dose group (benzo[a]pyrene, dibenz[a,b]anthracene, benz[a]anthracene and benzo[b]-fluoranthene) were 1, 1.7 and 3.0 µg benzo[a]pyrene and the benzo[a]pyrene

concentrations selected for the second dose group (phenanthrene, anthracene, fluoranthene, pyrene, chrysene, benzo[e]pyrene and benzo[g,h,i]perylene) were 1, 3, 9 and 27 µg benzo[a]pyrene (benzo[a]pyrene was not included in the group). A third dose group was created by combining the compounds in groups one and two; the relative benzo[a]pyrene concentrations selected for this dose group were 1, 1.7 and 3.0 µg benzo[a]pyrene. All compounds were dissolved in acetone. Four control groups that received only benzo[a]pyrene at doses of 1, 1.7 or 3.0 µg in acetone and a solvent control group were also employed. The shaved skins of 100 female NMRI mice/group were treated twice/week until natural death or development of an application site carcinoma (Schmahl et al., 1977).

The incidence of application site sarcomas in the solvent control group was 1/81; no other tumors were reported. In the benzo[a]pyrene control groups the carcinoma incidences were 10/77, 25/88 and 43/81 in the 1, 1.7 and 3 µg benzo[a]pyrene groups, respectively. The carcinoma incidences for the first dose group (benzo[a]pyrene, dibenz[a]anthracene, benz[a]anthracene and benzo[b]fluoranthene) were 25/81, 53/88 and 63/90 in the 1, 1.7 and 3 µg benzo[a]pyrene groups, respectively. The carcinoma incidences for the second dose group (phenanthrene, anthracene, fluoranthene, pyrene, chrysene, benzo[e]pyrene and benzo[g,h,i]perylene) were 1/85, 0/84, 1/88 and 15/86 in the 1, 3, 9 and 27 µg benzo[a]pyrene groups, respectively. In the third group (combination of groups one and two) the carcinoma incidences were 44/89, 54/93 and 64/93 in the 1, 1.7 and 3 µg benzo[a]pyrene groups, respectively. The reported papilloma and sarcoma incidences of all groups did not exceed 5% (Schmahl et al., 1977).

The cocarcinogenic potential of various substances found in cigarette smoke was studied by Van Düüren et al. (1973) and Van Düüren and Goldschmidt (1976). In the 1973 study, female ICR/Ha Swiss mice in groups of 50 were given repeated dermal applications of 5 µg benzo[a]pyrene in 0.1 mℓ acetone 3 times/week either alone or with pyrene, benzo[g,h,i]perylene or benzo[e]pyrene. Control groups received either no treatment or treatments of acetone, benzo[g,h,i]perylene, pyrene or benzo[e]pyrene alone. The experiment ran for 52 weeks. Cocarcinogenic activity was shown by the three PAHs (Table V-25). No skin tumors were found in the control groups. When benzo[a]pyrene was applied with the individual noncarcinogens, the number of mice with papillomas increased 1.5-2.6 times over the number observed when benzo[a]pyrene was applied alone. Likewise, the total number of papillomas was 2-6 times higher when the noncarcinogens were present as compared with benzo[a]pyrene alone. Moreover, the number of days from first application to appearance of the first tumor in the multiple treatment groups was similar to that in the benzo[a]pyrene group alone.

In a second study, Van Düüren and Goldschmidt (1976) assessed both the cocarcinogenic and tumor-promoting activity of benzo[g,h,i]perylene, fluoranthene, benzo[e]pyrene and pyrene in conjunction with benzo[a]pyrene. Again, female ICR/Ha Swiss mice in groups of 50 were used. Various dosages of the above noncarcinogenic PAHs alone and in conjunction with 5 µg benzo[a]pyrene were applied in 0.1 mℓ of acetone or in DMSO 3 times/week for $\leq$440 days. Pyrene, fluoranthene and benzo[e]pyrene were found to be potent cocarcinogens for benzo[a]pyrene, while benzo[g,h,i]perylene was determined to be "moderately cocarcinogenic". Pyrene and fluoranthene also proved to be weak tumor-promoting agents when an initiating dose of benzo-[a]pyrene was used. The results of the study are presented in Table V-26.

TABLE V-25

Cocarcinogenic Activity of Various PAHs with Benzo[a]pyrene in Mouse Skin[a]
(1 Year Exposure)

Carcinogen[b]	Cocarcinogen	Dose of Cocarcinogen[b] (µg)	No. of Mice with Papillomas/No. of Survivors at 52 Weeks
Benzo[a]pyrene	--	--	13/42
Benzo[a]pyrene	Benzo[e]pyrene	15	34/39
Benzo[a]pyrene	Benzo[g,h,i]perylene	21	20/37
Benzo[a]pyrene	Pyrene	12	27/41

[a]Source: Van Düüren et al., 1973

[b]Benzo[a]pyrene (5 µg) and/or cocarcinogen was applied as acetone solutions 3 times/week for 52 weeks. There were no tumors in the groups treated only with acetone, benzo[e]pyrene, benzo[g,h,i]perylene or pyrene.

TABLE V-26

Cocarcinogenic Activity of Various PAHs with Benzo[a]pyrene on Mouse Skin[a]

Carcinogen[b]	Cocarcinogen	Dose of Cocarcinogen (μg)	Duration of Experiment (days)	Days to First Papilloma	Mice with Papillomas/ Total Papillomas
Benzo[a]pyrene	Acetone	--	368	251	14/16
	Acetone	--	440	210	16/26
--	Benzo[e]pyrene	15	368	--	0
Benzo[a]pyrene	Benzo[e]pyrene	15	368	246	33/79
Benzo[a]pyrene	Benzo[e]pyrene	5	368	249	24/33
--	Benzo[g,h,i]perylene	21	368	--	0
Benzo[a]pyrene	Benzo[g,h,i]perylene	21	368	222	20/39
Benzo[a]pyrene	Benzo[g,h,i]perylene	7	368	238	19/31
--	Fluoranthene	40	440	--	0
Benzo[a]pyrene	Fluoranthene	40	440	99	39/126
--	Pyrene	40	440	--	0
--	Pyrene	12	368	--	0
Benzo[a]pyrene	Pyrene	40	440	229	35/66
Benzo[a]pyrene	Pyrene	12	368	186	26/42
Benzo[a]pyrene	Pyrene	4	368	250	12/14

[a]Source: Van Düüren and Goldschmidt, 1976

[b]Benzo[a]pyrene (5 μg/0.1 mℓ acetone or DMSO) was applied in the same solution as the cocarcinogen, 3 times/week.

Rice et al. (1984) also found fluoranthene and pyrene to have cocarcinogenic potential when combined with benzo[a]pyrene. When female CD-1 mice were given dermal applications of 150 µl [^{3}H] benzo[a]pyrene and either fluoranthene or pyrene, the occurrence of DNA adduct formation (after 24 hours) was 66 and 56%, respectively, above the level found for benzo[a]-pyrene alone. Phenanthrene co-application resulted in an average 17% decrease and formation of [^{3}H]benzo[a]pyrene-DNA adducts.

Slaga et al. (1979) examined the effects of several PAHs on skin tumor initiation by 7,12-dimethylbenz[a]anthracene (DMBA) and benzo[a]pyrene. Either benzo[e]pyrene, pyrene or fluoranthene in acetone was applied to the backs of female CD-1 mice (30/group) 5 minutes before initiation with DMBA or benzo[a]pyrene. Beginning 1 week after initiation mice received 10 µg of 12-O-tetradecanoylphorbol-13-acetate (TPA) in acetone twice weekly for 30 weeks. The incidences of both papillomas and carcinomas were observed weekly and removed at random for histologic verification. When applied before initiation, pyrene and benzo[e]pyrene slightly enhanced benzo[a]pyrene initiation whereas fluoranthene had a marginal effect. These same PAHs had an inhibitory effect on 7,12-dimethylbenz[a]anthracene skin tumor initiation. When pyrene and fluoranthene were applied after benzo[a]pyrene initiation, they had no tumor promoting effects.

Huang et al. (1986) investigated the ability of benzo[a]pyrene phenolic metabolites to interfere with the mutagenicity and tumorigenicity of benzo-[a]pyrene or the putative ultimate carcinogen, benzo[a]pyrene-7,8-diol-9,10-epoxide. Of 12 isomeric phenolic metabolites, 3-hydroxy-benzo[a]pyrene was the most potent antagonist of 7,8-diol-9,10-epoxide mutagenicity for

Salmonella typhimurium strain TA100; 3 μmol reduced mutagenicity by 50%. The 3-hydroxy-benzo[a]pyrene also decreased microsome-mediated mutagenicity of benzo[a]pyrene and benzo[a]pyrene-7,8-diol for TA100. Likewise, mutagenicity of the 7,8-diol-9,10-epoxide for mammalian cells (V79) was decreased by 50% upon addition of 8 μM 3-hydroxybenzo[a]pyrene. A 2500 μmole dose of 3-hydroxybenzo[a]pyrene was applied topically to the shaved backs of female CD-1 mice 5 minutes before addition of a tumor-initiating dose of 200 μmole of benzo[a]pyrene-7,8-diol-9,10-epoxide. This was followed by 16-20 weeks of promoting treatment with TPA. At the end of this time a 72-78% reduction in numbers of tumors/mouse and a 42-55% decrease in incidence was observed by comparison with animals treated with the 7,8-diol-9,10-epoxide and TPA alone. A less dramatic reduction (35-41% decrease in incidence, 40-52% inhibition in number of tumors/animal) was observed when benzo[a]-pyrene was the initiating agent.

The enhancing or inhibiting action of non-PAH materials on PAH carcinogenicity has also been a subject of study. El-Bayoumy (1985) showed that two synthetic organoselenium compounds, p-methoxybenzeneselenol and benzylselenocyanate, inhibited forestomach tumors in mice treated with benzo[a]-pyrene. As described in the oral carcinogenicity section, 9-week-old female CD-1 mice were fed a standard diet for 1 week. At the beginning of week 2, 25 mice/group were fed diets containing varying levels of the above two compounds or their nonselenium-containing congeners. A third pair of compounds, phenothiazine and phenoselenazine, were also tested. Beginning with week 3, mice were gavaged twice weekly for 4 weeks with 1 mg benzo[a]pyrene in corn oil. The test diets were continued for 1 week post-treatment and then standard diets were fed until week 28 when the mice were killed. Mice

receiving benzo[a]pyrene and standard diet had a forestomach tumor incidence of 85%. This was decreased to 25% in the high-dose p-methoxyphenol-treated group. Benzylselenocyanate and methoxybenzeneselenol produced insignificant reductions in tumor incidence but significant reductions in the number of tumors/mouse.

As noted in the nonoral carcinogenicity section, the pulmonary carcinogenicity of benzo[a]pyrene can be greatly enhanced by co-administration of particulate material. Pershagen et al. (1984) studied the pulmonary carcinogenicity of benzo[a]pyrene alone and in combination with arsenic trioxide (AS_2O_3) in male Syrian golden hamsters. The animals were divided into four groups: As_2O_3, benzo[a]pyrene, As_2O_3 plus benzo-[a]pyrene, and vehicle controls. At each of 15 weekly instillations, 3 mg/kg of arsenic and/or 6 mg/kg of benzo[a]pyrene was administered. All groups received a carrier dust (charcoal carbon), which increased the lung retention of arsenic. Carcinomas of the larynx, trachea, bronchi or lungs were found in 3/47, 17/40 and 25/54 animals examined in the As_2O_3, benzo[a]pyrene and As_2O_3 plus benzo[a]pyrene groups, respectively. No respiratory tract carcinomas were found in the 53 controls. The incidences of pulmonary adenomas, papillomas and adenomatoid lesions were markedly higher in the arsenic group than in the control group ($p<0.01$). There was also some evidence of a positive interaction between arsenic and benzo[a]-pyrene in relation to adenomatosis lung tumors. The authors noted that the presence of the carrier dust prevented rapid lung clearance of the As_2O_3. They proposed that the arsenic may have had an adverse effect on repair of benzo[a]pyrene-induced DNA damage (Pershagen et al., 1984).

Metivier et al. (1984) studied the effects of different levels of plutonium oxide (PuO_2) and benzo[a]pyrene on lung carcinogenesis in SPF Wistar rats in an effort to determine the cocarcinogenic effects of ionizing radiation and benzo[a]pyrene. Eight different experimental groups inhaled PuO_2 at four dose levels with and without benzo[a]pyrene (given in two doses of 5 mg by intratracheal instillation). The results are summarized in Table V-27. They show that the incidence of lung tumors increased as a function of both PuO_2 dose and benzo[a]pyrene exposure. The authors felt that a multiplicative relative risk model fit the observed effects.

Jones et al. (1984) investigated the effect of 1α,25-dihydroxycholecalciferol [1,25-$(OH)_2D_3$] on the transformation of cells pretreated with benzo[a]pyrene and benzo[e]pyrene. Treatment of Syrian hamster embryo cells with benzo[a]pyrene for 3 days followed by treatment with 1,25-$(OH)_2D_3$ for 4 days increased transformation of the cells. Benzo[e]pyrene, not a complete carcinogen by itself, induced cell transformation when followed by 1,25-$(OH)_2D_3$ application.

Rahimtula et al. (1977) examined the capabilities of nine antioxidants (ascorbate, butylated hydroxyanisole, butylated hydroxytoluene, ethoxyquin, glutathione, NNN'N'-tetramethyl-p-phenylenediamine dihydrochloride, nordihydroguariarotic acid, propyl gallate and pyrogallol) to affect benzo[a]pyrene hydroxylation by rat liver microsomal MFOs. All of the antioxidants tested reduced the bacterial mutagenicity of benzo[a]pyrene in the presence of rat liver microsomes and cofactors. They also found that several synthetic and naturally occurring flavones, when incorporated in the diet (3-5 mg/g) or applied to the skin, caused an increase in benzo[a]pyrene hydroxylase

TABLE V-27

Co-administration of PuO_2 and Benzo[a]pyrene by Inhalation*

PuO_2 Initial Lung Burden (Bq)	Benzo-[a]-pyrene (mg)	No. of Animals	Median Survival Time (days)	Median Lifetime Dose (Gy)	No. of Animals		
					With Pulmonary Malignancies		With Fatal Benign Tumors
					Fatal	Incidental	
0	0	89	864	0.0	0	0	0
220	0	89	820	3.3	4	13	0
630	0	30	798	9.4	6	8	0
6300	0	19	345	76.3	1	5	0
0	2x5	38	675	0	7	3	2
220	2x5	29	444	2.9	15	2	10
630	2x5	22	480	8.5	14	2	0
6300	2x5	19	330	75.4	18	1	0

*Source: Metivier et al., 1984

activity in the small intestine and skin, respectively. In addition, pulmonary adenoma formation resulting from oral administration of benzo[a]pyrene was totally prevented, and skin tumors initiated by benzo[a]pyrene application to mice were significantly reduced (>50%) by treatment with the synthetic flavone, β-naphthoflavone. Pulmonary tumor formation was also reduced 50% by incorporation of the naturally occurring flavone, quercetin pentamethyl ether, into the diet. Sullivan et al. (1978) demonstrated that BHA, BHT, phenothrazine, phenothrazine methosulfate and ethoxyquin all can reduce the quantitative yield of benzo[a]pyrene metabolites in incubations with rat liver microsomes.

Summary

Studies of the general and specific health effects in animals associated with exposure to PAHs tend to be limited in the number of PAHs considered and the route of administration. A wide variety of general toxic effects have been linked to exposures. Effects on the hematopoietic and lymphoid systems seem to predominate, such as anemia, hemolysis, leukemia and lymphocyte toxicity. Equally important to the nature of effects, the dosage eliciting such effects appears to vary by route of administration; for example, for compounds like fluoranthene, acute toxicity (LD_{50}) occurs at a lower dosage for oral versus other routes of exposure.

Some PAHs are known to be carcinogens for animals. In terms of specific health effects, relatively potent carcinogens like benzo[a]pyrene and dibenz[a,h]anthracene as well as moderate-to-strong cocarcinogens like fluoranthene and pyrene are found among the PAHs. Dose-response relationships for oral exposures have been documented for some of the carcinogenic

PAHs. The carcinogenic effect of some PAHs when administered to animals by other routes such as subcutaneous and intraperitoneal injections, inhalation and skin-painting has also been documented.

Environmental PAH exposures are thought to occur to mixtures rather than individual compounds. Experimental PAH mixtures research assessing carcinogenic activity has focussed on nonoral routes of administration; these include subcutaneous injection, dermal application and inhalation. When studied in combinations, inhibition, promotion and cocarcinogenic effects have been reported for PAHs. PAHs have also been reported to act as initiators of carcinogenesis when followed by repeated treatments with non-PAHs such as plutonium oxide. Mixtures, such as automobile exhaust condensate, that contain a variety of PAHs as well as other compounds, have also been shown to be carcinogenic in animals.

Reproductive/teratogenic effects have not been evaluated for most PAHs. However, there is some evidence suggesting that ingestion of benzo[a]pyrene reduces fertility and reproductive capacity and has deleterious effects on the offspring of mice so exposed.

Several PAHs have been extensively studied for genotoxic effects. Benzo[a]pyrene seems clearly to be mutagenic in both prokaryotic and mammalian cells in the presence of an activation system and causes various chromosomal effects in mammalian cells. Benzo[g,h,i]perylene, chrysene and dibenz[a,h]anthracene are mutagens in several systems, and the latter also damages the DNA of both prokaryotic and eukaryotic cells. Benz[a]anthracene, normally a weak carcinogen, can be activated to exhibit mutagenic

activities comparable to benzo[a]pyrene. For the remainder of the compounds under review, there are either too few data to draw firm conclusions, or the results have been mixed. Most of these compounds have exhibited genotoxicity in some systems.

VI. HEALTH EFFECTS IN HUMANS

Introduction

Human beings are likely to be exposed on a daily basis to various levels of PAHs in the ambient environment. Primary vehicles of exposure among the general population include inhalation of mainstream and sidestream tobacco smoke and exhaust products from the combustion of commonly used fuels. Certain occupational groups have the additional burden of exposure to extremely high levels of complex mixtures that contain PAHs as well as other toxic and tumorigenic compounds. Those considered at high risk for these exposures include steelworkers, coke oven workers, gas workers, foundry workers, aluminum reduction plant workers, roofers, chimney sweeps, and possibly auto mechanics. Scrotal cancer among chimney sweeps is considered to be the first and oldest identified occupational cancer (Pott, 1775). These occupational exposures occur almost exclusively via inhalation or direct dermal contact. It is from this body of scientific evidence that a causal relationship has been clearly established between PAH-containing mixtures and several specific cancer sites (IARC, 1984, 1985; WHO, 1988). These studies are briefly summarized at the end of this chapter, although their direct relevance to oral ingestion of PAHs in drinking water is unclear.

PAHs do occur in ingested media such as water and foods, but these exposures have not been well-characterized or evaluated. PAHs have been identified in smoked foods, charbroiled meats, raw vegetables, shellfish, and refined fats and oils. To study epidemiologically the relationship between any specific nutrient and a specific adverse event is very difficult, however, and extreme caution must always be exercised in

inferring causality. Howson et al. (1986) point out that, although an early study implicated ingestion of PAHs via charcoal broiled and smoked foods with an increased risk of gastric cancer, subsequent experimental research showed that PAHs were not good candidates for carcinogens of the glandular stomach and therefore probably were only indirectly associated with gastric cancer.

Cigarette smoking is strongly and consistently associated with cancer at several sites including the lung, larynx, oral cavity, esophagus and bladder (Schottenfeld and Fraumeni, 1982; Maclure and MacMahon, 1980). In addition to nitrosamines and other chemicals, 34 PAHs, some of which are recognized as carcinogens, have been identified in mainstream and sidestream cigarette smoke (IARC, 1983; Wynder, 1988; Appel et al., 1990). Inhalation of tobacco smoke is also considered to be a major risk factor for cardiovascular disease. However, the specific relationship of any of the compounds in tobacco smoke to the complex etiology of this disease is unclear. Albert et al. (1977) demonstrated in chickens that direct injection of PAHs [DMBA and B[a]P] into the aorta could induce atherosclerotic lesions. In humans, it is likely that other constituents of tobacco smoke, primarily carbon monoxide and nicotine, also are involved in the disease process and may possibly be the main culprits, not PAHs (Wynder, 1988). This will be briefly addressed later in the review of more recently published occupational studies (Hansen, 1983, 1989; Gustavsson et al., 1988), but a complete review of the smoking and human health literature is well beyond the scope of this document.

Clinical Case Studies

Oral. The only available case reports of oral ingestion of isolated PAHs are concerned with accidental ingestion of naphthalene. Acute hemolytic anemia is the most frequent manifestation of naphthalene poisoning in humans. Case reports have described the appearance of acute hemolytic anemia after naphthalene ingestion by children (Jacobziner and Raybin, 1964; Athreya et al., 1961; Gross et al., 1958; Zinkham and Childs, 1957, 1958; Haggerty, 1956; Chusid and Fried, 1985; Bregman, 1954; MacGregor, 1954; Abelson and Henderson, 1951; Mackell et al., 1951; Zuelzer and Apt, 1949) and adults (Anziulewicz et al., 1959; Zinkham and Childs, 1957, 1958; Gidron and Leurer, 1956).

The reported mechanisms and range of exposure to naphthalene in these case studies were: 1) chewing, sucking, or swallowing of mothballs (one to numerous) as a single incident or for periods up to 3 months; 2) ingestion of toilet bowl deodorant cakes (pure naphthalene) by a child over a period of a year; and 3) ingestion of naphthalene-containing deodorant in a diaper pail for an unspecified period. Doses of naphthalene were not generally reported in these case studies because of the poorly defined nature of the exposure. Tests to detect naphthalene derivatives in the urine of the anemic individuals were negative in some cases and positive in others.

Symptoms of naphthalene toxicity that frequently precede the diagnosis of acute hemolytic anemia in persons of all ages include mild to severe jaundice, dark urine (red, orange, or port wine colored), pallor, and lethargy (U.S. EPA, 1987a). Severe jaundice is often the reason for hospitalization, since the jaundice often develops before severe anemia

becomes manifest. However, it is clear that anemia and jaundice can develop in parallel as shown by a time-course study of hematologic changes in a 16-year-old girl who had ingested about 6 g of naphthalene in a suicide attempt (Gidron and Leurer, 1956). Vomiting and tachycardia are occasionally observed as preclinical signs of naphthalene poisoning in persons of all ages. Preclinical signs of naphthalene toxicity observed primarily in neonates or children include anorexia, cyanosis, shallow respiration or apnea, convulsions, and diarrhea. Preclinical symptoms of naphthalene poisoning reported by children or adults include fever, confusion, pain in abdominal or kidney region, pain at urination, nausea, headache, fainting, and vertigo (U.S. EPA, 1987a).

Frequent laboratory findings indicative of severe hemolytic anemia after naphthalene poisoning in persons of all ages include depressed hemoglobin, hematocrit, and erythrocyte count; elevated leukocyte and reticulocyte counts; erythrocyte anisocytosis, polychromatophilia, fragmentation, spherocytosis, and microspherocytosis; and occasional hemoglobinuria (U.S. EPA, 1987a). Additional laboratory findings indicative of severe hemolytic anemia after exposure of neonates or children to naphthalene include the following: erythrocyte poikilocytosis and microcytosis; elevated serum bilirubin; occasional observation of Heinz bodies, nucleated erythrocytes, and Howell-Jolly bodies; and occasional observation of methemoglobinuria (U.S. EPA, 1987a).

In most studies of persons who have developed severe hemolytic anemia after exposure to naphthalene, treatment with blood transfusions, treatment with blood transfusions plus alkali therapy, or observation without either

of these treatments has led to complete patient recovery with no observed complications. However, deaths have been observed after naphthalene-induced hemolytic anemia (U.S. EPA, 1987a), but it is unknown whether they were directly related to the exposure.

Other Routes. In addition to the cases of acute naphthalene poisoning following oral exposure, similar effects have been reported following exposure to naphthalene by other routes: combined dermal absorption and inhalation of naphthalene vapor by neonates (Grigor et al., 1966; Naiman and Kosoy, 1964; Valaes et al., 1963; Dawson et al., 1958; Cock, 1957; Schafer, 1951) and adults (Younis et al., 1957); inhalation of naphthalene vapor by neonates (Hanssler, 1964; Irle, 1964); inhalation of naphthalene vapor by a child and adults (Linick, 1983); and transplacental exposure of the fetus to naphthalene that had been ingested by the mother (Anziulewicz et al., 1959; Zinkham and Childs, 1957, 1958). These exposures occurred via combined dermal absorption and inhalation for a few days of naphthalene vapor from apparel and bed clothing that had been stored in mothballs; inhalation of vapor from a naphthalene-containing medication; inhalation of naphthalene vapor for several years from excessive numbers of mothballs kept throughout the home; and transplacental exposure, for about 3 months, of fetuses to naphthalene ingested by the mother.

Two groups of individuals have been shown to be especially susceptible to naphthalene-induced hemolytic anemia:

1. Persons whose erythrocytes are deficient in glucose 6-phosphate dehydrogenase (G6PDH) or persons in whom erythrocyte GSH is rapidly depleted by certain oxidant chemicals (U.S. EPA, 1987a). The precise mechanism by which GSH is depleted or a deficiency of G6PDH leads to naphthalene-induced hemolysis in these cases is not clear.

2. Neonates (U.S. EPA, 1987a). The sensitivity of neonates to naphthalene is explained in part by the same factors that confer sensitivity to children and adults; namely, G6PDH deficiency and/or diminished levels of GSH as described above. Additional naphthalene sensitivity in newborns may be conferred by the immaturity of pathways necessary for the conjugation and excretion of naphthalene metabolites (Valaes et al., 1963). Evidence for the latter hypothesis is suggested by the finding that glucuronide excretion by human newborn infants increased gradually during the first week of life and that the initial levels and the rate of increase were lower in the premature infant than in the full-term infant (Brown and Burnett, 1957).

Fanburg (1940) described the case of a man who had developed an allergic reaction to naphthalene from clothing that had been stored in mothballs. The reaction was an exfoliative dermatitis resembling mycosis fungoides. The elimination of naphthalene from the patient's environment resulted in prompt recovery, which lasted uninterruptedly during a 7-year period of observation.

Case studies that describe the presence of cataracts in persons exposed to naphthalene by the oral, dermal or inhalation routes have been summarized in an ambient water quality criteria documnt for naphthalene (U.S. EPA, 1980c). Ghetti and Mariani (1956) associated the occurrence of cataracts in 8/21 workers with naphthalene exposure in a manufacturing plant. Other cases of occupational instances of cataract formation have been described by Hollwich et al. (1975).

Possible preneoplastic epidermal changes in humans have been associated with dermal exposures to benzo[a]pyrene (Cottini and Mazzone, 1939; Klar, 1938; Rhoads et al., 1954). A group of 26 patients was given daily applications of a 1% solution of benzo[a]pyrene on protected (thigh) or unprotected (upper extremity) skin. These individuals were hospitalized for a variety

of conditions including pemphigus vulgaris, mycosis fungoides, prokeratosis, psoriasis, xeroderma pigmentosum, basal cell cancer, squamous cell cancer, lupus erythematosis, syphillis (in various stages) or ringworm. Treatment was for ~4 months and resulted in a series of progressive alterations in the skin proceeding from erythema and pigmentation to the development of lesions described by the authors as verrucae. The skin changes and lesions regressed within 2-3 months after cessation of treatment. The authors noted that the individual with xeroderma pigmentosum did not respond differently from the other subjects (Cottini and Mazzoni, 1939). Klar (1938) and Rhoads et al. (1954) reported potentially deleterious epidermal changes in men who had experienced accidental dermal exposures to benzo[a]pyrene.

Epidemiologic Studies

Oral. There are no studies of oral exposures to identified PAHs in the literature. PAHs are known to occur in some foods as by-products of the preparation process and in others as a consequence of environmental pollution, but their role as dietary causes or contributors to cancer can only be assessed indirectly in epidemiologic studies of nutrition. This body of data is not specific for PAHs and beyond the scope of this document.

Other Routes. Epidemiologic studies linking PAHs to human health effects have studied human exposures as they actually occurred. These studies can, therefore, be evaluated to determine the health effects of exposure only to mixtures of PAHs by inhalation and dermal contact. Occupational exposures to PAH-containing complex mixtures and 70 industrial processes that generate PAHs have been evaluated by the IARC Working Group. It is not possible to determine from these studies the effect of individual

PAHs. Toxic effects include a variety of skin lesions and noncancer lung diseases such as bronchitis.

The compiled case reports and large cohort studies of exposures to complex mixtures of PAHs have shown strong cause-effect relationships in that high rates of cancer can be associated with exposure, dose-response gradients can be demonstrated and results are consistent across time and geographic location. The data include thousands of case reports spanning over 200 years and cohort studies of cancer incidence and mortality from several countries. Data summarized in IARC (1984) monographs of polynuclear aromatic compounds demonstrate carcinogenicity to humans from industrial exposures to mixtures of PAHs in aluminum production, coal gasification, coke production and iron and steel founding. Bitumens, coal tars and derived products, shale oils and soots have also been evaluated for their human health effects (IARC, 1985). A detailed evaluation of PAH mixtures can be found in the document, "Carcinogen Assessment of Coke Oven Emissions" (U.S. EPA, 1984b). Evidence of human carcinogenicity based largely on the IARC review mentioned is summarized in Table VI-1. The extent to which the data derived from these types of exposure will be applicable to drinking water exposures is unknown and creates the major difficulty for the risk assessment.

Despite the high levels and large proportion of PAHs, other exposures to gases and metals are always present in these occupational environments. For example, coke oven emissions contain other carcinogenic agents including arsenic, beryllium, chromium, nickel, 2-naphthylamine and benzene. Asbestos is a possible exposure for chimney sweeps (soot). Cocarcinogens (compounds

TABLE VI-1

IARC Determinations of PAH Carcinogenesis Based on Human Data[a]

Agent or Process	Route[b]	Sites	Human[c]	Carcinogenic Factor[d]	Data Base
Coal gasification	Inhalation, dermal	lung	sufficient	Coal tar from the destructive distillation of coal.	Analysis of 3753 skin tumors, bladder cancer deaths by occupational group; cohort studies of gas industry.
		bladder	limited	Older coal gasification processes.	
		skin[e]	sufficient	Coal tar from the destructive distillation of coal.	
Coke production	Inhalation, dermal	lung	sufficient	Possible coal tar fume.	Analysis of 3753 skin tumors, bladder cancer deaths by occupational group; cohort studies in coking plant, in steel industry and coke oven workers. Cohort studies in five countries; two case-control studies.
		skin	sufficient	Coal tar from destructive distillation of coal.	
		kidney	limited	Coal tar from destructive distillation of coal.	
		intestinal pancreatic	inadequate	Coal tar from destructive distillation of coal.	
Aluminum production	Inhalation	lung	limited	Aluminum production industry, possibly pitch fume.	Cohort studies in aluminum production plants in USSR, USA, Canada, Norway (21,829 workers in USA).
		bladder	limited		
		pancreas	inadequate		
Bitumens	Inhalation, dermal	lung	inadequate	Bitumens only.	Cohort study of roofers, includes coal tar exposures.
		skin	inadequate		
Coal tars and derived products	Inhalation, dermal	skin	sufficient	Coal tars from destructive distillation of coal, coal tar pitches.	Numerous case reports of occupational and pharmaceutical exposures; cohort study of roofers.
		skin	limited	Creosotes.	

TABLE VI-1 (cont.)

Agent or Process	Route[b]	Sites	Human[c]	Carcinogenic Factor[d]	Data Base
Shale oils	inhalation, dermal	skin	sufficient	Shale oils.	Case reports; occupational mortality; cohort study of shale oil workers; mule-skinners cancer.
Soots (chimney origin)	inhalation, dermal	skin	sufficient	Soot.	Historic and contemporary case reports from various countries; cohort studies of sweeps in four countries.

[a]This table is summarized from IARC (1984, 1985).

[b]Both inhalation and dermal exposures are possible in most cases. Skin contact is most likely for coal tars and derivatives, shale oils and soots and in occupations where these products are used such as textile manufacturing (mule skinners).

[c]The preamble to IARC document states "The evidence for carcinogenicity from studies in humans is judged to fall into one of four groups, defined as follows: 1) ***Sufficient evidence*** of carcinogenicity indicates that there is a causal relationship between the exposure and human cancer; 2) ***Limited evidence*** of carcinogenicity indicates that a causal interpretation is credible, but that alternative explanations, such as chance, bias or confounding, could not adequately be excluded; 3) ***Inadequate evidence***, which applies to both positive and negative evidence, indicates that one of two conditions prevailed: (a) there are few pertinent data, or (b) the available studies, while showing evidence of association, do not exclude chance, bias or confounding; 4) ***No evidence*** applies when several adequate studies are available that do not show evidence of carcinogenicity.

[d]PAHs have been measured in the occupational setting. All processes listed included exposure to complex mixtures including at least seven of those PAHs of concern in this document (see Chapter II for occurrence).

[e]Case reports of PAH associated skin cancers include a large proportion of scrotal cancers.

that enhance tumor production in experimental animals when applied concurrently with carcinogens) and tumor promoters, both PAH and non-PAH, may also be components of the mixtures.

Data published since the IARC summary continue to provide evidence of the carcinogenicity of PAH mixtures from inhalation and dermal exposures. Hansen (1983) conducted a 5-year mortality follow-up among Danish chimney sweeps and noted a 2-fold overall excess compared with males employed in other occupations. When the excess was examined by age and cause, it was found that the high mortality of the sweeps in the older age group (45-74 years) was exclusively due to an excess number of deaths from cancer and ischemic heart disease, whereas the excess among the younger sweeps (15-44 years) was due to other causes. Those sweeps dying of cancer or ischemic heart disease had been occupationally exposed for an average of 30 years.

Auto mechanics are considered to be another group at risk of occupational exposure to PAH mixtures but have not been as thoroughly studied as chimney sweeps. The recent work of Hansen (1989) reports on the 10-year mortality among a cohort of Danish auto mechanics. Overall, a 21% increase in total mortality was observed in relation to a comparable population of skilled workers presumed to be unexposed to PAH mixtures. In addition to the moderate increase in cancer mortality, attributed primarily to an excess of pancreatic cancer, a statistically significant increase in ischemic heart disease deaths was found.

Gustavsson et al. (1988) conducted a study of cancer incidence in a cohort that comprised nearly all Swedish chimney sweeps employed any time

between 1918 and 1980. Earlier mortality analyses among the cohort had shown excess mortality from cancers of the lung, esophagus, liver, and from leukemia. Increased mortality from ischemic heart disease was also found. This study of incident tumors found a significant excess of cancers of the lung, esophagus, and bladder, which contributed to an overall excess incidence in the cohort. A nearly significant increase of hematopoetic malignancies that included both multiple myeloma and leukemia was also observed. Histologic examination attributed the lung cancer increase to an excess of squamous cell and undifferentiated/small cell carcinomas. The excess risk for the solid tumors depended mainly on an excess during followup for more than 30 years from start of exposure.

Siemiatycki et al. (1988) explored potential occupational exposures as risk factors for about 20 cancer sites in a population-based incident case-control study in Montreal. The study was designed to generate hypotheses for further analytic study. This particular report examined associations with occupational exposures to 10 types of exhaust and combustion products and the cancer sites of interest. The 10 exposures were classified as follows: four were exhaust products of internal combustion engines, distinguished by the type of engine fuel used (gasoline, diesel, jet fuel or propane), and the remaining six were products derived from "nonengine" combustion of coal, coke, wood, liquid fuels (heating oil, kerosene, naphtha and lamp oil), natural gas and propane. Among the many relationships examined in the study, several are of particular importance. A major finding was the association between squamous-cell lung cancer and both diesel and gasoline exhaust. Five other relationships were noted and considered by the investigators to warrant further study regarding the role

of PAH-containing mixtures in the etiology of human cancer. The most promising leads were the following: 1) the effects of exposure to gasoline and diesel exhaust on the occurrence of colorectal cancers; 2) the effects of exposure to gasoline exhaust on the occurrence of kidney cancer; 3) the effects of exposure to coal combustion products on the occurrence of pancreatic cancer and possibly on nonadenoma lung cancer; 4) the effects of exposure to combustion products of liquid fuels on the occurrence of prostatic cancer; and 5) the effects of exposure to natural-gas combustion products on the occurrence of bladder cancer (Siemiatycki et al., 1988).

The findings from these four studies provide further important evidence that occupational exposure to complex PAH mixtures, which occurs primarily via inhalation or dermal contact, may also result in cancers at sites other than those logically expected, i.e., lung and skin. It is unclear at this time as to what the mechanism of exposure to the target organs might involve. Gustavsson et al. (1988) propose that, for chimney sweeps, PAHs probably enter the body through direct resorption in the bronchi, or are expectorated, swallowed and absorbed through the gastric and intestinal mucosa. It is likely that both routes of uptake are relevant.

The reported increase in ischemic heart disease in three of these studies (Hansen, 1983, 1989; Gustavsson et al., 1988) is quite striking when viewed in light of the normal expectation of a "healthy worker effect". The usual expectation in an occupational study of morbidity and/or mortality is that there will be a depression of the standardized morbidity ratio (SMR) for cardiovascular diseases. This occurs due to pre-selection of the workforce for fitness for physical labor. Further studies specifically

designed to address the issue must be carried out to determine if this is indeed a true disease excess and not an artifact of the study methods.

Sensitive Populations

Data to determine what individuals within the general population may be more susceptible to PAH-induced toxicity are generally lacking. One factor that increases an individual's risk for lung cancer is cigarette smoking, which is suspected to act synergistically with other occupational exposures such as asbestos and uranium mine atmospheres. Although high exposure to sunlight and low level of skin pigmentation are well documented risk factors for skin cancer, no data exist to determine whether individuals with these risk factors are more susceptible to PAH-induced skin lesions.

Aryl hydrocarbon hydroxylase (AHH) is the term given to the enzyme or enzyme systems involved in the oxidative metabolism of PAHs, especially benzo[a]pyrene. Genetically determined individual differences in the ability to induce both increased levels and isozymes of AHH have been proposed as a possible reason for differences among humans in susceptibility to lung cancer (for further discussion see Chapters III and VII). Some early studies claimed that persons with lung cancer had higher inducibility of AHH in cultured lymphocytes, but subsequent studies have not all supported this claim (Perera, 1990). Busbee et al. (1980) present evidence supporting the need to analyze AHH in more than one tissue and recommend measuring inducibility in alveolar macrophages and lung tissue as well as lymphocytes. More research will be required to determine the sensitivity and specificity of this enzyme system as a predictor of human susceptibility to lung cancer.

Carcinogen-DNA adducts may ultimately prove to be helpful in identifying those persons at high risk of adverse outcome following exposures to PAH mixtures. It is currently possible to detect PAH-DNA adducts in several types of human tissue and sera in those known to be either occupationally or environmentally exposed (Herbert et al., 1990; Hemminki et al., 1990; Perera, 1990). However, the role of adducts in inducing cancer or other adverse outcomes is still a matter of much scientific discussion. Long-term validation studies are required in appropriate human populations before wide-scale monitoring for these biomarkers can be considered as a more refined and specific way of predicting who may or may not be at higher risk (i.e., more susceptible) of a particular outcome.

Summary

With the exception of naphthalene, which most commonly causes acute hemolytic anemia, no data were located directly pertaining to PAH toxicity to humans by the oral route of exposure. A large body of literature exists on toxicity and carcinogenicity to humans of a variety of mixtures containing PAHs. The summarized IARC data clearly demonstrate that inhalation and dermal exposures to complex mixtures containing PAHs result in lung, skin and other cancers. The most recent occupational studies continue to provide compelling evidence regarding the carcinogenicity of PAH mixtures. The increased risk of cardiovascular disease is noteworthy and must be studied further. In the occupational environments studied, some airborne PAHs may be cleared by mucociliary action and swallowed. This information may be useful for determining the potential carcinogenicity to humans of ingested PAHs.

One must be cautioned that using these data to predict the effects of exposure to any single PAH is inappropriate. Furthermore, most if not all PAH exposures occurring to humans are in the form of a complex mixture of the compounds, it seems both unrealistic and unnecessary to attempt to assess the potential role of each individual compound. Studies to date clearly implicate this chemical family as human carcinogens at relatively intense, high level exposures. Recent work has also more clearly delineated the role of PAHs and benzo[a]pyrene in particular as causal carcinogenic constituents in the highly complex mixture known as tobacco smoke (Wynder, 1988). While all the information summarized in this chapter is important for evaluating and clarifying the role of PAH mixtures and their relationship to cancer and cardiovascular disease in humans, the direct relevance of the information to the assessment of any adverse effect arising from ingestion of PAHs in drinking water remains to be determined.

VII. MECHANISMS OF TOXICITY

The carcinogenic potential of PAHs has been a subject of study for >50 years. They were among the first chemical agents shown to be tumorigenic, and their role in the causation of human cancers has been a subject of speculation almost since Pott's (1775) first description of "soot wart" in chimney sweeps in the 18th century. Noncarcinogenic health effects of PAHs have not been studied extensively; mechanisms of PAH toxicity apart from their ability to induce neoplasia are, thus, not well elucidated.

Mechanisms Involved in PAH Carcinogenicity

Structure Activity Relationships. Many physico-chemical and enzymatic parameters must be considered with respect to PAH carcinogenicity. Solubility and intracellular localization proximate to metabolic enzymes are likely to be important determinants of the carcinogenicity of a particular PAH.

Following the identification of the first carcinogenic hydrocarbon from soot, benzo[a]pyrene, an intensive effort was mounted to isolate the various active components of carcinogenic tars (IARC, 1973). It became apparent that carcinogenic PAHs are structurally derived from the simple angular phenanthrene nucleus (Pullman and Pullman, 1955; Arcos and Argus, 1974). Unsubstituted PAHs with fewer than four condensed rings that have been tested have so far not shown tumorigenic activity. Furthermore, of the six possible PAHs with arrangements with four benzene rings, only three of these compounds are active as carcinogens, namely benzo[c]phenanthrene, benz[a]anthracene and chrysene. The unsubstituted penta- and hexacyclic

aromatic hydrocarbons are generally the most potent carcinogens of the series. These include benzo[a]pyrene, dibenz[a,h]anthracene, dibenzo-[a,h]pyrene, dibenzo[a,i]pyrene, dibenzo[a,l]pyrene, dibenzo[a,e]pyrene, benzo[b]fluoranthene, and benzo[j]fluoranthene. Less potent carcinogens are the dibenzanthracenes and dibenzophenanthrenes. Only a few heptacyclic hydrocarbons show carcinogenic activity, including phenanthro[2',3':3,4']-pyrene, peropyrene, and dibenzo[h,rst]pentaphene. There are very few known carcinogenic hydrocarbons with more than seven unsubstituted aromatic rings (Santodonato et al., 1981).

Active carcinogens are also found among those PAHs containing a non-aromatic ring. Examples of this type of compound are cholanthrene, 1,12-ace-benz[a]anthracene, 8,9-cyclopentanobenz[a]anthracene, 6,7-acebenz-[a]anthracene, acenaphthanthracene, 1,2,5,6-tetrahydrobenzo[j]cyclo-pent[f,g]aceanthrylene, and "angular" steranthrene. All of these compounds contain an intact conjugated phenanthrene moiety.

Alkyl substitution at certain positions in the ring system of a fully aromatic hydrocarbon has been observed to confer carcinogenic activity or to enhance carcinogenic potency. Arcos and Argus (1974) noted that monomethyl substitution of benz[a]anthracene makes these dervatives potent carcinogens in mice. Potency depends on the position of substitution in the decreasing order position 7 > 6 > 8≈12 > 9. A further enhancement of carcinogenic activity is produced by appropriate dimethyl substitution of benz[a]anthra-cene. Carcinogenic compounds are produced by 6,8-dimethyl-, 8,9-dimethyl-8,12-dimethyl-, 7,8-dimethyl-, and 7,12-dimethyl-substitution. The last

compound is among the most potent PAH carcinogens identified in carcinogenesis bioassays. It has not been shown, however, to occur as a product of fossil fuel pyrolysis or to be a major environmental contaminant. Carcinogenic trimethyl- and tetramethyl-benz[a]anthracenes are known, and their relative potencies are comparable with the parent 7,12-dimethylbenz[a]anthracene. Methyl substitution in the angular ring of benz[a]anthracene tends to reduce the carcinogenic potential of the molecule; 4,5-dimethylbenz[a]anthracene may be an exception in this regard.

Alkyl substitution of partially aromatic condensed ring systems may also enhance the carcinogenicity of a compound. 3-Methylcholanthrene, a highly potent carcinogen, is the best example of this type.

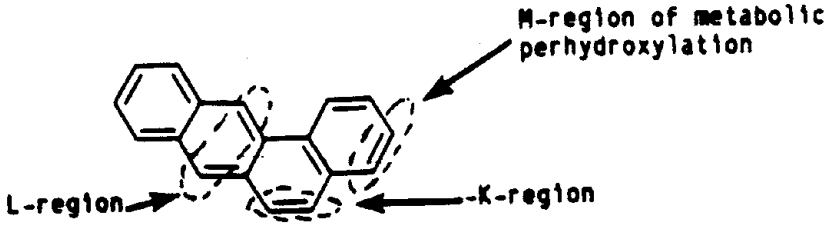
Data derived largely from skin painting studies have shown that the carcinogenicity of PAHs tends to decrease with alkyl substituents longer than methyl, possibly due to a decrease in transport through cell membranes. Benz[a]anthracene is especially sensitive to decreased carcinogenicity caused by the addition of bulky substituents at the 7-position. This observation lent credence to the view for most polycyclics that high reactivity of the mesophenanthrenic region (called the "K-region") was a critical determinant for carcinogenicity. Other studies, however, show that the K-region may not be involved in binding to DNA, a step considered important in the carcinogenic process (Blobstein et al., 1976; Weinstein et al., 1976; Moore et al., 1977).

Partial hydrogenation of the polycyclic aromatic skeleton has been observed to decrease the carcinogenic potency of some PAHs. This was noted

for hydrogenated derivatives of benzo[a]pyrene, benz[a]anthracene, and 3-methylcholanthrene. By constrast, the carcinogenicity of dibenz[a,h]anthracene, dibenzo[a,i]pyrene, and dibenzo[a,h]pyrene is not significantly altered by mesohydrogenation, probably because extensive resonance is preserved in the molecule. Moreover, 5,6-dihydrodibenz[a,h]anthracene actually displayed a 4-fold increase in carcinogenicity by comparison with the parent unsaturated hydrocarbon (Arcos and Argus, 1974), possibly due to the hydrophilicity and ease of intracellular transport of its dihydrodiol derivative.

For many years, investigators have sought a common molecular feature among PAH carcinogens that would serve to explain their biologic activity. The "electronic theory of carcinogenesis" has relied upon an analysis of the influence of electron density at specific molecular regions to explain unique reactivity with cellular constituents. A basic assumption arising from the work of the Pullmans and others (Pullman and Pullman, 1955) was that a mesophenanthrenic region ("K-region") of high π-electron density and with a propensity for addition reactions was a critical structural feature for polycyclic carcinogens. In an expansion of this hypothesis, further biologic significance was attributed to the concomitant presence of a rather unreactive meso-anthracenic region ("L-region") for high carcinogenicity. In addition, a region of comparatively low reactivity, which characteristically undergoes metabolic perhydroxylation (corresponding to the 3,4-positions of benz[a]anthracene), had been designated the M-region. According to the theory, only binding of the K-region to critical cellular sites would initiate tumor formation. Binding at the L-region could cause no tumorigenic effect, while inactivation would be produced by metabolic

perhydroxylation in the M-region. The three regions of reactivity are illustrated below in the benz[a]anthracene skeleton:



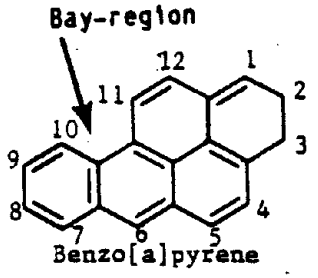
The electronic K-L theory of carcinogenic reactivity has encountered numerous inconsistencies, primarily because these relationships were derived from physico-chemical properties of the parent hydrocarbon and gave no consideration to the biologic effects of metabolites.

That many chemical carcinogens require metabolism to reactive electrophilic forms for their activity is generally accepted. PAHs are certainly in the class of those carcinogens requiring so-called "metabolic activation" for binding to critical macromolecules. The realization that this metabolic activation to reactive intermediates was a necessary first step has made possible an understanding of some of the inconsistencies encountered in structure-activity theories, such as K-region binding, that are based solely on a consideration of the parent compound.

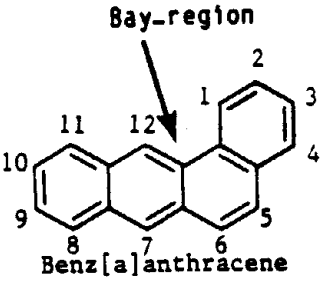
The metabolic processes undertaken by mammals exposed to PAHs are described in Chapter III. In general, they consist of oxidation steps catalyzed by cytochrome P-450-associated enzymes. Products include epoxide intermediates, dihydrodiols, phenols, quinones and combinations of these. These oxidized forms of PAHs have been shown to exhibit various biologic

activities. For example, various benzo[a]pyrene epoxides (including the K-region epoxide), phenols, quinones, diols and diol-epoxides have been shown to be mutagenic in several mammalian cell and bacterial assays (Gelboin, 1980; IARC, 1973; Schoeny et al., 1985; Chesis et al., 1984).

Research in recent years has focused attention on the potential reactivity of diol-epoxide metabolites of PAHs, and their ease of conversion to triol carbonium ions. The "bay-region" theory proposes that diol-epoxides, which are more readily converted to carbonium ions, will be better alkylating agents and thus mutagens and initiators of carcinogenesis (Jerina and Lehr, 1977; Wood et al., 1979). Examples of a bay-region in a polycyclic hydrocarbon are the regions between the 10 and 11 positions of benzo[a]pyrene and the 1 and 12 positions of benz[a]anthracene:



Benzo[a]pyrene



Benz[a]anthracene

The theory predicts that diol-epoxides in which the oxirane oxygen forms part of a bay-region (such as benzo[a]pyrene 7,8-diol-9,10-epoxide) will be more reactive and hence more carcinogenic than diol-epoxides in which the oxirane oxygen is not situated in a bay-region. The unique structural feature of the diol-epoxides appears to be that the epoxide is on a saturated angular benzo-ring, which forms part of a bay-region on the PAH. Perturbational molecular orbital calculations, which predict π-electron energy changes, indicate that the epoxides on saturated benzo-rings (which form part of the bay-region of a hydrocarbon) undergo ring opening to form a

carbonium ion much more easily than do nonbay-region epoxides (Pelkonen and Nebert, 1982). Synthetic bay-region diol-epoxides of benz[a]anthracene, benzo[a]pyrene, and chrysene have been shown to be more mutagenic in vitro and/or tumorigenic than other diol-epoxide metabolites, their precursor dihydrodiols, the parent hydrocarbons, or other oxidative metabolites. Moreover, quantum mechanical calculations provide support for the concept that reactivity at the bay-region is highest for all the diol epoxides derived from polycyclic hydrocarbons.

The bay-region concept has received enough confirmation to lead to suggestions that an analysis of theoretical reactivity in this manner may be useful in screening PAHs as potential carcinogens (Smith et al., 1978). Among several indices of theoretical reactivity examined, the presence of a bay-region for a series of PAHs displayed a high degree of correlation with positive carcinogenic activity (Table VII-1).

It is possible to predict what would be an ultimate carcinogenic form from the bay-region theory and a consideration of a PAH structure. There is, however, no way at the present time to predict whether a particular PAH will, in fact, be metabolized to a bay-region diol-epoxide in any given mammalian tissue. For example, benzo[e]pyrene is generally considered noncarcinogenic. Neither the parent compound nor the 9,10-dihydrodiol, the presumed bay-region diol-epoxide precursor, has significant initiating potency in mouse skin (IARC, 1983). It has been shown that neither cultured mammalian cells nor rat liver microsomes metabolize benzo[e]pyrene to the 9,10-dihydrodiol; furthermore, 9,10-dihydrodiol is not metabolized in these systems to the expected diol-epoxide. When synthetic benzo[e]pyrene 9,10-dihydrodiol was injected in newborn mice, hepatic tumors were observed,

TABLE VII-1

Reactivity Indices for Polycyclic Hydrocarbons[a]

Compound	K-region?	L-region?	Bay-Region	Carcinogenicity Index: Arcos and Argus, 1974	Carcinogenicity Index: Jerina and Lehr, 1977a,b
Naphthalene	-	-	-	0	-
Anthracene	-	+	-	0	-
Tetracene	-	+	-	0	-
Pentacene	-	+	-	0	-
Hexacene	-	+	-		
Benz[a]anthracene	+	+	+	5	+
Benzo[a]tetracene	+	+	+		-
Phenanthrene	+	-	+	0	-
Benzo[c]phenanthrene	+	-	+	4	+
Chrysene	+	-	+	3	+
Benzo[b]chrysene	+	+	+		-
Picene	+	-	+	0	-
Triphenylene	-	-	+	0	-
Benzo[g]chrysene	+	-	+	17	++
Dibenz[a,c]anthracene	-	+	+	3	+
Dibenz[a,j]anthracene	+	+	+	4	+
Dibenz[a,h]anthracene	+	+	+	26	++
Naphtho[2,3-b]pyrene	+	+	b/	27	++
Benzo[a]pyrene	+	-	+	73	++++
Benzo[e]pyrene	+	-	+	2	+
Dibenzo[a,l]pyrene	+	-	+	33	++[c]
Dibenzo[a,i]pyrene	+	-	+	74	++++
Dibenzo[a,e]pyrene	+	-	+	50	+++
Dibenzo[a,h]pyrene	+	-	+	70	++++
Tribenzo[a,e,i]pyrene	-	-	+	16	++

[a]Source: Smith et al., 1978

[b]This compound does not strictly possess a bay-region, but does contain a "pseudo" bay-region.

[c]Jerina and Lehr (1977b) have assigned this as ++++.

suggesting that the neonatal liver possessed some metabolic capacity not extant in mouse skin or *in vitro* systems (Buening et al., 1980). Synthetic *trans* and *cis* 9,10-diol-11,12-epoxides produced hepatic and pulmonary tumors, respectively, in newborn mice (Chang et al., 1981). Thus, a bay-region diol-epoxide of benzo[e]pyrene appears to be carcinogenic, but is not formed *in vivo* under normal circumstances.

Various researchers have attempted to refine and extend the K- or bay-region theories to allow for predictivity of carcinogenic potential from a consideration of PAH structure. For example, the reactivity index used by Mohammed (1983) established a correlation between the K-region reactivity and the bay-region theory to determine PAH metabolic products. A later development considered contribution of both sigma and pi-electronic systems when calculating bond superdelocalizability (Mohammed, 1985). The potential of PAHs for one-electron oxidation, as discussed by Cavalieri and Rogan (1983) is likely to be a factor in carcinogenicity and needs inclusion in a predictive quantitative structure activity scheme.

Tissue Specificity of PAH Metabolism. The capacity of mammalian liver to metabolize PAHs is well-documented. Other tissues may also be involved in the metabolism of a specific PAH to reactive forms. AHH, the primary benzo[a]pyrene metabolizing system, has been found in human liver, placenta, lymphocytes, monocytes and lung macrophages (Gelboin, 1980).

Cytochromes P-450 and associated enzymes are known to be present in mammalian lungs at lower concentrations than are generally found in the liver. Studies using lung microsomal preparations, cultured trachea and alveolar macrophages and isolated perfused lungs have shown that benzo[a]pyrene is

metabolized to the same oxidized forms as are produced by the liver. What differs is the proportion of classes of metabolites, the rate of metabolism and the ultimate tissue distribution of metabolites (Moore and Cohen, 1978; Santodonato et al., 1981). Co-administered particles or gases may also influence the measures (Warshawsky et al., 1981; Schoeny and Warshawsky, 1983).

Intestinal mucosa has been shown to have MFO activity and presumably to have the capacity to metabolize PAHs. It has also been observed that the susceptibility of mice to induction of forestomach tumors can be correlated with these enzymatic activities. Generally, levels of benzo[a]pyrene metabolizing enzymes in rodent small intestine and colon are rather low and not readily inducible to higher levels (Santodonato et al., 1981).

Genetic Control of PAH Metabolism. As noted in the preceding sections and in Chapter III, oxidative metabolism of PAHs is generally accomplished through a series of enzymes associated with cytochrome P-450. Both quantitative and qualitative changes in these enzymes can be induced in response to exposure to a variety of agents, including the PAHs themselves. PAHs can, thus, be responsible for inducing enzymes for their own metabolism, including activation to mutagenic and carcinogenic forms. The degree of inducibility as well as the spectrum of enzymes produced in response to a particular agent are not only tissue-specific, but also species/strain specific. In particular the enzyme system, loosely called AHH, that is involved in PAH metabolism (especially benzo[a]pyrene) has been shown to be under genetic control. The genetics of AHH induction has been well-characterized in mouse strains observed to be "responders" or "nonresponders" to

induction by PAH (usually 3-methylcholanthrene) (Nebert et al., 1981). The expression of the responsive trait is due to inheritance of an allele of the Ah locus, which codes for a cytosolic receptor. This receptor regulates expression of a set of cytochrome P-450 and associated enzymes. When PAH, 2,3,7,8-tetrachlorodibenzo-p-dioxin or other compounds are bound to the receptor, the sets of genes coding for the various metabolic enzymes are induced to higher levels of synthesis. Responsive mice have a high-affinity receptor that readily binds to a number of PAHs and is thus more easily induced in responders than in nonresponders, which have a low affinity receptor (Nebert et al., 1982; Eisen et al., 1983). A number of biologic effects observed in test animals as a consequence of PAH exposure can be shown to be affected by the responsiveness or nonresponsiveness of the strain. For example, mouse strains C3H/HeJ and C57Bl/6J, both responsive to dibenz[a,h]anthracene induction of AHH, were more susceptible to carcinogenesis after s.c. exposure to that compound than were two nonresponsive strains, AKR/J and DBA/2J. Incidences were 24/30 for C3H/HeJ and 16/30 for C57Bl/6J by comparison with 0/30 and 1/30 for AKR/J and DBA/2J, respectively (Lubet et al., 1983a). Similar results were reported by Kouri et al. (1983), who also showed that among progeny of a heterozygous responder (B6D2F1) x a homozygous nonresponder (D2) backcross, that susceptibility to subcutaneous tumor formation by dibenz[a,h]anthracene segregated with responsiveness.

Route of exposure plays a part in the extent of biologic effects produced by PAHs in responsive and nonresponsive mice. When benzo[a]pyrene is administered topically, s.c., i.p. or intratracheally, responsive mice are more likely than nonresponders to develop tumors or toxic responses at the site of application. When benzo[a]pyrene exposure is through the oral

route, nonresponsive mice are more likely to develop leukemia or bone marrow toxicity (Nebert, 1981; Legraverend et al., 1983). This is due to the effects of first pass metabolism and excretion of the PAH. Nebert et al. (1980) showed, for instance, that after oral exposure 10-20 times more [^{3}H] benzo[a]pyrene reaches the bone marrow and spleens of nonresponders than of responsive mice.

When fetal toxicity is being assessed there is the additional complication of the dam's responsiveness. This will determine to some extent the dose of maternally administered compound that is delivered to the fetuses, which will themselves be responders or nonresponders. For instance, when nonresponder dams received oral benzo[a]pyrene on days 2 and 10 of gestation, nonresponder fetuses showed a greater toxic response and more malformations than did responders. When heterozygous responder dams were similarly exposed, there was no segregation of toxic response with the nonresponder allele; that is, both nonresponder and responder fetuses were equally affected. In the case of the nonresponder dam, no induction of benzo[a]pyrene metabolism took place and the fetuses received a larger dose of the PAH. This allowed the genetic differences in the embryos to be detected; in this instance, greater amounts of toxic benzo[a]pyrene metabolites have been isolated from nonresponder fetuses than from responders. When the responsive dam was exposed, intestinal and hepatic metabolism of benzo[a]pyrene was increased. Fetuses received less PAH than in the above instance; thus there was less overall fetal toxicity, and the genetic differences among individual fetuses was not seen. The reverse of these effects was observed when dams were treated intraperitoneally (Legraverend et al., 1983).

It has been shown that ovarian AHH is inducible in responsive (e.g., C57B16/N) but not in nonresponder (e.g., DBA/2N) mouse strains. Responders have likewise been observed to be more sensitive to oocyte destruction by PAHs than nonresponders. The susceptibility to oocyte killing by PAHs, however, is not inherited as a simple autosomal dominant trait as is responsiveness. This suggests that other factors in addition to ovarian PAH metabolism are involved in oocyte destruction by PAH (Mattison et al., 1983).

An area of considerable uncertainty with regard to the carcinogenic hazard of PAHs to humans involves the relationship between AHH activity and cancer risk. Genetic variation in AHH inducibility has been implicated as a determining factor for susceptibility to lung and laryngeal cancer (Kellerman et al., 1973). It was suggested that the extent of AHH inducibility in lymphocytes was correlated with increasing susceptibility to lung cancer formation.

Paigen et al. (1977, 1978a,b) examined the question of genetic susceptibility to cancer and concluded that epidemiologic evidence supports this hypothesis. Moreover, they were able to show that AHH inducibility in lymphocytes segregates in the human population as a genetic trait. However, their studies failed to find a correlation between this inducibility and presumed cancer susceptibility, either among healthy progeny of cancer patients or in patients who had their cancer surgically removed. It is noteworthy that previous investigations on AHH inducibility were conducted in persons with active cancer.

In an attempt to elucidate sources of variability in human AHH inducibility, a study was undertaken using cultured monocytes obtained from 10 sets of monozygotic twins and 17 sets of dizygotic twins. Both benz[a]-anthracene-induced and basal levels of AHH were determined. Genetic factors were found to account for 50-66% of observed interindividual differences in AHH inducibility. The authors felt that a relatively few number of genes were involved in regulation of AHH induction (Okuda et al., 1977). It would seem that susceptibility to carcinogenesis by PAHs could be a genetically determined trait at the level of metabolism. The contradictory nature of studies in this area, however, point to the fact that many factors regarding PAH activation and subsequent steps in the carcinogenic process need to be elucidated.

Other Pathways Involved in Activation of PAH. PAHs are photoreactive compounds. Visible light can be absorbed by several PAHs at sufficient energy levels to result in photooxidation. Benzo[a]pyrene can be photo-oxidized to the 1,3-, 6,12- and 3,6-quinones as well as to dihydrodiols and phenols (Katz et al., 1979; Gibson and Smith, 1979). It is likely that quinone formation is through a phenoxy radical and that phenol and dihydrodiol formation may also proceed through radical formation (Inomata and Nagata, 1972; Jeftic and Adams, 1970; Greenstock and Wiebe, 1978). Benz[a]anthracene is also known to form quinones as a consequence of light exposure in aqueous media (Mill et al., 1981).

Cavalieri et al. (1985) described a 1-electron PAH oxidation pathway as opposed to the 2-electron mono-oxygenase pathway. This produces radical cations or radicals depending on the PAH. In addition to cytochrome P-450,

hydroperoxidases, peroxidases and prostaglandin synthetase can participate in these types of reactions. PAH free radicals (for example, 6-oxy-benzo[a]pyrene free radical) have been implicated in PAH binding to DNA (Santodonato et al., 1981).

Cobalt γ-irradiation of PAHs, including benz[a]anthracene, benzo[a]-pyrene and chrysene, has resulted in formation of mutagenic compounds. Likewise UV-irradiated benzo[a]pyrene was shown to be mutagenic for Salmonella typhimurium (Gibson et al., 1978). The potential for visible light and other electromagnetic radiation to activate PAHs has implications for mechanisms involved in PAH skin carcinogenesis.

PAH Involvement in Carcinogenic Processes. A description of the hypothetical mechanisms purported to be involved in the carcinogenic processes is beyond the scope of this document. Suffice it to say that PAHs may participate in many proposed carcinogenic steps. The majority of PAHs described in this document are mutagenic in one or more test systems as described in Chapter V. Many have been shown to be initiators in mouse skin initiation-promotion assays. PAHs can, thus, generally be described as capable of DNA-binding, or of causing DNA damage leading to mutations, which could be involved in the initiation phase of carcinogenesis.

Consideration must also be given to the fact that, in addition to the initiation of resting cells by a chemical carcinogen, a promotion phase involving cell proliferation is also involved in skin carcinogenesis (Yuspa et al., 1976). Promotion is likely to be a phase common in the carcinogenic process for most tissues. Certain PAHs may function only as initiators and have no promoting ability. It would appear, however, that

the more potent complete carcinogens also serve as promoters by interacting with cellular membranes, altering genetic expression, or in some other fashion causing cell proliferation. It should be noted, also, that as PAHs have been shown to have various immunosuppressive effects, they may impair the body's capacity for immune surveillance of neoplastic growths. PAHs may, thus, play a part in all steps of a carcinogenic process.

Mechanisms Involved in Noncarcinogenic Endpoints

The preferred target sites of PAHs appear to be rapidly proliferating tissues such as intestinal epithelium, bone marrow, lymphoid tissue and gonads. This has led investigators to the hypothesis that the toxic effects of PAHs are due to a specific attack on DNA of cells in the DNA synthesis or S phase of the cell cycle. Alterations in enzyme activity resulting from the direct attack on DNA may also have important significance to the adverse effects resulting from PAH exposure. Information on certain PAHs not considered in the rest of the document is presented here for illustrative purposes.

Hemolymphatic System. 7,12-Dimethylbenz[a]anthracene is well known for its effects on the hematopoietic system. Female Sprague-Dawley rats fed 112 or 133 mg/kg 7,12-dimethylbenz[a]anthracene developed pancytopenia due to a severe depression of hematopoietic and lymphoid precursors (Cawein and Sydnor, 1968). Maturation arrest occurred at the proerythroblast, promyloblast and promegakaryocytoblast levels; no injury occurred to stem cells or circulating formed elements. In another study, rats receiving 300 mg/kg (orally) and 50 mg/kg (i.v.) of 7,12-dimethylbenz[a]anthracene displayed extreme atrophy of hematopoietic elements, shrinkage of lymphoid organs,

agranulocytosis, lymphopenia and progressive anemia (Philips et al., 1973). Similar results have been noted in mice and rats given repeated injections of dibenz[a,h]anthracene. Dibenz[a,h]anthracene administered to mice in weekly subcutaneous injections for 40 weeks caused an increase in the number of lymph gland stem cells, an overall decrease in lymphoid cells, and dilation of lymphoid sinuses. The weights of the spleens of mice treated with dibenz[a]anthracene were also significantly less than those of both controls and animals treated with anthracene or benz[a]anthracene (Hoch-Ligeti, 1941).

In a similar study, rats given subcutaneous injections 5 times weekly for several weeks underwent changes in lymphoid tissue characterized by the presence of extravascular red blood cells in the lymph spaces and the presence of abnormal large pigmented cells (Lasnitski and Woodhouse, 1944). The noncarcinogen, anthracene, did not produce as dramatic a change in lymphoid tissue. These studies led investigators to believe that inhibition of DNA replication may be involved since only very rapidly proliferating hematopoietic elements were affected.

Acute hemolytic anemia is the most frequent manifestation of naphthalene poisoning in humans and has been described in newborn infants, children and adults (U.S. EPA, 1980c, 1987a). Pertinent information regarding the mechanism of naphthalene-induced hemotoxicity has been obtained by examining two groups of individuals that have been shown to be especially susceptible to naphthalene-induced hemolytic anemia.

The first group of naphthalene sensitive individuals comprises persons whose erythrocytes are deficient in glucose 6-phosphate dehydrogenase (G6PDH) or persons in whom erythrocyte GSH is rapidly depleted by certain oxidant chemicals (Grigor et al., 1966; Naiman and Kosoy, 1964; Valaes et al., 1963; Athreya et al., 1961; Dawson et al., 1958; Gross et al., 1958; Zinkham and Childs, 1958). The precise mechanism by which GSH is depleted or a deficiency of G6PDH leads to naphthalene-induced hemolysis in these cases is not clear. A deficiency of G6PDH will decrease the rate of conversion of nicotinamide adenine dinucleotide phosphate from its oxidized (NADP) to its reduced form (NADPH). One hypothesis for increased naphthalene sensitivity in G6PDH-deficient individuals is that the decreased availability of NADPH will decrease the conversion of oxidized glutathione to GSH, reduce the rate of conjugation and excretion of naphthalene metabolites and increase the accumulation of naphthalene metabolites in the body. A similar hypothesis may explain increased naphthalene sensitivity in individuals in which erythrocyte GSH can be rapidly depleted by certain oxidant chemicals (Naiman and Kosoy, 1964; Kellermeyer et al., 1962; Dawson et al., 1958; Gross et al., 1958; Zinkham and Childs, 1958). Gross et al. (1958) demonstrated a quantitative correlation between G6PDH deficiency and diminished levels of GSH in infants beyond 55 hours of age; however, diminished levels of erythrocyte GSH were observed in infants of less than 55 hours of age despite high levels of G6PDH activity. A second hypothesis for increased naphthalene sensitivity in G6PDH-deficient individuals is that the decreased availability of NADPH will, in the presence of oxidant metabolites of naphthalene, allow the accumulation of methemoglobin and products of its further irreversible oxidation (Kellermeyer et al., 1962).

The second group of naphthalene-sensitive individuals comprises neonates (Grigor et al., 1966; Naiman and Kosoy, 1964; Valaes et al., 1963; Dawson et al., 1958; Gross et al., 1958; Zinkham and Childs, 1958). The sensitivity of neonates to naphthalene is explained in part by the same factors that confer sensitivity to children and adults; namely, G6PDH deficiency and/or diminished levels of GSH as described above. Additional naphthalene sensitivity in newborns may be conferred by the immaturity of pathways necessary for the conjugation and excretion of naphthalene metabolites (Valaes et al., 1963). Evidence of the latter hypothesis is suggested by the finding that glucuronide excretion by human newborn infants increased gradually during the first week of life and that the initial levels and the rate of increase were lower in the premature infant than in the full-term infant (Brown and Burnett, 1957).

Cardiovascular System. Smoking is a known risk factor in atherosclerosis, and PAHs are a major component of cigarette smoke (McGill, 1977; Wald et al., 1973). Injections (i.m.) of pure PAHs into chickens has resulted in development of proliferative lesions bearing a close resemblance to human atherosclerotic plaques (Albert et al., 1977; Bond et al., 1981). It has been proposed that human atherosclerotic plaques are in fact benign hyperplastic lesions of mutagenic origin (Hartman, 1983). Majesky et al. (1983) undertook a study of PAH metabolism in two pigeon strains: atherosclerosis susceptible White Careneau (WC-2) and atherosclerosis resistant Show Racer (SR-39). After treatment with an enzyme inducer (3-methylcholanthrene) hepatic homogenates from the susceptible strain were more capable of benzo[a]pyrene metabolism than those from SR-39; furthermore 7,8-diol, a mutagenic/carcinogenic precursor, was increased. Assays of aortic homogenates showed that this tissue had an even greater capacity for

benzo[a]pyrene metabolism inducibility as a function of strain. It would appear that the responsive/nonresponsive concept may be applicable to nonrodent species and to noncancer endpoints.

Pulmonary System. Various investigators have observed that i.p. administration of naphthalene to rodents results in selective pulmonary bronchiolar epithelial cell (Clara) necrosis, but not hepatic or renal necrosis (Tong et al., 1981c, 1982; Warren et al., 1982; Mahvi et al., 1977; Reid et al., 1973). In an effort to determine the mechanism of action, numerous studies have focused on the biochemistry of naphthalene and the covalent binding characteristic of its metabolites.

Shank et al. (1980) found that mice pretreated with diethyl maleate prior to i.p. injection of naphthalene had three times the level of covalently-bound naphthalene metabolites in lung, liver, kidney and spleen. Studies with ^{14}C-naphthalene injected into mice revealed a similar binding pattern: binding was highest in the lung but low in the spleen. Increased binding corresponded to rapid and significant depletion of GSH in lung and liver, and to a lesser extent in the kidney. Covalent binding was dose-dependent and exhibited a threshold at dosages between 200 and 400 mg/kg. Warren et al. (1982) suggested that lung damage may be mediated by P-450 dependent metabolism and GSH depletion.

Buckpitt and Warren (1983) extended these studies, utilizing a variety of metabolic inhibitors. The results suggested that some of the metabolites involved in GSH depletion and covalent binding in extrahepatic tissues originated in the liver. Buckpitt et al. (1985, 1987) suggested that the difference in the rates of formation of specific metabolites between target

and nontarget tissues, and in different species, may reflect the stereochemistry of epoxidation by the tissue-specific P-450 isozymes. This may, in turn, relate to the selective pulmonary necrosis observed in mice.

Confirmation that P-450 was involved in pulmonary necrosis was obtained in the studies of Buckpitt et al. (1986). Liver microsomes from phenobarbitol-induced mice administered 300 mg naphthalene/kg i.p. exhibited 73% less covalent binding in the presence of piperonyl butoxide, a P-450 inhibitor, than controls. A similar degree of inhibition also was observed with SKF 525A. It was reported that piperonyl butoxide also blocked the pulmonary injury exhibited by naphthalene in controls.

Gastrointestinal System. Male and female rats were observed with injury to their intestinal epithelium after oral and intravenous administration of 7,12-dimethylbenz[a]anthracene (Philips et al., 1973). Further analysis demonstrated that the incorporation of ^{14}C-labeled thymidine into DNA of small and large intestine, spleen, cervical lymph nodes and other lymphatic structures was inhibited as much as 90% within 6 hours after dimethylbenz[a]anthracene administration. This finding indicates a strong inhibition of DNA synthesis and led the authors to believe that DNA in S phase cells is particularly susceptible to 7,12-dimethylbenz[a]anthracene and presumably to other PAH compounds as well. It has been proposed that somatic cell mutations, such as can be induced by PAH metabolites, may play a role in the formation of gastric polyps and chronic gastric ulcers (Hartman, 1983).

Gonads. Testicles and ovaries contain rapidly proliferating cells and thus, may be especially susceptible to damage by PAHs. Severe testicular

damage was induced in adolescent rats by a single intravenous injection of 7,12-dimethylbenz[a]anthracene (0.5-2.0 mg). Similar effects were produced in adult rats given 7,12-dimethylbenz[a]anthracene orally (20 mg) and intravenously (5 mg). Lesions involved destruction of spermatogonia and resting spermatocytes, both of which are the only testicular cells actively synthesizing DNA. The remaining germinal cells and Leydig cells were not damaged by 7,12-dimethylbenz[a]anthracene. No testicular damage was produced by a single feeding of 100 mg benzo[a]pyrene (Ford and Huggins, 1963).

In female mice, 7,12-dimethylbenz[a]anthracene was shown to cause destruction of small oocytes and a reduction in numbers of growing and large oocytes after oral administration. Mattison and Thorgeirsson (1977) have shown that destruction of primordial oocytes in mice following treatment with 3-methylcholanthrene was correlated with the genetic capability for PAH-induced increases in ovarian AHH activity. This indicates an apparent link between ovarian metabolism of PAH and ovatoxicity. This link is strengthened by the observation that the effective dose to kill 50% of small oocytes (ED_{50}) was less for responsive C57Bl/6N mice (3.38 μg/ovary) than for nonresponders DBA/2N (36.14 μg/ovary). An F_1 generation from mating of these two strains had an intermediate intraovarian injection ED_{50} of 8.27 μg/ovary.

Endocrine System. Few data are available concerning the mechanism of toxicity and the effect of PAHs on the endocrine system. Selective destruction of the adrenal cortex and induction of adrenal apoplexy by 7,12-dimethylbenz[a]anthracene has been demonstrated in rats after a single intragastric dose of 30 mg. The same amount of adrenal damage could be produced by a 5 mg dose of 7-hydroxymethol-12-methylbenz[a]anthracene, the principal

7,12-dimethylbenz[a]anthracene oxidative metabolite. No adrenal damage was noted with other 7,12-dimethylbenz[a]anthracene metabolites, thus indicating that a specific reactive intermediate may be responsible for this phenomenon (Santodonato et al., 1981).

Integumentary System. The integument is highly susceptible to agents that inhibit DNA synthesis as is evident in cancer patients receiving radiation treatment or chemotherapy. Such individuals show signs of alopecia, dermatitis and skin sloughing. Workers exposed to PAH-containing materials such as coal tar, mineral oil and petroleum waxes are known to develop chronic dermatitis and hyperkeratosis (Heuper, 1963; NAS, 1972a). It is well documented that the application of carcinogenic PAHs to mouse skin leads to destruction of sebaceous glands, skin ulcerations, hyperplasia and hyperkeratosis (Bock, 1964). Sebaceous glands undergo rapid cell turnover and are the most sensitive structures of the skin to PAH-induced toxicity.

Visual System. Ocular toxicity, particularly cataract formation, has long been associated with naphthalene administration in rodents and other laboratory animals as well as in humans (U.S. EPA, 1980c, 1987a).

Oral administration of naphthalene is believed to result in its metabolism in the liver and the metabolites then travel through the bloodstream to the eye where further metabolism takes place (van Heyningen, 1979). Evidence in rats and rabbits suggest that 1,2-dihydroxy naphthalene is enzymatically converted to 1,2-naphthoquinone which then reacts with eye proteins, resulting in damage (Pirie and van Heyningen, 1966; Rees and Pirie, 1967).

van Heyningen (1979), in her review of the literature, hypothesized that susceptibility to naphthalene-induced cataracts is more pronounced in rat and rabbit strains with lightly pigmented or dark eyes, due to the presence of polyphenol oxidase. This nonspecific enzyme, found only in pigmented tissues, catalyzes the formation of melanin from tyrosine.

van Heyningen and Pirie (1967) suggested that the toxic metabolite is 1,2-dihydroxy naphthalene. In gavage studies in which naphthalene was administered daily to 39 rabbits at 1 g/kg, they detected 1,2-dihydroxy naphthalene and 1,2-naphthoquinone in the eyes and three metabolites in blood that could be converted by different enzymes in the eye to 1,2-dihydroxynaphthalene. In more than half the rabbits, lens opacities and degeneration of the retina were observed. In addition, 1,2-naphthoquinone can oxidize ascorbic acid present in the aqueous and vitreous humors, resulting in oxalic acid formation as the ascorbic acid concentration decreases (van Heyningen, 1970a,b). Although ascorbic acid decreases in aqueous and vitreous humors, the level is maintained or increases in the eye lens itself (van Heynigen, 1970a). Presumably dehydroascorbic acid, formed by oxidation by naphthoquinones, penetrates the lens and is reduced to ascorbic acid. Ascorbic acid diffuses only slowly from the lens. Excessive depletion of ascorbic acid may account for the appearance of calcium oxalate crystals (Pirie and van Heyningen, 1966). GSH appears to be maintained at high levels in eye lens in spite of extensive oxidative reactions (van Heyningen, 1970a).

van Heyningen (1970b) found that the albino Wistar rat has only about 3% of the concentration of catechol reductase (an enzyme that catalyzes the interconversion of quinones and diols) found in the rabbit lens. The rat

also has less ascorbic acid in aqueous humor than the rabbit (van Heyningen, 1979). This would result in a higher level of 1,2-naphthoquinone. Thus, polyphenol oxidase may be the most important factor in the rat eye while catechol reductase may play a crucial role in ocular toxicity in the rabbit.

Rao and Pandya (1981) reported increased lipid peroxidation in the eyes of male albino rats administered 1 g napthalene daily for 10 days. Alkaline phosphatase showed a slight increase and aniline hydroxylase activity was not detected. Liver peroxide levels were elevated but serum lipid peroxides were not measured.

Lipid peroxides have been suggested as a causal factor in cataract formation. Yamauchi et al. (1986) investigated this aspect in relation to naphthalene. Naphthalene (1 g/kg) in acacia oil was administered to male Wistar rats daily for up to 18 days. GSH content in lens and serum and liver lipid peroxide levels were measured during interim sacrifice. Serum peroxide levels increased significantly on the fourth day and reached a maximum on the seventh day. Liver peroxide levels had a similar pattern. GSH content in lenses decreased to about 64% on the fourth day and remained depressed. The authors suggested that lipid peroxides are stable enough to reach the lens and cause ocular damage. Microscopic observation indicated slight cataractous changes in some rats on the 14th day when serum lipid peroxide levels were elevated (Yamauchi et al., 1986). It was suggested that peroxides may play a role in cataract formation, in addition to the role played by 1,2-naphthaquinone. A decrease in nonprotein sulfhydryl content in lens has previously been associated with naphthalene-induced cataracts in rabbits (Ikemoto and Iwata, 1978).

PAH-Induced Immunotoxicity

Numerous investigators have demonstrated that carcinogenic PAHs can produce an immunosuppressive effect. This effect was first observed by Malmgren et al. (1952) using high doses of 3-methylcholanthrene and dibenz[a,h]anthracene in mice. Subsequent studies established that single carcinogenic doses of 3-methylcholanthrene, 7,12-dimethylbenz[a]anthracene, and benzo[a]pyrene caused a prolonged depression of the immune response to sheep red blood cells (Stjernswärd, 1966, 1969). Noncarcinogenic hydrocarbons such as benzo[e]pyrene and anthracene reportedly had no immunosuppressive activity. In a review on immunosuppression and chemical carcinogenesis, substantial evidence was presented to indicate that the degree of immunosuppression was correlated with carcinogenic potency for PAHs (Baldwin, 1973). Both cell-mediated and humoral immune reactions are affected by PAHs.

The effects of three PAHs, 3-methylcholanthrene, dibenzo[a,h]anthracene and dibenzo[a,c]anthracene on the immune response was investigated in mice in relation to tumorigenesis (Lubet et al., 1984). The subcutaneous and oral routes were used in two strains of mice, C57B1/6 (B6N) and DBA/2N (D2N). The B6N strain is an AHH inducible species, and was more susceptible to tumor formation than was the noninducible D2N strain. Dibenz[a,h]anthracene at doses of 25 or 50 mg/kg produced an immunosuppression effect as measured by a modification of the Jerne plaque assay. This effect was more pronounced in the AHH inducible B6N mice than in the D2N mice. In contrast, dibenz[a,c]anthracene caused minimal effects in either strain. In general, it was noted that the more potent carcinogens produced greater immunosuppression than the noncarcinogens. The route of administration also influ-

enced the immunosuppressive effects. Following i.p. administration of high concentrations of PAHs striking immunosuppression was observed in both strains of mice. When the oral route was employed, the noninducible AHH mice (D2N) showed greater susceptibility than did the B6N mice. The authors suggested that in AHH inducible mice, orally administered PAHs are rapidly metabolized and are rapidly excreted. In the noninducible mice the lipophilic PAHs are absorbed and distributed to target organs. They concluded that AHH inducibility is an important factor in the immunosuppressive activity of PAHs.

A study by White et al. (1985) showed that suppression of an IgM response to SRBC in mice exposed to PAH was a function of the strain responsiveness. Responsive B6C3F1 mice did not experience the degree of immunosuppression following subcutaneous exposure as did D8A/2 nonresponsive mice. This study further showed a correlation between a PAH's capacity for immunosuppression and its carcinogenicity. Anthracene, chrysene, benzo[e]pyrene and perylene had no significant effect on immune response while benz[a]anthracene, benzo[a]pyrene, dibenz[a,c]anthracene and dibenz[a,h]anthracene exposure resulted in 55-91% suppression. Even greater immunosuppression was caused by 3-methylcholanthrene and 7,12-dimethylbenz[a]anthracene.

These results were contradicted to some extent in <u>in vitro</u> studies using the weak or noncarcinogen/carcinogen pair benzo[e]pyrene and benzo[a]pyrene (Blanton et al., 1986). These workers found that benzo[e]pyrene did suppress certain immune function but required higher doses than did benzo[a]pyrene. By contrast to the White et al. (1985) study, anthracene in this instance caused <u>in vitro</u> immunosuppressive effects. These studies also demonstrated that the immunosuppression by benzopyrenes is due to effects on

various cell types and is not attributable only to cytotoxicity. It has been shown that benzo[a]pyrene has potent effects on production of interleukin 1 by macrophages in vitro (Lyte and Bick, 1986).

Summary

The carcinogenesis-initiating potential of a PAH is dependent upon a number of factors: its lipid solubility and distribution to target organs, the presence of potentially reactive areas of its structure and its potential for metabolism to reactive electrophilic forms. This latter factor is tissue and species dependent and is related to some extent to the inducibility of higher levels and particular isozymes of cytochrome P-450-associated enzymes. Carcinogenic PAHs are generally mutagenic and can damage DNA. These activities are very likely to be involved in the compound's activity as an initiator of carcinogenic processes. Many PAHs are complete skin carcinogens, serving as their own promoters. Some are active as promoters or cocarcinogens for other initiating agents.

Target tissues for PAH-mediated toxicity other than carcinogenesis are generally actively engaged in DNA synthesis. These tissues include hematopoietic and immune systems, gonadal tissues and the lens of the eye. Mechanisms involving suppression of DNA synthesis or DNA damage have been proposed for PAH-induced toxicity to hematopoietic elements, the GI system and the cardiovascular system. In addition to the organ systems covered, PAH-induced lesions have been demonstrated in the lungs, liver and kidney. No evidence concerning precise mechanisms of toxicity has been found, but many toxic endpoints are linked to the species or strains capability for ready induction of PAH metabolizing enzymes.

Naphthalene appears to be metabolized in a different manner than other PAHs. Studies indicate that naphthalene is metabolized in the liver to 1,2-naphthaquinone. Administration of naphthalene causes an elevation in serum lipid peroxide and liver peroxide levels. It has been suggested that 1,2-naphthaquinone and elevated serum lipid peroxide levels may play a role in naphthalene induced cataract formation in rodents. Naphthalene injection has also caused bronchical epithelial cell necrosis in rodents and has caused acute hemolytic anemia in instances of human poisoning.

VIII. QUANTIFICATION OF TOXICOLOGIC EFFECTS

Introduction

The quantification of toxicologic effects of a chemical consists of separate assessments of noncarcinogenic and carcinogenic health effects. Chemicals that do not produce carcinogenic effects are believed to have a threshold dose below which no adverse, noncarcinogenic health effects occur, while carcinogens are assumed to act without a threshold.

In the quantification of noncarcinogenic effects, a Reference Dose (RfD), [formerly termed the Acceptable Daily Intake (ADI)] is calculated. The RfD is an estimate (with uncertainty spanning perhaps an order magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious health effects during a lifetime. The RfD is derived from a no-observed-adverse-effect level (NOAEL), or lowest-observed-adverse-effect level (LOAEL), identified from a subchronic or chronic study, and divided by an uncertainty factor(s) times a modifying factor. The RfD is calculated as follows:

$$\text{RfD} = \frac{\text{(NOAEL or LOAEL)}}{\text{[Uncertainty Factor(s) x Modifying Factor]}} = \_\_\_\_ \text{ mg/kg bw/day}$$

Selection of the uncertainty factor to be employed in the calculation of the RfD is based upon professional judgment, while considering the entire data base of toxicologic effects for the chemical. In order to ensure that uncertainty factors are selected and applied in a consistent manner,

the U.S. EPA (1991a) employs a modification to the guidelines proposed by the National Academy of Sciences (NAS, 1977, 1980) as follows:

Standard Uncertainty Factors (UFs)

- Use a 10-fold factor when extrapolating from valid experimental results from studies using prolonged exposure to average healthy humans. This factor is intended to account for the variation in sensitivity among the members of the human population. [10H]

- Use an additional 10-fold factor when extrapolating from valid results of long-term studies on experimental animals when results of studies of human exposure are not available or are inadequate. This factor is intended to account for the uncertainty in extrapolating animal data to the case of humans. [10A]

- Use an additional 10-fold factor when extrapolating from less than chronic results on experimental animals when there is no useful long-term human data. This factor is intended to account for the uncertainty in extrapolating from less than chronic NOAELs to chronic NOAELs. [10S]

- Use an additional 10-fold factor when deriving an RfD from a LOAEL instead of a NOAEL. This factor is intended to account for the uncertainty in extrapolating from LOAELs to NOAELs. [10L]

Modifying Factor (MF)

- Use professional judgment to determine another uncertainty factor (MF) that is greater than zero and less than or equal to 10. The magnitude of the MF depends upon the professional assessment of scientific uncertainties of the study and data base not explicitly treated above, e.g., the completeness of the overall data base and the number of species tested. The default value for the MF is 1.

The uncertainty factor used for a specific risk assessment is based principally upon scientific judgment rather than scientific fact and accounts for possible intra- and interspecies differences. Additional considerations not incorporated in the NAS/ODW guidelines for selection of an uncertainty factor include the use of a less than lifetime study for deriving an RfD, the significance of the adverse health effects and the counterbalancing of beneficial effects.

From the RfD, a Drinking Water Equivalent Level (DWEL) can be calculated. The DWEL represents a medium specific (i.e., drinking water) lifetime exposure at which adverse, noncarcinogenic health effects are not anticipated to occur. The DWEL assumes 100% exposure from drinking water. The DWEL provides the noncarcinogenic health effects basis for establishing a drinking water standard. For ingestion data, the DWEL is derived as follows:

$$\text{DWEL} = \frac{\text{(RfD) x (Body weight in kg)}}{\text{Drinking Water Volume in ℓ/day}} = \_\_\_\_ \text{ mg/ℓ}$$

where:

Body weight = assumed to be 70 kg for an adult
Drinking water volume = assumed to be 2 ℓ/day for an adult

In addition to the RfD and the DWEL, Health Advisories (HAs) for exposures of shorter duration (1-day, 10-day and longer-term) are determined. The HA values are used as informal guidance to municipalities and other organizations when emergency spills or contamination situations occur. The HAs are calculated using an equation similar to the RfD and DWEL; however, the NOAELs or LOAELs are identified from acute or subchronic studies. The HAs are derived as follows:

$$\text{HA} = \frac{\text{(NOAEL or LOAEL) x (bw)}}{\text{(UF) x (\_\_\_ ℓ/day)}} = \_\_\_\_ \text{ mg/ℓ}$$

Using the above equation, the following drinking water HAs are developed for noncarcinogenic effects:

1. 1-day HA for a 10 kg child ingesting 1 ℓ water per day.
2. 10-day HA for a 10 kg child ingesting 1 ℓ water per day.
3. Longer-term HA for a 10 kg child ingesting 1 ℓ water per day.
4. Longer-term HA for a 70 kg adult ingesting 2 ℓ water per day.

The 1-day HA calculated for a 10 kg child assumes a single acute exposure to the chemical and is generally derived from a study of <7 days duration. The 10-day HA assumes a limited exposure period of 1-2 weeks and is generally derived from a study of <30 days duration. The longer-term HA is derived for both the 10 kg child and a 70 kg adult and assumes an exposure period of ~7 years (or 10% of an individual's lifetime). The longer-term HA is generally derived from a study of subchronic duration (exposure for 10% of animal's lifetime).

The U.S. EPA categorizes the carcinogenic potential of a chemical, based on the overall weight-of-evidence, according to the following scheme:

> Group A: Human Carcinogen. Sufficient evidence exists from epidemiology studies to support a causal association between exposure to the chemical and human cancer.
>
> Group B: Probable Human Carcinogen. Sufficient evidence of carcinogenicity in animals with limited (Group B1) or inadequate (Group B2) evidence in humans.
>
> Group C: Possible Human Carcinogen. Limited evidence of carcinogenicity in animals in the absence of human data.
>
> Group D: Not Classified as to Human Carcinogenicity. Inadequate human and animal evidence of carcinogenicity or for which no data are available.
>
> Group E: Evidence of Noncarcinogenicity for Humans. No evidence of carcinogenicity in at least two adequate animal tests in different species or in both adequate epidemiologic and animal studies.

If toxicologic evidence leads to the classification of the contaminant as a known, probable or possible human carcinogen, mathematical models are used to calculate the estimated excess cancer risk associated with the ingestion of the contaminant in drinking water. The data used in these estimates usually come from lifetime exposure studies using animals.

In order to predict the risk for humans from animal data, animal doses must be converted to equivalent human doses. This conversion includes correction for noncontinuous exposure, less than lifetime studies and for differences in size. The factor that compensates for the size difference is the cube root of the ratio of the animal and human body weights. It is assumed that the average adult human body weight is 70 kg and that the average water consumption of an adult human is 2 ℓ of water per day.

For contaminants with a carcinogenic potential, chemical levels are correlated with a carcinogenic risk estimate by employing a cancer potency (unit risk) value together with the assumption for lifetime exposure from ingestion of water. The cancer unit risk is usually derived from a linearized multistage model with a 95% upper confidence limit providing a low dose estimate; that is, the true risk to humans, while not identifiable, is not likely to exceed the upper limit estimate and, in fact, may be lower. Excess cancer risk estimates may also be calculated using other models such as the one-hit, Weibull, logit and probit. There is little basis in the current understanding of the biological mechanisms involved in cancer to suggest that any one of these models is able to predict risk more accurately than any other. Because each model is based upon differing assumptions, the estimates derived for each model can differ by several orders of magnitude.

The scientific data base used to calculate and support the setting of cancer risk rate levels has an inherent uncertainty that is due to the systematic and random errors in scientific measurement. In most cases, only studies using experimental animals have been performed. Thus, there is uncertainty when the data are extrapolated to humans. When developing

cancer risk rate levels, several other areas of uncertainty exist, such as the incomplete knowledge concerning the health effects of contaminants in drinking water, the impact of the experimental animal's age, sex and species, the nature of the target organ system(s) examined and the actual rate of exposure of the internal targets in experimental animals or humans. Dose-response data usually are available only for high levels of exposure and not for the lower levels of exposure closer to where a standard may be set. When there is exposure to more than one contaminant, additional uncertainty results from a lack of information about possible synergistic or antagonistic effects. Since PAH occur only as mixtures in the environment, no epidemiologic data are found for individual PAH.

Noncarcinogenic Effects

For many of the PAHs experimental data on noncarcinogenic effects are either nonexistent or provide insufficient information on which to base criteria for drinking water exposure. In general, existing data are not suitable for criteria derivation for one or more of these reasons:

- Studies were designed to assess only carcinogenic potential
- Studies were designed to determine only lethal dose (LD_{50})
- Studies do not give dose/response data
- Studies contain only one dose level, at which severe health effects occurred
- Studies do not measure chronic exposure
- Studies were by other than oral exposure
- Measure of dose is not known
- Sample size is too small
- Test animals used do not provide relevant models for human health assessment.

Recently, the U.S. EPA (1988, 1989a,b,c) and Hazelton Laboratories America, Inc. (1989c, 1989d) conducted 90-day subchronic bioassays for acenaphthylene, anthracene, fluoranthene, fluorene and pyrene. These studies examined a variety of toxicologic endpoints and were of adequate design, thereby providing sufficient information on which to base criteria for drinking water exposure. These studies were insufficient to evaluate potential carcinogenicity of the above PAHs.

Short-term Studies in Animals

There were short-term studies wherein animals were treated by the oral route reported for only acenaphthylene, anthracene, fluoranthene and fluorene. For acenaphthylene there is an oral LD_{50} for rats and mice reported in abstract form (Knobloch et al., 1969). For anthracene there is a report in abstract form stating that a single oral dose of 17 g/kg of anthracene is not lethal to mice (Nagornyi, 1969). For fluoranthene the only data are from a range-finding study on more than 300 compounds; the study reported the oral LD_{50} for rats as 2000 mg/kg/day (Smyth et al., 1962). For fluorene, Kizer et al. (1985) reported that in rats fed 10.5 mg/kg/day in the diet for 3 weeks, no increases in either SGOT or hepatic microsomal epoxide hydrolase activity were observed. Thus, there are no data suitable for derivation of 1- or 10-day HAs for any PAH discussed in this document.

Longer-term Studies in Animals

No longer-term exposures of animals by the oral route were reported for the following: benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[g,h,i]-perylene, chrysene, indeno[1,2,3-cd]pyrene, phenanthrene and pyrene.

Acenaphthylene. Acenaphthylene was administered to groups of seven rats in doses of 600 mg/kg bw for 40 days or 2000 mg/kg bw for 32 days. Effects were reported on body weight, peripheral blood, renal function, kidney and liver morphology, and bronchitis was induced (Knobloch et al., 1969). The report is in abstract form; data are, thus, insufficient for derivation of criteria.

In a subchronic toxicity bioassay conducted by Hazelton Laboratories America, Inc. (1989c) acenaphthylene was administered to groups of 20 male and female CD-1 mice by gavage. Dose levels were 0, 100, 200 and 400 mg/kg/day. Criteria evaluated for compound-related effects were mortality, clinical signs, body weight, food consumption, opthalmology, hematology, clinical chemistry, organ weights, gross pathology and histopatholgy. Based on liver and kidney changes and deaths in females the LOAEL determined from this study is 100 mg/kg/day; no NOAEL was determined. Due to the high mortality observed in females receiving 100 mg/kg/day the data provided are considered insufficient for derivation of criteria.

Anthracene. Two bioassays for carcinogenicity of ingested anthracene have been conducted (Druckry and Schmähl, 1955; Schmähl and Reiter, n.d.). Neither study mentions noncancer health effects, nor were any tumors reported. The latter study has not been published. In a subchronic toxicity study, the U.S. EPA (1989e) administered anthracene to groups of 20 male and female CD-1 (ICR)BR mice by gavage. Dose levels were 0, 250, 500 and 1000 mg/kg/day. Criteria evaluated for compound-related effects were mortality, clinical signs, body weights, food consumption, opthalmology, hematology, clinical chemistry, organ weights, organ-to-body weight ratios,

gross pathology and histopathology. No treatment-related effects were noted; therefore, the NOAEL determined from this study is 1000 mg/kg/day.

Benz[a]anthracene. Klein (1963) published a report of benz[a]anthracene carcinogenicity for mice treated by gavage. Only one concentration was administered, and noncancer endpoints were not described. Thus, data are insufficient to derive criteria for benz[a]anthracene.

Benzo[a]pyrene. Benzo[a]pyrene was administered to mice at multiple concentrations in the diet in order to assess its carcinogenic potential (Rigdon and Neal, 1966, 1969; Neal and Rigdon, 1967). Treatment-related incidences of tumors of the forestomach and lung, and leukemias were observed. No noncancer health effects were reported, however. Rigdon and Neal (1965) also conducted a series of assays to determine if dietary benzo-[a]pyrene produced deleterious reproductive effects. Oral benzo[a]pyrene concentrations of 250, 500 or 1000 ppm over various time periods up to lifetime showed no treatment-related effects, except lack of weight gain related to feed unpalatability.

MacKenzie and Angevine (1981) dosed groups of 30 or 60 pregnant CD-1 mice with gavage preparations of benzo[a]pyrene to deliver 0, 10, 40 or 160 mg/kg bw/day. This was done only on days 7-16 of gestation. No maternal toxicity was noted, nor were there signs of fetal toxicity. Pups were culled to 8/litter and used in an F_1 mating study. By days 20 and 42, F_1 animals exposed to benzo[a]pyrene *in utero* were observed to have decreased body weights in comparison with controls. Gonadal weights of treated animals were also significantly reduced. The testes from animals

exposed in utero to the low benzo[a]pyrene dose weighed ~60% those of controls; testes from the 40 mg (middle dose) group weighed ~18% of controls. As most F_1 in utero exposed females had no ovaries or only remnants of ovarian tissues, weights were generally not recorded. Fertility was reduced among F_1 treated animals. Of control males 100% were fertile, 20/25 from the group exposed to 10 mg, 3/45 from the 40 mg group, and none of the males treated in utero with 160 mg benzo[a]pyrene/kg/day were fertile. Of treated females, 34% of the low-dose group produced a litter; none of the middle- and high-dose females produced a litter. This study, thus, demonstrates a LOAEL for gonadal weight decrease and loss of fertility of 10 mg/kg/day for benzo[a]pyrene.

Dibenz[a,h]anthracene. Dibenz[a,h]anthracene was carcinogenic to mice receiving the compound in an olive oil/drinking water emulsion (Lorenz and Stewart, 1947; Snell and Stewart, 1962a). Only one concentration of treatment suspension was administered. The only noncancer health effect reported was dehydration and emaciation of animals due to poor tolerance of the vehicle. There are, thus, no data suitable for derivation of criteria.

Fluoranthene. In a 13-week bioassay in mice, the U.S. EPA (1988) administered either 0, 125, 250 or 500 mg/kg/day of fluoranthene to groups of 20 male or female CD-1 mice by gavage. Body weights, food consumption and clinical signs of toxicity were monitored at regular intervals during the experimental period. At the end of the study period the animals were sacrificed and submitted for autopsy and hematologic and serum chemistry evaluations. A LOAEL of 250 mg/kg/day based on statistically significant ($p \leq 0.05$) changes in SGPT and absolute and relative liver weights, as well as

decreases in packed cell volume and red blood cell numbers (females only) and albumin/globulin ratios, was identified by the U.S. EPA (1991a); the corresponding NOAEL is 125 mg/kg/day.

Fluorene. Neither of two reports of bioassays for oral carcinogenic potential of fluorene gave indication of an increased tumor incidence (Morris et al., 1960; Wilson et al., 1947). The former study reported no effects related to treatment. In the latter study one set of rats was exposed to dietary concentrations of 0.062 or 1.0% fluorene for 104 days and a second set to 0.125, 0.25 or 0.5% fluorene for 453 days. The animals of the short-term group appeared normal in all respects except for treatment-related decreases in growth rate. The longer-term treated animals were observed to have significantly increased liver weights (two highest dose groups) and decreased spleen weights (all treated animals). While these changes were described as "significant", no numerical data were presented. This study is, therefore, unsuitable for criteria derivation.

In a 13-week subchronic bioassay in mice, the U.S. EPA (1989a) administered either 0, 125, 250 or 500 mg/kg/day fluorene, suspended in corn oil, to groups of 25 male and female CD-1 mice by gavage. Measures used to assess toxicity included food intake, body weight, clinical observations, hematology and serum chemistry, and gross and histopathologic changes. Using the data from U.S. EPA (1989a), the U.S. EPA (1991a) identified a LOAEL of 250 mg/kg/day for hematologic effects; the NOAEL is 125 mg/kg/day.

Pyrene. In a 13-week subchronic bioassay in mice, the U.S. EPA (1989b) administered either 0, 75, 125 or 250 mg/kg/day of pyrene to groups

of 20 male and 20 female CD-1 mice by gavage. Criteria used to assess toxicity included body and organ weights, food consumption, mortality, hematology amd serum chemistry, and gross and histopathology. Based on nephropathy, accompanied by statistically significant ($p \leq 0.01$) changes in absolute and relative kidney weights, the U.S. EPA (1991a) identified 125 mg/kg/day as the LOAEL; the NOAEL is 75 mg/kg/day.

Quantification of Noncarcinogenic Effects

Derivation of 1- and 10-Day Health Advisories. There were no data suitable for calculation of 1-day or 10-day HAs for children for any PAH covered in this document.

A number of short-term exposure studies have been reported wherein various immunologic endpoints have been measured. These are summarized in Tables VIII-1 and VIII-2. While it is important to consider immune deficits as indicators of early toxicity, it is not appropriate to base health advisories on the data presented in these two tables. First, none of the studies employed an oral route of administration, thereby decreasing the relevance of the data to drinking water health advisories. Secondly, it is not clear whether the immune deficits observed are, in fact, indicators of toxicity or are normal adaptive responses to stress.

For benzo[a]pyrene the data of MacKenzie and Angivine (1981) could also be considered as an indicator of noncancer toxic effects resulting from short-term exposure in utero. In view of the fact that this exposure encompassed an entire critical phase of the animal's lifespan, namely the fetal developmental phase, the data may be more suitable for derivation of a lifetime health advisory.

TABLE VIII-1

Immunotoxicity of Benzo[a]pyrene in Mice after Short-Term Exposure

Days Exposure	Strain	Route	LOAEL/NOEL (mg/kg)	Effect*	Reference
1	CBA	i.m.	33.3/ND	PFC	Stjernsward, 1966
1	C3H/ANF	in utero	150/ND	PFC GvH mixed lymph	Urso and Gengozian, 1984
1	B6C3F1	s.c.	252/ND	number of spleen cells	White et al., 1985
1	C57 C3H	i.p. i.p.	5.0/0.5 5.0/0.5	target cell killing	Wodjani et al., 1984
1	C3H	i.p.	18.0/1.8	INF	Griffin et al., 1986
1	C57B1/6 DBA/2	i.p. i.p.	25/- 50/25	PFC PFC	Lubet et al., 1984
10	B6C3F1	s.c.	5.0/-	AbT1 CPLPS	Dean et al., 1983
14	B6C3F1	s.c.	40.4/ND	spleen weight PFU	White et al., 1985

*All effects noted were decreases by comparison with controls in the measurements indicated. PFC = plaque-forming spleen cells; PFU = plaque-forming units; GvH = graft vs. host response; mixed lymph = mixed lymphocyte response; INF = interferon production; AbT1 = antibody production to a T-independent antigen; CPLPS = cell proliferation in response to lipopoly-saccharide mitogen.

ND = Not determined as only one dose was tested

- = No NOEL reported

TABLE VIII-2

Comparative Effects of PAH on Immune Functions in B6C3F1 Mice[a]

Compound	Dose[b] (mg/kg/day)	Effect
Anthracene	28.5	None
Benz[a]anthracene	36.5	Decreased antibody forming cells
Benzo[a]pyrene	40.4	Decreased antibody forming cells; decreased spleen weight
Chrysene	36.5	None
Dibenz[a,h]anthracene	42.7	Decreased antibody forming cells; decreased spleen weight

[a]Data from White et al. (1985)

[b]Animals were treated s.c. with 160 μmole/kg/day

Derivation of Longer-Term HA. There were no data suitable for calculation of longer-term HAs for children or adults for any PAH covered in this document.

Assessment of Lifetime Exposure and Derivation of DWELs. There were no data suitable for calculation of RfDs for the following: benz[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[g,h,i]perylene, chrysene, dibenz[a,h]anthracene, indeno[1,2,3-cd]pyrene or phenanthrene.

Acenaphthylene -- Quantitative data on the subchronic oral toxicity of acenaphthylene has been provided by Hazelton Laboratories America, Inc. (1989c). Acenaphthylene was administered to CD-1 mice (20/sex/group) at dosage levels of 0, 100, 200 or 400 mg/kg/day for at least 90 days. Effects examined included mortality, clinical signs, body weights, food consumption, opthalmology, clinical chemistry, organ weights, gross pathology and histopathology. Treatment-related effects were observed in all dosage groups. Due to the high mortality observed in all groups of treated females, the low dose is considered an FEL. Therefore, this study is insufficient for deriving criteria since the choice of mortality as the critical effect is not appropriate as the basis for an RfD.

Additionally, four subchronic studies on the toxicity of acenaphthylene were located in the literature: two oral studies (Rotenberg and Mashbits, 1965; Knobloch et al., 1969) and two inhalation studies (Rotenberg and Mashbits, 1965; Reshetyuk et al., 1970). However, deficiencies (no experimental controls, short duration and incomplete reporting of study design and/or results) preclude the use of any of these studies as a basis for an oral RfD.

Anthracene -- Quantitative data on the oral exposure to anthracene reported in U.S. EPA (1989e) failed to demonstrate treatment-related effects to male or female CD-1 (ICR)BR mice at doses up to 1000 mg/kg/day for at least 90 days. From these results, a NOAEL of 1000 mg/kg/day was identified. Using this NOAEL, the DWEL is derived as follows.

Step 1 - RfD Derivation

$$RfD = \frac{(1000\ mg/kg/day)}{3000} = 3.3\times10^{-1}\ mg/kg/day$$
(rounded to 3×10^{-1} mg/kg/day)

where:

1000 mg/kg/day = NOAEL reflecting no treatment-related effects in mice (U.S. EPA, 1989e)

3000 = combined uncertainty factors: 100 to account for intra- and interspecies extrapolation, 10 for the use of a subchronic study for RfD derivation, and 3 for the lack of reproductive/developmental and supporting chronic toxicity data

Step 2 - DWEL Derivation

$$DWEL = \frac{(3\times10^{-1}\ mg/kg/day)\ (70\ kg)}{(2\ \ell/day)} = 10.5\ mg/\ell$$

where:

3×10^{-1} mg/kg/day = RfD

70 kg = assumed body weight of an adult

2 ℓ/day = assumed volume of water consumed by an adult

Benzo[a]pyrene -- For benzo[a]pyrene an indicator of a noncancer toxic effect was the decrease in fertility and gonadal weight in mice exposed in utero. Dams were gavaged with 10 mg/kg/day benzo[a]pyrene in corn oil on days 7-16 of gestation (MacKenzie and Angevine, 1981). While this does not constitute a subchronic or chronic exposure it does cover the entire period of development most sensitive to insult of the test population. The study could, thus, be considered for use in estimating a longer-term risk.

For benzo[a]pyrene, available data show that the appearance of neoplastic effects occurs at lower doses than do indicators of systemic toxicity. For example a LOAEL based on the MacKenzie and Angevine (1981) data is 10 mg/kg/day, while significant increases in tumor incidence have been observed in mice receiving 6.5 mg/kg/day benzo[a]pyrene in the diet (Neal and Rigdon, 1967). It, thus, seems inadvisable at this time to propose any health advisories for benzo[a]pyrene based on noncancer effects.

Fluoranthene -- In a 13-week subchronic bioassay in mice the U.S. EPA (1988) identified a LOAEL of 125 mg/kg/day of fluoranthene. This LOAEL was based on statistically nonsignificant increases in clinical signs, serum chemistry and changes in liver and kidney pathology as well as significant increases in relative liver weights of male mice. In a reevaluation of U.S. EPA (1988), the U.S. EPA (1991a) identified the 125 mg/kg/day dose level as the NOAEL and the 250 mg/kg/day dose level as the LOAEL. The low dose was considered the NOAEL as clinical signs (i.e., salivation) were not dose-related effects, and changes in serum enzymes and kidney and liver histopathology were not considered adverse at 125 mg/kg/day. The 250 mg/kg/day dose is considered the LOAEL based on significant reductions in packed cell volume and red blood cell numbers in females, and albumin/globulin ratios and significant increases in SGPT and absolute and relative liver weights in both sexes. Based on a NOAEL of 125 mg/kg/day, the DWEL is derived as follows:

Step 1 - RfD Derivation

$$RfD = \frac{125\ mg/kg/day}{3000} = 4.17 \times 10^{-2}\ mg/kg/day$$
(rounded to 4×10^{-2} mg/kg/day)

where:

125 mg/kg/day = NOAEL reflecting the absence of dose-related effects in mice (U.S. EPA, 1988)

3000 = combined uncertainty factors: 100 to account for intra- and interspecies extrapolation, 10 for the use of a subchronic study for RfD derivation, and 3 for the lack of reproductive/developmental and supporting chronic toxicity data

Step 2 - DWEL Derivation

$$DWEL = \frac{(4\times10^{-2}\ mg/kg/day)\ (70\ kg)}{(2\ \ell/day)} = 1.4\ mg/\ell$$

where:

4×10^{-2} mg/kg/day = RfD

70 kg = assumed body weight of an adult

2 ℓ/day = assumed volume of water consumed by an adult

Fluorene -- In a 13-week subchronic bioassay in CD-1 mice (U.S. EPA, 1989a), quantitative data were reported on the adverse health effects associated with oral exposure to fluorene. From these results, the U.S. EPA (1991a) identified a LOAEL of 250 mg/kg/day for hematologic effects; the corresponding NOAEL is 125 mg/kg/day. Using this NOAEL, the DWEL is derived as follows.

Step 1 - RfD Derivation

$$RfD = \frac{(125\ mg/kg/day)}{3000} = 4.17\times10^{-2}\ mg/kg/day$$
(rounded to 4×10^{-2} mg/kg/day)

where:

125 mg/kg/day = NOAEL reflecting the absence of hematologic effects in mice (U.S. EPA, 1989a)

3000 = combined uncertainty factors: 100 to account for intra- and interspecies variability, 10 for the use of a subchronic study for RfD derivation, and 3 for the lack of reproductive/developmental and supporting chronic toxicity data

Step 2 - DWEL Derivation

$$DWEL = \frac{(4 \times 10^{-2}\ mg/kg/day)\ (70\ kg)}{(2\ \ell/day)} = 1.4\ mg/\ell$$

where:

4×10^{-2} mg/kg/day = RfD

70 kg = assumed body weight of an adult

2 ℓ/day = assumed volume of water consumed by an adult

Naphthalene -- Quantitative results on the chronic oral toxicity of naphthalene have been provided by Schmahl (1955). Groups of 28 BD I and BD II rats received naphthalene (estimated daily dose 10-20 mg) in the diet, starting when the rats were ~100 days old; the experiment was terminated on the 700th experimental day when a total dose of 10 g/rat was achieved. No treatment-related effects were observed. The use of this study to derive an oral RfD has been questioned by the U.S. EPA (1989c) because this study was designed to assess the carcinogenicity of naphthalene, small numbers of animals were used, and there is uncertainty about the actual dose administered.

Subchronic studies with mice and rats by NTP (1980a,b) and Shopp et al. (1984) support a lower chronic NOEL determined by the Schmahl (1955) study. In the NTP (1980a) study, mice were treated by gavage with 0, 12.5, 25, 50, 100 or 200 mg/kg/day naphthalene, 5 days/week for 13 weeks. Comprehensive histologic examination of the high-dose and control groups revealed no

treatment-related lesions. Clinical signs of toxicity occurred at or above the 100 mg/kg/day dose, but no effects were observed at or below the 50 mg/kg/day dose. No adverse effects on mortality, weight gain, immunologic effects, clinical chemistry and hematologic parameters or organ weights were observed in mice given a 53 mg/kg/day dose by gavage for 90 days (Shopp et al., 1984). Histologic examinations were not performed in this study. Subchronic NOELs from these studies were 35.7 mg/kg/day (NTP, 1980a) and 53 mg/kg/day (Shopp et al., 1984).

Derivation of quantitative data on the oral toxicity of naphthalene are not derived because concurrence on the most appropriate study on which to base the calculation has not been reached (U.S. EPA, 1989c).

Pyrene -- In a 13-week subchronic bioassay in CD-1 mice (U.S. EPA, 1989b), quantitative data were reported on the adverse health effects associated with oral exposure to pyrene. From these results, the U.S. EPA (1991a) identified a LOAEL of 125 mg/kg/day for nephropathy accompanied by changes in absolute and relative kidney weights; the corresponding NOAEL is 75 mg/kg/day. Using this NOAEL, the DWEL is derived as follows.

Step 1 - RfD Derivation

$$RfD = \frac{(75\ mg/kg/day)}{3000} = 2.5 \times 10^{-2}\ mg/kg/day$$
(rounded to 3×10^{-2} mg/kg/day)

where:

75 mg/kg/day = NOAEL reflecting the absence of nephropathy in mice (U.S. EPA, 1989b)

3000 = combined uncertainty factors: 100 to account for intra- and interspecies variability, 10 for the use of a subchronic study for RfD derivation, and 3 for the lack of reproductive/developmental and supporting chronic toxicity data.

Step 2 - DWEL Derivation

$$DWEL = \frac{(3 \times 10^{-2}\ mg/kg/day)\ (70\ kg)}{(2\ \ell/day)} = 1.05\ mg/\ell \text{ (rounded to 1 mg/}\ell\text{)}$$

where:

0.025 mg/kg/day = RfD

70 kg = assumed body weight of an adult

2 ℓ/day = assumed volume of water consumed by an adult

Carcinogenic Effects

The majority of health effects data for individual PAHs concerns their potential as carcinogens. The data base for these compounds consists entirely of animal studies. There are numerous case reports and epidemiologic investigations on human health effects of PAH-containing materials. These, however, have been reports on exposures to environmental or to occupationally generated mixtures containing PAHs and other compounds. There are no reports of exposures to individual PAHs with the exception of a case study of skin painting of benzo[a]pyrene. The exposed subjects developed verrucae, wart-like benign lesions that regressed upon cessation of treatment (Cottini and Mazzone, 1939). This observation is significant in that it is not unlike the process observed in animals skin painted with PAHs. In response to a carcinogenic PAH, animals generally develop non-malignant lesions (papillomas) that may regress upon cessation of treatment or may progress to carcinomas if treatment is continued or followed by a promoting treatment.

As reviewed in Chapter V, much of the data on PAH carcinogenicity comes from skin-painting bioassays, subcutaneous injection studies and to a lesser

extent from experiments wherein PAHs were administered intratracheally or by inhalation. There are comparatively few oral bioassays. The relevance of data from skin painting bioassays to evaluate the potential for induction of nonskin cancer in humans has been a subject of discussion. The example of benzo[a]pyrene may be instructive in this context. This PAH causes injection site sarcomas upon subcutaneous administration, skin tumors when applied topically, lung neoplasms when delivered intratracheally or by inhalation and forestomach tumors in rats when given orally. It appears that benzo[a]pyrene produces neoplastic growth at the site of delivery when at least two conditions are met: that the tissue is capable of metabolism of benzo[a]pyrene to reactive forms, and that the tissue is of a type that normally undergoes some degree of proliferation. The requirement for metabolism of benzo[a]pyrene is not a limiting factor for formation of distant site tumors; many metabolites can be transported in the blood and the majority of tissues assayed show some capacity for PAH metabolism.

The other PAHs in this document have been studied to a lesser extent and by fewer exposure routes than has benzo[a]pyrene. There are a few examples, however, to indicate that when a PAH is positive in skin painting bioassays, it will produce tumors when administered by other routes. Benz[a]anthracene and dibenz[a,h]anthracene, which produce skin tumors when applied topically, also produce neoplasms when delivered orally (Klein, 1963; Bock and King, 1959; Larinov and Soboleva, 1938; Lorenz and Stewart, 1947, 1948; Snell and Stewart, 1962a, 1962b). It appears justified to say that evidence of carcino- genicity from a PAH skin-painting bioassay is a cause for concern and should not be ignored when evaluating a PAH as a potential human carcinogen.

Classification of PAHs as to Potential for Human Carcinogenicity

Assignment of compounds to a classification based on their likelihood of producing carcinogenic effects in humans was done according to the U.S. EPA Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1986). Using these criteria the PAHs described in this document may be classified in the following two groups.

Group D. Not classifiable as to human carcinogenicity. This is due to a lack of specific human evidence and inadequate animal data for carcinogenicity.

Acenaphthylene. One skin painting biassay in mice showed no increase in tumor incidence. One assay for mutagenicity in Salmonella was not positive.

Anthracene. Studies wherein rats were administered anthracene orally did not result in tumor induction. Lung implantation and skin painting bioassays, and subcutaneous, intraperitoneal and intracerebral injection likewise have not shown a tumorigenic effect. Mutagenicity for Salmonella strain TA97 has been reported.

Benzo[g,h,i]perylene. A bioassay by intrapulmonary injection of rats was considered inadequate for evaluation of this PAH due to the presence of impurities. Two skin-painting studies for complete carcinogenicity and three initiation-promotion assays in mice were negative. There are data to suggest that this compound may act as a co-carcinogen for benzo[a]pyrene applied to mouse skin. Benzo[g,h,i]perylene was mutagenic for S. typhimurium.

Fluoranthene. Skin painting bioassays for complete carcinogenicity have not shown positive results. Initiation/promotion protocols wherein fluoranthene was used as the initiator, as well as a subcutaneous injection study, were negative. There is some evidence that fluoranthene served as a cocarcinogen for benzo[a]pyrene. Fluoranthene produced an increase in incidence in lung adenomas for males and females combined in a short-term *in vivo* bioassay generally considered not adequate for evaluation of carcinogenicity. Evidence for genetic toxicity of fluoranthene is equivocal.

Fluorene. Two oral bioassays reported no increase in tumors as a consequence of fluorene treatment. Both assays had deficiencies limiting their usefulness. IARC considered two apparently negative skin-painting bioassays to be inadequate for evaluation; a more recent initiation/promotion assay was not positive. Two subcutaneous administration studies were negative. Genetic toxicology data are limited but negative.

Naphthalene. Bioassays of naphthalene by the oral route and as a skin tumorigen were negative or inadequate for evaluation. An inhalation and an intraperitoneal injection study were negative, and a subcutaneous injection study was inadequate for evaluation. Supporting data for genetic toxicity are negative but limited.

Phenanthrene. An assay in which rats received a single oral treatment showed no increase in tumor incidence. Treatment of mice topically, subcutaneously and intraperitoneally has not resulted in tumor induction. One study reported phenanthrene to be an initiator of skin tumorigenesis in CD-1 mice when followed by high concentrations of TPA. This was countered

by negative studies in three other mouse strains. Phenanthrene and a diol-epoxide derivative were mutagenic for Salmonella typhimurium and human lymphoblast cells. Other genetic toxicology testing has not shown positive results.

Pyrene. Intratracheal instillation of pyrene and Fe_2O_3 particles did not induce tumors in hamsters. Skin painting assays in mice for complete carcinogenesis or initiating capacity have been negative or inconclusive. Mice injected either subcutaneously or intraperitoneally did not develop tumors, but there is evidence that pyrene enhances tumorigenicity of topically applied benzo[a]pyrene in mice. Both positive and negative results have been reported for assays of mutagenic effect; pyrene was not shown to transform mammalian cells.

Group B2. Probable human carcinogen. These judgments were based on sufficient animal evidence in the absence of human data for individual PAHs.

Benz[a]anthracene. Benz[a]anthracene produced tumors in mice treated orally, and in various mouse strains treated intraperitoneally, intravenously, topically, subcutaneously and intramuscularly. It was mutagenic for Salmonella, Drosophila and mammalian cells and produced DNA damage, SCE and morphologic transformation in cultured cell. Benz[a]anthracene was positive in a mouse lung adenoma assay.

Benzo[a]pyrene. Orally administered benzo[a]pyrene was carcinogenic to three mouse strains. It produced tumors when administered intratracheally to rats and hamsters. Benzo[a]pyrene delivered intraperitoneally has

induced tumors in mice and rats. It is the best documented experimental skin carcinogen producing tumors in mice, rats, rabbits and guinea pigs. It was carcinogenic when administered subcutaneously to mice, rats, hamsters, guinea pigs and some primates. Benzo[a]pyrene has produced positive responses in a number of genetic toxiology assays in bacterial and mammalian cells.

Benzo[b]fluoranthene. Exposure of rats by lung implantation resulted in tumor formation as did intraperitoneal exposure of newborn mice. A total of three skin painting and initiation/promotion studies in mice were positive as was an assay by subcutaneous injection of mice. Benzo[b]fluoranthene was mutagenic in a forward assay in Salmonella.

Benzo[k]fluoranthene. Lung implantation produced tumors in rats, and initiation/promotion protocols in two mouse strains resulted in increased tumor incidence. Intraperitoneal injection in newborn mice produced equivocal results. Benzo[k]fluoranthene was mutagenic in a forward assay in Salmonella.

Chrysene. Chrysene produced tumors in several mouse strains when applied topically in assays for complete skin carcinogenicity or in initiation/promotion protocols. Several early studies employing intramuscular or subcutaneous injection of mice and rats produced negative or equivocal results. Three studies wherein neonatal mice of two strains were exposed intraperitoneally reported increased tumor incidence in liver and other sites. Chrysene produced mutations in Salmonella and chromosome aberrations and morphologic transformation in mammalian cells.

Dibenz[a,h]anthracene. Mice of various strains have been shown to develop tumors as a consequence of oral exposure to dibenz[a,h]anthracene. Mice were also reported to develop tumors after pulmonary, intratracheal, intravenous or topical treatment. Injection site tumors (intramuscular or subcutaneous) have been observed in mice, rats, guinea pigs, pigeons and unspecified fowl. Results of DNA damage, mutation and morphologic transformation assays have been positive.

Indeno[1,2,3,-cd]pyrene. Lung implantation of indeno[1,2,3-cd]pyrene produced tumors in rats. Skin painting assays for complete carcinogenicity and initiating ability were positive in two mouse strains, and injection site tumors were reported after subcutaneous exposure of a third strain. Limited data indicate mutagenicity for Salmonella.

Quantification of Carcinogenic Effects

Compounds classified as Group A, B and C carcinogens are generally regarded as suitable for quantitative risk assessment. There are studies sufficient to classify seven PAHs in this document in group B2. Many of these studies, however, do not provide a suitable basis for dose-response assessment for the following reasons:

- Studies do not give dose/response data.
- Studies contain only one dose level.
- Studies use exposure other than oral exposure (such as skin painting).
- Sample size is too small.

No carcinogenicity bioassays using the oral route of exposure have been reported for benzo[b]fluoranthene, benzo(k)fluoranthene, chrysene and indeno[1,2,3-cd]pyrene.

Benz[a]anthracene. In a study by Klein (1963), male B6AF1 mice were gavaged with a 3% solution of benz[a]anthracene 3 times/week for 5 weeks and observed for either 437 or 547 days. Incidences of both lung adenomas and liver tumors were increased at both observations. As there was only one dose of compound administered, and exposure was of short duration, derivation of a quantitative risk estimate based on this study is inappropriate.

Benzo[a]pyrene. Several studies have reported increased incidence of alimentary tract tumors in rodents as a consequence of oral benzo[a]pyrene exposure. In the majority of these, there was a single gavage exposure (e.g., Huggins and Yang, 1962; McCormick et al., 1981), or only one gavage or dietary dose was employed (Berenblum and Haran, 1955; Gibel, 1964; Chu and Malmgren 1965; Biancifiori et al., 1967; Wattenberg, 1972, 1974; El-Bayoumy, 1985). Triolo et al. (1977) observed forestomach tumor induction in female mice (9/group) fed 200 and 300 ppm benzo[a]pyrene in the diet. Treatment was for a relatively small percentage of the animals' usual lifespan; that is, 12 weeks. Effective numbers of animals were not reported. In part of the Wattenberg (1972) study, mice receiving two dietary doses of benzo[a]pyrene were observed to develop forestomach tumors. Treatment was for <1 year, and no concurrent controls were reported.

A quantitative assessment for oral exposure to benzo[a]pyrene regarding its carcinogenic effects can be based on the experiments reported by Neal and Rigdon (1967), in which benzo[a]pyrene at doses ranging between 1 and 250 ppm in the diet was fed to strain CFW mice for $\leq$197 days. No tumors were found in the control group nor in the groups treated with 1, 10 or 30 ppm benzo[a]pyrene. The incidence of tumors, however, increased between the

40 and 250 ppm benzo[a]pyrene dosages. Stomach tumors, which were mostly squamous cell papillomas but also some carcinomas, appeared with an incidence significantly higher than controls ($p<0.001$, Fisher Exact Test) at several doses. Quantitative risk estimates for human cancer incidence were developed by U.S. EPA (1980d) using the linearized multistage procedure and more recently by Clement Associates (1988, 1990a) using a two-stage model. Krewski and Murdock (1990) also applied the Armitage-Doll and a two-stage model to these data. These approaches appear below.

U.S. EPA (1980d) Approach. The Neal and Rigdon (1967) data, with adjustments to approximate daily lifetime doses (Table VIII-3), were used in calculation of a quantitative risk estimate for human lifetime cancer incidence by use of the linearized multistage procedure. Tumor incidence data at the highest three doses were not used in the extrapolation due to lack of fit to the multistage model. [A discussion of the fit of data to the multistage model appears in Human Health Methodology Appendix to the October 1980 Federal Register (45 FR 79379).]

A carcinogenic potency factor for humans (q_1^*) was determined to be 11.53 $(mg/kg/day)^{-1}$. An estimate of the cancer risk from consuming 1 µg benzo[a]pyrene/ℓ water (unit risk) could be calculated as follows:

$$\text{Unit risk} = \frac{11.53\ (mg/kg/day)^{-1} \times 2\ \ell/day \times 0.001\ mg/\mu g}{70\ kg} = 3.29 \times 10^{-4}/\mu g/\ell$$

where:

70 kg = assumed weight of adult human

2 ℓ = assumed water consumption of adult human

TABLE VIII-3

Incidence of Tumors in Mice Treated Intragastrically with Benzo[a]pyrene[a,b]

Experimental Dose (ppm diet)	Transformed Dose (mg/kg/day)	Incidence[c] No. Responding/No. Tested
0	0.0	0/289
1	0.1	0/25
10	1.3	0/24
20	2.6	1/23
30	3.9	0/37
40	5.2	1/40
45	5.9	4/40
50	6.5	24/34
100	13.0	19/23
250	32.5	66/73

[a]Source: Neal and Rigdon, 1967

[b]Length of exposure = 110 days; length of the experiment = 183 days; lifespan of mouse = 630 days; average weight of mouse = 0.034 kg

[c]The incidences at the highest three doses were not used in the extrapolation due to lack of fit of the multistage model.

There are substantial difficulties with the procedure used to derive a risk estimate from the Neal and Rigdon (1967) data as given in Table VIII-3. Among the factors that render this data set problematic are the following:

1. Exposure time was variable in the higher dose groups (Table VIII-4). The exposure time was reported as a range, and individual animal data are now unavailable.
2. Animals were started on test at differing ages.
3. A very sharp increase in tumor incidence was noted in those animals fed 50 ppm by comparison with those fed 45 ppm.
4. There was an apparent leveling off of tumor incidence among the highest dose groups.

In the U.S. EPA (1980d) use of the multistage model the peculiarities of the data set were handled by using one estimate of exposure time (110 days) and by discarding data at the highest three doses as they did not fit the model. This precluded use of the highest incidence data and resulted in the odd situation of eliminating a dose group (50 ppm) essentially equivalent to one (45 ppm) that was used in the modeling procedure.

Clement Associates (1988). An alternate approach was proposed by Clement Associates (1988) in which the Neal and Rigdon (1967) data were fit to a two-stage dose-response model. This two-stage model is a special case of the Moolgavkar and Knudson (1981) and Moolgavkar (1986) cancer risk model as adapted by Thorslund et al. (1987) to account for exposure to known levels of carcinogens. According to the two-stage model, the population of cells at risk for induction of cancer consists of stem cells. These cells can divide, undergo differentiation to terminal, nondividing cells, die, or undergo changes that result in a preneoplastic state. It is assumed that this last option includes a mutation or heritable change at a critical site.

TABLE VIII-4

Forestomach Tumors in Mice Fed Benzo[a]pyrene*

Exposure B[a]P ppm x	First Subgroup			Second Subgroup			No. with Forestomach Tumors/No. of Mice r/n
	Age First Exposed t_{01}	Age Last Exposed t_{f1}	Age Last Observed t_1	Age First Exposed t_{02}	Age Last Exposed t_{f2}	Age Last Observed t_2	
0	300	300	300				0/289
1	30	140	140				0/25
10	30	140	140				0/24
20	116	226	226				1/23
30	33	143	143	67	177	177	0/37
40	33	143	143	101	211	211	1/40
45	31	141	143	71	181	183	4/40
50	17	124	124	22	219	219	24/34
100	20	118	118	24	146	146	19/23
250	18	88	88	20	185	185	66/73
250	49	50	155				0/10
250	56	58	162				1/9
250	49	53	155				1/10
250	62	67	168				4/9
250	49	56	155				3/10
250	91	121	198				26/26
100	74	81	182				0/10
100	48	78	156				12/18
5000	98	99	209	180	181	294	17/33

*Source: Adapted from Neal and Rigdon (1967) by Clement Associates (1990a)

The preneoplastic cell then has the options of division, differentiation, death, or further change to a fully transformed or cancer cell. This cancer cell, if permitted to proliferate, will form the basis of a tumor. In the Moolgavkar and Knudson (1981) model, these processes are described mathematically by postulating specific exposure-dependent rates for cell changes. A simplified version of the model can be expressed in the following manner:

$$I(t) = M_0 M_1 \int_0^t C_0(v)\ [\exp(B-D)(t-v)]dv \qquad (8\text{-}1)$$

where

$I(t)$ = age-specific cancer incidence at age t

M_0 = transition rate from stem to preneoplastic cell

M_1 = transition rate from preneoplastic to cancerous cell

$C_0(v)$ = number of susceptible stem cells per individual target organ at age v

B = birth rate or rate of cell proliferation of preneoplastic cells

D = death rate of preneoplastic cells

It is likely that at least some of the increased incidence is attributable to the fact that the 50 ppm dosed animals were younger when exposure was begun. It is, nevertheless, appropriate to use as much of the data as feasible in calculating the quantitative estimate. It is reasonable to assume that the biologic processes described by the above equation can be affected by exposure to carcinogens and that the likelihood of their occurrence is a function of exposure time. Thorslund et al. (1987) thus developed a version of the model that incorporated time and exposure dependence.

In applying the two-stage model to the Neal and Rigdon (1967) data in Table VIII-5, some modifications were made by Clement Associates (1988) and some parameters were estimated. In the absence of experimental information about cell stages and differences in exposure over time, it was not possible to estimate the individual background and exposure-induced mutation rates for preneoplastic and transformed cells using tumor rate data. Nor could the relative transition rates that correspond to each stage be identified. Two exposure-induced relative transition rates and a background transition rate were estimated from bioassay data. If these transition rates are linear functions of dose (which is likely at low doses), they may be expressed as $M_0 = \alpha_0 + \beta_0 x$ and $M_1 = \alpha_1 + \beta_1 x$ where α is the background transition rate and β is the PAH-induced transition rate per unit of exposure for each stage. It was assumed that the factors influencing the background transition rates and the PAH-induced transition rates have the same relative effectiveness for each stage. Under this assumption, $\beta_0/\alpha_0 = \beta_1/\alpha_1 = S$, the relative transition rate, so that $M_0 M_1 = \alpha_0\alpha_1(1+Sx)^2 = M(1+Sx)^2$, where $M = \alpha_0\alpha_1$.

In this application of the model it was assumed that promotional effects result from multiple molecular interactions. Thus, the growth rate of preneoplastic cells G at low doses is virtually independent of exposure level so that $G = B - D$. If this assumption is violated at higher doses, the shape of the curve will have greater curvature than quadratic, and the quadratic model will be rejected.

Finally, as a first approximation it was assumed that the number of stem cells post-maturity, $C_0(v)$, is relatively constant and may be taken to be unity [i.e., $C_0(v) \simeq C_0 \simeq 1$].

TABLE VIII-5

Variable Exposure Data Used to Estimate Parameters in the Ingestion Dose-Response Model for Benzo[a]pyrene[a]

Dose (ppm) x	Age First Exposed (days) t_s	Age Sacrificed (last exposed) (days) t	Number of Animals Exposed n	Number of Forestomach Tumors	
				Observed	Expected[b]
0	--	300	289	0	0.000
1	30	140	25	0	0.002
10	30	140	24	0	0.232
20	116	226	23	1	0.875
30	33 67	143 177	37	0	3.091
40	33 101	143 211	40	1	5.746
45	31 71	141 181	40	4	7.560
50	17 22	124 219	34	24	19.692
100	20 24	118 146	23	19	14.220
250	18 20	88 185	73	66	64.259

[a]Source: Neal and Ridgon (1967) as adapted by Clement Associates (1988)

[b]It was assumed that one-half the animals in each group were exposed during each of the intervals reported, t_s to t. $x^2 = 17.12$, d.f. = 8, $p \simeq 0.03$

Substituting these expressions [G, M, S, $C_0(v)$] into equation 8-1 and integrating yields a cumulative hazard function H(x,t), so that the probability that a tumor will develop by time t as a result of exposure to a level of genotoxic agent x can be expressed as:

$$P(x,t) = 1 - \exp[-H(x,t)] = 1 - \exp[-M(1+Sx)^2][\exp(Gt) - 1 - Gt]/G^2 \quad (8\text{-}2)$$

where

M = background tumor rate parameter

S = fractional increase in the transition rate between cell stages per unit dose, assumed to be the same for each stage

G = B - D and is the exposure-independent growth rate of preneoplastic cells

t = the time (or age) at which risk is evaluated

The level of agent at the target tissue was assumed to be directly related to the administered dose. This was based on observations of experiments discussed earlier in which the rate of formation of the major benzo[a]pyrene diol epoxide-DNA adduct was found to be linearly related with respect to dose in the forestomach, lung, and skin (Pereira et al., 1979; Adriaenssens et al., 1983).

The specific dose-response model derived for benzo[a]pyrene (equation 8-2) was thus a restricted form of the model developed by Armitage and Doll (1957), Moolgavkar and Knudson (1981), and Thorslund et al. (1987). It was restricted by assuming that G is independent of treatment (x) and that the two transition rates are linear functions of x with proportional coefficients. The consequence of these assumptions is that at constant t the dose-response function has only two parameters:

$$P(x) = 1 - \exp\text{-}A(1 + Sx)^2 \quad (8\text{-}3)$$

where $A = M[\exp(Gt)-1-Gt]/G^2$ and t = age at last observation.

Use of this modification of the two-stage model for quantitative risk estimation for benzo[a]pyrene has several advantages.

1. At low doses, the model converges to a linear, nonthreshold form.
2. Only two parameters need to be estimated.
3. A stable point estimate of risk could be obtained directly.
4. The mathematical form of the two-stage model is based on an acceptable theory of cancer induction.
5. The model is consistent with what is known regarding the mechanism of tumor induction by PAHs, including benzo[a]pyrene (e.g., exposure to cigarette smoke).
6. Data from all dose groups reported in Neal and Rigdon (1967) could be used in the risk estimation.

As indicated earlier, the animals in the Neal and Rigdon (1967) study were exposed at various ages and for varying times. In order to describe the dose-response relationship for the ingestion of benzo[a]pyrene and tumor incidence for each exposure group, estimates for each of the exposure variables were made as indicated in Table VIII-5. As a first approximation, each mouse was assumed to be exposed to one of two exposure patterns:

(1) those that were youngest at first exposure and were sacrificed at the earliest age (e.g., for the group exposed to 0.04 mg benzo[a]pyrene in Table VIII-4, were first exposed at age 33 days and last exposed at age 143 days), and

(2) those that were oldest at first exposure and were sacrificed at the oldest age (e.g., for the 0.04 mg/group, were first exposed at 67 days and last exposed at 177 days).

One-half of the mice in each exposure group were assumed to have been exposed to each exposure pattern. Using the maximum likelihood method, the data in Table VIII-5, and the assumption that the tumors are incidental, the parameters in the hypothesized risk equation were estimated as outlined in

Gart et al. (1986). The resulting best-fitting model has the parameters estimates $\gamma\alpha^2 \to 0$, G = 0.194, and $\gamma\beta^2$ = 0.2142. This mathematical model gives a statistically adequate fit (i.e., the resulting χ^2 has a p value >0.01, which is the criterion used for the multistage model by the U.S. EPA). This fit is shown in Table VIII-5.

The resulting model is a quadratic function of dietary concentration at high exposure levels. This model is assumed to be valid over the longest exposure duration employed for a group of animals; that is, $t-t_s=(219-22)/7=28.143$ weeks. The parameter value for G (0.194), however, is incompatible with increases in age-specific cancer death rates that have been observed. This value for G implies that the rates increased at about the 15th to 16th powers of age (since $h(t)=\exp Gt-1 \sim \alpha\, t^{15.5}$). This is greater than the highest reported rate, which is that for human prostate cancer (k=13). Values for k are usually between 4 and 8. This large value of G suggested to the authors of Clement Associates (1988) that benzo[a]pyrene exerts a promotional effect, which was not incorporated into the model.

To calculate a lifetime risk estimate for humans it was assumed that a mouse consumes 13% of its weight/day as food, a standard surface area adjustment was made for differences between species and 70 kg was assumed for human body weight. The low dose linear term for humans was calculated to be 3.22 per(mg/kg)/day.

Krewski and Murdoch (1990). Krewski and Murdoch (1990) also made an attempt to make greater use of the available data in Neal and Rigdon (1967) shown in Table VIII-4. Appropriate adjustments were made for start and

duration of exposure and age at sacrifice. Both the Armitage-Doll and a two-stage model adapted from Moolgavkar (1986) with exponential expansion of preneoplastic cells, were fit to the data. As is shown in Table VIII-6, however, neither model was very successful in predicting the observed tumor incidences. The authors suggested that this failure to fit the data may be due to an underlying deviation of the theoretical basis of the models from reality, but may also represent inadequate experimental technique in the generation of data or the use of the approximate mathematical forms of the model. Alternative explanations for the failure of the model to fit the data are the following:

- use of an inappropriate method of adjusting for differences in the exposure interval within an exposure group;
- lack of an appropriate exposure-dependent growth rate of preneoplastic cell growth rate expression;
- background tumor rates poorly defined by the data;
- lack of data on whether observed tumors are papillomas or carcinomas.

Clement Associates (1990a). More recently a model was developed incorporating a different assumption as to how to treat the exposure groups containing animals with different durations of exposure. This model differs from the earlier Clement Associates (1988) report in that both transition rates and the growth rate of preneoplastic cells were considered to be exposure-dependent. To this end a simple saturation assumption was used to define the functional form for the dose-dependence of the preneoplastic growth rate.

The cumulative hazard function for the two-stage model for exposure constant at level x over the interval t_0 to t_f for $t=t_f$ is discussed by Thorslund et al. (1987).

TABLE VIII-6

Forestomach Tumors in Mice Subjected to Variable Exposures of Benzo[a]pyrene[a]

Dose Group	Dose d (ppm)	Exposure Period t_1-t_2 days	Time at Sacrifice T days	Number of Animals Exposed	Number of Animals with Tumors		
					Observed	Predicted	
						A-D Model	M-V-K Model
1	0	--	300	289	0	0.0	0.0
2	1	20-140	140	25	0	0.2	0.2
3	10	30-140	140	24	0	1.9	1.4
4	20	116-226	226	23	1	3.6	2.7
5	30	50-160	160	37	0	8.3	6.3
6	40	67-177	177	40	1	11.4	8.8
7	45[b]	51-161	163	40	4	13.0	10.4
8	50	20-172	172	34	24	18.8	23.8
9	100	22-132	132	23	19	13.3	11.1
10	250	19-137	137	73	66	67.0	66.6
11	250	49-50	155	10	0	0.4	0.4
12	250	56-58	162	9	1	0.6	0.8
13	250	49-53	155	10	1	1.3	1.6
14	250	62-67	168	9	4	1.5	1.7
15	250	49-56	155	10	3	2.2	2.5
16	250	91-121	198	26	26	16.1	15.5
17	100	74-81	182	10	0	1.0	1.1
18	100	48-78	156	18	12	5.8	5.6
19	5000	139-140	252	33	17	17.9	21.9
					x^2	50.9	44.7
					d.f.	16	15
					p	2×10^{-5}	9×10^{-5}

[a]Source: Adapted from Krewski and Murdoch (1990)
[b]Corrected from 0.05 (rounded value) given by authors

To fit the model it was assumed that the exposure groups are made up of two subpopulations with different exposure durations of one of two ranges as shown in Table VIII-4. It was also assumed that the first and second subpopulations in any exposure group j are a proportion of the total of γ_j and $1-\gamma_j$; γ_j may be considered as an unknown parameter. The proportion of animals responding in exposure group j is:

$$P(x,t) = \gamma_j[1-\exp-H(x,t_{01},t_{f1},t_1)]+(1-\gamma_j)[1-\exp-H(t_{02},t_{f2},t_2)]$$

When $\gamma=1$ (animals exposed under uniform conditions),

$$P(x,t)=1-\exp-H(x,t_{01},t_{f1},t_1).$$

The data in Table VIII-4 were fit to the model with the addition of two zero dose groups. The control groups reported by Neal and Rigdon (1967) showed no incidence of forestomach tumors. Using zero incidence data in the model may result in some cases in unstable parameter estimates; it was thus decided to include some nonzero historical control data. Mice used by Neal and Rigdon (1967) were described as "an inbred CFW strain kept in this laboratory for 8 years". The CFW mouse is, in fact, an outbred strain from Carworth Farm (CF) and was derived from Swiss Webster. The outbred stock is still maintained by Charles River Breeding Laboratories (Crl). It is believed that the inbred mice from the testing laboratory are not likely to be genetically identical to other inbred CFW colonies or to other Swiss Webster mice. However, forestomach tumor incidences for untreated animals considered to be similar to the test population are given in Table VIII-7.

TABLE VIII-7

Historical Control Incidence Data for Forestomach Tumors in Strains of Swiss (Webster) Bred Mice

Strain	Sex	Age (days)	Tumor Type	Incidence	Reference
Swiss random-bred	M	--	papilloma	1/99	Toth et al., 1976
	F	--		0/99	
CFW	M	--	--	0/100	Sher, 1974
	F	--	--	0/203	
SPF Swiss (Webster)	M	--	--	0/28	Prejean et al., 1973
	F	--	--	0/38	
SWR/J Swill	M	700	squamous cell carcinoma	2/268	Rabstein et al., 1973
	F	608	squamous cell carcinoma	1/402	
CFW Texas colony	unknown	300	--	0/289	Neal and Rigdon, 1967

These data indicate that forestomach tumor incidence was quite low in Swiss-derived mice in the 1970s. The data chosen for inclusion were those for SWR/J mice reported by Rabstein et al. (1973). These data have the advantages of being from mice of a known average age and of having a nonzero incidence of squamous cell carcinomas in both males and females. The SWR/J incidence data are consistent with those reported for the test population at 300 days of age.

Two forms of the model were used. The first used the simplifying assumption that each of the two subpopulations of the exposure groups contained half the animals (γ_j=1/2). The second assumption made was that the preneoplastic cell growth rate reverts to background after exposure; in other words, G*(x)=G(0). The growth rate was defined to be of a general functional form:

$$G(x) = G(0)\left[1 + \frac{bx}{1+Cx}\right]^m$$

where m is a given integer, here taken to be 1 as a conservative assumption; b and C are unknown parameters to be estimated from the data.

To obtain maximum likelihood estimates of the unknown parameters, the general approach discussed by Gart et al. (1986) was used (Table VIII-8). No adequate fit to the full data set was obtained. If, however, the data were restricted to the lower dose, longer exposure groups (presumably those most relevant for low-dose continuous human exposure estimates) a reasonable fit was obtained. Table VIII-9 presents comparisons of estimates derived by Clement Associates (1988, 1990a), and Krewski and Murdoch (1990).

TABLE VIII-8

Maximum Likelihood Parameter Estimates for Two-Stage Model with Saturation of Growth Rate Function

Parameter	Physical Meaning	Estimated Numerical Values for All Continuous Exposure Data
A	product of background transition rates	2.4569×10^{-10}
G(0)	background growth rate of preneoplastic cells	1.1995×10^{-2}
S	relative cell transition rates per unit of exposure (mg/g in food)	845.56
b	relative increase of preneoplastic cell growth rate per unit of target dose equivalent exposure from mg/g in food	159.32
C	saturation coefficient exposure (mg/g food)	16.303
$u(x,t)=P(x,t)-P(o,t)$ $t=730$	low-dose risk	
$x=1 \times 10^{-5}$		3.00×10^{-4}
1×10^{-6}		2.97×10^{-5}

Model

$$p(x,t)=1-\exp -H(x,t)$$

$$H(x,t) = \frac{A(1+Sx)^2}{G(x)} \{\exp[G(x)t]-G(x)t-1\}$$

$$G(x) = G(0) \cdot \left[1 + \frac{bx}{1+Cx}\right]$$

TABLE VIII-9

Forestomach Tumors in Mice Subjected to Exposures of Benzo[a]pyrene for at least 70 Days[a]

Dose Group	Dose d (ppm)	No. of Animals Exposed	Number of Animals with Tumors					
			Observed	Predicted				
				Krewski and Murdoch		Clement Associates		
				A-D Model[b]	M-V-K Model[c]	M-V-K:Initiation		M-V-K: Initiation and Promotion
						1988	1990	
1	0	289	0	0.0	0.0	0.0	0.0	0.0
2	1	25	0	0.2	0.2	0.0	0.0	0.0
3	10	24	0	1.9	1.4	0.2	0.2	0.0
4	20	23	1	3.6	2.7	0.9	0.9	0.2
5	30	37	0	8.3	6.3	3.1	3.1	0.7
6	40	40	1	11.4	8.8	5.7	5.8	2.4
7	45	40	4	13.0	10.4	7.6	7.2	3.7
8	50	34	24	18.8	23.8	19.7	19.4	18.8
9	100	23	19	13.3	11.1	14.2	14.2	18.9
10	250	73	66	67.0	66.6	64.3	64.9	66.1
11	0	268	2	--	--	--	2.6	2.0
12	0	402	1	--	--	--	0.4	1.0
				x^2		9.94	18.08	
				degrees of freedom		7	9	
				p		0.192	0.034	

[a]Source: Clement Assocites (1990a)

[b]Armitage-Doll model

[c]Moolgavkar-Venson-Knudson model

A difficulty with the previous estimate by Clement Associates (1988) was that the value of G was unrealistically large. This difficulty was addressed by Clement Associates (1990a) by deriving a $G_{(0)}$ for humans. It was assumed that the human G_0 is equal to the mouse G_0 times a constant equal to the mouse lifespan of 2 years divided by a human lifespan of 76 years. The result is G_0=0.1152. To relate this value to a power of age, they solved for the value of k (the number of stages in a multistage model) that will cause the same relative increase in the age-specific risk for an individual at one-half lifespan compared with full lifespan. For G=0.1152 and lifespan =76, Clement Associates (1990) found that k=7.33. As indicated in an earlier section, most human values for k fall between 4 and 8; for the U.S. population the value for k for stomach cancer is -7. They concluded that the value of $G_{(0)}$ for humans of 0.1152 is in a reasonable range.

A maximum likelihood estimate for humans was obtained. Under an additive risk model the risks become a linear function of dose as x approaches 0. The additional risk over background was expressed as u(x,t)=P(x,t)-P(o,t), where P(x,t) is the probability of cancer by age t given an exposure level of x. As the exposure x becomes small, one can derive the approximation u(x,)~L(t).x, where L(t) is the low-dose linear term. Extensive application of the chain rule of derivatives for the model in Table VIII-8 resulted in the following equation:

$$L(t) = \frac{A}{G(0)^2} \exp[G(0)t] \cdot [2(S-b)+bG(0)t][1-P(o,t)]$$

Substituting the maximum likelihood parameter estimates in Table VIII-8 into this formula provides a value of L(t)=29.63 for t=730 days. To change the estimate into units of mg/kg/day, the assumption was made that a mouse consumes 13% of its body weight per day.

Thus, the low-dose linear term for mice

$$= 29.63 \times 0.001/0.13$$

$$= 0.2278 \text{ per(mg/kg)/day}$$

To obtain the low-dose linear term for humans a standard assumption of surface area equivalence between mice and humans was made and the resulting maximum-likelihood estimate of the low dose linear was found:

$$0.2278 \sqrt[3]{\frac{70 \text{ kg}}{0.034 \text{ kg}}} = 2.90 \text{ per(mg/kg)/day}$$

An approximate co[illegible]tional upper bound was then calculated by Clement Associates (1990a). The low-dose linear term was considered to be a linear combination of exposure-related parameters b and S. By assuming that the age specific rate function for controls was known (that is, the parameters A and G(0) were fixed at their maximum likelihood estimates), the low-dose linear term could be expressed as

$$L(t)=H(o,t) \cdot [1-P(o,t)] \{2S+b\}[G(0)t-2]\}$$

where $P(o,t) = 1-\exp-H(o,t)$

and $H(o,t) = \frac{A}{G(0)2} \{\exp[G(0)t]-G(0)t-1\}$

The equation was solved using t=730, $A=2.4599 \times 10^{-9}$ and G(0)=0.11992 from Table VIII-8. An upper 95% bound on L(t) was obtained by substituting values for S (relative transition rate) and b (promotional effect) that maximized L(t), subject to certain constraints. In this procedure the upper bound on the promotion parameter, b, was reduced from its maximum likelihood value by about 2-fold. The human upper bound of the low-dose linear term thus obtained was 5.88 per(mg/kg)/day.

In U.S. EPA (1991b) issue was taken with the manner in which the linear slope and the upper bound were calculated. The authors' interpretation of the above slope factor is that it represents an lower bound rather than an upper bound; it is the slope or tangent at dose 0. They further objected to the following: (1) not all parameters were included in the process of obtaining the upper bound, but rather only two of the dose-dependent parameters; (2) it was not considered biologically reasonable to require a more than 50% reduction in the cell growth rate in order to obtain an upper bound; and (3) the historical background tumor incidence used was from a mouse strain not used in the bioassay.

To these authors the inability to derive an upper bound by starting from the parameter values associated with the point estimate of dose response suggests a peculiarity of the model. It can be shown that a 50% decrease in the cell growth rate value can significantly alter the shape of the dose response model.

As an alternative, they provided an upper bound by extrapolating linearly from the 10% response point to the background of an empirically fitted dose-response curve. It was noted in cases wherein the fitted dose-response curve is not linear at low doses that the potency slope or upper bound can be defined as a secant from a point on the dose-response curve to the zero dose point. Similar concepts and approaches have been proposed by Krewski et al. (1986, 1991) and Gaylor and Kodell (1980). Their results indicate that potency slopes thus calculated are comparable to those obtained from a linearized multistage procedure for the majority of compounds investigated.

The upper bound calculated in U.S. EPA (1991b) on the Neal and Rigdon (1967) data as modeled by Clement Associates (1990) is 9.3 per(mg/kg)/day.

U.S. EPA (1991b). The authors of this report chose a model to reflect the exposure pattern in the Neal and Rigdon (1967) study; that is, partial lifetime exposure over different (presumably developmentally varied) portions of their lifetime. To this end they used a Weibull-type dose-response model that could accomodate partial lifetime exposure. To derive this model it was asssumed that the hazard rate for tumor occurrence induced by benzo[a]pyrene can be given by

$$h_1(t) = \int_0^t (t-s)^{k-2} D(s)\,ds$$

where $D(s) = f(d)$

$= a_1 d + a_2 d^2 + \ldots + a_m d^m$ for some positive integer m if $t_0 < s < t_1$ and

$D(s) = 0$ otherwise

and d = dose given to animal at time s.

The dose-related hazard function $h_1(t)$ implies that the early benzo[a]-pyrene exposure contributes more to the hazard rate than does the later exposure. This assumption is consistent with the observation that a single dose of benzo[a]pyrene of a certain magnitude is sufficient to induce papillomas after a sufficiently lengthy observation period.

The cumulative hazard by time T for background can be given by

$$H_0(T) = \int_0^T at^{k-1}dt$$

$$= (1/k)aT^k$$

The cumulative hazard for $T \geq t$ for the hazard induced by benzo[a]pyrene can then be expressed as

$$H_1(T) = \frac{f(d)}{(k-1)k}\,[(T-t_0)^k-(T-t_1)^k].$$

Thus, the probability of tumor observation by time t (given that animals are exposed only to benzo[a]pyrene during the time interval $[t_0, t_1]$) has the form

$$P(d,t) = 1-\exp\{-(q_0+q_1d+\ldots+q_md^m)[(t-t_0)^k-(t-t_1)^k]\}.$$

This model was considered to be equivalent to the multistage model that would be used when animals are exposed to benzo[a]pyrene for their entire lifetimes. A quadratic model with m=2 was found to be adequate to fit the Neal and Rigdon (1967) data. The results of the modeling are given in Table VIII-10. U.S. EPA (1991b) reported that data fit was adequate for all points except those from the 50 and 100 ppm treatment groups. They point out that the poor fit may be attributable to biologic factors rather than to statisitical reasons; they note that the animals in these dose groups were started on test at a younger age ($\leq$20 days) than were animals in other groups.

TABLE VIII-10

Predicted vs. Observed Tumor Incidence and Data Used to Calculate the Dose-Response Model[a,b]

Dose (mg/g diet)	t_0 (day)	t_1 (day)	t (day)	Observed[c]	Predicted Incidence Rate
0.000	0	300	300	0/289 (0.00)	0.00
0.001	30	140	140	0/25 (0.00)	0.00
0.01	30	140	140	0/24 (0.00)	0.00
0.02	116	226	226	1/23 (0.05)	0.02
0.03	50	160	160	0/37 (0.00)	0.04
0.04	67	177	177	1/40 (0.03)	0.08
0.045	50	160	162	4/40 (0.10)	0.11
0.05	20	172	172	24/34 (0.70)	0.30[d]
0.10	22	132	132	19/23 (0.82)	0.40[d]
0.25	19	137	137	66/73 (0.90)	0.98

[a]Source: U.S. EPA, 1991b

[b]t_0 = age of mice (in days) when exposure began, t_1 = age of mice (in days) when exposure was terminated, t = age of mice (in days) when the study was terminated. Data on the control and the first nine low-dose groups were taken from Table 1 of Neil and Rigdon (1967). Data from other groups were not included because animals in these groups were exposed to high doses of B[a]P for only very short periods.

[c]Observed number of animals with tumors (incidence rate)

[d]The predicted value lies outside the 95% confidence interval calculated from the observed response. This crude test is used to determine whether or not the model reasonably predicts the observed response.

Parameters were estimated by the maximum likelihood method as follows:

$$q_0 = q_1 = 0$$
$$q_2 = 2.1 \times 10^{-5}$$
$$k = 3.13.$$

A slope factor at the 10% point (d= 2.4 ppm or 0.3 mg/kg/day) was calculated by setting $t_0 = 0$, $t_1 = 730$, and $t = 730$. The human upper bound thus obtained was 4.5 per(mg/kg)/day.

Brune et al. (1981). The study by Brune et al (1981) is the only reported benzo[a]pyrene ingestion study of 2 years duration. Benzo[a]pyrene was administered to Sprague-Dawley rats in either the diet or by gavage in a solution of 1.5% caffeine. Effective numbers were not reported; it was assumed that the number of animals started on test (32 males and 32 females) constituted the group examined for tumors. Incidence rates for males and females combined are given in Table VIII-11.

A linearized multistage procedure was used to calculate an upper bound slope factor (q1*) for benzo[a]pyrene from these data. A body surface equivalence assumption was used for interpecies conversion; that is, the animal slope was multiplied by a factor equal to

$$(W_h/W_a)^{1/3}$$

where

W_h = 70 kg (assumed adult human weight)

W_a = 0.4 kg (assumed rat weight)

and dose is expressed in mg/kg/day

TABLE VIII-11

Incidence to Forestomach Only and Total Contact Site Tumors in Sprague-Dawley Rats Exposed to Benzo[a]pyrene by Gavage or in the Diet[a]

Dose (mg/year)	Median Survival Time (days)	Tumor Incidence	
		Forestomach Only	Total Contact Site[b]
Gavage			
0	102	3/64	6/64
6	112	11/64	13/64
18	113	25/64	26/64
39	87	14/64[c]	14/64[c]
Diet			
0	129	2/64	3/64[d]
6	128	1/64	3/64[d]
39	131	9/64	10/64[d]

[a]Source: Adapted from Brune et al., 1981

[b]Includes forestomach, larynx and esophagus

[c]Data from this group were not used in calculation due to the shorter survival time. No adjustment was done as time-to-death data were not available.

[d]The incidence of total tumors in males for the control, low- and high-dose groups was 3/32, 3/32 and 8/32, respectively.

Higher slope factors were obtained by U.S. EPA (1991b) from the gavage data (27.1 per(mg/kg)/day based on total contact-site tumors and 27.5 per(mg/kg)/day for forestomach tumors only) than were calculated from the dietary intake study [4.7 per(mg/kg)/day based on total tumors and 3.8 per(mg/kg)/day for forestomach tumors]. It is not possible to determine from the Brune et al. (1981) paper whether the enhanced tumor response is due to the effects of gavage (potential irritation), to the co-carcinogenic activity of caffeine, or to some combination of both factors.

In the dietary, but not in the gavage component of the study, more total contact-site tumors (larynx, esophagus and forestomach) were observed in the males than in the females. Although it was possible to separate total contact-site tumors into incidences for males and females, information in the paper was not adequate for the same adjustment for forestomach tumor incidence. In order to derive a potency estimate based on male rat data only, the slope factor calculated by U.S. EPA (1991b) on the combined male and female data was multiplied by a factor of 1.5. The 50% increase was used to adjust for the risk in cancer risk for male-only total tumors. Slope factors based on male rat total contact-site tumors or forestomach tumors only were 7.1 and 5.7 per(mg/kg)/day respectively.

Chouroulinkov et al. (1967). Chouroulinkov et al. (1967) was another relatively long-term study (14 months) wherein albino mice of unspecified strain were administered a total estimated dose of 8 mg of benzo[a]pyrene mixed with olive oil in the diet. The incidence of forestomach tumors was 0/81 for the controls and 5/81 for the treated animals. As there was only

one non-zero dose the slope factor was calculated in U.S. EPA (1991b) by direct extrapolation from the observed response of 0.062 at 0.63 mg/kg/day to the background rate of 0:

$$(0.062/063) \times (70 \text{ kg}/0.03 \text{ kg})^{1/3} \times (24 \text{ months}/14 \text{ months})^{3}$$
$$= 6.5 \text{ per(mg/kg)/day}$$

where $(24/14)^3$ is a factor to adjust for less-than-lifetime observation.

Choice of a Quantitative Estimate for Benzo[a]pyrene

Slope factors described in the foregoing text are presented for comparison in Table VIII-12. Note that the slope factors obtained from the gavage portions of the Brune et al. (1981) study are not included. This is because of the uncertainty as to magnitude of co-carcinogenic effect presented by the caffeine vehicle and the potential irritation component of gavage. Only results of dietary studies are considered in this instance for basis of the quantitative risk estimate. Also eliminated from consideration at this point is the slope factor at dose 0 (maximum likelihood estimate) derived in Clement Associates (1988), in order to facilitate comparison among like estimates (upper bounds).

As indicated in Table VIII-12, these estimates span less than an order of magnitude. Each is based on a less-than-optimal but acceptable data set from studies in two species of outbred rodents. Each estimate is based on a low dose extrapolation procedure that entails the use of multiple assumptions and default procedures.

TABLE VIII-12

Slope Factors for Humans Based on Benzo[a]pyrene Feeding Studies

Study	Slope Factor per(mg/kg)/day	Comments
Neil and Rigdon, 1967	11.5	Linearized multistage, highest points dropped (U.S. EPA, 1980d)
	5.9	Two-stage, conditional upper bound (Clement Associates, 1990)
	9.0	Clement two-stage, slope from 10% response (U.S. EPA, 1991b)
	4.5	Weibull-type model (U.S. EPA, 1991b)
Brune et al., 1981	4.7	Larynx, esophagus, forestomach tumors, male and female rats
	7.1	Larynx, esophagus, forestomach tumors, males only
	3.8	Forestomach tumors only, male and female rats
	5.7	Forestomach tumors only, male rats only
Chouroulinkov et al., 1967	6.5	Extrapolated from the observed response.

The least acceptable of the estimates is that presented by U.S. EPA (1980d) of 11.53 per(mg/kg)/day based on the Neal and Rigdon (1967) data. This application of the linearized multistage model does not account for the variable times of exposure or the varying ages of exposure of the dose groups. In fitting the model all data from exposures >45 ppm were dropped. In 1987 an Agency work group, the Carcinogen Risk Assessment Verification Endeavor (CRAVE), found sufficient reason to revise this estimate in the light of improved risk quantitation procedures. It was decided at this time not to include the 1980 quantitative estimate on the Integrated Risk Information System (IRIS).

The use of simplified two-stage Moolgavkar-Venson-Knudsen models, has permitted the use of more of the data reported by Neal and Rigdon (1967). The two-stage model is described as being biologically based. It is consistent with current thinking that PAHs act as carcinogens by a multistage process with a small number of stages and allows for incorporation of terms that may model other than initiating activity. In the form of the model presented by Clement Associates (1990a), a term to permit modeling data for benzo[a]pyrene acting as its own promoter was included. This is not inconsistent with the observation of benzo[a]pyrene's activity as a complete carcinogen in skin. The modified two-stage model is relatively simple requiring the estimation of a limited number of parameters. As applied it is linear at low doses as long as the background incidence is not zero. This application has, however, necessitated the use of control data of a historical nature from related but not identical strains of mice.

The simplified two-stage model does not accurately predict tumor incidence from high-dose short-term exposures. It may be that exposure of this sort results in tumor induction by mechanisms sufficiently different from chronic low-dose exposure that similar models could not be expected to apply. A potential problem discussed by Clement Associates (1990a) is that the approximation for the exact hazard function used to generate mathematical expressions may not be sufficiently accurate. They suggested that improved accuracy would be possible using the exact model if both birth and death rates of pre-neoplastic cells were available.

The use of available forms of the two-stage model for generating risk assessments has come under some criticism. It would appear that this type of model is best defined as biologically based when the several parameters given biologic meaning can be estimated from specific data. In the case of the Neal and Rigdon (1967) study, the data are inadequate to this application. A proposed future direction is to investigate the use of human epidemiologic data for the calculation of estimates of parameters dealing with background transition rates and background growth rates of preneoplastic cells.

For the Neal and Rigdon (1967) data it can only be said that the two-stage model provides a convenient curve fitting tool, and that its use is not contraindicated by information on potential carcinogenic mechanisms for benzo[a]pyrene.

The use of the Weibull type model in a curve-fitting procedure also allows for the consideration of the variable exposure time of the animals in Neal and Rigdon (1967). A difficulty is the lack of fit to the 50 and 100 ppm dose group data. In a test of this model to several other benzo[a]-pyrene data sets it was shown that it accurately predicted the tumor response in mice in many dietary studies but not in those wherein administration was by gavage. There are two plausible explanations for this discrepancy: (1) benzo[a]pyrene given by gavage induces greater incidences of gastric tumors than does ingestion; (2) or the effect of PAH continues after exposure. The latter hypothesis is supported by observation of tumor induction as a consequence of a single dose of benzo[a]pyrene.

The Brune et al. (1981) dietary exposure data provide a more limited dose range than do those of Neal and Rigdon (1967). For the lower dose of the two treated groups the incidence of both total contact-site and forestomach tumors are equal to or less than the controls. The data are not reported as single sex incidences for forestomach tumors. This adds some additional uncertainty, as it appears from inspection of the report that there was essentially no effect in the females. It should be noted that this study was done in rats.

The Weibull model, with parameters that fit the Neal and Rigdon (1967) dietary exposure data, tends to underestimate the tumor incidence observed in the gavage studies.

The Chouroulinkov (1967) study offers the twin disadvantages of only one non-zero dose and less-than-lifetime exposure and observation.

There is little basis on which to make a recommendation of a single slope factor from those listed in Table VIII-12. One way to present a quantitative risk estimate for benzo[a]pyrene is as a range, excluding those estimates considered inappropriate. As discussed previously, use of the 11.5 per(mg/kg)/day estimate based on Neal and Rigdon (1967) is not recommended as the higher doses (and tumor incidences) were excluded from the modeling procedure nor were duration differences included. The three upper bound estimates from two different modeling procedures applied to these data all have both pros and cons, and represent reasonable estimates from problematic data.

It is reasonable to exclude the slope factor of 6.5 per(mg/kg)/day as this was obtained from direct extrapolation from the observed data (Chouroulinkov et al., 1967). This is a rather unusual approach and is not directly comparable with the other calculations. Furthermore, there are no special circumstances that dictate the desirability of choosing this unusual approach. There is considerable uncertainty in applying a linearized multistage procedure to this data set: there is only one non-zero dose point and there is uncertainty regarding the duration extrapolation factor. The Brune et al. (1981) and other data indicate that for benzo[a]pyrene gavage and dietary exposure may not be strictly comparable.

For the Brune et al. (1981) data, the most appropriate approach is to use the majority of the data. Total contact site tumors, including esophagus, pharynx, etc., are a reasonable basis for comparison with the data obtained for mice, which developed only forestomach tumors. The estimate derived by applying the linearized multistage procedure to the data

for total contact site tumor for both males and females is 4.7 per(mg/kg)/day.

The range of the most acceptable data sets is, thus, 4.5-9.0 per(mg/kg)/day. The median is 6.25 per(mg/kg)/day.

There is also precedent for recommending a combined risk estimate by using a geometric mean (or other combination technique) to calculate a single number from several slope factors of equal merit. As discussed by Stiteler and Schoeny (1991) the use of a geometric mean offers some advantages over other central tendency calculations for values derived from linearized multistage models. They further recommend that, when possible, data sets be combined before a modeling procedure when a combined estimate is desired. For the data sets in question this latter approach seems impractical in view of the very dissimilar experimental protocols employed. Vater and Schoeny (1991) have set out a series of biologic considerations to be weighed when evaluating data sets for combination. These criteria include judgments as to study quality, common mechanisms of action, similarity of tumor types and sites. Among commonalities of the data sets for benzo[a]pyrene are the following: mode of administration, tumor site, tumor types, and presumed mechanisms of action.

There are precedents for using multiple data sets from different studies using more than one sex, strain and species; for example, quantitative risk estimates for DDT (U.S. EPA, 1985) and carbon tetrachloride (U.S. EPA, 1984d).

There is less precedent for use of combined risk estimates based on a mean of slope factors obtained by differing modeling procedures. In the interest of using more of the available data, the slope factor of 5.79 per(mg/kg)/day, which is the geometric mean of the four estimates chosen for the range, is recommended. A unit risk for drinking water is calculated as follows:

$$\frac{5.79 \text{ per(mg/kg)/day} \times 2 \text{ ℓ/day} \times 0.001 \text{ mg/µg}}{70 \text{ kg}} = 1.65 \times 10^{-4} \text{ per µg/ℓ}$$

where:

70 kg = assumed weight of adult human and

2 ℓ/day = assumed water consumption of adult human

Concentrations of benzo[a]pyrene coresponding to lifetime risks of 10^{-5}, 10^{-6} and 10^{-7} calculated from the above unit risk are 6.0×10^{-2}, 6.0×10^{-3} and 6.0×10^{-4} µg/ℓ, respectively.

Dibenz[a,h]anthracene. Two studies involving oral exposure to dibenz-[a,h]anthracene have been reported (Lorenz and Stewart, 1947; Snell and Stewart, 1962a). The former study was designed to compare tumor susceptibil- ity of various mouse strains and to test the suitability of the olive oil-water emulsions as a vehicle.

The Snell and Stewart (1962a) study reports four sets of tumor incidences; alveolar carcinoma in male and female mice, hemangioendothelioma for males and mammary carcinoma for females. Exposure duration was reported only as a range for each group, presenting a difficulty in the use of these data for calculation of quantitative risk estimates.

In the Errata: PAH Ambient Water Quality Criterion for the Protection of Human Health (U.S. EPA, 1982), a midpoint of the exposure duration range was used to calculate quantitative risk estimates using the linearized multistage procedure. These are presented in Table VIII-13. It should be emphasized that the estimates in this table were prepared for purposes of comparison with risk estimates for other PAHs and were not considered suitable for calculation of criteria.

Special Considerations

Toxicity Equivalence Factor Approach for PAHs in Group B2. It would be very useful in the hazard assessment of a diverse but related group of compounds such as PAHs to be able to prepare quantitative estimates of biologic effects by comparison with a well-studied type compound. Various attempts have been made, for example, to compare carcinogenic activity of individual PAHs with that of benzo[a]pyrene. If such comparisons were proven valid then it would become feasible, for example, to estimate carcinogenic potency of a mixture of identified PAHs by applying a suitable model if all activities were additive. Another potential use would be in regulating less well-studied compounds by comparison with the standard. The authors of the Errata: PAH Ambient Water Quality Criterion for the Protection of Human Health (U.S. EPA, 1982) used data sets for several PAHs administered in skin bioassays to generate comparative potency estimates. Results of these calculations based on skin painting bioassays are shown in Table VIII-14.

TABLE VIII-13

Cancer Potency Estimates for Excess Risk of 10^{-5} from Lifetime Exposure Based on Oral Exposure Data for Dibenz[a,h]anthracene*

Species/Sex	Slope Factor per(mg/kg)/day	Tumor Type
Mouse/M	0.57	hemangioendothelioma
Mouse/M	0.75	alveolar carcinoma
Mouse/F	1.24	alveolar carcinoma
Mouse/F	2.36	mammary carcinoma

*Source: U.S. EPA, 1982

TABLE VIII-14

Potency Indices for the Carcinogenic PAH Compounds Based on Skin Painting Data[a]

Compound	Q_1^*	ED_{10} (and 95% C.L.)	q_1^*	ed_{10} (and 95% C.L.)	Reference
Benzo[a]pyrene	470	2.98×10^{-3} (8.62×10^{-4}, 5.98×10^{-3})	152.49	9.33×10^{-4} (6.54×10^{-4}, 1.21×10^{-3})	Wynder et al., 1957
Benzo[a]pyrene	435	1.43×10^{-3} (3.68×10^{-4}, 2.49×10^{-3})	67.62	1.71×10^{-3} (8.51×10^{-4}, 2.57×10^{-3})	Wynder and Hofmann, 1959b
Benzo[a]pyrene	NA	NA	20.83	1.43×10^{-2} (4.24×10^{-3}, 2.44×10^{-2})	Bingham and Falk, 1969
Dibenz[a,h]-anthracene	299.62	6.34×10^{-4} (3.24×10^{-4}, 9.44×10^{-4})	292.81	6.16×10^{-4} (3.28×10^{-4}, 9.04×10^{-4})	Wynder and Hofmann, 1959a
Benzo[b]-fluoranthene	35.64	5.0×10^{-3} (2.75×10^{-3}, 7.25×10^{-3})	11.57	1.29×10^{-2} (8.54×10^{-3}, 1.73×10^{-2})	Wynder and Hofmann, 1959b
Benzo[k]-fluoranthene	NA	NA	0.30	No point estimate (0.35, none)	Wynder and Hofman, 1959b
Chrysene[b]	0.53	0.35 (0.23, 0.47)	0.88	0.21 (0.34, 1.12)	Wynder and Hofmann, 1959a
Benz[a]anthracene	NA	NA	0.28	0.73 (0.34, 1.12)	Bingham and Falk, 1969
Indeno[1,2,3,cd]-pyrene	NA	NA	1.16	0.15 (0.08, 0.22)	Wynder and Hofmann, 1959a

[a]Source: U.S. EPA, 1982; Chu and Chen, 1984

[b]Since there is only one dose group for chrysene, Q_1^* is calculated by using Kaplan-Meier survival analysis and the assumption that the control group has a zero response.

NA = Not available

Q_1^* = Estimate of carcinogenic potency based on the incidence data combined with the time-to-tumor data. It is the 95% confidence upper bound for the linear coefficient in the multistage model.

q_1^* = Estimate of carcinogenic potency based on the incidence data alone. It is the 95% confidence upper bound for the linear coefficient in the multistage model.

ED_{10} = Estimate of carcinogenic potency based on the incidence data combined with the time-to-tumor data as the effective dose associated with a risk of 10%.

ed_{10} = Estimate of carcinogenic potency based on the incidence data alone as the effective dose associated with a risk of 10%.

NOTE: for Q_1^* and q_1^*, higher numbers indicate increased potency whereas for ED_{10} and ed_{10}, lower numbers indicate increased potencies.

For a complete discussion of the caveats and assumptions involved in the generation of these estimates, refer to U.S. EPA (1982). The authors note that the potency estimates in Table VIII-14 are not directly comparable. The q_1^* value is the slope of 95% upper limit on the linear coefficient in the multistage model (as revised by Howe and Crump, 1982) when tumor incidence data are used. By contrast the Q_1^* is the 95% confidence upper-bound for the linear coefficient in the multistage model developed by Daffer et al. (1980) and evaluated at t=12 months. This model incorporates time-to-tumor data for individual animals dying in the course of the study. The ed_{10} is the dose level corresponding to the 10% incremental tumor response when incidence data are used; ED_{10} is similarly defined for use with time-to-tumor data. The Q_1^* and q_1^* reflect estimates of carcinogenic potency at low doses outside the experimental range. By contrast, the ED_{10} and ed_{10} reflect carcinogenic potency within the experimental range, and presumably are not as model-dependent as the other two indices. The authors of U.S. EPA (1982) ranked the PAHs based on the potencies computed by the four methods as given in Table VIII-15.

It is interesting to compare these rankings with a set of rankings based on unmodeled data. The data in Table VIII-16 were prepared by Santodonato (1986) from published data of Conney (1982). As can be seen from both tables, the rank assigned to a carcinogenic PAH depends on the type of model-based procedure applied, the carcinogenic endpoint measured, and whether nonmodeled data are used. What is not apparent, but is discussed in U.S. EPA (1982), is that variations in study protocol for a given route and endpoint change the overall outcome of the test and, thus, potency estimates

TABLE VIII-15

Ranking[a] of PAHs Based on Estimates of Potency in Skin Painting Bioassay[b]

Q_1*	q_1*	ED_{10}	ed_{10}
Benzo[a]pyrene	Dibenz[a,h]anthracene	Dibenz[a,h]anthracene[c]	Dibenz[a,h]anthracene[c]
Dibenz[a,h]anthracene	Benzo[a]pyrene	Benzo[a]pyrene[c]	Benzo[a]pyrene[c]
Benzo[b]fluoranthene	Benzo[b]fluoranthene	Benzo[b]fluoranthene	Benzo[b]fluoranthene
Chrysene	Indeno[1,2,3-cd]pyrene[c]	Chrysene	Benz[a]anthracene[d]
	Chrysene[c]		Indeno[1,2,3-cd]pyrene[d]
	Benz[a]anthracene[c]		Chrysene[d]

[a]Ranking is most potent first to least potent last.

[b]Source: U.S. EPA, 1982

[c,d]Compounds with the same superscript are indistinguishable in potency.

TABLE VIII-16

The Carcinogenic Potency of Various PAHs Measured in Two Different Animal Bioassay Systems[a]

	Skin Study[b] (tumors/μmol)	Rank	Newborn Study[c] (tumors/μmol)	Rank
Benzo[a]pyrene	22.5	1	4.5	2
Benz[a]anthracene	0.15	4	0.57	3
Chrysene	0.36	3	0.26	4
Dibenz[a,h]anthracene	13.4	2	111.7	1
Phenanthrene	0.03	5	0.14	5

[a]Source: Santodonato, 1986

[b]Mice received a single topical application of compound followed 7 days later by twice weekly applications of TPA for 16-25 weeks (Conney, 1982).

[c]Mice were given i.p. injections of 1/7, 2/7 and 4/7 of the total dose of compound on the first, 8th and 15th days of life, respectively. The animals were killed at 22-42 weeks of age (Conney, 1982)

and ranks. Variations in use of solvents, animals of varying susceptibility, and both length of exposure and overall study duration further complicate attempts to compare carcinogenic potency.

While the absolute rank will vary as a function of the considerations outlined above, certain commonalities can be seen. In general, three groups of PAHs can be distinguished. The most potent carcinogens are invariably benzo[a]pyrene and dibenz[a,h]anthracene. A second group of carcinogens having intermediate potency consists of benzo[b]fluoranthene and generally (but not invariably) benz[a]anthracene, chrysene and indeno[1,2,3-cd]-pyrene. The third group consists of weak carcinogens or PAHs that have not shown significant carcinogenicity including phenanthrene, pyrene and benzo[k]- fluoranthene.

An alternative, more quantitative approach to risk estimation for PAHs has been proposed by Clement Associates (1988, 1990b) and other authors. This entails deriving a numerical estimate of the carcinogenic potency of a particular PAH by comparison with that of benzo[a]pyrene. This method is not unlike the generation of toxicity equivalence factors proposed for risk estimation of chlorinated dibenzodioxins and dibenzofurans proposed by U.S. EPA (1987c) and recently revised (U.S. EPA, 1989d).

What is done in this approach is to calculate a benzo[a]pyrene-equivalent dose using data from studies (such as skin painting bioassays) generally not considered suitable for quantitative risk estimation. In the Clement Associates (1988, 1990b) reports only those data sets were included wherein benzo[a]pyrene was tested concurrently with one or more PAH. This

was done in order to account for inter-laboratory variations, varying susceptibility to carcinogenic activity of the different test animals, varying metabolic capacity of these animals, and differences in protocols and endpoints measured. For each report considered, the comparison was made between benzo[a]pyrene activity and the activity of a particular PAH in that same report.

In the Clement Associates (1988) report all risk estimates were generated using the two-stage model. The low-dose terms or maximum likelihood estimates are amenable to comparison whereas it is felt that comparison of upper bounds has less meaning. In calculating the estimated transition rates from the two-stage model on which comparisons were based, the assumption was made that papillomas and other benign tumors are observable clones of first stage cells. It was further assumed that carcinomas can develop from papillomas, and thus the observation of either a papilloma or carcinoma serves as evidence of at least one transformation or stage. In those instances wherein benign and malignant tumors were combined, the simple form of the model equation was used:

$$P(\chi) = 1 - \exp - A(1+S\chi)$$

The form of the model employing two stages was used for data in which malignant tumors were reported separately:

$$P(\chi) = 1 - \exp - A(1+S\chi)^2$$

To derive the potency for each PAH relative to benzo[a]pyrene, it was assumed that the PAH and benzo[a]pyrene have similar dose-response curves, but that it takes a proportionately larger concentration of nonbenzo[a]pyrene materials to induce the same response. The relative potency, Rj, is simply the ratio of estimated transition rates, with that of benzo[a]pyrene taken as 1.0.

The result of a series of calculations was a range of relative potency estimates. For example, for benzo[b]fluoranthene individual relative potencies from the three data sets were 1.067, 0.874 and 0.232. In a similar fashion the ranges of relative potencies presented in Table VIII-17 were generated using data from studies described in Chapter V. Using benzo-[b]fluoranthene as an illustration, a range of relative potencies spanning an order of magnitude was obtained.

Clement Associates (1988) proposed that selection of the most appropriate relative potency be based on a consideration of the qualitative differences among the studies. Criteria used in selection of the most suitable studies included the following:

1. Relevance of route of administration in the bioassay to presumed human exposure
2. Duration of exposure approximating natural lifespan
3. Sample size
4. Inclusion of a concurrent vehicle control
5. Observance of a dose-response relationship consistent with the model (linear-quadratic)
6. Extent to which observed responses cover the possible response range
7. Absence of complicating variables such as administration of promoting agents

TABLE VIII-17

Summary of Relative Potency Estimates for Indicator PAHs[a]

Compound	Test System				
	Mouse Skin Carcinogenesis	Subcutaneous Injection into Mice	Intrapulmonary Administration to Rats[b]	Initiation-Promotion on Mouse Skin	Intraperitoneal Injection in Newborn Mice
Benzo[a]pyrene	1.0	1.0	1.0	1.0	1.0
Benz[a]anthracene	0.145[c]				0.057, 0.524, 0.496[d]
Benzo[b]fluoranthene	0.167[e]		0.140	0.258[f], 0.125[g]	0.232, 1.067, 0.874[h]
Benzo[k]fluoranthene	0.020[e]		0.066	0.022[f]	0.040, 0.097, 0.044[h]
Benzo[ghi]perylene	0.015[i]		0.022	0.005[i]	
Chrysene	0.0044[j]			0.040[g]	0.125, 0.33[d]
Dibenz[ah]anthracene	1.11[j]	2.82[k], 4.50[l]			
Indeno[1,2,3-cd]pyrene	0.021[e], 0.089[i]		0.232	0.074[i]	0.013[h]
Pyrene					0.081, 0.050, 0.586[d]

[a]Where more than one potency estimate is shown, they were derived from the same study using different tumor types as endpoints.

[b]Deutsch-Wenzel et al., 1983

[c]Bingham and Falk, 1969

[d]Wislocki et al., 1986

[e]Habs et al., 1980

[f]LaVoie et al., 1982a

[g]Van Duuren et al., 1966

[h]LaVoie et al., 1987

[i]Hoffmann and Wynder, 1966

[j]Wynder and Hoffmann, 1959a

[k]Pfeiffer, 1977

[l]Bryan and Shimkin, 1943

Based on application of these criteria single relative potency estimates were proposed as given in Table VIII-18. Only those PAHs classified as B2, probable human carcinogen, are included in this listing as it is inappropriate to calculate a quantitative risk estimate for those in group D, not classifiable.

Other approaches for obtaining a single estimate are also feasible. A weighted average or some other numerical combination procedure could be used in those instances wherein a range of relative potencies is available. Other criteria than those described above (or different emphasis on certain criteria) could result in choices other than those in Table VIII-18. It should be noted in this context that the Deutsch-Wenzel et al. (1983) study was thought to be less reliable than more standard skin painting studies because of the unusual route of exposure (lung implantation of wax pellets). This experimental protocol also may entail confounding variables such as trauma or irritation (which could have promoting effects) or changes in metabolism of the implanted PAH. It is worthy to note that this is the only assay wherein indeno[1,2,3-cd]pyrene had an elevated response by comparison with the generally more carcinogenic PAH benzo[b]fluoranthene. A more appropriate method of study and relative potency selection may be to employ an objective method such as that described by DuMouchel and Harris (1983).

In 1990 a modified TEF approach was proposed (Clement Associates, 1990b). In order to increase the precision of the relative potency estimates, the analysis incorporated time-to-tumor information, vehicle controls from multiple experiments and historical controls. A form of the two-stage model was used that was extended to accomodate possible saturation

TABLE VIII-18

Summary of PAHs Relative Potencies

Compound	Relative Potency	Reference
Benzo[a]pyrene	1.0	
Benzo[e]pyrene		Deutsch-Wenzel et al., 1983
Benz[a]anthracene	0.145	Bingham and Falk, 1969
Benzo[b]fluoranthene	0.140	Deutsch-Wenzel et al., 1983
Benzo[j]fluoranthene	0.061	
Benzo[k]fluoranthene	0.066	Deutsch-Wenzel et al., 1983
Chrysene	0.0044	Wynder and Hoffman, 1959a
Dibenz[a,h]anthracene	1.11	Wynder and Hoffman, 1959a
Indeno[1,2,3-cd]pyrene	0.232	Deutsch-Wenzel et al., 1983

of processes for converting PAH to reactive metabolites. Data recommended for this approach were exclusively from a series of experiments on induction of lung epidermoid carcinomas by implantation of PAH in the lungs of female Osborne-Mendel rats. These data were collected by a single group of investigators. Published data on background rates of lung carcinomas in female Osborne-Mendel rats were used in addition to assay vehicle controls to provide a non-zero incidence for the zero dose used in the two-stage model. The relative potencies obtained by comparison of maximum likelihood estimates are given in Table VIII-19. Relative potencies for PAH-containing mixtures were used to test additivity assumptions.

Rugen et al. (1989) assessed relative carcinogenic potencies of PAH as a basis for proposing acceptable exposure levels (AEL) in drinking water. They used the published slope factor of 11.53 per(mg/kg)/day for benzo-[a]pyrene (U.S. EPA, 1980d) and a 10^{-5} risk level to determine a 0.028 µg/ℓ concentration as the AEL. Relative potencies or relative tumor doses (RTD) were determined as follows:

$$RTD = (d_1/n_1)/(d_2/n_2)$$
$$= (d_1 n_2/d_2 n_1)$$
$$= (d_1/d_2) \times (n_2/n_1)$$

where:

d_1 = dosage of chemical 1

n_1 = tumor frequency after m months of exposure to d_1

d_2 = dosage of chemical 2

n_2 = tumor frequency after m months of exposure to d_2

TABLE VIII-19

Relative Potencies for PAH and PAH-Containing Mixtures[a,b]

Material	Relative Potency
Benzo[a]pyrene	1.0
Benzo[b]fluoranthene	0.1228
Benzo[j]fluoranthene	0.0523
Benzo[k]fluoranthene	0.0532
Benzo[e]pyrene	0.0070
Indeno[1,2,3-c,d]pyrene	0.2780
Benzo[g,h,i]perylene	0.0212
Anthanthrene	0.3160
Flue gas from coal-fired furnace	0.0542
Diesel engine exhaust	0.0028
Gasoline engine exhaust	0.0217
Sidestream cigarette smoke	0.0030

[a]Relative potencies based on maximum likelihood estimates from lung implantation data.

[b]Table adapted from Clement Associates (1990b)

Direct comparisons of dose and tumor frequency were made for several skin-painting assays. No model was applied to the data but rather single doses were chosen as the basis for comparison. Table VIII-20 lists the RTD values and data on which they were based.

One end use of a series of relative potency would be in the evaluation of PAH-containing mixtures (a TEF approach). This is an alternative to assuming that all carcinogenic PAHs are equipotent with benzo[a]pyrene. All the means of comparing PAHs described in this chapter indicate that benzo-[a]pyrene and dibenz[a,h]anthracene are of comparable potency as carcinogens and the other PAHs described in this document less active. In the absence of synergistic interactions risk estimates of PAH mixtures based on one-to-one benzo[a]pyrene equivalency will be overestimates.

A critical review and analysis of the above has not yet been done by U.S. EPA. This will be necessary before a TEF or other comparative approach to the derivation of quantitative estimates for PAH can be recommended. At this time it is thus recommended that a quantitative cancer risk estimate be proposed only for benzo[a]pyrene.

Interactions With Other Chemicals. As reviewed in the Synergism and/or Antagonism Section in Chapter V, several of the PAHs have been determined to be affected by the presence of other PAHs or other substances. Much of the research in this area has focused on the promotion or inhibitory effect of noncarcinogens such as pyrene on a known animal carcinogen, often benzo[a]pyrene. The route of administration is typically nonoral (dermal, subcutaneous injection or inhalation). There are data for both enhancement

TABLE VIII-20

Comparison of Carcinogenic Potency of PAH Using Relative Tumor Dose*

PAH	Observation Time (months)	Concentration (%)	Tumor Incidence (%)	Relative Tumor Dose	Reference
Benzo[a]pyrene	7	0.01	75	1	Wynder and Hoffman, 1959a
Dibenz[a,h]anthracene	7	0.01	45	1.67	Wynder and Hoffman, 1959a
Benzo[a]pyrene	8	0.01	85	1	Wynder and Hoffman, 1959a
Benzo[j]fluoranthene	8	0.1	65	13.1	Wynder and Hoffman, 1959b
Benzo[b]fluoranthene	8	0.1	20	42.5	Wynder and Hoffman, 1959b
Benzo[a]pyrene	10	0.005	50	1	Wynder and Hoffman, 1959a
Indeno[1,2,3-c,d]pyrene	10	0.5	30	167	Wynder and Hoffman, 1961
Benzo[a]pyrene	18	0.02	50	1	Bingham and Falk, 1969
Benz[a]anthracene	18	1.0	14	179	Bingham and Falk, 1969
Benzo[a]pyrene	15	0.001	33	1	Wynder and Hoffman, 1959a
Benz[a]anthracene	15	0.02	2.5	264	Bingham and Falk, 1969

*Table adapted from Rugen et al., 1989

and inhibition of biologic activity when PAHs are tested as simple mixtures and as components of complex mixtures. The outcomes are dependent upon such variables as strain and species, whether the PAHs are given concurrently (tests for cocarcinogenesis) or applied sequentially (tests for initiation and promotion) and the route employed.

It has been postulated that PAHs act by similar mechanisms as initiators of carcinogenicity, which allows an assumption of simple similar action and dose additivity. This assumption, however, does not take into account the data, albeit limited, on both PAH promotional and inhibitory effects. Presentation of any PAH risk assessment based on additivity must, therefore, be qualified; theoretically both under- and over-estimation of risk could result from an assumption of additivity.

Existing Guidelines, Recommendations and Standards

A drinking water standard for PAHs as a class has been developed (U.S. EPA, 1980d). The World Health Organization European Standards for Drinking Water recommends a concentration of PAHs not to exceed 0.2 µg/ℓ (WHO, 1970). This recomendation is based on the composite analysis of six PAHs in drinking water. Fluoranthene, benzo[a]pyrene, benzo[g,h,i]perylene, benzo-[b]fluoranthene, benzo[k]fluoranthene and indeno[1,2,3-cd]pyrene were used. These six were not chosen on the basis of potential health effects, but because they were considered useful indicators of the presence of PAH pollutants (Borneff and Knute, 1969). A quantitative risk estimate of 11.53 $(mg/kg/day)^{-1}$ based on the data of Neal and Rigdon (1967) was derived in U.S. EPA (1980d). From this, the criterion for the ambient water quality for PAHs associated with human lifetime cancer risk of 10^{-5} was estimated to be 28 ng/ℓ (U.S. EPA, 1982).

In the occupational environment, a Federal standard has been promulgated for coke oven emissions, based primarily on the presumed effects of the carcinogenic PAHs contained in the mixture as measured by the benzene soluble fraction of total particulate matter (U.S. EPA, 1984b). Similarly, the ACGIH (1988) recommends a workplace exposure limit for coal tar pitch volatiles, based on the benzene-soluble fraction containing carcinogenic PAHs. NIOSH has also recommended a workplace criterion for coal tar products (coal tar, creosote and coal tar pitch), based on measurements of the cyclohexane extractable fraction. These criteria are summarized below:

Substance	Exposure Limit	Agency
Coke oven emissions	0.15 mg/m³ (soluble fraction), 8-hour time-weighted average	NIOSH (1973b) OSHA (1985)
Coal tar products	0.1 mg/m³, 10-hour time-weighted average	NIOSH (1973a, 1977)
Coal tar pitch Volatiles	0.2 mg/m³, (benzene soluble fraction), 8-hour time-weighted average	ACGIH (1988)

Special Groups at Risk

There are several human subpopulations that are considered to be at increased risks from exposure to PAHs. The largest subpopulation comprises those who are occupationally exposed to one or more of the PAHs. Coke oven emissions and coal tar pitch volatiles include PAHs such as naphthalene, acenaphthene and fluoranthene. Industries that expose workers to pesticides, coal tar, creosote and fossil fuel oils (i.e., steelworkers, roofers and auto mechanics) appear to be at higher risk. Persons living in industrial areas, or where exposure to auto exhaust is greater and smokers are also considered to be at an increased risk from PAH exposure.

It is known that PAHs are dependent upon metabolism to reactive electrophiles for their activity and that individuals of a species, humans included, vary in their capacity to undertake this metabolism. It is also known that the inducibility of AHH in human tissues is genetically determined. The degree to which inducibility or noninducibility of AHH figures as a risk factor for human carcinogenesis, however, is not clear.

Another genetic predisposing factor may be the individual's capacity to repair certain types of DNA damage that can be caused by PAH metabolites in a fashion that is error-free and does not lead to fixation of mutagenic events.

Summary

There were no data reported suitable for calculation of 1-day, 10-day or longer-term health advisories. DWELs for several PAHs were determined on the basis of 90-day studies. They are the following: anthracene, 10.5 mg/ℓ; fluoranthene, 1.4 mg/ℓ; fluorene, 1.4 mg/ℓ; pyrene, 1.0 mg/ℓ.

Weight-of-evidence determinations for likelihood of human carcinogenicity were these: Group D, not classifiable as to human carcinogenicity -- acenaphthylene, anthracene, benzo[g,h,i]perylene, fluoranthene, fluorene, naphthalene, phenanthrene and pyrene; group B_2, probable human carcinogen -- benz[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]-pyrene, chrysene, dibenz[a,h]anthracene and indeno[1,2,3-cd]pyrene.

Data from three studies wherein benzo[a]pyrene was given orally were modeled by several procedures. Both a two-stage and the linearized multistage procedures were used to calculate upper bound risk estimates from the data of Neal and Rigdon (1967). Data from both male and female rats as well as various subsets of the published data from the dietary portion of the study by Brune et al. (1981) were used. An extrapolation was also done from the observed response in Chouroulinkov et al. (1967). The upper bounds judged to be most acceptable range from 4.5 per(mg/kg)/day to 9.0 per(mg/kg)/day. A geometric mean of the acceptable upper bound estimates is 5.8 per(mg/kg)/day or 1.65×10^{-4} per µg/ℓ. Concentrations of benzo-[a]pyrene corresponding to lifetime risks of 10^{-5}, 10^{-6} and 10^{-7} were determined to be 6.0×10^{-2}, 6.0×10^{-3} and 6.0×10^{-4} µg/ℓ, respectively.

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