PUBLIC HEALTH GOALS FOR CHEMICALS IN DRINKING WATER

BENZO(a)PYRENE

September 2010

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Public Health Goal for
Benzo(a)pyrene
in Drinking Water

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September 2010

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PREFACE

Drinking Water Public Health Goals
Pesticide and Environmental Toxicology Branch
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This Public Health Goal (PHG) technical support document provides information on health effects from contaminants in drinking water. PHGs are developed for chemical contaminants based on the best available toxicological data in the scientific literature. These documents and the analyses contained in them provide estimates of the levels of contaminants in drinking water that would pose no significant health risk to individuals consuming the water on a daily basis over a lifetime.

The California Safe Drinking Water Act of 1996 (Health and Safety Code, Section 116365) requires the Office of Environmental Health Hazard Assessment (OEHHA) to perform risk assessments and adopt PHGs for contaminants in drinking water based exclusively on public health considerations. The Act requires that PHGs be set in accordance with the following criteria:

1. PHGs for acutely toxic substances shall be set at levels at which no known or anticipated adverse effects on health will occur, with an adequate margin of safety.
2. PHGs for carcinogens or other substances that may cause chronic disease shall be based solely on health effects and shall be set at levels that OEHHA has determined do not pose any significant risk to health.
3. To the extent the information is available, OEHHA shall consider possible synergistic effects resulting from exposure to two or more contaminants.
4. OEHHA shall consider potential adverse effects on members of subgroups that comprise a meaningful proportion of the population, including but not limited to infants, children, pregnant women, the elderly, and individuals with a history of serious illness.
5. OEHHA shall consider the contaminant exposure and body burden levels that alter physiological function or structure in a manner that may significantly increase the risk of illness.
6. OEHHA shall consider additive effects of exposure to contaminants in media other than drinking water, including food and air, and the resulting body burden.
7. In risk assessments that involve infants and children, OEHHA shall specifically assess exposure patterns, special susceptibility, multiple contaminants with toxic mechanisms in common, and the interactions of such contaminants.

8. In cases of insufficient data for OEHHA to determine a level that creates no significant risk, OEHHA shall set the PHG at a level that is protective of public health with an adequate margin of safety.
9. In cases where scientific evidence demonstrates that a safe dose response threshold for a contaminant exists, then the PHG should be set at that threshold.
10. The PHG may be set at zero if necessary to satisfy the requirements listed above in items seven and eight.
11. PHGs adopted by OEHHA shall be reviewed at least once every five years and revised as necessary based on the availability of new scientific data.

PHGs are not regulatory requirements, but instead represent non-mandatory goals. Using the criteria described above, PHGs are developed for use by the California Department of Public Health (DPH) in establishing primary drinking water standards (State Maximum Contaminant Levels, or MCLs). Thus, PHGs are not developed as target levels for cleanup of ground or ambient surface water contamination, and may not be applicable for such purposes, given the regulatory mandates of other environmental programs.

Whereas PHGs are to be based solely on scientific and public health considerations, drinking water standards adopted by DPH are to consider economic factors and technical feasibility. Each primary drinking water standard adopted by DPH shall be set at a level that is as close as feasible to the corresponding PHG, with emphasis on the protection of public health. Each primary drinking standard adopted by DPH is required to be set at a level that is as close as feasible to the corresponding PHG, with emphasis on the protection of public health. MCLs established by DPH must be at least as stringent as the federal MCL, if one exists.

Additional information on PHGs can be obtained at the OEHHA web site at www.oehha.ca.gov.

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PUBLIC HEALTH GOAL FOR BENZO(A)PYRENE

SUMMARY

The Office of Environmental Health Hazard Assessment (OEHHA) has developed a Public Health Goal (PHG) of 0.007×10^{-3} mg/L, 0.007 µg/L or parts per billion (ppb) for benzo(a)pyrene (BaP). The existing California and federal Maximum Contaminant Level (MCL) for BaP is 0.2×10^{-3} mg/L, or 0.2 ppb, and the existing PHG is 0.004 µg/L or ppb.

This revised PHG for BaP is based on carcinogenic effects observed in animals. Significant increases in tumors have been observed in the forestomach of female mice and the liver and forestomach of male and female rats administered BaP by the oral route. BaP was shown to be genotoxic in studies *in vivo* and *in vitro*. DNA adducts have been observed in animals following exposure to BaP. Studies of BaP metabolism are extensive, and while still incomplete, link the genotoxic and carcinogenic activity of BaP with its metabolism to reactive forms. Evidence that specifically links exposure to BaP to an increased incidence of cancer in humans is lacking. BaP is a member of a family of structurally-related chemicals called polyaromatic hydrocarbons (PAHs). Human exposures to mixtures of PAHs such as diesel exhaust, cigarette smoke and coal tar are linked to increased incidences of cancer.

A time-to-tumor model was employed to obtain a lower-bound estimate of the dose associated with a 10 percent increased incidence of tumors of the oral cavity or forestomach and tumors of the liver in male and female rats, and tumors of the oral cavity or forestomach of female mice. Tumors in the female mouse oral cavity or forestomach yielded the lowest lower-bound dose associated with a 10 percent increased incidence of tumors, which was therefore used to estimate cancer potency. A cancer potency for BaP of 1.7 $(mg/kg\text{-}day)^{-1}$ was derived using the lower-bound dose associated with a 10 percent increased incidence of oral cavity or forestomach tumors and an assumed linear relationship between dose and response at low doses. The potency was increased by 1.7 fold to 2.9 $(mg/kg\text{-}day)^{-1}$ based on correction for early-in-life exposures (i.e., treatment of the rodents in the critical study began at weaning, whereas human exposures to environmental chemicals would start *in utero*, and therefore the fetal and infant exposures are not accounted for in the cancer bioassay). The PHG was derived employing this cancer potency and exposure to 0.052 L/kg-day of water, an age-adjusted upper 95^{th} percentile of drinking water consumption matching the age ranges for the cancer potency correction.

A health-protective level was also derived based on non-carcinogenic effects. A lowest-observed-adverse-effect level (LOAEL) of 5 mg/kg-day was identified from a subchronic study, based on renal toxicity. A health protective level of 4 µg/L or ppb was derived employing an uncertainty factor of 3,000, a relative source contribution of 0.1, and the ingestion of 0.044 L/kg-day of water.

The existing PHG for BaP of 0.004 ppb was based on the findings of an older, lower-quality cancer bioassay. The revised PHG in this document relies on two new well-

conducted cancer bioassays. The California Safe Drinking Water Act of 1996 (amended Health and Safety Code, Section 116365), the legislation that authorized the development of PHGs, anticipated the availability of new studies with time and the need to change a PHG based on new information, as in this case.

INTRODUCTION

The purpose of this document is to re-evaluate current scientific information on BaP in order to update the health-protective estimate for BaP concentration in drinking water. PHGs are based on a comprehensive analysis of information on the toxicology of the compounds, and are based solely on protection of public health without regard to cost impacts or other factors. PHGs for carcinogens are set at a *de minimis* risk level of one in a million (10^{-6}), assuming a lifetime of exposure to the chemical in the drinking water. PHGs for non-carcinogens are based on levels estimated to be without risk of any adverse effects for exposures up to a lifetime, to the general population as well as any significant identifiable sensitive subpopulations.

BaP is one of the polycyclic aromatic hydrocarbons (PAHs) formed when gasoline, garbage, or any animal or plant materials burn incompletely. It was first isolated from coal tar. BaP and the other PAHs are ubiquitous environmental contaminants. People may be exposed to BaP from air, water, soil, cigarette and other plant product smoke, food and some work environments, through inhalation, ingestion and skin contact.

Exposure to PAHs may cause harmful health effects. Mixtures of PAHs that include BaP (e.g., coal tar) were shown to be dermal carcinogens in animals as early as 1918. BaP has caused tumors in laboratory animals when administered in the diet, when applied to their skin or when inhaled for a long period of time. Humans exposed to mixtures of PAHs and related compounds at high concentrations over long periods of time can also develop cancer. Mice administered high levels of BaP in the diet during pregnancy had difficulty reproducing and so did their offspring. The offspring from pregnant mice administered BaP in the diet also exhibited birth defects and decreased body weights.

This document represents an update of an earlier health risk assessment of BaP conducted by OEHHA in 1997 that provided technical support for the development of a PHG for BaP (OEHHA, 1997). The 1997 document was based on an earlier risk assessment for BaP by this office (OEHHA, 1994) which provided part of the technical support to list BaP as a toxic air contaminant and as a Proposition 65 carcinogen.

The revision incorporates the higher values for drinking water consumption described by U.S. EPA (2004), in order to be more protective of the entire population. This update also incorporates an Age Sensitivity Factor (ASF) adjustment for cancer potency for exposure to BaP during infancy, as described earlier by OEHHA (2009). The revised procedure takes into account information which suggests that children can be especially susceptible to carcinogens (OEHHA, 2001). Weighting factors utilized to calculate cancer risks from exposures of infants, children and adolescents reflect their anticipated special sensitivity to carcinogens. Cancer risk is weighted by a factor of 10 for exposures that occur from the third trimester of pregnancy to 2 years of age, and by a factor of 3 for exposures that occur from 2 years through 15 years of age. This approach applies to all

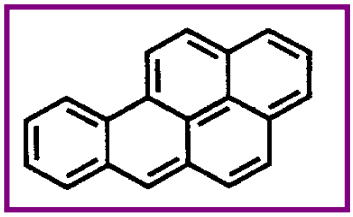
carcinogens unless chemical-specific data exist that could be used to make more specific adjustments to risk. Drinking water consumption values appropriate for the postnatal age ranges above (OEHHA, 2010) are also incorporated into the calculation.

CHEMICAL PROFILE

Chemical Identity

BaP is a five-ring PAH. The chemical formula, structure, synonyms and identification numbers shown in Table 1 are adopted from two Agency for Toxic Substances and Disease Registry (ATSDR) documents (ATSDR, 1990a, 1995).

Table 1. Chemical Identity of Benzo(a)pyrene (ATSDR, 1990a, 1995; IARC, 1983)

Chemical name	Benzo(a)pyrene
Synonyms	Benzo(d,e,f)chrysene; 3,4-benzopyrene; 3,4-benzpyrene; 3,4-benzylpyrene; 3,4-benz(a)pyrene; 6,7-benzopyrene; benzpyrene; benz(a)pyrene; 3,4-bp; BP; BaP; BαP; benzo(alpha)pyrene
Chemical formula	$C_{20}H_{12}$
Wiswesser line notation	L D6 B6666 2AB TJ (HSDB, 1997)
Chemical structure	
Identification numbers	
Chemical Abstracts Service (CAS) Registry No:	50-32-8
NIOSH Registry of Toxic Effects of Chemical Substances (RTECS) No:	DJ3675000
U.S. EPA Hazardous Waste No:	U022
Oil and Hazardous Materials/Technical Assistance Data System (OHM/TADS) No:	8200129
Hazardous Substances Data Bank (HSDB) No:	2554

Sources and Uses

BaP is a well-studied PAH. PAHs, also known as polynuclear aromatic hydrocarbons, are a class of compounds which possess two or more annellated rings. They are not manufactured commercially in the United States but are ubiquitous in the environment. PAHs are formed during the incomplete burning of coal, oil, gas, wood, garbage, complex petroleum products, products of coal liquefaction processes or other organic substances such as tobacco, any plant materials, charbroiled meat or any animal materials. PAHs can be synthetic or occur naturally. BaP is not produced commercially in the U.S. in quantities greater than research levels and the commercial production is not a significant source of BaP in the environment. There is no known use for BaP except as a research chemical.

The principal natural sources of BaP are forest fires, volcanic eruptions, and peat fires, while anthropogenic sources include the incomplete combustion of fossil fuels, coke oven emissions, aluminum smelters, coal combustion and conversion industries, incinerators, vehicle exhausts and cigarette, cigar and marijuana smoke. BaP typically found in smoke and soot combines with dust particles in the air and is carried into water and soil and onto crops. It is also found in creosote, a substance used to preserve wood.

Physical and Chemical Properties

Important physical and chemical properties of BaP are given in Table 2. BaP is a lipophilic (fat-soluble) solid, only slightly soluble in water, and is poorly volatile.

ENVIRONMENTAL OCCURRENCE AND HUMAN EXPOSURE

The estimated annual U.S. emission of BaP is 500 tons (ATSDR, 1990a). Most of the direct releases of BaP to the environment are first to the atmosphere, from both natural and anthropogenic sources. Emissions from human activities predominate. In air, BaP is predominantly sorbed to particulates but also appears as a gaseous vapor. The low solubility, low vapor pressure, and high octanol-water partition coefficient (K_{ow}) of BaP result in its partitioning mainly to soil (82 percent) and sediment (17 percent), with approximately one percent partitioning into water and less than one percent into air, suspended sediment and biota. BaP is identified as one of the eleven most persistent toxic chemicals in the Great Lakes, the largest inland body of fresh water on this planet (Hicks, 1996).

BaP may bioconcentrate in aquatic organisms that cannot metabolize it, including plankton, oysters and some fish. The bioconcentration factors (BCFs) have been reported in the range from 0 in mosquito fish to 9 in clams and to 134,248 in cladoceran water fleas for various periods of exposure. Some levels of accumulation of BaP in aquatic species have been recorded. However, most fish and shellfish can metabolize BaP and the elimination of BaP takes only days. Biomagnification in food chains has not been reported (U.S. EPA, 1980; ATSDR, 1995). Nevertheless, food is the major source of human exposure to BaP which predominantly originates from environmental pollution,

food packaging, cooking and processing (Santodonato *et al*., 1981; de Vos *et al*., 1990; Buckley *et al*., 1995).

Table 2. Physical and Chemical Properties of BaP

Property	Value	References
Molecular weight	252.3 g/mol	IARC (1983)
Color	Pale yellow; fluoresces yellow-green in UV light	IARC (1983) Weast (1987)
Physical state	Solid, plates or needles (recrystallized from benzene/ligroin)	Weast (1987)
Odor	Faint aromatic odor	ATSDR (1995)
Odor threshold	No data	
Melting point	179-179.3°C	Weast (1987)
Boiling point	310-312°C (at 10 mm Hg)	Weast (1987)
	495°C (at 760 mm Hg)	Aldrich (1996)
Flash point, flammability limits, autoignition temp	Unknown	
Solubility		
Water	3.8×10^{-6} g/L (25°C)	U.S. EPA (1982)
	2.3×10^{-3} mg/L	ATSDR (1995)
Organic solvents	Sparingly soluble in ethanol and methanol; soluble in benzene, toluene, xylene, acetone, DMSO, and ether	IARC (1983)
Specific gravity	1.351	U.S. EPA (1991)
Partition coefficients		
Octanol-water (K_{ow})	1.15×10^{6}	U.S. EPA (1982)
Log K_{ow}	6.06 (25°C)	U.S. EPA (1982)
Soil-organic carbon-water (K_{oc})	5.5×10^{6}	U.S. EPA (1982)
Log K_{oc}	6.74 (25°C)	U.S. EPA (1982)
Vapor pressure	5.6×10^{-9} mm Hg (20°C)	U.S. EPA (1982)
	7.47×10^{-7} mm Hg (25°C)	WHO (1996)
Henry's law constant	4.9×10^{-7} atm-m^3/mol	U.S. EPA (1982)
Conversion factors[1]	1 ppm = 10.32 mg/m^3	Verschueren (1983)

[1] Calculated based on the ideal gas law, PV= *n*RT at 25°C: ppm = mg/m^3 x 24.45 x 1,000/MW.

The most important degradation processes for BaP in water are photooxidation, chemical oxidation and biodegradation by aquatic microorganisms. BaP in water is oxidized by chlorination and ozonation. BaP in air is degraded through photolysis, reaction with nitrogen oxides, hydroxides, ozone, sulfur oxides and peroxyacetyl nitrate with estimated half-lives of approximately 40 minutes to seven days when adsorbed to particles and exposed to sunlight. Microbial metabolism and breakdown by sunlight are the major

degradation processes for BaP in soil. The estimated half-life of BaP in soil is about 229 to 309 days. Bioremediation is emerging as a practical alternative to traditional disposal techniques (ATSDR, 1995).

Air

In 1970, ambient BaP concentrations in 120 U.S. cities were between 0.2 and 19.3 ng/m^3 while BaP concentrations in non-urban areas ranged from 0.1 to 1.2 ng/m^3. Airborne BaP in New York City was recorded at levels up to 50 ng/m^3 in 1972 (ATSDR, 1995). The annual average concentration of BaP in London has decreased from 26 to 39 ng/m^3 in 1962 to less than 1 ng/m^3 in 1997 (Williams and Maynard, 1997). In polluted Lahore, Pakistan, the 1992 yearly average of BaP was 9 ng/m^3 (Smith *et al*., 1996). The inhaled BaP intake from air was estimated as 10 to 44 ng/day (Santodonato *et al*., 1981).

In addition to certain industrial activities, traffic exhaust is a major source of BaP in cities, especially for nonsmokers (Pastorelli *et al*., 1996; Zheng *et al*., 1997). In Moscow, BaP content varied from 1 ng/m^3 in parks and forests to 10 ng/m^3 in the city center, and 20 to 30 ng/m^3 in the area adjacent to large factories and crossroads with heavy traffic (Khesina *et al*., 1996). BaP concentrations were 4.4 ng/m^3 in air from a street at Central Copenhagen with only diesel buses and petrol cars, and 1.3 to 1.4 ng/m^3 in a nearby city park during January to March, 1992 and March, 1993 (Nielsen, 1996). The traffic to rural ratio of BaP varied between 7.07 and 17.6 in the fine particle mode, and between 5.32 and 16.9 in the coarse particle mode in a southern Taiwan study (Sheu *et al*., 1996). In diesel exhaust BaP was detected at 680 ng/g particulate in Sweden (Soontjens *et al*., 1997). BaP was detected in burning biomass while conditions during the burning affected the types and the amounts of PAHs produced (Jenkins *et al*., 1996).

In 1988, BaP was measured at 1.3 ng/m^3 in urban southern Guangzhou City, GuangDong Province, China (Simoneit *et al*., 1991). BaP was 0.25 to 17.26 ng/m^3 in Beicun countryside of Datong City, an industrialized area with twice the usual liver and lung cancer mortality, versus 0.1 to 4.6 ng/m^3 in rural areas 30 kilometers north of Datong City, ShanXi Province, China (Han *et al*., 1995). In Xuan Wei, YunNan Province, a high lung cancer rate area in China, women burning smoky coal without a chimney were exposed to 383 ng/m^3 BaP; those burning smoky coal with a chimney were exposed to 184 ng/m^3; and women burning wood or natural gas in Beijing had no detectable exposure (Mumford *et al*., 1993, 1995). The BaP contribution from kitchens using the characteristic Chinese cooking process can be significant (Zheng *et al*., 1997). Exposure to complex PAH and other toxic emissions from cookstoves in developing countries may pose health risks (Zhang and Smith, 1996).

In four New Jersey areas, BaP levels in 1985 ranged from 0.11 to 0.23 ng/m^3 in urban areas and from 0.04 to 0.06 ng/m^3 in rural areas during summer, and from 0.69 to 1.63 ng/m^3 urban and from 0.17 to 0.32 ng/m^3 rural during winter. At 27 New Jersey sites, urban BaP was 0.6 ng/m^3 and rural was 0.3 ng/m^3 (ATSDR, 1995). It was estimated that 97 percent of the annual BaP emissions in New Jersey occurred during the November to March five-month heating season (ATSDR, 1990a). The largest primary source contributors to fine particle mass concentrations in Los Angeles included diesel engine

exhaust, paved road dust and gasoline-powered vehicle exhaust, plus emissions from food cooking and wood smoke, with smaller contributions from tire dust, plant fragments, natural gas combustion aerosol and cigarette smoke (Schauer *et al*., 1996). BaP appeared to be associated primarily with the fine aerosol fraction (Allen *et al*., 1996).

BaP concentrations from 800 to 23,100 ng/m^3 have been reported in the work room air of coke oven operations in the U.K. and in Sweden (ATSDR, 1995). A level of 900 ng/m^3 BaP has been detected in a Norwegian electrode paste plant (Ovrebo *et al*., 1994), from 170 to 4,880 ng/m^3 BaP has been measured in a Swiss carbon anode plant (Petry *et al*., 1996), and from 2 to 60 ng/m^3 BaP has been determined in an American iron foundry (Santella *et al*., 1993). The personal air samples of Estonian cookery workers in an oil shale processing plant had a mean BaP concentration of 5,700 ng/m^3 with 18 percent of the samples exceeding 10,000 ng/m^3, in addition to skin contamination of about 1.2 ng/cm^2 (Kuljukka *et al*., 1996). Iron and steel workers in Anshan, AnHwei Province, China, were exposed to a range from 840 to greater than 3,200 ng/m^3 BaP (Xu *et al*., 1996). Exposure to eight-hour time-weighted average (TWA) concentrations from 16 to 45 ng/m^3 of BaP was reported in Dutch fire-fighting trainers (Feunekes *et al*., 1997).

Soil

Atmospheric deposition after local and long-term transport is believed to be the major source of BaP in soil since BaP has low mobility in soil. Approximately 52 percent of the BaP in air returns to surface soil and water via dry deposition. Typical concentrations of BaP in soils of the world are between 100 and 1,000 μg/kg; values as high as 650,000 μg/kg near a German soot plant and up to 500,000 μg/kg near oil refineries have been reported (ATSDR, 1990a). The background concentration of BaP is about 2 to 1,300 μg/kg in rural soil, about 4.6 to 900 μg/kg in agricultural soil, and about 165 to 220 μg/kg in urban soil (ATSDR, 1995). BaP has been detected in sludge ranging from 3 to 1,330 μg/kg, and in freeze-dried sewage sludge ranging from 540 to 13,300 μg/kg (U.S. EPA, 1991). The soil half-life for BaP ranges from 0.16 to 1.5 years (Borgert *et al*., 1995).

Water

BaP, a lipophilic carcinogen, has relative low water solubility. Atmospheric deposition is believed to be the major source of BaP in surface water, with smaller amounts contributed by refinery effluents, municipal waste water, urban runoff and rivers. BaP in raw water tends to adsorb onto particulates and can be removed by filtration before reaching the tap. Contamination of raw water supplies from natural and anthropogenic sources, and leachate from coal tar and asphalt linings in water storage tanks and distribution lines contribute the major sources of BaP in drinking water. BaP in tap water is mainly caused by the presence of PAH-containing materials in water storage and distribution systems. Granular activated charcoal is the U.S. Environmental Protection Agency (U.S. EPA)-approved treatment method for removing BaP and other PAHs in drinking water. It was estimated that one to two tons of BaP were released from municipal sewage effluents and 0.1 to 0.4 tons of BaP were released from petroleum

refinery waste water in the U.S. in 1977 (ATSDR, 1990a). BaP was detected in the water soluble fraction of partially combusted crude oil in Kuwait after the 1991 Gulf War (Gundersen *et al*., 1996). BaP in the ground water from five U.S. wood treatment facilities was reported to have an average concentration of 57,000 parts per trillion (ppt) (ATSDR, 1995). Mean concentrations of BaP in the Great Lakes have been between 0.03 and 0.7 ppt in water (Environment Canada, 1991).

BaP has been detected in surface water ranging from 0.2 to 13,000 ppt, tap water ranging from 0.2 to 1,000 ppt, rain water ranging from 2.2 to 7.3 ppt, subterranean water ranging from 0.4 to 7 ppt and waste water ranging from 0.001 to 6,000 ppb (U.S. EPA, 1991), in two ground water sites at 0.3 and 4 ppt, respectively, in treated surface water used as drinking water ranging from 0.3 to 2 ppt and in untreated water ranging from 0.6 to 210 ppt (ATSDR, 1990a). Of the 6,074 sites sampled from 10 states including California, 0.26 percent had detectable BaP but all were below the MCL of 0.2 ppb (U.S. EPA, 1997). BaP levels of less than 1 ppt were found in six American drinking water systems (NRC, 1982), and in several American studies including multiple cities, levels ranged from 0.1 to 2.1 ppt with an average of about 1 ppt (HSDB, 1997).

BaP was detected in the source drinking water collected from stomach cancer-prevalent areas of Zanhuang County, HoPei Province, China, at 1.48 to 3.05 ppt (Zhang *et al*., 1995). BaP concentrations averaging 2 and 3 ppt and maximizing at 15 ppt in tap water were reported in European water distribution systems (HSDB, 1997). BaP ranged from 0.01 to 0.95 ppt in Austrian mineral water and from 0.05 to 2.2 ppt in drinking water (Tiefenbacher *et al*., 1982). The oral BaP intake from water was estimated as 1 ng/day (Santodonato *et al*., 1981).

Food and Other Sources

Humans may also be exposed to BaP in food, tobacco and other plant smoke, and some occupational environments, and through contacts with BaP-containing products such as coal tar, coal tar-based shampoo, asphalt and creosote-treated wood. BaP has been detected in grains, fruits, vegetables and seafood. It was estimated at an average of 9,000 ng/kg BaP in charcoal-broiled steak (ATSDR, 1990a, 1995). U.S. EPA (1985) estimated a daily BaP intake from food of 50 ng. A daily median total ingested dose of 176 ng of BaP was also estimated, based on a urinary biomarker study of 14 adult human volunteers in New Jersey over 14 consecutive days, exceeding by 16-fold the winter inhalation dose of 11 ng/day and by 122-fold the summer/fall inhalation dose of 2.3 ng/day (Buckley *et al*., 1995).

The daily oral PAH intake from food *per capita* was estimated as 1,600 to 16,000 ng including 30 percent carcinogenic PAHs, and 160 to 1,600 ng/day was estimated as the BaP intake from food (Santodonato *et al*., 1981). A matching study in the Netherlands estimated the oral PAH intake from food *per capita* as 1,100 to 22,500 ng/day with BaP ranging from 30 to 350 ng/day (Vaessen *et al*., 1988). The daily BaP intake from European total diets was estimated in the range of 120 to 290 ng (de Vos *et al*., 1990). Similar estimates for BaP intake from food included 50 ng/day in Japan, 250 ng/day in the U.K. and 3,400 ng/day in Austria (Pfannhauser, 1991).

Food is a significant source of BaP in Europe due to PAHs in oils, fats and cereals which represent a high percent of the European diets (Guillen *et al*., 1996). Legislation in Germany, Austria and Poland in 1987 established a maximum limit of 500 ng/kg for BaP in smoked meat. The German food industries recommended in 1990 a limit of BaP in refined fats and oils of below 5,000 ng/kg, even though levels as high as 68,600 ng/kg had been found in rape seed oil. In Brazil, mean levels of BaP in oils from sunflower, rice, palm, soybean and corn were 200, 1,800, 2,100, 2,200 and 10,800 ng/kg, respectively. BaP levels in garlic and rape seed oil were below the detection limit of 500 ng/kg (Pupin and Toledo, 1996).

In Germany, BaP measured in 27 smoked fish products ranged from 200 to 4,100 ng/kg (ppt) with a mean of 1,200 ng/kg, and the total carcinogenic PAHs averaged 9,000 ng/kg (Karl and Leinemann, 1996). BaP ranged from 10 to 19,110 ng/kg in various Austrian food including fruits, vegetables, smoked meat and fish products, oils, fats, grilled meats and spices (Tiefenbacher *et al*., 1982), and from 100 to 1,300 ng/kg in various Dutch food including breads, biscuits, rice, wheat products, potatoes, fruits, vegetables, meat and fish products, milk and dairy products, poultry and eggs, oils, fats, nuts and drinks (de Vos *et al*., 1990). In Spain, mean BaP content of 15 smoked sausage samples was around 20 ng/kg, and all but two samples had BaP below the 30 ng/kg limit imposed in the European legislation for smoking-flavor agents (Garcia Falcon *et al*., 1996).

Cigarette smoke was reported to contain about 5 to 80 ng BaP per cigarette in mainstream smoke while a much greater amount of about 25 to 200 ng BaP per cigarette was in the sidestream smoke. The BaP in a cigarette smoke-polluted environment can be from 400 to 760,000 ng/m^3. Cigar and pipe smoke can contain from 18 to 51 ng/g and 85 ng/g of BaP, respectively. BaP has also been detected in the Gulf War zone, in areas with leaked or spilled crude oil, incineration emissions and ashes, pulp mill and other industrial effluents and indoor air (ATSDR, 1990a, 1995). BaP in 28 pine needle samples from the U.K. ranged from 0.49 to 7.9 ng/g dry weight (Tremolada *et al*., 1996).

METABOLISM AND PHARMACOKINETICS

Absorption

Benzo(a)pyrene is absorbed following exposure by the oral, inhalation or dermal route.

Oral Route

Over 30 percent of a 1 mg dose of BaP that was administered in corn oil intraduodenally in combination with bile to male Sprague-Dawley rats was excreted in the bile after 48 hours (BaP was recovered from a cannulated bile duct; bile was introduced separately into the duodenum) (Rahman *et al.*, 1986). Only seven percent of the dose of BaP was recovered in the bile when no bile was introduced in the duodenum.

Biliary excretion of BaP and BaP metabolites (from a cannulated bile duct) were measured in male Sprague Dawley rats intraduodenally or intravenously administered a low dose (4-10 pmol/min intravenous; or 9-15 pmol/min intraduodenal) of BaP (Foth,

1988). Approximately forty percent of the intravenous dose was recovered after four hours and 20 percent of the intraduodenally administered dose was recovered in the bile suggesting that 50 percent of the intraduodenal dose was absorbed. Incidentally, because of the cannulation of the bile duct, bile did not appear to be available to aid absorption in the small intestine in this study. After large doses of BaP (100 mg/kg) were administered orally or intravenously to male Fischer-344 rats, comparison of area under the blood concentration time curve indicated that approximately 40 percent of the oral dose of BaP was absorbed (Ramesh *et al.*, 2001b).

Inhalation Route

BaP (0.1, 1.0 or 2.5 mg/m^3) administered on carbon black particles to male and female F-344 rats for 4 hours by inhalation (nose only) was rapidly absorbed (Ramesh *et al.*, 2001a). Plasma levels of BaP and its metabolites peaked one hour post exposure and were at or near background levels four hours post exposure. Lung BaP-metabolite levels mirrored what was observed in the plasma, peaking at one hour and returning to near background levels 4 hours post-exposure. Other studies have also demonstrated rapid clearance of BaP from the lung with the rate of clearance appearing to be governed by the size of the particle used to deliver the toxicant (WHO, 1998).

Dermal Route

Percutaneous absorption of BaP has been investigated in several species *in vivo* or *in vitro* and with different vehicles (Kao *et al.,* 1985; Sanders *et al.,* 1986; Yang *et al.,* 1986, 1989; Wester *et al*., 1990; Storm *et al.,* 1990; Ng *et al.,* 1992; Moody *et al.,* 1995). Depending on how absorption is measured (e.g., in *in vitro* studies absorption can be defined as amount recovered in receiver fluid or amount of BaP extracted from skin plus amount in receiver fluid; the latter is the basis of the following discussion), absorption ranged from a few percent to 50-90 percent of the applied dose. The vehicle used to apply BaP to skin had a large effect on the absorption. Absorption *in vivo* in the monkey ranged from 10 percent when applied in soil to 50 percent when applied neat (Wester *et al.,* 1990). BaP absorption *in vitro* using human skin ranged from 1 percent from soil to 24 percent when applied neat (Wester *et al.,* 1990).

Metabolism

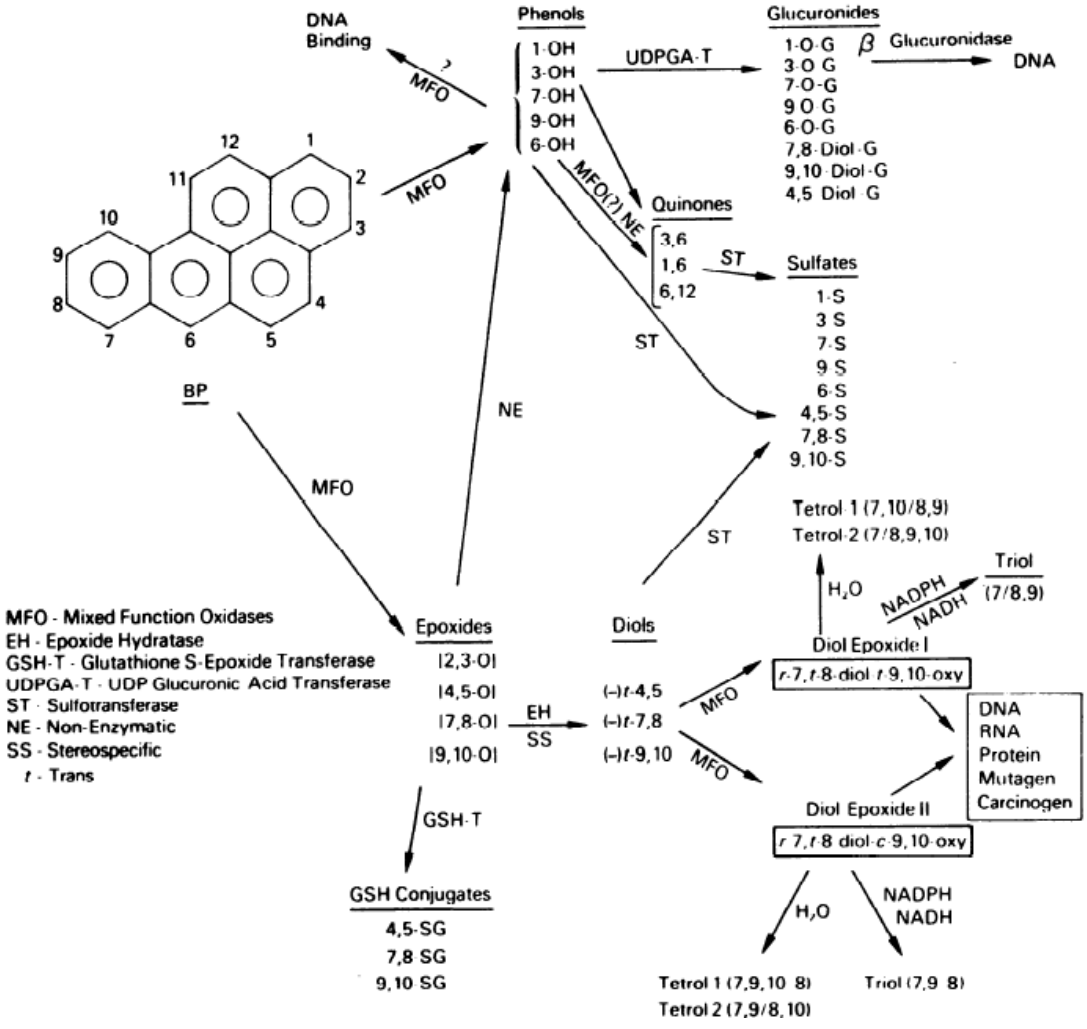
The metabolism of the polycyclic aromatic hydrocarbons including BaP has been painstakingly studied for over 50 years and is the subject of numerous reviews (Gelboin, 1980; ATSDR, 1995; WHO, 1998). As shown in Figure 1, BaP metabolism is quite complex, resulting in a host of products and a number of enzymatic and non-enzymatic reactions. While numerous products are formed during metabolism, reactions that produce diol epoxides, in particular the 7,8-diol-9,10 epoxide isomer, have been the focus of metabolic studies because these metabolites are considered the ultimate carcinogens.

BaP metabolism begins by oxidization by microsomal enzymes (the mixed function oxidases (MFO)) and the cytochrome P450s (CYP1A1, CYP1A2 and others). Arene oxides (epoxides) are formed at several positions on the BaP moiety which then hydrate spontaneously or enzymatically by epoxide hydrase to form dihydrodiols (other reactions form other products). The diol can undergo further oxidation by MFO to yield a diol

epoxide. While many other products are formed (see Figure 1), the 7,8-diol-9,10 epoxide isomer is highly reactive with nucleophiles (DNA, RNA, proteins) and is believed to be the ultimate carcinogen. BaP reactive products are detoxified by conjugation with glucuronic acid, glutathione or sulfate which are water soluble and readily eliminated from the body (Figure 1).

The cytochrome P450s have been detected in many tissues and species. The specificity and rate of metabolism differ for different forms of the enzyme, in different tissues and in different species. CYP1A1 activity is very low in the liver of mice not induced by Ah receptor agonists (Uno *et al*., 2006). The cytochrome P450s are induced by BaP (inducing its own metabolism), other PAHs, and by many other chemicals. BaP induces its own metabolism by binding to the cytosolic Ah receptor which is translocated into the nucleus where mRNA synthesis is induced.

Figure 1. Benzo(a)pyrene Metabolism



From Gelboin, 1980

Distribution

BaP and its metabolites have been detected in a number of tissues after intravenous, oral, inhalation, or intratracheal administration (reviewed by ATSDR, 1995; WHO, 1998).

Following intravenous administration (2, 6 or 15 mg/kg) to male Wistar rats, BaP levels were measured in blood, adipose, kidney, liver and lung (Moir *et al.*, 1998). BaP levels in the lung were higher than in the other tissues and persisted in the lung much longer than in other tissues. Following intratracheal installation (1 µg/kg) in male Sprague-Dawley rats, BaP levels in the lung rapidly decreased while much of the dose was detected in the liver and intestine (Weyand and Bevan, 1986). Over 5 percent of the dose was detected in the stomach with approximately 1 percent of the dose detected in the testes.

Excretion

Less than 1 percent of a dose of BaP (2, 6, 20 or 60 µmol/kg) was recovered in the urine (as BaP metabolites) of male Sprague-Dawley rats following intravenous, percutaneous, or gavage administration (Bouchard and Viau, 1997). Eighty percent of an oral dose of BaP (1 mmol/kg) that was administered to male Wistar rats was recovered in the feces over five days (van de Wiel *et al.,* 1993). Sixty percent of the oral dose administered to female rats was recovered in their feces. Because the dose was administered orally, it is unclear how much of the dose was absorbed and excreted in the bile or simply not absorbed from the gut.

Following intravenous administration of BaP (2, 6 or 15 mg/kg) to male Wistar rats, over 50 percent was recovered in the feces after 32 hours, while 6 to 7 percent of the administered dose was recovered in the urine (Moir *et al.*, 1998). Roughly 40 percent of the dose of BaP infused in the femoral vein was recovered in the bile after 3 hours (Foth *et al.,* 1988). The investigators reported that only 2 percent of the dose was recovered in the urine after 36 hours. Seventy-four percent of the dose of BaP (1 µg/kg) instilled into the trachea of male Sprague-Dawley rats was recovered in the bile within 6 hours of administration (Weyand and Bevan, 1986). Only 5 percent of the dose was in the lung after 6 hours.

BaP extraction capability was determined for the lung and liver by measuring differences in blood levels following BaP infusion in the anaesthetized rat (Foth *et al.,* 1988). The lung extracted roughly 10 to 15 percent of the infused dose while the liver extracted roughly 40 to 50 percent of the infused dose. These findings indicated systemic availability of BaP following oral exposure (no first pass metabolism) given that the liver only extracted 40 percent of the infused dose. The ability of isolated liver and lungs from control and cytochrome P450 induced animals to remove BaP from perfusion media indicated that at perfusion rates consistent with physiological conditions, the lung may clear about the same amount of BaP as the liver even though the liver has a much higher ability to metabolize BaP (Roth and Vinegar, 1990). It appears that blood flow may limit the rate of clearance by the liver. Other studies in rat (Wiersma and Roth, 1983) and in

isolated perfused lung and liver also indicate that at least half of the BaP in the portal circulation escapes first pass metabolism by the liver (Foth *et al.,* 1984).

BaP clearance was measured in knockout and wild type male C57BL/6J mice (Uno *et al.,* 2006). Blood BaP levels in knockout mice with no CYP1A1 remained elevated for three times as long (15 vs 5 hours) compared to wild type mice while peak blood levels were nearly equivalent. CYP1A1 activity is almost undetectable initially in wild type mice but is rapidly induced by the BaP treatment. Pretreatment with TCDD, which induced CYP1A1 levels, markedly reduced peak BaP levels in wild type but not knockout mice (no CYP1A1 to induce). Removal of other P450s enzymes (CYP1B1, CYP1A2) in other knockout mice lines had little effect on BaP blood levels. Knockout mice missing both CYP1A1 and CYP1B1 had somewhat greater peak blood BaP levels compared to the single CYP1A1 knockout mice, suggesting a role for CYP1B1 in BaP metabolism.

The findings of the aforementioned toxicokinetic studies, in which BaP was distributed and metabolized in the lung and intestine in addition to the liver, and the inability of the liver to extract all BaP from the blood (no first pass metabolism), are consistent with the detection of tumors in extrahepatic tissues in cancer bioassays in which BaP was administered orally.

TOXICOLOGY

Toxicological Effects in Animals

Benzo(a)pyrene is a well-known carcinogen and has been a major focus of cancer research. Consequently, few studies have investigated non-carcinogenic effects of BaP.

Acute Toxicity

Male and female F-344 rats were administered one dose of BaP (0, 100, 600 or 1000 mg/kg (the maximum amount that could be dissolved in the vehicle) by gavage (Knuckles *et al.,* 2001). Liver to body weight ratios were significantly increased in male (100 mg/kg or greater) and female rats (600 mg/kg or greater) 14 days after treatment. Blood hemoglobin levels (100 mg/kg or greater) were increased and white blood cell counts (600 mg/kg or greater) were decreased in male rats. No histopathology or adverse effects on blood chemistry were observed.

Subchronic Toxicity

Male and female F-344 rats were given 0, 5, 50 or 100 mg/kg BaP in the diet for up to 90 days (Knuckles *et al.*, 2001). Food consumption and body weight were decreased in high dose males after 60 and 90 days. Liver to body weight ratios were significantly increased in high dose male but not female rats after 90 days. Hematocrit and hemoglobin levels were significantly decreased in both males and females that received the high dose and in female rats receiving 50 mg/kg at 60 but not 90 days. Increased abnormalities (e.g.,

increased tubular casts) were observed in the male kidney at all doses (5 mg/kg or greater) and the occurrence of the abnormalities appeared to be dose-dependent.

Knockout mice missing CYP1A1 were more sensitive to BaP toxicity (exposed to 125 mg/kg-day for 18 days) than wild type mice, suggesting an important role of CYP1A1 in detoxifying BaP at relatively high doses (Uno *et al.*, 2006). Toxicity was indicated by marked weight loss, atrophy of the spleen and thymus and increased liver weight, elevated plasma AST and ALT levels and other effects in red and white blood cells. Unexpectedly, higher levels of DNA adducts in the small intestine, spleen and bone marrow (and liver in mice treated with 12.5 mg/kg-day) were detected in the CYP1A1 knockout mice treated with 125 mg/kg-day BaP compared to wild type mice, suggesting a role for other cytochrome P450 isozymes in generating DNA-reactive metabolites.

Genetic Toxicity

Benzo(a)pyrene has been studied in a multitude of genotoxicity bioassays and is often employed as a positive control because it is active in both *in vivo* and *in vitro* assays. Reviews by IARC (1983), ATSDR (1995) and WHO (1998) provide detailed discussion of genotoxicity studies of BaP and other PAHs. Evidence of genetic damage produced by BaP includes gene mutations, DNA binding/adduct formation, DNA single strand breaks, chromosomal alterations, micronuclei, unscheduled DNA synthesis, and sister chromatid exchange. Effects have been observed in bacterial and eukaryote cells, in cultured human cells, and *in vivo* studies in animals.

Developmental and Reproductive Toxicity

Pregnant CD-1 mice were administered BaP (0, 10, 40 or 160 mg/kg-day) by gavage on days 7-16 of gestation (MacKenzie and Angevine, 1981). Administration of 160 mg/kg-day of BaP resulted in a reduced percentage of mice that were pregnant, reduced number of viable litters at parturition, and reduced mean pup weight at 20 and 42 days post-parturition. Mean pup weight was also significantly reduced at 20 or 42 days post-parturition in females that received 10 mg/kg-day or greater of BaP.

Effects on male and female mice in the F_1 generation were more dramatic. Male offspring of dams treated with 10, 40 or 160 mg/kg-day and then bred with untreated females yielded decreased fertility (10 mg/kg or greater), with almost no pregnant females when the males were exposed to either 40 or 160 mg/kg-day *in utero*. Similarly, fertility was reduced in female mice exposed to 10 mg/kg-day *in utero* and no pregnancy was observed in females exposed *in utero* to the two higher doses. Female mice exposed to 10 mg/kg-day *in utero* produced fewer litters and smaller litters than control (unexposed) mice. Marked effects were observed in the gonad tissues of male and female mice exposed to 40 mg/kg *in utero*.

The following excerpt presents a good review of the reproductive and developmental effects of BaP. It is from Appendix C-1 of The Prioritization of Toxic Air Contaminants – Children's Environmental Health Protection Act, Final Report (OEHHA, 2001):

Intraperitoneal injection of benzo[*a*]pyrene, at doses between 50 and 300 mg/kg body weight given at day 7 or 10 of gestation, causes *in utero* toxicity and teratogenicity in mice (Shum *et al.*, 1979). A reduction in the number of surviving offspring (resulting both from resorptions and stillbirths) was observed in all cases. The severity of the effect was correlated with the ability of the fetus and maternal systems to metabolize benzo[*a*]pyrene, which is influenced by induction of aryl hydrocarbon hydroxylase (AHH). Mice (and other mammals) are described as genetically "responsive" when AHH activity is induced by exposure to PAHs and other activators of the *Ah* receptor. A greater impact on pre-and post-natal mortality was observed in C57BL/6 mice which are responsive to AHH induction than in non-responsive AKR inbred mice. Malformations were noted in the responsive mice only; these included club foot, hemangio-endothelioma, cleft palate and various other anomalies of the skeleton and soft tissues. Representative results from this study are shown in Table 3.

Table 3. Impact of benzo[*a*]pyrene treatment on fetal and newborn survival and malformations in responsive and non-responsive mice (Shum *et al.*, 1979)

Dosed on Gestation Day	Strain	# Litters	# Implant-ations	# Stillborn	# Resorbed	# Malformed	Percent of all effects
7	B6	7	48	2	19	17	79
6	AK	6	43	0	9	0	21
10	B6	10	62	0	11	18	47
11	AK	11	78	1	8	0	12
7	B6	7	47	1	20	3	51
5	AK	5	45	0	6	0	13
31	B6	31	187	2	33	0	19
12	AK	12	107	0	6	0	6.5

B6 = C57BL/6 mice, responsive to AHH induction.
AK = AKR mice, non-responsive to AHH induction.
Dose given was 200 mg/kg benzo[*a*]pyrene i.p.
Using AKR x (C57BL/6) (AKR)F_1 and (C57BL/6) (AKR)F_1 x AKR backcrosses, allelic differences at the fetal *Ah locus* could be correlated with dysmorphogenesis. If the mother is non-responsive *(Ah^d/Ah^d)*, the Ah^b/Ah^d genotype in the fetus is associated with more stillborns and resorptions, decreased fetal weight, increased congenital anomalies, and enhanced P_1-450-mediated covalent binding of BP metabolites to fetal protein and DNA, compared with the Ah^d/Ah^d genotype in the fetus from the same uterus. If the mother is responsive *(Ah^b/Ah^d)*, however, none of these parameters can be distinguished between Ah^b/Ah^d and Ah^d/Ah^d individuals in the same uterus, presumably because enhanced BP metabolism in maternal tissues and placenta cancels out these differences between individual fetuses.

Infertility was observed in CD-1 mice after exposure *in utero* to benzo[*a*]pyrene (McKenzie and Angevine, 1981). Groups of 30 or 60 pregnant female mice were

given doses of 10, 40 or 160 mg/kg/day benzo[*a*]pyrene in 0.2 ml corn oil on days 7 - 16 of gestation; controls received corn oil only. There was no maternal toxicity or embryolethality at any dose level, although pregnancy maintenance was impaired at 160 mg/kg/day. Mean pup weight was reduced in the litters of all treated dams, with the effect becoming more noticeable with age. As adults, offspring which were exposed to benzo[*a*]pyrene *in utero* showed loss of fertility in controlled breeding studies with untreated partners: at the higher doses this included complete infertility, and histological abnormalities of the gonads. Treated pup weights, and results of the breeding studies with the F_1 mice are shown in Table 4.

Table 4. Pup weight and reproductive performance of male and female F_1 mice exposed prenatally to benzo[*a*]pyrene (MacKenzie and Angevine, 1981)

	Benzo[*a*]pyrene (mg/kg/day)[a]			
	0	**10**	**40**	**160**
Treated Pup Weight				
Mean pup weight at 4 days (g)	2.7 ± 0.02	2.8 ± 0.04	2.5 ± 0.02	2.2 ± 0.04
Mean pup weight at 20 days (g)	11.2 ± 0.1	11.6 ± 0.1	10.4 ± 0.1**	9.7 ± 0.2**
Mean pup weight at 42 days (g)	29.9 ± 0.2	28.2 ± 0.3**	28.0 ± 0.2**	26.8 ± 0.4**
F_1 Male breeding study				
Number of F_1 males tested[b]	45	25	45	20
Fertility index[c]	80.4	52.0*	4.7**	0.0**
Mean litter size	11.0 ± 0.1[d]	10.7 ± 0.2	10.8 ± 0.6	-
F_1 Female breeding study				
Number of F_1 females tested[e]	35	35	55	20
Fertility index	100.0	65.7**	0.0**	0.0**
Mean litter size	12.9 ± 0.2	10.4 ± 0.4**	-	-

a Mice were exposed prenatally to benzo[a]pyrene on days 7 through 16 of gestation.
b Beginning at 7 weeks of age, each F_1 male was exposed to 10 untreated females over a period of 25 days.
c Fertility index: Females pregnant/females exposed to males x 100.
d Mean ± SEM.
e Beginning at 6 weeks of age, each F_1 female was cohabitated continuously with an untreated male for 6 months.
* Significantly different from controls ($P<0.05$).
** Significantly different from controls ($P<0.01$).

Thus, *in utero* exposure to benzo[*a*]pyrene interfered with the development of the reproductive organs. The severity of the effects seen in this experiment are notable: males exposed to 40 mg/kg benzo[*a*]pyrene showed severely atrophied and essentially aspermic seminiferous tubules. Exposed females showed hypoplastic

ovaries with very few follicles or corpora lutea; most of the animals exposed to the higher doses had no identifiable ovaries or only remnants of ovarian tissue. The endocrine effects of such changes are likely to be substantial throughout postnatal growth and development as well as in the adult. The observation in this experiment of low pup weight as a trend of marginal significance immediately after birth, but becoming more noticeable and statistically significant in older (20 or 42 day old) pups may be indicative of endocrine effects.

Similar reductions in fertility of female NMRI mice were observed by Kristensen *et al.* (1995) after exposure *in utero* to 10 mg/kg/day oral benzo[*a*]pyrene on days 7-16 of pregnancy.

Urso and Gengozian (1982), Urso and Johnson (1988) and Urso *et al.* (1992) reported a series of experiments in mice which demonstrated that a single exposure to benzo[*a*]pyrene (by injection) during pregnancy results in immunosuppression in the offspring which is noticeable not only in the neonates but also later in life, and also changes in the maternal immune system which may impact the maintenance of pregnancy and the subsequent immunological status of the offspring. (They suggested that the effects in the offspring might be related to the later development of tumors at a large number of sites in these mice.) The immune responses were measured as the degree of anti-sheep erythrocyte plaque-forming response, mixed lymphocyte response of cultured lymphocytes, and measures of T-cell function. A typical experiment reported by Urso *et al*. (1992), in which mice were treated at mid-pregnancy with a single intraperitoneal injection of 150 mg/kg benzo[*a*]pyrene, is shown in Table 5.

Table 5. Progeny and maternal mixed lymphocyte response

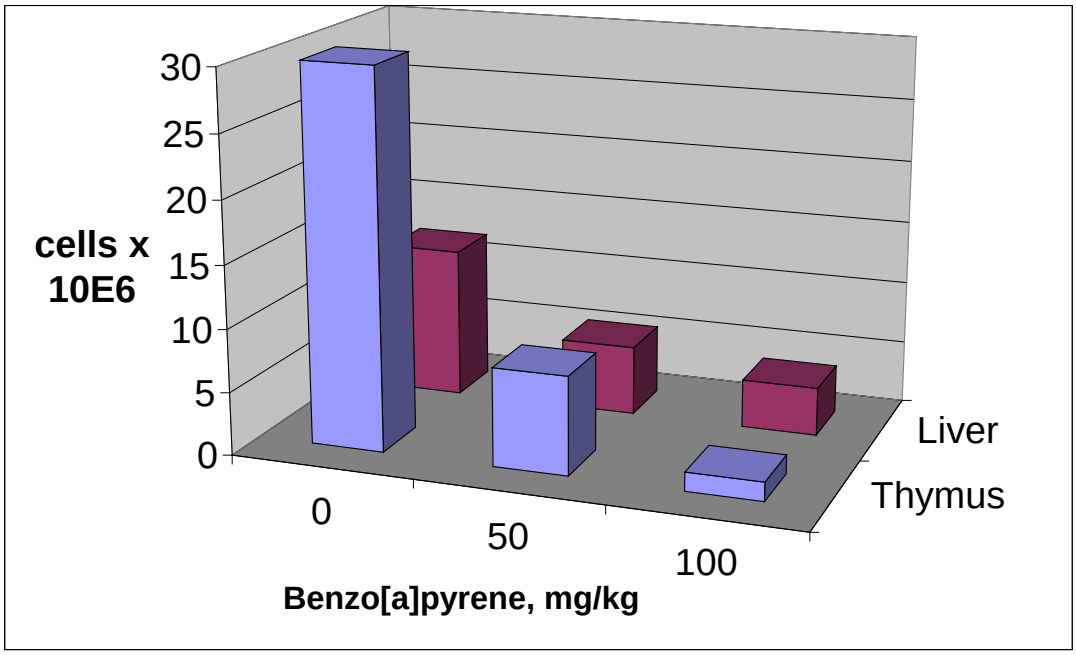
	MLR expressed as percent controls*			
	Progeny		**Maternal**	
Time after treatment	**Spleen**	**Thymus**	**Spleen**	**Thymus**
17 days gestation (G)		95	50	47
19 days G		105	51	15
1 day postnatal (P)		61		
3 days P		60		14
7 days P	55	26	40	8
4 weeks P	13			
20 weeks P	44			
53 weeks P	60			
104 weeks P	43			

Data from Urso *et al.* (1992).

* MLR, mixed lymphocyte response by responder cells cultured for 4 days with allogeneic stimulator cells (mitomycin C inactivated) after a [^{3}H]-thymidine pulse. Each value represents 6-10 determinations; fetal thymus value is a pool of tissue from fetuses (3-6) of one mother. Values above 95 percent, not significant; all others significant at $P < 0.05$ to $P < 0.0001$ (Student's t-test).

Holladay and Smith (1994) demonstrated severe depletion of both thymus and liver cells of $B6C3F_1$ mice exposed to benzo[*a*]pyrene (maternal dose 50, 100 or 150 mg/kg/day) on days 13-17 of gestation. Numbers of thymocytes and fetal liver cells (obtained by mechanical disruption, resuspension and washing in lysing solution to remove erythrocytes) determined by Coulter counter are shown in Figure 2.

Figure 2. Fetal thymus and liver cellularity in $B6C3F_1$ mice exposed to benzo[*a*]pyrene *in utero*



(Graphic from OEHHA, 2001; data from Holladay and Smith, 1994.)

Differences in the proportions of various classes of surface antigens (CD4, CD8 and HSA) were also noted in perinatally isolated thymocytes. The authors concluded that the changes were suggestive of impaired maturation in the surviving thymocytes, and that these and other changes observed were consistent with the long-term immunosuppression seen in mice exposed to benzo[*a*]pyrene *in utero.* Similar effects of benzo[*a*]pyrene on development of T-cells, with long-term consequences for development of the immune system, have been reported by others, including (Rodriguez *et al.,* 1999).

Immunotoxicity

Immunotoxicity of BaP has been investigated principally in *in vitro* studies involving cell cultures and *in vivo* where the compound was administered to mice by injection (see review of WHO, 1998). Immunotoxicity was indicated in these studies based on a variety of indicators: organ size, morphology, cellularity, lymphoproliferative response or

functional tests. Developmental studies have also demonstrated immunotoxic effects in the F_1 generation exposed *in utero* (see preceding reproductive and developmental effects section).

Male Wistar rats were administered BaP (0, 3, 10, 30, 90 mg/kg) by gavage 5 days a week for 7 weeks (De Jong *et al.*, 1999). Thymus weights were significantly decreased in animals that received 10 mg/kg or more of BaP and decreased lymph node weights were observed at 90 mg/kg. Morphometric analysis of the thymus revealed a decrease in thymus weight at 10, 30 or 90 mg/kg, decrease in thymus cortex weight at 30 and 90 mg/kg, and decrease in thymus medulla weight and cortex surface area in the 90 mg/kg group. Significant decreases in splenic total cell and B cell numbers and serum IgA levels were observed in animals treated with 90 mg/kg. General indicators of toxicity including decreased body weight, kidney weight, increased liver weight, and abnormal hematological parameters (WBC, RBC, Hb, MCV, MCH levels) were observed in animals treated with 90 mg/kg BaP.

Neurotoxicity

Using a battery of functional endpoints, neurotoxicity was investigated in male F344 rats administered a single large dose of BaP (0, 25, 50, 100, 200 mg/kg) by gavage (Saunders *et al.*, 2002). Mobility (total distance moved) was significantly decreased in animals receiving 50 mg/kg of BaP or more (and perhaps 25 mg/kg). In a subsequent study, Saunders and coworkers observed decreased motor activity at 25 mg/kg (Saunders *et al*., 2006). Animals receiving higher doses displayed lower mobility, which remained decreased for longer periods of time post-treatment. Other functional measures including gait, forelimb and hindlimb grip strength, and landing foot splay were also affected at 50 mg/kg or more of BaP, and higher doses resulted in neurotoxicity for longer time periods. Sensorimotor functions such as click response and tail pinch were also affected at doses of 50 mg/kg or more.

Chronic Toxicity/Carcinogenicity

Benzo(a)pyrene has been studied in numerous cancer bioassays in various laboratory animals (rat, mouse, hamster) in which the compound was administered orally, percutaneously, via inhalation of particulates, via intratracheal or intrabronchial installation, and by injection. The focus of the following discussion is studies in which BaP was administered by the oral route. Older studies are first reviewed, then two well-conducted modern studies (Culp *et al.*, 1998; Kroese *et al*., 2001) are discussed.

Oral route

Neal and Rigdon, 1967. Benzo(a)pyrene (0, 1, 10, 20, 30, 40 or 45 ppm) was administered in food to groups of male and female CFW mice for 110 days (Neal and Rigdon, 1967). Based on the reported date of sacrifice, it appears that the mice were exposed for 110 days and observed for the same 110 days. In a second study, groups of male and female mice were administered higher doses of BaP (50, 100 or 250 ppm) in the diet for 70 to 197 days. As with the low dose group, the animals appeared to be observed

during the time BaP was administered (and no longer), whereupon the animals were sacrificed. Statistically significant increases in papillomas and squamous cell carcinomas in the "squamous portion of the stomach" (forestomach) were observed in the highest four dose groups of mice (Table 6). No information regarding tumors at other sites was provided.

The protocol employed in this older study is not comparable with more modern cancer bioassays (although the study included an unusually large number [nine] of dose groups). The low doses and the high dose groups appeared to be tested at different times. In the study, BaP was administered to combined groups of male and female mice, not to separate groups of male or female mice. The number of mice in each dose group was variable. Information on the purity of the test compound was not presented, nor was food consumption monitored. In the three highest dose groups, animals began the study at different ages (ranging from 18 to 116 days of age) and were sacrificed at different ages (ranging from day 70 to day 226). In the three high dose groups, the only groups where marked increases in tumors were observed, the animals were exposed to and observed for markedly different time periods (ranging from 70 to 197 days).

Table 6. Gastric Tumors in Mice Administered BaP[a]

BaP in Diet (ppm)	0	1	10	20	30	40	45	50	100	250
Forestomach Tumors	0/289	0/25	0/24	1/23	0/37	1/40	4/40[b]	24/34[b]	19/23[b]	66/73[b]

[a]From Neal and Rigdon, 1967

[b]Statistically significant increase in tumors (Fisher's Exact Test, $p < 0.05$)

The number of mice in the control group was unusually large for a cancer bioassay. The investigator reported "none of the 289 mice fed the control ration had a gastric tumor. There were 171 males and 118 female in this group and their ages varied from 70 to 300 days." It is unclear if this group was a matched control treated in an identical manner as the animals in the long term cancer studies, or also included untreated animals matched to shorter duration exposure studies (not discussed here). If a portion of this large group was matched to other parts of the study, one would consider them more as "historical controls" than a matched control group. The number of animals in the control group is important because it influences the statistical power of the study and the findings of a dose-response analysis.

The marked differences in the length of exposure within and between dose groups would be expected to influence the incidence of tumors in the animals. The exposure and observation periods were as long as 197 days (high dose group) and 110 days (low dose groups), which is considerably less than the lifespan of a mouse. The lack of an observed increase of tumors in the low dose groups may reflect the short duration of the exposure, or the relatively small number of animals in these groups. The statistical significance of the increase in tumors in animals receiving 45 ppm may be related to the very large control group.

Brune *et al.*, 1981. BaP was administered in the diet or by gavage in an aqueous caffeine (1.5 percent) vehicle to groups of 32 male and 32 female Sprague-Dawley rats (treatment groups were composed of both sexes) (Brune *et al.,* 1981). Several different schedules were used to administer the agent in this study. Animals administered BaP by gavage (0.15 mg/kg) five days a week until death (mean survival time of 87 weeks) displayed 14 papillomas of the forestomach while animals that received the same dose every 3rd day until death (mean survival time of 113 weeks) displayed 25 papillomas and one carcinoma of the forestomach.

Animals administered the same dose every 9th day until death (mean survival time of 112 weeks) displayed 11 papillomas and one carcinoma in the forestomach. Two animals in the control group, administered the vehicle alone, displayed papillomas of the forestomach. The increases in tumors were statistically significant (author's analysis) in animals that received BaP 5 times a week or every third day ($p<0.05$) (Brune *et al.*, 1981).

In accompanying studies by Brune *et al.* (1981), BaP was administered in the rats' feed. One group received the BaP five times a week for their lifetimes. Nine animals displayed a papilloma of the forestomach. In contrast, only one animal displayed a papilloma in animals that received BaP in the feed every ninth day. Neither increase was statistically significant according to the authors' analysis; the control group displayed two papillomas.

Sharma *et al.*, 1997. A single 3 mg dose of BaP was administered by gavage to small groups of male and female CD-1 mice which were sacrificed 240 days later (Sharma *et al.,* 1997). A protocol known to induce lung tumors in CD-1 mice was employed to investigate sex-related sensitivity to the agent. The diet of half the mice was supplemented with t-butylated hydroxyanisole (BHA) to induce glutathione-S-transferase levels (although this agent also has other effects including production of tumors). Some animals receiving BHA exhibited evidence of gastritis or severe hemorrhage and therefore vitamin K was added to the diets of all animals, which appeared to effectively ameliorate the toxicity.

No lung tumors were observed in the male (0/8) or female (0/8) mice control groups that received both vehicle and the control diet. In animals that received vehicle and the BHA supplemented diet, no tumors were observed in male mice (0/5), while 2/6 female mice exhibited lung tumors. After BaP and the control diet, lung tumors were observed in 14/26 female mice, but only in 5/24 males. The sex-related difference was statistically significant. A marked decrease in tumors was observed in female mice (2/26) treated with BaP when BHA was included in the diet, compared to the control diet. Tumor incidence in male mice (5/17) was not significantly different when BHA was added to the diet, compared to the control diet.

While previous studies have shown that GST levels are higher in male mice and BHA treatment induces GST levels in female but not male CD-1 mice, it is not clear if this is the mechanism that reduced BaP induced tumors in female mice. Female mice that received the control diet did appear to be more sensitive to BaP under the conditions of the study.

Weyand *et al.*, 1995. Benzo(a)pyrene was administered (0, 0.0406, 0.257 mg/kg-day, based on dietary intake) in a gel diet to female A/J mice for 260 days (Weyand *et al.*, 1995). Statistically significant and dose-related increases in both lung and forestomach tumors were observed (Table 7).

Table 7. Increase in Lung and Forestomach Tumors Following 260 Days of Dietary Benzo(a)pyrene Administration (Weyand *et al.*, 1995)

Treatment (mg/kg-day)	Lung tumors	Forestomach tumors
0	4/21[a]	0/21[a]
0.0406	9/25	5/25[b]
0.257	14/27[b]	27/27[b]

[a]Statistically significant, Mantel-Haenszel test for trend ($p<0.05$).
[b]Statistically significant, Fisher's Exact Test ($p<0.05$).

In a separate study, a single dose of benzo(a)pyrene (1.8 mg) was administered to female mice by intraperitoneal injection (i.p.). Lung tumors were observed in 100 percent of mice administered benzo(a)pyrene, compared to 37 percent of mice receiving vehicle control. Forestomach tumors were observed in 83 percent of mice administered BaP, while no forestomach tumors were observed in vehicle controls. Food consumption and weight gain appeared to be unaffected by benzo(a)pyrene treatment.

Singh *et al.*, 1998. Using an animal model designed to be sensitive to chemical carcinogens, ten male and female A/J mice were administered three doses of 0.5 or 1.5 mg of BaP by gavage, one dose every 14 days (Singh *et al.*, 1998). The animals were sacrificed twenty-six weeks after the first dose. Lung and forestomach tissues were examined for histopathology.

At the low dose, no tumors were observed in the lung or forestomach of male mice, while approximately 40 percent of the female mice displayed tumors in the lung or forestomach. At the high dose 80 percent of female and 40 percent of males displayed a lung tumor and 60 percent of males and female displayed a forestomach tumor. This study suggests that female mice may be more sensitive than male mice to BaP. The investigators observed higher levels of isoenzymes of glutathione-S-transferase (an enzyme that detoxifies BaP) in the liver, forestomach and lung of male mice, perhaps explaining the higher sensitivity in female mice.

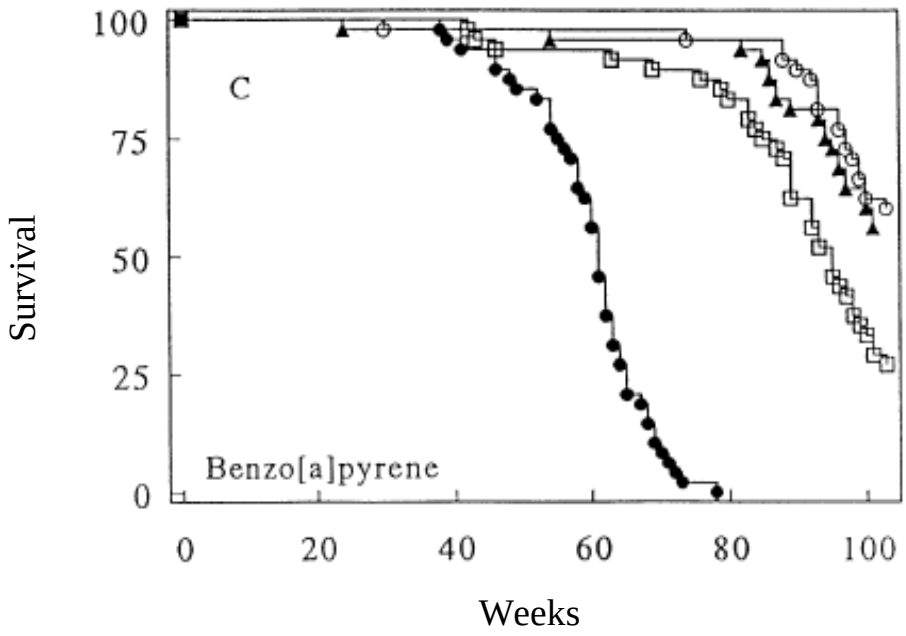
While female mice displayed statistically significant increases in lung tumors at both doses, significant increases in lung tumors were only observed in male mice at the high dose ($p <0.05$), and at a lower incidence than observed in high dose females. Similar to what was observed in the lung, female mice displayed statistically significant increases in forestomach tumors at both doses while male mice displayed increases in forestomach tumors only at the high dose. The incidence of forestomach tumors in high dose animals was similar in male and female mice.

The investigators also explored a possible mechanism of action by measuring glutathione-S-transferase (GST) isoenzyme levels in the liver, lung and forestomach of male and female mice administered BaP. Differences in certain GST isoenzyme levels were observed in male and female mice, particularly in the liver, while the differences were less pronounced in the target tissues. The higher levels of certain GST isoenzymes in males could account for the higher incidence of tumors observed in female mice (particularly at the lower dose, which may be below the level of enzyme saturation), because this enzyme is involved in detoxifying the active forms of BaP.

Culp *et al.,* 1998. Female $B6C3F_1$ mice were administered benzo(a)pyrene (0, 5, 25, or 100 ppm) in the diet for two years in a study aimed primarily at evaluating the carcinogenicity of coal tar. BaP treatment groups were included in this study to evaluate the relative potency of a PAH mixture relative to the potency of a known carcinogenic PAH. The findings of animals treated with coal tar are not presented here. Only female mice were used in this study. The investigators stated that only females were included in this study "because of their low spontaneous liver tumors lifetime incidence."

Based on measured food consumption and body weight (OEHHA calculation), the female mice received time weighted average doses of 0.0, 0.65, 3.5 and 15.2 mg/kg-day of BaP, respectively. Survival in the animals receiving 100 ppm benzo(a)pyrene was significantly reduced, with all animals dead or sacrificed due to morbidity by week 80 (Figure 3). The survival among mice that received 25 ppm BaP also was significantly decreased, while survival in the 5 ppm group was similar to controls.

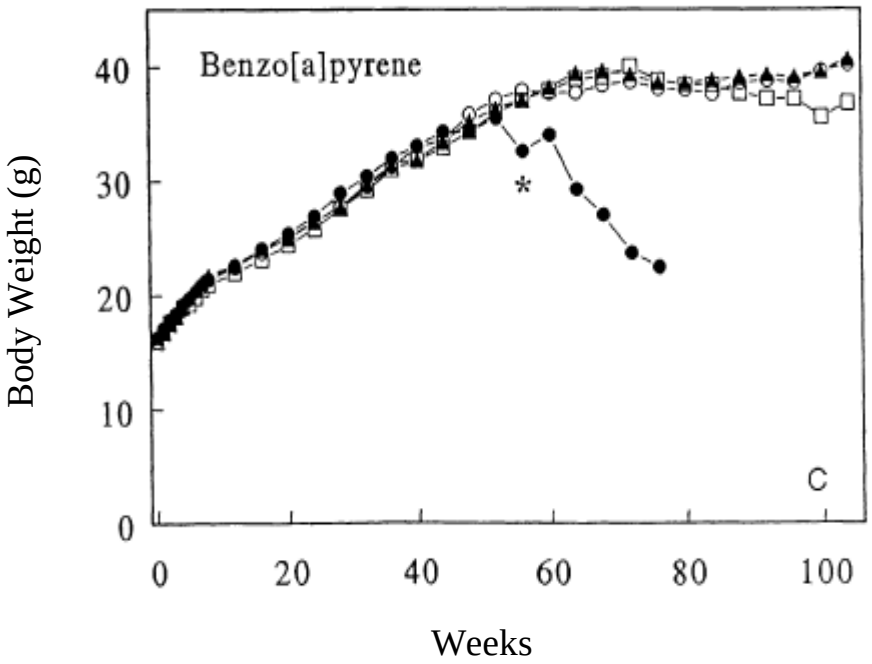
Figure 3. Survival of Mice Administered Benzo(a)pyrene



Key: Solid circle, 100 ppm BaP; Square, 25 ppm BaP; Triangle, 5 ppm BaP; Open circle, 0 ppm BaP. Adapted from Figure 2 in Culp *et al.*, 1998.

Average body weights of all BaP treatment groups were similar to control until week 50 when the mean body weight of the high dose group began to decrease rapidly (Figure 4). Body weight of the other BaP groups remained near control levels.

Figure 4. Body Weight of Mice Administered Benzo(a)pyrene



Key: Solid circle, 100 ppm BaP; Square, 25 ppm BaP; Triangle, 5 ppm BaP; Open circle, 0 ppm BaP. Adapted from Figure 1 in Culp *et al.*, 1998.

Neoplasms

BaP administration resulted in statistically significant and dose related increases in adenomas or carcinomas of the forestomach, esophagus or tongue (Table 8). Liver tumors and lymphomas (combined mixed cell lymphomas, undifferentiated lymphomas, lymphocytic lymphomas and lymphomas) decreased with dose, probably a consequence of a shortening of lifespan at higher doses due to tumors at other sites. The incidence of squamous cell neoplasms of the tongue, esophagus, forestomach or larynx were combined as indicated by (McConnell *et al.*, (1986) (shown in Table 8). Forty-six of the 48 mice treated with 100 ppm of BaP displayed a tumor while 37 of 48 treated with 25 ppm BaP displayed a tumor at one or more of the sites. In mice treated with 5 ppm of BaP, three of 48 exhibited a tumor at the combined sites (Culp *et al.*, 1998). The combined incidence of tumors roughly paralleled those for forestomach tumors. All mice but one that developed a non-forestomach tumor also displayed a forestomach tumor (Table 8).

The study's author provided supplemental information on the tumors and causes of death. In many animals, multiple tumors were observed in the forestomach and often tumors had metastasized to other tissues. "Forestomach squamous epithelial neoplasm caused the death or contributed to the death of 22 mice in the 25 ppm group and 46 mice in the 100

ppm group" (supplemental data provided by Culp). None of the mice had multiple hepatic adenomas and "none died as the result of the neoplasm" (from supplemental data provided by Culp).

Table 8. Incidence of Adenomas or Carcinomas in Mice Administered Benzo(a)pyrene[a] (Culp *et al.*, 1998)

Site	0 ppm	5 ppm	25 ppm	100 ppm
Liver	2/48	7/48	5/47	0/45
Lung	5/48	0/48	4/45	0/48
Lymphomas	9/48	14/48	6/47	0/48
Forestomach[c]	1/48	3/47	36/46[b]	46/47[b]
Esophagus[c]	0/48	0/48	2/45	27/46[b]
Tongue[c]	0/48	0/48	2/46	23/48[b]
Larynx[c]	0/35	0/35	3/34	5/38
Combined: Forestomach, Esophagus, Tongue[c]	1/48	3/48	37/48 [b]	46/48 [b]

[a]Adenoma or carcinomas observed in tissue.

[b] Statistically significant, $p < 0.05$ (Kodell *et al.*, 1983).

[c]Test for trend, $P<0.05$ (Kodell *et al.*, 1983).

Toxicity

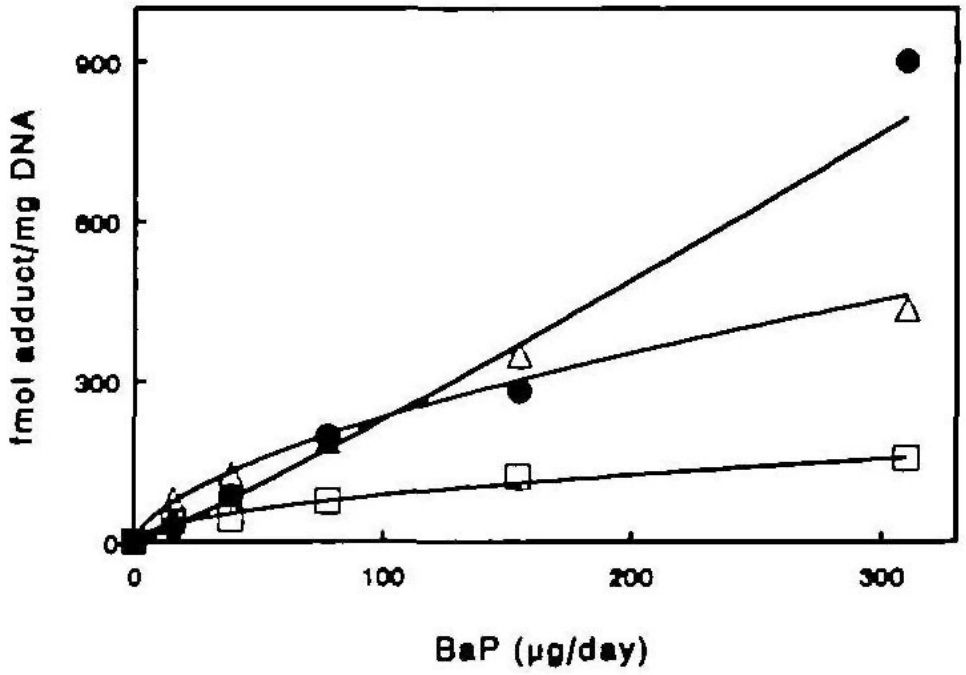
While mice in the higher dose groups (100 and 25 ppm) died early, it appears that the premature deaths in both treatment groups and the marked decrease in body weight in the 100 ppm group were a consequence of the tumors. Other effects that were reported included basal cell hyperplasia of the esophageal epithelium and epithelial hyperplasia, hyperkeratosis and micro abscesses of the forestomach (Pathology Associates, 1995). Few other lesions were reported. Given that cause of death and the other indicators of toxicity (weight loss, hyperplasia) appeared to be a consequence of the tumors, it does not appear that the maximum tolerated dose was exceeded in this study.

DNA Adducts

In a separate study, male $B6C3F_1$ mice were administered BaP (0, 0.016, 0.039, 0.078, 0.155 or 0.310 mg/day) in the diet for 21 days (Culp and Beland, 1994). This study in males employed lower doses than the Culp *et al.* (1998) two-year cancer bioassay in females (the high dose was half the high dose in the two-year cancer bioassay). DNA adducts were assayed in lung, liver and forestomach, and detected down to the lowest doses administered with no suggestion of a threshold for effect (Figure 5). At low doses, adduct formation in the liver and forestomach was essentially equivalent. Formation of adducts in the forestomach and lung appeared to plateau, perhaps indicating saturation, while adducts in the liver were linear with dose with no evidence of saturation. While

tumors were detected in the forestomach in the two year cancer bioassay, no statistically significant increases in tumors were detected in the lung or liver. However, adduct formation was measured in male mice while the bioassay was conducted in female mice, making direct comparison of adduct formation with tumor incidence problematic.

Figure 5. DNA Adduct Levels Following BaP Treatment in Male Mice

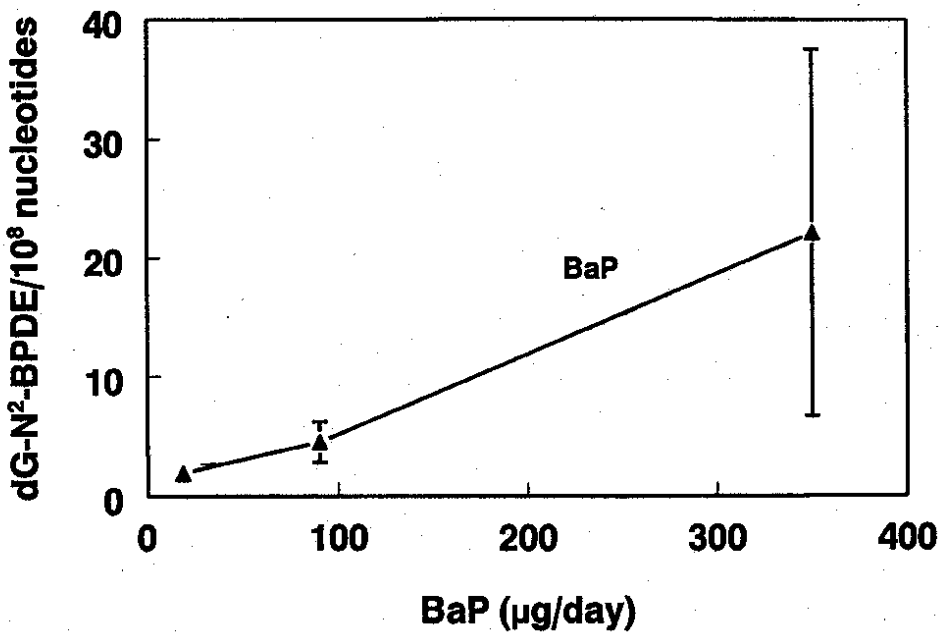


Key: circle-liver; square-lung; triangle–forestomach. From Culp and Beland, 1994.

In another study, investigators measured DNA adducts after BaP treatment in female mice (Culp *et al.*, 2000). Adduct formation in the forestomach was roughly linear with dose (Figure 6). There was no evidence of a threshold for effect nor a plateau (saturation) in the range of doses tested, which was similar to what was used in the male mouse adduct study. Adducts were not measured in lung or liver in mice receiving BaP treatment. Sixty eight percent of forestomach tumors sampled from animals treated with BaP displayed a K-*ras* mutation, about 43 percent in codon 12 and 62 percent in codon 13. Ten percent of the tumors from BaP treated animals displayed H-*ras* or *p*53 mutations.

BaP adducts were also measured (in only two animals per group) in the lung and forestomach of male and female mice administered BaP (50 ppm) in the diet for 94 or 185 days (Weyand *et al.*, 1994). Adduct levels in the forestomach were low in male and female mice. A modest elevation in adduct levels was observed in the lung of female but not male mice. Length of exposure had little effect.

Figure 6. DNA Adducts in Forestomach of Mice Treated with BaP



Amount of BaP consumed per day versus the concentration of dG-N^2-BPDE in the forestomachs of mice fed BaP (▲)

Adapted from Figure 3 of Culp *et al*., 2000.

Sensitivity of female mice

Culp and associates justified the testing of only female mice in their 1998 cancer bioassay based on "their low spontaneous liver tumors lifetime incidence" (Gaylor *et al.*, 2000). Background incidence, however, does not necessarily indicate that male mice are less sensitive than female mice.

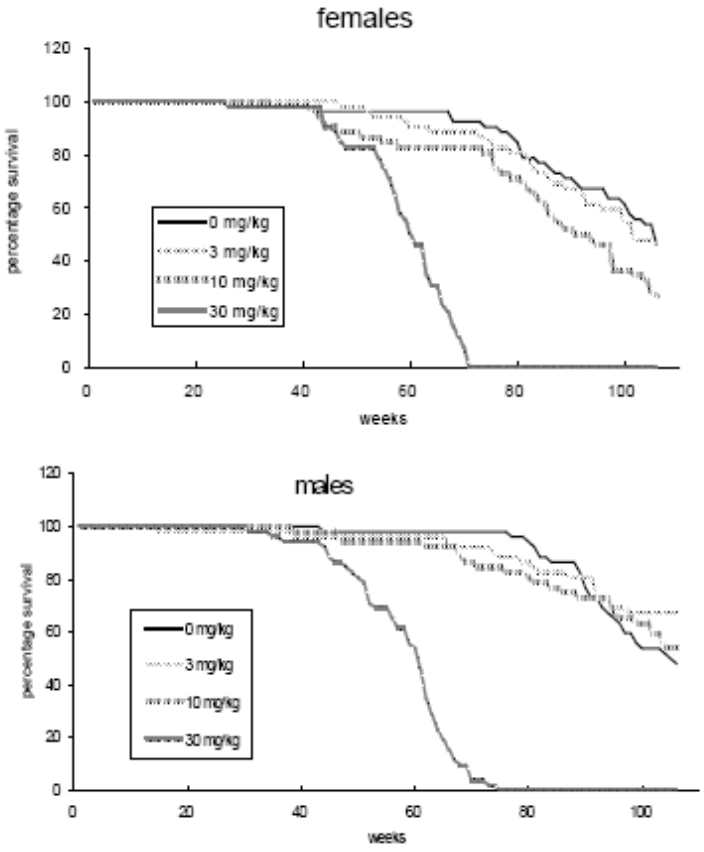
Studies of Singh *et al.* (1998) and Sharma *et al.* (1997) indicate that female mice may be more sensitive to the carcinogenic activity of BaP, particularly at lower doses. Female A/J mice administered three doses of BaP (0.5 or 1.5 mg/mouse) by gavage every 14 days displayed a higher tumor incidence at the lower dose and equivalent incidence at the higher dose compared to male mice (Singh *et al.*, 1998). The investigators detected higher glutathione-S-transferase isozymes levels in the liver, forestomach and lung of male mice, suggesting that at lower doses male mice may be conjugating (detoxifying) BaP more readily. At the high dose, detoxification of BaP may have become saturated, which may explain the similar incidence of tumors in male and female mice at the higher dose.

The incidence of lung tumors in CD-1 mice administered one dose of BaP (3 mg/mouse) by gavage was greater in female mice (14/26) compared to male mice (5/24) (Sharma *et al.*, 1997). Control male and female mice exhibited no lung tumors. Other studies by these investigators showed that glutathione-S-transferase activity in the liver of female mice was roughly half of that detected in male mice (Singh, 1998).

Kroese *et al.*, 2001. Groups of 52 male and female Riv:Tox Wistar rats were administered BaP (0, 3, 10 or 30 mg/kg by gavage, five days per week) for 106-109 weeks (Kroese *et al.*, 2001). Body weight, food and water consumption were monitored at regular intervals. Separate groups of 10 male and female rats were treated with BaP at the same doses and sacrificed after 90 days to assess the health status of the animals. DNA adducts were measured in separate groups of six male and female rats treated with BaP for four or five months. An additional low dose group of 0.1 mg/kg-day was included in the adduct study.

The survival of the animals was good (Figure 7). In the control group, the main cause of death was the occurrence of pituitary tumors. Early deaths in the high dose BaP male and female groups were mainly attributed to liver and forestomach tumors.

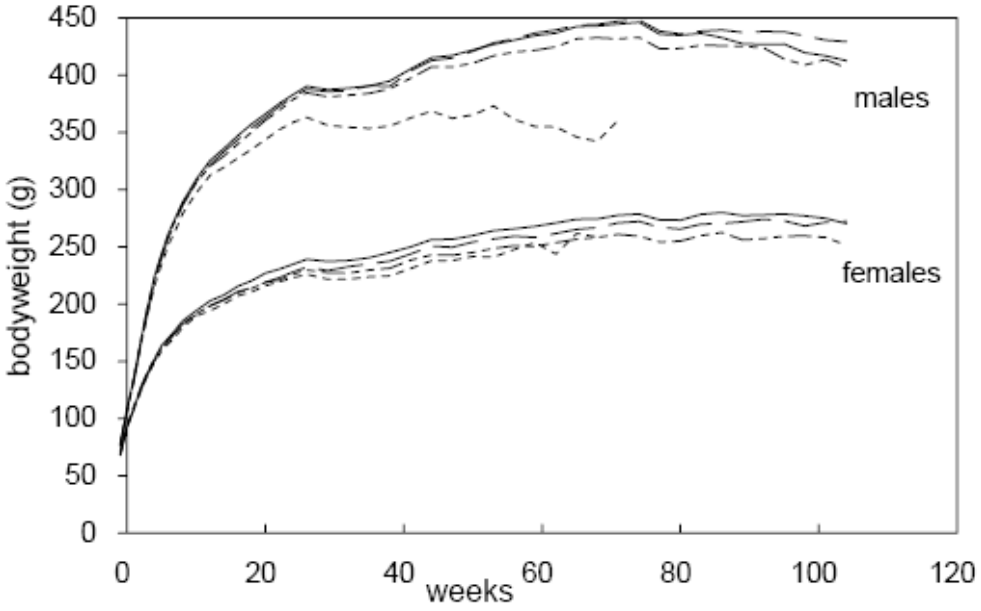
Figure 7. Survival of Wistar Rats Administered Benzo(a)pyrene



From Kroese *et al.*, 2001

Body weight and food consumption were not markedly affected by BaP treatment except in high dose males (Figures 8 and 9). Water consumption was elevated in the two highest dose groups in female rats and high dose males prior to sacrifice (data not shown).

Figure 8. Changes in Body Weight in Wistar Rats Administered Benzo(a)pyrene



Body weights of female and male rats during oral exposure to B[a]P at the indicated dose levels for 104 weeks. Control (—), 3 mg/kg bw (— —), 10 mg/kg bw (— – –), and 30 mg/kg bw (– – – –).

From Kroese *et al.*, 2001

Non-neoplastic lesions in male and female rats did not indicate substantial toxicity. Increased liver and decreased thymus weight were observed in animals treated with the high dose of BaP for three months. After ninety days of exposure, the epithelium of the forestomach in the high dose groups "demonstrated minimal changes that could be summarized as 'basal cell disturbance.'" Statistically significant and dose related increases in the incorporation of BrdU in the nuclei of forestomach epithelium of male and female rats were observed following 90 days of exposure to BaP. While some fatty vacuolation was observed in the livers of "a significant portion of both male and females," no compound related necrosis or inflammation was apparent. Serum enzyme levels did not indicate hepatocellular toxicity.

Maximum tolerated dose. The early death and decreased weight gain observed in the high dose group appeared to be a consequence of B(a)P-induced tumors. Otherwise, little toxicity was observed in the high dose group after ninety days of exposure. Therefore, there is little evidence that the maximum tolerated dose was exceeded in any of the dose groups in this study, and the induction of tumors should not be considered secondary to any frank (non-cancer) toxic effect.

Figure 9. Food Consumption in Wistar Rats Administered Benzo(a)pyrene

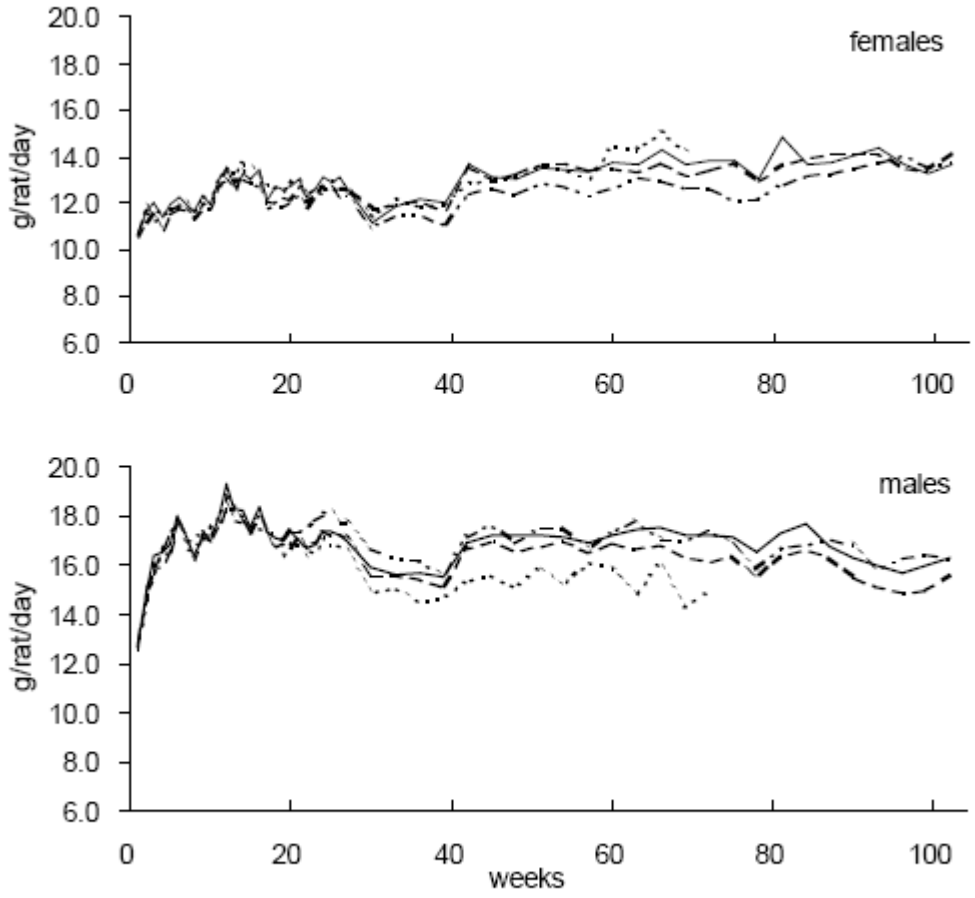


Figure 1. Food consumption of female and male rats during oral exposure to B[a]P at the indicated dose levels for 104 weeks. Control (——), 3 mg/kg bw (— —), 10 mg/kg bw (— -- —), and 30 mg/kg bw (----).

From Kroese *et al.*, 2001

Neoplasms

The administration of BaP to rats resulted in an increased incidence of tumors in a number of tissues. Marked, dose-related increases in liver and forestomach tumors were observed following chronic oral exposure to BaP, which had not been previously reported in the rat. Tumors of the small intestine or auditory canal had not previously been associated with benzo(a)pyrene administration to rodents.

The most prominent carcinogenic effects, dose-related increases in tumors of the forestomach and liver and dose-related decreases in tumors of the pituitary, were

observed in both male and female animals (Tables 9 and 10). When multiple tumor types (adenomas, carcinomas) in the liver or forestomach were observed, the investigators reported the most progressed lesion. The investigators described the scoring of liver tumors this way: "…Histologically only the most progressed lesion was scored, thereby encompassing overdiagnosing. Thus, since tumours were usually multiple, if carcinoma was scored, this implies the concomitant presence of adenomas and foci of cellular alteration." Consequently, in the liver, dose levels with a high incidence of carcinomas were characterized by a reduced incidence of adenomas. The investigators described the scoring of forestomach tumors as: "…the diagnosis of squamous cell carcinoma implicitly signifies the presence of both papilloma and basal cell hyperplasia, and the diagnosis of papilloma implicitly includes the presence of basal cell hyperplasia." Pituitary adenomas or carcinomas occurred with great frequency in the male and female control group. The frequency of pituitary tumors decreased with increasing dose of BaP. This was probably due to early death of the rats from liver or stomach tumors.

Table 9. Incidence of Tumors in Female Rats (Kroese *et al.*, 2001)

Site		**0 mg/kg**	**3 mg/kg**	**10 mg/kg**	**30 mg/kg**
Oral Cavity[a]	Papilloma	0/19	0/21	0/9	9/31 [c]
	Squamous cell carcinoma	1/19	0/21	0/9	9/31
Forestomach	Papilloma[b]	1/52	3/51	20/51[c]	25/52[c]
	Squamous[b] cell carcinoma	0/52	3/51	10/51[c]	25/52[c]
Combined: Oral cavity and Forestomach	Adenoma or Carcinoma[b]	2/52	6/51	30/51 [c]	50/52 [c]
Liver	Hepatocellular adenoma	0/52	2/52	7/52[c]	1/52
	Hepatocellular[b] carcinoma	0/52	0/52	32/52[c]	50/52[c]
	Carcinoma or adenoma[b]	0/52	2/52	39/52 [c]	51/52 [c]
Auditory Canal[a]	Papilloma	0/0	0/1	0/0	1/20
	Carcinoma	0/0	0/1	0/0	13/20
Pituitary	Adenoma	22/46	17/52	11/47	8/52 [c]
	Carcinoma	8/46	7/52	1/47 [c]	0/52 [c]

[a]Tissues that did not display abnormalities upon macroscopic examination were not examined for histopathology.
[b]Statistically significant (Mantel-Haenszel test for trend, $P<0.05$)
[c]Statistically significant (Fisher's Exact, $P<0.05$).

Tumors of the liver were most responsible for mortality and morbidity among BaP treated animals, according to the authors.

Table 10. Incidence of Tumors in Male Rats (Kroese *et al.*, 2001).

Site		**0 mg/kg**	**3 mg/kg**	**10 mg/kg**	**30 mg/kg**
Oral Cavity[a]	Papilloma[b]	0/24	0/24	2/37	10/38 [c]
	Squamous cell carcinoma[b]	1/24	0/24	5/37	11/38 [c]
Forestomach	Papilloma[b]	0/52	7/52[c]	18/52[c]	17/52[c]
	Squamous cell[b] carcinoma	0/52	1/52	25/52[c]	35/52[c]
Combined: Oral cavity and Forestomach	Adenoma or carcinoma[b]	1/52	8/52 [c]	45/52 [c]	52/52 [c]
Liver	Hepatocellular adenoma	0/52	3/52	15/52[c]	4/52
	Hepatocellular[b] carcinoma	0/52	1/52	23/52[c]	45/52[c]
	Carcinoma or adenoma[b]	0/52	4/52	37/52 [c]	49/52 [c]
Auditory Canal[a]	Adenoma	0/1	0/0	0/7	4/33
	Carcinoma	0/1	0/0	2/7	19/33
Pituitary	Adenoma[b]	29/51	29/52	20/51	1/50
	Carcinoma	3/51	2/52	3/51	0/50
Jejunum	Adenocarcinoma[b]	0/51	0/50	0/51	8/49[c]

[a]Tissues that did not display abnormalities upon macroscopic examination were not examined for histopathology
[b]Statistically significant (Mantel-Haenszel test for trend, $P<0.05$)
[c]Statistically significant (Fisher's Exact, $P<0.05$).

DNA Adducts

Separate groups of animals were administered BaP for four or five months in a study of DNA adduct formation. After four or five months of treatment, four major adducts were detected in a number of tissues. Adduct levels were comparable after four or five months, indicating levels had reached steady state. Interestingly, substantial adduct levels were detected in tissues where tumors occurred (liver, forestomach) as well as in tissues where no statistically significant increases in tumors were detected (lung, heart, kidney) suggesting that adducts were not involved in the etiology of the tumors.

However adduct formation only at specific DNA sites may be responsible for the occurrence of tumors in certain tissues. Adduct formation in the liver and forestomach was detected at the lowest dose (0.1 mg/kg-day), nearly 100 fold below the dose associated with statistically significant tumor increases in these tissues. This observation is consistent with a lack of threshold, or a threshold well below the doses associated with statistically significant increases in tumors from exposure to BaP in this study.

Adduct levels were determined in the liver and lung of male and female Wistar rats following the administration of BaP (0, 5, 50, 100 mg/kg) in the diet for up to 90 days (Ramesh and Knuckles, 2006). The strain of rat and the doses employed were similar to those in the Kroese *et al.* (2001) study. Adduct levels in the liver were similar in male rats receiving 50 or 100 mg/kg-day for 30 or 60 days. After 90 days, adduct levels were markedly elevated at the two highest doses, suggesting possible overwhelming of BaP detoxification or DNA repair mechanism(s). In female rats, liver adducts in the high dose group were markedly increased after 60 and 90 days and in the 50 mg/kg-day dose group after 90 days of exposure. Adduct levels in the two high dose groups were slightly higher in female rats than in males after 90 days of exposure. One explanation for these findings in both male and female rats is that at the highest BaP dose(s), detoxification may have become saturated (glutathione-S-transferase activity, particularly in the male rat) or that DNA repair capability may be exhausted. A similar pattern was observed in the lung, although adduct levels were considerably higher in the lung.

In a separate study, rats were treated with BaP (0, 5, 50, 100 mg/kg) for 90 days, the exposure stopped and then the animals were sacrificed up to thirty days later to evaluate the persistence of adducts (Ramesh and Knuckles, 2006). The persistence of adducts was similar in the lungs of male and female rats and the livers of male and female rats. However, in the liver, a more rapid decrease in adduct levels was observed in animals that received a higher dose of BaP.

Both the study of Ramesh and Knuckles (2006) and the study of Kroese *et al.* (2001) observed high levels of adducts in the liver and lung, but increased incidences of tumors were not observed in the lungs of Wistar rats by Kroese *et al.* (2001).

Inhalation

The administration of BaP (0, 2.2, 9.5 or 46.5 mg/m^3) condensed onto sodium chloride particulates by the inhalation route (nose only, restrained animals) to hamsters for up to 109 weeks resulted in a statistically significant dose-related increase in tumors of the larynx and pharynx (Thyssen *et al.,* 1981)**.** Tumors were also observed in the nasal cavity, trachea and forestomach, but no tumors were observed in the lung.

Percutaneous

The extensive effort focused on investigating BaP's carcinogenic activity associated with application to mouse skin has been reviewed elsewhere (ATSDR, 1995; WHO, 1998; IARC, 1983). Very briefly, the application of BaP to mouse skin has resulted in statistically significant increases in papillomas and/or carcinomas in multiple studies. BaP acts as an initiator and as a complete carcinogen (*i.e.*, no promoter is needed to produce significant increases in skin tumors). Certain mouse strains are more susceptible to tumors than others and the choice of solvent influences the incidence of tumors.

Other routes/models

Because BaP is a well-known carcinogen, it has been employed in a number of experimental models that involve different species, routes and duration of exposure to investigate its mechanism of action. As these studies have been reviewed elsewhere, the following is a very brief summary of the findings of some of the animal models used to study benzo(a)pyrene carcinogenesis (IARC, 1983; WHO, 1998; U.S. EPA, 1991).

Intratracheal instillation of BaP in hamsters and rats resulted in increased respiratory tumors. Subcutaneous injection of BaP induced localized sarcomas in rats, hamsters, guinea pigs and mice. Intraperitoneal injection of mice and rats with BaP resulted in localized tumors in mice and mammary and uterine tumors in rats. Intrabronchial implantation resulted in localized tumors in rats. Newborn mice that received BaP by subcutaneous or ip injection developed hepatic and lung tumors. Subcutaneous or intraperitoneal injection of pregnant mice resulted in an increased incidence of lung adenomas in their offspring.

The following review of possible age-related sensitivity to the carcinogenic effects of BaP is excerpted from Appendix C-1 of The Prioritization of Toxic Air Contaminants – Children's Environmental Health Protection Act, Final Report (OEHHA, 2001):

> Vesselinovitch *et al.* (1975) studied the carcinogenicity of benzo[*a*]pyrene in two hybrid strains of mice exposed by a single intraperitoneal injection at 1, 15 or 42 days old. Tumors were observed at several sites, and the relationship between tumor incidence and age at exposure varied from site to site. In the case of liver and lung tumors, young mice were more sensitive than older mice, showing both higher tumor incidence and shorter time before appearance of tumors. Incidence of tumors was generally higher for liver and lung tumors than for other sites.
>
> The strains used were C57BL/6J x C3HeB/FeJ F_1 ("B6C3F_1") and C3HeB/FeJ x A/J F_1 ("C3AF_1"); treated group sizes varied from 30 to 62 animals whereas control groups contained 98 to 100 animals. Doses of 75 or 150 μg benzo[*a*]pyrene were dissolved in trioctanoin. Control animals had low mortality; controls included two groups, in one of which survivors were necropsied at age 90 weeks and in the other survivors were necropsied at age 142 weeks. Incidences of all tumors in controls were low, except for lung tumors in the C3AF_1 mice (Males: 49/97 at 90 weeks; 60/100 at 142 weeks. Females: 26/100 at 90 weeks; 50/100 at 142 weeks). Treated animals were examined regularly for tumors throughout life; mortality immediately after dosing was virtually zero, but later survival was impacted by the appearance of lethal tumors.
>
> Results are presented in Table 11. For liver tumors in both strains, the incidence was greater, and the average age of tumor appearance was lower, in animals treated at 1 day than at 15 days. This trend continued when comparing those treated at 42 days. The authors judged these differences to be significant ($P < 0.01$) using the χ^2 test for incidence comparisons and Student's t-test for comparing averages at which tumors were detected at autopsy. Similar trends were observed for lung tumors, although the high background incidence of this tumor in the C3AF_1 mice reduced the extent and statistical significance of the differences.

Table 11. Incidence of Lung and Liver Tumors in Mice Treated with Benzo[*a*]pyrene at Various Ages

		Liver tumors				Lung tumors					
Dose:		**75 mg/kg**		**150 mg/kg**		**75 mg/kg**			**150 mg/kg**		
***Strain*/ Sex**	**Age when dosed[a] (days)**	**percent tumors[b]**	**Time to tumor[c] (weeks)**	**percent tumors[b]**	**Time to tumor[c] (weeks)**	**percent tumors[b]**	**Time to tumor[c] (weeks)**	**Multi-plicity[d]**	**percent tumors[b]**	**Time to tumor [c] (weeks)**	**Multi-plicity[d]**
B6C3F$_1$											
Males	1	55	86	81	81	43	103	3	59	84	4
	15	60	93	58	81	25	103	2	36	82	2
	42	13	108	9	87	36	119	2	38	95	2
Females	1	7	129	18	121	49	126	3	62	112	4
	15	7	116	7	90	33	122	2	40	101	3
	42	0		0		26	131	2	17	118	3
C3AF$_1$											
Males	1	34	80	46	69	93	78	6	92	70	8
	15	27	90	23	77	93	87	5	94	75	6
	42	0		3	79	93	91	5	87	85	6
Females	1	2	91	2	70	93	82	7	93	73	7
	15	2	102	2	62	94	98	5	91	79	6
	42	0		0		87	93	5	90	83	6

(from OEHHA, 2001; data from Vesselinovitch *et al.*, 1975)

a. Age (in days) at which animals received ip injections of BaP at the stated dose level, dissolved in trioctanoin.

b. Number of mice bearing liver or lung tumors / effective number exposed, expressed as a percentage.

c. Average age (in weeks) at which tumors were observed.

d. Average number of grossly visible lung tumors per whole lung.

Toxicological Effects in Humans

No studies were identified that specifically link exposure to BaP with adverse effects in humans. However, a number of studies, the subject of several reviews (ATSDR, 1995; IARC, 1983; WHO, 1998**)**, have investigated possible adverse effects associated with exposure to PAH mixtures containing BaP in occupational or environmental settings.

Acute/Subchronic Toxicity

No studies were located regarding death, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, neurological, reproductive, developmental, dermal or ocular effects in humans following specific inhalation, ingestion, or dermal exposure to BaP. No studies were located regarding acute and subchronic effects in humans (ATSDR, 1995).

Genetic Toxicity

Iron factory workers exposed to 0.5 to 20 ng/m^3 BaP had DNA adducts and exhibited increased mutation rates in peripheral lymphocytes (Perera *et al*., 1988, 1993). DNA adducts and urine metabolites had been detected at higher levels than in controls in Xuan Wei women exposed to 184 to 383 ng/m^3 of BaP (Mumford *et al*., 1993, 1995), and in workers in an electrode paste plant exposed to 900 ng/m^3 (Ovrebo *et al.,* 1994). BaP diol epoxide adducts with hemoglobin (Hb) were detected in newspaper vendors exposed to traffic exhaust in the city of Milan, Italy, in the summer of 1993. Adduct concentration was not different for low and high density traffic-exposed smokers. Among the nonsmokers, Hb adducts were detectable in 60 percent of high-exposure subjects and in 28 percent of those with low exposure (Pastorelli *et al*., 1996).

Chronic Toxicity

Following chronic inhalation exposure to particulate matter that contained BaP, respiratory adverse effects were observed in workers at a rubber factory (Gupta *et al.*, 1993, 1994). In Poland, male iron foundry workers chronically exposed to 200 to 500,000 ng/m^3 BaP in complex mixtures on top of the coke oven showed depressed serum immunoglobins (Szczeklik *et al*., 1994). In Austria, chronic exposure to 651 or 5,396 ng/m^3 BaP in PAH mixtures induced immunosuppressive effects in coke oven workers (Winker *et al*., 1997). Fetal Hb, found in men employed in a cooking plant of the steel mill "Huta Sendzimira" in Krakow, southern Poland and chronically exposed to BaP at concentrations from 900 to 388,900 ng/m^3, might be a marker of susceptibility to industrial pollutants (Stepniewski *et al*., 1996). Several biomarkers in plasma, blood and urine samples from coke oven workers were evaluated as indicators of exposure to PAHs (Ovrebo *et al*., 1995).

Carcinogenicity

The first instance of occupational cancer ever to be described (by Sir Percival Pott in 1775) was scrotal cancer in chimney sweeps who had been exposed since childhood to contact with soot from coal fires containing BaP and other PAHs. Epidemiological evidence suggests that workers intimately exposed to the products of combustion or distillation of bituminous coal containing BaP are at an increased risk for cancers of the skin, respiratory tract, bladder and kidney. Skin cancer in man is well known to have occurred following exposure to poorly refined lubricating and cutting oils containing BaP and PAHs (HSDB, 1997). Epidemiological studies have shown that machinists have an increased risk of lung cancer and bladder cancer, and a biomarker study indicates that dermal uptake of PAHs is a major route of exposure (Moen *et al*., 1996).

Epidemiological studies have shown increased mortality due to lung cancer in humans exposed to coke oven emissions, roofing-tar emissions, fuel pump emissions and cigarette smoke, all of which are likely to contain BaP. Excess risks of bladder cancer among aluminum smelter workers exposed to coal tar pitch involving BaP have been reported (ATSDR, 1995). Cancer risk due to occupational exposure to PAHs in Canada has been analyzed by Nadon *et al*. (1995), who concluded that BaP is associated with lung, bladder, esophagus, stomach, pancreas and prostate cancers in some workers.

Long-term iron and steel workers in Anshan, AnHwei Province, China, exhibited a 40 percent increased risk for lung and stomach cancers with exposure to complex mixtures including BaP levels from 840 to greater than 3,200 ng/m^3 (Xu *et al*., 1996). A man who had been exposed to BaP for three weeks while he was carrying out an experiment in mice had persistent nodules which were diagnosed as squamous epithelioma (reported in 1938 and reviewed in the IARC Monograph, 1983).

Mechanism of Action

Benzo(a)pyrene has been a focus of considerable research aimed at understanding the mechanism of action of carcinogenic agents. Because the findings of decades of research aimed at eliciting the mechanism of action of BaP have been the subject of a number of reviews (Conney *et al.*, 1994; WHO, 1998; ATSDR, 1995; Miller and Ramos, 2001), only a very brief synopsis of this research follows.

BaP requires metabolic activation to elicit its carcinogenic effects. Phase 1 metabolism, i.e., metabolism to active metabolites, occurs primarily via the mixed function oxidase system that involves a family of related enzymes, the cytochrome P450s. Metabolism occurs in the liver and also in most other tissues. The ability to metabolize BaP to an active metabolite and the ability to detoxify BaP (phase two metabolism) in animals is linked to the occurrence and incidence of cancer. The putative ultimate carcinogen is benzo(a)pyrene-7,8-diol-8,10 epoxide, which is highly susceptible to nucleophilic attack.

BaP is active as both an initiator and a complete carcinogen (*i.e.*, not requiring the application of a promoter) in skin painting studies. BaP is active in *in vivo* and *in vitro* mutagenicity bioassays, indicating a genotoxic mechanism of action. DNA adducts, particularly reactions associated with guanine, have been detected in animals treated with

BaP or in humans highly exposed to PAHs. Interestingly, in some studies, adducts are detected in tissues where tumors have not been observed.

Possible carcinogenesis mechanisms other than genotoxicity have also been investigated, including effects on cell proliferation, cell signaling, inhibition of the immune system and alterations in cell-to-cell communication.

Examination of Evidence of BaP Carcinogenicity

Animal studies

BaP has been evaluated in a number of animal bioassays using a variety of routes of exposure and experimental protocols. Statistically significant increases in tumors have been observed in male and female, immature and mature animals. Tumors have been observed in several tissues (lung, liver, forestomach and skin) and in a number of species (rats, mice, monkeys, hamsters). Statistically significant and often dose-related increases in tumors have been observed following the administration of BaP by the oral and inhalation route, by percutaneous application, by subcutaneous or intraperitoneal injection, and by installation into the trachea. Tumors have been observed following chronic or subchronic administration or after a few doses in certain protocols.

Human studies

Epidemiological studies have associated exposure to mixtures of PAHs with increases in cancer. Cigarette smoke, diesel exhaust, coke oven and roofing-tar emissions, all of which contain PAHs, have been identified as human carcinogens. Given that BaP is a prominent component of the PAH mixtures, these studies are considered suggestive of BaP carcinogenicity in humans. No studies are available which can specifically link human exposure to BaP with an increase in cancer because exposure always occurs to a mixture of PAHs.

Genotoxicity Studies

BaP displayed genotoxic activity in a host of *in vitro* and *in vivo* assays in bacteria and mammalian cells. Because it is generally recognized as a genotoxic agent, BaP is often employed as a positive control substance in genotoxicity bioassays. Numerous studies have demonstrated the formation of DNA adducts following BaP administration, further indicating a genotoxic mechanism.

Mechanism

Considerable effort has been focused on understanding the mechanism of action of BaP carcinogenesis. Most of the effort has focused on a genotoxic mechanism. BaP's metabolism is complex, involving a number of enzymes generating highly reactive metabolites that covalently link to DNA. Metabolism occurs in multiple tissues, and adducts have been identified in a number of tissues. Interestingly, high adduct levels

have also been detected in tissues where increases in tumors have not been observed. Investigators have attempted to understand whether specific adducts, and /or specific sites are linked to the occurrence of tumors. Studies have also investigated other mechanisms of action including stimulation of cell proliferation, suppression of the immune system, and changes in intercellular communication. The precise mechanism of action of BaP remains unclear.

Conclusion

Given the strong evidence of carcinogenic activity in experimental animals, suggestive evidence of carcinogenicity in humans, substantial evidence of genotoxicity, and substantial information concerning the possible mechanism(s) of action of BaP, it is prudent to conclude that BaP presents a carcinogenic risk if present in drinking water. Therefore, the PHG for this agent will be developed based on this toxic endpoint.

Sensitive Populations

The metabolism of BaP to active metabolites by several of the microsomal cytochrome P450 isoenzymes (principally CYP1A1 in the mouse liver) is believed to be a necessary condition for BaP carcinogenesis (Jerina *et al*, 1976; WHO, 1998). A population with higher cytochrome P450 isozyme activity that metabolizes BaP to an active metabolite would be anticipated to be a potentially sensitive population. Similarly, populations with increased ability to enzymatically inactivate reactive BaP metabolites by conjugation with sulfate, glutathione or glucuronic acid would be expected to be relatively insensitive.

CYP1A1 appears to be the enzyme most responsible for activating BaP to the putative ultimate carcinogen, benzo(a)pyrene-7,8-diol-9,10 epoxide. This enzyme is constitutively low in the mouse liver but is rapidly induced by various agents including BaP itself (Uno *et al.*, 2006). The recent cancer bioassays of Culp *et al.* (1998) and Kroese *et al.* (2001) were long-term rodent studies in which BaP administration would have been expected to induce its own metabolism. Therefore, observed increases in tumors in the mouse and the rat appear to have occurred in induced or "sensitive animal populations."

Humans are routinely exposed to many substances that induce the synthesis of mixed function oxidases. While differences in the rate of BaP metabolism by human liver *in vitro* have been reported (see review by Conney *et al.*, 1994), it is unclear whether there are specific human populations with increased ability to metabolize BaP to an active metabolite(s).

The bioassay in mice was only conducted in female mice (Culp *et al.*, 1998). Female mice appear to be a "sensitive population" because studies in mice have linked higher levels of tumors in female mice with a lower ability to conjugate BaP reactive metabolites when compared to male mice. Whether there are analogous sensitive human population(s) with reduced ability to conjugate reactive BaP metabolites is unclear.

There is very little information regarding possible sensitivity to early life exposure to BaP. Studies in which a single dose of BaP was administered by i.p. injection resulted in

a higher incidence of tumors in animals exposed an earlier age (Vesselinovitch *et al.*, 1975). These data are suggestive but not conclusive that younger animals are more sensitive to the chronic carcinogenic effects of BaP, given that the animals were exposed to a single large dose administered by injection.

Chemical Interactions

Benzo(a)pyrene is a member of a large class of structurally related toxicants, the polycyclic aromatic hydrocarbons, that occur in complex mixtures in the environment. Toxicological interactions among members of this class of toxicants and with other toxicants have been investigated (reviewed in ATSDR, 1995). What little that is known regarding the interactions indicates the interactions are complex.

Studies that investigated initiation or complete carcinogen activity following the application of BaP in combination with other PAHs (typically to mice skin) indicate that BaP's cancer potency was increased (Hoffman and Wynder, 1963; Van Duuren and Goldschmidt, 1976; Schmetz, 1978), decreased (Hoffman and Wynder, 1963; Schmetz, 1978, Falk *et al*., 1964) or unchanged (Roe, 1962; Falk *et al.*, 1964; Pfeiffer, 1977; Grant and Roe, 1963). Administration of complex mixtures of PAHs in combination with benzo(a)pyrene generally decreased or had little effect on the potency associated with BaP (Falk *et al.*, 1964).

DOSE-RESPONSE ASSESSMENT

Noncarcinogenic Effects

Several studies have investigated non-carcinogenic effects of BaP (Table 12). Knuckles and associates conducted both acute and subchronic studies of BaP in F-344 rats (Knuckles *et al.*, 2001). Adverse effects on the liver and white blood cells were observed in animals receiving a single dose of 100 mg/kg, the lowest dose employed in the acute study. Effects on white blood cells and liver were observed in male and female rats at doses as low as 50 mg/kg in subchronic studies (Knuckles *et al.*, 2001). Adverse effects on the kidney were reported at 5 mg/kg, the lowest dose administered in the subchronic study (Table 12).

Reduced fertility was observed following exposure of male and female mice to 10 mg/kg-day or greater *in utero* (Table 12) (MacKenzie and Angevine, 1981). Similar reductions in fertility of female NMRI mice were observed by Kristensen *et al.* (1995) after exposure *in utero* to 10 mg/kg-day BaP on days 7-16 of pregnancy. Immunotoxicity, indicated by reduced thymus and lymph node weights, was observed in male Wistar rats administered 10 mg/kg-day BaP (De Jong *et al.*, 1999). Neurotoxicity was observed in animals receiving one dose of 25 mg/kg-day or greater (Saunders *et al.*, 2002, 2006). Administration of BaP to rats for three months resulted in increased liver weight at 7 and 21 mg/kg-day and decreased thymus weight at 21 mg/kg-day (Kroese *et al.*, 2001). Studies in rats and mice revealed toxicity following subchronic exposure at doses as low as 5 mg/kg-day and a NOAEL for immunotoxicity of 3 mg/kg-day.

Table 12. Non-Carcinogenic Toxic Effects of BaP*

Study Length	Dose (mg/kg-day)	Endpoint	Toxic Effect	Reference
Subchronic	5	LOAEL	Renal toxicity	Knuckles *et al.*, 2001
Subchronic	10	LOAEL	Reproductive toxicity	MacKenzie and Angevine, 1981
Subchronic	10	LOAEL	Reproductive toxicity	Kristensen *et al*., 1995
Subchronic	3	NOAEL	Immunotoxicity	De Jong *et al.*, 1999
Acute	25	LOAEL	Neurotoxicity	Saunders *et al.*, 2006
Subchronic	7	LOAEL	Hepatotoxicity	Kroese *et al*., 2001

*Lowest dose where toxicity was observed/not-observed.

Carcinogenic Effects

Two well-conducted modern studies were judged best suited for deriving a dose-response relationship for BaP. One study was conducted in female mice (Culp *et al.*, 1998) and the other in male and female rats (Kroese *et al*., 2001). These studies were judged by OEHHA to be much higher in quality than the study of Neal and Rigdon (1967) that provided the basis of previous dose-response relationships derived by OEHHA (1993), Collins *et al.* (1991) and others (U.S. EPA, 2008). The study of Neal and Rigdon (1967) was deficient because combined groups of males and females were employed, the number of animals in each group was variable, BaP administration began at different ages, and treatment occurred for different time intervals.

Culp *et al*., 1998. The administration of BaP to female mice resulted in statistically significant and dose related increases in combined tumors of the forestomach, tongue, or esophagus (sites with statistically significant and marked increases in tumors), as well as at each of these sites individually (neoplasms were combined at these sites per McConnell *et al.*, 1986). At other sites (liver, lymphoma), the incidence of tumors in the high-dose groups were lower than in the lower-dose group(s). This probably was due to a shortened survival of the animals in the higher dose groups, which may have prevented the occurrence of late-developing tumors (although shortened survival would not alter the incidence of a tumor that caused the death of the animal). The incidence of late-occurring tumors would be expected to be influenced by marked decreases in survival.

Forestomach neoplasms occurred in nearly 100 percent of the animals that received 100 ppm of BaP. This finding complicates the development of a dose-response relationship because the notable shortening of lifespan, particularly in the high dose group, would be expected to influence the incidence of tumors at some sites. In addition, a nearly 100 percent response in the high dose group does not provide much information for developing a dose-response relationship.

Because of the differences in survival of the different dose groups and a near 100 percent tumor response in the high dose group, a time-to-tumor model would appear to be appropriate for estimating the potency of BaP. The time-to-tumor model is valuable because the model accounts for both how many tumors occurred and when they occurred. As discussed in The National Research Council Report, Drinking Water and Health, Volume 3 (1980), "…These response times permit the formulation of mathematical models that relate the dose level to the probability distribution of times to response. These models may then be used to estimate the expected number of responders in the population at risk at any time."

Culp and associates published and provided supplemental information describing the types of tumors that were observed and when they were detected in each animal. They also noted if a given tumor caused the death of the animal. These data allowed the specification of whether a given tumor was the cause of or incidental to the death of each animal, as required by the time-to-tumor model.

A multistage-in-dose Weibull-in-time model was employed using Tox_Risk v. 5.2, software to develop a dose-response relationship for combined incidence of tumors of the esophagus, forestomach or tongue**.** This model uses the formula below to determine the probability of tumor by time t as:

$$P(t,d) = 1 - \exp[-(q_0 + q_1 d + ... + q_j d^j)(t - t_0)^k]$$

with

$$q_i \geq 0, \text{ for all i, and } 0 \leq t_0 < t$$

where d is dose, t_0 is commonly interpreted as the latency period, and k is the age exponent. In this case, carcinogenic potency for animals is derived by applying a maximum likelihood modeling approach to estimate the parameters (q_i, t_0, and k) and generate the distribution of q_1. The animal cancer potency, q_{animal}, is defined as the upper 95 percent confidence bound on q_1 estimated at 104 weeks, the assumed standard lifetime for mice.

The dose in mice was scaled to humans based on the ratio of human and animal body weights to the ¾ power (30 g female mouse, 70 kg human).

The lower-bound estimate of the dose associated with a 10 percent increase in cancer (LED_{10}) was 0.059 mg/kg-day, based on lifetime (70 years) exposure (Table 13). An upper-bound estimate of cancer potency (q_1*) from Tox_Risk was 1.67 $(mg/kg\text{-}day)^{-1}$. The slope associated with a (LED_{10}) of 0.059 mg/kg-day is:

$$0.1 \div 0.059 \text{ mg/kg-day} = 1.7 \text{ (mg/kg-day)}^{-1}$$

For comparative purposes, a dose-response relationship for combined tumors at the three sites was also determined using a linearized multistage (LMS) model. The goodness of fit for combined tumors of the esophagus, forestomach, or tongue in the mouse was very poor ($p < 10^{-6}$). After the highest dose was removed the fit of the LMS model was acceptable, p = 0.8. The lower-bound estimate of the dose associated with a 10 percent cancer risk (LED_{10}) was 0.08 mg/kg-day, based on lifetime (70 years) exposure.

Table 13. The Cancer Potencies and Doses Associated with 10 Percent Incidence of Tumors in Mice and Rats Administered BaP[a]

	Male		Female	
	Liver	**Forestomach/oral cavity**	**Liver**	**Forestomach/oral cavity**
Mouse (Culp *et al*., 1998)				
Mean estimate (mg/kg-day)[b]				1.22
95 percent lower bound (mg/kg-day)[b]				0.059
q_1* (mg/kg-day)$^{-1}$				1.67
Rat (Kroese *et al.*, 2001)				
Mean estimate (mg/kg-day)[b]	0.63	0.48	0.63	0.62
95 percent lower bound (mg/kg-day)[b]	0.42	0.27	0.52	0.30
q_1* (mg/kg-day)$^{-1}$	0.21	0.36	0.10	0.33

[a]Results from time-to-tumor model.
[b]Dose associated with 10 percent increase in tumors.

Although the potency estimates of the two models are derived using different dose groups (time-to-tumor model was derived using all doses, while the LMS modeling only used the lowest three dose groups), the time-to-tumor model yielded a higher estimate of potency than the LMS model, as would be expected. The time-to-tumor model accounts for the early death in the animals that prevented the observation of late-occurring tumors.

Kroese *et al.*, 2001. The administration of BaP to male and female Wistar rats resulted in statistically significant, dose-related increases in liver tumors and combined tumors of the oral cavity or forestomach (per McConnell *et al.*, 1986, none of the esophageal tumors were epithelial). In high dose groups, nearly 100 percent of female and male rats exhibited both forestomach and liver tumors, indicating sufficient time to develop tumors at these sites despite a shortened lifespan mainly due to the occurrence of liver tumors.

Because of dose-related differences in survival and a near 100 percent tumor response rate in the two highest dose groups, a time-to-tumor model would appear to be useful for estimating BaP potency. The time-to-tumor model is valuable because it accounts for both how many tumors occurred and when they occurred in developing a dose-response relationship. Kroese and associates provided supplemental information on the types of tumors observed and when they were detected in each animal. They also noted if the tumor caused death of the animal. These data allowed the determination of whether a given tumor was the cause of or incidental to the death of each animal, as required by the time-to-tumor model.

The same type of multistage Weibull time-to-tumor model in Tox_Risk v. 5.2 as used for the Culp study was employed to develop a dose-response relationship for the incidence of

adenomas or carcinomas of the liver or combined oral or forestomach adenomas or carcinomas (Table 10)**.** The dose in animals was adjusted to reflect daily exposure to BaP (multiplied by 5/7) and scaled to humans based on the human and animal body weight ratio (rat 0.35 kg, human 70 kg) to the ¾ power. The q_1*s and lower bound estimates of the doses associated with a 10 percent incidence of liver and combined oral cavity or forestomach tumors from the time-to-tumor model are shown in Table 13.

The lower bound estimate of the dose associated with a 10 percent increase in tumors (0.3 mg/kg-day) is based on combined oral or forestomach tumors (LED_{10}) in male or female rats and is based on lifetime (70 years) exposure. The slope factor (q_1*) associated with an LED_{10} of 0.3 mg/kg-day is:

$$0.1 \div 0.3 \text{ mg/kg-day} = 0.33 \text{ (mg/kg-day)}^{-1}$$

For comparative purposes, a dose-response relationship for combined tumors of the oral cavity and forestomach in female rats was also determined using a linearized multistage (LMS) model. The lower-bound estimate of the dose associated with a 10 percent cancer risk (LED_{10}) was 1.3 mg/kg-day, based on lifetime (70 years) exposure. As expected, the LMS yielded a lower estimate of potency than the time-to-tumor model. A dose-response relationship for combined tumors of the oral cavity and forestomach in male rats was also determined using a linearized multistage (LMS) model. Because of an unacceptable fit, the highest dose was removed, whereupon the model yielded an acceptable fit. The lower-bound estimate of the dose associated with a 10 percent cancer risk (LED_{10}) was 1.5 mg/kg-day, based on lifetime (70 years) exposure.

Cancer potency is corrected by an Age Sensitivity Factor (ASF), as defined earlier (OEHHA, 2009). The procedure for application of cancer potency factors has been revised to take into account information which suggests that children can be especially susceptible to carcinogens (OEHHA, 2001). Weighting factors are utilized to calculate cancer risks from exposures of infants, children and adolescents, to reflect their anticipated special sensitivity to carcinogens. Cancer risk is weighted by a factor of 10 for exposures that occur from the third trimester of pregnancy to <2 years of age, and by a factor of 3 for exposures that occur from ≥2 years through <16 years of age. This approach applies to all carcinogens, regardless of purported mechanism of action, unless chemical-specific data exist that could be used to make more specific adjustments to risk.

The increase in risk associated with using the ASF is derived by adjusting the cancer slope factor, which increases the 10^{-6} risk associated with a lifetime exposure to a carcinogen to 1.7×10^{-6}, as follows:

Risk	*ASF*	*Duration*	*Risk*[a]
R (third trimester to age <2 yrs)	10	2.25/70	3.2×10^{-7}
R (age ≥2 to age <16 yrs)	3	14/70	6.0×10^{-7}
R (age ≥16 to 70 yrs)	1	54/70	7.7×10^{-7}
Lifetime Risk			1.7×10^{-6}

[a] $=10^{-6}$ x duration

Using the ASF, the slope factors described above are adjusted by multiplying by 1.7 as follows:

Slope factor (mouse) $= 1.7\ (mg/kg\text{-}day)^{-1} \times 1.7 = 2.9\ (mg/kg\text{-}day)^{-1}$

Slope factor (rat) $= 0.33\ (mg/kg\text{-}day)^{-1} \times 1.7 = 0.56\ (mg/kg\text{-}day)^{-1}$

CALCULATION OF PHG

Noncarcinogenic Effects

For estimation of a health-protective concentration of a chemical in drinking water, an acceptable daily dose of the chemical from all sources will first be calculated. This involves incorporation of appropriate estimates of uncertainty in the extrapolation of the critical toxic dose from human or animal studies to the estimation of a lifetime daily dose that is unlikely to result in any toxic effects. For this purpose, the following equation will be used:

$$ADD = \frac{\text{NOAEL/LOAEL in mg/kg-day}}{UF}$$

where,

ADD = an estimate of the maximum daily dose which can be consumed by humans for an entire lifetime without toxic effects;

NOAEL/LOAEL = no-observed-adverse-effect level or lowest-observed-adverse-effect level in the critical study;

UF = combined uncertainty factor, often composed of factors of 10 for extrapolation from a LOAEL to a NOAEL, for extrapolation from a less-than-lifetime study, for interspecies extrapolation, and for variability among humans, limited to a maximum of 3,000.

Calculation of a public health-protective concentration (C, in mg/L) for a chemical in drinking water uses the following equation for noncarcinogenic endpoints:

$$C = \frac{\text{ADD mg/kg-day x RSC}}{\text{L/kg-day}}$$

RSC = relative source contribution (usually 20 to 80 percent, 0.20 to 0.80);

L/kg-day = volume of daily water consumption, the upper 95th percentile of water consumption for the relevant population group(s) (U.S. EPA, 2004).

For calculation of ADD, the LOAEL of 5 mg/kg-day based on renal toxicity (Knuckles *et al.*, 2001) is chosen for risk assessment because it is the lowest LOAEL. Supporting studies include that of De Jong *et al.* (1999) with a subchronic LOAEL of 10 mg/kg-day and a NOAEL of 3 mg/kg-day based on immunotoxicity, and the subchronic LOAEL of 7 mg/kg-day of Kroese *et al*. (2001) based on hepatotoxicity (see Table 12). The combined uncertainty factor can be composed of factors of 10 for extrapolation from a LOAEL to a NOAEL, 10 for a less-than-lifetime study, 10 for interspecies extrapolation, and 10 for variability among humans; it is then limited to 3,000. Thus,

$$\text{ADD} = \frac{5 \text{ mg/kg-day}}{3{,}000} = 0.0017 \text{ mg/kg-day}$$

To estimate the health-protective concentration for non-carcinogenic effects, an RSC is utilized which acknowledges that drinking water is a very small source of BaP, as described above in the environmental occurrence section. Therefore RSC has been set at 0.1 in this case. For drinking water consumption, the general population upper 95th percentile value from U.S. EPA (2004) is used. Thus,

$$C = \frac{0.0017 \text{ mg/kg-day} \times 0.1}{0.044 \text{ L/kg-day}} = 0.004 \text{ mg/L (4 } \mu\text{g/L or 4 ppb)}$$

Based on these calculations, the health-protective level for non-cancer effects is estimated to be 4 ppb.

Carcinogenic Effects

Because BaP is lipophilic, having relatively low water solubility, it associates with particulate matter in ambient water. As such, little BaP is available for dermal absorption or will partition into the air during showering. Therefore, the estimated health protective concentration (C) is based on exposure to BaP due to drinking water ingestion only. The health-protective concentration (C), for oral exposure to a carcinogen is derived using the following general equation:

$$C = \frac{R}{P_{oral} \text{ (mg/kg-day)}^{-1} \times L_{ingest}/\text{kg-day}}$$

where:

R = *de minimis* lifetime excess individual cancer risk (a default of 10^{-6} or one additional tumor in 1 million people exposed to BaP for their lifetimes);

P_{oral} = oral cancer potency $(\text{mg/kg-day})^{-1}$;

L_{ingest}/day = daily amount of water ingested (L/kg-day), based on the upper 95th percentile exposures for the populations at risk.

Drinking water exposure is estimated for this calculation for the post-natal age ranges used above in the Dose Response section for the cancer potency correction for early life exposures. The drinking water consumption values utilized are the upper 95th percentile values estimated by OEHHA (2010), which are adjusted for exposure duration by multiplying the consumption values by the fraction of a lifetime and summing the products, as follows:

Age range (years)	Duration correction factor	Upper 95th percentile (L/kg-day)	Exposure contribution (L/kg-day)
0-<2	2/70	0.196	0.0056
≥2-<16	14/70	0.061	0.0122
≥16-70	54/70	0.045	0.0347
		Weighted Sum:	0.0525

Tumors of the gastrointestinal tract occurred in both mice and rats given BaP orally, but the compound was more potent in the mouse (slope factor of 2.9 $(mg/kg\text{-}day)^{-1}$) than in the rat (slope factor 0.56 $(mg/kg\text{-}day)^{-1}$. Therefore the slope factor of 2.9 $(mg/kg\text{-}day)^{-1}$, which is more health protective, was employed to derive the PHG. The resulting equation is as follows:

$$C = \frac{10^{-6}}{2.9\ (mg/kg\text{-}day)^{-1} \times 0.0525\ L/kg\text{-}day}$$

$$= 0.007 \times 10^{-3}\ mg/L = 0.007\ \mu g/L\ (ppb)$$

The PHG for BaP is therefore calculated to be 0.007 x10^{-3} mg/L, or 0.007 μg/L or ppb, representing a lifetime cancer risk of one in one million. Other toxic effects associated with BaP were observed at much higher exposure levels. A PHG for BaP that is protective of carcinogenic effects will be protective against these other toxic effects.

Given the lack of specific information regarding populations that might be especially sensitive to toxic effects of BaP and utilizing the correction and exposure factors for early-in-life exposures, the PHG of 0.007 ppb is considered protective of the entire population against lifetime exposure to BaP. Levels of BaP in drinking water associated with a lifetime theoretical extra cancer risk of 10^{-4} or 10^{-5} are 0.7 and 0.07 ppb, respectively.

RISK CHARACTERIZATION

The PHG for BaP of 0.007 μg/L or ppb is based on risk associated with the ingestion of BaP in drinking water. Various sources of uncertainty regarding the development of health-protective criteria are discussed.

Hazard Identification

There is extensive evidence that exposure to BaP results in increased incidences of cancer in experimental animals. Two recent cancer bioassays in which BaP was administered by the oral route revealed statistically significant dose-related increases of tumors in the oral cavity/forestomach of female mice and the liver and oral cavity/forestomach of male and female rats (Culp *et al.*, 1998; Kroese *et al*., 2001).

BaP is positive in genotoxicity assays and DNA adducts have been detected in various tissues in rodents following exposure to BaP. Human exposures to PAHs have been linked to increases in cancer and DNA adducts. The metabolism of BaP has been extensively studied. BaP metabolism generates highly reactive metabolites that covalently link to DNA. Metabolism occurs in a number of tissues.

Taken together, these data provide sufficient reason for concern regarding the carcinogenic potential of this toxicant in humans.

Recent studies have investigated non-carcinogenic effects of BaP. Reproductive, developmental, immunotoxic and neurotoxic effects have been observed by various investigators. Therefore a health protective concentration is also derived for BaP based on non-carcinogenic effects.

Dose response – non-cancer endpoint

Recent studies that have investigated non-carcinogenic adverse effects of BaP were judged to be sufficient to develop dose response relationships, namely the identification of a NOAEL or a LOAEL for BaP. Only studies where the compound was administered by the oral route were utilized. The lowest LOAEL was for renal toxicity at 5 mg/kg-day in the subchronic study of Knuckles *et al.* (2001). There were two other similarly low LOAELs, for hepatotoxicity at 7 mg/kg-day in the study of Kroese *et al*. (2001) and for immunotoxicity at 10 mg/kg-day in the study of De Jong *et al.* (1999). Because the lowest dose studied resulted in toxic effects in both the study of Knuckles *et al.* (2001) and Kroese *et al.* (2001), a NOAEL must be extrapolated. A 10-fold factor is customarily applied for extrapolation to a NOAEL from the LOAEL, although 3-fold might be used if the endpoint is judged to be of marginal toxicological significance. In this case the endpoint of renal toxicity, supported by toxic effects on other organs or tissues at similar doses in two other studies, a factor of 10 seems most appropriate to be applied to the LOAEL of Knuckles *et al.* (2001). The aggregate uncertainty factor was limited to 3,000, which is generally considered the maximum value, based on recommendations of California's Risk Assessment Advisory Committee (1996) and the U.S. EPA (2002).

Dose Response – cancer endpoint

Oral exposure - The available human studies provided insufficient information to develop a dose-response relationship for BaP. The findings of Kroese *et al*. (2001) and Culp *et al.* (1998) yielded sufficient information to develop dose-response relationships for BaP. In the male and female rat, marked statistically significant increases in tumors were observed in the liver and forestomach/oral cavity. A time-to-tumor model was employed to develop dose response relationships for tumors at both sites in both sexes.

In the female mouse, marked statistically significant increases in tumors of the forestomach were observed. A time-to-tumor model was also employed to develop a dose-response relationship for tumors of the forestomach/oral cavity of the mouse. The resulting potency was similar in male and female rats and female mice. The findings in female mice were employed to develop the PHG because they yielded a slightly higher, more health-protective cancer potency estimate.

Exposure Assessment

The estimated upper 95th percentile daily community water consumption value for the general population (whole-life) recently derived by the U.S. EPA (2004) was chosen for the non-cancer calculation because this exposure is not broken down into critical life stages, for the chronic effects presumed.

The cancer risk assessment, on the other hand, incorporates available information concerning altered sensitivity to cancer-causing chemicals with life stage (OEHHA, 2009), so this risk assessment also includes a specific calculation for the drinking water consumption at the corresponding age ranges (OEHHA, 2010). No sensitive populations have been identified for any specific effects. The contribution of exposure routes other than oral was judged minimal for BaP in drinking water, given the low volatility of the compound and its propensity to associate with particulates, which will limit inhalation and dermal exposures and absorption.

The non-cancer health-based criterion also reflects a relative source contribution (RSC) of 10 percent of the total exposure coming from drinking water, because (in non-smokers) the predominant exposure to BaP is from the diet. Inhalation of BaP and other PAHs by smokers further decreases their RSC from drinking water. While OEHHA generally uses the defaults of 0.2 to 0.8 recommended by the U.S. EPA (2000), we consider it more appropriate to use an alternative value when the data support it. WHO (2003) recommends a default RSC of 0.1 and a value of 0.01 for highly lipophilic chemicals (i.e., chlordane, diethylhexyl phthalate, and lindane) but this is a matter of policy, not strictly data-driven. We think that the low value of 0.01 is not realistic in this case.

RSC is not used in the cancer calculation because the cancer risk is calculated as “extra” risk, which would be in addition to other sources of exposure to the chemical.

Risk Characterization

The various sources of uncertainty attendant in the hazard identification, dose response, and exposure assessment are reflected in the estimates of risk for BaP. As better studies aimed at understanding the mechanism(s) of the carcinogenicity of BaP, better methods to characterize the dose-response relationship, and better methods to characterize exposure become available, the uncertainties associated with the risk assessment can be reduced. This is the major reason for the difference between the PHG calculated in the current BaP risk assessment and the earlier PHG (OEHHA, 1997).

With the presently available information, the risk associated with exposure to BaP may have been under- or overestimated. To address this uncertainty, the risk assessment

utilized an upper-bound estimate of potency in the development of the health-based criteria, to ensure that risk is not markedly underestimated.

No sensitive populations were identified, although a correction factor was incorporated to adjust for early-in-life exposures. OEHHA believes that pregnant women and their fetuses, infants, the elderly, and other potentially sensitive populations are adequately protected by the revised PHG.

OTHER REGULATORY STANDARDS

OEHHA developed a PHG for BaP of 4 ppt (0.004 ppb) in 1997 (OEHHA, 1997; Collins *et al*, 1991). This PHG published in 1997 was based on the findings of the study of Neal and Rigdon (1967), a marginal study at best, as discussed in the animal toxicity/cancer section of this document. The California MCL for BaP is 0.2 ppb, established in 1994 based upon the U.S. EPA MCL, also 0.2 ppb, established in 1992.

BaP has been classified as a Group B2 probable human carcinogen by the U.S. EPA (U.S. EPA, 2008; IRIS, last updated 11/01/1994). The International Agency for Research on Cancer (IARC) has classified BaP as a Group 1 carcinogen, "carcinogenic to humans." In toxicology laboratory testing, BaP has been used as a positive control for mutagenicity and carcinogenicity and usually is administered at a single dose level, which makes the quantitation of cancer risk difficult. The U.S. Department of Health and Human Services (DHHS) has also determined that BaP is a known animal carcinogen. Under DHHS, ATSDR has prepared toxicological profiles for BaP (ATSDR, 1990a) and PAHs (ATSDR, 1990b, 1995) under the Comprehensive Environmental Response, Compensation and Liability Act of 1980 (CERCLA) since BaP is one of the 54 PAHs which have been identified in at least 600 of the National Priorities List hazardous waste sites. The World Health Organization (WHO) has published drinking water guidelines for BaP of 7, 0.7 and 0.07 ppb (or 70 ppt) corresponding to theoretical excess lifetime individual cancer risks of 10^{-4}, 10^{-5} and 10^{-6}, respectively, for stomach tumors (WHO, 1996) based on the Neal and Rigdon (1967) feeding study in mice. The risk quantification estimate was prepared by Clement Associates (1990) for the U.S. EPA (1991b).

In addition to the drinking water standards, U.S. EPA has provided estimates of levels of total cancer-causing PAHs including BaP in lakes and streams associated with a risk of human cancer development (U.S. EPA, 1980; ATSDR, 1995). U.S. EPA must be notified within a 24-hour period of time if one pound or more of BaP is released to the environment. BaP levels in various kinds of food also have been regulated or monitored worldwide. The Netherlands National Institute of Public Health and the Environment has developed maximum permissible concentrations for BaP of 0.05 ppb (0.05 μg/L or 50 ppt) in water, 0.26 mg/kg in soil, and 2.7 mg/kg in sediment as Environmental Quality Objectives (Kalf *et al*., 1997).

For a work environment involving exposure to BaP, the U.S. National Institute for Occupational Safety and Health (NIOSH) has established a recommended occupational exposure limit, time-weighted average (REL-TWA) for coal tar products of 0.1 mg of PAHs per cubic meter of air (0.1 mg/m^3) for a 10-hour work day within a 40-hour work

week, due to the risks of lung and skin cancer in workers. The American Conference of Governmental Industrial Hygienists (ACGIH) has, since 1991, recommended an occupational exposure limit for coal tar products of 0.2 mg/m^3 for an eight-hour work day within a 40-hour work week. In 1993, the Occupational Safety and Health Administration (OSHA) established a legally enforceable limit of 0.2 mg/m^3 averaged over an eight-hour exposure period. The OSHA Permissible Exposure Limit (PEL) for mineral oil mist, which has an IARC classification of 1 for sufficient evidence of carcinogenicity in humans, has been 5 mg/m^3 averaged over an eight-hour exposure period. NIOSH has established an REL-TWA for mineral oil mist of 5 mg/m^3 for a 10-hour work day, 40-hour work week, with a 10 mg/m^3 short-term exposure limit (STEL) (ATSDR, 1995).

REFERENCES

ATSDR (1990a). Toxicological Profile for Benzo(a)pyrene. Prepared by ICF-Clement under Contract No. 68-02-4235 for ATSDR, in collaboration with the U.S. EPA and Oak Ridge National Laboratory. Agency for Toxic Substances and Disease Registry, U.S. Public Health Service, Atlanta, GA. ATSDR/TP-88/05.

ATSDR (1990b). Toxicological Profile for Polycyclic Aromatic Hydrocarbons. Prepared by Clement International Corp for the ATSDR. Agency for Toxic Substances and Disease Registry, U.S. Public Health Service, Atlanta, GA. ATSDR/TP-90/20.

ATSDR (1995). Toxicological Profile for Polycyclic Aromatic Hydrocarbons (PAHs) (Update). Prepared by Research Triangle Institute for ATSDR. Agency for Toxic Substances and Disease Registry, U.S. Public Health Service, Atlanta, GA.

Aldrich (1996). Benzo(a)pyrene. Aldrich Catalog Handbook of Fine Chemicals. Aldrich Chemical Company, Milwaukee, Wisconsin.

Allen JO, Dookeran NM, Smith KA, Sarofim AF, Taghizadeh K, Lafleur AL (1996). Measurement of polycyclic aromatic hydrocarbons associated with size-segregated atmospheric aerosols in Massachusetts. Environ Sci Technol 30(3):1023-31.

Borgert CJ, Roberts SM, Harbison RD, James RC (1995). Influence of soil half-life on risk assessment of carcinogens. Regul Toxicol Pharmacol 22:143-51.

Bouchard M, Viau C (1997). Urinary excretion of benzo[a]pyrene metabolites following intravenous, oral, and cutaneous benzo[a]pyrene administration. Can J Physiol Pharmacol 75:185-92.

Brune H, Deutsch-Wenzel RP, Habs M, Ivankovic S, Schmahl D (1981). Investigation of the tumorigenic response to benzo(a)pyrene in aqueous caffeine solution applied orally to Sprague-Dawley rats. J Cancer Res Clin Oncol 102:153-7.

Buckley TJ, Lioy PJ (1992). An examination of the time course from human dietary exposure to polycyclic aromatic hydrocarbons to urinary elimination of 1-hydroxypyrene. Br J Ind Med 49(2):113-24.

Buckley TJ, Waldman, JM, Dhara R, Greenberg, A, Ouyang Z, Lioy PJ (1995). An assessment of a urinary biomarker for total human environmental exposure to benzo(a)pyrene. Int Arch Occup Environ Health 67(4):257-66.

Clement Associates (1990). Ingestion dose-response model to benzo(a)pyrene. Report prepared by TW Thorslund of Clement International Corp, Fairfax, VA, for the Office of Health and Environmental Assessment, U.S. Environmental Protection Agency, Washington, D.C., under EPA Contract No. 68-02-4601.

Collins JF, Brown JP, Dawson SV, Marty MA (1991). Risk assessment for benzo(a)pyrene. Regul Toxicol Pharmacol 13:170-84.

Conney AH, Chang RL, Jerina DM, Wei SJ (1994). Studies on the metabolism of benzo[a]pyrene and dose-dependent differences in the mutagenic profile of its ultimate carcinogenic metabolite. Drug Metab Rev 26:125-63.

Culp SJ, Beland FA (1994). Comparison of DNA adduct formation in mice fed coal tar or benzo[a]pyrene. Carcinogenesis 15:247-52.

Culp SJ, Gaylor DW, Sheldon WG, Goldstein LS, Beland FA. (1998). A comparison of the tumors induced by coal tar and benzo[a]pyrene in a 2-year bioassay. Carcinogenesis 19:117-24.

Culp SJ, Warbritton AR, Smith BA, Li EE, Beland FA (2000). DNA adduct measurements, cell proliferation and tumor mutation induction in relation to tumor formation in B6C3F1 mice fed coal tar or benzo[a]pyrene. Carcinogenesis 21:1433-40.

De Jong WH, Kroese ED, Vos JG, Van Loveren H (1999). Detection of immunotoxicity of benzo[a]pyrene in a subacute toxicity study after oral exposure in rats. Toxicol Sci 50:214-20.

De Vos RH, van Dokkum W, Schouten A, de Jong-Berkhout P (1990). Polycyclic aromatic hydrocarbons in Dutch total diet samples (1984-1986). Food Chem Toxicol 28(4):263-8.

Environment Canada (1991). Toxic Chemicals in the Great Lakes and Associated Effects: Vol I. Contaminant Levels and Trends. Dept of Fisheries and Oceans, Environment Canada, Health and Welfare Canada.

Falk HL, Kotin P, Thompson S (1964). Inhibition of carcinogenesis. The effect of hydrocarbons and related compounds. Arch Environ Health 9:169-79.

Feunekes FD. Jongeneelen, FJ, Laan HV, Schoonhof FHG (1997). Uptake of polycyclic aromatic hydrocarbons among trainers in a fire-fighting training facility. Am Ind Hyg Assoc J 58:23-8.

Foth H, Kahl R, Kahl GF (1988). Pharmacokinetics of low doses of benzo[a]pyrene in the rat. Food Chem Toxicol 26:45-51.

Foth H, Molliere M, Kahl R, Jahnchen E, Kahl GF (1984). Covalent binding of benzo(a)pyrene in perfused rat lung following systemic and intratracheal administration. Drug Metab Dispos 12:760-6.

Garcia Falcon MS, Gonzalez Amigo S, Lage Yusty MA, Lopez de Alda Villaizan MJ, Simal Lozano J (1996). Enrichment of benzo(a)pyrene in smoked food products and determination by high-performance liquid chromatography-fluorescence detection. J Chromatogr A 753:207-15.

Gaylor DW, Culp SJ, Goldstein LS, Beland FA (2000). Cancer risk estimation for mixtures of coal tars and benzo(a)pyrene. Risk Anal 20:81-5.

Gelboin HV (1980). Benzo[alpha]pyrene metabolism, activation and carcinogenesis: role and regulation of mixed-function oxidases and related enzymes. Physiol Rev 60:1107-66.

Grant G, Roe FJC (1963). The effect of phenanthrene on tumour induction by 3,4-benzopyrene administration to newly born mice. Brit J Cancer 17:261-5.

Guillen MD, Sopelana P, Cid C, Partearroyo MA (1996). Presence of polycyclic aromatic hydrocarbons in the foods that form part of the diet of different European countries. Alimentaria 34(278):41-7.

Gundersen DT, Kristanto SW, Curtis LR, Al-Yakoob SN, Metwally MM, Al-Ajmi D (1996). Subacute toxicity of the water-soluble fraction of Kuwait crude oil and partially combusted crude oil on Menidia beryllina and Palaemonetes pugio. Arch Environ Contam Toxicol 31:1-8.

Han, CZ, Guo Y, Jing JX, Chao HW, Lee C, Miao L, Ma XL, Chow X (1995). A study on the relationship between malignant tumor mortality and environmental pollution in Beicun countryside of Datong City. Chung Hua Liu Hsin Ping Hsueh Tsa Chih (China Environmental Epidemiology Journal) 16(2):101-4.

Hicks HE (1996). The Great Lakes: A historical overview. Toxicol Ind Health 12(2/3):303-13.

Hoffmann D, Wynder EL (1963). Studies on gasoline engine exhaust. J Air Pollut Control Assoc 13:322-7.

Holladay SD, Smith BJ (1994). Fetal hematopoietic alterations after maternal exposure to benzo[a]pyrene: a cytometric evaluation. J Toxicol Environ Health 42:259-73.

HSDB (1997). Benzo(a)pyrene. Hazardous Substances Data Bank, National Library of Medicine, National Toxicology Program, Bethesda, Maryland.

IARC (1983). Polynuclear Aromatic Compounds. Part. 1. Chemical, Environmental and Experimental Data. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Vol 32. International Agency for Research on Cancer, World Health Organization, Lyon, France.

IARC (2008). Air Pollution, Part 1, Some Non-heterocyclic Polycyclic Aromatic Hydrocarbons and Some Related Industrial Exposures. Vol. 92. International Agency for Research on Cancer, World Health Organization, Lyon, France.

Jenkins, BM, Jones AD, Turn SQ, Williams RB (1996). Particle concentrations, gas-particle partitioning and species intercorrelations for polycyclic aromatic hydrocarbons (PAHs) emitted during biomass burning. Atmos Environ 30(22):3825-35.

Jerina DM, Lehr RE, Yagi H, Hernandez O *et al.* (1976). Mutagenicity of benzo *[a]*pyrene derivatives and the description of a quantum mechanical model which predicts the ease of carbonium ion formation from diol epoxides. In: *In vitro* Metabolic Activation in Mutagenesis Testing. De Serres FJ, Fouts JR, Bend JR, ed. New York, Elsevier North Holland, pp 159-78.

Kalf DF, Crommentuijn T, van de Plassche EJ (1997). Environmental quality objectives for 10 polycyclic aromatic hydrocarbons (PAHs). Ecotoxicol Environ Safety 36:89-97.

Kao J, Patterson FK, Hall J (1985). Skin penetration and metabolism of topically applied chemicals in six mammalian species, including man: an in vitro study with benzo[a]pyrene and testosterone. Toxicol Appl Pharmacol 81:502-16.

Karl H, Leinemann M (1996). Determination of polycyclic aromatic hydrocarbons in smoked fishery products from different smoking kilns. Z Lebensm Unters Forsch 202:458-64.

Khesina AY, Kolyadich MN, Krivosheeva LV, Sokol'sksya NN, Shcherbak NP, Levinskii SS (1996). Assessment of pollution of the Moscow city air with carcinogenic polycyclic aromatic hydrocarbons (PAHs) and N-nitrosamines. Ekaperimental'naya Onkologiya (Russia Experimental Oncology, Kiev) 18(1):14-8.

Knuckles ME, Inyang F, Ramesh A (2001). Acute and subchronic oral toxicities of benzo[a]pyrene in F-344 rats. Toxicol Sci 61:382-8.

Kodell RL, Haskin MG, Shaw GW, Gaylor DS (1983) CHRONIC: A SAS procedure for statistical analysis of carcinogenesis studies. J Statist Comput Simul 16:287-310.

Kristensen P, Eilertsen E, Einarsdottir E, Haugen A, Skaug V, Ovrebo S (1995). Fertility in mice after prenatal exposure to benzo[a]pyrene and inorganic lead. Environ Health Perspect 103:588-90.

Kroese E, Muller JJA, Mohn GR, Dortant PM, Wester PW (2001). Tumorigenic effects in Wistar rats orally administered benzo(a)pyrene for two years (gavage studies). Implication for human cancer risk associated with oral exposure to polycyclic aromatic hydrocarbons. Report No 6658603 010. RIVM, Bilthoven, Netherlands.

Kuljukka T, Vaaranrinta R, Veidebaum T, Sorsa M, Peltonen K (1996). Exposure to polycyclic aromatic hydrocarbon (PAH) compounds among cookery workers in the oil shale industry. Environ Health Perspect 104(Suppl 3):539-41.

MacKenzie KM, Angevine DM (1981). Infertility in mice exposed in utero to benzo(a)pyrene. Biol Reprod 24:183-91.

McConnell EE, Solleveld HA, Swenberg JA, Boorman GA (1986). Guidelines for combining neoplasms for evaluation of rodent carcinogenesis studies. J Natl Cancer Inst 76:283-9.

Miller KP, Ramos KS (2001). Impact of cellular metabolism on the biological effects of benzo[a]pyrene and related hydrocarbons. Drug Metab Rev 33:1-35.

Moir D, Viau A, Chu I, Withey J, McMullen E (1998). Pharmacokinetics of benzo[a]pyrene in the rat. J Toxicol Environ Health A 53:507-30.

Moody RP, Nadeau B, Chu I (1995). In vivo and in vitro dermal absorption of benzo[a]pyrene in rat, guinea pig, human and tissue-cultured skin. J Dermatol Sci 9:48-58.

Mumford JL, Li X, Hu F, Lu XB, Chuang JC (1995). Human exposure and dosimetry of polycyclic aromatic hydrocarbons in urine from Xuan Wei, China with high lung cancer mortality associated with exposure to unvented coal smoke. Carcinogenesis 16(12):3031-6.

Mumford JL, Li X, Lewtas J, Young TL, Santella RM (1993). DNA adducts as biomarkers for assessing exposure to polycyclic aromatic hydrocarbons in tissues from Xuan Wei women with high exposure to coal combustion and high lung cancer mortality. Environ Health Perspect 99:83-7.

Neal J, Rigdon RH (1967). Gastric tumors in mice fed benzo(a)pyrene: a quantitative study. Texas Rep Biol Med 25:553-7.

Ng KM, Chu I, Bronaugh RL, Franklin CA, Somers DA (1992). Percutaneous absorption and metabolism of pyrene, benzo[a]pyrene, and di(2-ethylhexyl) phthalate: comparison of in vitro and in vivo results in the hairless guinea pig. Toxicol Appl Pharmacol 115:216-23.

Nielsen T (1996). Traffic contribution of polycyclic aromatic hydrocarbons in the center of a large city. Atmos Environ 30(20):3481-90.

NRC (1980). Drinking Water and Health, Vol 3. National Research Council, National Academy of Sciences. National Academy Press, Washington, D.C.

NRC (1982). Drinking Water and Health, Vol 4. National Research Council, National Academy of Sciences. National Academy Press, Washington, D.C.

OEHHA (1993). Benzo(a)pyrene as a Toxic Air Contaminant, Part B, Health Assessment: Health Effects of Benzo(a)pyrene. Prepared by JF Collins and GV Alexeeff, Office of Environmental Health Hazard Assessment for the Air Resources Board, California Environmental Protection Agency, Sacramento, CA.

OEHHA (1997). Benzo(a)pyrene. Public Health Goals for Chemicals in Drinking Water. Office of Environmental Health Hazard Assessment, California Environmental Protection Agency, Berkeley and Sacramento, CA.

OEHHA (2001). Prioritization of Toxic Air Contaminants Under the Children's Environmental Health Protection Act. Final Report. Office of Environmental Health Hazard Assessment, California Environmental Protection Agency, Sacramento, CA. Accessed at: http://www.oehha.ca.gov/air/toxic_contaminants/pdf_zip/SB25 percent20TAC percent20prioritization.pdf.

OEHHA (2009). Air Toxics Hot Spots Risk Assessment Guidelines Part II: Technical Support Document for Cancer Potency Factors. (May 2009). Office of Environmental Health Hazard Assessment, California Environmental Protection Agency, Sacramento, CA. Accessed at: http://www.oehha.ca.gov/air/hot_spots/tsd052909.html.

OEHHA (2010). Water Intake Exposure Pathway, Chapter 8, Exposure Assessment and Stochastic Analysis - DRAFT. Air Toxics Hot Spots Program Risk Assessment Guidelines. Office of Environmental Health Hazard Assessment, Oakland, CA.

Ovrebo S, Haugen A, Fjeldstad, PE, Hemminki K, Szyfter K (1994). Biological monitoring of exposure to polycyclic aromatic hydrocarbons in an electrode paste plant. J Occup Med 36(3):303-10.

Pastorelli R, Restano J, Guanci M, Maramonte M, Magagnotti C, Allevi R, Lauri D, Fanelli R, Airoldi L (1996). Hemoglobin adducts of benzo(a)pyrene diol epoxide in newspaper vendors: association with traffic exhaust. Carcinogenesis 17(11):2389-94.

Pathology Associates (1995). Pathology Report. Chronic Bioassay of Two Composite Samples from Selected Manufactured Gas Plant Waste Sites, National Center for Toxicology Research, Experiment 6722.02.

Petry T, Schmid P, Schlatter C (1996). Airborne exposure to polycyclic aromatic hydrocarbons (PAHs) and urinary excretion of 1-hydroxypyrene of carbon anode plant workers. Ann Occup Hyg 40(3):345-57.

Pfannhauser W (1991). Polycyclic aromatic hydrocarbons (PAH) in food and on selected samples of vegetables in Austria. Mitt Gebiete Lebensm Hyg 82:66-79.

Pfeiffer EH (1977). Oncogenic Interaction of Carcinogenic and Non-carcinogenic Polycyclic Aromatic Hydrocarbons in Mice. In: Air Pollution and Cancer in Man. Mohr U, Schmahl D, Tomatis L, Eds. Proc. Second Hanover International Carcinogenesis Meeting. IARC Sci Publication No. 16, Lyon, France.

Pupin AM, Toledo MCF (1996). Benzo(a)pyrene in Brazilian vegetable oils. Food Addit Contam 13(6):639-46.

Rahman A, Barrowman JA, Rahimtula A (1986). The influence of bile on the bioavailability of polynuclear aromatic hydrocarbons from the rat intestine. Can J Physiol Pharmacol 64:1214-8.

Ramesh A, Knuckles ME (2006). Dose-dependent benzo(a)pyrene [B(a)P]-DNA adduct levels and persistence in F-344 rats following subchronic dietary exposure to B(a)P. Cancer Lett 240:268-78.

Ramesh A, Greenwood M, Inyang F, Hood DB (2001a). Toxicokinetics of inhaled benzo[a]pyrene: plasma and lung bioavailability. Inhal Toxicol 13:533-55.

Ramesh A, Inyang F, Hood DB, Archibong AE, Knuckles ME, Nyanda AM (2001b). Metabolism, bioavailability, and toxicokinetics of benzo(alpha)pyrene in F-344 rats following oral administration. Exp Toxicol Pathol 53:275-90.

Risk Assessment Advisory Committee (1996). A Review of the California Environmental Protection Agency's Risk Assessment Practices, Policies, and Guidelines. Office of Environmental Health Hazard Assessment, California Environmental Protection Agency, Sacramento, California.

Rodriguez JW, Kirlin WG, Wirsiy YG, Matheravidathu S, Hodge TW, Urso P (1999). Maternal exposure to benzo[a]pyrene alters development of T lymphocytes in offspring. Immunopharmacol Immunotoxicol 21:379-96.

Roe FJC (1962). Effect of phenanthrene on tumor-initiation by 3,4-benzopyrene. Brit J Cancer 16:503-6.

Roth RA, Vinegar A (1990). Action by the lungs on circulating xenobiotic agents, with a case study of physiologically based pharmacokinetic modeling of benzo(a)pyrene disposition. Pharmacol Therap 48:143-55.

Sanders CL, Skinner C, Gelman RA (1986). Percutaneous absorption of 7,10 ^{14}C-benzo[a]pyrene and 7,12 ^{14}C-dimethylbenz[a]anthracene in mice. J Environ Pathol Toxicol Oncol 7:25-34.

Santella RM, Hemminki K, Tang DL, Paik M, Ottman R, Young TL, Savela K, Vodickova L, Dickey C, Whyatt R, Perera FP (1993). Polycyclic aromatic hydrocarbon-DNA adducts in white blood cells and urinary 1-hydroxypyrene in foundry workers. Cancer Epidemiol Biomarkers Prev 2:59-62, January-February.

Santodonato J, Howard P, Basu D (1981). Health and ecological assessment of polynuclear aromatic hydrocarbons. J Environ Pathol Toxicol 5:1-376.

Saunders CR, Das SK, Ramesh A, Shockley DC, Mukherjee S (2006). Benzo(a)pyrene-induced acute neurotoxicity in the F-344 rat: role of oxidative stress. J Appl Toxicol 26:427-38.

Saunders CR, Ramesh A, Shockley DC (2002). Modulation of neurotoxic behavior in F-344 rats by temporal disposition of benzo(a)pyrene. Toxicol Lett 129:33-45.

Schauer JJ, Rogge WF, Hildemann LM, Mazurek MA, Cass GR (1996). Source apportionment of airborne particulate matter using organic compounds as tracers. Atmospheric Environ 30(22):3837-55.

Schmetz I, Tosk J, Hilfrich J, Hirota D, Hoffman D, Wynder EL (1978). Bioassay of naphthalene and alkylnaphthalenes for co-carcinogenic activity. Relation to tobacco carcinogenesis. In: Carcinogenesis, Vol. 3. PW Jones, RI Freudenthal, eds. Raven Press, New York.

Sharma R, Haque AK, Awasthi S, Singh SV, Piper JT, Awasthi YC (1997). Differential carcinogenicity of benzo[a]pyrene in male and female CD-1 mouse lung. J Toxicol Environ Health 52:45-62.

Sheu HL, Lee WJ, Tsai JH, Fan YC, Su CC, Chao HR (1996). Particle size distribution of polycyclic aromatic hydrocarbons in the ambient air of a traffic intersection. J Environ Sci Health Part A, 31(6):1293-316.

Shum S, Jensen NM, Nebert DW (1979). The murine Ah locus: in utero toxicity and teratogenesis associated with genetic differences in benzo[a]pyrene metabolism. Teratology 20:365-76.

Simoneit BR, Sheng G, Chen X, Fu J, Zhang J, Xu Y (1991). Molecular marker study of extractable organic matter in aerosols from urban areas of China. Atmospheric Environ 25A(10):2111-29.

Singh SV, Benson PJ, Hu X, Pal A, Xia H, Srivastava SK, Awasthi S, Zaren HA, Orchard JL, Awasthi YC (1998). Gender-related differences in susceptibility of A/J mouse to benzo[a]pyrene-induced pulmonary and forestomach tumorigenesis. Cancer Lett 128:197-204.

Smith DJT, Harrison RM, Luhana L, Pio AA, Castro LM, Tariq MN, Hayat S, Quraishi T (1996). Concentrations of particulate airborne polycyclic aromatic hydrocarbons and metals collected in Lahore, Pakistan. Atmos Environ 30(23):4031-40.

Soontjens CD, Holmberg K, Westerholm RN, Rafter JJ (1997). Characterization of polycyclic aromatic compounds in diesel exhaust particulate extract responsible for aryl hydrocarbon receptor activity. Atmos Environ 31(2):219-25.

Storm JE, Collier SW, Stewart RF, Bronaugh RL (1990). Metabolism of xenobiotics during percutaneous penetration: role of absorption rate and cutaneous enzyme activity. Fundam Appl Toxicol 15:132-41.

Thyssen J, Althoff J, Kimmerle G, Mohr U (1981). Inhalation studies with benzo[a]pyrene in Syrian golden hamsters. J Natl Cancer Inst 66:575-7.

Tiefenbacher K, Pfannhauser W, Woidich H (1982). Investigation on contamination of food by polycyclic aromatic hydrocarbons. In: Recent Developments in Food Analysis, Proc First European Conf on Food Chemistry, Vienna, Austria, Feb 17-20, 1981, B Baltes, PB Czedik-Eysenberg, W Pfannhauser, eds. Verlag Chemie, Deerfield Beach, FL, pp. 76-82.

Tremolada P, Burnett V, Calamari D, Jones KC (1996). Spatial distribution of PAHs in the U.K. atmosphere using pine needles. Environ Sci Technol 30:3570-7.

U.S. EPA (1980). Ambient Water Quality Criteria for Polycyclic Aromatic Hydrocarbons, October, 1980. Office of Water Regulations and Standards, Criteria and Standards Division, U.S. Environmental Protection Agency, Washington, D.C. EPA-440/5-80-069.

U.S. EPA (1982). Aquatic Fate Process Data for Organic Priority Pollutants. Prepared by WR Mabey, JH Smith, RT Podoll, *et al*. Office of Water Regulations and Standards, U.S. Environmental Protection Agency, Washington, D.C. EPA-440/4-81-014.

U.S. EPA (1985). An Exposure and Risk Assessment for Benzo(a)pyrene and Other Polycyclic Aromatic Hydrocarbons. Vol. IV. Prepared by J Perwak, *et al*., AD Little, Inc., Cambridge, MA, October, 1982. Monitoring and Data Support Division, Office of Water Regulations and Standards, U.S. Environmental Protection Agency, Washington, D.C. EPA/440/4-85-020-V4.

U.S. EPA (1991). Drinking Water Criteria Document for Polycyclic Aromatic Hydrocarbons (PAHs), Final Report, December, 1991. Environmental Criteria and Assessment Office, Office of Health and Environmental Assessment, U.S. Environmental Protection Agency, Washington, D.C. EPA/600/X-92/015.

U.S. EPA (1997). 40 CFR Parts 141 and 142, Drinking Water Monitoring Requirements for Certain Chemical Contaminants - Chemical Monitoring Reform (CMR) and Permanent Monitoring Relief (PMR); Proposed Rule. U.S. Environmental Protection Agency. Fed Reg 62, 128, pp. 36100-36. Thursday, July 3.

U.S. EPA (2000). Methodology for deriving ambient water quality criteria for the protection of human health. Office of Water, U.S. Environmental Protection Agency, Washington, D.C. EPA-822-B-00-004, Oct., 2000.

U.S. EPA (2002). A review of the reference dose and reference concentration processes. Final Report. Prepared by the Risk Assessment Forum for U.S. Environmental Protection Agency, Washington, D.C. EPA/630/)-02/002F, December 2002. Accessed at: http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=55365.

U.S. EPA (2004). Estimated Per Capita Water Ingestion and Body Weight in the United States - An Update. U.S. Environmental Protection Agency, Washington, D.C. EPA-822-R-00-001.

U.S. EPA (2008). Benzo [a] pyrene (BaP) (CASRN 50-32-8). (Last updated 11/01/1994) Integrated Risk Information System, U.S. Environmental Protection Agency, Washington, D.C. Accessed at: www.epa.gov/ncea/iris/subst/0136.htm.

Uno S, Dalton TP, Dragin N, Curran CP, Derkenne S, Miller ML, Shertzer HG, Gonzalez FJ, Nebert DW (2006). Oral benzo[a]pyrene in Cyp1 knockout mouse lines: CYP1A1 important in detoxication, CYP1B1 metabolism required for immune damage independent of total-body burden and clearance rate. Molec Pharmacol 69:1103-14.

Urso P, Gengozian N (1982). Alterations in the humoral immune response and tumor frequencies in mice exposed to benzo[a]pyrene and X-rays before or after birth. J Toxicol Environ Health 10:817-35.

Urso P, Johnson RA (1988). Quantitative and functional change in T cells of primiparous mice following injection of benzo(a)pyrene at the second trimester of pregnancy. Immunopharmacol Immunotoxicol 10:195-217.

Urso P, Zhang W, Cobb JR (1992). Immunological consequences from exposure to benzo(a)pyrene during pregnancy. Scan J Immunol 11:203-6.

Vaessen, HAMG, Jekel AA, Wilbers AAMM (1988). Dietary intake of polycyclic aromatic hydrocarbons. Toxicol Environ Chem 16:281-94.

van de Wiel JA, Fijneman PH, Duijf CM, Anzion RB, Theuws JL, Bos RP (1993). Excretion of benzo[a]pyrene and metabolites in urine and feces of rats: influence of route of administration, sex and long-term ethanol treatment. Toxicology 80:103-15.

Van Duuren BL, Goldschmidt BM (1976). Cocarcinogenic and tumor-promoting agents in tobacco carcinogenesis. J Natl Cancer Inst 56:1237-42.

Verschueren K (1983). Handbook of Data on Organic Chemicals, 2nd ed. Van Nostrand Reinhold Co., New York, NY.

Vesselinovitch SD, Kyriazis AP, Mihailovich N, Rao KV (1975). Conditions modifying development of tumors in mice at various sites by benzo(a)pyrene. Cancer Res 35:2948-53.

Weast RC, ed. (1987). CRC Handbook of Chemistry and Physics, 68th ed. CRC Press, Boca Raton, FL.

Wester RC, Maibach HI, Bucks DA, Sedik L, Melendres J, Liao C, DiZio S (1990). Percutaneous absorption of [^{14}C]DDT and [^{14}C]benzo[a]pyrene from soil. Fundam Appl Toxicol 15:510-6.

Weyand EH, Bevan DR (1986). Benzo(a)pyrene disposition and metabolism in rats following intratracheal instillation. Cancer Res 46:5655-61.

Weyand EH, Chen YC, Wu Y, Koganti A, Dunsford HA, Rodriguez LV (1995). Differences in the tumorigenic activity of a pure hydrocarbon and a complex mixture following ingestion: benzo[a]pyrene vs. manufactured gas plant residue. Chem Res Toxicol 8:949-54.

Weyand EH, Wu Y, Patel S, Goldstein L (1994). Biochemical effects of manufactured gas plant residue following ingestion by B6C3F1 mice. J Toxicol Environ Health 42:89-107.

WHO (1996). Polycyclic Aromatic Hydrocarbons. Guidelines for Drinking Water Quality, Vol 2, Health Criteria and Other Supporting Information, pp. 495-506.

International Programme on Chemical Safety, World Health Organization, Geneva, Switzerland.

WHO (1998). Selected non-Heterocyclic Polycyclic Aromatic Hydrocarbons. Environmental Health Criteria 202. International Programme on Chemical Safety, World Health Organization, Geneva, Switzerland.

WHO (2003). Guidelines for drinking water quality. World Health Organization, Geneva, Switzerland. Accessed at: www.who.int/docstore/water_sanitation_health/GDWO/draftchemicals/list.htm.

Wiersma DA, Roth RA (1983). Total body clearance of circulating benzo(a)pyrene in conscious rats: effect of pretreatment with 3-methylcholanthrene and the role of liver and lung. J Pharmacol Exp Ther 226:661-7.

Williams ML, Maynard RL (1997). Exposure to benzo(a)pyrene. Lancet 349:652.

Xu Z, Brown LM, Pan GW, Liu TF, Gao GS, Stone BJ, Cao RM, Guan DX, Sheng JH, Yan ZS, Dosemeci M, Fraumeni JF, Blot WJ (1996). Cancer risks among iron and steel workers in Anshan, China, part II: Case-control studies of lung and stomach cancer. Am J Ind Med 30:7-15.

Yang JJ, Roy TA, Mackerer CR (1986). Percutaneous absorption of benzo[a]pyrene in the rat: comparison of in vivo and in vitro results. Toxicol Indust Health 2:409-16.

Yang JJ, Roy TA, Krueger AJ, Neil W, Mackerer CR (1989). In vitro and in vivo percutaneous absorption of benzo[a]pyrene from petroleum crude-fortified soil in the rat. Bull Environ Contam Toxicol 43:207-14.

Zhang J, Smith KR (1996). Hydrocarbon emissions and health risks from cookstoves in developing countries. J Expo Anal Environ Epidemiol 6(2):147-61.

Zhang X, Wang F, Xie T (1995). Detection of carcinogens in source drinking water in stomach cancer prevalent areas of Zanhuang County. Chung Hua Yu Fang I Hsueh Tsa Chih (China Preventive Medicine Journal) 29(3):149-52.

Zheng M, Wan TSM, Fang M, Wang F (1997). Characterization of the non-volatile organic compounds in the aerosols of Hong Kong - identification, abundance and origin. Atmos Environ 31(2):227-37.