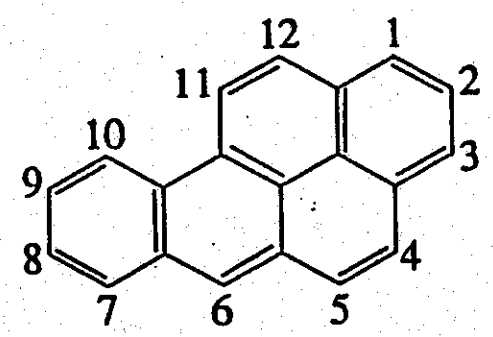
California Environmental Protection Agency

Air Resources Board

Benzo[a]pyrene

as a Toxic Air Contaminant



Part B
Health Assessment

July 1994

Health Effects of Benzo(a)pyrene

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Office of Environmental Health Hazard Assessment
California Environmental Protection Agency

PART B

HEALTH EFFECTS OF BENZO(a)PYRENE

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1. SUMMARY

The health effects of benzo(a)pyrene have been reviewed and evaluated to determine if benzo(a)pyrene may be a toxic air contaminant as defined by California Health and Safety Code Section 39655. At ambient temperatures benzo(a)pyrene is present largely in particulate form. Once absorbed, benzo(a)pyrene is rapidly distributed throughout the body. Acute and chronic exposure leads to reproductive system toxicity and bone marrow toxicity. At current ambient levels of benzo(a)pyrene, however, no acute or noncarcinogenic chronic effects are expected.

Benzo(a)pyrene has the ability through its metabolites to arylate DNA (form DNA adducts), causes gene mutations in both prokaryotic and eukaryotic cells, induces sister chromatid exchanges in mammalian cells, and produces unscheduled DNA synthesis in mammalian cells. Several types of tumors have been induced in rodents by benzo(a)pyrene. Feeding induces tumors of the stomach, topical application induces skin tumors, and both inhalation exposure and intratracheal instillation induce respiratory tract tumors. The steepest dose-dependent response has been seen for gastric tumors in mice. Available epidemiologic studies of people occupationally exposed to benzo(a)pyrene are not usable for risk assessment. In these studies the benzo(a)pyrene was only one component of a mixture of carcinogenic and noncarcinogenic polycyclic aromatic hydrocarbons and therefore a precise exposure assessment specific to benzo(a)pyrene was not made. This coupled with confounding exposures complicate the analysis and interpretation of the epidemiological data.

The International Agency for Research on Cancer (IARC) has concluded that there is sufficient evidence for the carcinogenicity of benzo(a)pyrene in experimental animals; IARC found no adequate data to evaluate the carcinogenicity in humans. Overall, based on both the animal and human data, IARC considers that benzo(a)pyrene is probably carcinogenic in humans (class 2A). Using classification criteria based on weight of evidence, the USEPA considers benzo(a)pyrene a possible human carcinogen (Group B2), with sufficient evidence of animal carcinogenicity and inadequate human evidence. OEHHA staff concurs with these conclusions. In addition, OEHHA staff has found no evidence for a carcinogenic threshold level for benzo(a)pyrene.

The OEHHA staff recommends that the range of risks for ambient exposures to benzo(a)pyrene be based on the upper 95% confidence limits predicted from fitting a multistage model to the two best animal data sets, gastric tumors induced in mice by feeding benzo(a)pyrene and respiratory tract tumors induced in hamsters by inhalation of benzo(a)pyrene. The excess theoretical lifetime cancer risk is the risk incurred by an individual from 24-hour-per-day exposure for a 70 year lifetime to ambient airborne concentrations of benzo(a)pyrene in California. This risk is estimated to be 0.5 to 1.5 cases per million persons exposed. Exposure to the average ambient value of 5.3×10^{-4} $\mu g/m^3$ could result in 16 to 48 excess lifetime cancers (Upper 95% Confidence Limit) among the 28 million residents of California. The unit risk is estimated to range from 0.0011 to 0.0033 $(\mu g/m^3)^{-1}$ of benzo(a)pyrene and is based on lifetime exposure.

The range of risk values results from several sources of uncertainty, including statistical uncertainty due to the number of animals in the

experiments to which the model was applied. For example, the inhalation experiment in hamsters had only 25-27 animals in each of the 4 exposure groups. Other general sources of uncertainty, include the extent of absorption of benzo(a)pyrene by various routes, variability of response to benzo(a)pyrene in different species, variability of the thoroughness of the pathological examination of the animals, the choice of the animal-to-human scaling factors, the choice of the low dose extrapolation model, and the large range of extrapolation (five orders of magnitude) from the benzo(a)pyrene concentrations used in the animal experiments to current ambient levels.

Based on the findings of carcinogenicity and the results of the risk assessment, OEHHA staff finds that ambient benzo(a)pyrene is an air pollutant which may cause or contribute to an increase in mortality or an increase in serious illness, or which may pose a present or potential hazard to human health.

Benzo(a)pyrene is one of more than one hundred polycyclic aromatic hydrocarbon (PAH) compounds identified. IARC has classified some mixtures containing PAHs as known human carcinogens (class 1) and many PAH compounds as probable or possible human carcinogens. The USEPA has classified several PAHs as possibly carcinogenic. Generally there are insufficient data to perform a complete health effects evaluation of these other PAHs. To evaluate the impacts of other PAHs, they are often considered to be as carcinogenic as benzo(a)pyrene or they are ignored. For many PAHs there are data establishing their genotoxicity and carcinogenicity. Consequently many PAHs can be ranked for their relative carcinogenic potency. Such a ranking

system was developed for 25 compounds. This ranking system is included as part of the benzo(a)pyrene health effects assessment.

2. EVALUATION HIGHLIGHTS

I. Exposure Sources

A. Air levels

1. Ambient levels: The population-weighted mean concentration of benzo(a)pyrene (BaP), based on measurements at air-monitoring stations around California, was previously estimated to be 4.6×10^{-4} $\mu g/m^3$. Measurements for 1989 resulted in a revised estimate of 5.3×10^{-4} $\mu g/m^3$.

2. Ambient levels measured in "hot spots:" There are no BaP production facilities, since BaP is a byproduct of (incomplete) combustion, but BaP occurs in fossil fuels and thus elevated levels are possible near refineries, foundries, smelters, etc.

3. Indoor air: BaP concentrations are variable depending on the quantity released, the time since release, and the size and ventilation rate of the room. Median indoor air in U.S. homes ranged from 0.0007 to 0.0135 $\mu g/m^3$. The maximum concentration measured in homes was 0.0607 $\mu g/m^3$ (McCann et al., 1987). Contamination by BaP originates, for the most part, from sources within the home. The use of combustive processes in kitchen stoves, wood stoves, fireplaces, kerosene heaters, and tobacco products increases the concentrations within homes.

B. Reported levels in water

1. National data: Untreated water in the Ohio River near heavy industry had levels as high as 210 ng/liter (EPA, 1980). Drinking water sources had concentrations of BaP from 0.1 to 2.1 ng/liter (EPA, 1980). BaP is likely to be broken down in soil and groundwater.

2. California drinking water: No data available.

C. Reported levels in food: The highest levels in food were reported in charcoal-broiled and smoked foods. Concentrations up to 289 $\mu g/kg$ have been found (IARC, 1983).

II. Metabolism: The principal metabolites in humans and animals are epoxy and hydroxy derivatives of BaP at several sites on the parent molecule. In humans, at least 80% of BaP is metabolized. Metabolism of BaP is probably complete within 24 hrs, but the metabolites remain in the body bound to protein and DNA.

III. Quantitative risk assessment for cancer

A. Shape of the dose-response curve

1. Animal: The carcinogenic dose-response curves for mouse and hamster tumors are not linear. (The studies used show anomalies and thus imply some uncertainty about the shape of the curve.)

2. Human: Studies are not applicable for BaP.

B. Range of extrapolation for animal to human exposure in air for lifetime daily exposure

1. Experimental to ambient: approximately 10^5 (5 orders of magnitude)

2. Experimental to "hot spots:" Data are not available.

C. Range of risks: The unit risk for a continuous, lifetime exposure, based on an inhalation and a feeding study in animals ranged from 3.8 to 11.5 $(mg/kg/day)^{-1}$ or from 1.1×10^{-3} to 3.3×10^{-3} $(\mu g/m^3)^{-1}$.

IV. National and International Evaluation

A. U.S. Environmental Protection Agency (EPA)

1. Genotoxicity tests: Metabolic activation is required to obtain positive responses. Pure BaP is an indirect mutagen; its metabolites are highly mutagenic in both bacterial and mammalian cells, such that BaP is one of the most potent mutagens.

2. Animal carcinogenicity tests: Sufficient evidence of carcinogenicity in animals.

3. Human evidence: Insufficient data to assess human carcinogenicity.

4. Cancer potency: USEPA is using an oral potency of 7.3 $(mg/kg/day)^{-1}$ for BaP on its Integrated Risk Information System (IRIS) database, a value somewhat lower than the value of 11.5 $(mg/kg/day)^{-1}$ developed by EPA in 1984.

5. Inhalation Unit Risk: In 1984 the USEPA developed an inhalation potency of 6.1 $(mg/kg/day)^{-1}$ for BaP which corresponds to an inhalation unit risk of 1.7×10^{-3} $(\mu g/m^3)^{-1}$. No value is available on IRIS.

6. Conclusions: Based on EPA's proposed cancer guidelines, the overall evidence for BaP results in its classification as a possible human carcinogen (group B2) (EPA, 1984).

B. International Agency for Research on Cancer (IARC)

1. Animal carcinogenicity assays: sufficient evidence of animal carcinogenicity

2. Human evidence: no adequate data available to evaluate human carcinogenicity

3. METABOLISM, DISTRIBUTION, AND KINETICS

3.1 Absorption

Data on absorption of BaP by animals and humans through the respiratory, gastrointestinal, and dermal routes have been summarized recently (ATSDR, 1987). In humans there are no quantitative data on rate and extent of absorption of BaP after inhalation, but the presence of urinary metabolites of BaP and of DNA adducts in white blood cells of industrial workers, both smokers and nonsmokers (Becher and Bjorseth, 1983; Shamsuddin et al., 1985), indicates that the chemical is absorbed. Data from rats indicate that only a fraction of the BaP inhaled is deposited on respiratory membranes. Of that which is deposited essentially all is absorbed. Approximately 20% of inhaled radioactive BaP (bound to Ga_2O_3 particles) is deposited on respiratory membranes, whereas only 10% of inhaled radioactive BaP not bound to particles is deposited (Sun et al., 1982). Approximately 80% to 90% of BaP administered to rats by gavage in peanut oil is absorbed in the gastrointestinal tract (and metabolized) since less than 15% of the radioactivity recovered in feces and urine is unchanged BaP (Hecht et al., 1979). By the dermal route approximately 6% of radioactive BaP (administered in acetone) is absorbed from the site of application on mouse skin in 1 hour while 40% is removed in 24 hours (Sanders et al., 1986). In vitro studies (Kao et al., 1985) suggest a wide variation in BaP permeability (24 hr) in animal skin ranging from 0.1% with guinea pig skin to 10% with mouse skin; human skin gives an intermediate value of 3%.

3.2 Distribution and Kinetics

In general, whole animal studies of BaP kinetics have been largely descriptive, measuring loss of radioactivity from organs after inhalation or intratracheal instillation, without analysis of metabolic activity. Mitchell (1982) performed inhalation studies in groups of rats using nose-only exposures to 500 μg ^{3}H-BaP/L (mass median diameter, 1 to 2 μm) for 1 hr. Radioactivity was measured at 0.5, 3, 6, 12, 24, and 48 hr post exposure in nasal turbinates, larynx, trachea, lungs, blood, tracheobronchial lymph nodes, liver, GI tract, kidneys, spleen, brain, and testes. Radioactivity appeared immediately in nearly all tissues, particularly the small intestines, stomach, and respiratory tract. Loss of radioactivity from the respiratory tract was biphasic with half-lives of ~2 hr for the rapid phase and 40 hr for the slow phase. In contrast, the caecum, large intestine, kidneys, brain, and testes continued to accumulate radioactivity through 6 hr. Levels of radioactivity in brain, testes, and spleen were comparatively quite low throughout the period of study. Excretion was primarily via the feces with about 10% of radiolabel appearing in the urine. (Fecal elimination can be enhanced several fold by increased dietary fiber (Mirvish et al., 1981)). Mitchell's subsequent study (1983) showed similar results, with liver producing more water-soluble metabolites and less covalent binding.

Medinsky and Kampcik (1985) exposed rats by intratracheal instillation to doses of 16, 90, or 6400 ng ^{14}C-BaP/rat in order to study pulmonary retention over 7 days. Once instilled, BaP clearance from the respiratory tract was biphasic and the rate constants varied with dose. As the dose

increased, the proportion of clearance by the rapid component rose while the proportion by the slower component fell sharply from 11.3% to 0.24% (Medinsky and Kampcik, 1985), suggesting that the slower component is easily saturated at higher doses. The saturability of this slower component may be partially responsible for the relatively constant level of covalent binding of BaP equivalents measured in the lung irrespective of dose. This phenomenon could lead to underestimation of lung concentrations of BaP from low-level environmental exposure if the estimate is based on linear extrapolation from controlled studies at higher (saturating) doses. Weyand and Bevan (1986) instilled 200 to 250 ng ^{3}H-BaP per rat and found a rapid, biphasic elimination; half-times were 5 minutes and 116 minutes.

Because the water solubility of BaP is very low, clearance of BaP from the lungs to the blood requires non-covalent binding to carriers such as erythrocytes (Smith and Doody, 1981), plasma proteins (McKenzie et al., 1978; Shu and Bymun, 1983; Aarstad et al., 1987), lipoproteins (Smith and Doody, 1981; Shu and Nichols, 1981; Busbee et al., 1982; Aarstad et al., 1987), and triglycerides (Yoo et al., 1984). Yoo et al. (1984) have related BaP uptake by serum to total serum lipid concentration and, more specifically, to serum triglyceride levels. This suggests that increases in serum triglyceride levels could contribute to increased levels of circulating BaP. Aarstad et al. (1987), in comparing BaP uptake by serum components from rats and humans, found that BaP binds primarily to the low density lipoproteins (LDL) in humans but to the high density lipoproteins (HDL) in rats. They also related overall higher uptake in humans relative to rats to higher cholesterol levels, although Yoo et al. (1984) saw no such correlation. Shu and Nichols (1981) demonstrated that the BaP

metabolites, 3-hydroxy-BaP and BaP-7,8-dihydrodiol, are also taken up by plasma lipoproteins. Moreover, 3-hydroxy-BaP is taken up by HDL to a greater extent than is BaP itself. Similarly, Busbee et al. (1982) showed that anti-BPDE (r-7,t-8-dihydroxy-t-9,10-oxy-7,8,9,10-tetrahydro-BaP) is capable of binding to LDL. The latter observation is significant, since cells can release reactive metabolites into the extra-cellular space (Harris et al., 1978; Merrick et al., 1981). This may be a mechanism whereby reactive metabolites are transported from metabolically competent tissues (i.e., liver) to metabolically less competent target organs (i.e., skin). Ginsberg and Atherholt (1989) reported in vitro studies which indicated that mouse serum bound BPDE and protected it from hydrolysis. Busbee et al. (1982) also showed that LDL-bound anti-BPDE was taken up by cells and became bound to nucleic acids *in vitro*. Cellular uptake is a rate-limiting factor in this process because its rate constant is 2 orders of magnitude lower than that for BaP desorption from LDL (Plant et al., 1987) and BaP uptake can be effectively inhibited by such ubiquitous polyamines as putrescine, spermine, or spermidine (Kowitz and Zeeck, 1985).

Pulmonary absorption is slowed significantly when BaP is adsorbed onto airborne particles. Environmental exposure typically involves BaP adsorbed to particles (Li et al., 1984). In an effort to understand the kinetics of PAH absorption from inhaled particles, studies have been conducted in which radiolabeled BaP was adsorbed onto inorganic or organic particles. When rats are exposed for 1 hr by nose-only inhalation to ^{3}H-BaP adsorbed onto diesel engine exhaust particles (4 to 6 μg/L air; 0.14 μm mass median diameter), clearance from the lung is biphasic (Sun et al., 1984). During the first phase approximately 50% of the radioactivity is cleared with a

half time of less than one hour. The remainder clears with a half-time of 18 ± 2 days. Analysis of tissue samples indicate that the major portion of the diesel particle-associated radiolabel is parent BaP (65 to 76%). There are also detectable amounts of BaP-phenol (13 to 17%) and BaP-quinones (5 to 18%), which indicates that BaP is desorbed, metabolized by the lungs, then readsorbed onto particles.

Similar studies have been conducted with radiolabeled BaP adsorbed onto gallium oxide (Ga_2O_3) (Sun et al., 1982), since a substantial portion of environmental BaP is condensed onto inorganic particulates. A substantial portion of the ^{3}H-BaP on Ga_2O_3 particles is removed by mucociliary clearance and ingested. Particulate binding of ^{3}H-BaP therefore increases the proportion of the dose of BaP that is delivered to the stomach, liver, and kidney and increases the contact time with these organs. By comparison, inhaled ^{3}H-BaP aerosol is cleared primarily by absorption directly into the blood, although mucociliary clearance may also occur (Mitchell, 1982). Kinetic analysis indicates that particulate-associated BaP is cleared more slowly from the lung (1 day compared to 4 hours for 90% clearance of BaP alone), apparently due to the rate-limiting process of desorption from the particles. Particles are also taken up by pulmonary macrophages (Autrup et al., 1979), which increases BaP retention in the lung (Schoeny and Warshawsky, 1983). Adsorption to particles may also increase the conversion of BaP to reactive metabolites in the lung and reduce the production of inactive, water-soluble metabolites (Warshawsky et al., 1983; Warshawsky et al., 1984).

Some kinetic studies have also been performed *in vitro*. Induction by phenobarbital increases production and excretion of polar metabolites of BaP which increases overall clearance in isolated perfused rat liver (Forti and Trieff, 1980). However, mutagenicity of bile extracts is also greater in induced animals. Klaus et al. (1982) placed an isolated perfused liver in series with a lung preparation and exposed the lung to radiolabeled BaP metabolites generated by the liver. Relative to direct pulmonary exposure to BaP, this arrangement decreased covalent binding to lung macromolecules; use of liver induced with 5,6-benzoflavone enhanced this protective effect. Molliere et al. (1987) suggested that induction by phenobarbital and 5,6-benzoflavone increases the conjugating capacity of the liver, which leads to lower concentrations of BaP-dihydrodiols in the lung. Wiersma and Roth (1983) predicted kinetic parameters for liver and lung using kinetic data from microsomal studies and tested the predictions in isolated, perfused organs. The liver had greater BaP metabolism ("intrinsic free clearance") than lung and 3MC induction caused a significant drop in liver K_m (increased binding for BaP) compared to lung. However, differences in overall clearance indicated that the lung would be equally competent as the liver in clearing BaP equivalents in induced animals.

3.3 Metabolism

The requirement of metabolic activation of BaP for toxicity is well established (Gelboin, 1980; Pelkonen and Nebert, 1979: Conney, 1982; Phillips, 1983). The biotransformation of BaP has been studied in a wide range of systems, both *in vivo* and *in vitro*, and is summarized in Figure 3-1. The scheme is relatively complex and represents a compilation of all

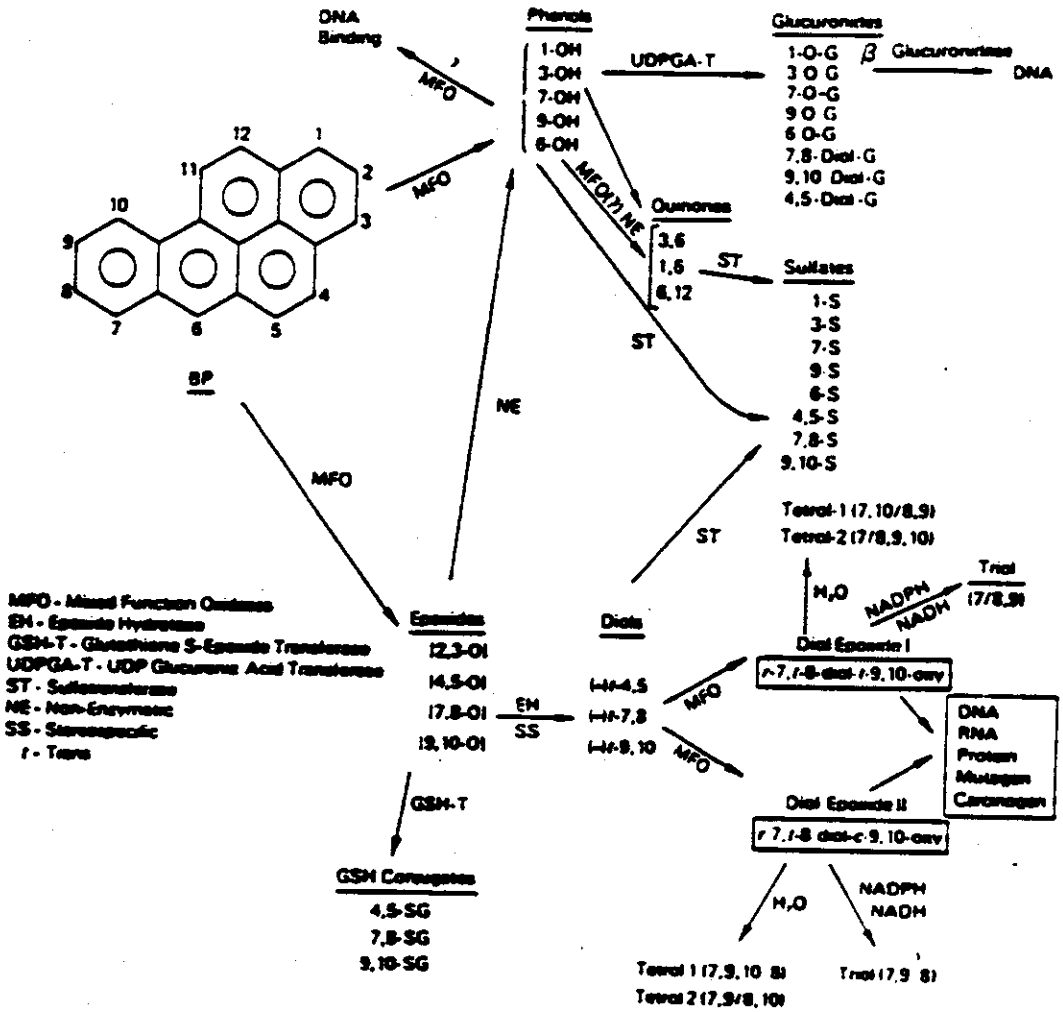

Figure 3-1. Metabolism and activation of BaP. (Adapted from Gelboin, 1980).

known metabolic pathways, but does not indicate quantitative differences among species or experimental models. For example, because of their relative simplicity, *in vitro* systems tend to produce a narrower spectrum of metabolites than is typically found *in vivo*. Many workers (Sabadie et al., 1981; Ekstrom et al., 1982; Sipal et al., 1979) have noted far greater interindividual variability among humans with respect to metabolic capacity compared to other animal species, and have speculated that this may account for some of the variability in cancer susceptibility in human populations (Gurtoo et al., 1984). With few exceptions (Moore et al., 1983), species and strain differences in susceptibility to BaP toxicity have been related to differential metabolic activating ability (Skelly and Shertzer, 1985) and to genetic differences in aromatic hydrocarbon (Ah) receptor affinity (Legraverend et al. 1983; Legraverend et al. 1984). In spite of such differences, there is considerable qualitative similarity in metabolism among model systems.

Among the most frequently detected BaP metabolites, particularly *in vitro*, are various phenols and quinones (Cavalieri et al., 1988). These simple oxidation products are generated by the action of cytochrome P450-dependent monooxygenases (Holder et al., 1974) and may be metabolized further to glucuronide and/or sulfate conjugates (Cohen et al., 1976; Nemoto et al., 1977). Although mixed function oxidases are capable of hydroxylating BaP directly, the more usual route of phenol formation is via the production of one of four epoxides which may then rearrange non-enzymatically to form phenols or be hydrated to the corresponding trans-dihydrodiols by epoxide hydrolase (Yang et al., 1977a; Yang et al., 1977b). Of the latter, the (±) trans-7,8-dihydrodiols can be "recycled" through cytochrome P450 to produce

four diol-epoxide isomers (Grover et al., 1976: Yang et al., 1977b), which are the ultimate adducting species. Under physiological conditions, however, the (-) enantiomer of trans-7,8-dihydrodiol is preferentially produced (Yang et al., 1977b), although the ratio of syn- to anti-diol epoxide products depends on the nature of the tissue and enzyme preparation under study. The r-7,t-8-dihydroxy-c-9,10-oxy-7,8,9,10-tetrahydro-BaP (syn-BaP diol-epoxide or syn-BPDE) (Figure 3-2) is extremely unstable chemically and is capable of alkylating cellular constituents. Nevertheless, once syn-BPDE is protonated to form its triol cation, it generally reacts with water to form a tetrol or with a reducing equivalent from NADH or NADPH to form a 7,8,9-triol. The r-7,t-8-dihydroxy-t-9,10-oxy-7,8,9,10-tetrahydro-BaP (anti-BaP diol-epoxide or anti-BPDE) is slightly less labile than the syn isomer. For this reason it does not form the triol cation as readily and therefore survives in the cell long enough to form adducts with intra- and extracellular macromolecules.

At the cellular level BaP binds to a cytosolic Ah receptor, as do a variety of planar PAH, and the BaP-receptor complex is translocated into the nucleus where it mediates induction of cytochromes P450 (Tukey et al., 1982). The binding affinity of the Ah receptor for BaP, a genetically determined characteristic, has been linked to a variety of toxic effects (Legraverend et al., 1983; Legraverend et al., 1984). The receptor has been isolated in mice and rats, and an apparently similar protein has been detected in man (Brown et al., 1987). BaP also binds with high affinity to a separate polycyclic aromatic hydrocarbon binding protein (PBP) which exists in the cytosol of liver and other organs of several species (Barton and Marletta, 1988).

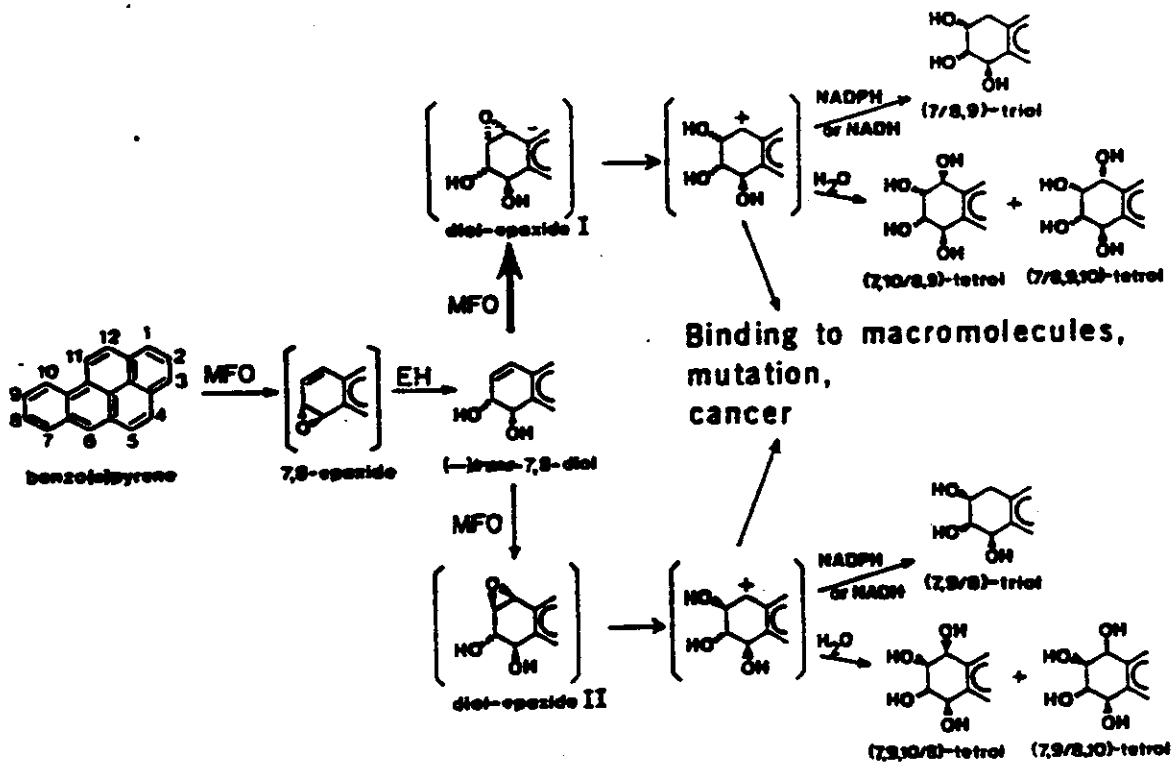

Figure 3-2. Mechanism and Stereospecificity of Activation of BaP to BPDE. MFO = Mixed Function Oxidase. EH = Epoxide Hydratase. (From Yang et al., 1977a).

Aryl hydrocarbon hydroxylase (AHH) activities necessary for the activation of BaP have been found in sub-cellular fractions including smooth and rough endoplasmic reticulum, plasma membrane, mitochondria, and nuclei (Oesch et al., 1985) of a wide variety of tissues in nearly every species studied. Although the liver is the organ with the greatest xenobiotic transforming capabilities, it is not a target organ for BaP-induced carcinogenesis. Furthermore, while the liver is the primary site of BaP metabolism, studies in isolated perfused organ systems have shown that there is less protein binding than in the lung (Klaus et al., 1982; Molliere et al., 1987). The lungs, as well as other portions of the respiratory system, can metabolically activate BaP (Mehta and Cohen, 1979; Autrup et al., 1983; Bond, 1983; Bond et al., 1988). This probably plays a role in BaP-induced carcinogenesis of the lung.

Human bronchial mucosal cells (Harris et al., 1977; Hukkelhoven et al., 1982; Teel et al., 1986) and explants (Autrup et al., 1982; Selkirk et al., 1982; Stoner et al., 1982; Daniel et al., 1983; Mass and Genta, 1985) in culture have inducible AHH activity and produce most major metabolites. While Siegfried et al. (1986) found similar metabolic profiles in cultures of human bronchial epithelial cells, they found wide individual variation in metabolism which could not be directly related to the even wider variations in susceptibility of the cells to toxicity. Pulmonary alveolar macrophages are also capable of BaP activation (Harris et al., 1978; Bond et al., 1984), but both metabolic activity and inducibility are highly variable among cell cultures from different individuals (Marshall et al., 1979). Type II alveolar cells in culture have produced 3-hydroxy-BaP

(Devereux and Fouts, 1981) as well as 7,8- and 9,10-BaP-dihydrodiols and glucuronide conjugates of 9-hydroxy-BaP (Bond et al., 1983).

Placental microsomes from smoking mothers are capable of producing some of the major BaP metabolites (Namkung and Juchau, 1980; Juchau et al., 1982), including anti-BPDE (Pelkonen and Saarni, 1980), while microsomes from non-smokers are almost devoid of activity. Gurtoo et al. (1983) have related induction level of AHH to both number of cigarettes smoked per day and to formation of active metabolites; saturation of the inductive effect occurs at consumption levels beyond 20 to 25 cigarettes/day. Paradoxically, Manchester and Jacoby (1984) noted a higher incidence of abnormalities, particularly anencephaly, in children of smoking mothers whose placentas had developed lower levels of induction, as measured by apparent K_m toward ethoxyresorufin. This may indicate a genetic alteration in induction of enzyme with consequent teratogenic effects due to accumulation of xenobiotic.

Other tissues in which BaP metabolism has been detected *in vitro* include: epidermis (Kao et al., 1985), nasal mucosa (Dahl et al., 1985; Petridou-Fischer et al., 1988), bronchus (Daniel et al., 1983; Garner et al., 1985), buccal mucosa (Moore and Gould, 1984; Autrup et al., 1985), colon (Garner et al., 1985), kidney (Prough et al., 1979), bladder (Daniel et al., 1983), ovary and adrenal (Bengtsson et al., 1983), endometrium (Kulkarni et al., 1986), mammary epithelium (Moore et al., 1986), and aorta (Bond et al., 1980).

In humans, BaP metabolism has been detected in hair follicles (Vermorken et al., 1979; Hukkelhoven et al., 1983), epidermis (Selkirk et al., 1983; Weston et al., 1983; Bickers et al., 1984; Kao et al., 1985; Hall and Grover, 1988), fibroblasts (Fox et al., 1975; Yamasaki et al., 1977), epidermal and esophageal keratinocytes (Heimann and Rice, 1983), buccal mucosa (Moore and Gould, 1984; Autrup et al., 1985), esophagus (Autrup et al., 1982; Selkirk et al., 1983), pancreatic duct (Harris et al., 1977), duodenum (Autrup et al., 1982), colon (Autrup et al., 1982; Mayhew et al., 1983; Garner et al., 1985), endometrium (Mass et al., 1981; Kaufman et al., 1983; Kulkarni et al., 1986), mammary epithelium (Stampfer et al., 1981; Moore et al., 1986), lymphocytes (Marshall et al., 1980; Okano et al., 1979; Feo et al., 1987), monocytes (Okano et al., 1979; Salmon et al., 1981; Nowak et al., 1988), bladder (Stoner et al., 1982; Daniel et al., 1983; Selkirk et al., 1983), and pulmonary alveolar macrophages (Marshall et al., 1979).

In addition to the monooxygenase-produced metabolites from phase I reactions, phase II reactions convert a number of metabolites to conjugates of glucuronic acid (Gelboin, 1980), sulfate (Cohen et al., 1976), and glutathione (Gelboin, 1980). Glutathione-S-transferase, which catalyzes conjugation of epoxides and therefore prevents accumulation of dihydrodiols (Singh et al., 1985), probably represents one of the major detoxification pathways for BaP. Moreover it has been postulated that the anti-neoplastic effects of butylated hydroxytoluene (BHT) and other anti-oxidants may be due to induction of glutathione-S-transferases (Singh et al., 1987).

3.4 DNA Adducts

Since carcinogenesis induced by BaP has not been established in humans, other experimental models have been used in an effort to obtain dose-response information for risk assessment purposes. It is important to obtain information on the metabolites formed and to determine the carcinogenic potential of each metabolite. Since monooxygenase-catalyzed oxidation of BaP involves several competing pathways, qualitative and quantitative differences between the experimental model and the target organ in humans must be identified. This information is difficult to obtain in any experimental system since anti-BPDE, generally regarded to be the ultimate carcinogenic form of BaP, is unstable and therefore not amenable to analysis. However, information on potential biological activity may be inferred by evaluating BaP-derived metabolites that covalently bind to DNA. This method has the advantage of elimination of metabolites that fail to bind to DNA. Binding of BaP results from a number of metabolites (Figure 3-1) which form adducts with purine and pyrimidine bases at several reactive centers within each base. Since it is difficult to obtain experimental data that are predictive of BaP-induced tumor development, DNA adduct profiles rather than specific DNA adducts are compared (Watson et al., 1987). A comprehensive analysis of adduct profiles and stability is needed.

In male Swiss mice binding of BaP metabolites to DNA is linearly related (on a log-log scale) to orally administered BaP from 10 ng to 1 mg per animal (Dunn, 1983). In female A/HEJ mice binding to DNA is nearly linearly related to dose over the range of 11 μg to 7.5 mg per animal

(Adriaenssens et al., 1983). Thus, at least in mice, the internal dose to DNA can be predicted from the dose of BaP administered to the animal.

A great deal of effort has gone into isolation and characterization of BaP-derived adducts to DNA. The work of Baird and Brooks (1973) provided a chromatographic method, using Sephadex LH-20 resin, for isolation of adducted nucleosides. Studies conducted with radiolabeled BaP (^{3}H or ^{14}C) provide a means of tracking the extremely small amounts of adducts formed: approximately 1 ng/mouse skin or one adduct per 10^{6} nucleotides of DNA. Further separation of the adducted nucleoside fraction by high performance liquid chromatography indicates that the majority (60 to 90%) of the radioactivity chromatographs as a single component. Chemical characterization studies show that the major adduct is produced by the reaction of anti-BPDE and the N-2 exocyclic amino group of deoxyguanosine (dGuo) to yield the structure shown in Figure 3-3. The second most prevalent adduct is derived from the syn-isomer of BPDE which also binds to the amino group of dGuo. Subsequent studies have identified BPDE adducts to deoxyadenosine as well as other bases. The majority of this work was conducted in the 1970's and has been extensively reviewed (Jeffery, 1985; Gelboin, 1980).

Carcinogenicity studies with metabolites of BaP have demonstrated that monohydroxy-BaP, BaP dihydrodiol, and BPDE metabolites are all carcinogenic; the anti-BPDE is the most active (Table 3-1). The syn-BPDE, BaP tetrols, and monohydroxy-BaP metabolites have little, if any, carcinogenic activity. These results provide strong evidence that anti-BPDE is the major ultimate carcinogen. However, any conclusions about the

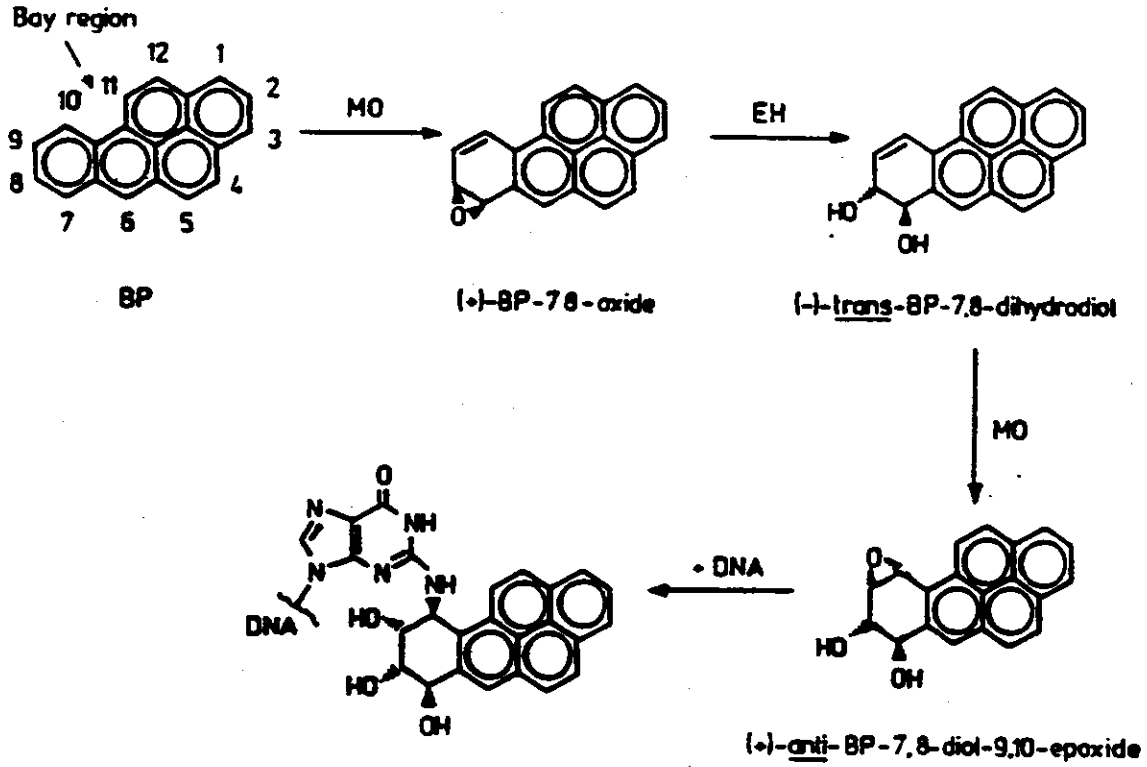

Figure 3-3. Activation and macromolecular binding of BaP. (From Gelboin, 1980).

Table 3-1. Biological reactivity of different benzo-ring metabolites of benzo[a]pyrene

Benzo-ring Derivative	Mutagenicity		Malignant Transformation	Carcinogenicity
	Bacteria	Animal Cells		
(+)-Trans-7,8-dihydrodiol		+		+
(-)-Trans-7,8-dihydrodiol		++		+++
7,8-Diol-9,10-epoxide (syn)	+++	+	+	-
7,8-Diol-9,10-epoxide (anti)	++	++	++	+++
(+)-Anti-isomer (7β,8α-dihydroxy)	+	+++		+++
(-)-Syn-isomer (7β,8α-dihydroxy)	++	+		+
(-)-Anti-isomer (7α,8β-dihydroxy)	+	+		±
(+)-Syn-isomer (7α,8β-dihydroxy)	+	+		±
9,10-Diol-7,8-epoxide (syn)	+	-	-	
9,10-Diol-7,8-epoxide (anti)	+	-	-	
7,8-Diacetyl				++
7,8-Catechol				-
7,8-Dihydro	++		+	++
9,10-Dihydro	+		-	-
7,8-Quinone	-			
7,8,9,10-Tetrahydro	-			
7,8-Dihydroxy-7,8,9,10-tetrahydro	-			

Data taken directly from Pelkonen and Nebert (1982).

relative importance of the individual DNA adducts cannot be made. For other PAHs, adducts with bases other than dGuo may be important in tumor initiation (Balmain and Pragnell, 1983).

In an effort to improve extrapolation experimental models to humans, BaP-DNA binding and adduct levels have been evaluated in endometrial tissue from mice, rats, hamsters, and humans (Kulkarni et al., 1986). Total binding was highest in humans and lowest in rats. Less than 12% of the radiolabel bound to DNA eluted as polar uncharacterized adducts in mice, hamster, and human preparations, whereas in rats approximately 50% eluted in this fraction. Nevertheless, anti-BPDE-dGuo adducts predominated in all four species.

Measurement of adducts to hemoglobin has been proposed as a method of monitoring exposure to BaP and other PAH. In mice, hemoglobin-BaP adducts are derived only from the anti-BPDE metabolite of BaP (Shugart and Kao, 1985). Surprisingly, most of the BaP adduct is attached to the heme group rather than the protein (globin) portion of the molecule where adducts generally bind (Lee and Santella, 1988). Protein adducts may provide information on exposure and may correlate with carcinogenic response. However, confirmatory data are needed in this area.

It is generally accepted that chemical carcinogens and cocarcinogens are responsible for a significant number of cancers in humans. Chemical carcinogenesis induced by initiating agents may begin with covalent binding of the compound (or a metabolite) to DNA. This event, initiation, is followed by promotion and progression until a tumor develops. The

carcinogenic potency of various PAH correlates reasonably well with their ability to form DNA-adducts (Lutz, 1979). Because of this association several methods have been proposed to determine the extent of exposure for people in certain occupations. For example, Shamsuddin et al. (1985) used enzyme-linked immunosorbent assay and ultra-sensitive radioimmunoassays to determine the concentration of BPDE-DNA in white blood cells from exposed roofers and foundry workers. Fourteen of the 48 workers tested positive for BPDE-DNA adducts while two (both cigarette smokers) of nine control (volunteers, laboratory personnel) were positive. The lower limit of detection of 2 fmol BPDE/50 μg DNA is equivalent to 1 BPDE adduct in 7.5 x 10^7 bases. (A femtomole, abbreviated fmol, equals 10^{-15} moles.) Substantial individual variability occurs, with adduct levels ranging from 2 to 124 fmol/50 μg DNA. This molecular epidemiological approach may lead to methods for monitoring exposed populations and, once relationships between adduct levels and cancer risk are established, it may provide a predictive tool for high-risk individuals such as smokers.

Several studies using mouse skin have demonstrated that DNA adducts reach maximum levels approximately 24 hr after dermal application of BaP. Adduct levels decreased rapidly; by 7 and 21 days binding values were 10 and <5%, respectively, of their values at 24 hr (e.g., Ashurst et al., 1983). Similar results were obtained with radiolabeled BaP and with antiserum to BPDE adducts (Nakayama et al., 1984). Using ^{14}C-thymidine and ^{3}H-BaP, DiGiovanni et al. (1985) demonstrated that, after the initial rapid loss of adducts between 24 and 48 hours, the rate of disappearance of adducts paralleled cell turnover rates. These results suggest that DNA repair

occurs within the first few days after exposure and that some adducts persist for the life of the cell.

Stomach, lung, and skin are considered to be the target organs for BaP-induced neoplasia (Ioannou et al., 1982). Anderson et al. (1981) treated mice with BaP under conditions that induce pulmonary adenomas and found that binding to lung DNA is approximately 20 pmol/mg. This level of binding is nearly equivalent to that found in mouse skin following application of tumorigenic doses (DiGiovanni et al., 1985; Nakayama et al., 1984; Springer et al., 1989). Even though liver is not a target organ, binding to hepatic DNA is approximately 10-fold greater than for lung. Nevertheless, hepatic BaP-DNA adducts are removed more rapidly than those from lung (Stowers and Anderson, 1985) or skin, and this removal, through DNA repair and possibly cell turnover, may be the mechanism which prevents development of hepatic tumors. Analysis of DNA from BaP-treated animals demonstrated that the major DNA adduct from these three organs co-chromatograph with anti-BPDE-dGuo. Smaller amounts of syn-BPDE-dGuo are also present.

The ultrasensitive ^{32}P-postlabeling assay for DNA adducts has been used by Seidman et al. (1988) to determine whether BaP-derived adducts are formed in the rat and in human mammary epithelial cells. Their results demonstrate that both in vivo (rats) and in vitro (human mammary cells) the major adduct is anti-BPDE-dGuo. However, when mammary epithelial cells from human donors were screened for DNA adducts formed in situ, distinct adduct patterns were observed and the anti-BPDE-dGuo adduct was absent (Seidman et al., 1988).

4. GENOTOXICITY

Benzo(a)pyrene itself is not mutagenic, but the ability of its metabolites to induce mutation is well established (EPA, 1979; EPA, 1980; IARC 1983). Very recently the Agency for Toxic Substances and Disease Registry (ATSDR) tabulated a very large number of reports which demonstrate the mutagenicity of BaP metabolites (ATSDR, 1987). This chapter is a selective review of some aspects of BaP genotoxicity.

A number of short-term *in vitro* and *in vivo* tests have been developed to screen chemicals for potential carcinogenic and mutagenic activity (Brusick, 1987; Hollstein, 1979; Hollstein et al., 1979; Rinkus and Legator, 1979). Many of these assays employ a host (bacteria, cultured mammalian cells, or an intact animal) and measure the effects of the test chemical on the host after short periods of time (hours to weeks). The end points for these assays are mutagenesis, cell transformation, and DNA damage to the host. Most of the assays employ an activation system that consists of a subcellular enzyme preparation and co-factors such as NADPH, NADH, lipids, and metals. Commonly used preparations for PAH activation consist of S-9 fractions of liver homogenates and of mammalian cells in culture. Homogenates and microsomal preparations from lung, skin, or kidney, and highly purified microsomal fractions have also been used.

BaP and its metabolites have been extensively studied in both bacterial and mammalian assays. Data from these assays have assisted in the identification of biologically active BaP metabolites, in the clarification

of the metabolic pathways of BaP, and in the prediction of mutagens (i.e., PAH) which may be potential environmental hazards.

4.1 Mutagenicity Assays in Bacteria

The Salmonella histidine reversion assay developed by Ames and associates (1973) has provided a simple and inexpensive test system to evaluate many potential chemical carcinogens. In this assay several histidine-requiring (his^-) strains of Salmonella may undergo back mutation to His^+ wild type when exposed to a chemical carcinogen (Ames et al., 1973). Each tester strain contains a different type of mutation in the histidine operon. Other mutations, such as the rfa mutation, which enhance the permeability of large molecules into bacterial cells (Maron and Ames, 1983), may be present. BaP and other PAH are used as positive control mutagens to routinely check the genetic integrity of each strain. BaP at 1 μg/plate exhibits mutagenic activity with all of the currently recommended tester strains (TA97, TA98, TA100, and TA102) in the presence of rat liver S9. BaP exhibits the most mutagenic activity with tester strain TA100, which is sensitive to base-pair substitution mutations.

Several extensive studies using the histidine reversion assay have been conducted with BaP and its metabolites (Wood et al., 1976; Nagao and Sugimura, 1978; Wislocki et al., 1976). Wood and co-workers studied four benzoepoxide derivatives of BaP for mutagenic activity in three tester strains: TA1538, TA98, and TA100. All four compounds, anti-BPDE, syn-BPDE, 7,8-epoxy-7,8,9,10-tetrahydro-BaP, and 9,10-epoxy-7,8,9,10-tetrahydro-BaP, were mutagenic in all three strains. A dose response was observed over

limited concentration ranges. Anti-BPDE was 1.5 to 4 times more potent as a mutagen than syn-BPDE. The mutagenicity of the two tetrahydro-epoxides was destroyed upon coincubation with purified epoxide hydrolase, while the mutagenic activity of the diol epoxides was not altered in the presence of this enzyme.

Nagao and Sugimura (1978) studied BaP and its metabolites using TA98 as the tester strain. Syn- and anti-BPDE were equipotent, direct-acting mutagens (i.e., mutagenic without metabolic activation) and were the most mutagenic of the forms tested. Other investigators (Levin et al., 1978) reported that in bacterial systems syn-BPDE was more potent than anti-BPDE. All twelve isomeric phenols, 8 dihydrodiols, BaP-9,10-epoxide, and BaP-11,12-epoxide were characterized by these studies as weak, direct-acting mutagens in the bacterial system. Both Wislocki et al. (1976) and Nagao et al. (1978) reported that K-region epoxides were considerably less mutagenic than non-K region epoxides; BaP diol epoxide was more mutagenic than BaP 4,5-epoxide in strains TA98 and TA100. The same investigators also found that the phenols were either weakly-mutagenic or non-mutagenic without metabolic activation. Only 1-, 2-, 3- and 9-hydroxy-BAP showed significant mutagenicity with metabolic activation, whereas other monohydroxy-BaP isomers were essentially inactive. Some of the diols of BaP, which include the 4,5-, 7,8-, and 9,10-diol, have been examined by Nagao and Sugimura (1978). Their results indicated that after metabolic activation the 7,8-diol was the most mutagenic diol. Wislocki et al. (1976) also demonstrated that the cis- and trans- isomers were not mutagenic without metabolic activation. Nagao et al. (1978) reported that the (-) trans-7,8-diol is a fairly strong direct-acting mutagen to Salmonella TA100 but not to TA98,

suggesting that the biological activity of this intermediate is dependent on further metabolism to diol epoxides. Both (-) and (+) trans-7,8-diol of BaP were reported to be highly mutagenic in the presence of activating enzymes (Nagao et al., 1978) . The quinones formed from BaP and the BaP tetrols and triols showed little mutagenic activity in the presence or absence of metabolic activation. Of the methylated BaP forms, the 6-hydroxymethyl-BaP was more mutagenic than the 6-methyl-BaP.

The Ames mutagenesis assay is useful not only for detecting potential carcinogens but also for assaying body fluids or excretions for mutagens. Several studies have detected mutagens in urine from cigarette smokers and coke plant workers, in whom BaP exposure would be expected. Urine samples from non-smoking coke workers were more mutagenic in strain TA1538 in the presence of a liver metabolic system than samples from a control group of non-smoking office and laboratory workers (Kriebel et al., 1983). Moller and Dybing (1980) demonstrated that XAD-2 resin extracts of urine samples from smokers were significantly more mutagenic in tester strain TA98 than samples from non-smoking coke plant workers. Furthermore, there was no significant difference between exposed coke workers and controls. Falck et al. (1980) reported that urine samples from smokers and non-smokers in the rubber industry were more mutagenic than controls. These studies showed that humans exposed to BaP can metabolize the carcinogen to reactive metabolites. Thus, this simple and inexpensive assay has become a tool to detect exposure. Unfortunately, a quantitative relationship between BaP exposure and the concentration of urinary mutagens has not been established.

4.2 Mutagenicity Assays in Mammalian Cells

A number of mammalian mutagenesis assays have been used to study BaP and its K- and non-K-region epoxide derivatives. Huberman et al. (1976) tested BaP and 15 of its derivatives including phenols, BaP-4,5-epoxide, dihydrodiols, the two isomeric 7,8-diol-9,10-epoxides (syn- and anti-BPDE), 6-methyl BaP, and 6-hydroxymethyl BaP in Chinese hamster V79 cells. Irradiated, cultured Syrian hamster embryo tissue was used for activation. Mutations were characterized by resistance to ouabain or 8-azaguanine. All the tested phenols, 4,5-diols, trans-9,10-diol, 6-methyl, and 6-hydroxmethyl BaP showed little or no mutagenicity in this system with or without metabolic activation. However, in the absence of metabolic activation, anti-BPDE showed a 2000- and 270-fold higher mutation frequency manifested as ouabain and 8-azaguanine resistance, respectively, than the K-region BaP-4,5-epoxide at equimolar concentrations of 0.7 μM. At 0.7 μM, the mutagenic potency of anti-BPDE was 26-fold (ouabain resistance) and 22-fold (8-azaguanine resistance) greater than the isomeric syn-BPDE. The 7,8-diol was more active than the parent hydrocarbon, BaP, in cell-mediated mutagenesis. The mutagenic activity of the compounds was reduced 80 to 90% by 7,8-benzoflavone, an inhibitor of microsomal oxygenase, which indicates activation by mixed-function oxygenase.

Transformation of normal hamster cells by BaP and six of its metabolites (the trans-4,5-, 7,8-, and 9,10-dihydrodiols, the 4,5-epoxide, and the two stereoisomers of the Bay region diol epoxides, syn- and anti-BPDE) was reported by Mager et al. (1977). Of the dihydrodiols, the trans-7,8-diol was the most active in inducing transformation and cytotoxicity in these

cells. All three epoxides induced transformation of normal hamster cells; anti-BPDE was the most active. At 3.0 μg/ml anti-BPDE induced 1.4% transformed colonies as compared to 0.2% and 0.1% for syn-BPDE and the K-region epoxide, respectively. These results correlate with other studies in mammalian cells with respect to relative potency (Huberman et al., 1976; Yang et al., 1976).

4.3 Sister Chromatid Exchange

Studies of sister chromatid exchange (SCE) induction by BaP *in vitro* have been performed with many different cells using a variety of experimental protocols. Comparative studies have shown that SCE frequency is dependent upon the cell system used (Baker et al., 1983; Hopkin and Perry, 1980; Tomkins et al., 1982; Mehnert et al., 1984). The usual cultures employed for SCE induction are whole blood or purified lymphocytes. Fundamental differences with respect to metabolic activity exist between these two systems. Mehnert et al. (1984) compared SCE induction in these systems, with and without metabolic activation, after exposure to both direct and indirect mutagens, including BaP. BaP produced a significant increase in frequency of SCE in whole blood cells (mainly lymphocytes) and purified lymphocyte cultures without activation after 2- or 24-hour exposures. No significant difference in response to BaP was seen between the whole blood cultures and the purified lymphocyte cultures.

In most *in vitro* tests, BaP increases SCE frequency without exogenous activation. A review of the literature on whole blood culture shows divergent findings with regard to SCE induction. Some researchers have

reported negative results (Madle, 1981; White and Hesketh, 1980) which may be explained by inadequate incubation time or exposure time. Other researchers (Waalkens et al., 1981; Norppa et al., 1983) have shown distinct SCE induction. These contradictory findings are probably due to differences in experimental conditions.

Craig-Holmes and Shaw (1977) reported an increase in the frequency of SCE in cultured human lymphocytes exposed to BaP. This increase was dependent upon time of exposure rather than upon concentration. They suggested that BaP may be both an inducer and a substrate for the monooxygenase activation system. Inoue et al. (1983) demonstrated only slight increases in SCE frequency at higher concentrations (10^{-4} M) of BaP in the absence of an S9 activating system. With the S9 activating system, a gradual increase in SCE frequency was observed with increasing concentrations of BaP after 1 hour of exposure. At 10^{-4} M BaP the SCE frequency was approximately two-fold greater than the solvent control. Takehisa and Wolff (1977) reported that BaP, without an exogenous activating system, increased SCE frequency slightly in Chinese hamster ovary cells. The addition of S9 activating system SCE increased the frequency of SCE greatly.

Although several investigators have reported an increase in SCE with many mutagenic and carcinogenic agents, the relationships among an increased frequency of SCE, the chromosome breakage seen in cancer cells, and the process of carcinogenesis are not yet defined (Craig-Holmes and Shaw, 1977; Kato and Shimada 1975; Latt, 1974; Perry and Evans, 1975).

4.4 Unscheduled DNA Synthesis

Unscheduled DNA synthesis (UDS), a measure of excision repair of damaged DNA, was proposed by San and Stich (1975) as a rapid bioassay for chemicals which may act by damaging DNA. Investigators of UDS have employed several types of cellular systems including cultured human skin fibroblasts, rat and human hepatocytes, human urothelial cells, and human skin epithelial cells (San and Stich, 1975; Lake et al., 1978; Safe and Wong, 1981; Belitsky and Budanova, 1983; and Bem et al., 1987). Human skin fibroblast cell lines are used most frequently for this assay, but are limited by their low metabolic capacity, and thus require an exogenous metabolic activation system. Other cells, such as human epithelial cells or rat liver cells have sufficient cytochrome P450-dependent monooxygenase activity and thus do not require an exogenous source of metabolic activation. Frequently cells are incubated with arginine-free medium supplemented with hydroxyurea to suppress the scheduled (replicative) synthesis of DNA. The magnitude of UDS is determined autoradiographically on the basis of non-S-phase, nuclear ^{3}H-thymidine incorporation. Primary rat hepatocyte cultures, exposed to 5 nmoles/ml of BaP, demonstrated an approximately 10-fold increase in UDS over background (Probst et al., 1981). Lake et al. (1978) reported that BaP induces detectable UDS in human skin epithelial cells without exogenous metabolic activation.

4.5 Relationship of BaP-diol Adducts and Genotoxic Effects

The relationship between BaP-diol epoxide adduct levels and genotoxic effects has been studied in several mammalian cell systems (Arce et al.,

1987; Recio et al., 1987). Arce et al. (1987) measured BaP adducts in several systems and quantified the mutagenic and cell-transforming effects induced by BaP. The mammalian cells included Chinese hamster V79, mouse lymphoma, primary hamster embryo, Syrian hamster embryo (SHE), and mouse embryo C3H10T1/2 cells.

Chinese hamster V79 cells co-cultured with irradiated SHE cells were treated with BaP for 24 hours prior to measurement of cytotoxicity, TG^r (6-thioguanine resistant) mutants, SCE, and DNA binding. At a concentration of 0.125 μg BaP per ml a slight decrease in survival was observed but no further decrease in survival occurred up to 15 μg BaP/ml. Maximal mutational frequencies of 150 and 300 TG^r mutants per 10^6 survivors were obtained at 0.5 μg/ml in two independent experiments. Above this concentration (up to 10 μg/ml), the mutant frequency declined and reached a plateau. The frequency of SCE, expressed as SCE/cell, was 20 to 25 up to a concentration of 0.5 μg/ml. No increases in the frequency of SCE were observed from 2 to 10 μg BaP/ml. At 1 μg/ml, the BaP-DNA binding ranged from 8.3 to 10 μmol BaP/mol DNA.

Similar results for adduct formation were observed by Recio et al. (1987) using the hypoxanthine-guanine phosphoribosyltransferase (CHO/HGPRT) mutation assay in Chinese hamster ovary cells. The number of BPDE-adducts increased from 21 to 260 adducts/10^6 nucleotide base pairs with increasing concentrations of BaP-diol from 1.4 to 7.0 μM. A linear relationship between the number of BPDE-adducts and mutagenicity (89 to 606 mutants/10^7 clonable cells) was observed over the concentration range assayed.

Arce et al. (1987) also observed cytotoxicity in BaP-treated mouse C3H10T1/2 embryo cells, in which colony survival was decreased by 50% at 0.3 μg/ml. Survival of the mouse lymphoma (6.0 μg/ml) and SHE cells (1.0 μg/ml) was reduced by 12% and 75%, respectively.

In mouse lymphoma cells, chromosome aberrations increased nonlinearly with BaP concentrations. At 6.0 μg BaP/ml, approximately 40 to 50 aberrations per 100 cells were observed The BaP-DNA binding levels reached a maximum of 30 to 50 μmol BaP/mol DNA at 4 to 4.5 μg BaP/ml (Arce et al., 1987).

The enhancement of SA7 viral transformation of SHE cells by BaP increased 1.5 to 7.0 fold over the concentration range of 0.2 to 1.0 μg/ml and was observed at doses as low as 0.2 lg/ml BaP. BaP-DNA binding in SHE cells showed a linear dose response relationship up to 1.0 μg/ml (Arce et al., 1987).

Morphological transformation of the C3H10T1/2 cells, measured by the appearance of types II and III foci, increased with concentration over the range of 0.2 to 1.5 μg BaP/ml to a maximum of approximately 12 types II and III foci/10^3 cells. Similarly, BaP-DNA binding levels in C3H10T1/2 cells increased with concentration up to 1.2 μg/mL in all experiments. Maximum binding levels were 45 μmol BaP/mol DNA (Arce et al., 1987).

Separation of BaP-DNA adducts by HPLC showed the predominant adduct to be anti-BPDE-dGuo in all the mammalian systems. These data show that: (1) DNA binding and genotoxic responses vary significantly among assays; (2) each genetic endpoint is induced with a differing efficiency on a per adduct

basis; and (3) there are linear relationships between mutagenic activity, frequency of transformation and amount of BaP-DNA bound.

4.6 Metabolic Activation Systems by Isolated Hepatocytes

A number of studies have been conducted using isolated hepatocytes in combination with the Salmonella typhimurium histidine reversion assay to study the potential genotoxicity of suspect chemicals. Neis and co-workers (1984, 1985, 1986a, 1986b) have reported substantial interspecies differences among isolated rat, hamster, guinea pig, dog, and human hepatocytes in their capacity to activate certain chemicals to their mutagenic forms. They compared the mutagenic activity of some known genotoxic agents (i.e., BaP) in the Salmonella histidine assay after activation by isolated hepatocytes from monkey (Macaca fasicularis) and man (Neis et al., 1986a). Mutagenic potency of BaP was 16-fold higher toward tester strain TA100 when monkey hepatocytes were used than when human hepatocytes served as the metabolic activating system. BaP was weakly mutagenic in TA100 in another study which used isolated human hepatocytes (Neis et al., 1986b). Monkey hepatocytes may have either a greater capacity to activate BaP or a lower glutathione-S-transferase activity, which in turn would influence the detoxification rate of BaP.

4.7 Mutagenicity in vivo

Treatment of pregnant mice with 2 mg/kg BaP orally during days 7 through 10 of gestation resulted in somatic mutations at several loci for coat color.

When BaP was administered several times during that period, 20 to 25% of the offspring showed mutations (Davidson and Dawson, 1977).

5. WHOLE ANIMAL TOXICOLOGY

5.1 Acute Toxicity

The LD_{50} for mice after intraperitoneal injection is 250 mg BaP/kg body weight (Salamone, 1981). Little data exists on acute toxicity of BaP.

5.2 Noncarcinogenic Subchronic and Chronic Toxicity

5.2.1 Miscellaneous Effects

Data on noncarcinogenic chronic toxic effects of BaP are limited (IARC, 1983; ATSDR, 1987). Studies in mice which are either highly inducible (Ah^b) or poorly inducible (Ah^d) for aryl hydrocarbon hydroxylase (AHH) indicate that chronic administration of BaP leads to inhibition of bone marrow proliferation and to aplastic anemia in mice where the detoxification system has been circumvented (Robinson et al., 1975). Chronic subcutaneous administration of PAH fractions which include BaP leads to liver damage in mice (Meiss et al., 1982). BaP has been shown to stimulate cell proliferation in rat trachea (IARC, 1983).

In an inhalation study of BaP with hamsters, Thyssen et al. (1980) noted that the control group gained weight while hamsters exposed to the lower dose of BaP used (9.8 mg/m^3) did not gain weight during exposure for 4.5 h/d, 5 d/wk for 16 weeks. The hamsters weighed 77 g. Based on the formula

$I = 0.5\ W^{0.9017}$ (EPA, 1988), they would inhale 0.053 m^3/day. Thus a LOAEL for BaP based on lack of weight gain of 0.9 mg/kg/d can be calculated. {[9.8 mg/m^3 x 0.053 m^3/d x 4.5 h/24 h x 5 d/7 d] / 0.077 kg = 0.9 mg/kg/d} Some limited information on thresholds based on reproductive endpoints are given in Sections 5.4 and 5.5.

5.2.2 Cardiovascular Toxicity

Heart disease is responsible for more deaths in the U.S. than cancer. Cancer of the heart is very rare but atherosclerosis has some attributes of cancer. Atherosclerotic plaques are similar to tumors in that plaques are monoclonal in origin, i.e., they arise from a single cell (Benditt and Benditt, 1973). Another attribute of plaques, similar to tumors, is the ability of human coronary artery plaque DNA to transform tissue culture cells (NIH 3T3). The transformed cells subsequently cause tumors when injected into athymic nude (T cell immunity deficient) mice (Penn et al., 1986).

Possible environmental causes of heart disease are coming under increasing scrutiny (Penn, 1989; Glantz and Parmley, 1991; Shaw et al., 1992; Glantz, 1993). PAHs, including BaP, have been implicated in heart disease, in part due to their presence in tobacco smoke (Glantz and Parmley, 1991; Penn and Snyder, 1993). Chimney sweeps, in addition to their increased risk of cancer due to PAH exposure in soot, also exhibit increased heart disease (Hansen, 1983).

Animal studies in pigeons and chickens indicate a role for PAH in atherosclerosis. Enzymes that are inducible by inducers of cytochrome P450 and that convert BaP to metabolites which bind covalently to DNA have been demonstrated in chicken aorta (Bond et al., 1980). The walls of arteries in pigeons contain monooxygenase (cytochrome P450) enzymes that metabolize BaP (Majesky et al., 1983). The enzymes are induced by a greater amount in atherosclerotic-susceptible (White Carneau) pigeons than in atherosclerotic-resistant (Show Racer) pigeons (Majesky et al., 1983), indicating that BaP metabolites might be responsible for the atherosclerotic effects. Both types of pigeons have appreciable levels of baseline (uninduced) activity. Atherosclerotic-resistant pigeons actually have higher baseline activity than atherosclerotic-susceptible pigeons. The enzyme results imply that monooxygenase levels are not the only factors involved in susceptibility.

Statistically significant increases in aortic plaque size have been reported in male White Carneau (atherosclerotic-susceptible) pigeons injected intramuscularly with 0.1, 10, and 100 ppm (mg/kg) BaP weekly for 6 months (Revis et al., 1984). Plaque number was also significantly increased at 0.1 and 10 ppm, but not at 100 ppm. In pigeons, the carcinogenic PAH 7,12-dimethylbenzanthracene and the noncarcinogenic PAH benzo(e)pyrene did not affect plaque size or number (Revis et al., 1984). It was not clear from the report how many animals were in each group but it may have been as few as four.

More recently Hough et al. (1993) studied BaP-enhanced atherosclerosis in the thoracic aorta and the brachiocephalic arteries of both

atherosclerotic-susceptible and atherosclerotic-resistant pigeons using weekly intramuscular injections of 10 mg/kg BaP. Females were much more susceptible than males to the effects of BaP on increased plaque size (as measured by area) and number. Since there were also effects on fertility in females, it was hypothesized that BaP exerted estrogenic effects on the arterial wall. The lesser effect of BaP in (atherosclerotic-susceptible) males in their study compared to that observed in Revis et al. (1984) was attributed by Hough et al. to housing stress (Hough et al. housed their pigeons in cages) with resulting increased lesions in the control (no BaP) groups. Unexpectedly, under these conditions of higher stress in the Hough et al. study, the females of the so-called atherosclerotic-resistant strain also showed increased plaque size and number due to BaP treatment. The main difference between atherosclerotic-susceptible and atherosclerotic-resistant pigeons in the Hough et al. study was that the atherosclerotic lesions in the atherosclerotic-susceptible controls were significantly larger than those in the atherosclerotic-resistant controls. No effects (relative to the controls) were seen in pigeons injected with the non-carcinogenic PAH benzo(e)pyrene.

Based on the study of Revis et al.(1984), 0.1 mg/kg/week (equivalent to 0.014 mg/kg/day) is a LOAEL for BaP for cardiovascular effects in pigeons.

Intramuscular injections of 50 mg/kg BaP in chickens for 13 to 18 weeks also resulted in significant increases in the size of atherosclerotic lesions (plaque) of the abdominal aorta relative to controls (Albert et al., 1977). In chickens, unlike pigeons, the carcinogenic PAH, 7,12-

dimethylbenzanthracene, was active in increasing plaque size (Albert et al., 1977).

Studies of the effects of pure BaP on plaque formation have not been carried out in mammals. However, injection (route unspecified) of the carcinogenic PAH 3-methylcholanthrene into mice led to a dose-dependent increase in the number and sizes of aortic lesions (Paigen et al., 1985). There is also indirect evidence that similar effects could occur in mammals exposed to BaP. For example, the application of cigarette smoke condensate, which contains BaP, to the skin of mice resulted in adducts in the DNA of heart tissue which chromatographed similarly to adducts derived from benzo(a)pyrene and other PAHs (Randerath et al., 1988). Thus, heart is a target tissue for BaP. There is need for a study of the effects of BaP on plaque development in a mammalian species.

5.3 Carcinogenesis Studies

BaP is carcinogenic by intratracheal, inhalation, and dermal exposure, by intraperitoneal injection, and when given in the diet.

5.3.1 Inhalation and Intratracheal Exposures

An important route of exposure to toxic air contaminants is via inhalation. Intratracheal administration of chemicals is often used as a model of inhalation exposure. Although it has limitations as a surrogate for that exposure, e.g., a less uniform distribution than inhalation (Brain et al., 1976; Phalen, 1984), the dose of chemical to the target organ is known. In

addition chronic inhalation exposures, especially with particulates, are technically difficult to perform. Thus, for BaP there is a large number of experiments demonstrating carcinogenicity by the intratracheal route. There is one inhalation experiment showing significant increases in lung tumors at two concentrations of BaP (Thyssen et al., 1981).

Early experiments, summarized by Saffiotti et al. (1968), indicated that PAHs are at least weakly carcinogenic to the respiratory tract after intrabronchial and intratracheal administration, provided that the compounds are suitably administered. Subsequently, Saffiotti et al. (1968) administered a mixture of BaP (3 mg) and Fe_2O_3 (hematite) (3 mg) in a saline suspension to Syrian golden hamsters by intratracheal instillation. The median particle size of the hematite was approximately 0.25 μm. Animals were dosed once per week for 15 weeks; controls were untreated or were exposed to Fe_2O_3 only. Eleven of 30 males (37%) and 21 of 30 females (70%) survived 15 weeks of exposure. Most animals receiving BaP plus Fe_2O_3 developed tumors of the respiratory tract (14/19 males and 21/21 females), whereas those receiving Fe_2O_3 only or those receiving no treatment did not develop tumors. One hundred percent of the animals that survived 15 weeks of treatment developed tumors. Tumors developed rapidly since all males were dead by the 45th week of the experiment; similarly, all females were dead by the 60th week of the experiment. The most common tumor type observed was bronchogenic carcinoma. Data are shown in the Tables 5.1 and 5.2.

Subsequently, Saffiotti et al. (1972) determined the carcinogenic dose-response relationship after intratracheal instillation of a suspension of

BaP and Fe_2O_3 in male and female Syrian golden hamsters. Test materials were administered once weekly for 30 weeks at 2.0, 1.0, 0.5, and 0.25 mg BaP/animal and an equivalent weight of Fe_2O_3 (hematite) as particulate carrier. Controls either received 2.0 mg Fe_2O_3/animal/dose or were untreated. Survival rates were shortened in the two highest BaP exposure groups. Tumors were not present in animals receiving ferric oxide or in untreated controls. Respiratory tract tumors (including squamous cell carcinomas of the larynx, of the trachea, and of the bronchi, adenocarcinomas of the bronchi, and adenomas of the bronchi and of the bronchioles and alveoli) developed in all groups of BaP/Fe_2O_3 treated animals; the response was dose related (Table 5.3). Tumors appeared most frequently in the bronchi and trachea. Other lesions present in the respiratory tract included: 1) patches of squamous metaplasia in the trachea, 2) areas of peripheral broncho-alveolar squamous metaplasia, and 3) peripheral proliferative lesions. An increase in papillomas of the forestomach was also observed.

Thyssen et al. (1980) conducted an inhalation study in which male Syrian golden hamsters were exposed to a BaP condensation aerosol (in 0.1% saline) with particle size ranging from 0.2 to 1.5 μm. Eight week old animals were exposed for 4.5 hr/day, 5 days/wk for 16 weeks at concentrations of 9.8 mg BaP/m^3. Another group (14 weeks of age) was exposed for 10 weeks to 44.8 mg BaP/m^3. Unexposed 14-week-old hamsters served as controls. The animals were observed for life. Mean survival times for the two exposed groups were not different from controls. Neoplastic changes in the respiratory tract were not seen even though the estimated doses were 2 to 10 times higher than the amounts received by hamsters in studies where carcinogenic

Table 5.1. Tumor Induction in Syrian Golden Hamsters following Intratracheal Instillation of BaP[a]

Group	Treatment[b]	Sex	Initial no. hamsters	Hamsters autopsied	Total hamsters with Respiratory tract tumors	Total hamsters with Other tumors
1	BaP + Hematite	M	30	28	14	1
		F	30	27	21	3
2	Hematite alone	M	24	20	0	2
		F	24	21	0	2
3	None	M	100	90	0	20
		F	100	86	0	13

[a] Adapted from Saffiotti et al. (1968).

[b] Intratracheal instillations were given once weekly for 15 weeks. Group 1: each dose consisted of 3 mg benzo[a]pyrene + 3 mg hematite in 0.2 ml saline. Group 2: each dose consisted of 3 mg hematite in 0.2 ml saline.

Table 5.2. Tumors of the Respiratory Tract in Syrian Golden Hamsters given Benzo[a]pyrene Intratracheally for 15 Weeks[a]

Tumor Incidence		Males	Females
Initial number of animals		30	30
Effective number of animals[b]		19	21
Survivors at the end of the exposure (16 weeks)		11	21
Tumor-bearing animals	Total number	14	21
	Percent of effective no. of animals	73%	100%
	Percent of survivors after 16 weeks	100%	100%
Total number of tumors		28	38
Sites and types of lesions			
Larynx	Carcinoma, squamous cell	2	0
Trachea	Squamous metaplasia alone	8	6
	Papilloma, squamous cell	3	8
	Polyp	1	0
	Carcinoma, squamous cell	5	7
	Carcinosarcoma	0	1
	Fibrosarcoma	1	1
Bronchi	Squamous metaplasia alone	5	3
	Carcinoma Squamous cell	4	12
	Anaplastic	6	4
	Adenocarcinoma	3	3
	Adenoma	3	2
	Adenomatoid lesion	13	13
Alveoli	Alveolar squamous metaplasia	1	0

[a] Adapted from Saffiotti et al. (1968).

[b] Animals that died in the first 6 weeks of treatment or that were lost because of post-mortem changes or cannibalism were discarded.

Table 5.3. Tumor Induction in Syrian Golden Hamsters after Intratracheal Administration of BaP and Fe_2O_3[a]

	Treatment[b] BaP:Fe_2O_3(mg)	Sex	Respiratory Tract Tumors[c] Effective no. of hamsters	Tumor-bearing animals	Total no. of tumors	Forestomach Tumors[d] Papilloma bearing animals	Total no. of papillomas
1M	2.0:2.0	M	28	17	28	13	17
1F		F	29	17	30	8	12
2M	1.0:1.0	M	33	22	31	8	11
2F		F	34	20	28	9	12
3M	0.5:0.5	M	33	10	11	5	7
3F		F	30	9	11	2	2
4M	0.25:0.25	M	47	6	6	2	2
4F		F	41	4	4	4	4
5M	0:2.0	M	47	0	0	2	2
5F		F	45	0	0	0	0
6M	Untreated	M	97	0	0	5	5
6F		F	96	0	0	2	2

[a] Adapted from Saffiotti et al. (1972).

[b] Intratracheal instillations were given once weekly for 30 weeks.

[c] Chi-square tests on groups 1M-5M (males) and 1F-5F (females) yielded $p<.001$ for each sex, which indicates a significant association between incidence of respiratory tract tumors and dose (Gad and Weil, 1986).

[d] Chi-square tests on groups 1M-5M (males) and 1F-5F (females) yielded $p<.001$ for each sex, which indicates a significant association between incidence of forestomach tumors and dose.

effects were observed after BaP was administered by intratracheal administration. However, the duration of exposure may have been too short for tumor induction.

In a subsequent experiment Thyssen et al. (1981) exposed male Syrian golden hamsters to BaP condensed onto sodium chloride particles at BaP concentrations of 2.2, 9.5, and 46.5 mg BaP/m^3. Controls were unexposed. Survival time was significantly decreased from 96 weeks for controls to 60 weeks for animals in the highest BaP exposure group; survival times were not altered in the other exposure groups. Tumors were not observed in the respiratory tract of the control group or the group that received 2.2 mg/m^3. The incidence of tumors in this organ system increased in a dose dependent manner for the 9.5 and 46.5 mg/m^3 exposure groups (34.6% and 52% respectively)(Table 5.4). In the animals exposed to 9.5 and 46.5 mg/m^3, tumors were seen in the nasal cavity, larynx, trachea, pharynx, esophagus, and forestomach; however, lung tumors were absent. The tumor types included papillomas, papillary polyps, and squamous cell carcinomas.

5.3.2 Feeding Studies

Feeding of pelletized chow containing BaP to male and female CFW mice caused gastric tumors (papillomas and squamous cell carcinomas), pulmonary adenomas, and leukemia (Rigdon and Neal, 1966; 1969; Neal and Rigdon, 1967). The pulmonary adenomas, gastric tumors, and leukemia occurred independently of each other (Rigdon and Neal, 1969). These experiments showed that BaP can cause tumors distal to the point of application. Incidences of gastric tumors of 70% or higher were seen in male and female

mice fed 50 to 250 ppm BaP for 4 to 6 months (Neal and Rigdon, 1967)(Table 5.5). A 50% incidence of stomach tumors was seen in mice fed 0.5% BaP for one day. Tumor incidences above background were seen in groups of mice fed 40 to 45 ppm BaP for 110 days. The overall data strongly suggest a positive carcinogenic effect since there were 0 gastric tumors in 289 control mice while 178 out of 454 mice fed various levels of BaP had gastric tumors (Neal and Rigdon, 1967). The pathological evaluation of the animals did not seem very thorough which could lead to an underestimation of the tumorigenic effect.

5.3.3 Dermal Application

BaP has been shown in many experiments to be carcinogenic by dermal application and is often used as a positive control in such experiments (ATSDR, 1987). Unfortunately it is often difficult to determine the dose given due to the method of application of the carcinogen. In 1933 BaP was shown to cause skin tumors in mice (Cook et al., 1933). Wynder and associates demonstrated a positive dose-response relationship for BaP-induction of skin tumors in Swiss and C57BL in mice and showed a tumor response at doses as low as 0.001% BaP applied topically in acetone every 2 weeks for up to 2 years (Wynder and Hoffmann, 1959, Wynder et al., 1957; 1960). In addition incidences of 95% for papillomas and carcinomas of the skin were obtained by chronic administration (3 times weekly for 1 year) of 0.01% BaP to Swiss mice (Wynder and Hoffman, 1959). Extensive experiments conducted by Conney and associates demonstrated the tumor initiating activity of BaP and several of its epoxide and hydroxy derivatives. For example, the administration of 0.15 μmol benzo(a)pyrene-7,8-dihydrodiol

Table 5.4. Respiratory Tract Tumors from Benzo(a)pyrene Inhalation in Male Syrian Golden Hamsters[a]

Exposure (mg/m^3)	Tumor Incidence[b]
0	0/27
2.2	0/27
9.5	9/26
46.5	13/25

[a] Adapted from Thyssen et al. (1981) and EPA (1984).

[b] A Chi-square test yielded $p<.001$, which indicates a significant association between incidence of respiratory tract tumors and dose.

Table 5.5. Gastric Tumors in Mice from Feeding Benzo(a)pyrene[a]

Exposure (ppm)	Incidence of Gastric Tumors[b,c]
0	0/289
1	0/25
10	0/24
20	1/23
30	0/37
40	1/40
45	4/40
50	24/34
100	19/23
250	66/73

[a] Adapted from Neal and Rigdon (1967).

[b] Number responding with gastric papillomas or carcinomas over number exposed to given food concentration.

[c] A Chi-square test yielded $p<.001$, which indicates a significant association between incidence of gastric tumors and dose of BaP.

(ATSDR, 1987). Unfortunately it is often difficult to determine the dose given due to the method of application of the carcinogen. In 1933 BaP was shown to cause skin tumors in mice (Cook et al., 1933). Wynder and associates demonstrated a positive dose-response relationship for BaP-induction of skin tumors in Swiss and C57BL in mice and showed a tumor response at doses as low as 0.001% BaP applied topically in acetone every 2 weeks for up to 2 years (Wynder and Hoffmann, 1959, Wynder et al., 1957; 1960). In addition incidences of 95% for papillomas and carcinomas of the skin were obtained by chronic administration (3 times weekly for 1 year) of 0.01% BaP to Swiss mice (Wynder and Hoffman, 1959). Extensive experiments conducted by Conney and associates demonstrated the tumor initiating activity of BaP and several of its epoxide and hydroxy derivatives. For example, the administration of 0.15 μmol benzo(a)pyrene-7,8-dihydrodiol topically to mice once every 2 weeks for 60 weeks resulted in skin tumors in 100% of the mice (summarized by EPA, 1979 and by Conney, 1982).

5.4 Teratology and Developmental Effects

Treatment of pregnant mice with an intraperitoneal injection of 200 mg BaP/kg at 7, 10, or 12 days of gestation resulted in intrauterine mortality (Shum et al., 1979). This effect was most pronounced in mouse strains genetically responsive at the Ah locus. In addition, malformations were observed only in the genetically responsive strain. These effects may be due to allelic differences at a single genetic locus which controls the production of cytosolic receptors that bind certain xenobiotics including BaP. This xenobiotic-receptor complex activates the genes responsible for the biosynthesis of cytochrome P450-dependent monooxygenase (aryl

hydrocarbon hydroxylase or AHH) that converts BaP to reactive intermediates. Non-responsive mice have either smaller amounts of receptor or the receptor has a decreased affinity for BaP. The results of Shum et al. (1979) indicate that the responsive animals, those that are inducible for monooxygenase activity, have the ability to convert BaP to reactive intermediates and that these BaP metabolites are responsible for the observed *in utero* toxicity and malformations.

The question of whether in utero toxicity, malformations, and cross-placental carcinogenesis result from maternal metabolic capability or from fetal exposure to parent BaP with subsequent fetal metabolism was evaluated by Shum et al. (1979). A portion of Ah locus heterozygous fetuses from BaP-treated (20 mg/kg), non-responsive homozygous dams were resorbed; those that survived had increased incidences of congenital anomalies. In contrast, fetuses homozygous for the nonresponsive gene had few, if any, congenital anomalies. These results provide strong evidence for the involvement of fetal rather than maternal metabolism of BaP. Furthermore, these results are consistent with data suggesting that BaP moves freely across the placenta (Kelman and Springer, 1982). BaP metabolism and induction were first measured directly in fetal tissue homogenates in the 1970's (Juchau et al., 1972). Detection of production of all three trans-dihydrodiols in several fetal tissues (Pelkonen and Karki, 1975) suggested the presence of epoxide hydrolase activity in fetal tissues. Blanck et al. (1983) suggested that fetal AHH activity might undergo induction by BaP, since fetal microsomes of smoking mothers metabolized BaP more rapidly than those from non-smokers, but the data were not conclusive due to small

sample size. Fetal epoxide hydratase and AHH activities and inducibility were described by Peng et al. (1984).

Namkung and Juchau (1980) demonstrated that the proportion of BaP converted to dihydrodiol and monohydroxy metabolites by the placenta is concentration-dependent. At a low BaP concentration (2.7 μM), similar to that from ambient exposure, proportionally more dihydrodiol metabolites were produced. The results indicated that a higher proportion of 7,8-dihydrodiol, the predominant carcinogenic and mutagenic form of BaP, may be generated *in vivo* by the placenta at typical conditions of (low level) exposure such as cigarette smoking. Pelkonen and Saarni (1980) reported that incubation of placental microsomes with radiolabeled BaP and purified DNA resulted in covalent binding of BPDE, as well as other metabolites, to DNA. Others have reported that human fetal liver and human placental microsomal preparations from non-smoking mothers form few or no BaP metabolites, while similar preparations from smoking mothers produce 7,8-dihydrodiol as well as other metabolites of BaP (Gurtoo et al., 1983). Fetal liver microsomes convert BaP to monohydroxy metabolites only (Juchau et al., 1972).

5.5 Reproductive Effects

Exposure of animals to a variety of PAH, including BaP, results in adverse reproductive effects in females. Intraperitoneal and intraovarian injection of various PAH, including BaP, results in dose-, time-, strain-, and species-dependent destruction of primordial oocytes in mice (Mattison and Nightingale, 1980; Mattison and Thorgeirsson, 1979; Takizawa et al.,

1984). Effects were seen with single doses of 80 or 100 mg BaP/kg or with a total of 100 mg/kg divided evenly among 4 or 10 daily doses. The last yields a LOAEL for this endpoint of 10 mg/kg/d based on a 10 day exposure. Studies with AHH-inducible (C57BL/6N) mice and non-inducible (DBA/2N) mice demonstrated that the effective dose (ED_{50}) of BaP for oocyte destruction after intraovarian injection is 3.38 μg/ovary and 36.14 μg/ovary for inducible and noninducible strains, respectively; threshold doses for oocyte effects were similar between the two strains at 0.05 μg/ovary (Takizawa et al., 1984). Unilateral intraovarian injection of BaP caused destruction of primordial oocytes in the treated ovary but not in the untreated ovary, suggesting that the ovary has the metabolic capability for enzymatic conversion of the BaP to ovotoxic reactive intermediates (Mattison and Nightingale, 1980). This hypothesis is supported by decreased ovotoxicity when mice are pretreated with alpha-naphthoflavone, a compound that competes with BaP for the monooxygenase active site (Mattison and Thorgeirsson, 1979). In addition, intrauterine BaP exposure resulted in induction of AHH activity in the inducible strain, but not in the noninducible strain, without induction of hepatic AHH activity or increases in cytochrome P450 levels (Mattison and Nightingale, 1980). Using the intraovarian injection protocol, Takizawa et al. (1984) administered BaP-7,8-diol, BaP-diol-epoxide (BPDE), and BaP and found that the rank order for doses required for small oocyte destruction was BaP $\simeq$ BaP-diol $\gg$ BPDE, demonstrating that BPDE is the most toxic metabolite.

Bengtsson et al. (1983) demonstrated that PAH, including BaP, are metabolized in the ovary by microsomal cytochrome P450-dependent monooxygenases and that other detoxification enzymes such as glutathione-S-

transferase and epoxide hydratase are also present in ovaries. Comparison of BaP metabolism in rat ovaries and adrenals indicates that the predominant products are diols and monohydroxy derivatives for both organs. By comparison, liver produces these metabolites, along with quinones, which are not produced in the ovary and adrenal preparations. Kinetic data demonstrate that ovarian AHH has a 20- and 200- fold higher affinity (lower K_m) for BaP than adrenal and liver AHH, respectively. These results suggest that at low PAH exposure, where concentrations are non-saturating, the ovaries and adrenals may be substantially more efficient at bioactivation than liver, thereby increasing the potential for toxic effects in these organs.

MacKenzie and Angevine (1981) exposed CD-1 mice in utero, during days 7 to 16 of gestation, to 0, 10, 40, or 160 mg BaP/kg maternal body weight. Ninety-seven percent of the offspring (both male and female) of dams exposed to 40 and 160 mg/kg were sterile. Both impaired fertility and histological alterations in reproductive tissues were noted in those F_1 mice exposed to 10 mg BaP/kg during gestation. From this study, a LOAEL of 10 mg/kg was determined for reproductive and developmental toxicity (ATSDR, 1987). Thus BaP causes reproductive tissue toxicity both by direct injection and by more usual toxicological test protocols.

6. HUMAN STUDIES

The predominant sources of airborne BaP are combustion processes. Thus, this compound rarely enters the environment alone but rather is associated with additional PAHs and other components frequently present in particulate form. Available epidemiological information, therefore, is from persons exposed to mixtures such as tobacco smoke, diesel exhaust, air pollutants, synthetic fuels, or other similar materials. IARC (1987) lists several mixtures containing PAHs which are carcinogenic to humans (Table 6.1). Those in class 1 are classified as known human carcinogens based on epidemiologic studies of cancer in people. One of these, soot, has been known to cause cancer in people since 1775 when Percival Pott described scrotal cancer in chimney sweeps. In addition other processes, in which PAH exposure occurs but which also include exposure to other carcinogens, processes such as iron and steel founding and aluminum production, are also known human carcinogens (IARC, 1987). Several IARC volumes have been dedicated to the analysis of cancer in processes which involve exposure to polynuclear aromatic compounds (i.e., PAHs) (IARC, 1983; 1984a; 1984b; 1985). The types of cancer reported are often consistent with the exposure pathway: scrotal cancer and lung cancer in chimney sweeps exposed to soot; skin cancer (including scrotal cancer) where shale oils are used; and lung cancer where airborne exposure of PAHs occurs, such as in iron and steel foundries.

Because it is difficult to determine the relative contribution of individual carcinogenic agents in studies with mixtures, molecular approaches to estimate exposure are being developed to aid epidemiologic

Table 6.1. IARC Groupings of Mixtures with PAHs

Group 1	Group 2A	Group 2B
Coal-tar pitches	Creosotes	Carbon black extracts
Coal-tars		
Coke production		
Mineral oils (untreated and mildly treated)		
Shale-oils		
Soots		
Tobacco smoke		

Abstracted from IARC Supplement 7 (1987).
Group 1: carcinogenic to humans.
Group 2A: probably carcinogenic to humans.
Group 2B: possibly carcinogenic to humans.

studies. In addition, DNA-adduct levels reflect the fraction of the dose that escapes detoxification and binds to DNA. While these methods are usually developed with animal models, recent reports describe their application to human studies.

Shamsuddin and Gan (1988) examined several human tissues collected at surgery or autopsy using rabbit high-specificity antibody to BPDE-DNA adducts and light immunocytochemistry. Immunological methods can be highly specific for specific BaP metabolites present in DNA adducts (Santella et al., 1985). Antigenicity was detected by Shamsuddin and Gan (1988) in a number of tissues including lung, ovary, placenta, uterine cervix, and white blood cells. Their results indicated that the tissue concentration of adducts varies substantially in the human population. One of five specimens showed BPDE-DNA antigenicity in bronchial epithelial cells, whereas levels were moderately high in oocytes of all the smokers examined. Placental specimens were positive for BPDE-DNA adducts for both smokers and non-smokers with the chorionic villi showing the strongest response. This response was not unexpected since the placenta is known to have high levels of AHH activity. Results from this study (and others as shown in Chapter 3) indicate that BPDE-DNA adducts can be detected in human tissues by immunochemical techniques.

Other studies have also demonstrated that immunological methods have the selectivity and sensitivity to detect BPDE-DNA adducts in human tissues. Five of twelve human lung samples obtained at surgery, from smokers or former smokers, showed positive antigenicity for BPDE-DNA adducts with values ranging from 4 to 12 fmoles BPDE/mg DNA (Garner et al., 1988).

The ^{32}P-postlabeling method has been used to study BaP adducts in human tissues (Randerath et al., 1986; Hemminki et al., 1988). This method is highly sensitive in that it will detect one adduct in 10^8 to 10^{10} DNA nucleotides; however, the method does not provide structural information about the adducting species, only that the adduct co-chromatographs with known adducts. In iron foundry workers in Finland higher DNA-adduct levels were detected in the white blood cells of workers with jobs in high PAH exposure areas than in the white blood cells of workers with jobs in low PAH exposure areas (Perera et al., 1988; Hemminki et al., 1990). In a subsequent report 61 workers were classified as high, medium, or low BaP exposure. There was a highly significant correlation between BaP exposure and DNA-adduct levels (Reddy et al., 1991). Perera et al. (1993) also found a correlation in foundry workers between PAH-DNA adduct levels and mutation frequency at the hypoxanthine guanine phosphoribosyl transferase (HPRT) locus in their lymphocytes. In another study of worker exposure, that around coke ovens, Ovrebo et al. (1992) divided workers into high, medium, and low estimated exposure and found a correlation between estimated exposure and PAH-DNA adducts. Perera et al. (1993) extended the technique to look at environmental exposures in a highly industrialized and polluted area of Poland. They found that PAH adducts were higher in an industrialized area in winter than both in a more rural area in winter and in the same urban area in summer (when less burning of fuel would occur).

Several investigators are now looking at PAH-derived adducts bound to serum protein which is more abundant and easier to obtain than DNA from cells in the blood. One study found somewhat higher levels of PAH-albumin adducts in foundry workers and in roofers than in their respective reference groups

(Lee et al., 1991). Another study compared 45 foundry workers with 45 controls for levels of BaP-derived adducts bound to serum protein (Sherson et al., 1990). Smokers had higher levels than non-smokers and workers in high BaP exposure areas had 2 to 3 times the levels of workers in low exposure areas.

Studies with human placental tissues have shown that AHH activity is several times higher in smokers than non-smokers and that this activity increases in a sigmoidal manner with increased numbers of cigarettes smoked (Gurtoo et al., 1983). Moreover, the interindividual variability for AHH activity is high (by as much as 1000-fold), suggesting that genetic factors contribute to this variability and, ultimately, to susceptibility of individuals to tumor development and reproductive effects (Manchester and Jacoby, 1984).

Human lung tissue collected at autopsy was analyzed for BaP (Weisz, 1971; Weisz et al., 1971). Tissues were digested chemically and the digest was extracted with an organic solvent. BaP was separated by chromatography, then quantitated by fluorescence. BaP concentrations ranged from 0.3 to 15.6 μg/lung and were not correlated with sex, occupation, or other known risk factors. Unfortunately these other known factors, which should include cigarette smoking, were not provided in the report.

7. QUANTITATIVE RISK ASSESSMENT

7.1. Noncancer Health Effects

In part A of this document, the California Air Resources Board estimates that the annual average ambient air level of benzo(a)pyrene in California is 5.3×10^{-4} $\mu g/m^3$. A "standard man" weighing 70 kg and breathing 20 m^3 of air daily will have an intake of 1.1×10^{-2} μg BaP per day (5.3×10^{-4} $\mu g/m^3$ x 20 m^3) or 1.1×10^{-5} mg per day. Dividing by the 70 kg body weight yields a dose of 1.6×10^{-7} mg/kg/day. At this level noncancer health effects are unlikely to occur, since this expected dose to humans is on the order of one-millionth that which causes adverse effects on fertility, oocyte destruction, and weight gain in mammals and cardiovascular effects in pigeons (LOAELs of 10, 10, 0.9, and 0.014 mg/kg/day, respectively) (see Chapter 5). However, NOAELs and LOAELs for BaP effects in animals are very scarce and are non-existent for humans. Such scarcity of data constitutes a data gap for BaP. Data for additional toxicologic endpoints are needed.

7.2. Carcinogenic Risks

A very large number of experiments have demonstrated that BaP causes tumors at several sites, by several routes of administration, in both sexes, and in several animal species. (See Chapter 5; Zeise and Crouch, 1984; Jones and Walsh, 1985; IARC, 1983). Many studies, however, are very limited in scope or in data reported and are not suitable for risk assessment (Zeise and Crouch, 1984).

OEHHA guidelines prescribe that risk assessments use the most sensitive sex, site, and species where a significant increase in cancer incidence is observed (California Department of Health Services, 1985). For BaP this indicates the use of data on gastric tumors (papillomas and squamous cell carcinomas) observed in male and female mice due to feeding of BaP (Neal and Rigdon, 1967). In addition, large numbers of mice (319 experimental, 289 control) were used to establish the dose-response curve. Potency estimates were also derived from the data for respiratory tract tumors in hamsters from the inhalation bioassay of Thyssen et al. (1981) and from data obtained after intratracheal administration of BaP (Saffiotti et al., 1972; Feron et al., 1973). Cancer risk associated with exposure to ambient levels of BaP is estimated by extrapolating approximately five orders of magnitude from the experimental data to ambient levels by means of the best fitting linearized multistage model GLOBAL86 (Howe and van Landingham, 1986). In addition other models have been fit to the data for comparison. In its risk assessment, the EPA used the data for stomach tumors from oral exposure to benzo(a)pyrene in mice and the data for respiratory tract tumors from inhalation exposure in hamsters to estimate cancer potency and unit risks associated with exposure to BaP (EPA, 1984).

7.2.1. Thresholds

A threshold dose of a toxicant is one below which a specified outcome does not occur. While threshold models for carcinogenesis have been proposed based on various mechanisms such as saturation of detoxification enzymes, the existence of DNA repair mechanisms, and recurrent toxicity, none has been convincingly demonstrated. An "epigenetic" mechanism, that could in theory embody

threshold doses, has been invoked to explain the carcinogenic action of substances that do not directly produce genetic damage in short-term tests. However, for benzo(a)pyrene there is compelling evidence of genotoxicity. Metabolites of benzo(a)pyrene bind to DNA and mutagenic responses have been observed in a wide variety of prokaryotic and eukaryotic systems (Chapter 4). There is experimental evidence that benzo(a)pyrene acts as an initiator of tumorigenesis (Chapter 5). Tumors have been noted after a single high dose of BaP (e.g., Neal and Rigdon, 1967). These are not considered to be threshold phenomena. Therefore, OEHHA staff treats benzo(a)pyrene-induced carcinogenesis as a nonthreshold phenomenon and, as such, applies a nonthreshold, linear extrapolation model for cancer potency estimation.

7.2.2. Animal Data and the Multistage Model

Since there is no adequate information regarding the carcinogenicity of BaP to humans from epidemiological studies, data from animal bioassays are extrapolated to estimate human cancer risk. The linearized multistage model developed by Crump and colleagues, utilized in the computerized form as GLOBAL86 (Howe and van Landingham, 1986), is used to estimate the cancer potency of BaP. This model and its earlier versions such as GLOBAL82 have been used in previous risk assessments by the OEHHA/Department of Health Services and is the model preferred by EPA (Anderson et al., 1983; EPA, 1986). The model uses animal tumor incidence data to compute maximum likelihood estimates (MLE) and upper 95% confidence limits (UCL) of risk associated with a particular dose. The UCL is regarded as the upper limit of the true risk. The true risk is very unlikely to be greater than the upper limit, may be lower than the upper limit, and could be zero. The linearized multistage

model yields upper bound estimates of risk which are a linear function of dose at low doses and are used frequently as a basis for regulation. They are more stable statistically and more protective of public health than are MLEs.

The linearized multistage model is based on several assumptions about the process of carcinogenesis. Cancer is assumed to be an irreversible process which originates in a single cell and involves a number of biological events or stages. The rate of occurrence of each stage varies linearly with dose. In addition the incidences of background and chemically-induced cancer are assumed to be additive.

The multistage model may be expressed mathematically as:

$$P(d) = 1 - e^{-(q_0 + q_1 d + q_2 d^2 + \ldots + q_k d^k)} \qquad \text{[Eq. 1]}$$

where P(d) is the lifetime probability of developing a tumor at a given dose d of carcinogen, q_0 is a constant that accounts for the background incidence of cancer (i.e., occurring in the absence of the carcinogen under consideration), and q_1, q_2, ... q_k are coefficients that allow the data to be expressed to various powers of the dose of carcinogen in order to obtain the best fit of the model to the data. In order to determine the extra risk above the background rate at dose d or:

$$P_e(d) = [P(d) - P(0)]/[1-P(0)] \qquad \text{[Eq. 2]}$$

the equation takes the form:

$$P_e(d) = 1 - e^{-(q_1 + q_2 d^2 + \ldots + q_k d^k)} \qquad \text{[Eq. 3]}$$

At low doses, the extra risk is approximated by:

$$P_e(d) = q_1 d \qquad \text{[Eq. 4]}$$

The goodness-of-fit of the model to the data in the observed range is tested using the asymptotic Chi-square distribution of the log-likelihood ratio; values of Chi-square which give a p value > 0.01 are considered by the USEPA and the developers of the program to indicate an acceptable fit. In recent documents OEHHA has considered p > 0.05 to be acceptable. As shown below the p values of the fits in the present analysis are greater than 0.05.

Using the computer software GLOBAL86, the linearized multistage model was fit to the dose-response data from the studies of gastric tumors in mice and respiratory tract tumors in hamsters given in Tables 7.1 and 7.2, respectively (see Chapter 5).

For the mouse data, to obtain a statistically acceptable fit of the model to the data, the data in the highest three dosage groups (Table 7.1) were not used (EPA, 1984). This step is unfortunate since all the data are not used and the data which are not used show a very high tumor incidence (Table 7.1). (The strong carcinogenic response at the high doses adds to the weight of evidence for the carcinogenicity of BaP.) However, with this change the p value for goodness of fit for the multistage model is an acceptable 0.37. The mouse gastric tumor data yield a maximum likelihood estimate (MLE) for q_1 (the linear or slope term, which relates the probability of cancer to the dose of carcinogen administered) of 0.006 $(mg/kg/day)^{-1}$ and a q_0 equal

Table 7.1. Gastric Tumors in Mice from Feeding Benzo(a)pyrene[a]

Exposure (ppm)	Calculated Daily Dose[b] (mg/kg/day)(animal)	Incidence of Gastric Tumors[c]
0	0	0/289
1	0.078	0/25
10	0.781	0/24
20	1.563	1/23
30	2.344	0/37
40	3.126	1/40
45	3.516	4/40
[50	3.908	24/34][d]
[100	7.815	19/23][d]
[250	19.538	66/73][d]

[a] Adapted from Neal and Rigdon (1967) and EPA (1984).

[b] Calculation based on a 0.034 kg mouse consuming 13% of its body weight in food daily for 110 days during a 183 day experiment.

[c] Number responding with gastric papillomas or carcinomas over number exposed to given food concentration.

[d] Use of these exposure concentrations does not result in a statistically acceptable fit of the GLOBAL 86 multistage model to the data set (see text).

Table 7.2. Respiratory Tract Tumors from Benzo(a)pyrene Inhalation[a]

Exposure (mg/m^3)	Hamster Dose (mg/kg/day)[b] based on I = $0.037\ m^3/day$[c]	$0.063\ m^3/day$[d]	Tumor Incidence
0	0	0	0/27
2.2	0.089	0.152	0/27
9.5	0.385	0.655	9/26
[46.5			13/25][e]

[a] Adapted from Thyssen et al. (1981) and EPA (1984).

[b] Based on the various exposure conditions in Thyssen et al. (1981). The calculation is presented in EPA (1984). For the lowest dose, $2.2\ mg/m^3 \times [(10wk/95.2\ wk \times 4.5h/24h) + (85.2wk/95.2wk \times 3h/24h)] \times 0.037m^3/d\ /\ 0.12kg = 0.089\ mg/kg/day$.

[c] Inhalation rate assumed in EPA (1984) based on a standard hamster size of 0.120 kg and an allometric equation based on rat data.

[d] Based on $I = 0.5\ W^{0.9017} = 0.5\ (0.1^{0.9017}) = 0.063m^3/day$ (EPA), 1988. Thyssen et al. (1981) used 0.1 kg hamsters.

[e] These data were not used due to shortened lifespan of the hamsters in the exposure group. (The carcinogenic response, however, is apparent.)

to 0. An Upper 95% Confidence Limit (UCL) on q_1, q_1^*, equal to 0.0222 $(mg/kg/day)^{-1}$, also known as the carcinogenic potency, was obtained from the data. The values for q presented here are the same values EPA obtained using the same data (EPA, 1984).

Several numerical adjustments must be made to convert the q_1^* calculated from the animal data to a q_1^* relevant to humans. The experimental, less-than-lifetime exposure period L_e (183 days for the Neal and Rigdon mouse bioassay) was adjusted to an equivalent lifetime exposure L, estimated by the EPA (1984) to be equal to 630 days for mice, by dividing L_e by L. This term was then raised to the third power, based on the assumption that cancer incidence increases with the third power of age. A surface area scaling factor, the human to animal body weight ratio raised to the 1/3 power, was then applied to relate the experimental animal doses to equivalent human doses. Thus:

$$q_1^* \text{ (human)} = q_1^* \text{ (animal)} \times (L/L_e)^3 \times (\text{human bw/animal bw})^{1/3}$$

$$q_1^* \text{ (human)} = 0.0222 \times (630/183)^3 \times (70/0.034)^{1/3} = 11.5 \text{ (mg/kg/d)}^{-1}$$

If a 2 year (730 day) lifespan is used for mice, q_1^* is 17.9 $(mg/kg/d)^{-1}$.

The value of 11.5 $(mg/kg/day)^{-1}$ obtained using the Neal and Rigdon data is consistent with other estimates for BaP carcinogenicity. In an unpublished study, Zeise and Crouch (1984) examined all existing multiple dose data sets of BAP carcinogenicity in rodents (principally mice) induced by feeding, by gavage, and through the drinking water, including the Neal and Rigdon (1967) data. Many data sets were faulty due to lack of controls or lack of explicit

information on the conduct of the experiment in the paper. Multistage models were fit to the data sets. Most animal cancer potency value estimates, expressed as coefficients of dose to the first power, were in the range of 0.2 to 1.3 $(mg/kg/day)^{-1}$. Multiplying by a mouse to man surface area correction factor of $(70/0.03)^{1/3} = 13.3$ yields a range of human potency estimates of 2.6 to 17.2 $(mg/kg/day)^{-1}$.

Assuming that the percentage of benzo(a)pyrene absorbed by the respiratory tract after inhalation is similar for mice and humans (Chapter 3) and using an average human body weight of 70 kg and an average inhalation rate of 20 m^3 per day, a dose of 1 mg/kg/day benzo(a)pyrene is equivalent to breathing air with a BaP concentration of 3500 $\mu g/m^3$. Using the latter units, the 95% UCL for q_1, q_1^*, equals 3.3×10^{-3} $(\mu g/m^3)^{-1}$. This is the (lifetime) unit risk value for inhalation. Since benzo(a)pyrene generally occurs in particulate matter, calculations in units of $(ppm)^{-1}$ or $(ppb)^{-1}$ are not appropriate.

The multistage model was also fit to data for respiratory tract tumors resulting from inhalation exposure of hamsters to benzo(a)pyrene (Table 7.2). The data from the highest dose group were not used since these animals had an appreciably shortened lifespan (59 weeks versus 96 weeks in other groups) (Thyssen et al. 1981; EPA, 1984). By considering the conditions of exposure given in the report and using an inhalation rate of 0.037 m^3/day and a "standard" body weight of 0.12 kg for hamsters (EPA, 1988), a dose of benzo(a)pyrene in mg/kg/day can be estimated (Table 7.2) (EPA, 1984). From the hamster inhalation data, MLE values of $q_0 = 0$, $q_1 = 0$ and $q_2 = 2.68$ $(mg/kg/day)^{-1}$ were obtained. The p value for goodness of fit was 0.43. For 95% UCLs, the model yields a $q_1^* = 0.73$ and a $q_2^* = 0.78$. For practical

purposes, at low doses such as those encountered by breathing ambient air which are 5 orders of magnitude below the experimental doses, only q_1^* is usually important mathematically. The q_1^* obtained using the animal data can then be converted to a q_1^* (human) by multiplying by (human bw/hamster bw)$^{1/3}$, the surface area extrapolation factor, to obtain a q_1^* (human) = 6.11 (mg/kg/day)$^{-1}$. This potency estimate was obtained by EPA (1984) and is within a factor of 2 of that obtained using mouse stomach tumor data. The unit risk derived from this data set is 1.7×10^{-3} (μg/m^3)$^{-1}$.

The inhalation rate for hamsters of 0.037 m^3/day used by the EPA (1984) is a low estimate (Marty Miller, New York Department of Health, personal communication). EPA has recently published a handbook with recommended values for biological parameters used in risk assessment (EPA, 1988). The allometric formula given for the inhalation rate of hamsters is $I = 0.5\ W^{0.9017}$. Hamsters weighing 100 g (0.1 kg), as actually used by Thyssen et al. (1981) in their studies, would thus inhale 0.063 m^3 per day. With this higher inhalation rate and lower body weight, 70% higher doses of BaP are estimated (Table 7.2) and a q_1^* (animal) equal to 0.43 (mg/kg/day)$^{-1}$ is obtained. Multiplying by the interspecies surface area correction factor of $(70/0.1)^{1/3}$ yields a human equivalent q_1^* = 3.8 (mg/kg/day)$^{-1}$ for ingestion and one of 1.1×10^{-3}(μg/m^3)$^{-1}$ for inhalation.

Several studies have used intratracheal instillation of BaP to demonstrate its carcinogenicity (Section 5.3.2). While this method of dosing is radically different from inhalation, a larger percentage of the dose gets to the lower respiratory tract than with inhalation. There are several data sets for tumors due to intratracheal instillation which are available for carcinogenic

potency determination. Two experiments were selected and evaluated since they involved a relatively large number of doses and animals. Saffiotti et al. (1972) dosed groups of male and female hamsters with 0.25, 0.5, 1, and 2 mg BaP (with an equal weight of ferric oxide as particulate carrier) weekly for 30 weeks and observed the animals for their lifetimes. Some animals in each dose group showed respiratory tract tumors (Table 7.3). Applying surface area extrapolation and correction factors as described in the footnotes of Table 7.3 to the results of the multistage model yields a q_1^* (human) of 16.9 $(mg/kg/day)^{-1}$ from the data on males and 15.7 $(mg/kg/day)^{-1}$ from the data on females. In another experiment Feron et al. (1973) gave male Syrian golden hamsters intratracheal doses of 0, 0.625, 0.125, 0.5, or 1 mg BaP weekly for 52 weeks. A variety of tumors were produced throughout the respiratory tract. Data on bronchoalveolar adenomas and carcinomas were selected for risk assessment (Table 7.4). The data yielded a human equivalent q_1^* of 15.3 $(mg/kg/day)^{-1}$, in good agreement with other estimates. The potencies obtained from the several data sets with GLOBAL86 are summarized in Table 7.5.

7.2.3. Risk Estimate using the Gaylor-Kodell Approach

The application of the multistage model requires the use of a computer program to fit the model to the data and extrapolation into the range below the lowest dose tested. Another approach was introduced by Gaylor and Kodell (Gaylor and Kodell, 1980; Williams and Burson, 1985). In this method a model is fit to the observed responses, then the 95% upper confidence limit (UCL) of the value predicted from the model is determined for the lowest dose of risks result from not using the incidence data for the 20 ppm dose (Table 7.1). Clement Associates calculated dose coefficients of 3.22 and 5.74 $(mg/kg/day)^{-1}$

Table 7.3. Respiratory Tract Tumors from Intratracheal Instillation of Benzo(a)pyrene in Hamsters- 30 Week Exposure[a]

Weekly Dose[b] (mg)	Average Daily Dose (mg)	Lifetime Adj. Daily Dose[c] (mg/kg/day)	Human Equivalent[d] Dose (mg/kg/day)	Tumor Incidence[e] Males	Females
0	0	0	0	0/47	0/45
0.25	0.036	0.119	0.013	6/47	4/41
0.5	0.071	0.239	0.027	10/33	9/30
1.0	0.143	0.477	0.054	22/33	20/34
[2.0	0.286	0.953	0.107	17/28	17/29][f]

[a] Adapted from Saffiotti et al. (1972).

b All groups received an equal amount of benzo(a)pyrene and ferric oxide except the 0 control which received only 2 mg ferric oxide.

[c] Calculated by dividing the weekly dose by 7 days and 0.1 kg body weight and multiplying by (30 weeks exposure/90 week lifetime). There was a dose-related decrease in survival in the experiment.

[d] The animal daily dose was divided by $(70\ kg/0.1\ kg)^{1/3}$.

[e] Animals with respiratory tract tumors over effective number of exposed animals (i.e., those not cannibalised and not showing post-mortem autolysis). There was an additional control group which was untreated. No respiratory tract tumors were noted among 97 males and 96 females. A dose-dependent increase in gastric tumors was also noted in this experiment.

[f] Data group was not used since exposure started 7 weeks after other groups.

Table 7.4. Bronchoalveolar Tumors from Intratracheal Instillation of Benzo(a)pyrene in Hamsters- 52 Week Exposure[a]

Weekly Dose (mg)	Average Daily Dose (mg)	Lifetime Adj. Daily Dose[b] (mg/kg/day)	Human Equivalent[c] Dose (mg/kg/day)	Tumor Incidence[d]
0	0	0	0	0/29
0.0625	0.009	0.0495	0.0059	1/30
0.125	0.018	0.0989	0.0118	4/30
0.25	0.036	0.198	0.0237	6/30
0.5	0.071	0.395	0.0473	17/30
1.0	0.143	0.791	0.0947	19/30

[a] Adapted from Feron et al. (1973).

[b] Calculated by dividing the weekly dose by 7 days and 0.12 kg and multiplying by (52 week exposure/78 week length of experiment).

[c] Calculated by dividing the animal daily dose by $(70\ kg/0.12\ kg)^{1/3}$.

[d] Animals bearing bronchoalveolar adenomas and/or carcinomas over the number of animals examined.

Table 7.5 Cancer Potency Estimates obtained from GLOBAL86 using Various Data Sets

Data Set	Tumor Type[a]	Variable	95% UCL $(mg/kg/d)^{-1}$	95% UCL $(\mu g/m^3)^{-1}$
Neal & Rigdon (1967)	gastric		11.5	3.3×10^{-3}
Thyssen et al. (1981)	respiratory	$I=.037 m^3/d$	6.1	1.7×10^{-3}
Thyssen et al. (1981)	respiratory	$I=.063 m^3/d$	3.8	1.1×10^{-3}
Saffioti et al.(1972)	respiratory	(males)	16.9	4.8×10^{-3}
Saffioti et al.(1972)	respiratory	(females)	15.7	4.5×10^{-3}
Feron et al. (1973)	respiratory		15.3	4.4×10^{-3}

[a] For more details on tumor type see Chapter 5.

(Clement Associates, 1987, 1988 respectively) using the two stage model. For comparison, the multistage model yields a q_1^* of 6.23 $(mg/kg/day)^{-1}$ when the 20 ppm dose data are dropped. In the case of the inhalation-induced tumors (Table 7.2), the lower risks result from not using a surface area extrapolation factor (Clement Associates, 1988). These authors calculated a dose coefficient of 0.45 $(mg/kg/day)^{-1}$ whereas the multistage model yields a q_1^* of 0.43 $(mg/kg/day)^{-1}$ prior to the surface area adjustment. Thus, although the two-stage model is intended to be more relevant chemical for which cancer incidence is increased over background. This UCL is then extrapolated linearly ("interpolated") to the background incidence to determine an upper boundary line on risk. Under the assumption of strict linearity (not just at low doses), the true risk is predicted to be at or below this line with 95% probability. For gastric tumors in mice (Neal and Rigdon, 1967), the 95% UCL of risk calculated from the multistage model fit at the 45 ppm feeding dose was 0.21. Since the background incidence of respiratory tumors is 0, the excess risk is 0.21. The animal daily dose from 45 ppm BaP of 3.516 mg/kg/day (Table 7.1) can be converted to a human equivalent dose by dividing the animal dose by $(70\ kg/0.034\ kg)^{1/3}$ or 12.7. Thus 3.516/12.7 = 0.28 mg/kg/day. Dividing the excess risk of 0.21 by 0.28 mg/kg/day yields a potency of 0.75 $(mg/kg/day)^{-1}$. This converts to an inhalation risk equal to 2.1×10^{-4} $(\mu g/m^3)^{-1}$. For the estimated ambient level of 5.3×10^{-4} $\mu g/m^3$, the individual risk is 1.2×10^{-7}. This is a low estimate since a factor to correct for the less than lifetime experiment was not used. The factor of $(630/183)^3$ used above equals 40.8. Therefore a corrected risk would be 0.75 x 40.8 = 30.6 $(mg/kg/day)^{-1}$.

Application of the Gaylor-Kodell approach to the hamster inhalation data (Table 7.2) resulted in a potency of 7.4 $(mg/kg/d)^{-1}$ and an inhalation risk of 2.1×10^{-3} $(\mu g/m^3)^{-1}$. In this instance the 95% UCL on the model fit at the 9.5 mg/m^3 exposure was 0.54 (using I = 0.063 m^3/d) and the human equivalent dose was 0.0738 mg/kg/d $(0.655 / [70/0.1]^{1/3})$.

A biologically-based, two stage model of cancer has been proposed with equations containing variables for interstage transitions from normal cell to precancerous cell (first stage) and from precancerous cell to malignant cell (second stage), as well as variables for organ cell numbers, cell birth and death rates, carcinogen dose, and time (Moolgavkar and Knudson, 1981; Clement Associates, 1987; 1988; Charnley and Thorslund, 1988). At the very low doses expected from environmental exposures, this model, like the multistage model, reduces to a linear function of dose.

$$P(x) = 1 - e^{-A(1 + Sx)^2} = 1 - e^{-A(1 + 2Sx + S^2x^2)} \approx 1 - e^{-A(1 + 2Sx)}$$

Instead of the coefficient q_1 in the multistage model, the coefficient in the two stage model at low doses is 2S, where S is the exposure-dependent transition rate for conversion of normal cells to precancerous cells and precancerous cells to malignant cells and A is the background transition rate between cell stages. The model has been applied to the BaP data of Neal and Rigdon (1967) and Thyssen et al. (1981) and yields lower risks than the multistage model (Clement Associates, 1987; 1988; Charnley and Thorslund, 1988). However, in the case of feeding-induced gastric tumors, the lower risks result from not using the incidence data for the 20 ppm dose (Table 7.1). Clement Associates calculated dose coefficients of 3.22 and 5.74

$(mg/kg/day)^{-1}$ (Clement Associates, 1987, 1988 respectively) using the two stage model. For comparison, the multistage model yields a q_1^* of 6.23 $(mg/kg/day)^{-1}$ when the 20 ppm dose data are dropped. In the case of the inhalation-induced tumors (Table 7.2), the lower risks result from not using a surface area extrapolation factor (Clement Associates, 1988). These authors calculated a dose coefficient of 0.45 $(mg/kg/day)^{-1}$ whereas the multistage model yields a q_1^* of 0.43 $(mg/kg/day)^{-1}$ prior to the surface area adjustment. Thus, although the two-stage model is intended to be more relevant biologically, in this instance it does not give risk numbers for BaP significantly different from the multistage model.

7.2.4. Uncertainty in Risk Assessment Estimates

The range of risk values results from several sources of uncertainty, including statistical uncertainty due to the number of animals in the experiment to which the model was applied. For example, the Thyssen et al. study had only 25-27 hamsters per group. Other sources of uncertainty, include the extent of absorption of benzo(a)pyrene by various routes, variability of response to benzo(a)pyrene in different species, the choice of the animal-to-human scaling factors, the choice of the extrapolation model, and the large range of extrapolation (five orders of magnitude) from the benzo(a)pyrene concentrations used in the animal experiments to current ambient levels. In the Neal and Rigdon study the animals began BaP exposures at different ages including as late as 4 months of age and the pathology protocol described was not rigorous. The latter could actually lead to an underestimation of tumors. The GLOBAL86 extrapolation model could not be fit to the high dose data in the Neal and Rigdon experiment, data which clearly

indicated a carcinogenic effect. In addition there is the possibility in light of the absence of an epidemiological connection between exposure to BaP and cancer that the risks in mice and hamsters may not be applicable to humans. Since many mixtures containing BaP are known human carcinogens, this is unlikely. While a portion of the population is exposed to concentrations of BaP greater than 0.53 ng/m^3, others will be exposed to less and thus have a lower risk.

7.2.5. Estimate of Cancer Incidence in California

Air measurements of benzo(a)pyrene in California have yielded an estimated ambient air concentration of 5.3×10^{-4} $\mu g/m^3$. Using the q_1^* of 1.1×10^{-3} and 3.3×10^{-3} $(\mu g/m^3)^{-1}$ derived from hamster inhalation (Table 7.2) and mouse feeding (Table 7.1) data sets respectively, the two best data sets available, a range for individual risk from:

$$1.1 \times 10^{-3} \ (\mu g/m^3)^{-1} \times 5.3 \times 10^{-4} \ (\mu g/m^3) = 5.9 \times 10^{-7}$$

to

$$3.3 \times 10^{-3} \ (\mu g/m^3)^{-1} \times 5.3 \times 10^{-4} \ (\mu g/m^3) = 1.7 \times 10^{-6}$$

can be estimated. (These values are for what has often been called extra risk, [P(d) - P(0)] / [1 - P(0)], which assumes that the environmental carcinogen causes cancer independently of "background" causes.) The upper 95% confidence limit estimate of excess cancers in the California population of 28×10^6 persons due to a lifetime exposure by inhalation to current ambient levels of benzo(a)pyrene ranges from:

$$5.9 \times 10^{-7} \times 28 \times 10^{6} = 16 \text{ excess cancers}$$

to

$$1.7 \times 10^{-6} \times 28 \times 10^{6} = 48 \text{ excess cancers}$$

These theoretical cancer cases would occur among the currently expected 6 to 8 million cases of cancer in the state's population of 28 million persons, based on cancer incidence for Los Angeles County for the years 1972 through 1977 and on estimated cancer incidence in California for 1988 (World Health Organization, 1982; Silverberg and Lubera, 1988).

7.2.6 Selection of Best Values for Risk Assessment

Because of the limited amount of data currently available for risk assessment of benzo(a)pyrene, the inhalation unit risk of 1.1×10^{-3} $(\mu g/m^3)^{-1}$ based on respiratory tract tumors in hamsters (Table 7.5) must be used as a best value for inhalation exposures. For exposures to benzo(a)pyrene by other routes the potency of 11.5 $(mg/kg/d)^{-1}$ based on gastric tract tumors in mice (Table 7.5) can be used.

7.2.7. Relative Potencies of Other PAHs

Benzo[a]pyrene is the most studied PAH, but it is only one of more than one hundred PAH compounds known. IARC considers several purified PAHs and PAH derivates to be probable (Group 2A) or possible (Group 2B) human carcinogens (IARC, 1987) (Table 7.6). Some mixtures containing PAHs are known human carcinogens (Group 1) (Table 7.6). The USEPA has classified several PAHs in

Table 7.6. IARC Groupings of PAHs, Mixtures with PAHs, and Derivatives

Group 1	Group 2A	Group 2B
Coal-tar pitches	Benz[a]anthracene	Benzo[b]fluoranthene
Coal-tars	Benzo[a]pyrene	Benzo[j]fluoranthene
Coke production	Creosotes	Benzo[k]fluoranthene
Mineral oils	Dibenz[a,h]anthracene	Carbon black extracts
Shale-oils		Dibenz[a,h]acridine
Soots		Dibenz[a,j]acridine
Tobacco smoke		7H-Dibenzo[c,g]carbazole
		Dibenzo[a,e]pyrene
		Dibenzo[a,h]pyrene
		Dibenzo[a,i]pyrene
		Dibenzo[a,l]pyrene
		Indeno[1,2,3-cd]pyrene
		5-Methylchrysene
		5-Nitroacenaphthene
		1-Nitropyrene
		4-Nitropyrene
		1,6-Dinitropyrene
		1,8-Dinitropyrene
		6-Nitrochrysene
		2-Nitrofluorene

Abstracted from IARC Supplement 7 (1987) and IARC Volume 46 (1989).
Group 1: carcinogenic to humans.
Group 2A: probably carcinogenic to humans.
Group 2B: possibly carcinogenic to humans.

Group B2, possibly carcinogenic to humans (Table 7.7). IARC (1987, 1989) has classified a large number of PAHs in Group 3, a class of chemicals for which there are no human data but limited or inadequate data in animals (Table 7.8).

While the studies available for carcinogenic risk assessment of BaP are not ideal for risk assessment, those for practically all other individual PAHs are less complete for risk assessment. However, there are extensive data establishing the genotoxicity, and in some cases the carcinogenicity, of many PAHs or their genotoxic metabolites. In other cases, some PAHs are not considered carcinogens. Several authors have used mutagenicity and various tests of carcinogenicity to rank several PAHs for their relative carcinogenicity (e.g., Deutsch-Wenzel et al., 1983; Bingham and Falk, 1969; Habs et al., 1980; Wynder and Hoffman, 1959; Wislocki et al., 1986) and their relative genotoxicity (Brown, 1989). Many of these comparisons were summarized by Clement Associates (1988) (Table 7.9) and Krewski et al. (1989). In these analyses dibenz(a,h)anthracene has been shown to be more potent than BaP, while other PAHs tested were less or much less potent. These comparisons indicate that considering all PAHs to be equivalent in potency to BaP would overestimate the cancer potency of a PAH mixture, but such an assumption would be health protective and is likely to be helpful in a screening estimate of PAH risks.

The mutagenicity of nitro derivatives, such as 2-nitrofluoranthene and the nitropyrenes, can be greater than their parent PAHs. Such compounds can be formed during combustion or through atmospheric interaction with NOx emissions. IARC (1987; 1989) has classified several nitro PAHs, including

Table 7.7. USEPA Groupings of PAHs

Group B2	Group D
Benz[a]anthracene	Acenaphthylene
Benzo[a]pyrene	Anthracene
Benzo[b]fluoranthene	Benzo[e]pyrene*
Benzo[j]fluoranthene*	Benzo[g,h,i]perylene
Benzo[k]fluoranthene	Fluorene
Chrysene	Naphthalene
Dibenz[a,h]anthracene	Phenanthrene
Indeno[1,2,3-c,d]pyrene	

Abstracted from IRIS (1993). Group B2: possibly carcinogenic to humans. Group D: unclassifiable as to carcinogenicity.
* risk assessment under review as of April 1993

Table 7.8. IARC Group 3 PAHs and PAH derivatives

Chemical	Animal Evidence
Acridine orange	inadequate
5-Aminoacenaphthene	inadequate
2-Aminoanthraquinone	limited
Anthanthrene	limited
Anthracene	inadequate
Benz[a]acridine	inadequate
Benz[c]acridine	limited
Benzo[ghi]fluoranthene	inadequate
Benzo[ghi]perylene	inadequate
Benzo[c]phenanthrene	inadequate
Benzo[e]pyrene	inadequate
Carbazole	limited
Chrysene	limited
Cyclopenta[c,d]pyrene	limited
Dibenz[a,c]anthracene	limited
Dibenz[a,j]anthracene	limited
Dibenzo[a,e]fluoranthene	limited
Dibenzo[h,rst]pentaphene	limited
3,7-Dinitrofluoroanthene	limited
3,9-Dinitrofluoroanthene	limited
1,3-Dinitropyrene	limited
Fluoranthene	inadequate
Fluorene	inadequate
1-Methylchrysene	inadequate
2-Methylchrysene	limited
3-Methylchrysene	limited
4-Methylchrysene	limited
6-Methylchrysene	limited
2-Methylfluoranthene	limited
1-Methylphenanthrene	inadequate
1,5-Naphthalenediamine	limited
9-Nitroacenaphthene	limited
9-Nitroanthracene	no adequate data
7-Nitrobenz[a]anthracene	limited
6-Nitrobenzo[a]pyrene	limited
3-Nitrofluoranthene	inadequate
1-Nitronaphthalene	inadequate
2-Nitronaphthalene	inadequate
3-Nitroperylene	inadequate
2-Nitropyrene	inadequate
Perylene	inadequate
Phenanthrene	inadequate
N-Phenyl-2-naphthylamine	limited
Pyrene	inadequate
Triphenylene	inadequate

Abstracted from IARC Supplement 7 (1987) and IARC volume 46 (1989). Group 3: have either limited or inadequate evidence in animals and are not classifiable as to their carcinogenicity in humans due to no adequate data.

TABLE 7.9 SUMMARY OF RELATIVE POTENCY ESTIMATES FOR INDICATOR PAHs

	Test System						
Compound	Mouse Skin Carcinogenesis	Subcutaneous Injection into Mice[m]	Intrapulmonary Administration to Rats[g]	Initiation-Promotion on Mouse Skin	Intraperitoneal Injection in Newborn Mice[m]	DNA Adduct Formation	Genotoxic Ranking[n]
Benzo(a)pyrene	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Benz(a)anthracene	0.145[a]				0.057,0.524,0.496[k]	0.07	0.62
Dibenz(ah)anthracene	1.11[d]	2.82[e],4.50[f]				0.56	0.47
Benzo(e)pyrene			0.004				0.42
Chrysene	0.0044[d]			0.040[i]	0.125,0.33[k]		0.37
Cyclopentadieno(cd)pyrene	0.023[b]						0.26
Benzo(b)fluoranthene	0.167[b]		0.140	0.258[h],0.125[i]	0.232,1.067,0.874[j]		0.20
Benzo(j)fluoranthene	0.061[b]			0.048[h]	0.320,0.471,0.450[j]		
Benzo(k)fluoranthene	0.020[b]		0.066	0.022[h]	0.040,0.097,0.044[j]		
Pyrene					0.081,0.050,0.586[k]		0.20
Indeno(1,2,3-cd)pyrene	0.021[b],0.189[c]		0.232	0.074[c]	0.013[j]		0.14
Benzo(ghi)perylene	0.015[c]		0.022	0.005[c]			0.08
Anthanthrene			0.320				0.06

[a]Bingham and Falk (1969).
[b]Habs et al. (1980).
[c]Hoffmann and Wynder (1966).
[d]Wynder and Hoffmann (1959).
[e]Pfeiffer (1977).
[f]Bryan and Shimkin (1943).
[g]Deutsch-Wenzel et al. (1983).
[h]LaVoie et al. (1982).
[i]Van Duuren et al. (1966).
[j]LaVoie et al. (1987).
[k]Wislocki et al. (1986).
[l]Phillips et al. (1979).
[m]Where more than one potency estimate is shown, they were derived from the same study using different tumor types as end points.
[n]Brown (1989).
Modified from Clement Associates (1988).

1-nitropyrene, 4-nitropyrene, 1,6-dinitropyrene, 1,8-nitropyrene, 6-nitrochrysene, 2-nitrofluorene, and 5-nitroacenaphthene, as possible human carcinogens (class 2B) (Table 7.6). The contribution to carcinogenicity of nitro derivatives has not been as extensively studied and has not been evaluated in previous comparisons. They have not been systematically evaluated by the USEPA. Note that nitro derivatives are not included in Table 7.9.

If one assumes that PAHs are as carcinogenic as they are genotoxic, then their hazard relative to BaP would be dependent on their concentration in the environment. If PAHs other than BaP are much more prevalent than BaP itself, then they could contribute a substantial portion of risk to humans. That is, they may be of lesser potency, but of greater concentration. As indicated by Brown (1989), several PAHs may be greater genotoxic risks than BaP in urban ambient environments due to their much higher concentration. But BaP appears, in light of the limited information available on other PAHs, to remain an important representative or surrogate for this important group of chemically diverse air pollutants.

7.2.8 Selection of Risk Values for Other PAHs

The original intent in the toxic air contaminant identification process was to examine polycyclic aromatic hydrocarbons as a class. However, BaP was chosen as the primary representative of the class because of the large amount of toxicological data available on BaP (versus the relatively incomplete database for other PAHs), the availability of monitoring techniques for BaP, and the significant exposure expected (and found). But, as indicated above, a number of PAHs and PAH derivatives, e.g. nitroPAHs, are considered to be potentially carcinogenic to humans. Thus there is a need to consider the impact of not only BaP but also of a large number of related PAHs and PAH derivatives. In order to address this issue, a recent paper (LaGoy and Nisbet, 1991) presented a Toxic Equivalency Factor (TEF) scheme for 17 PAHs. The paper was an extension of earlier work by other investigators (Clement Associates 1987, 1988; Krewski et al., 1989) which is shown in Table 7.9. Along similar lines OEHHA has developed a Potency Equivalency Factor (PEF) procedure to assess the relative potencies of PAHs and PAH derivatives as a group. This would allow the ARB to address the impact of carcinogenic PAHs in ambient air since they are usually present together.

Due to the variety of data available on the carcinogenicity and mutagenicity of PAHs, an order of preference for the use of available data in assessing relative potency was developed. If a health effects evaluation and quantitative risk assessment leading to a cancer potency value have been conducted on a specific PAH, then these values should be given the highest preference. Such an analysis for BaP has been carried out in this report, in earlier USEPA reports, and has been published by Collins et al. (1991).

Unfortunately no similar risk assessments have been carried out for other PAHs although suitable data, at least from ingestion, are available for some. Second in the order of preference would be a risk assessment carried out using standard risk assessment methodology and valid tumor incidence data. One example would be the expedited cancer potency values developed for the implementation of Proposition 65 using the cancer potency (TD50) database of Gold et al. (1984, 1986, 1987, 1989, 1990) and the multistage model. As of November, 1992, such risk assessments had been carried out for dibenz(a,h)anthracene, 7,12-dimethylbenz(a)anthracene, 3-methylcholanthrene, and 5-nitroacenaphthene. These methods would yield actual carcinogenic potencies which are listed in Table 7.10. The documentation for each chemical is discussed in Appendix A. If potency values have not been developed for specific compounds, a carcinogenic activity relative to BaP, rather than a true potency, can be developed. These relative activity values will be referred to as Potency Equivalency Factors (PEFs). Such factors are similar to Toxic Equivalency Factors (TEFs) developed for dioxin (CDHS, 1986). However, PEFs are on stronger scientific grounds for the prediction of cancer than the TEFs for dioxin since nearly all PEFs are based on cancer bioassay information while the dibenzodioxin and dibenzofuran TEFs are mainly based on acute toxicity, structure-activity relationships, and short term tests such as aryl hydrocarbon hydroxylase induction plus some limited cancer bioassay data. For air contaminants, relative potency to BaP based on data from inhalation studies would be optimal. Otherwise, intrapulmonary or intratracheal administration, such as those published by Deutsch-Wenzel et al. (1983), would be most relevant, since such studies are in the target organ of interest. Next in order of preference is information on activity by the oral route which is available for several compounds including BaP. This information would be

Table 7.10. Potencies of PAHs and Derivatives

Chemical	Potency[a]	Unit Risk[a]
benzo[a]pyrene	11.5	1.1×10^{-3}
dibenz[a,h]anthracene	4.1	3.9×10^{-4}
7,12-dimethylbenzanthracene	250	2.4×10^{-2}
3-methylcholanthrene	22	2.1×10^{-3}
5-nitroacenaphthene	0.13	1.1×10^{-5}

[a] Units are $(mg/kg\text{-}day)^{-1}$ for potency and $(\mu g/m^3)^{-1}$ for unit risk. The numbers for benzo[a]pyrene were developed in this document while the other potencies are from expedited risk assessments for implementing Proposition 65. It is assumed that unit risks for inhalation have the same relative activities as cancer potencies for oral intake.

useful in establishing PEFs since BaP causes tumors by the oral route. Data on skin painting are also available. These studies involve tumor production by PAHs following either repeated dosing with the PAH of interest or initiation with the PAH of interest followed by promotion with, for example, phorbol esters. Skin is also a target organ of concern with PAH exposure and the skin does have P450 activity to activate and deactivate PAHs. Intraperitoneal and subcutaneous administration rank at the bottom of the in vivo tests considered useful for PEF development because of their lack of relevance to environmental exposures. However, they do measure the ability of a PAH to cause cancer. Next in decreasing order of preference are genotoxicity data which exist for a large number of compounds. In many cases genotoxicity information is restricted to mutagenicity data. These data are of interest but, since the compounds we are considering have been classified as potential carcinogens based on actual cancer tests, their usefulness in applied situations is supplemental. Also the relative mutagenicities of nitroPAHs compared to PAHs may be much greater than their relative carcinogenicities. For example, 1,8-dinitropyrene can be as much as 10^5 times as mutagenic as BaP in the Ames test, yet it is at most ten times as carcinogenic and, based on the best available data, appears to be less carcinogenic than BaP. Finally there are data on structure-activity relationships among PAH compounds. Structure-activity considerations may help identify a PAH as carcinogenic, but at this time have not been established as predictors of carcinogenic potency.

Using this order of preference (Table 7.11), PEFs have been derived for a number of PAHs and are presented in Table 7.12. These PEFs are based primarily on chronic internal dosing experiments and skin painting studies.

Table 7.11. Scheme for PEF Selection

1. Complete quantitative risk assessment
2. "Expedited" quantitative risk assessment
3. Tumor data from inhalation exposure
4. Tumor data from intratracheal or intrapulmonary administration
5. Tumor data from oral administration
6. Tumor data from skin painting studies
7. Tumor data from subcutaneous or intraperitoneal administration
8. Genotoxicity data
9. Structure activity information

Table 7.12. OEHHA'S PEF Weighting Scheme for PAHs

PAH or derivative	Suggested PEF
benzo[a]pyrene[ARB]	1.0 (index compound)
benz[a]anthracene	0.1
benzo[b]fluoranthene[ARB]	0.1
benzo[j]fluoranthene	0.1
benzo[k]fluoranthene[ARB]	0.1
dibenz[a,j]acridine	0.1
dibenz[a,h]acridine	0.1
7H-dibenzo[c,g]carbazole	1.0
dibenzo[a,e]pyrene	1.0
dibenzo[a,h]pyrene	10
dibenzo[a,i]pyrene	10
dibenzo[a,l]pyrene	10
indeno[1,2,3-c,d]pyrene[ARB]	0.1
5-methylchrysene	1.0
1-nitropyrene	0.1
4-nitropyrene	0.1
1,6-dinitropyrene	10
1,8-dinitropyrene	1.0
6-nitrochrysene	10
2-nitrofluorene	0.01
chrysene	0.01

ARB designates PAHs with ARB monitoring data. The nitroPAHs are those listed as IARC class 2B. Although chrysene is an IARC class 3 carcinogen, EPA classifies it as B2. The justification for each PEF is detailed in Appendix A.

Explanation of the derivation of each PEF, the type of data used in the derivation, and the relevant references are given in Appendix A. As indicated in Table 7.12, PEF values have been rounded to the nearest factor of 10. This was done to retain the uncertainty in these estimates.

In summary this analysis presents potency equivalency factors relative to BaP for 21 PAHs and PAH derivatives (Table 7.12). Cancer potency values in units of $(mg/kg/day)^{-1}$ are presented for 4 additional PAH compounds (Table 7.10). The focus of the evaluation has been on those chemicals with demonstrated carcinogenicity in bioassays. Unfortunately, potency estimates could not be developed for all potential PAH carcinogens. In addition, a much larger number of PAHs and PAH derivatives are considered mutagenic or genotoxic, but these compounds are not considered in this evaluation. Furthermore, structure-activity analysis may suggest that additional PAHs are carcinogenic. As a result, additional PAHs are likely to be identified as potential human carcinogens and these substances may need to be considered at a later date.

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Appendix A.

Potency and Potency Equivalency Factors (PEFs) for PAHs and Derivatives

1. Benzo[a]pyrene. Benzo[a]pyrene (BaP) is the index compound for relative potency and for Potency Equivalency Factors (PEFs) for PAHs and derivatives. The calculations of its cancer potency of 11.5 $(mg/kg\text{-}day)^{-1}$ and of its inhalation unit risk of 1.1×10^{-3} $(\mu g/m^3)^{-1}$ are presented in Chapter 7 of this report. For the potency equivalency scheme, it is assigned a PEF of 1.

2. Dibenz[a,h]anthracene. An expedited potency of 4.1 $(mg/kg\text{-}day)^{-1}$ has been derived using the linearized multistage model with the only dose response data set available - a drinking water study (Snell et al. 1962) which reported alveolar carcinomas of the lung in male DBA/2 mice due to dibenz[a,h]anthracene (incidence = 14/21 at 28.3 mg/kg-day versus 0/25 in controls). An inhalation unit risk can be obtained from a potency under the assumptions that the chemicals are equally absorbed and are equally potent by oral and inhalation routes and that a 70 kg person inhales 20 cubic meters of air per day. When the potency in units of $(mg/kg\text{-}day)^{-1}$ is divided by 3500 (70 kg * 1000 $\mu g/mg/20\ m^3$), an inhalation unit risk is obtained in units of $(\mu g/m^3)^{-1}$.

3. 7,12-Dimethylbenzanthracene. An expedited potency of 250 $(mg/kg\text{-}day)^{-1}$ has been derived. The only study listed in the Gold et al. cancer potency (TD50) database (Gold et al. 1984, 1986, 1987, 1989, 1990) is the feeding study by Chouroulinkov et al. (1967) in female albino mice. Significant increases in malignant angioendotheliomas of the mesenteric intestine and papillomas of the forestomach were observed in animals treated with 0.39 mg/kg-day of 7,12-dimethylbenzanthracene. Cancer potency is based on the angioendotheliomas of the mesenteric intestine (incidence = 49/75 vs 0/40 in controls).

4. 3-Methylcholanthrene. An expedited potency of 22 $(mg/kg\text{-}day)^{-1}$ has been derived. Results of 3 studies in male Long Evans rats, one study in an unspecified strain of female rats, and 10 studies in female Wistar rats are included in the Gold et al. database. All studies in female rats found highly significant increases in tumors of the mammary gland. The cancer potency for 3-methylcholanthrene is taken as the geometric mean of cancer potencies estimated from 9 of the 10 studies in female rats (Shay et al., 1962; Gruenstein et al., 1964; Shay et al., 1961). The upper bound on potency could not be estimated from one of the studies by Shay et al. (1961), because 100% of the treated animals developed mammary gland tumors.

5. 5-Nitroacenaphthene. An expedited potency of 0.13 $(mg/kg\text{-}day)^{-1}$ has been derived based on the combined incidence of benign and malignant tumors of the ear canal in female rats. Usable studies are feeding studies by Takemura et al. (1974) in female Syrian golden hamsters and by the National Cancer Institute (NCI) (1978) in male and female B6C3F1 mice and F344 rats. The compound 5-nitroacenaphthene induced increases in tumor incidences at multiple sites in rats and female mice. Rats are the most sensitive species; the sensitivity of males is similar to that of females.

6. Benzo[b]fluoranthene. Benzo[b]fluoranthene has been assigned a PEF of 0.1. Clement Associates (1988) applied both a two stage model, discussed in Section

7.2.3, and the multistage model to various data sets for several PAHs (see Table 7.8.). The two models generally gave similar results for relative potency. In order to verify the results, OEHHA staff used GLOBAL86 to fit the multistage model to the tumor data used by Clement Associates and obtained relative cancer potencies similar to those obtained by Clement Associates. Clement Associates (1988) used the mouse skin carcinogenesis data obtained by Habs et al. (1980) and the intrapulmonary administration to rats by Deutsch-Wenzel et al. (1983) to estimate a cancer potency for benzo[b]fluoranthene relative to BaP. As an example of the type of data used, Deutsch-Wenzel et al. obtained pulmonary tumor incidences of 0, 2.9, and 25.7% after intrapulmonary administration of 0.1, 0.3, and 1 mg benzo[b]fluoranthene, respectively, whereas they obtained 11.8, 60.0, and 94.3% tumor incidences after the same doses of benzo[a]pyrene. Clement Associates estimated a relative cancer potency for benzo[b]fluoranthene of 0.140 after fitting the two stage model to the data and 0.105 after fitting the multistage model. Using the data of Habs et al. a relative cancer potency of 0.167 was obtained with the two stage model and 0.201 with the multistage model. The results from the multistage model were averaged, then rounded (down) to 0.1 to obtain the PEF. OEHHA obtained a relative potency of 0.208 for benzo[b]fluoranthene fitting the multistage model to the data from Habs et al. OEHHA staff were also able to reproduce the calculations for the 2 stage model in the case of the hamster inhalation data. Since the multistage model is the accepted model for cancer risk assessment in California, results from the multistage model have been used to obtain PEFs although the 2 models usually gave the same PEF.

7. Benzo[j]fluoranthene. Benzo[j]fluoranthene has been assigned a PEF of 0.1. Clement Associates (1988) used the mouse skin carcinogenesis data obtained by Habs et al. (1980) to estimate a cancer potency relative to BaP of 0.0648. OEHHA staff estimated 0.065 using the same data. This was rounded to 0.1 to obtain the PEF. Clement Associates did not use the data of Deutsch-Wenzel et al. (1983) on benzo[j]fluoranthene to calculate a relative potency but Deutsch-Wenzel et al. found that it was very similar in tumorigenic activity to benzo[k]fluoranthene.

8. Benzo[k]fluoranthene. Benzo[k]fluoranthene has been assigned a PEF of 0.1. Clement Associates (1988) used mouse skin carcinogenesis data obtained by Habs et al. (1980) to obtain a cancer potency relative to BaP of 0.0235 and the intrapulmonary administration to rats by Deutsch-Wenzel et al. (1983) to estimate one of 0.085. Because the latter was obtained by the pulmonary route it was chosen to be the basis of the PEF. The value was rounded to 0.1 to obtain the PEF.

9. Benz[a]anthracene. Benz[a]anthracene has been assigned a PEF of 0.1. In the case of benz[a]anthracene, mouse skin carcinogenesis data obtained by Bingham and Falk (1969) were used by Clement Associates (1988) to calculate potencies for benz[a]anthracene. For this chemical the multistage model gave a relative potency of 0.0137. Using the 2 stage model a higher cancer potency of 0.145 relative to BaP was obtained. In the Wislocki et al. (1986) report, in which lung adenomas were induced in newborn mice, benz[a]anthracene (2.8 micromoles) was less carcinogenic (12/71 or 17% versus 7/138 or 5% in controls) relative to 0.56 micromoles BaP (24/64 or 38% versus 7/138 in controls). The relative potency was 0.08, which rounds to 0.1. Since the U.S. EPA is at least provisionally using 0.1 for this PAH (EPA, 1993b) and the

data from the Wislocki study are consistent with a PEF of 0.1, the value of 0.1 was selected by OEHHA.

10. Dibenz[a,j]acridine. Dibenz[a,j]acridine has been assigned a PEF of 0.1. Warshawsky et al. (1992) compared the tumor-initiating ability of dibenz[a,j]acridine to benzo[a]pyrene in mouse skin. Two hundred nanomoles of each compound were applied to groups of 30 mice, then the skin lesion was promoted with a phorbol ester for 24 weeks. Twenty-seven out of 30 BaP mice (90%) had skin papillomas, while 17 of 30 (57%) of the dibenz[a,j]acridine mice had skin papillomas. The multistage model was fit to both sets of data and the ratio of upper 95% confidence limits on the linear coefficient was 0.36. This is rounded to a PEF of 0.1.

11. Dibenz[a,h]acridine. Dibenz[a,h]acridine has also been assigned a PEF of 0.1. Its carcinogenic classification by IARC was based on studies published in 1940 and earlier and the studies did not appear appropriate for estimation of a PEF. Since its structure is similar to dibenz[a,j]acridine, it has been assigned the same PEF as dibenz[a,j]acridine until usable compound-specific bioassay data are available.

12. 7H-Dibenzo[c,g]carbazole. 7H-dibenzo[c,g]carbazole has been assigned a PEF of 1.0. Warshawsky et al. (1992) compared the tumor-initiating ability of 7H-dibenzo[c,g]carbazole to benzo[a]pyrene in mouse skin. Two hundred nanomoles of each compound were applied to 30 mice, then promoted with a phorbol ester for 24 weeks. Twenty-seven out of 30 BaP-treated mice (90%) had skin papillomas, while 26 of 30 (87%) of the dibenz[a,j]acridine-treated mice had skin papillomas for a relative tumorigenic activity of 0.97. This is rounded to a PEF of 1.

13. Dibenzo[a,l]pyrene. Dibenzo[a,l]pyrene has been assigned a PEF of 10. Cavalieri et al. (1989, 1991) studied the tumor-initiating and dose-response tumorigenicity of 4 dibenzo[a]pyrenes in mouse skin and rat mammary gland. BaP was used as a reference compound in some experiments. Dibenzo[a,l]pyrene was the most potent member of the group. Several levels of PAHs were tested. When results from 33.3 nanomoles of dibenzo[a,l]pyrene as a skin tumor initiator (with promotion by a phorbol ester) were compared to results using the same amount of BaP, dibenzo[a,l]pyrene induced skin tumors in 23/24 (96%) of the animals while BaP induced tumors in 10/23 (43%) which resulted in a relative potency of 5.8. Dibenzo[a,l]pyrene induced approximately 5 times as many tumors per tumor bearing animal. In a second experiment 4 nanomoles of each chemical were compared. Ninety-two percent (22/24) of the dibenzo[a,l]pyrene-treated mice had tumors but only 4% (1/24) of the BaP animals which yielded a relative potency of 25.1. In a third experiment 100 nanomoles were compared without promotion. Twenty-nine percent (7/24) of the dibenzo[a,l]pyrene-treated mice had tumors but only 4% (1/24) of the BaP animals for a relative potency of 4. Finally, with direct application to the mammary gland 0.25 and 1.0 micromoles dibenzo[a,l]pyrene led to tumors in all the rats treated (19 and 20 per group, respectively) whereas only 1 animal in the 0.25 micromole BaP group showed a tumor for a relative potency greater than 100. Based on its much greater tumorigenic activity than BaP in the above tests dibenzo[a,l]pyrene is assigned a PEF of 10.

14. Dibenzo[a,h]pyrene. Dibenzo[a,h]pyrene has been assigned a PEF of 10 since, in the experiments by Cavalieri et al. (1989) in which all 4

dibenzo[a]pyrenes were studied, its tumor causing activity was similar to dibenzo[a,l]pyrene. For example, when used to initiate tumors in mouse skin, 18 of 24 (75%) of mice treated with dibenzo[a,h]pyrene had tumors compared to 22 of 24 (92%) with dibenzo[a,l]pyrene. Controls showed skin tumors in 2 of 23 mice (9%).

15. Dibenzo[a,i]pyrene. Dibenzo[a,i]pyrene has been assigned a PEF of 10 since, in the experiments by Cavalieri et al. (1989) in which all 4 dibenzo[a]pyrenes were studied, its tumor-causing activity was similar to dibenzo[a,l]pyrene. For example, when used to initiate tumors in mouse skin, 15 of 24 (63%) of mice treated with dibenzo[a,i]pyrene had tumors compared to 22 of 24 (92%) with dibenzo[a,l]pyrene. Controls showed skin tumors in 2 of 23 mice (9%).

16. Dibenzo[a,e]pyrene. Dibenzo[a,e]pyrene has been assigned a PEF of 1.0. Dibenzo[a,e]pyrene was the weakest member of the 4 dibenzo[a]pyrenes studied by Cavalieri et al. (1989, 1991). In the experiments in which all 4 dibenzo[a]pyrenes were compared (Cavalieri et al., 1989), its tumor-causing activity was approximately one-tenth to one-twentieth that of dibenzo[a,l]pyrene.

17. Indeno[1,2,3-c,d]pyrene. Indeno[1,2,3-c,d]pyrene has been assigned a PEF of 0.1. Clement Associates (1988) used the mouse skin carcinogenesis data obtained by Habs et al. (1980) and by Hoffman and Wynder (1966) and the lung tumor data obtained by Deutsch-Wenzel et al. (1983) after intrapulmonary administration to estimate cancer potencies relative to BaP of 0.0302, 0.0292, and 0.246, respectively. These were averaged and rounded to obtain a PEF of 0.1. Note that rounding just the lung tumor data gives the same result.

18. 5-Methylchrysene. 5-Methylchrysene has been assigned a PEF of 1.0. The activity of 5-methylchrysene relative to BaP has been studied by Hecht et al. (1976) using skin tumor initiation with phorbol ester (tetradecanoyl phorbol acetate) promotion as well as skin tumor induction in mice. In the skin tumor induction test the tumorigenic activities of 5-methylchrysene and BaP were comparable enough so that a PEF of 1.0 was selected for 5-methylchrysene. Weekly application of 0.01% 5-methylchrysene led to skin carcinomas in 10 of 15 mice treated for up to 62 weeks, while 0.01% BaP led to skin carcinomas in 14 of 18 mice. The results for 0.005% of the 2 chemicals were 6 of 9 and 7 of 10, respectively.

19. 1-Nitropyrene. 1-Nitropyrene has been assigned a PEF of 0.1. In the Wislocki et al. (1986) report, in which lung tumors were induced in newborn mice, 1-nitropyrene (0.7 micromoles) was weakly carcinogenic in males (6/34 or 18% versus 4/45 or 9% in controls) and not carcinogenic in females (3/50 or 6% versus 2/34 or 6% in controls) relative to 0.56 micromoles BaP (13/37 or 35% in males versus 1/28 or 4% in control males and 13/27 or 48% in females versus 0/31 in control females). The relative potency was 0.348 in males and 0.076 in females. A PEF of 0.1 was assigned based on the experiment.

20. 4-Nitropyrene. 4-Nitropyrene has been assigned a PEF of 0.1. Wislocki et al. (1986) compared the lung tumorigenicity of nitrated derivatives of pyrene to BaP in a newborn mouse assay. The background incidences were 4% in males and 0% in females. The administration of 2.8 micromoles of 4-nitropyrene gave a net incidence of 34% tumors in males and 31% in females, while 0.56

micromoles BaP gave 31% tumors in males and 48% in females. The potency of 4-nitropyrene relative to BaP was 0.23 in males and 0.12 in females. These are averaged and rounded to a PEF of 0.1.

21. 1,6-Dinitropyrene. 1,6-Dinitropyrene has been assigned a PEF of 10. In the Wislocki et al. (1986) report, 1,6-dinitropyrene (0.2 micromoles) was weakly carcinogenic in inducing lung tumors in females (2/29 versus 0/31 in controls) and essentially not carcinogenic in males (1/25 versus 1/28 in controls) relative to 0.56 micromoles BaP (see 1-nitropyrene above for BaP data). However the weak response combined with the low dose of 1,6-dinitropyrene (0.2 micromoles) relative to BaP (0.56 micromoles), the relative potency was 0.52 in females and 0.54 in males. In an intratracheal injection experiment (Takayama et al., 1985) hamsters were given 26 weekly instillations of 0.5 mg BaP. All 10 males and 9 of 10 females developed respiratory tract tumors. An unit risk of 2.9×10^{-2} $(\mu g/m^3)^{-1}$ was obtained from the female data which is 6.4 times the unit risks obtained from intratracheal studies using BaP and 26 times that using inhalation data (Table 7.5). In a study by Iwagawa et al. (1989) using several doses of 1,6-dinitropyrene or BaP implanted directly into the lungs a relative potency of 5.1 was obtained from the resulting lung cancer data. In light of the 2 experiments showing high relative potency and of 1,6-dinitropyrene's strong mutagenicity, a PEF of 10 appears to be more appropriate than 1.0.

22. 1,8-Dinitropyrene. 1,8-Dinitropyrene has been assigned a PEF of 1.0. In the Wislocki et al. (1986) report, 1,8-dinitropyrene (0.2 micromoles) was weakly carcinogenic in females (2/29 versus 0/31 in controls) and not carcinogenic in males (1/31 versus 1/28 in controls) relative to 0.56 micromoles BaP. However, due again to the low dose of 1,8-dinitropyrene chosen, the relative potency was 0.46 in females and 0.41 in males. In view of the high PEF of 1,6-dinitropyrene derived above and the very high mutagenicity of 1,8-dinitropyrene, the default PEF of 1.0 is assigned to 1,8-dinitropyrene until better in vivo data become available to derive a PEF.

23. 6-Nitrochrysene. 6-Nitrochrysene has been assigned a PEF of 10. In the Wislocki et al. (1986) report, 0.7 micromoles of 6-nitrochrysene gave a net incidence of 76% lung tumors in males (28/33 versus 4 of 45 in controls) and 84% in females (36/40 versus 2/34 in controls). The potency of 6-nitrochrysene relative to BaP was 3.27 in males and 2.50 in females. In the newborn mouse assay of Busby et al. (1988), "(t)he ED50 for total lung tumors was 0.02 μmol for 6-NC and 0.2 μmol for BaP, thus showing a 10-fold higher potency for 6-NC compared with the 25-fold difference noted with tumor multiplicity." In a subsequent report (Busby et al., 1989), 0.03 micromoles of 6-nitrochrysene caused lung adenomas and adenocarcinomas in 19/26 males and 13/22 females (versus controls of 13/91 in males and 7/101 in females) while 0.24 micromoles BaP caused lung adenomas and adenocarcinomas in 13/28 males and 19/27 females (against the same controls). The relative potencies were 17.51 for males and 6.17 for females. Based on the several experiments a PEF of 10 is selected.

24. 2-Nitrofluorene. 2-Nitrofluorene has been assigned a PEF of 0.01. Miller et al. (1955) fed 2-nitrofluorene at a level of 1.62 mmol(215 mg)/kg diet to rats. This is estimated to give an animal dose of 33.1 mg/kg/day and a human equivalent dose of 4.7 mg/kg/day. In one experiment 17 of 20 male rats (85%) developed forestomach tumors by 12 months. In another experiment 4 of 9

female rats (44%) developed mammary tumors by 10 months. These experiments yielded cancer potencies of 0.25 and 0.62 $(mg/kg\text{-}day)^{-1}$, approximately 0.02 and 0.05 that of BaP obtained in this risk assessment. The values of 0.02 and 0.05 were averaged and rounded to obtain a PEF of 0.01.

25. Chrysene. Chrysene has been assigned a PEF of 0.01. Clement Associates (1988) used the mouse skin carcinogenesis data obtained by Wynder and Hoffman (1959) to estimate a cancer potency relative to BaP of 0.0132. This was rounded to obtain a PEF of 0.01.

7. QUANTITATIVE RISK ASSESSMENT

7.1. Noncancer Health Effects

In part A of this document, the California Air Resources Board estimates that the annual average ambient air level of benzo(a)pyrene in California is 5.3×10^{-4} $\mu g/m^3$. A "standard man" weighing 70 kg and breathing 20 m^3 of air daily will have an intake of 1.1×10^{-2} μg BaP per day (5.3×10^{-4} $\mu g/m^3$ x 20 m^3) or 1.1×10^{-5} mg per day. Dividing by the 70 kg body weight yields a dose of 1.6×10^{-7} mg/kg/day. At this level noncancer health effects are unlikely to occur, since this expected dose to humans is on the order of one-millionth that which causes adverse effects on fertility, oocyte destruction, and weight gain in mammals and cardiovascular effects in pigeons (LOAELs of 10, 10, 0.9, and 0.014 mg/kg/day, respectively) (see Chapter 5). However, NOAELs and LOAELs for BaP effects in animals are very scarce and are non-existent for humans. Such scarcity of data constitutes a data gap for BaP. Data for additional toxicologic endpoints are needed.

7.2. Carcinogenic Risks

A very large number of experiments have demonstrated that BaP causes tumors at several sites, by several routes of administration, in both sexes, and in several animal species. (See Chapter 5; Zeise and Crouch, 1984; Jones and Walsh, 1985; IARC, 1983). Many studies, however, are very limited in scope or in data reported and are not suitable for risk assessment (Zeise and Crouch, 1984).

OEHHA guidelines prescribe that risk assessments use the most sensitive sex, site, and species where a significant increase in cancer incidence is observed (California Department of Health Services, 1985). For BaP this indicates the use of data on gastric tumors (papillomas and squamous cell carcinomas) observed in male and female mice due to feeding of BaP (Neal and Rigdon, 1967). In addition, large numbers of mice (319 experimental, 289 control) were used to establish the dose-response curve. Potency estimates were also derived from the data for respiratory tract tumors in hamsters from the inhalation bioassay of Thyssen et al. (1981) and from data obtained after intratracheal administration of BaP (Saffiotti et al., 1972; Feron et al., 1973). Cancer risk associated with exposure to ambient levels of BaP is estimated by extrapolating approximately five orders of magnitude from the experimental data to ambient levels by means of the best fitting linearized multistage model GLOBAL86 (Howe and van Landingham, 1986). In addition other models have been fit to the data for comparison. In its risk assessment, the EPA used the data for stomach tumors from oral exposure to benzo(a)pyrene in mice and the data for respiratory tract tumors from inhalation exposure in hamsters to estimate cancer potency and unit risks associated with exposure to BaP (EPA, 1984).

7.2.1. Thresholds

A threshold dose of a toxicant is one below which a specified outcome does not occur. While threshold models for carcinogenesis have been proposed based on various mechanisms such as saturation of detoxification enzymes, the existence of DNA repair mechanisms, and recurrent toxicity, none has been convincingly demonstrated. An "epigenetic" mechanism, that could in theory embody

threshold doses, has been invoked to explain the carcinogenic action of substances that do not directly produce genetic damage in short-term tests. However, for benzo(a)pyrene there is compelling evidence of genotoxicity. Metabolites of benzo(a)pyrene bind to DNA and mutagenic responses have been observed in a wide variety of prokaryotic and eukaryotic systems (Chapter 4). There is experimental evidence that benzo(a)pyrene acts as an initiator of tumorigenesis (Chapter 5). Tumors have been noted after a single high dose of BaP (e.g., Neal and Rigdon, 1967). These are not considered to be threshold phenomena. Therefore, OEHHA staff treats benzo(a)pyrene-induced carcinogenesis as a nonthreshold phenomenon and, as such, applies a nonthreshold, linear extrapolation model for cancer potency estimation.

7.2.2. Animal Data and the Multistage Model

Since there is no adequate information regarding the carcinogenicity of BaP to humans from epidemiological studies, data from animal bioassays are extrapolated to estimate human cancer risk. The linearized multistage model developed by Crump and colleagues, utilized in the computerized form as GLOBAL86 (Howe and van Landingham, 1986), is used to estimate the cancer potency of BaP. This model and its earlier versions such as GLOBAL82 have been used in previous risk assessments by the OEHHA/Department of Health Services and is the model preferred by EPA (Anderson et al., 1983; EPA, 1986). The model uses animal tumor incidence data to compute maximum likelihood estimates (MLE) and upper 95% confidence limits (UCL) of risk associated with a particular dose. The UCL is regarded as the upper limit of the true risk. The true risk is very unlikely to be greater than the upper limit, may be lower than the upper limit, and could be zero. The linearized multistage

model yields upper bound estimates of risk which are a linear function of dose at low doses and are used frequently as a basis for regulation. They are more stable statistically and more protective of public health than are MLEs.

The linearized multistage model is based on several assumptions about the process of carcinogenesis. Cancer is assumed to be an irreversible process which originates in a single cell and involves a number of biological events or stages. The rate of occurrence of each stage varies linearly with dose. In addition the incidences of background and chemically-induced cancer are assumed to be additive.

The multistage model may be expressed mathematically as:

$$P(d) = 1 - e^{-(q_0 + q_1 d + q_2 d^2 + \ldots + q_k d^k)} \qquad \text{[Eq. 1]}$$

where P(d) is the lifetime probability of developing a tumor at a given dose d of carcinogen, q_0 is a constant that accounts for the background incidence of cancer (i.e., occurring in the absence of the carcinogen under consideration), and q_1, q_2, ... q_k are coefficients that allow the data to be expressed to various powers of the dose of carcinogen in order to obtain the best fit of the model to the data. In order to determine the extra risk above the background rate at dose d or:

$$P_e(d) = [P(d) - P(0)]/[1-P(0)] \qquad \text{[Eq. 2]}$$

the equation takes the form:

$$P_e(d) = 1 - e^{-(q_1 + q_2 d^2 + \ldots + q_k d^k)} \qquad \text{[Eq. 3]}$$

At low doses, the extra risk is approximated by:

$$P_e(d) = q_1 d \qquad \text{[Eq. 4]}$$

The goodness-of-fit of the model to the data in the observed range is tested using the asymptotic Chi-square distribution of the log-likelihood ratio; values of Chi-square which give a p value > 0.01 are considered by the USEPA and the developers of the program to indicate an acceptable fit. In recent documents OEHHA has considered p > 0.05 to be acceptable. As shown below the p values of the fits in the present analysis are greater than 0.05.

Using the computer software GLOBAL86, the linearized multistage model was fit to the dose-response data from the studies of gastric tumors in mice and respiratory tract tumors in hamsters given in Tables 7.1 and 7.2, respectively (see Chapter 5).

For the mouse data, to obtain a statistically acceptable fit of the model to the data, the data in the highest three dosage groups (Table 7.1) were not used (EPA, 1984). This step is unfortunate since all the data are not used and the data which are not used show a very high tumor incidence (Table 7.1). (The strong carcinogenic response at the high doses adds to the weight of evidence for the carcinogenicity of BaP.) However, with this change the p value for goodness of fit for the multistage model is an acceptable 0.37. The mouse gastric tumor data yield a maximum likelihood estimate (MLE) for q_1 (the linear or slope term, which relates the probability of cancer to the dose of carcinogen administered) of 0.006 $(mg/kg/day)^{-1}$ and a q_0 equal

Table 7.1. Gastric Tumors in Mice from Feeding Benzo(a)pyrene[a]

Exposure (ppm)	Calculated Daily Dose[b] (mg/kg/day)(animal)	Incidence of Gastric Tumors[c]
0	0	0/289
1	0.078	0/25
10	0.781	0/24
20	1.563	1/23
30	2.344	0/37
40	3.126	1/40
45	3.516	4/40
[50	3.908	24/34][d]
[100	7.815	19/23][d]
[250	19.538	66/73][d]

[a] Adapted from Neal and Rigdon (1967) and EPA (1984).

[b] Calculation based on a 0.034 kg mouse consuming 13% of its body weight in food daily for 110 days during a 183 day experiment.

[c] Number responding with gastric papillomas or carcinomas over number exposed to given food concentration.

[d] Use of these exposure concentrations does not result in a statistically acceptable fit of the GLOBAL 86 multistage model to the data set (see text).

Table 7.2. Respiratory Tract Tumors from Benzo(a)pyrene Inhalation[a]

Exposure (mg/m^3)	Hamster Dose (mg/kg/day)[b] based on I = 0.037 m^3/day[c]	0.063 m^3/day[d]	Tumor Incidence
0	0	0	0/27
2.2	0.089	0.152	0/27
9.5	0.385	0.655	9/26
[46.5			13/25][e]

[a] Adapted from Thyssen et al. (1981) and EPA (1984).

[b] Based on the various exposure conditions in Thyssen et al. (1981). The calculation is presented in EPA (1984). For the lowest dose, 2.2 mg/m^3 x [(10wk/95.2 wk x 4.5h/24h) + (85.2wk/95.2wk x 3h/24h)] x 0.037m^3/d / 0.12kg = 0.089 mg/kg/day.

[c] Inhalation rate assumed in EPA (1984) based on a standard hamster size of 0.120 kg and an allometric equation based on rat data.

[d] Based on $I = 0.5\ W^{0.9017} = 0.5\ (0.1^{0.9017}) = 0.063 m^3/day$ (EPA), 1988. Thyssen et al. (1981) used 0.1 kg hamsters.

[e] These data were not used due to shortened lifespan of the hamsters in the exposure group. (The carcinogenic response, however, is apparent.)

to 0. An Upper 95% Confidence Limit (UCL) on q_1, q_1^*, equal to 0.0222 $(mg/kg/day)^{-1}$, also known as the carcinogenic potency, was obtained from the data. The values for q presented here are the same values EPA obtained using the same data (EPA, 1984).

Several numerical adjustments must be made to convert the q_1^* calculated from the animal data to a q_1^* relevant to humans. The experimental, less-than-lifetime exposure period L_e (183 days for the Neal and Rigdon mouse bioassay) was adjusted to an equivalent lifetime exposure L, estimated by the EPA (1984) to be equal to 630 days for mice, by dividing L_e by L. This term was then raised to the third power, based on the assumption that cancer incidence increases with the third power of age. A surface area scaling factor, the human to animal body weight ratio raised to the 1/3 power, was then applied to relate the experimental animal doses to equivalent human doses. Thus:

$$q_1^* \text{ (human)} = q_1^* \text{ (animal)} \times (L/L_e)^3 \times (\text{human bw/animal bw})^{1/3}$$

$$q_1^* \text{ (human)} = 0.0222 \times (630/183)^3 \times (70/0.034)^{1/3} = 11.5 \text{ (mg/kg/d)}^{-1}$$

If a 2 year (730 day) lifespan is used for mice, q_1^* is 17.9 $(mg/kg/d)^{-1}$.

The value of 11.5 $(mg/kg/day)^{-1}$ obtained using the Neal and Rigdon data is consistent with other estimates for BaP carcinogenicity. In an unpublished study, Zeise and Crouch (1984) examined all existing multiple dose data sets of BAP carcinogenicity in rodents (principally mice) induced by feeding, by gavage, and through the drinking water, including the Neal and Rigdon (1967) data. Many data sets were faulty due to lack of controls or lack of explicit

information on the conduct of the experiment in the paper. Multistage models were fit to the data sets. Most animal cancer potency value estimates, expressed as coefficients of dose to the first power, were in the range of 0.2 to 1.3 $(mg/kg/day)^{-1}$. Multiplying by a mouse to man surface area correction factor of $(70/0.03)^{1/3} = 13.3$ yields a range of human potency estimates of 2.6 to 17.2 $(mg/kg/day)^{-1}$.

Assuming that the percentage of benzo(a)pyrene absorbed by the respiratory tract after inhalation is similar for mice and humans (Chapter 3) and using an average human body weight of 70 kg and an average inhalation rate of 20 m^3 per day, a dose of 1 mg/kg/day benzo(a)pyrene is equivalent to breathing air with a BaP concentration of 3500 $\mu g/m^3$. Using the latter units, the 95% UCL for q_1, q_1^*, equals 3.3×10^{-3} $(\mu g/m^3)^{-1}$. This is the (lifetime) unit risk value for inhalation. Since benzo(a)pyrene generally occurs in particulate matter, calculations in units of $(ppm)^{-1}$ or $(ppb)^{-1}$ are not appropriate.

The multistage model was also fit to data for respiratory tract tumors resulting from inhalation exposure of hamsters to benzo(a)pyrene (Table 7.2). The data from the highest dose group were not used since these animals had an appreciably shortened lifespan (59 weeks versus 96 weeks in other groups) (Thyssen et al. 1981; EPA, 1984). By considering the conditions of exposure given in the report and using an inhalation rate of 0.037 m^3/day and a "standard" body weight of 0.12 kg for hamsters (EPA, 1988), a dose of benzo(a)pyrene in mg/kg/day can be estimated (Table 7.2) (EPA, 1984). From the hamster inhalation data, MLE values of $q_0 = 0$, $q_1 = 0$ and $q_2 = 2.68$ $(mg/kg/day)^{-1}$ were obtained. The p value for goodness of fit was 0.43. For 95% UCLs, the model yields a $q_1^* = 0.73$ and a $q_2^* = 0.78$. For practical

purposes, at low doses such as those encountered by breathing ambient air which are 5 orders of magnitude below the experimental doses, only q_1^* is usually important mathematically. The q_1^* obtained using the animal data can then be converted to a q_1^* (human) by multiplying by (human bw/hamster bw)$^{1/3}$, the surface area extrapolation factor, to obtain a q_1^* (human) = 6.11 (mg/kg/day)$^{-1}$. This potency estimate was obtained by EPA (1984) and is within a factor of 2 of that obtained using mouse stomach tumor data. The unit risk derived from this data set is 1.7×10^{-3} (μg/m^3)$^{-1}$.

The inhalation rate for hamsters of 0.037 m^3/day used by the EPA (1984) is a low estimate (Marty Miller, New York Department of Health, personal communication). EPA has recently published a handbook with recommended values for biological parameters used in risk assessment (EPA, 1988). The allometric formula given for the inhalation rate of hamsters is $I = 0.5\ W^{0.9017}$. Hamsters weighing 100 g (0.1 kg), as actually used by Thyssen et al. (1981) in their studies, would thus inhale 0.063 m^3 per day. With this higher inhalation rate and lower body weight, 70% higher doses of BaP are estimated (Table 7.2) and a q_1^* (animal) equal to 0.43 (mg/kg/day)$^{-1}$ is obtained. Multiplying by the interspecies surface area correction factor of $(70/0.1)^{1/3}$ yields a human equivalent q_1^* = 3.8 (mg/kg/day)$^{-1}$ for ingestion and one of 1.1×10^{-3}(μg/m^3)$^{-1}$ for inhalation.

Several studies have used intratracheal instillation of BaP to demonstrate its carcinogenicity (Section 5.3.2). While this method of dosing is radically different from inhalation, a larger percentage of the dose gets to the lower respiratory tract than with inhalation. There are several data sets for tumors due to intratracheal instillation which are available for carcinogenic

potency determination. Two experiments were selected and evaluated since they involved a relatively large number of doses and animals. Saffiotti et al. (1972) dosed groups of male and female hamsters with 0.25, 0.5, 1, and 2 mg BaP (with an equal weight of ferric oxide as particulate carrier) weekly for 30 weeks and observed the animals for their lifetimes. Some animals in each dose group showed respiratory tract tumors (Table 7.3). Applying surface area extrapolation and correction factors as described in the footnotes of Table 7.3 to the results of the multistage model yields a q_1^* (human) of 16.9 $(mg/kg/day)^{-1}$ from the data on males and 15.7 $(mg/kg/day)^{-1}$ from the data on females. In another experiment Feron et al. (1973) gave male Syrian golden hamsters intratracheal doses of 0, 0.625, 0.125, 0.5, or 1 mg BaP weekly for 52 weeks. A variety of tumors were produced throughout the respiratory tract. Data on bronchoalveolar adenomas and carcinomas were selected for risk assessment (Table 7.4). The data yielded a human equivalent q_1^* of 15.3 $(mg/kg/day)^{-1}$, in good agreement with other estimates. The potencies obtained from the several data sets with GLOBAL86 are summarized in Table 7.5.

7.2.3. Risk Estimate using the Gaylor-Kodell Approach

The application of the multistage model requires the use of a computer program to fit the model to the data and extrapolation into the range below the lowest dose tested. Another approach was introduced by Gaylor and Kodell (Gaylor and Kodell, 1980; Williams and Burson, 1985). In this method a model is fit to the observed responses, then the 95% upper confidence limit (UCL) of the value predicted from the model is determined for the lowest dose of risks result from not using the incidence data for the 20 ppm dose (Table 7.1). Clement Associates calculated dose coefficients of 3.22 and 5.74 $(mg/kg/day)^{-1}$

Table 7.3. Respiratory Tract Tumors from Intratracheal Instillation of Benzo(a)pyrene in Hamsters- 30 Week Exposure[a]

Weekly Dose[b] (mg)	Average Daily Dose (mg)	Lifetime Adj. Daily Dose[c] (mg/kg/day)	Human Equivalent[d] Dose (mg/kg/day)	Tumor Incidence[e] Males	Females
0	0	0	0	0/47	0/45
0.25	0.036	0.119	0.013	6/47	4/41
0.5	0.071	0.239	0.027	10/33	9/30
1.0	0.143	0.477	0.054	22/33	20/34
[2.0	0.286	0.953	0.107	17/28	17/29][f]

[a] Adapted from Saffiotti et al. (1972).

b All groups received an equal amount of benzo(a)pyrene and ferric oxide except the 0 control which received only 2 mg ferric oxide.

[c] Calculated by dividing the weekly dose by 7 days and 0.1 kg body weight and multiplying by (30 weeks exposure/90 week lifetime). There was a dose-related decrease in survival in the experiment.

[d] The animal daily dose was divided by $(70\ kg/0.1\ kg)^{1/3}$.

[e] Animals with respiratory tract tumors over effective number of exposed animals (i.e., those not cannibalised and not showing post-mortem autolysis). There was an additional control group which was untreated. No respiratory tract tumors were noted among 97 males and 96 females. A dose-dependent increase in gastric tumors was also noted in this experiment.

[f] Data group was not used since exposure started 7 weeks after other groups.

Table 7.4. Bronchoalveolar Tumors from Intratracheal Instillation of Benzo(a)pyrene in Hamsters- 52 Week Exposure[a]

Weekly Dose (mg)	Average Daily Dose (mg)	Lifetime Adj. Daily Dose[b] (mg/kg/day)	Human Equivalent[c] Dose (mg/kg/day)	Tumor Incidence[d]
0	0	0	0	0/29
0.0625	0.009	0.0495	0.0059	1/30
0.125	0.018	0.0989	0.0118	4/30
0.25	0.036	0.198	0.0237	6/30
0.5	0.071	0.395	0.0473	17/30
1.0	0.143	0.791	0.0947	19/30

[a] Adapted from Feron et al. (1973).

[b] Calculated by dividing the weekly dose by 7 days and 0.12 kg and multiplying by (52 week exposure/78 week length of experiment).

[c] Calculated by dividing the animal daily dose by $(70\ kg/0.12\ kg)^{1/3}$.

[d] Animals bearing bronchoalveolar adenomas and/or carcinomas over the number of animals examined.

Table 7.5 Cancer Potency Estimates obtained from GLOBAL86 using Various Data Sets

Data Set	Tumor Type[a]	Variable	95% UCL $(mg/kg/d)^{-1}$	95% UCL $(\mu g/m^3)^{-1}$
Neal & Rigdon (1967)	gastric		11.5	3.3×10^{-3}
Thyssen et al. (1981)	respiratory	$I=.037 m^3/d$	6.1	1.7×10^{-3}
Thyssen et al. (1981)	respiratory	$I=.063 m^3/d$	3.8	1.1×10^{-3}
Saffioti et al.(1972)	respiratory	(males)	16.9	4.8×10^{-3}
Saffioti et al.(1972)	respiratory	(females)	15.7	4.5×10^{-3}
Feron et al. (1973)	respiratory		15.3	4.4×10^{-3}

[a] For more details on tumor type see Chapter 5.

(Clement Associates, 1987, 1988 respectively) using the two stage model. For comparison, the multistage model yields a q_1^* of 6.23 $(mg/kg/day)^{-1}$ when the 20 ppm dose data are dropped. In the case of the inhalation-induced tumors (Table 7.2), the lower risks result from not using a surface area extrapolation factor (Clement Associates, 1988). These authors calculated a dose coefficient of 0.45 $(mg/kg/day)^{-1}$ whereas the multistage model yields a q_1^* of 0.43 $(mg/kg/day)^{-1}$ prior to the surface area adjustment. Thus, although the two-stage model is intended to be more relevant chemical for which cancer incidence is increased over background. This UCL is then extrapolated linearly ("interpolated") to the background incidence to determine an upper boundary line on risk. Under the assumption of strict linearity (not just at low doses), the true risk is predicted to be at or below this line with 95% probability. For gastric tumors in mice (Neal and Rigdon, 1967), the 95% UCL of risk calculated from the multistage model fit at the 45 ppm feeding dose was 0.21. Since the background incidence of respiratory tumors is 0, the excess risk is 0.21. The animal daily dose from 45 ppm BaP of 3.516 mg/kg/day (Table 7.1) can be converted to a human equivalent dose by dividing the animal dose by $(70\ kg/0.034\ kg)^{1/3}$ or 12.7. Thus 3.516/12.7 = 0.28 mg/kg/day. Dividing the excess risk of 0.21 by 0.28 mg/kg/day yields a potency of 0.75 $(mg/kg/day)^{-1}$. This converts to an inhalation risk equal to 2.1×10^{-4} $(\mu g/m^3)^{-1}$. For the estimated ambient level of 5.3×10^{-4} $\mu g/m^3$, the individual risk is 1.2×10^{-7}. This is a low estimate since a factor to correct for the less than lifetime experiment was not used. The factor of $(630/183)^3$ used above equals 40.8. Therefore a corrected risk would be 0.75 x 40.8 = 30.6 $(mg/kg/day)^{-1}$.

Application of the Gaylor-Kodell approach to the hamster inhalation data (Table 7.2) resulted in a potency of 7.4 $(mg/kg/d)^{-1}$ and an inhalation risk of $2.1x10^{-3}$ $(\mu g/m^3)^{-1}$. In this instance the 95% UCL on the model fit at the 9.5 mg/m^3 exposure was 0.54 (using I = 0.063 m^3/d) and the human equivalent dose was 0.0738 mg/kg/d $(0.655 / [70/0.1]^{1/3})$.

A biologically-based, two stage model of cancer has been proposed with equations containing variables for interstage transitions from normal cell to precancerous cell (first stage) and from precancerous cell to malignant cell (second stage), as well as variables for organ cell numbers, cell birth and death rates, carcinogen dose, and time (Moolgavkar and Knudson, 1981; Clement Associates, 1987; 1988; Charnley and Thorslund, 1988). At the very low doses expected from environmental exposures, this model, like the multistage model, reduces to a linear function of dose.

$$P(x) = 1 - e^{-A(1 + Sx)^2} = 1 - e^{-A(1 + 2Sx + S^2x^2)} \approx 1 - e^{-A(1 + 2Sx)}$$

Instead of the coefficient q_1 in the multistage model, the coefficient in the two stage model at low doses is 2S, where S is the exposure-dependent transition rate for conversion of normal cells to precancerous cells and precancerous cells to malignant cells and A is the background transition rate between cell stages. The model has been applied to the BaP data of Neal and Rigdon (1967) and Thyssen et al. (1981) and yields lower risks than the multistage model (Clement Associates, 1987; 1988; Charnley and Thorslund, 1988). However, in the case of feeding-induced gastric tumors, the lower risks result from not using the incidence data for the 20 ppm dose (Table 7.1). Clement Associates calculated dose coefficients of 3.22 and 5.74

$(mg/kg/day)^{-1}$ (Clement Associates, 1987, 1988 respectively) using the two stage model. For comparison, the multistage model yields a q_1^* of 6.23 $(mg/kg/day)^{-1}$ when the 20 ppm dose data are dropped. In the case of the inhalation-induced tumors (Table 7.2), the lower risks result from not using a surface area extrapolation factor (Clement Associates, 1988). These authors calculated a dose coefficient of 0.45 $(mg/kg/day)^{-1}$ whereas the multistage model yields a q_1^* of 0.43 $(mg/kg/day)^{-1}$ prior to the surface area adjustment. Thus, although the two-stage model is intended to be more relevant biologically, in this instance it does not give risk numbers for BaP significantly different from the multistage model.

7.2.4. Uncertainty in Risk Assessment Estimates

The range of risk values results from several sources of uncertainty, including statistical uncertainty due to the number of animals in the experiment to which the model was applied. For example, the Thyssen et al. study had only 25-27 hamsters per group. Other sources of uncertainty, include the extent of absorption of benzo(a)pyrene by various routes, variability of response to benzo(a)pyrene in different species, the choice of the animal-to-human scaling factors, the choice of the extrapolation model, and the large range of extrapolation (five orders of magnitude) from the benzo(a)pyrene concentrations used in the animal experiments to current ambient levels. In the Neal and Rigdon study the animals began BaP exposures at different ages including as late as 4 months of age and the pathology protocol described was not rigorous. The latter could actually lead to an underestimation of tumors. The GLOBAL86 extrapolation model could not be fit to the high dose data in the Neal and Rigdon experiment, data which clearly

indicated a carcinogenic effect. In addition there is the possibility in light of the absence of an epidemiological connection between exposure to BaP and cancer that the risks in mice and hamsters may not be applicable to humans. Since many mixtures containing BaP are known human carcinogens, this is unlikely. While a portion of the population is exposed to concentrations of BaP greater than 0.53 ng/m^3, others will be exposed to less and thus have a lower risk.

7.2.5. Estimate of Cancer Incidence in California

Air measurements of benzo(a)pyrene in California have yielded an estimated ambient air concentration of 5.3×10^{-4} $\mu g/m^3$. Using the q_1^* of 1.1×10^{-3} and 3.3×10^{-3} $(\mu g/m^3)^{-1}$ derived from hamster inhalation (Table 7.2) and mouse feeding (Table 7.1) data sets respectively, the two best data sets available, a range for individual risk from:

$$1.1 \times 10^{-3} \ (\mu g/m^3)^{-1} \times 5.3 \times 10^{-4} \ (\mu g/m^3) = 5.9 \times 10^{-7}$$

to

$$3.3 \times 10^{-3} \ (\mu g/m^3)^{-1} \times 5.3 \times 10^{-4} \ (\mu g/m^3) = 1.7 \times 10^{-6}$$

can be estimated. (These values are for what has often been called extra risk, [P(d) - P(0)] / [1 - P(0)], which assumes that the environmental carcinogen causes cancer independently of "background" causes.) The upper 95% confidence limit estimate of excess cancers in the California population of 28×10^6 persons due to a lifetime exposure by inhalation to current ambient levels of benzo(a)pyrene ranges from:

$$5.9 \times 10^{-7} \times 28 \times 10^{6} = 16 \text{ excess cancers}$$

to

$$1.7 \times 10^{-6} \times 28 \times 10^{6} = 48 \text{ excess cancers}$$

These theoretical cancer cases would occur among the currently expected 6 to 8 million cases of cancer in the state's population of 28 million persons, based on cancer incidence for Los Angeles County for the years 1972 through 1977 and on estimated cancer incidence in California for 1988 (World Health Organization, 1982; Silverberg and Lubera, 1988).

7.2.6 Selection of Best Values for Risk Assessment

Because of the limited amount of data currently available for risk assessment of benzo(a)pyrene, the inhalation unit risk of 1.1×10^{-3} $(\mu g/m^3)^{-1}$ based on respiratory tract tumors in hamsters (Table 7.5) must be used as a best value for inhalation exposures. For exposures to benzo(a)pyrene by other routes the potency of 11.5 $(mg/kg/d)^{-1}$ based on gastric tract tumors in mice (Table 7.5) can be used.

7.2.7. Relative Potencies of Other PAHs

Benzo[a]pyrene is the most studied PAH, but it is only one of more than one hundred PAH compounds known. IARC considers several purified PAHs and PAH derivates to be probable (Group 2A) or possible (Group 2B) human carcinogens (IARC, 1987) (Table 7.6). Some mixtures containing PAHs are known human carcinogens (Group 1) (Table 7.6). The USEPA has classified several PAHs in

Table 7.6. IARC Groupings of PAHs, Mixtures with PAHs, and Derivatives

Group 1	Group 2A	Group 2B
Coal-tar pitches	Benz[a]anthracene	Benzo[b]fluoranthene
Coal-tars	Benzo[a]pyrene	Benzo[j]fluoranthene
Coke production	Creosotes	Benzo[k]fluoranthene
Mineral oils	Dibenz[a,h]anthracene	Carbon black extracts
Shale-oils		Dibenz[a,h]acridine
Soots		Dibenz[a,j]acridine
Tobacco smoke		7H-Dibenzo[c,g]carbazole
		Dibenzo[a,e]pyrene
		Dibenzo[a,h]pyrene
		Dibenzo[a,i]pyrene
		Dibenzo[a,l]pyrene
		Indeno[1,2,3-cd]pyrene
		5-Methylchrysene
		5-Nitroacenaphthene
		1-Nitropyrene
		4-Nitropyrene
		1,6-Dinitropyrene
		1,8-Dinitropyrene
		6-Nitrochrysene
		2-Nitrofluorene

Abstracted from IARC Supplement 7 (1987) and IARC Volume 46 (1989).
Group 1: carcinogenic to humans.
Group 2A: probably carcinogenic to humans.
Group 2B: possibly carcinogenic to humans.

Group B2, possibly carcinogenic to humans (Table 7.7). IARC (1987, 1989) has classified a large number of PAHs in Group 3, a class of chemicals for which there are no human data but limited or inadequate data in animals (Table 7.8).

While the studies available for carcinogenic risk assessment of BaP are not ideal for risk assessment, those for practically all other individual PAHs are less complete for risk assessment. However, there are extensive data establishing the genotoxicity, and in some cases the carcinogenicity, of many PAHs or their genotoxic metabolites. In other cases, some PAHs are not considered carcinogens. Several authors have used mutagenicity and various tests of carcinogenicity to rank several PAHs for their relative carcinogenicity (e.g., Deutsch-Wenzel et al., 1983; Bingham and Falk, 1969; Habs et al., 1980; Wynder and Hoffman, 1959; Wislocki et al., 1986) and their relative genotoxicity (Brown, 1989). Many of these comparisons were summarized by Clement Associates (1988) (Table 7.9) and Krewski et al. (1989). In these analyses dibenz(a,h)anthracene has been shown to be more potent than BaP, while other PAHs tested were less or much less potent. These comparisons indicate that considering all PAHs to be equivalent in potency to BaP would overestimate the cancer potency of a PAH mixture, but such an assumption would be health protective and is likely to be helpful in a screening estimate of PAH risks.

The mutagenicity of nitro derivatives, such as 2-nitrofluoranthene and the nitropyrenes, can be greater than their parent PAHs. Such compounds can be formed during combustion or through atmospheric interaction with NOx emissions. IARC (1987; 1989) has classified several nitro PAHs, including

Table 7.7. USEPA Groupings of PAHs

Group B2	Group D
Benz[a]anthracene	Acenaphthylene
Benzo[a]pyrene	Anthracene
Benzo[b]fluoranthene	Benzo[e]pyrene*
Benzo[j]fluoranthene*	Benzo[g,h,i]perylene
Benzo[k]fluoranthene	Fluorene
Chrysene	Naphthalene
Dibenz[a,h]anthracene	Phenanthrene
Indeno[1,2,3-c,d]pyrene	

Abstracted from IRIS (1993). Group B2: possibly carcinogenic to humans. Group D: unclassifiable as to carcinogenicity.
* risk assessment under review as of April 1993

Table 7.8. IARC Group 3 PAHs and PAH derivatives

Chemical	Animal Evidence
Acridine orange	inadequate
5-Aminoacenaphthene	inadequate
2-Aminoanthraquinone	limited
Anthanthrene	limited
Anthracene	inadequate
Benz[a]acridine	inadequate
Benz[c]acridine	limited
Benzo[ghi]fluoranthene	inadequate
Benzo[ghi]perylene	inadequate
Benzo[c]phenanthrene	inadequate
Benzo[e]pyrene	inadequate
Carbazole	limited
Chrysene	limited
Cyclopenta[c,d]pyrene	limited
Dibenz[a,c]anthracene	limited
Dibenz[a,j]anthracene	limited
Dibenzo[a,e]fluoranthene	limited
Dibenzo[h,rst]pentaphene	limited
3,7-Dinitrofluoroanthene	limited
3,9-Dinitrofluoroanthene	limited
1,3-Dinitropyrene	limited
Fluoranthene	inadequate
Fluorene	inadequate
1-Methylchrysene	inadequate
2-Methylchrysene	limited
3-Methylchrysene	limited
4-Methylchrysene	limited
6-Methylchrysene	limited
2-Methylfluoranthene	limited
1-Methylphenanthrene	inadequate
1,5-Naphthalenediamine	limited
9-Nitroacenaphthene	limited
9-Nitroanthracene	no adequate data
7-Nitrobenz[a]anthracene	limited
6-Nitrobenzo[a]pyrene	limited
3-Nitrofluoranthene	inadequate
1-Nitronaphthalene	inadequate
2-Nitronaphthalene	inadequate
3-Nitroperylene	inadequate
2-Nitropyrene	inadequate
Perylene	inadequate
Phenanthrene	inadequate
N-Phenyl-2-naphthylamine	limited
Pyrene	inadequate
Triphenylene	inadequate

Abstracted from IARC Supplement 7 (1987) and IARC volume 46 (1989). Group 3: have either limited or inadequate evidence in animals and are not classifiable as to their carcinogenicity in humans due to no adequate data.

TABLE 7.9 SUMMARY OF RELATIVE POTENCY ESTIMATES FOR INDICATOR PAHs

	Test System						
Compound	Mouse Skin Carcinogenesis	Subcutaneous Injection into Mice[m]	Intrapulmonary Administration to Rats[g]	Initiation-Promotion on Mouse Skin	Intraperitoneal Injection in Newborn Mice[m]	DNA Adduct Formation	Genotoxic Ranking[n]
Benzo(a)pyrene	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Benz(a)anthracene	0.145[a]				0.057,0.524,0.496[k]	0.07	0.62
Dibenz(ah)anthracene	1.11[d]	2.82[e],4.50[f]				0.56	0.47
Benzo(e)pyrene			0.004				0.42
Chrysene	0.0044[d]			0.040[i]	0.125,0.33[k]		0.37
Cyclopentadieno(cd)pyrene	0.023[b]						0.26
Benzo(b)fluoranthene	0.167[b]		0.140	0.258[h],0.125[i]	0.232,1.067,0.874[j]		0.20
Benzo(j)fluoranthene	0.061[b]			0.048[h]	0.320,0.471,0.450[j]		
Benzo(k)fluoranthene	0.020[b]		0.066	0.022[h]	0.040,0.097,0.044[j]		
Pyrene					0.081,0.050,0.586[k]		0.20
Indeno(1,2,3-cd)pyrene	0.021[b],0.189[c]		0.232	0.074[c]	0.013[j]		0.14
Benzo(ghi)perylene	0.015[c]		0.022	0.005[c]			0.08
Anthanthrene			0.320				0.06

[a]Bingham and Falk (1969).
[b]Habs et al. (1980).
[c]Hoffmann and Wynder (1966).
[d]Wynder and Hoffmann (1959).
[e]Pfeiffer (1977).
[f]Bryan and Shimkin (1943).
[g]Deutsch-Wenzel et al. (1983).
[h]LaVoie et al. (1982).
[i]Van Duuren et al. (1966).
[j]LaVoie et al. (1987).
[k]Wislocki et al. (1986).
[l]Phillips et al. (1979).
[m]Where more than one potency estimate is shown, they were derived from the same study using different tumor types as end points.
[n]Brown (1989).
Modified from Clement Associates (1988).

1-nitropyrene, 4-nitropyrene, 1,6-dinitropyrene, 1,8-nitropyrene, 6-nitrochrysene, 2-nitrofluorene, and 5-nitroacenaphthene, as possible human carcinogens (class 2B) (Table 7.6). The contribution to carcinogenicity of nitro derivatives has not been as extensively studied and has not been evaluated in previous comparisons. They have not been systematically evaluated by the USEPA. Note that nitro derivatives are not included in Table 7.9.

If one assumes that PAHs are as carcinogenic as they are genotoxic, then their hazard relative to BaP would be dependent on their concentration in the environment. If PAHs other than BaP are much more prevalent than BaP itself, then they could contribute a substantial portion of risk to humans. That is, they may be of lesser potency, but of greater concentration. As indicated by Brown (1989), several PAHs may be greater genotoxic risks than BaP in urban ambient environments due to their much higher concentration. But BaP appears, in light of the limited information available on other PAHs, to remain an important representative or surrogate for this important group of chemically diverse air pollutants.

7.2.8 Selection of Risk Values for Other PAHs

The original intent in the toxic air contaminant identification process was to examine polycyclic aromatic hydrocarbons as a class. However, BaP was chosen as the primary representative of the class because of the large amount of toxicological data available on BaP (versus the relatively incomplete database for other PAHs), the availability of monitoring techniques for BaP, and the significant exposure expected (and found). But, as indicated above, a number of PAHs and PAH derivatives, e.g. nitroPAHs, are considered to be potentially carcinogenic to humans. Thus there is a need to consider the impact of not only BaP but also of a large number of related PAHs and PAH derivatives. In order to address this issue, a recent paper (LaGoy and Nisbet, 1991) presented a Toxic Equivalency Factor (TEF) scheme for 17 PAHs. The paper was an extension of earlier work by other investigators (Clement Associates 1987, 1988; Krewski et al., 1989) which is shown in Table 7.9. Along similar lines OEHHA has developed a Potency Equivalency Factor (PEF) procedure to assess the relative potencies of PAHs and PAH derivatives as a group. This would allow the ARB to address the impact of carcinogenic PAHs in ambient air since they are usually present together.

Due to the variety of data available on the carcinogenicity and mutagenicity of PAHs, an order of preference for the use of available data in assessing relative potency was developed. If a health effects evaluation and quantitative risk assessment leading to a cancer potency value have been conducted on a specific PAH, then these values should be given the highest preference. Such an analysis for BaP has been carried out in this report, in earlier USEPA reports, and has been published by Collins et al. (1991).

Unfortunately no similar risk assessments have been carried out for other PAHs although suitable data, at least from ingestion, are available for some. Second in the order of preference would be a risk assessment carried out using standard risk assessment methodology and valid tumor incidence data. One example would be the expedited cancer potency values developed for the implementation of Proposition 65 using the cancer potency (TD50) database of Gold et al. (1984, 1986, 1987, 1989, 1990) and the multistage model. As of November, 1992, such risk assessments had been carried out for dibenz(a,h)anthracene, 7,12-dimethylbenz(a)anthracene, 3-methylcholanthrene, and 5-nitroacenaphthene. These methods would yield actual carcinogenic potencies which are listed in Table 7.10. The documentation for each chemical is discussed in Appendix A. If potency values have not been developed for specific compounds, a carcinogenic activity relative to BaP, rather than a true potency, can be developed. These relative activity values will be referred to as Potency Equivalency Factors (PEFs). Such factors are similar to Toxic Equivalency Factors (TEFs) developed for dioxin (CDHS, 1986). However, PEFs are on stronger scientific grounds for the prediction of cancer than the TEFs for dioxin since nearly all PEFs are based on cancer bioassay information while the dibenzodioxin and dibenzofuran TEFs are mainly based on acute toxicity, structure-activity relationships, and short term tests such as aryl hydrocarbon hydroxylase induction plus some limited cancer bioassay data. For air contaminants, relative potency to BaP based on data from inhalation studies would be optimal. Otherwise, intrapulmonary or intratracheal administration, such as those published by Deutsch-Wenzel et al. (1983), would be most relevant, since such studies are in the target organ of interest. Next in order of preference is information on activity by the oral route which is available for several compounds including BaP. This information would be

Table 7.10. Potencies of PAHs and Derivatives

Chemical	Potency[a]	Unit Risk[a]
benzo[a]pyrene	11.5	1.1×10^{-3}
dibenz[a,h]anthracene	4.1	3.9×10^{-4}
7,12-dimethylbenzanthracene	250	2.4×10^{-2}
3-methylcholanthrene	22	2.1×10^{-3}
5-nitroacenaphthene	0.13	1.1×10^{-5}

[a] Units are $(mg/kg\text{-}day)^{-1}$ for potency and $(\mu g/m^3)^{-1}$ for unit risk. The numbers for benzo[a]pyrene were developed in this document while the other potencies are from expedited risk assessments for implementing Proposition 65. It is assumed that unit risks for inhalation have the same relative activities as cancer potencies for oral intake.

useful in establishing PEFs since BaP causes tumors by the oral route. Data on skin painting are also available. These studies involve tumor production by PAHs following either repeated dosing with the PAH of interest or initiation with the PAH of interest followed by promotion with, for example, phorbol esters. Skin is also a target organ of concern with PAH exposure and the skin does have P450 activity to activate and deactivate PAHs. Intraperitoneal and subcutaneous administration rank at the bottom of the in vivo tests considered useful for PEF development because of their lack of relevance to environmental exposures. However, they do measure the ability of a PAH to cause cancer. Next in decreasing order of preference are genotoxicity data which exist for a large number of compounds. In many cases genotoxicity information is restricted to mutagenicity data. These data are of interest but, since the compounds we are considering have been classified as potential carcinogens based on actual cancer tests, their usefulness in applied situations is supplemental. Also the relative mutagenicities of nitroPAHs compared to PAHs may be much greater than their relative carcinogenicities. For example, 1,8-dinitropyrene can be as much as 10^5 times as mutagenic as BaP in the Ames test, yet it is at most ten times as carcinogenic and, based on the best available data, appears to be less carcinogenic than BaP. Finally there are data on structure-activity relationships among PAH compounds. Structure-activity considerations may help identify a PAH as carcinogenic, but at this time have not been established as predictors of carcinogenic potency.

Using this order of preference (Table 7.11), PEFs have been derived for a number of PAHs and are presented in Table 7.12. . These PEFs are based primarily on chronic internal dosing experiments and skin painting studies.

Table 7.11. Scheme for PEF Selection

1. Complete quantitative risk assessment
2. "Expedited" quantitative risk assessment
3. Tumor data from inhalation exposure
4. Tumor data from intratracheal or intrapulmonary administration
5. Tumor data from oral administration
6. Tumor data from skin painting studies
7. Tumor data from subcutaneous or intraperitoneal administration
8. Genotoxicity data
9. Structure activity information

Table 7.12. OEHHA'S PEF Weighting Scheme for PAHs

PAH or derivative	Suggested PEF
benzo[a]pyrene[ARB]	1.0 (index compound)
benz[a]anthracene	0.1
benzo[b]fluoranthene[ARB]	0.1
benzo[j]fluoranthene	0.1
benzo[k]fluoranthene[ARB]	0.1
dibenz[a,j]acridine	0.1
dibenz[a,h]acridine	0.1
7H-dibenzo[c,g]carbazole	1.0
dibenzo[a,e]pyrene	1.0
dibenzo[a,h]pyrene	10
dibenzo[a,i]pyrene	10
dibenzo[a,l]pyrene	10
indeno[1,2,3-c,d]pyrene[ARB]	0.1
5-methylchrysene	1.0
1-nitropyrene	0.1
4-nitropyrene	0.1
1,6-dinitropyrene	10
1,8-dinitropyrene	1.0
6-nitrochrysene	10
2-nitrofluorene	0.01
chrysene	0.01

ARB designates PAHs with ARB monitoring data. The nitroPAHs are those listed as IARC class 2B. Although chrysene is an IARC class 3 carcinogen, EPA classifies it as B2. The justification for each PEF is detailed in Appendix A.

Explanation of the derivation of each PEF, the type of data used in the derivation, and the relevant references are given in Appendix A. As indicated in Table 7.12, PEF values have been rounded to the nearest factor of 10. This was done to retain the uncertainty in these estimates.

In summary this analysis presents potency equivalency factors relative to BaP for 21 PAHs and PAH derivatives (Table 7.12). Cancer potency values in units of $(mg/kg/day)^{-1}$ are presented for 4 additional PAH compounds (Table 7.10). The focus of the evaluation has been on those chemicals with demonstrated carcinogenicity in bioassays. Unfortunately, potency estimates could not be developed for all potential PAH carcinogens. In addition, a much larger number of PAHs and PAH derivatives are considered mutagenic or genotoxic, but these compounds are not considered in this evaluation. Furthermore, structure-activity analysis may suggest that additional PAHs are carcinogenic. As a result, additional PAHs are likely to be identified as potential human carcinogens and these substances may need to be considered at a later date.

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Appendix A.

Potency and Potency Equivalency Factors (PEFs) for PAHs and Derivatives

1. Benzo[a]pyrene. Benzo[a]pyrene (BaP) is the index compound for relative potency and for Potency Equivalency Factors (PEFs) for PAHs and derivatives. The calculations of its cancer potency of 11.5 $(mg/kg\text{-}day)^{-1}$ and of its inhalation unit risk of 1.1×10^{-3} $(\mu g/m^3)^{-1}$ are presented in Chapter 7 of this report. For the potency equivalency scheme, it is assigned a PEF of 1.

2. Dibenz[a,h]anthracene. An expedited potency of 4.1 $(mg/kg\text{-}day)^{-1}$ has been derived using the linearized multistage model with the only dose response data set available - a drinking water study (Snell et al. 1962) which reported alveolar carcinomas of the lung in male DBA/2 mice due to dibenz[a,h]anthracene (incidence = 14/21 at 28.3 mg/kg-day versus 0/25 in controls). An inhalation unit risk can be obtained from a potency under the assumptions that the chemicals are equally absorbed and are equally potent by oral and inhalation routes and that a 70 kg person inhales 20 cubic meters of air per day. When the potency in units of $(mg/kg\text{-}day)^{-1}$ is divided by 3500 (70 kg * 1000 $\mu g/mg/20\ m^3$), an inhalation unit risk is obtained in units of $(\mu g/m^3)^{-1}$.

3. 7,12-Dimethylbenzanthracene. An expedited potency of 250 $(mg/kg\text{-}day)^{-1}$ has been derived. The only study listed in the Gold et al. cancer potency (TD50) database (Gold et al. 1984, 1986, 1987, 1989, 1990) is the feeding study by Chouroulinkov et al. (1967) in female albino mice. Significant increases in malignant angioendotheliomas of the mesenteric intestine and papillomas of the forestomach were observed in animals treated with 0.39 mg/kg-day of 7,12-dimethylbenzanthracene. Cancer potency is based on the angioendotheliomas of the mesenteric intestine (incidence = 49/75 vs 0/40 in controls).

4. 3-Methylcholanthrene. An expedited potency of 22 $(mg/kg\text{-}day)^{-1}$ has been derived. Results of 3 studies in male Long Evans rats, one study in an unspecified strain of female rats, and 10 studies in female Wistar rats are included in the Gold et al. database. All studies in female rats found highly significant increases in tumors of the mammary gland. The cancer potency for 3-methylcholanthrene is taken as the geometric mean of cancer potencies estimated from 9 of the 10 studies in female rats (Shay et al., 1962; Gruenstein et al., 1964; Shay et al., 1961). The upper bound on potency could not be estimated from one of the studies by Shay et al. (1961), because 100% of the treated animals developed mammary gland tumors.

5. 5-Nitroacenaphthene. An expedited potency of 0.13 $(mg/kg\text{-}day)^{-1}$ has been derived based on the combined incidence of benign and malignant tumors of the ear canal in female rats. Usable studies are feeding studies by Takemura et al. (1974) in female Syrian golden hamsters and by the National Cancer Institute (NCI) (1978) in male and female B6C3F1 mice and F344 rats. The compound 5-nitroacenaphthene induced increases in tumor incidences at multiple sites in rats and female mice. Rats are the most sensitive species; the sensitivity of males is similar to that of females.

6. Benzo[b]fluoranthene. Benzo[b]fluoranthene has been assigned a PEF of 0.1. Clement Associates (1988) applied both a two stage model, discussed in Section

7.2.3, and the multistage model to various data sets for several PAHs (see Table 7.8.). The two models generally gave similar results for relative potency. In order to verify the results, OEHHA staff used GLOBAL86 to fit the multistage model to the tumor data used by Clement Associates and obtained relative cancer potencies similar to those obtained by Clement Associates. Clement Associates (1988) used the mouse skin carcinogenesis data obtained by Habs et al. (1980) and the intrapulmonary administration to rats by Deutsch-Wenzel et al. (1983) to estimate a cancer potency for benzo[b]fluoranthene relative to BaP. As an example of the type of data used, Deutsch-Wenzel et al. obtained pulmonary tumor incidences of 0, 2.9, and 25.7% after intrapulmonary administration of 0.1, 0.3, and 1 mg benzo[b]fluoranthene, respectively, whereas they obtained 11.8, 60.0, and 94.3% tumor incidences after the same doses of benzo[a]pyrene. Clement Associates estimated a relative cancer potency for benzo[b]fluoranthene of 0.140 after fitting the two stage model to the data and 0.105 after fitting the multistage model. Using the data of Habs et al. a relative cancer potency of 0.167 was obtained with the two stage model and 0.201 with the multistage model. The results from the multistage model were averaged, then rounded (down) to 0.1 to obtain the PEF. OEHHA obtained a relative potency of 0.208 for benzo[b]fluoranthene fitting the multistage model to the data from Habs et al. OEHHA staff were also able to reproduce the calculations for the 2 stage model in the case of the hamster inhalation data. Since the multistage model is the accepted model for cancer risk assessment in California, results from the multistage model have been used to obtain PEFs although the 2 models usually gave the same PEF.

7. Benzo[j]fluoranthene. Benzo[j]fluoranthene has been assigned a PEF of 0.1. Clement Associates (1988) used the mouse skin carcinogenesis data obtained by Habs et al. (1980) to estimate a cancer potency relative to BaP of 0.0648. OEHHA staff estimated 0.065 using the same data. This was rounded to 0.1 to obtain the PEF. Clement Associates did not use the data of Deutsch-Wenzel et al. (1983) on benzo[j]fluoranthene to calculate a relative potency but Deutsch-Wenzel et al. found that it was very similar in tumorigenic activity to benzo[k]fluoranthene.

8. Benzo[k]fluoranthene. Benzo[k]fluoranthene has been assigned a PEF of 0.1. Clement Associates (1988) used mouse skin carcinogenesis data obtained by Habs et al. (1980) to obtain a cancer potency relative to BaP of 0.0235 and the intrapulmonary administration to rats by Deutsch-Wenzel et al. (1983) to estimate one of 0.085. Because the latter was obtained by the pulmonary route it was chosen to be the basis of the PEF. The value was rounded to 0.1 to obtain the PEF.

9. Benz[a]anthracene. Benz[a]anthracene has been assigned a PEF of 0.1. In the case of benz[a]anthracene, mouse skin carcinogenesis data obtained by Bingham and Falk (1969) were used by Clement Associates (1988) to calculate potencies for benz[a]anthracene. For this chemical the multistage model gave a relative potency of 0.0137. Using the 2 stage model a higher cancer potency of 0.145 relative to BaP was obtained. In the Wislocki et al. (1986) report, in which lung adenomas were induced in newborn mice, benz[a]anthracene (2.8 micromoles) was less carcinogenic (12/71 or 17% versus 7/138 or 5% in controls) relative to 0.56 micromoles BaP (24/64 or 38% versus 7/138 in controls). The relative potency was 0.08, which rounds to 0.1. Since the U.S. EPA is at least provisionally using 0.1 for this PAH (EPA, 1993b) and the

data from the Wislocki study are consistent with a PEF of 0.1, the value of 0.1 was selected by OEHHA.

10. Dibenz[a,j]acridine. Dibenz[a,j]acridine has been assigned a PEF of 0.1. Warshawsky et al. (1992) compared the tumor-initiating ability of dibenz[a,j]acridine to benzo[a]pyrene in mouse skin. Two hundred nanomoles of each compound were applied to groups of 30 mice, then the skin lesion was promoted with a phorbol ester for 24 weeks. Twenty-seven out of 30 BaP mice (90%) had skin papillomas, while 17 of 30 (57%) of the dibenz[a,j]acridine mice had skin papillomas. The multistage model was fit to both sets of data and the ratio of upper 95% confidence limits on the linear coefficient was 0.36. This is rounded to a PEF of 0.1.

11. Dibenz[a,h]acridine. Dibenz[a,h]acridine has also been assigned a PEF of 0.1. Its carcinogenic classification by IARC was based on studies published in 1940 and earlier and the studies did not appear appropriate for estimation of a PEF. Since its structure is similar to dibenz[a,j]acridine, it has been assigned the same PEF as dibenz[a,j]acridine until usable compound-specific bioassay data are available.

12. 7H-Dibenzo[c,g]carbazole. 7H-dibenzo[c,g]carbazole has been assigned a PEF of 1.0. Warshawsky et al. (1992) compared the tumor-initiating ability of 7H-dibenzo[c,g]carbazole to benzo[a]pyrene in mouse skin. Two hundred nanomoles of each compound were applied to 30 mice, then promoted with a phorbol ester for 24 weeks. Twenty-seven out of 30 BaP-treated mice (90%) had skin papillomas, while 26 of 30 (87%) of the dibenz[a,j]acridine-treated mice had skin papillomas for a relative tumorigenic activity of 0.97. This is rounded to a PEF of 1.

13. Dibenzo[a,l]pyrene. Dibenzo[a,l]pyrene has been assigned a PEF of 10. Cavalieri et al. (1989, 1991) studied the tumor-initiating and dose-response tumorigenicity of 4 dibenzo[a]pyrenes in mouse skin and rat mammary gland. BaP was used as a reference compound in some experiments. Dibenzo[a,l]pyrene was the most potent member of the group. Several levels of PAHs were tested. When results from 33.3 nanomoles of dibenzo[a,l]pyrene as a skin tumor initiator (with promotion by a phorbol ester) were compared to results using the same amount of BaP, dibenzo[a,l]pyrene induced skin tumors in 23/24 (96%) of the animals while BaP induced tumors in 10/23 (43%) which resulted in a relative potency of 5.8. Dibenzo[a,l]pyrene induced approximately 5 times as many tumors per tumor bearing animal. In a second experiment 4 nanomoles of each chemical were compared. Ninety-two percent (22/24) of the dibenzo[a,l]pyrene-treated mice had tumors but only 4% (1/24) of the BaP animals which yielded a relative potency of 25.1. In a third experiment 100 nanomoles were compared without promotion. Twenty-nine percent (7/24) of the dibenzo[a,l]pyrene-treated mice had tumors but only 4% (1/24) of the BaP animals for a relative potency of 4. Finally, with direct application to the mammary gland 0.25 and 1.0 micromoles dibenzo[a,l]pyrene led to tumors in all the rats treated (19 and 20 per group, respectively) whereas only 1 animal in the 0.25 micromole BaP group showed a tumor for a relative potency greater than 100. Based on its much greater tumorigenic activity than BaP in the above tests dibenzo[a,l]pyrene is assigned a PEF of 10.

14. Dibenzo[a,h]pyrene. Dibenzo[a,h]pyrene has been assigned a PEF of 10 since, in the experiments by Cavalieri et al. (1989) in which all 4

dibenzo[a]pyrenes were studied, its tumor causing activity was similar to dibenzo[a,l]pyrene. For example, when used to initiate tumors in mouse skin, 18 of 24 (75%) of mice treated with dibenzo[a,h]pyrene had tumors compared to 22 of 24 (92%) with dibenzo[a,l]pyrene. Controls showed skin tumors in 2 of 23 mice (9%).

15. Dibenzo[a,i]pyrene. Dibenzo[a,i]pyrene has been assigned a PEF of 10 since, in the experiments by Cavalieri et al. (1989) in which all 4 dibenzo[a]pyrenes were studied, its tumor-causing activity was similar to dibenzo[a,l]pyrene. For example, when used to initiate tumors in mouse skin, 15 of 24 (63%) of mice treated with dibenzo[a,i]pyrene had tumors compared to 22 of 24 (92%) with dibenzo[a,l]pyrene. Controls showed skin tumors in 2 of 23 mice (9%).

16. Dibenzo[a,e]pyrene. Dibenzo[a,e]pyrene has been assigned a PEF of 1.0. Dibenzo[a,e]pyrene was the weakest member of the 4 dibenzo[a]pyrenes studied by Cavalieri et al. (1989, 1991). In the experiments in which all 4 dibenzo[a]pyrenes were compared (Cavalieri et al., 1989), its tumor-causing activity was approximately one-tenth to one-twentieth that of dibenzo[a,l]pyrene.

17. Indeno[1,2,3-c,d]pyrene. Indeno[1,2,3-c,d]pyrene has been assigned a PEF of 0.1. Clement Associates (1988) used the mouse skin carcinogenesis data obtained by Habs et al. (1980) and by Hoffman and Wynder (1966) and the lung tumor data obtained by Deutsch-Wenzel et al. (1983) after intrapulmonary administration to estimate cancer potencies relative to BaP of 0.0302, 0.0292, and 0.246, respectively. These were averaged and rounded to obtain a PEF of 0.1. Note that rounding just the lung tumor data gives the same result.

18. 5-Methylchrysene. 5-Methylchrysene has been assigned a PEF of 1.0. The activity of 5-methylchrysene relative to BaP has been studied by Hecht et al. (1976) using skin tumor initiation with phorbol ester (tetradecanoyl phorbol acetate) promotion as well as skin tumor induction in mice. In the skin tumor induction test the tumorigenic activities of 5-methylchrysene and BaP were comparable enough so that a PEF of 1.0 was selected for 5-methylchrysene. Weekly application of 0.01% 5-methylchrysene led to skin carcinomas in 10 of 15 mice treated for up to 62 weeks, while 0.01% BaP led to skin carcinomas in 14 of 18 mice. The results for 0.005% of the 2 chemicals were 6 of 9 and 7 of 10, respectively.

19. 1-Nitropyrene. 1-Nitropyrene has been assigned a PEF of 0.1. In the Wislocki et al. (1986) report, in which lung tumors were induced in newborn mice, 1-nitropyrene (0.7 micromoles) was weakly carcinogenic in males (6/34 or 18% versus 4/45 or 9% in controls) and not carcinogenic in females (3/50 or 6% versus 2/34 or 6% in controls) relative to 0.56 micromoles BaP (13/37 or 35% in males versus 1/28 or 4% in control males and 13/27 or 48% in females versus 0/31 in control females). The relative potency was 0.348 in males and 0.076 in females. A PEF of 0.1 was assigned based on the experiment.

20. 4-Nitropyrene. 4-Nitropyrene has been assigned a PEF of 0.1. Wislocki et al. (1986) compared the lung tumorigenicity of nitrated derivatives of pyrene to BaP in a newborn mouse assay. The background incidences were 4% in males and 0% in females. The administration of 2.8 micromoles of 4-nitropyrene gave a net incidence of 34% tumors in males and 31% in females, while 0.56

micromoles BaP gave 31% tumors in males and 48% in females. The potency of 4-nitropyrene relative to BaP was 0.23 in males and 0.12 in females. These are averaged and rounded to a PEF of 0.1.

21. 1,6-Dinitropyrene. 1,6-Dinitropyrene has been assigned a PEF of 10. In the Wislocki et al. (1986) report, 1,6-dinitropyrene (0.2 micromoles) was weakly carcinogenic in inducing lung tumors in females (2/29 versus 0/31 in controls) and essentially not carcinogenic in males (1/25 versus 1/28 in controls) relative to 0.56 micromoles BaP (see 1-nitropyrene above for BaP data). However the weak response combined with the low dose of 1,6-dinitropyrene (0.2 micromoles) relative to BaP (0.56 micromoles), the relative potency was 0.52 in females and 0.54 in males. In an intratracheal injection experiment (Takayama et al., 1985) hamsters were given 26 weekly instillations of 0.5 mg BaP. All 10 males and 9 of 10 females developed respiratory tract tumors. An unit risk of 2.9×10^{-2} $(\mu g/m^3)^{-1}$ was obtained from the female data which is 6.4 times the unit risks obtained from intratracheal studies using BaP and 26 times that using inhalation data (Table 7.5). In a study by Iwagawa et al. (1989) using several doses of 1,6-dinitropyrene or BaP implanted directly into the lungs a relative potency of 5.1 was obtained from the resulting lung cancer data. In light of the 2 experiments showing high relative potency and of 1,6-dinitropyrene's strong mutagenicity, a PEF of 10 appears to be more appropriate than 1.0.

22. 1,8-Dinitropyrene. 1,8-Dinitropyrene has been assigned a PEF of 1.0. In the Wislocki et al. (1986) report, 1,8-dinitropyrene (0.2 micromoles) was weakly carcinogenic in females (2/29 versus 0/31 in controls) and not carcinogenic in males (1/31 versus 1/28 in controls) relative to 0.56 micromoles BaP. However, due again to the low dose of 1,8-dinitropyrene chosen, the relative potency was 0.46 in females and 0.41 in males. In view of the high PEF of 1,6-dinitropyrene derived above and the very high mutagenicity of 1,8-dinitropyrene, the default PEF of 1.0 is assigned to 1,8-dinitropyrene until better in vivo data become available to derive a PEF.

23. 6-Nitrochrysene. 6-Nitrochrysene has been assigned a PEF of 10. In the Wislocki et al. (1986) report, 0.7 micromoles of 6-nitrochrysene gave a net incidence of 76% lung tumors in males (28/33 versus 4 of 45 in controls) and 84% in females (36/40 versus 2/34 in controls). The potency of 6-nitrochrysene relative to BaP was 3.27 in males and 2.50 in females. In the newborn mouse assay of Busby et al. (1988), "(t)he ED50 for total lung tumors was 0.02 μmol for 6-NC and 0.2 μmol for BaP, thus showing a 10-fold higher potency for 6-NC compared with the 25-fold difference noted with tumor multiplicity." In a subsequent report (Busby et al., 1989), 0.03 micromoles of 6-nitrochrysene caused lung adenomas and adenocarcinomas in 19/26 males and 13/22 females (versus controls of 13/91 in males and 7/101 in females) while 0.24 micromoles BaP caused lung adenomas and adenocarcinomas in 13/28 males and 19/27 females (against the same controls). The relative potencies were 17.51 for males and 6.17 for females. Based on the several experiments a PEF of 10 is selected.

24. 2-Nitrofluorene. 2-Nitrofluorene has been assigned a PEF of 0.01. Miller et al. (1955) fed 2-nitrofluorene at a level of 1.62 mmol(215 mg)/kg diet to rats. This is estimated to give an animal dose of 33.1 mg/kg/day and a human equivalent dose of 4.7 mg/kg/day. In one experiment 17 of 20 male rats (85%) developed forestomach tumors by 12 months. In another experiment 4 of 9

female rats (44%) developed mammary tumors by 10 months. These experiments yielded cancer potencies of 0.25 and 0.62 $(mg/kg\text{-}day)^{-1}$, approximately 0.02 and 0.05 that of BaP obtained in this risk assessment. The values of 0.02 and 0.05 were averaged and rounded to obtain a PEF of 0.01.

25. Chrysene. Chrysene has been assigned a PEF of 0.01. Clement Associates (1988) used the mouse skin carcinogenesis data obtained by Wynder and Hoffman (1959) to estimate a cancer potency relative to BaP of 0.0132. This was rounded to obtain a PEF of 0.01.