2. HEALTH EFFECTS

2.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective of the toxicology of polycyclic aromatic hydrocarbons (PAHs). It contains descriptions and evaluations of toxicological studies and epidemiological investigations and provides conclusions, where possible, on the relevance of toxicity and toxicokinetic data to public health.

A glossary and list of acronyms, abbreviations, and symbols can be found at the end of this profile.

2.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

To help public health professionals and others address the needs of persons living or working near hazardous waste sites, the information in this section is organized first by route of exposure-inhalation, oral, and dermal; and then by health effect-death, systemic, immunological, neurological, reproductive, developmental, genotoxic, and carcinogenic effects. These data are discussed in terms of three exposure periods-acute (14 days or less), intermediate (15-364 days), and chronic (365 days or more).

Levels of significant exposure for each route and duration are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. LOAELs have been classified into “less serious” or “serious” effects. “Serious” effects are those that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death). “Less serious” effects are those that are not expected to cause significant dysfunction or death, or those whose significance to the organism is not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an end point should be classified as a NOAEL, “less serious” LOAEL, or “serious” LOAEL, and that in some cases, there will be insufficient data to decide whether the effect is indicative of significant dysfunction. However, the Agency has established guidelines and policies that are used to classify these end points. ATSDR believes that there is sufficient merit in this approach to warrant an attempt

at distinguishing between “less serious” and “serious” effects. The distinction between “less serious” effects and “serious” effects is considered to be important because it helps the users of the profiles to identify levels of exposure at which major health effects start to appear. LOAELs or NOAELs should also help in determining whether or not the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health.

The significance of the exposure levels shown in the Levels of Significant Exposure (LSE) tables and figures may differ depending on the user’s perspective. Public health officials and others concerned with appropriate actions to take at hazardous waste sites may want information on levels of exposure associated with more subtle effects in humans or animals (LOAELs) or exposure levels below which no adverse effects (NOAELs) have been observed. Estimates of levels posing minimal risk to humans (Minimal Risk Levels or MRLs) may be of interest to health professionals and citizens alike.

Levels of exposure associated with carcinogenic effects (Cancer Effect Levels, CELs) of PAHs are indicated in Tables 2-1, 2-2, and 2-3 and Figures 2-l and 2-2. Because cancer effects could occur at lower exposure levels, Figure 2-2 also shows a range for the upper bound of estimated excess risks, ranging from a risk of 1 in 10,000 to 1 in 10,000,000 (10^{-4} to 10^{-7}), as developed by EPA.

Estimates of exposure levels posing minimal risk to humans (Minimal Risk Levels or MRLs) have been made for PAHs. An MRL is defined as an estimate of daily human exposure to a substance that is likely to be without an appreciable risk of adverse effects (noncarcinogenic) over a specified duration of exposure. MRLs are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration within a given route of exposure. MRLs are based on noncancerous health effects only and do not consider carcinogenic effects. MRLs can be derived for acute, intermediate, and chronic duration exposures for inhalation and oral routes. Appropriate methodology does not exist to develop MRLs for dermal exposure.

Although methods have been established to derive these levels (Barnes and Dourson 1988; EPA 1990), uncertainties are associated with these techniques. Furthermore, ATSDR acknowledges additional uncertainties inherent in the application of the procedures to derive less than lifetime MRLs. As an example, acute inhalation MRLs may not be protective for health effects that are delayed in development or are acquired following repeated acute insults, such as hypersensitivity reactions,

asthma, or chronic bronchitis. As these kinds of health effects data become available and methods to assess levels of significant human exposure improve, these MRLs will be revised.

A User's Guide has been provided at the end of this profile (see Appendix A). This guide should aid in the interpretation of the tables and figures for Levels of Significant Exposure and the MRLs.

PAHs are a group of chemicals that are formed during the incomplete burning of coal, oil, gas, wood, garbage, or other organic substances, such as tobacco and charbroiled meat. PAHs can either be synthetic or occur naturally. Most of these chemicals as individual compounds (i.e., not as part of a combustion product) have no known use except for research purposes. A few PAHs are used in medicines and to make dyes, plastics, and pesticides. Others are contained in asphalt used in road construction. They are found throughout the environment in the air, water, and soil. There are more than 100 different PAH compounds and the health effects of the individual PAHs are not exactly alike.

Fifty-four PAHs have been identified at one or more NPL hazardous waste sites. These 54 are acenaphthene, acenaphthylene, 2-acetoaminofluorene, anthracene, 9, 10-anthracenedione, benz[a]anthracene, benzo[a]pyrene, benzo[e]pyrene, benzo[a]fluoranthene, benzo[b]fluoranthene, benzo[b]fluorene, benzofluoranthene, benzo[j]fluoranthene, benzo[k]fluoranthene, benzo[g,h,i]fluoranthene, benzoperylene, benzo[g,h,i]perylene, benzophenanthrene, benzopyrene, benzothiophene, benzo[b]thiophene, chrysene, 4H-cyclopenta[d,e,f]phenanthrene, dibenz[a,j]anthracene, dibenz[a,h]anthracene, 7,12-dimethylbenz[a]anthracene, 2,7-dimethylbenzo[b]thiophene, 1,4-dimethoxyanthracene, dimethyl phenanthrene, 2,5dimethyl phenanthrene, dodecachlorodecahydrotrim, fluoranthene, fluorene, indeno[1,2,3-c,d] pyrene, 12-methylbenz[a]anthracene, methyl anthracene, 9-methylanthracene, 3-methylcholanthrene, methylfluorene, methylphenanthrene, 2-methylphenanthrene, 1-methylphenanthrene, 4-methylphenanthrene, methylpyrene, phenanthrene, phenanthridine, phenanthroline, pyrene, perylene, 6,7-tetrahydropyrene, tetramethylphenanthrene, 3,4,5,6-tetramethylphenanthrene, and trimethylphenanthrene.

However, only 17 PAHs are discussed in this profile. These 17 PAHs are:

- acenaphthene
- acenaphthylene
- anthracene

- benz[a]anthracene
- benzo[a]pyrene
- benzo[e]pyrene
- benzo[b]fluoranthene
- benzo[j]fluoranthene
- benzo[g,h,i]perylene
- benzo[k]fluoranthene
- chrysene
- dibenz[a,h]anthracene
- fluoranthene
- fluorene
- indeno[l,2,3-c,d]pyrene
- phenanthrene
- pyrene

These 17 PAHs were selected using the following four criteria:

(1) toxicity
(2) potential for human exposure
(3) frequency of occurrence at NPL hazardous waste sites
(4) extent of information available.

The 17 PAHs were combined into one profile to avoid repetition across multiple profiles on the individual PAHs since these chemicals often occur together in the environment and many have similar toxicological effects, environmental fate, etc. Instances in which it is known that the various PAHs differ with regard to toxicological effects or environmental fate will be pointed out. For example, PAHs can be classified as “alternant” (e.g., benzo[a]pyrene, benz]a]anthracene, chrysene, dibenz[a,h]anthracene) or “nonalternant” (e.g., fluoranthene, benzo[k]fluoranthene, benzol[j]fluoranthene, indeno[1,2,3-c,d]pyrene). This distinction is based on the electron density associated with the molecule. Altemant PAHs have an equally distributed electron density, whereas nonalternant PAHs behave almost as if they were two different molecules because of an uneven distribution of electron density from one portion of the molecule to another. The toxicological significance of this difference is that alternant and nonalternant PAHs appear to behave differently, for example, with regard to how they are metabolized to ultimate carcinogens (see Section 2.3.3, Metabolism).

Reliable health-based and environmental information exists on only a few of the 17 PAHs discussed in this profile, and the potential health effects of the other less well-studied PAHs must be inferred from this information. By combining all 17 PAHs in one profile, these comparisons and inferences can

easily be made. Although a large toxicity database exists on complex mixtures that contain PAHs (such as crude oils, various high boiling point distillates, complex petroleum products, coal tars, creosote, and the products of coal liquification processes), these data generally have not been used in this profile. It is difficult to ascertain the toxicity of the component PAHs in these mixtures because of the potential interactions that could occur and the presence of other toxic substances in the mixtures. Furthermore, ATSDR has developed a profile on one of these complex mixtures, creosote, and the reader is referred to this profile for information on this complex mixture (ATSDR 1994). However, most of the available information on the health effects of PAHs in humans must be inferred from studies that reported the effects of exposure to complex mixtures that contain PAHs. Several epidemiologic studies have shown increased mortality due to lung cancer in humans exposed to coke oven emissions, roofing-tar emissions, and cigarette smoke. Each of these mixtures contains benzo[a]pyrene, chrysene, benz[a]anthracene, benzo[b]fluoranthene, and dibenz[a,h]anthracene as well as other potentially carcinogenic PAHs and other carcinogenic and potentially carcinogenic chemicals, tumor promoters, initiators, and co-carcinogens such as nitrosamines, coal tar pitch, and creosote. It is thus impossible to evaluate the contribution of any individual PAH to the total carcinogenicity of these mixtures in humans because of the complexity of the mixtures and the presence of other carcinogens. Despite these limitations, reports of this nature provide qualitative evidence of the potential for mixtures containing PAHs such as benzo[a]pyrene, chrysene, benz[a]anthracene, benzo[b]fluoranthene, and dibenz[a,h]anthracene to cause cancer in humans. For this reason, and also because of the lack of data on the effects of individual PAHs in humans, such information has been included in this profile on PAHs.

2.2.1 Inhalation Exposure

2.2.1.1 Death

No studies were located regarding death in humans following inhalation exposure to any of the 17 PAHs discussed in this profile. However, a dose-related decrease in survival was noted in hamsters after 60 weeks of inhalation exposure to 46.5 mg/m^3 benzo[a]pyrene for 109 weeks (Thyssen et al. 1981). The authors attributed this reduced survival in part to toxic and carcinogenic effects induced by this PAH (e.g., tumors in the pharynx and larynx that could have inhibited food intake).

2.2.1.2 Systemic Effects

No studies were located regarding cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, dermal, or ocular effects in humans or animals following inhalation exposure to any of the 17 PAHs discussed in this profile. The systemic effects observed after inhalation exposure are discussed below.

The highest NOAELs for respiratory and renal effects in each species and duration category are recorded in Table 2-1 and plotted in Figure 2-1.

Respiratory Effects. Only one study was located regarding respiratory effects in humans following inhalation exposure to PAHs, specifically, benzo[a]pyrene. The respiratory health of 667 workers in a rubber factory was investigated (Gupta et al. 1993). Respiratory health was evaluated and examined for correlations to length of employment at the factory. In addition, total suspended particulate matter and benzo[a]pyrene concentrations were monitored in various parts of the factory and examined for possible correlation with the respiratory health of the workers in the same area of the factory. Statistically significant decrements in ventilatory function occurred following prolonged exposure as assessed by duration of employment. When different sections of the factory were considered, workers in the compounding section were the most affected, which was associated with the highest exposure to particulate matter and benzo[a]pyrene. Workers in the compounding section exhibited radiographic abnormalities including patch opacities, prominent bronchiovascular markings, and pleural effusions. Other symptoms included bloody vomit, breathing problems, chest pains, chest irritation, throat irritation, and cough. Workers in other areas of the plant exposed to lower levels of particulate matter and benzo[a]pyrene were similarly affected although to a lesser degree and in fewer numbers (Gupta et al. 1993). No attempt was made to separate the effects of exposure to benzo[a]pyrene and particulate matter, or to identify possible simultaneous exposure to other toxic chemicals.

Groups of 40 Fischer-344/Crl rats/sex were exposed nose-only to an aerosol of benzo[a]pyrene (7.7 mg/m^3) 2 hours/day, 5 days/week, for 4 weeks (Wolff et al. 1989a). Nasal and left lung sections were examined histopathologically. No treatment-related lesions were noted in the lungs or nasal cavities of the animals exposed to benzo[a]pyrene. Although this was a well-conducted inhalation toxicity study, it is not appropriate for use in risk assessment because only one concentration was

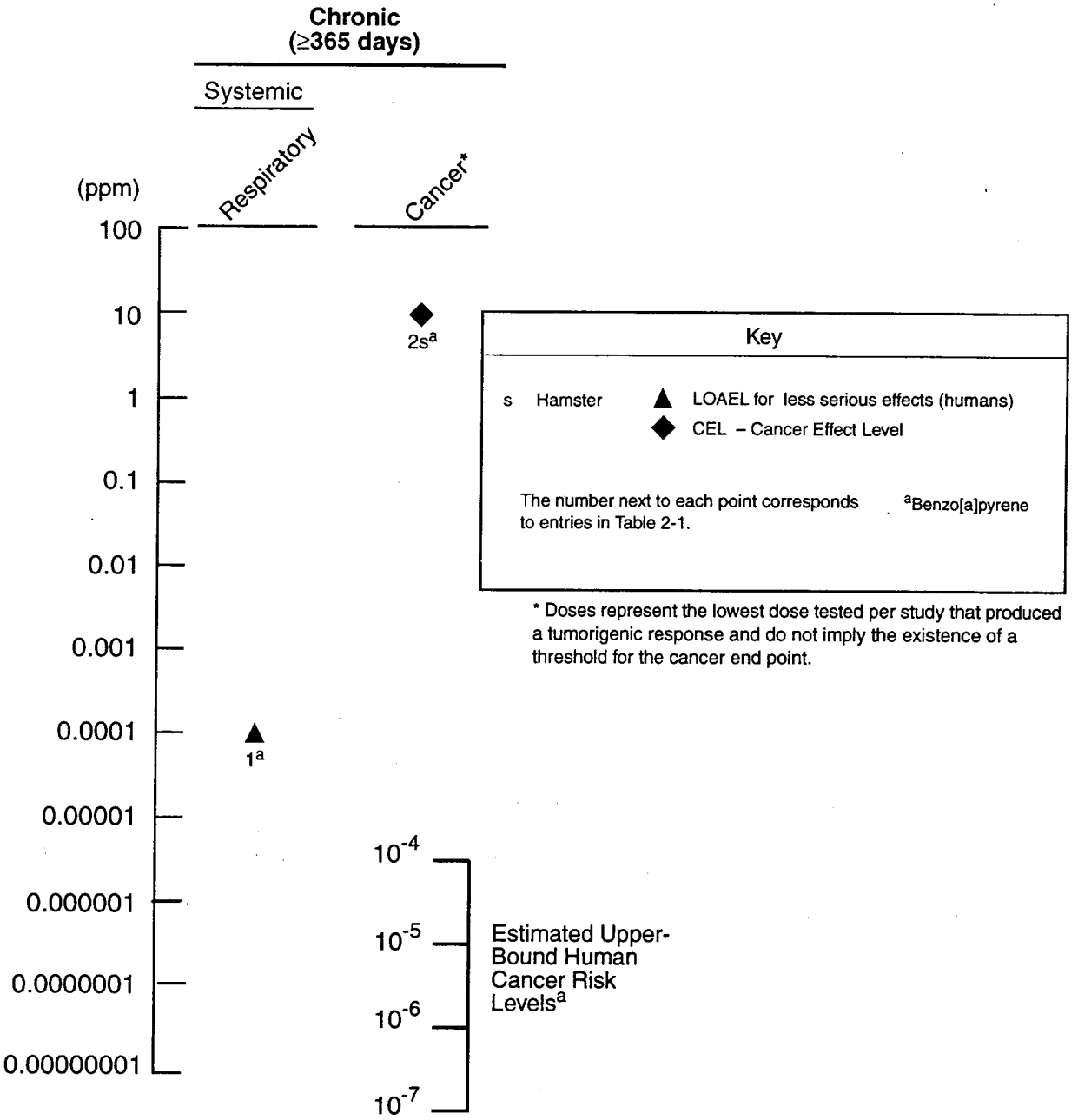
TABLE 2-1. Levels of Significant Exposure to Polycyclic Aromatic Hydrocarbons - Inhalation

Key to figure[a]	Species/ (strain)	Exposure/ duration/ frequency	System	NOAEL (mg/m3)	LOAEL Less serious (mg/m3)	LOAEL Serious (mg/m3)	Reference
CHRONIC EXPOSURE							
Systemic							
1	Human	6 mo - >6 yr	Resp			0.0001 NS (reduced lung function, abnormal chest x-ray, cough, bloody vomit, throat and chest irritation.	Gupta et al. 1993 benzo[a]pyrene
Cancer							
2	Hamster (Syrian golden)	109 wk 7 d/wk 3-4.5 hr/d				9.5 M (CEL: 34.6% increase in respiratory tract tumors; neoplasms of the upper digestive tract in 26.9%)	Thyssen et al. 1981 benzo[a]pyrene

[a]The number corresponds to entries in Figure 2-1.

CEL = cancer effect level; d = day(s); hr = hour; LOAEL = lowest-observed-adverse-effect level; M = male; mo = month; NOAEL = no-observed-adverse-effect level; NS = not specified; Resp = respiratory; wk = week(s); yr = year(s)

Figure 2-1. Levels of Significant Exposure to Polycyclic Aromatic Hydrocarbons – Inhalation



studied (thereby precluding the assessment of a dose-response relationship); no adverse treatment-related effects were observed; and the only parts of the respiratory tract examined histopathologically were the lungs and nose.

Renal Effects. No studies were located regarding renal effects in humans following inhalation exposure to any of the 17 PAHs discussed in this profile.

Groups of 40 Fischer-344/Crl rats/sex were exposed nose-only to an aerosol of benzo[a]pyrene 2 hours/day, 5 days/week, for 4 weeks (Wolff et al. 1989a). Kidney sections were examined histopathologically. No treatment-related lesions were noted in the kidneys of the animals exposed to benzo[a]pyrene.

2.2.1.3 Immunological and Lymphoreticular Effects

Humoral immunity was monitored in male iron foundry workers in Poland (Szczeklik et al. 1994). Coke oven workers (199) were compared to cold-rolling mill workers (76). The groups were similar with respect to age, length of employment, and smoking habits. The results showed that coke oven workers, exposed to high concentrations of atmospheric PAHs, including fluoranthene, perylene, pyrene, benzo[a]pyrene, chrysene, benz[a]anthracene, dibenz[a,h]anthracene, and benzo[g,h,i]perylene, had reduced levels of serum immunoglobins. The workers most exposed to PAHs worked at the topside area of the coke ovens. Benzo[a]pyrene exposure was used as a reference point. Coke oven workers, exposed to 0.0002-0.50 mg/m^3 benzo[a]pyrene, were compared to cold-rolling mill workers, whose exposure to benzo[a]pyrene was 3-5 orders of magnitude less. Average length of employment was 15 years. IgG, IgA, IgM, and IgE concentrations were measured. Coke oven workers exhibited a marked depression of mean serum IgG and IgA, compared to mill workers. IgM tended to decrease, whereas IgE tended to increase in the coke oven workers. The biological significance of this finding is unclear and is not addressed by the authors. However, the authors suggest that serum immunoglobulin levels may be a useful biomarker for PAH exposure. The authors note, however, that the coke oven workers were exposed to higher levels of sulfur dioxide and carbon monoxide than were the cold-rolling mill workers, and they suggest that this additional exposure may have potentiated the effects of the PAH exposure. The potential contribution of the smoking habits of the subjects was not investigated.

No studies were located regarding the following effects in humans or animals following inhalation exposure to any of the 17 PAHs discussed in this profile:

2.2.1.4 Neurological Effects

2.2.1.5 Reproductive Effects

2.2.1.6 Developmental Effects

2.2.1.7 Genotoxic Effects

Becher et al. (1984) evaluated urine and blood samples from 15 aluminum plant workers (average age, 29 years; average years employed, 3.8) exposed to an estimated total PAH concentration of 1 mg per 8-hour work shift. The main PAH components identified by air sampling and also detected in the urine samples included phenanthrene, fluoranthene, pyrene, benz[a]anthracene, chrysene, benzo[e]pyrene, and benzo[a]pyrene. Results of the cytogenetic analysis of peripheral lymphocytes of the exposed workers indicated that the frequency of sister chromatid exchange was not influenced by the presence of large amounts of PAHs. These findings were reported to be consistent with the negligible increase in lung cancer found in epidemiological studies of aluminum workers. The investigators, therefore, questioned the relevance of PAH air monitoring as a measure of the occupational hazards associated with PAH exposure. Alternatively, it is possible that there are no occupational hazards associated with PAH exposure at these levels. Similar results were obtained with iron factory workers (length of employment: 2 46 years) exposed to 0.0005-0.00 mg/m^3 benzo[a]pyrene (Perera et al. 1993), who exhibited an increased rate of mutations in peripheral lymphocytes that were not correlated with PAH exposure. These authors suggest that both biomonitoring and personal monitoring may be necessary to evaluate exposure.

The high lung cancer rate in Xuan Wei, China, is associated with smokey coal use in unvented homes, but not with wood or smokeless coal use (Mumford et al. 1993). Smoky coal combustion emits higher PAH concentrations than wood combustion. This study evaluated PAH-DNA adducts in placentas, and in peripheral and cord white blood cells (WBC) from Xuan Wei women burning smoky coal or wood and from Beijing women using natural gas. Exposures were based on benzo[a]pyrene concentrations determined by personal monitors. Women in Xuan Wei burning smoky coal without a chimney were

exposed to 0.383 mg/m^3 benzo[a]pyrene, those burning smoky coal with chimneys were exposed to 0.184 mg/m^3, and women burning wood or using natural gas (Beijing) had no detectable exposure to benzo[a]pyrene. Positive results (detection of PAH-DNA adducts) were found in 58, 47, and 5% of the placentas from Xuan Wei women burning smoky coal without a chimney, with a chimney, and Beijing women using natural gas, respectively. Positive results were found in 46, 6.5, 56, and 25% of placentas from Xuan Wei women who lived in houses without and with chimneys, Xuan Wei women burning wood, and Beijing controls, respectively. Peripheral WBC samples were positive in 7 of 9, 8 of 9, and 3 of 9 for the Xuan Wei women who lived in houses without and with chimneys and Beijing women, respectively. No dose-response relationship was observed between the air benzo[a]pyrene concentrations and DNA adduct levels or percentage of detectable samples. However, using the fluorescent color assay, there was a significant association between DNA adduct detection in the placenta and cooking methods. Moreover, individual comparisons of the data revealed a significant difference between both smokey coal groups (chimney, no chimney) and natural gas cooking. The results of this study suggest that DNA adducts can be used as a biomarker to assess human exposure to combustion emissions.

Thirty-four workers in an electrode paste plant were monitored for response to exposure (Ovrebo et al. 1994). Exposure to benzo[a]pyrene was 0.9 μg/m^3; exposure to pyrene was 3.5μg/m^3. 1-Hydroxypyrene was measured in the urine, and PAH-DNA adducts were measured in white blood cells to demonstrate their relationship to the exposure. Results from these workers were compared to two reference control groups: research and development (R&D) workers and nickel refinery workers. Mean values of PAH-DNA adducts in the white blood cells from randomly selected participants in the three groups were only marginally different, with the exception of two smokers in the electrode plant, who had the highest levels. Mean PAH-DNA adduct levels were 10.9 adducts per 108 nucleotides for the electrode workers, 10.8 adducts per 10^8 nucleotides for the R&D personnel, and 10.0 adducts per 10^8 nucleotides for nickel plant workers not occupationally exposed to PAHs. No correlation was found between PAH-DNA adducts and 1-hydroxypyrene in the urine.

In an ongoing comprehensive evaluation of biological markers, workers in or near an iron foundry with varying exposures to PAHs were examined for response to exposure (Santella et al. 1993). Exposure to benzo[a]pyrene, determined by personal monitors, was 2-60 ng/m^3, which are the lowest levels yet analyzed in foundry workers. 1-Hydroxypyrene was measured in the urine, and PAH-DNA adducts were measured in white blood cells to demonstrate their relationship to the exposure.

Cigarette smoking, but not age or charbroiled food, influenced the level of 1-hydroxypyrene but not PAH-DNA adducts. When workers were classified into three categories of exposure (low, <.0005 mg/m^3; medium, 0.0005-0.0012 mg/m^3; high, >0.0012 mg/m^3), PAH-DNA adducts showed an increasing trend, with exposure from 5.2 to 6.2-9.6 adducts per 10^8 nucleotides in the low-, medium, and high-exposure groups, respectively. However, the three exposure groups did not differ significantly from each other, and no independent control group was used.

In order to evaluate the correlation between peripheral blood leukocyte DNA adducts as an indicator of exposure to PAHs and the airborne contamination of PAH at the workplaces, a survey of 69 coke oven workers was carried out (Assennato et al. 1993). In each workplace, total PAH and specific (benz[a]anthracene, benzo[a]pyrene, chrysene) PAH airborne concentrations were measured. Job titles included supervisor, door maintenance, machine operator, gas regulators, temperature operators, and top side workers. For the workplaces evaluated, the range of airborne concentrations (μg/m^3) for benz[a]anthracene, benzo[a]pyrene, and chrysene, respectively, were: supervisor (0.41, 0.29, 0.32), door maintenance (4.26-14.79, 2.31-6.37, 2.34 6.53), machine operator (0.11-33.19, 0.08-13.17, 0.03-12.63), gas regulators (0.21-2.10, 0.12-1.61, 0.13-1.60), temperature operators (1.77-10.07, 1.37-5.03, 0.98 4.78), and top side workers (0.45-3.40, 0.47-4.73, 0.23-2.42). Mean values (fmol/μg DNA) for PAH-DNA adducts in leukocytes by job title were: supervisor (0.059), door maintenance (0.174) machine operator (0.065), gas regulators (0.081), and temperature operators (0.071). Levels of exposure were correlated with PAH-DNA adduct formation. However, the differences were not statistically significant. The major limitations of the study included no record of length of exposure, no independent control group, no reporting of expected background levels of adducts, and no estimation of the length of time individual workers were exposed to particular levels of the PAHs. Other genotoxicity studies are discussed in Section 2.4.

2.2.1.8 Cancer

No studies were located regarding cancer in humans following inhalation exposure to any of the 17 PAHs discussed in this profile. However, epidemiologic studies have shown increased mortality due to lung cancer in humans exposed to coke oven emissions (Lloyd 1971; Mazumdar et al. 1975; Redmond et al. 1976), roofing-tar emissions (Hammond et al. 1976), and cigarette smoke (Maclure and MacMahon 1980; Wynder and Hoffmann 1967). Each of these mixtures contains benzo[a]pyrene, chrysene, benz[a]anthracene, benzo[b]fluoranthene, and dibenz[a,h]anthracene as well as other

potentially carcinogenic PAHs and other carcinogenic and potentially carcinogenic chemicals, tumor promoters, initiators, and co-carcinogens such as nitrosamines, coal tar pitch, and creosote. It is thus impossible to evaluate the contribution of any individual PAH to the total carcinogenicity of these mixtures in humans because of the complexity of the mixtures and the presence of other carcinogens. Furthermore, the levels of individual or total PAHs were not quantified in any of these reports. Despite these limitations, reports of this nature provide qualitative evidence of the potential for mixtures containing PAHs such as benzo[a]pyrene, chrysene, benz[a]anthracene, benzo[b]fluoranthene, and dibenz[a,h]anthracene to cause cancer in humans.

Several inhalation studies for animals given benzo[a]pyrene were located. Shulte et al. (1993) found a significant increase in all lung tumors and a dose-dependent increase in malignant lung tumors for mice exposed to PAH-enriched exhausts containing 0.05 or 0.09 mg/m^3 benzo[a]pyrene. The chronic study of Thyssen et al. (1981) provides clear-cut evidence of a dose-response relationship between inhaled benzo[a]pyrene particles (99% of the benzo[a]pyrene particles were between 0.2 and 0.54 microns in diameter) and respiratory tract tumorigenesis. Respiratory tract tumors were induced in the nasal cavity, pharynx, larynx, and trachea in a dose-related manner in hamsters exposed to 9.5 mg/m^3 or 46.5 mg/m^3 for 109 weeks. No lung tumors were found, and the reason for the absence of lung tumors is not known. Furthermore, the particle sizes were reported to be within the respirable range (0.2-0.5 microns in diameter). Tumors were also observed following exposure to 46.5 mg/m^3 in the esophagus and forestomach (presumably as a consequence of mucocilliary particle clearance) (Thyssen et al. 1981). These tumor types consisted of papillomas, papillary polyps, and squamous cell carcinomas.

The CEL from the Thyssen et al. (1981) study is recorded in Table 2-1 and plotted in Figure 2-l.

2.2.2 Oral Exposure

2.2.2.1 Death

No studies were located regarding death in humans after oral exposure to any of the 17 PAHs discussed in this profile.

Oral exposure to 120 mg/kg/day benzo[a]pyrene has resulted in decreased survival time in two strains of mice (DBA/2N and AKR/N) whose hepatic aryl hydrocarbon hydroxylase (AHH) activity is not induced by PAHs ("nonresponsive" mice) (Robinson et al. 1975). AHH is a microsomal enzyme believed to be responsible for the metabolism of benzo[a]pyrene. All of the mice in the treatment group died, with at least half the deaths occurring within 15 days of dosing. Only three mice in the control group died. Death appeared to be caused by bone marrow depression (aplastic anemia, pancytopenia), leading to hemorrhage or infection. In contrast, only 6 of 90 (7%) mice with inducible AHH activity ("responsive" mice) similarly exposed to benzo[a]pyrene died over the same period of time. The authors concluded that the decreased survival in the nonresponsive mice was associated with a single gene difference encoding aromatic hydrocarbon responsiveness and was dependent on route of exposure. Benzo[a]pyrene was not as rapidly metabolized by the liver and excreted following oral administration in nonresponsive mice as in responsive mice. Therefore, more benzo[a]pyrene was available to reach the target tissue (i.e., bone marrow) in the nonresponsive mice, resulting in bone marrow depression and death.

A LOAEL for death for intermediate-duration exposure in mice is recorded in Table 2-2 and plotted in Figure 2-2.

2.2.2.2 Systemic Effects

No studies were located regarding respiratory, cardiovascular, hematological, musculoskeletal, hepatic, dermal, or ocular effects in humans following oral exposure to any of the 17 PAHs discussed in this profile. The systemic effects observed in humans or animals after oral exposure are discussed below.

The highest NOAEL values and all LOAEL values from each reliable study for each species and duration category are recorded in Table 2-2 and plotted in Figure 2-2.

Respiratory Effects. Male and female mice were exposed to 0, 175, 350, or 700 mg/kg/day acenaphthene by gavage for 13 weeks (EPA 1989c). No signs of respiratory distress were seen during life for any dose group, and no gross or microscopic damage was seen upon necropsy. Similar findings were reported after 13-week administration of 1,000 mg/kg/day anthracene, and 500 mg/kg/day fluoranthene, or 500 mg/kg/day fluorene (EPA 1988e, 1989d, 1989e).

TABLE 2-2. Levels of Significant Exposure to Polycyclic Aromatic Hydrocarbons - Oral

Key to figure[a]	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
	ACUTE EXPOSURE						
	Systemic						
1	Rat (Wistar/Af/Ha n/Mol/ Kuo)	4 d 1 x/d (G)	Gastro	150 M			Nouslainen et al. 1984 benz[a]anthracene
			Hepatic	150 M			
			Renal	150 M			
2	Rat (Wistar/Af/Ha n/Mol/ Kuo)	4 d 1 x/d (G)	Gastro	150 M			Nouslainen et al. 1984 benzo[a]pyrene
			Hepatic	150 M			
			Renal	150 M			
	Reproductive						
3	Mouse (CD-1)	10 d Gd 7-16 (G)		40 F		160 F (reduced pregnancy)	Mackenzie and Angevine 1981 benzo[a]pyrene
	Developmental						
4	Mouse (B6AKF1, AKR/J)	8 d Gd 2-10 (F)				120 F (fetal resorption in Ahd/Ahd)	Legraverend et al. 1984 benzo[a]pyrene
5	Mouse (CD-1)	10 d Gd 7-16 (G)		10 F		40 F (reduced pup weight at 20 days)	Mackenzie and Angevine 1981 benzo[a]pyrene
	Cancer						
6	Mouse (CFW Swiss)	1-7 d ad lib (F)		13.3		33.3 (CEL: gastric neoplasms)	Neal and Rigdon 1967 benzo[a]pyrene

TABLE 2-2. Levels of Significant Exposure to Polycyclic Aromatic Hydrocarbons - Oral (continued)

Key to figure[a]	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
	INTERMEDIATE EXPOSURE						
	Systemic						
7	Mouse (CD-1)	13 wk 1 x/d (GO)	Resp	700			EPA 1989c acenaphthene
			Cardio	700			
			Gastro	700			
			Hemato	700			
			Musc/skel	700			
			Hepatic		175[b]	(increased relative liver weight)	
			Renal	700			
			Endocr	700			
			Dermal	700			
			Ocular	700			
			Bd Wt	700			
8	Mouse (CD-1)	13 wk 1 x/d (GO)	Resp	1000			EPA 1989d anthracene
			Cardio	1000			
			Gastro	1000			
			Hemato	1000			
			Musc/skel	1000			
			Hepatic	1000[c]			
			Renal	1000			
			Endocr	1000			
			Dermal	1000			
			Ocular	1000			
			Bd Wt	1000			

TABLE 2-2. Levels of Significant Exposure to Polycyclic Aromatic Hydrocarbons - Oral (continued)

Key to figure[a]	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
9	Mouse (DBA/2,ARR/ N); (C57B1/b, C3H/HeN, BALB/cAnN)	6 mo (F)	Hemato			120 (aplastic anemia)	Robinson et al. 1975 benzo[a]pyrene
			Hepatic		120 (increased liver weight)		
10	Mouse (CD-1)	13 wk 1 x/d (GO)	Resp	500			EPA 1988e fluoranthene
			Cardio	500			
			Gastro	500			
			Hemato	125 F 500 M	250 F (decrease in packed cell volume)		
			Musc/skel	500			
			Hepatic		125[d] M (increased relative liver weight)		
				125 F	250 F (increased relative liver weight; centrilobular pigmentation, increased enzymes)		
			Renal	250 M 125 F	500 M (renal tubular 250 F regeneration; interstitial lymphocytic infiltrates and/or fibrosis)		
			Endocr	500			
			Dermal	500			
			Ocular	500			
			Bd Wt	500			

TABLE 2-2. Levels of Significant Exposure to Polycyclic Aromatic Hydrocarbons - Oral (continued)

Key to figure[a]	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
11	Mouse (CD-1)	13 wk 1 x/d (GO)	Resp	500			EPA 1989e fluorene
			Cardio	500			
			Gastro	500			
			Hemato	125	250 (decreased PCV and MCHC in males; decreased RBC, PCV, MCH, and MCHC in females)		
			Musc/skel	500			
			Hepatic		125[e] (increased relative liver weight)		
			Renal	250 M 500 F	500 M (increased absolute and relative kidney weight)		
			Endocr	500			
			Dermal	500			
			Ocular	500			
			Bd Wt	500 M 250 F	500 F (increased body weight)		
	Immunological/Lymphoreticular						
12	Mouse (CD-1)	13 wk 1 x/d (GO)		700			EPA 1989c acenaphthene
13	Mouse (CD-1)	13 wk 1 x/d (GO)		1000			EPA 1989d anthracene
14	Mouse (CD-1)	13 wk 1 x/d (GO)		500 F	500 M (increased serum globulin values)		EPA 1988e fluoranthene

TABLE 2-2. Levels of Significant Exposure to Polycyclic Aromatic Hydrocarbons - Oral (continued)

Key to figure[a]	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
15	Mouse (CD-1)	13 wk 1 x/d (GO)		125	250 M (increased spleen weight)		EPA 1989e fluorene
	Neurological						
16	Mouse (CD-1)	13 wk 1 x/d (GO)		700			EPA 1989e acenaphthene
17	Mouse (CD-1)	13 wk 1 x/d (GO)		1000			EPA 1989d anthracene
18	Mouse (CD-1)	13 wk 1 x/d (GO)		500			EPA 1988 fluoranthene
19	Mouse (CD-1)	13 wk 1 x /d (GO)		500			EPA 1989e fluorene
	Reproductive						
20	Mouse (CD-1)	13 wk 1 x/d (GO)		700 M 350 F	700 F (decreased ovary weights correlated with increased incidence and degree of inactivity of the ovary and uterus)		EPA 1989c acenaphthene
21	Mouse (CD-1)	13 wk 1 x/d (GO)		1000			EPA 1989d anthracene

TABLE 2-2. Levels of Significant Exposure to Polycyclic Aromatic Hydrocarbons - Oral (continued)

Key to figure[a]	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)		Reference
22	Mouse (White Swiss)	19-29 d ad lib (F)		133.3 F				Rigdon and Neal 1965 benzo[a]pyrene
23	Mouse (CD-1)	13 wk 1 x/d (GO)		500				EPA 1988 fluoranthene
24	Mouse (CD-1)	13 wk 1 x/d (GO)		500				EPA 1989e fluorene
	Cancer							
25	Mouse (CFW Swiss)	30-197 d ad lib (F)		1.3		2.6	(CEL: gastric tumor)	Neal and Rigdon 1967 benzo[a]pyrene
26	Mouse (Swiss)	23-238 d ad lib (F)				33.3	(CEL: papillomas; squamous cell carcinomas)	Rigdon and Neal 1966 benzo[a]pyrene

TABLE 2-2. Levels of Significant Exposure to Polycyclic Aromatic Hydrocarbons - Oral (continued)

Key to[a] figure	Species/ (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL				Reference
					Less Serious (mg/kg/day)		Serious (mg/kg/day)		
27	Mouse (Swiss)	80-140 d ad lib (F)					33.3	(CEL: tumors of the forestomach in 69/108)	Rigdon and Neal 1969 benzo[a]pyrene

[a]The number corresponds to entries in Figure 2-2.

[b]Used to derive an intermediate-duration oral minimal risk level (MRL) of 0.6 mg/kg/day for acenaphthene; dose divided by an uncertainty factor of 300 (3 for use of a LOAEL, 10 for extrapolation from animals to humans and 10 for human variability)

[c]Used to derive an intermediate-duration oral MRL of 10 mg/kg/day for anthracene; dose obtained by dividing the NOAEL value by 100 (10 for extrapolation from animals to humans and 10 for human variability)

[d]Used to derive an intermediate-duration oral MRL of 0.4 mg/kg/day for fluoranthene; dose divided by an uncertainty factor of 300 (3 for use of a minimal LOAEL, 10 for extrapolation from animals to humans and 10 for human variability)

[e]Used to derive an intermediate-duration oral MRL of 0.4 mg/kg/day for fluorene; dose divided by an uncertainty factor of 300 (3 for use of a minimal LOAEL, 10 for extrapolation from animals to humans and 10 for human variability)

ad lib = ad libitum; BaP = benzo(a)pyrene; Bd Wt = body weight; Cardio = cardiovascular; CEL = cancer effect level; d = day(s); Endocr = endocrine; F = female; (F) = feed; (G) = gavage; Gastro = gastrointestinal; Gd = gestation day(s); (GO) = gavage (oil); Hemato = hematological; LOAEL = lowest-observed-adverse-effect level; M = male; MCH = mean cell hemoglobin; MCHC = mean cell hemoglobin concentration; mo = month(s); Musc/skel = musculoskeletal; NOAEL = no-observed-adverse-effect level; PCV = packed cell volume; RBC = red blood cells; Resp = respiratory; TPA = tetradecanoyl phorbol acetate; wk = week(s); x = time(s)

Figure 2-2. Levels of Significant Exposure to Polycyclic Aromatic Hydrocarbons – Oral

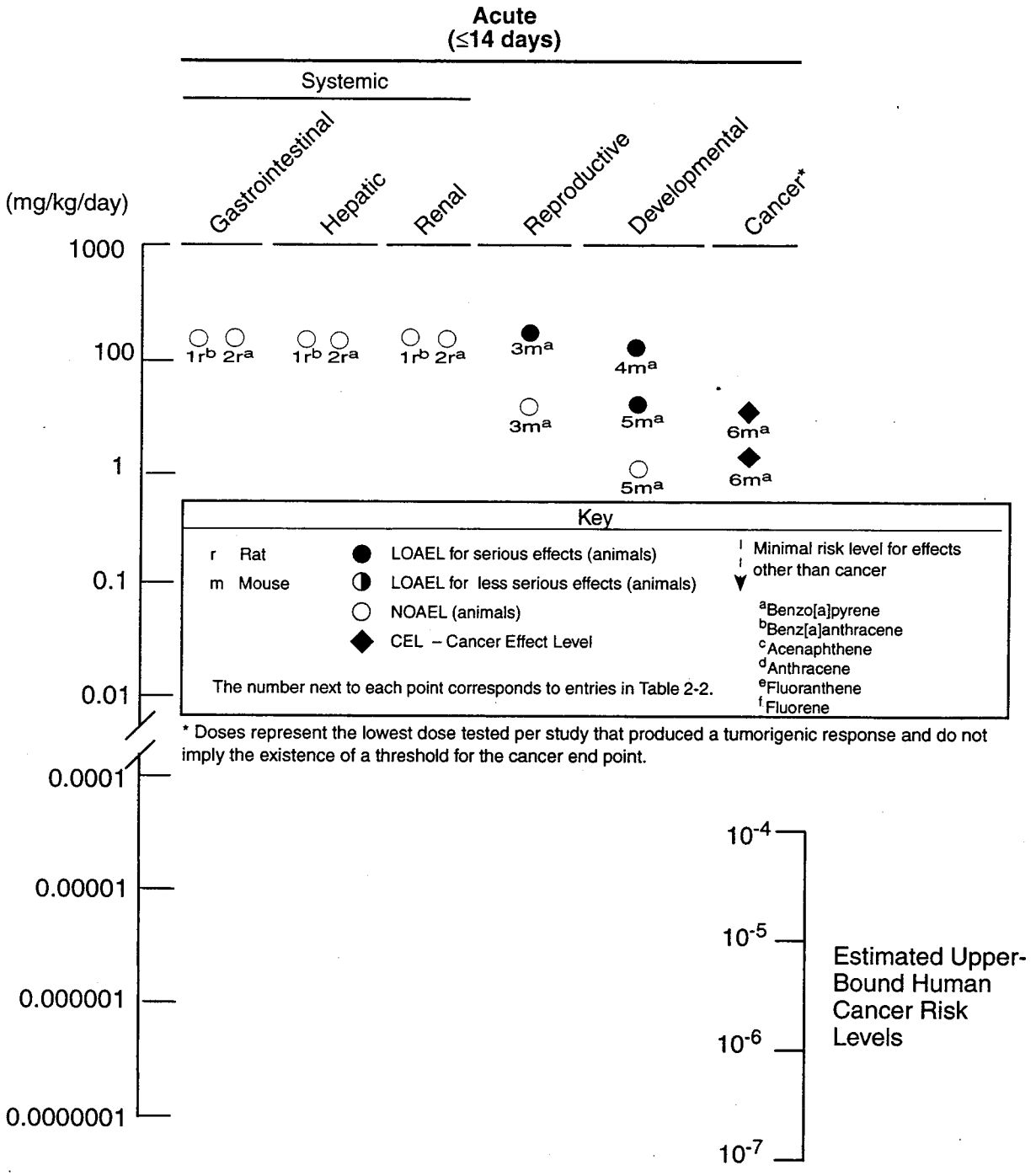


Figure 2-2. Levels of Significant Exposure to Polycyclic Aromatic Hydrocarbons – Oral (continued)

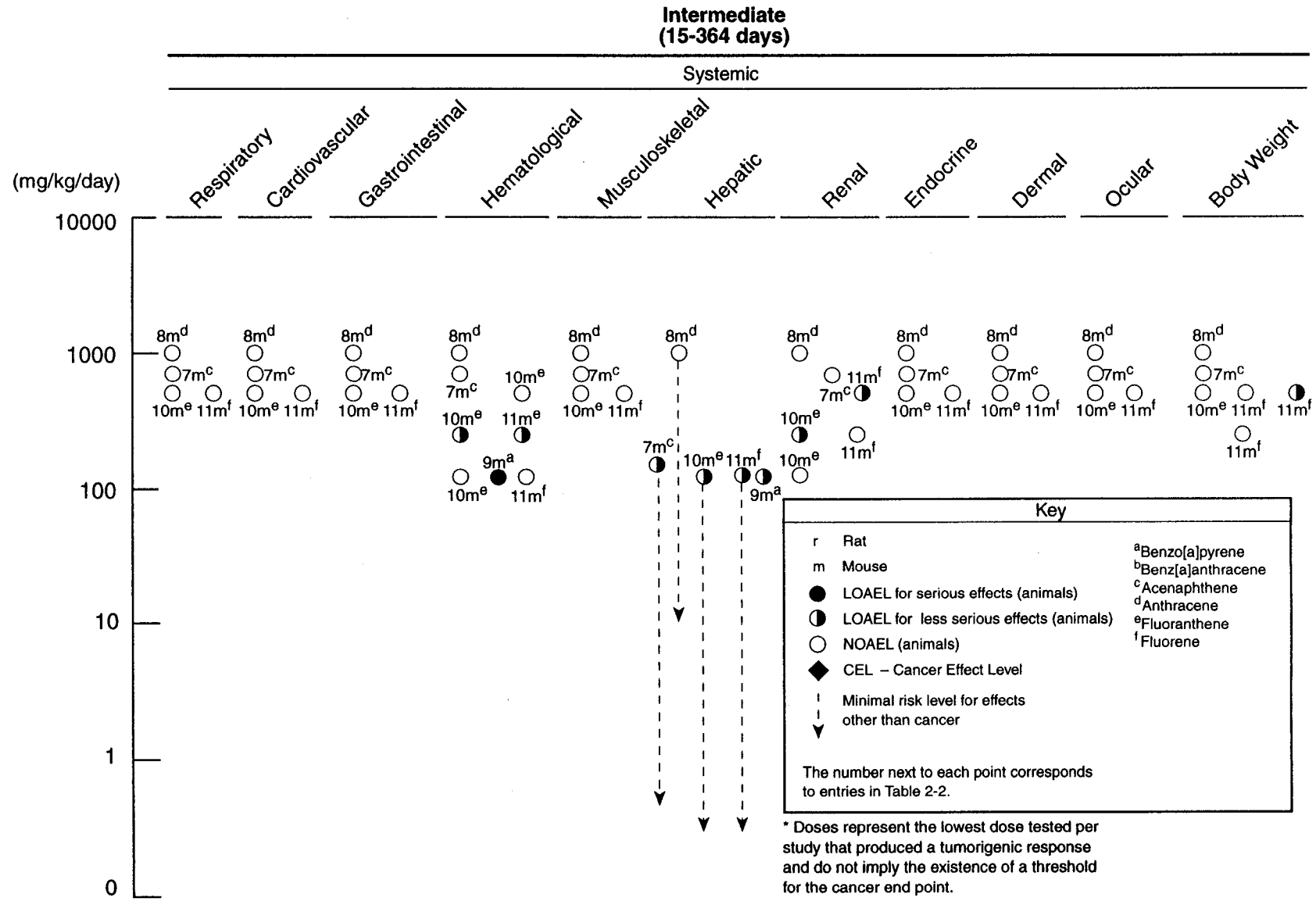
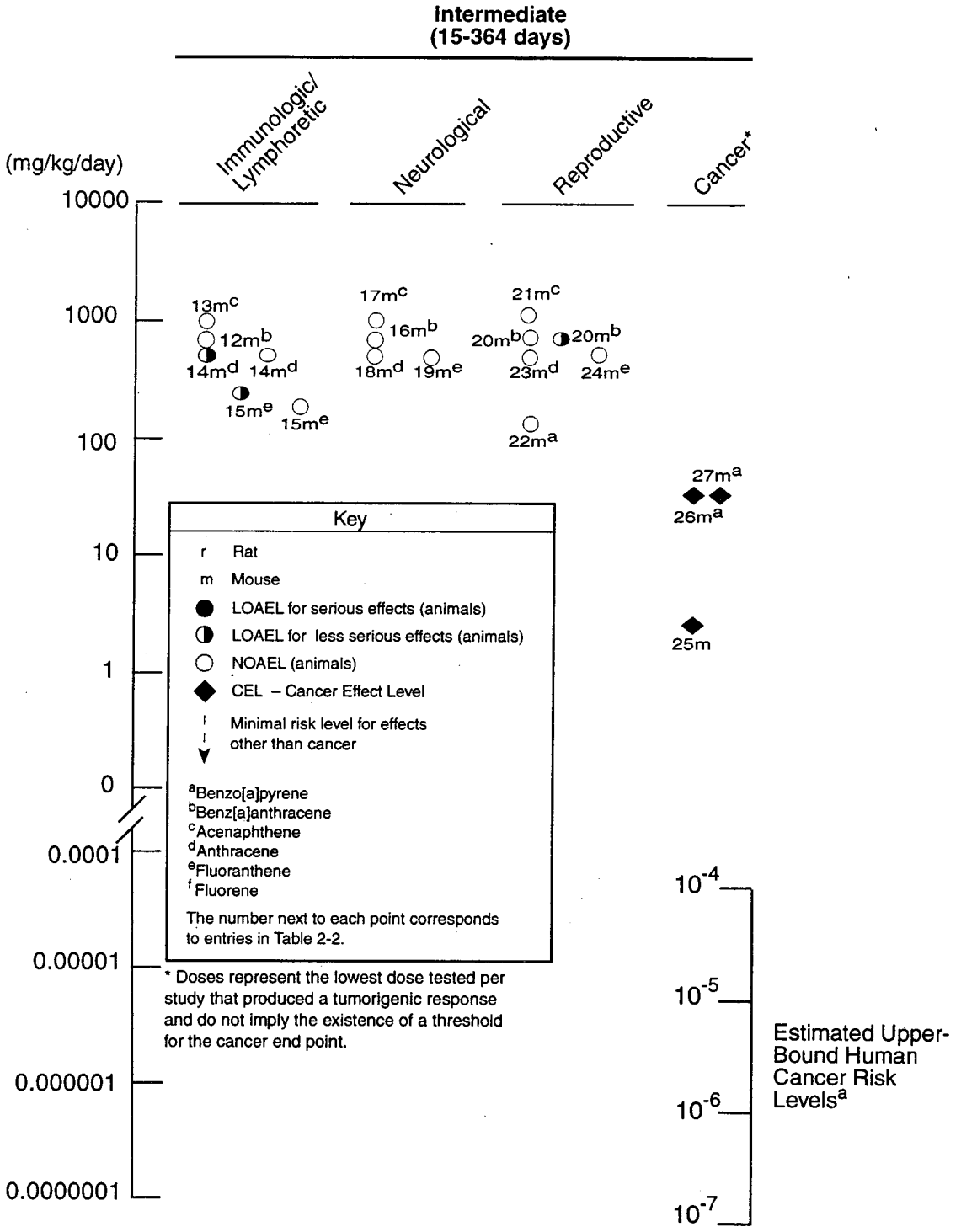


Figure 2-2. Levels of Significant Exposure to Polycyclic Aromatic Hydrocarbons – Oral (continued)



Cardiovascular Effects. Male and female mice were exposed to 0, 175, 350, or 700 mg/kg/day acenaphthene by gavage for 13 weeks (EPA 1989c). No signs of cardiovascular distress were seen during life for any dose group, and no gross or microscopic damage was seen upon necropsy. Similar findings were reported after 13-week administration of 1,000 mg/kg/day anthracene, and 500 mg/kg/day fluoranthene, or 500 mg/kg/day fluorene (EPA 1988e, 1989d, 1989e).

Gastrointestinal Effects. Minimal information is available on the gastrointestinal effects of human oral exposure to PAHs. In one study, humans that consumed anthracene-containing laxatives (the anthracene concentration was not specified) for prolonged periods of time were found to have an increased incidence of melanosis of the colon and rectum (i.e., unusual deposits of black pigments in the colon and rectum) compared to patients who did not consume anthracene laxatives. However, no definitive conclusions can be drawn from these results because of study limitations that include possible misclassification of patients with respect to the level of anthracene laxative use over 30 years and no accounting for other factors involved in the pathogenesis of melanosis (Badiali et al. 1985).

Enzyme alterations in the mucosa of the gastrointestinal tract have been observed in animals acutely exposed to anthracene, benz[a]anthracene, benzo[a]pyrene, or phenanthrene. In rats, acute intragastric administration of 50 or 150 mg/kg/day benz[a]anthracene or benzo[a]pyrene, respectively, for 4 days resulted in suppression of carboxylesterase activity in the intestinal mucosa (reduction of activity by 30% and 44%, respectively); rats exposed to 100 mg/kg/day of anthracene or phenanthrene exhibited carboxylesterase activity that was increased by 13% and 30%, respectively (Nousiainen et al. 1984). Enzyme alteration in the absence of other signs of gastrointestinal toxicity is not considered an adverse health effect, but it may precede the onset of more serious effects. Based on this very limited information, it would appear that acute ingestion of anthracene, benz[a]anthracene, benzo[a]pyrene, or phenanthrene at these doses may not adversely affect the gastrointestinal tract of animals; however, exposed animals exhibited biochemical changes and it is possible that more serious effects could occur at high doses.

Male and female mice were exposed to 0, 175, 350, or 700 mg/kg/day acenaphthene by gavage for 13 weeks (EPA 1989c). No adverse effects on the gastrointestinal system were seen during life for any dose group, and no gross or microscopic damage was seen upon necropsy. Similar findings were reported after 13-week administration of 1,000 mg/kg/day anthracene, 500 mg/kg/day fluoranthene, or mg/kg/day fluorene (EPA 1988e, 1989d, 1989e).

Hematological Effects. Male and female mice were exposed to 0, 175, 350, or 700 mg/kg/day acenaphthene by gavage for 13 weeks (EPA 1989). No hematological effects were seen during life for any dose group, and no gross or microscopic damage was seen upon necropsy. Similar findings were reported after 13-week administration of 1,000 mg/kg/day anthracene (EPA 1989d). Administration of 250 mg/kg/day fluoranthene by gavage for 13 weeks to mice resulted in decreased packed cell volume in females, but not in males, given doses up to 500 mg/kg/day (EPA 1988e). Both male and female mice exposed to 250 mg/kg/day fluorene exhibited hematologic effects, including decreased packed cell volume and hemoglobin content (EPA 1989e).

Adverse hematopoietic effects (e.g., aplastic anemia, pancytopenia) that ultimately led to death were reported in the Ah-nonresponsive strains of mice, DBA/2N and AKR/N, following oral exposure to 120 mg benzo[a]pyrene/kg/day for 180 days. Death was attributed to hemorrhage or infection that resulted from pancytopenia (Robinson et al. 1975). Similar results were obtained by Legraverend et al. (1983). The Ah gene encodes a cytosolic receptor (Ah receptor) that regulates the induction of the cytochrome P-450 enzymes. Differences in this gene locus determine whether the Ah receptor will be "high-affinity" (i.e., will allow for the induction of the cytochrome P-450 enzymes [more specifically, AHH] and is found in responsive mice) or "low-affinity" (i.e., does not allow for the induction of the AHH and is found in nonresponsive mice). Mice with a high-affinity Ah receptor (i.e., responsive mice) were administered 120 mg/kg/day benzo[a]pyrene in the diet for 3 weeks and exhibited no myelotoxicity. However, all nonresponsive mice that were treated according to the same regimen died from myelotoxic effects within 3 weeks (Legraverend et al. 1983). These results support the results of Robinson et al. (1975).

Musculoskeletal Effects. Male and female mice were exposed to 0, 175, 350, or 700 mg/kg/day acenaphthene by gavage for 13 weeks (EPA 1989c). No signs of musculoskeletal effects were seen during life for any dose group, and no gross or microscopic damage was seen upon necropsy. Similar findings were reported after 13-week administration of 1,000 mg/kg/day anthracene, 500 mg/kg/day fluoranthene, or 500 mg/kg/day fluorene (EPA 1988e, 1989d, 1989e).

Hepatic Effects. The induction of foci of altered hepatocytes is often seen in rats and mice that also develop liver tumors. These foci have altered enzyme activities and higher rates of cell proliferation than normal hepatocytes. A l-day intragastric administration of 200 mg/kg of benzo[a]pyrene or dibenz[a,h]anthracene, or of 180 mg/kg benz[a]anthracene to rats was followed by a

diet containing 2-acetylaminofluorene (2-AAF) and carbon tetrachloride induced gamma-GT foci (Tsuda and Farber 1980). Partially hepatectomized rats and sham hepatectomized rats were used, to provide proliferating and non-proliferating hepatocytes, respectively. Partially hepatectomized rats were more responsive to treatment than the sham-operated animals. For partially hepatectomized rats, benzo[a]pyrene was a more potent foci inducer than either benz[a]anthracene or dibenz[a,h]anthracene. Increased relative liver weight was seen in male mice and increased absolute and relative liver weight was seen in female mice given 175 mg/kg/day acenaphthene daily by gavage for 13 weeks; these effects were unaccompanied by other hepatic effects (EPA 1989c). Increased absolute and relative liver weight correlated with hepatocellular hypertrophy was seen in male and female mice given 350 mg/kg/day acenaphthene daily by gavage for 13 weeks (EPA 1989c). Increased serum cholesterol was also seen in females receiving 350 mg/kg/day acenaphthene (EPA 1989c). Increased liver weight and dose-related centrilobular pigmentation accompanied by an increase in liver enzymes were observed in both male and female mice receiving 250 mg/kg/day fluoranthene by gavage for 13 weeks (EPA 1988e). Male mice exposed to 125 mg/kg/day fluoranthene exhibited a slight increase in centrilobular pigmentation, and an increase in relative liver weight (EPA 1988e). Increased relative liver weight was observed in all treated groups, whereas increased absolute and relative liver weight was observed in the mid- and high-dose animals receiving 0, 125, 250, and 500 mg/kg/day fluorene for 13 weeks (EPA 1989e). However, there were no accompanying histopathological changes. No statistically significant effects of treatment were reported after 13-week administration of 1,000 mg/kg/day anthracene (EPA 1989d)

The ability to induce aldehyde dehydrogenase (ADH) in animals has been correlated with carcinogenic potency. Rats that were intragastrically administered 100 mg/kg/day of benzo[a]pyrene, benz[a]anthracene, anthracene, chrysene, or phenanthrene for 4 days exhibited cytosolic ADH induction (Torronen et al. 1981). However, benzo[a]pyrene and benz[a]anthracene were much more effective than phenanthrene, chrysene, or anthracene. Exposure to benzo[a]pyrene and benz[a]anthracene also increased the relative liver weights by 27% and 19%, respectively (Torronen et al. 1981). The authors concluded that anthracene, phenanthrene, and chrysene, which have been characterized as either noncarcinogens or equivocal carcinogens (see Section 2.2.2.8), are poor ADH inducers (Torronen et al. 1981).

The induction of carboxylesterase activity has also been observed in animals exposed to PAHs (Nousiainen et al. 1984). Benzo[a]pyrene, benz[a]anthracene, and chrysene were moderate inducers of

hepatic carboxylesterase activity in rats that were intragastrically administered 50, 100, and 150 mg/kg/day (100 mg/kg/day for chrysene), respectively, for 4 days. However, rats administered 100 mg/kg/day anthracene or phenanthrene did not exhibit induction of hepatic carboxylesterase activity. Induction of hepatic microsomal enzymes generally results in enhanced biotransformation of other xenobiotics (to either more or less toxic forms).

Increases in liver weight following partial hepatectomy have also been examined following acute oral exposure to various PAHs. Partially hepatectomized rats were fed diets containing various PAHs for 10 days. Administration of 51.4 mg/kg/day acenaphthene or 180 mg/kg/day fluorene resulted in statistically significant increases in liver weight compared to controls, which may have indicated an effect on regeneration, although rates of cell proliferation were not determined. Administration of 15.4 mg/kg/day acenaphthene, 51.4 mg/kg/day benzo[a]pyrene, or 51.4 mg/kg/day pyrene, anthracene, or phenanthracene had no effect. Diets containing 51.4 mg/kg/day acenaphthene or dibenz[a,h]anthracene, 180 mg/kg/day anthracene or phenanthracene, or 437 mg/kg/day pyrene produced no increase in the liver-to-body-weight ratio. Rats that were fed a diet containing 514 mg/kg/day chrysene exhibited equivocal results: in one trial, a significant increase in liver weight gain was noted, while in another trial, no increase in. liver-to-body-weight ratio was observed (Gershbein 1975). Thus, both suspected carcinogenic and noncarcinogenic PAHs can affect liver weights, although much higher doses are required for noncarcinogenic PAHs. The livers of rats administered single doses of ,fluorene by gavage in dimethyl sulfoxide (DMSO) were evaluated for the promotion of growth (i.e., cell proliferation as determined by organ weight and mitotic index) (Danz et al. 1991). The authors claimed that liver weight was increased in a dose-dependent manner to 20% over control values, and that the mitotic index of the hepatocytes was increased by 6-fold after 48 hours. However, the organ weight data were not presented, and the mitotic index data presented graphically in the text do not indicate a 6-fold increase over controls.

Ah-responsive strains of mice (C57BL/6, C3H/HeN, BALB/cAnN) that were orally administered 120 mg benzo[a]pyrene/kg/day in their diet for 180 days exhibited a 13% increase in relative liver weights (Robinson et al. 1975).

The hepatic effects observed in animals following oral exposure to PAHs are generally not considered serious. However, the enzyme alterations, gamma-GT foci induction, liver regeneration, and increased liver weight may precede the onset of more serious hepatic effects.

Renal Effects. The kidney microsomal carboxylesterase activity of rats was moderately induced by 50-150 mg/kg of benzo[a]pyrene following 4 days of intragastric administration; however, rats administered 100 mg/kg/day of anthracene or phenanthrene and 50-150 mg/kg benz[a]anthracene did not exhibit increased activity. The authors conclude that anthracene, phenanthrene, and benz[a]anthracene are not inducers of kidney carboxylesterase activity (Nousiainen et al. 1984). Enzyme induction is considered an adverse effect when observed concurrently with more serious effects such as impaired renal function and/or histopathological changes of the kidney.

Increasing dietary doses of pyrene ranging from 1,000 mg/kg food (127 mg/kg/day) up to 25,000 mg/kg food (917 mg/kg/day) for a mean dose of 426.6 mg/kg/day over a 25-day study produced dilation of the renal tubules in an unspecified number of mice. This effect was not observed until the highest dose was administered (Rigdon and Giannukos 1964). The limitations of this study (e.g., doses changed throughout exposure period and no statistical analyses performed) render these results of questionable toxicological significance.

Male and female mice were exposed to 0, 175, 350, or 700 mg/kg/day acenaphthene by gavage for 13 weeks (EPA 1989c). No signs of renal toxicity were seen during life for any dose group, and no gross or microscopic damage was seen upon necropsy. Similar findings were reported after 13-week administration of 1,000 mg/kg/day anthracene (EPA 1989d). Increased absolute and relative kidney weight was observed in males, but not females receiving 500 mg/kg/day fluorene for 13 weeks (EPA 1989e). Renal tubular regeneration, and interstitial lymphocytic infiltrates and/or fibrosis were observed after 13-week oral administration of fluoranthene to female mice at 250 mg/kg/day, and male mice at 500 mg/kg/day (EPA 1988e).

Endocrine Effects. Male and female mice were exposed to 0, 175, 350, or 700 mg/kg/day acenaphthene by gavage for 13 weeks (EPA 1989c). No signs of endocrine imbalance were seen during life for any dose group, and no gross or microscopic damage was seen upon necropsy. Similar findings were reported after 13-week administration of 1,000 mg/kg/day anthracene, 500 mg/kg/day fluoranthene, or 500 mg/kg/day fluorene (EPA 1988e, 1989d, 1989e).

Dermal Effects. Male and female mice were exposed to 0, 175, 350, or 700 mg/kg/day acenaphthene by gavage for 13 weeks (EPA 1989c). No signs of derrnal effects were seen during life for any dose group, and no gross or microscopic damage was seen upon necropsy. Similar findings

were reported after 13-week administration of 1,000 mg/kg/day anthracene, 500 mg/kg/day fluoranthene, or 500 mg/kg/day fluorene (EPA 1988e, 1989d, 1989e).

Ocular Effects. Male and female mice were exposed to 0, 17.5, 350, or 700 mg/kg/day acenaphthene by gavage for 13 weeks (EPA 1989c). No signs of ocular toxicity were seen during life for any dose group, and no gross or microscopic damage was seen upon necropsy. Similar findings were reported after 13-week administration of 1,000 mg/kg/day anthracene, 500 mg/kg/day fluoranthene, or 500 mg/kg/day fluorene (EPA 1988e, 1989d, 1989e).

Body Weight Effects. Male and female mice were exposed to 0, 175, 350, or 700 mg/kg/day acenaphthene by gavage for 13 weeks (EPA 1989c). No adverse effects on body weight were seen during life or upon necropsy. Similar findings were reported after 13-week administration of 1,000 mg/kg/day anthracene, and 500 mg/kg/day fluoranthene (EPA 1988e, 1989d). After administration of 500 mg/kg/day fluorene for 13 weeks; however, female mice exhibited increased body weight, although male mice showed no effect at the same dose level (EPA 1989e).

Other Systemic Effects. The number of thymic glucocorticoid receptors in 6-week-old rats treated once with 2 mg/kg benzo[a]pyrene was measured (Csaba et al. 1991). It is assumed that administration was by ora gavage, but this was never explicitly stated. The number of these receptors was decreased by 40% in females and unaffected in males relative to the vehicle control animals. The statistical significance of these effects was not indicated, nor was the functional consequences of a decrease in receptor number assessed by examination of functional parameters.

2.2.2.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological effects in humans following oral exposure to any of the 17 PAHs discussed in this profile.

A single gavage dose of 150 mg/kg fluorene to male Sprague-Dawley rats had no effect on thymus or spleen weight (Danz and Brauer 1988). Little useful information can be obtained from this study as only one dose was tested (thereby precluding assessment of the validity of the negative response) and no tests of immune function were evaluated. Male and female mice exposed to 0, 175, 350, or 700 mg/kg/day acenaphthene by gavage for 13 weeks showed no effect of treatment on splenic weight

or histopathology (EPA 1989c). Similar findings were reported after 13-week administration of 1,000 mg/kg/day anthracene, and 500 mg/kg/day fluoranthene (EPA 1988e, 1989d). After administration of 2.50 mg/kg/day fluorene for 13 weeks, however, increased absolute and relative spleen weight was seen in both sexes (EPA 1989e).

Lee and Strickland (1993) looked for antibodies specific to PAH-DNA adducts in the serum of BALB/c mice treated orally twice per week for 8 weeks with 0.5 or 5 mg/kg benz[a]anthracene, benzo[a]pyrene, benzo[b]fluoranthene, chrysene, dibenz[a,h]anthracene, or fluoranthene. Increased antibody response was noted in animals treated with the low dose of benz[a]anthracene and benzo[b]fluoranthene, but not any of the other PAHs.

2.2.2.4 Neurological Effects

No studies were located regarding neurological effects in humans following oral exposure to any of the 17 PAHs discussed in this profile.

Male and female mice exposed to 0, 175, 350, or 700 mg/kg/day acenaphthene by gavage for 13 weeks showed no effect of treatment on behavior, or histopathologic effects on nerve or brain samples (EPA 1989c). Similar findings were reported after 13-week administration of 1,000 mg/kg/day anthracene, and 500 mg/kg/day fluoranthene (EPA 1988e, 1989d). After administration of 500 mg/kg/day fluorene for 13 weeks, however, increased brain weight was observed in females, but not in males (EPA 1989e). No histopathologic changes were observed.

2.2.2.5 Reproductive Effects

No studies were located regarding reproductive effects in humans following oral exposure to the PAHs discussed in this profile. Three animal studies were located that evaluated the reproductive effects of benzo[a]pyrene in animals. The results of two oral studies in mice (Mackenzie and Angevine 1981; Rigdon and Neal 1965) and one in rats (Rigdon and Rennels 1964) indicate that benzo[a]pyrene induces reproductive toxicity in animals. The incidence and severity of these effects depends on the strain, method of administration, and dose levels used. In one study, benzo[a]pyrene administered by gavage to pregnant CD-l mice decreased the percentage of pregnant females that reached parturition and produced a high incidence of sterility in the progeny (Mackenzie and Angevine 1981). In

contrast, benzo[a]pyrene administered in the diet caused no adverse effects on fertility of Swiss mice (Rigdon and Neal 1965) but reduced the incidence of pregnancy in female rats (Rigdon and Rennels 1964). Based on these studies, the LOAEL for benzo[a]pyrene-induced reproductive toxicity in parental mice was 160 mg/kg/day, and the LOAEL for these effects in the progeny of exposed animals was 10 mg/kg/day (Mackenzie and Angevine 1981). Because only the parental doses are quantifiable, these are the only data presented in Table 2-2.

When CD-l mice were administered benzo[a]pyrene by gavage daily for 10 days during gestation, there was a significant reduction in the percentage of pregnant females to reach parturition at 160 mg/kg/day, the highest dose tested (Mackenzie and Angevine 1981). When F_1 progeny were bred with untreated animals, the fertility index decreased significantly in all treatment groups. At 10 mg/kg/day, the lowest dose tested, the reduced fertility noted was associated with significant alterations in gonadal morphology and germ cell development. The treatment at higher doses resulted in total sterility. Contrary to these results, no adverse effects on reproduction were observed in Swiss mice fed benzo[a]pyrene in the diet at ≤133 mg/kg/day over varying time spans during mating, gestation, and parturition (Rigdon and Neal 1965). The apparent discrepancy in the results of the two studies may be attributable to the method of benzo[a]pyrene administration and metabolic differences in the two strains of mice used.

Dietary administration of benzo[a]pyrene for 28 days revealed no treatment-related effects on the estrous cycle of female rats. These rats experienced no significant adverse effects on their fertility when bred to untreated male rats (Rigdon and Rennels 1964). In another series of experiments, when benzo[a]pyrene-fed male and female rats were bred, only two of seven females became pregnant (as compared to 3 of 6 controls); the offspring of one rat were stillborn while those of others were resorbed (Rigdon and Rennels 1964). Although the data suggest that benzo[a]pyrene may induce reproductive toxicity in rats, they are inconclusive because of the use of a single dose level, small number of animals, and inadequate reporting of data.

Male mice exposed to 0, 175, 350, or 700 mg/kg/day acenaphthene by gavage for 13 weeks showed no effect of treatment on reproductive organ weight or histology (EPA 1989c). Female mice, however, exhibited decreased ovary weights correlated with an increase of inactivity of the ovary and uterus (EPA 1989c). No adverse effects on reproductive organs were reported after 13-week

administration of 1,000 mg/kg/day anthracene, 500 mg/kg/day fluoranthene, or 500 mg/kg/day fluorene to male and female mice (EPA 1988e, 1989d, 1989e).

The available information from animal studies suggests that benzo[a]pyrene may have the potential to produce adverse reproductive effects in exposed humans. The highest NOAEL and all LOAEL values from each reliable study for reproductive effects following acute- and intermediate-duration exposures are reported in Table 2-2 and plotted in Figure 2-2.

2.2.2.6 Developmental Effects

No studies were located regarding developmental effects in humans following oral exposure to PAHs. Three animal studies were reviewed that assessed developmental effects of benzo[a]pyrene in inbred strains of rats and mice. The data from these studies indicate that prenatal exposure to benzo[a]pyrene produced reduced mean pup weight during postnatal development and caused a high incidence of sterility in the F_1 progeny of mice (Mackenzie and Angevine 1981). Using Ah-responsive and Ah-nonresponsive strains of mice, the increased incidences of stillboms, resorptions, and malformations observed correlated with the maternal and/or embryonal genotype (Legraverend et al. 1984). In another study, negative results were obtained when benzo[a]pyrene was administered to Swiss (responsive) mice (Rigdon and Neal 1965).

Benzo[a]pyrene was administered by gavage to pregnant CD-l mice during gestation at doses of 10, 40, and 160 mg/kg/day. The viability of litters at parturition was significantly reduced in the highest dose group (Mackenzie and Angevine 1981). The mean pup weight was significantly reduced in all treatment groups by 42 days of age. The F_1 progeny that were exposed prenatally to benzo[a]pyrene (10, 40, and 160 mg/kg/day) were bred with untreated animals and further studied for postnatal development and reproductive function. The F_1 progeny from the 10-mg/kg/day group experienced decreased fertility with associated alterations in gonadal morphology and germ-cell development. Because only the parental doses are quantifiable, these are the only data presented in Table 2-2. Therefore, the LOAEL of 10 mg/kg/day noted in the F_1 progeny discussed above is not presented in Table 2-2. Higher doses produced total sterility. This study provides good evidence for the occurrence of developmental effects following *in utero* exposure to benzo[a]pyrene.

The effect of genetic differences in metabolism of orally administered benzo[a]pyrene on *in utero* toxicity and teratogenicity was evaluated in mice that either metabolize benzo[a]pyrene readily (Ah-responsive) or not (Ah-nonresponsive) (Legraverend et al. 1984). Pregnant mice, either B6AKFl (Ah-responsive) or AKR/J (Ah-nonresponsive), were fasted prior to a diet containing 120 mg/kg/day benzo[a]pyrene on days 2-10 of gestation. The mice were killed on day 18 of gestation. On day 16 of gestation, intraperitoneal injections of napthoflavone were administered to distinguish between fetuses with different Ah-genotypes (Ahb/Ahd and Ahd/Ahd). Oral administration of benzo[a]pyrene to the pregnant AKR/J mice (non-responsive) caused more stillbirths, decreased weight gain, resorptions, and birth defects among Ahd/Ahd (Ah-nonresponsive) than among Ahb/Ahd (Ah-responsive) embryos. However, no differences in *in utero* toxicity or teratogenicity were observed in Ah-genetically different embryos (Ahd/Ahd and Ahb/Ahd) of B6AKFl mothers (responsive). The authors concluded that differences in *in utero* toxicity and teratogenicity are specific to the route of administration and can be attributed to "first pass" liver metabolism occurring with oral dosing. They also concluded that *in utero* toxicity and teratogenicity are directly related to the maternal and/or embryonal genotype controlled by the Ah-locus; that is, both maternal metabolism as well as target organ metabolism (embryo/fetus) were important in determining susceptibility to developmental toxicity. Specifically, metabolism by a responsive mother reduces *in utero* toxic effects in the fetus. Similarly, responsive fetuses in the uterus of a non-responsive mother show fewer *in utero* toxic effects. Non-responsive fetuses in the uterus of a non-responsive mother show the highest incidence of *in utero* toxic effects. Although the study emphasizes the importance of administrative route in benzo[a]pyrene metabolism and resulting toxicity, it had the following limitations: 1) only one dose was evaluated; 2) no quantitative comparisons between treated groups and corresponding control animals were presented for any of the reported *in utero* toxicity or teratogenic effects; 3) small sample size; 4) purity of benzo[a]pyrene was not specified.

In another study, negative results were obtained when the potential developmental effects of benzo[a]pyrene were studied in mice (Rigdon and Neal 1965). Dietary administration of this chemical to mice at concentrations equivalent to 33.3, 66.7, or 133.3 mg/kg/day at various times before and after mating elicited no adverse effects on the developing embryos. Maternal weight gain was reduced in the mice administered the higher levels of benzo[a]pyrene, but this effect was reversed when the animals were changed to the control ration. Limitations of this study that preclude its inclusion in Table 2-2 consist of the use of an inconsistent protocol, varying number of animals, and varying time of gestation exposure.

The highest NOAEL values and all LOAEL values from each reliable study for developmental effects in mice for acute-duration exposure is recorded in Table 2-2 and plotted in Figure 2-2.

2.2.2.7 Genotoxic Effects

No studies were located regarding genotoxic effects in humans following oral exposure to any of the 17 PAHs discussed in this profile.

Pregnant *Erythrocebus patas* monkeys were treated once on gestation day (Gd) 50, 100, or 150 (term = 160 days) with 5-50 mg/kg benzo[a]pyrene (Lu et al. 1993). Fetuses were removed by Cesarean section l-50 days after treatment and fetal organs, placentae, and maternal livers were assayed for DNA adducts. Benzo[a]pyrene-DNA adducts were high in fetal organs, placentae, and maternal livers in all three trimesters of gestation. Adduct levels were higher in mid-gestation compared to early or late gestation. dG-N2-BPDE was the major adduct detected. The adduct levels in fetal tissues increased with benzo[a]pyrene dose, but at a much lower rate that placentae or maternal livers. Preference in binding to DNA of various fetal tissues was more apparent in early gestation compared to late gestation, and at lower doses compared to higher doses. During early gestation and at lower doses, benzo[a]pyrene produced a similar level of DNA binding in fetal lung, liver, maternal liver, and placenta. Individual fetal organ adduct levels correlated significantly with placental adduct levels, indicating placental and/or maternal contribution to adduct formation in fetuses. Evidence of fetal contribution to adduct formation was also found. DNA adduct levels in fetal skin were lowest of all fetal organs tested and less affected by gestational stage at time of treatment. In contrast, DNA adduct levels in fetal liver exhibited distinct gestation stage specificity with higher adduct, levels attained during mid-gestation compared to other stages of gestation. Adduct levels decreased at a much faster rate during the first 10-15 days compared to 15-50 days after treatment. However, 10% of the DNA adducts persisted 50 days after treatment in all organs studied. Together, the results suggest that placental adduction accurately indicates fetal exposure.

Male B6C3F_1 mice were fed 0, 0.325, 0.1825, 1.625, 3.25, or 6.5 mg/kg/day benzo[a]pyrene for 21 days (Culp and Beland 1994). Animals were killed and the liver, lung, and forestomach DNA extracted and analyzed for benzo[a]pyrene-DNA adducts. The major adduct, dG-N2-BPDE, was quantified. Adduct levels in liver and lung increased in a linear manner. Adduct levels in the forestomach appeared to plateau at the highest dose. At doses below the highest, adduct levels were in

the order of forestomach > liver > lung, with the values of average slopes being 3.0 + 0.59, 2.1 + 0.17, 1.3 + 0.37 fmol adduct/mg DNA/μg benzo[a]pyrene/day, respectively. At these doses, the lung and the forestomach were not significantly different. At the high dose, liver > forestomach > lung, and each tissue was significantly different from the other.

DNA binding of coal tar components in male mice was investigated following the ingestion of coal tar obtained from a manufactured coal plant (Weyand et al. 1991). One of four different samples (A-D) of coal tar or a mixture of four equal portions of the four samples was administered in a gel diet which contained 0.25% coal tar. The coal tar contained phenanthrene, fluoranthene, pyrene, benz[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[1,2,3-c,d]pyrene, and benzo[g,h,i]perylene, among other PAHs. In addition, a diet containing benzo[a]pyrene at the same level as the 0.25% diet prepared with Sample C was administered; animals consuming this diet ingested 0.01-0.02 mg benzo[a]pyrene per day. The diets were administered for 15 days. Chemical-DNA adduct formation was evaluated in animals following 14 days of treatment. Chemical-DNA adduct formation was also evaluated in animals maintained on a 0.1, 0.2, 0.5, and 1.0% coal tar diet prepared with one of the coal tar samples (C). Chemical-DNA adduct formation in animals dosed with 0.l-1.0% Sample C indicated a dose-related effect in lung DNA adduct formation, but no dose-related effect was observed for forestomach tissue. In addition, overall adduct levels in lung tissue were considerably higher than forestomach levels for animals on the 0.5 or 1% diet. In contrast, adduct levels were highest in the forestomach of animals on diets lower in coal tar content (0.1 or 0.2%). Chemical-DNA adducts of coal tar components evaluated for Samples A-D and the mixture of the four coal tar samples at 0.25% in the diet administered for 15 days indicated adducts in the lung, liver, and spleen of all animals. Adduct patterns were similar, but quantitative differences were observed between coal tar samples and tissue sites. The highest adduct levels were detected in lung DNA. Adduct formation in animals fed the benzo[a]pyrene diet, could not account for the differences in the adduct levels observed in animals given the mixtures. Also, adduct formation in animals fed the coal tar mixtures correlated with benzo[a]pyrene content in the coal tar, indicating the adducts arose from a variety of PAHs in the coal tar mixtures. The levels of 1-hydroxypyrene in the urine of these animals correlated with the pyrene content of these coal tars.

The DNA binding of manufactured gas plant residue (MGP) components in male B6C3Fl mice was investigated following oral administration (Weyand and Wu 1994). Male mice were fed a gel diet containing manufactured gas plant (MGP) residue (coal tar) at 0.3% for 28 days, or the corresponding

control diet. Two mixtures of MGP residue were used: Mix of 3 combining equal amounts of samples from three different MGP plant sites, and Mix of 7 combining equal amounts of samples from seven different MGP plant sites, including those used in the Mix of 3. The mixtures contained pyrene, benz[a]anthracene, chrysene, benzo[b]fluorene, benzo[k]fluorene, benzo[a]pyrene, indeno[l,2,3-cd] pyrene, dibenz[a,h]anthracene, benzo[g,h,i]perylene. Data was presented in terms of pyrene consumed. Animals were sacrificed on the twenty-ninth day and lung and forestomach were excised and DNA isolated. Chemical-DNA adduct formation was evaluated. Ingestion of the adulterated diets resulted in a relatively low level of DNA adducts in the forestomach in comparison with the lung (one-tenth the level). PAH-DNA adduct levels in the lung of mice maintained on the Mix of 3 (1.4 mg/kg/day pyrene) were two times greater than the level induced by the Mix of 7 (1.2 mg/kg/day pyrene) suggesting that the composition of the MGP residue may have influence PAH absorption or DNA adduct formation.

Oral exposure to a total dose of 10 mg/kg benzo[a]pyrene produced gene mutations in the mouse coat color spot test (Davidson and Dawson 1976, 1977). Dose-related increases in the frequency of micronuclei were seen in bone marrow cells harvested from MS/Ae and CD-l male mice (four mice/strain/dose) 48 hours after administration of a single oral dose of benzo[a]pyrene ranging from 62.5 to 500 mg/kg (Awogi and Sato 1989). Although the response appeared to be stronger in the MS/Ae strain, the reduction in polychromatic erythrocytes, indicative of target cell toxicity at all levels in the CD-1 strain, limited the comparative evaluation of strain specificity.

In another study, a dose of benzo[a]pyrene (150 mg/kg) known to induce a clastogenic response was orally administered to groups of five adult males and females, pregnant females, and fetal ICR mice. An increased incidence of micronuclei in bone marrow cells harvested from the various groups of adult animals and also in the livers of the fetuses was observed (Harper et al. 1989). Genetic damage was most severe in the fetuses. The approximately 7-fold increase in micronuclei in fetal livers as compared to maternal bone marrow suggests that the transplacentally-induced genotoxicity was probably associated with the immature detoxification processes of fetal liver as compared to adult bone marrow. It would, nevertheless, appear that the fetus may be at an increased risk.

Data showing that orally administered benzo[a]pyrene induces micronuclei were confirmed in subsequent studies (Shimada et al. 1990, 1992) using rats (Sprague-Dawley) and mice (CD-l and BDF_1), different dosing regimes (single, double, or triple doses), and different target cells (bone

marrow and peripheral blood reticulocytes). A single oral gavage dose of 63 mg/kg benzo[a]pyrene significantly ($p<0.0l$) increased the yield of chromosomes with abnormal morphology in bone marrow cells collected from hybrid $lC3F_1$ male mice (Adler and Ingwersen 1989).

There is conflicting evidence that the genetic damage induced by benzo[a]pyrene is partially controlled by the expression of structural genes for benzo[a]pyrene-specific cytochromes P-450. In one study, two inbred strains of mice differing in AHH inducibility (AHH-inducible strain C57BL/6 and AHH-noninducible strain DBA/2) received two consecutive daily doses of either 10 or 100 mg/kg of the test material (Wielgosz et al. 1991). Animals were sacrificed 5 days postexposure, and bone marrow and spleen cells were examined for sister chromatid exchange and DNA adducts. Results showed a marked increase in sister chromatid exchange induction and the formation of DNA adducts in bone marrow and spleen cells recovered from the DBA/2 mice (AHH-noninducible) in both dose groups compared to the C57BL/6 (AHH-inducible) mice. However, no clear correlation between AHH inducibility and the positive clastogenic response induced by 150 mg/kg benzo[a]pyrene was found in adult male and female mice with genetically determined differences in AHH induction (Adler et al. 1989). Similarly, the transplacental exposure of 11-day-old homozygous and hybrid embryos (dams received a single oral gavage dose of 150 mg/kg and embryos were sampled 15 hours after treatment) to benzo[a]pyrene showed that the clastogenic response was independent of genetic constitution.

In contrast to the relatively uniform evidence that benzo[a]pyrene is a genotoxin in whole animals, the test material failed to induce unscheduled DNA synthesis (UDS) in the parenchymal liver cells of Brown Norway rats exposed by oral gavage to 12.5 mg/mL (Mullaart et al. 1989). There was, however, a clear increase in single-strand DNA breaks in cells from the two major centers of metabolism (the parenchymal liver and intestinal cells) of the treated animals that was not apparent in the nonparenchymal liver cells.

Significant ($p<0.05$), but marginal, increases in the frequency of abnormal sperm were found in CD-l mice (8-12/group) exposed via oral gavage to benzo[a]pyrene doses ranging from 360 to 432 mg/kg (Salamone et al. 1988). The effect, however, was not clearly dose related, and the wide variation in the background frequency rendered the data inconclusive. Comparable doses produced no adverse effects in $B6C3F_1$ mice. Similarly, the evaluation of pyrene (241-844 mg/kg) in this study yielded uniformly negative results.

Orally administered fluoranthene (400 and 750 mg/kg) did not increase the sister chromatid exchange frequency in mice (Palitti et al. 1986). Gene mutations were not produced in bacteria or yeast in a host-mediated assay in which anthracene, benzo[a]pyrene, chrysene, or fluoranthene were administered to mice by gavage; positive results were produced in bacteria in the same test system in which mice were exposed to benz[a]anthracene and injected intraperitoneally with the bacteria (Simmon et al. 1979). Other genotoxicity studies are discussed in Section 2.4.

2.2.2.8 Cancer

No studies were located regarding cancer in humans following oral exposure to the 17 PAHs discussed in this profile. The animal studies discussed in this section are presented first by exposure duration (acute, intermediate, and chronic), and within each duration category the information on individual PAHs is discussed in alphabetical order. PAHs for which no information was available for specified exposure durations were omitted.

Acute-Duration Exposure. Mice acutely administered 1.5 mg/day benz[a]anthracene by oral gavage two times over 3 days exhibited increased incidences of hepatomas and pulmonary adenomas (80% and 85%, respectively) as compared to control incidences (10% and 30% for hepatomas and pulmonary adenomas, respectively) after 568 days of observation (Klein] 1963). No malignant tumors were observed in this study.

Mice fed benzo[a]pyrene in the diet at a concentration equivalent to 33.3 mg/kg/day exhibited forestomach neoplasms following 2 or more days of consumption. However, a lower concentration of benzo[a]pyrene (equivalent to 13.3 mg/kg/day) administered for up to 7 days did not produce forestomach tumors (Neal and Rigdon 1967) (see Table 2-2). Hamsters have also been observed to develop papillomas and carcinomas of the alimentary tract in response to gavage or dietary exposure to benzo[a]pyrene (Chu and Malmgren 1965). A 77% mammary tumor incidence was observed 90 weeks after a single oral dose of 50 mg benzo[a]pyrene (100 mg/kg) was administered to rats, as compared to a 30% incidence in untreated animals (McCormick et al. 1981).

A single dose of 0.05 mg/kg dibenz[a,h]anthracene in polyethylene glycol (PEG)-400 failed to induce tumors in male Swiss mice after 30 weeks. However, forestomach papillomas were found in 10% of mice administered a single dose of 0.05 mg/kg dibenz[a,h]anthracene followed by 30 weekly doses of

PEG alone, and in 21% of the mice when the dibenz[a,h]anthracene dose was followed by 30 weekly doses of PEG plus 3% croton oil (Berenblum and Haran 1955). Treatment with croton oil alone yielded a 14-16% tumor incidence. These results suggest that the carcinogenic activities of croton oil and dibenz[a,h]anthracene are additive in the mouse forestomach.

Intermediate-Duration Exposure. One intermediate-duration study was located that evaluated the carcinogenic potential of acenaphthene. Male and female mice exposed to 0, 175, 350, or 700 mg/kg/day acenaphthene by gavage for 13 weeks showed no evidence of tumorigenesis at necropsy (EPA 1989c).

Similarly, only one intermediate-duration study was located that evaluated the carcinogenic potential of anthracene. Male and female mice exposed to 0, 250, 500, 1,000 mg/kg/day anthracene by gavage for 13 weeks showed no evidence of tumorigenesis at necropsy (EPA 1989d).

One intermediate-duration study was located that evaluated the carcinogenic potential of benz[a]anthracene. Mice that received intermittent gavage doses of 1.5 mg/kg/day benz[a]anthracene for 5 weeks (Klein 1963). Mice were sacrificed at a median age of 437 or 547 days. The treated mice killed at 437 days exhibited a 95% incidence of pulmonary adenomas at an average of 3 per lung and a 46% incidence of hepatomas, with an average of 2.1 per tumor-bearer. Forestomach papillomas were found in 5% of the mice. Control animals killed after 441 days exhibited a 10% incidence of pulmonary adenomas. Treated mice sacrificed after 547 days exhibited a 95% pulmonary adenoma incidence, as was observed in the group sacrificed earlier, but an increased hepatoma incidence of 100%. Control animals sacrificed after 600 days had 30 and 10% incidences of pulmonary adenomas and hepatomas, respectively. This study was not adequately reported; it did not include complete histopathology, adequate treatment durations, large enough sample sizes, or statistical analysis. Although this study is inconclusive because of methodological limitations, it does provide some qualitative evidence for the potential carcinogenicity of benz[a]anthracene by the oral route.

Intragastric doses of 67-100 mg/kg benzo[a]pyrene have been shown to elicit pulmonary adenomas and forestomach papillomas in mice (Sparnins et al. 1986; Wattenberg and Leong 1970). Intermittent gavage exposure of mice to 67-100 mg/kg benzo[a]pyrene resulted in increased forestomach (100%) and pulmonary tumor incidences relative to controls at 30 weeks of age (Sparnins et al. 1986;

Wattenberg and Leong 1970). The study by Wattenberg and Leong (1970) involved gavage administration of approximately 1.0 mg of benzo[a]pyrene once a week for 8 weeks.

The incidence of forestomach tumors (papillomas and carcinomas) in mice was related to the duration of oral exposure to benzo[a]pyrene following intermediate-duration administration of dietary benzo[a]pyrene at various doses up to 250 ppm (33.3 mg/kg/day) for 30-197 days (Neal and Rigdon 1967, see Table 2-2). The tumor incidence also increased with increasing dose. In the same study, mice fed 250 ppm (33 mg/kg/day) for periods of 1-7 days exhibited increased forestomach tumor incidences following 2 or more days of benzo[a]pyrene exposure (total dose of 2 mg), while mice fed 10 ppm (13.3 mg/kg/day) for 110 days (total dose of 4.48 mg) did not develop tumors. The authors suggest that these findings provide evidence that there are no cumulative carcinogenic effects of benzo[a]pyrene or its metabolites in mice. These data suggest that differences in susceptibility may be strongly influenced by the age of the mice at the time that they were initially exposed. This study provides the best dose-response information available for the oral route of exposure despite the irregular protocol employed, although the relevance of forestomach tumors in rodents to human cancer is the subject of some controversy because humans lack a forestomach.

An association between dietary benzo[a]pyrene and the development of leukemia and tumors of the forestomach and lung has been observed in mice. Tumor incidence was related to both dose and length of exposure (except in the case of leukemia). Mice administered dietary doses of up to 1,000 ppm (up to 133 mg/kg/day) for intermediate lengths of time (23-238 days) exhibited an increased incidence of forestomach tumors (papillomas and carcinomas) (Rigdon and Neal 1966, 1969) (see Table 2-2). Mice administered 250 ppm (33.3 mg/kg/day) benzo[a]pyrene developed papillomas or carcinomas of the forestomach (64%) and all the mice in the 1,000-ppm (133 mg/kg/day) group exhibited forestomach tumors after 86 days of benzo[a]pyrene consumption. A similar relationship was observed for the incidence of lung tumors: mice fed 250 ppm (33.3 mg/kg/day) benzo[a]pyrene exhibited an increased lung adenoma incidence. The occurrence of leukemia was related to the ingestion of 250 ppm (33.3 mg/kg/day) benzo[a]pyrene; 37% of the treated mice developed leukemias (Rigdon and Neal 1969) (see Table 2-2). The lack of consistent protocol in these experiments and the short exposure duration and observation periods preclude the assessment of a dose-response relationship. Furthermore, because tumors were reported as combined papillomas and carcinomas, no distinction between these benign and malignant tumors can be made.

Mammary tumors have also been observed following intermediate-duration exposure to benzo[a]pyrene in rats. Eight weekly oral doses of 6.25 mg benzo[a]pyrene (12.5 mg/kg) administered to rats resulted in a 67% increase in the incidence of mammary tumors in female rats after 90 weeks of observation (McCormick et al. 1981). A 30% incidence of these tumors was observed in the control animals.

Two intermediate-duration studies investigated the carcinogenicity of dibenz[a,h]anthracene in animals following oral exposure. Mammary carcinomas were observed in 5% of the female BALB/c mice dosed with 0.5% dibenz[a,h]anthracene after 15 weeks of dosing; however, no control group was included (Biancifiori and Caschera 1962). In the other study, male and female rats were administered an emulsion of aqueous olive oil and dibenz[a,h]anthracene in place of their drinking water for up to 200 days (Snell and Stewart 1963). Pulmonary adenomatosis, alveologenic carcinoma, mammary carcinoma, and hemangioendotheliomas were observed in the treated rats. These tumors were not observed in the control animals. However, extensive dehydration and emaciation occurred because the animals did not tolerate the vehicle well, which lead to early death and the need to periodically remove the animals from the treatment vehicle. Neither of these studies was adequately reported: they did not perform appropriate histopathologic evaluations, treatment or study durations were inadequate, and the sample size was inadequate. Despite these methodological limitations, these studies do provide some evidence of dibenz[a,h]anthracene's carcinogenicity by the oral route.

One intermediate-duration study was located that evaluated the carcinogenic potential of fluoranthene. Male and female mice exposed to 0, 125, 250, or 500 mg/kg/day fluoranthene by gavage for 13 weeks showed no evidence of tumorigenesis at necropsy (EPA 1988e).

Similarly, only one intermediate-duration study was located that evaluated the carcinogenic potential of fluorene. Male and female mice exposed to 0, 125, 250, or 500 mg/kg/day fluorene by gavage for 13 weeks showed no evidence of tumorigenesis at necropsy (EPA 1989e).

Chronic-Duration Exposure. Benzo[a]pyrene was administered in the diet of 32 Sprague-Dawley rats/sex/group either every 9th day or 5 times/week at a dose of 0.15 mg/kg until the animals were either moribund or dead (Brune et al. 1981). An untreated control group consisted of 32 animals/sex. There was no treatment-related effect on survival and no treatment-related increase in tumors at any one site. However, a statistically significant increase in the proportion of animals with tumors of the forestomach, esophagus, and larynx combined was noted among animals receiving treatment

5 times/week (the combined incidence of animals with these tumors was 3/64, 10/64, and 3/64 in the controls, the group fed benzo[a]pyrene 5 times/week, and the group fed benzo[a]pyrene every 9th day). In the same study, groups of 32 Sprague-Dawley rats/sex were administered 0.15 mg/kg benzo[a]pyrene by gavage in a 1.5% caffeine solution either every 9th day (Group 3), every 3rd day (Group 2), or 5 times/week (Group 1) until the animals were moribund or dead, resulting in average annual doses of 6, 18, or 39 mg/kg, respectively. Survival was adversely affected only in Group 3 (mean survival.time = 87 weeks versus 102 weeks in the controls). Treatment with benzo[a]pyrene significantly increased the proportion of animals with tumors of the forestomach, esophagus, and larynx (the combined tumor incidence was 3/64, 6/64, 13/64, 2664, and 14/64 for the untreated controls, the gavage controls, and Groups 3, 2, and 1, respectively).

Summary. These results indicate that benz[a]anthracene, benzo[a]pyrene, dibenz[a,h]anthracene, and possibly other PAHs are carcinogenic to rodents following oral exposure at high doses.

All reliable CELs in mice for acute- and intermediate-duration exposure are recorded in Table 2-2 and plotted in Figure 2-2.

2.2.3 Dermal Exposure

2.2.3.1 Death

No studies were located regarding death in humans or animals after dermal exposure to the 17 PAHs discussed in this profile.

2.2.3.2 Systemic Effects

No studies were located regarding respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, or ocular effects in humans or animals after dermal exposure to any of the 17 PAHs discussed in this profile. Other systemic effects observed after dermal exposure are discussed below.

The highest NOAEL values and all LOAEL values from each reliable study for each species and duration category are recorded in Table 2-3.

TABLE 2-3. Levels of Significant Exposure to Polycyclic Aromatic Hydrocarbons - Dermal

Species/ (Strain)	Exposure/ Duration/ Frequency/ (Specific Route)	System	NOAEL	LOAEL			Reference
				Less Serious		Serious	
ACUTE EXPOSURE							
Systemic							
Mouse (C57BL/6)	1-2 d 1 x/d	Dermal	0.05 M mg/c m2				Iwata et al. 1981 anthracene
Mouse (C57BL/6)	1-2 d 1 x/d	Dermal	0.001 M mg/c m2	0.005 M mg/cm 2	(induction of melanocytes)	0.025 M (substantial melanocytes)	Iwata et al. 1981 benzo[a]pyrene
Mouse (C34/HeN)	5 d 2 x/5d	Dermal		120 mg F	(contact hypersensitivity)		Klemme et al. 1987 benzo[a]pyrene
Mouse (C57BL/6)	1-2 d 1 x/d	Dermal		0.0125 M mg/cm 2	(slight increase melanocytes)		Iwata et al. 1981 chrysene
Mouse (C57BL/6)	1-2 d 1 x/d	Dermal	0.05 M mg/c m2				Iwata et al. 1981 fluoranthene
Mouse (C57BL/6)	1-2 d 1 x/d	Dermal	0.05 M mg/c m2				Iwata et al. 1981 fluorene
Mouse (C57BL/6)	1-2 d 1 x/d	Dermal	0.05 M mg/c m2				Iwata et al. 1981 pyrene
Immunological/Lymphoreticular							
Mouse (C34/HeN)	5 d 2 x/5d			120 F	(contact hypersensitivity)		Klemme et al. 1987 benzo[a]pyrene

TABLE 2-3. Levels of Significant Exposure to Polycyclic Aromatic Hydrocarbons - Dermal (continued)

Species/ (Strain)	Exposure/ Duration/ Frequency/ (Specific Route)	System	NOAEL	LOAEL Less Serious	LOAEL Serious	Reference
Cancer						
Mouse (CD-1)	20 d 10 x				10.1µg F [CEL: 35% (7/20) tumor incidence]	Weyand et al. 1993b benzo[b]fluoranthene
INTERMEDIATE EXPOSURE						
Immunological/Lymphoreticular						
Gn pig (Hartley)	2-3 wk 2 x			0.001% F (slight contact sensitivity)	1.0% F (contact sensitivity)	Old et al. 1963 benzo[a]pyrene
Cancer						
Mouse (C3H/HeJ)	6 mo 2 x/wk				0.05 mg M (CEL: 1/13 (8%) had a papilloma with coadministration of 0.0005 mg BaP)	Warshawshy et al. 1993 anthracene
Mouse (CD-1)	1 d 1 x/d, then 25 wk 3 d/wk (TPA)		0.09 F mg/kg		0.57 F mg/kg (CEL: 36% skin tumor incidence)	Levin et al. 1984 benz[a]anthracene
Mouse (SENCAR)	once then 23 wk 2 d/wk (TPA)				0.2 mg F (CEL: 6 papillomas/mouse)	Cavalieri et al. 1988b benzo[a]pyrene
Mouse (Swiss)	20 wk 2 x/wk 1 x/d				0.025 F mg (CEL: tumors in 90%)	Cavalieri et al. 1988b benzo[a]pyrene

TABLE 2-3. Levels of Significant Exposure to Polycyclic Aromatic Hydrocarbons - Dermal (continued)

Species/ (Strain)	Exposure/ Duration/ Frequency/ (Specific Route)	System	NOAEL	LOAEL			Reference
				Less Serious	Serious		
Mouse (Crl:CD-1)	20 d every other day				0.01 mg F	(CEL: 35% developed skin tumors)	LaVoie et al. 1993a benzo[b]fluoranthene
Mouse (CD-1)	20 d every other day				0.006 mg F	(CEL: 5% developed skin tumors)	LaVoie et al. 1993b benzo[j]fluoranthene
Mouse (C3H/HeJ)	6 mo 2 x/wk				0.05 mg M	(CEL: 1/15 (7%) had a papilloma from chrysene alone; 3/13 (23%) having papillomas or malignancies with coadministration of 0.0005 mg BaP)	Warshawshy et al. 1993 chrysene
Mouse (C3H/HeJ)	6 mo 2 x/wk				0.05 mg M	(CEL: 1/12 (8%) had papillomas with coadministration of 0.0005 mg BaP)	Warshawsky et al. 1993 fluoranthene
Mouse (CD-1)	20 d 1 x/2d, then 22 wks (TPA)				100 mg F	(CEL: 80% incidnece of tumors)	Rice et al. 1985a indeno(1,2,3-c,d)pyr
Mouse (Swiss)	12 mo 3 x/wk		50 F		100 µg F	(CEL: 6/20 had papillomas, 3/20 had carcinomas)	indeno(1,2,3-cd)pyre

TABLE 2-3. Levels of Significant Exposure to Polycyclic Aromatic Hydrocarbons - Dermal (continued)

Species/ (Strain)	Exposure/ Duration/ Frequency/ (Specific Route)	System	NOAEL	LOAEL			Reference
				Less Serious	Serious		
Mouse (C3H/HeJ)	6 mo 2 x/wk				0.05 mg M	(CEL: 3/13 (23%) had a tumor (papillomas and malignant) from the mixture alone; 8/17 (47%) with coadministration of 0.0005 mg BaP)	Warshawsky et al. 1993 mix
Mouse (C3H/HeJ)	6 mo 2 x/wk				0.05 mg M	(CEL: 1/12 (8%) had papillomas from phenanthrene alone; 1/17 (6%) had a malignant tumor with coadministration of 0.0005 mg BaP)	Warshawsky et al. 1993 phenanthrene
Mouse (C3H/HeJ)	6 mo 2 x/wk				0.05 mg M	(CEL: 1/13 (8%) had a papillomas from pyrene alone)	Warshawsky et al. 1993 pyrene
CHRONIC EXPOSURE							
Cancer							
Mouse (NMRI)	17-22 mo 2 d/wk 1 x/d				2 mg F	(CEL: 45% developed skin tumors)	Habs et al. 1984 benzo[a]pyrene
Mouse (C3H/HrJ)	99 wk 2 d/wk 1 x/d				12.5 µg M	(CEL: malignant tumors in 47/50)	Warshawsky and Barkley 1987 benzo[a]pyrene
Mouse (Swiss)	lifetime 3 d/wk 1 x/d				0.01% F	(CEL: papillomas in 5%)	Wynder and Hoffmann 1959b benzo[b]fluoranthene

TABLE 2-3. Levels of Significant Exposure to Polycyclic Aromatic Hydrocarbons - Dermal (continued)

Species/ (Strain)	Exposure/ Duration/ Frequency/ (Specific Route)	System	NOAEL	LOAEL		Reference
				Less Serious	Serious	
Mouse (NMRI)	19-20 mo 2 d/wk 1 x/d				15 µg F (CEL: skin carcinomas in 1/20)	Habs et al. 1984 mix

BaP = benzo(a)pyrene; CEL = cancer effect level; d = day(s); F = female; LOAEL = lowest-observed-adverse-effect level; M = male; mo = month(s); NOAEL = no-observed-adverse-effect level; TPA = tetradecanoyl phorbol acetate; wk = week(s); x = time(s)

Dermal Effects. Mixtures of carcinogenic PAHs cause skin disorders in human and animals; however, specific effects in humans of individual PAHs, except for benzo[a]pyrene, have not been reported. Mixtures of PAHs are also used to treat some skin disorders in humans. From these patients comes much of the data describing dermal effects of PAH exposure.

Regressive verrucae (i.e., warts) was reported following up to 120 dermal applications of 1% benzo[a]pyrene in benzene to human skin over 4 months (Cottini and Mazzone 1939). Although reversible and apparently benign, the changes were thought to represent neoplastic proliferation.

Adverse dermal effects have been noted in humans following intermediate-duration dermal exposure to benzo[a]pyrene in patients with the preexisting dermal conditions of pemphigus vulgaris (acute or chronic disease characterized by occurrence of successive crops of blisters) and xeroderma pigmentosum (a rare disease of the skin marked by disseminated pigment discolorations, ulcers, and cutaneous and muscular atrophy) (Cottini and Mazzone 1939). A 1% benzo[a]pyrene solution topically applied to patients with pemphigus resulted in local bullous eruptions characteristic of the disease. Patients with xeroderma pigmentosum exposed to 1% benzo[a]pyrene slightly longer than the pemphigus patients exhibited only pigmentary and slight verrucous effects. Similarly treated patients with preexisting active skin lesions due to squamous cell cancer showed a general improvement and/or retardation of the lesion. The severity of abnormal skin lesions appeared to be related to age; those in the lowest age range exhibited fewer and less-severe effects than those in the mid-range groups. No such age relationship of effects involving those patients with normal or preexisting skin lesions was noted.

Adverse dermal effects have also been ohserved in animals following both acute- and intermediate duration dermal exposure to various PAHs. For example, acute topical application of benzo[a]pyrene, benz[a]anthracene, or dibenz[a,h]anthracene applied to the shaved backs of Swiss mice were all reported to suppress sebaceous glands (Bock and Mund 1958). However, controls were not employed; therefore, it is not possible to determine if the effects seen were due to the solvent and/or the application procedures.

Benzo[a]pyrene was applied once weekly to the skin of female ICR/Harlan mice (43-50/group) at doses of 16, 32, or 64 μg per application for 29 weeks (Albert et al. 1991b). Cell cycle kinetics and

morphometrics were evaluated. Evidence of epidermal cytotoxicity and death followed by regeneration was seen in animals administered 64 μg benzo[a]pyrene beginning the first weeks of exposure and later in the lower dose groups. This evidence included dose-related epidermal thickening and vertical nuclei stacking, increased mitotic labeling (2-4-fold with increasing dose), increased incidence of pyknotic and dark cells, and a pronounced inflammatory response in the dermis. The increase in cell proliferation was accompanied by only a minor increase in the size of the epidermal cell population, indicating that the proliferation was a regenerative response.

An acute (96-hour) dermal application of anthracene to the backs of hairless mice followed by ultraviolet radiation exposure for 40 minutes resulted in enhanced dermal inflammation compared to mice exposed exclusively to ultraviolet radiation. However, this effect was reversed within 48 hours (Forbes et al. 1976). Anthracene thus potentiates the skin damage elicited by sunlight exposure and may be considered a photosensitizer in hairless mice.

In animals, dermal application of 1% benzo[a]pyrene to the skin of hairless mice resulted in epidermal cell growth alterations (Elgjo 1968). Increases were observed in mitotic rates, mitotic counts, and mitotic duration and the author suggested that these were indicative of a regenerative reaction. However, concurrent controls were not utilized. The authors concluded that the alterations in the kinetics of epidermal cell growth produced by benzo[a]pyrene were more sustained than after application of croton oil. The study is limited for drawing conclusions concerning the dermal toxicity of benzo[a]pyrene because experimental data were compared with historical controls only, no acetone control was evaluated, and the statistical significance of the increased values was not determined.

2.2.3.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological effects in humans following dermal exposure to the 17 PAHs discussed in this profile.

Benzo[a]pyrene can elicit an immune response when applied dermally to the skin of animals. In mice, acute application of 120 μg benzo[a]pyrene elicited an allergic contact hypersensitivity in C3H mice that was antigen specific (Klemme et al. 1987). Slight contact hypersensitivity was also observed in guinea pigs following two dermal applications of 0.001% benzo[a]pyrene given over a period of 2-3 weeks. This response was more severe at a dose of 1.0% benzo[a]pyrene (Old et al. 1963).

In addition to eliciting a contact hypersensitivity response, benzo[a]pyrene has been shown to suppress this response to other sensitizers. The effects of dermally applied benzo[a]pyrene (alone or following dermal pretreatment with the prostaglandin synthetase inhibitor, indomethacin) on contact hypersensitivity (cell-mediated immunity), and production of antibodies to dinitrophenol (DNP) (humoral immunity) were studied in male BALB/c mice treated for 6 weeks to 6 months (Andrews et al. 1991a). A group of mice treated with acetone served as controls. Benzo[a]pyrene alone caused a significant reduction ($p<0.01$) in the contact hypersensitivity response to dinitrofluorobenzene (DNFB) as measured by increases in ear thickness when compared to the vehicle controls. However, indomethacin pretreatment prevented the benzo[a]pyrene-induced contact hypersensitivity response. Benzo[a]pyrene also reduced antibody titres to DNP in treated mice. This suppressive effect on humoral immune function was not restored by pretreatment with indomethacin. These findings led the authors to conclude that the mechanism of benzo[a]pyrene-induced suppression of cell-mediated immunity involved prostaglandins, whereas benzo[a]pyrene-induced suppression of humoral immunity operated via a mechanism independent of prostaglandins. In a subsequent experiment, the effects of dermally applied benzo[a]pyrene (alone or following subcutaneous implantation of the prostaglandin synthetase inhibitor, indomethacin) on Langerhans cells and on skin prostaglandin (PGE_2) levels were studied in male BALB/c mice treated for 3 weeks (Andrews et al. 1991b). Langerhans cells are antigen-presenting cells involved in cell-mediated immunity in skin. A group of mice treated with indomethacin served as controls. Benzo[a]pyrene alone caused a significant increase in the number of skin Langerhans cells, but reduced the percentage of Langerhans cells with dendritic morphology. Skin PGE_2 levels were also significantly increased by benzo[a]pyrene. Indomethacin attenuated the increase in Langerhans cell number and the changes in their morphology, and increased PGE_2 levels, such that all of these parameters were similar to those measured in the control animals. Based on these results, the authors suggested that benzo[a]pyrene induces increases in skin PGE_2 that in turn alter Langerhans cell number and morphology such that the cell-mediated immune response to skin antigens is suppressed.

An earlier study also demonstrated that benzo[a]pyrene affects epidermal Langerhans cells and dermal immunological responses. Female BALB/c mice were administered dermal applications on the dorsal skin of 0.5% benzo[a]pyrene in acetone twice weekly for up to 6 months (Ruby et al. 1989). Animals treated with acetone served as controls. The density, area, perimeter, and morphology of epidermal Langerhans cells were evaluated, along with the contact hypersensitivity response to DNFB. Benzo[a]pyrene treatment caused an increase in the number of epidermal Langerhans cells (as

determined by Ia antigens and -glucuronidase) from week 2 to week 5 of treatment and after weeks 10 and 18. The area and perimeter of these cells were unaffected by benzo[a]pyrene treatment, but the morphology was altered in that the dendrites appeared shortened. The contact hypersensitivity response to DNFB was significantly reduced in the benzo[a]pyrene-treated mice from 4 to 24 weeks of treatment. The authors propose that benzo[a]pyrene alters Langerhans cell number and morphology such that the cell-mediated immune response to skin antigens is suppressed. Skin tumors appeared in 20% of the benzo[a]pyrene-treated mice after 18 weeks of treatment, and 35% of the mice had l-3 tumors after 24 weeks of treatment. The tumors were squamous papillomas (58%) and squamous cell carcinomas (42%). The changes in Langerhans cell number, distribution, and morphology coincided with the onset of tumors and other nonneoplastic skin lesions that were observed (epidermal hyperplasia and cellular atypia).

All reliable LOAELs from each reliable study for immunological effects for each species and duration category are recorded in Table 2-3.

No studies were located regarding the following health effects in humans or animals following dermal exposure to the 17 PAHs discussed in this profile:

2.2.3.4 Neurological Effects

2.2.3.5 Reproductive Effects

2.2.3.6 Developmental Effects

2.2.3.7 Genotoxic Effects

No studies were located regarding genotoxic effects in humans following dermal exposure to the 17 PAHs discussed in this profile. A single topical application of benzo[a]pyrene (0.5-500 μg/mouse) or chrysene (50-1,000 μg/mouse) to groups of HRA/Skh hairless mice (four mice/dose/group) resulted in significantly increased frequencies of micronucleated keratinocytes (He and Baker 1991). In the same study, micronuclei were not induced in the mouse skin cells following application of 2.5-2,500 μg/pyrene per mouse. Male SENCAR mice receiving two topical applications of 20 μg benzo[a]pyrene at 72-hour intervals exhibited increased DNA adduct formation in both epidermal and

lung tissue (Mukhtar et al. 1986). Following a single topical application, 100 μg benzo[b]fluoranthene, benzo[j]fluoranthene, benzo[k]fluoranthene, and indeno[1,2,3-c,d]pyrene were reported to bind to DNA in CD-l mouse skin (Weyand et al. 1987). The relative extent of binding was benzo[b]fluoranthene > benzo[j]fluoranthene > benzo[k]fluoranthene > indeno[1,2,3-c,d]pyrene. Covalent binding of chemicals to DNA can result in strand breaks and DNA damage, ultimately leading to mutations.

Benzo[a]pyrene (62.5 or 500 μg) was applied once to the shaved backs of male C57BL/6 mice (Bjelogrlic et al. 1994). Mice were killed at different time intervals after the treatment. DNA was isolated from the skin, purified, and analyzed for benzo[a]pyrene-7,8-diol-9,10-epoxide-DNA adducts. Skin was also evaluated for monoclonal antibody binding to mouse p53 protein, which has been shown to increase in response to DNA damage. Alterations in p53 are the most frequently observed mutations in human cancer. Benzo[a]pyrene-7,8-diol-9,10-epoxide-DNA adducts reached their maximum concentration 24 hours after the treatment, and decreased sharply within 1 week, regardless of the dose. An increase in p53 protein was seen only after treatment with 500 μg benzo[a]pyrene.

Benzo[j]fluoranthene, benzo[j]fluoranthene-4,5-diol, and benzo[j]fluoranthene-9,10-diol were applied to the shaved backs of CD-l mice and the DNA adducts were isolated and separated using multidimensional thin-layer chromatography (TLC) and reverse-phase high performance liquid chromatography (HPLC) (Weyand et al. 1993a). The highest level of adducts was observed with benzohlfluoranthene-4,5-diol, which resulted in the formation of 383 mol of DNA adducts/mg DNA. This level of DNA modification was more than 2 orders of magnitude greater than that observed with benzo[j]fluoranthene. In contrast, the major DNA adducts detected with benzo[j]fluoranthene-9,10-diol had chromatographic properties distinctly different than the adducts formed from either benzo[j]fluoranthene or B[j]F-4,5-diol. The adducts of the diols corresponded to DNA adducts produced *in vitro* from the respective diolepoxides. In a companion study, benzo[b]fluoranthene, benzo[b]fluoranthene-9,10 diol, 6-hydroxy-benzo[b]fluoranthene-9,10-diol, or 5-hydroxybenzo[b]fluoranthene-9,10-diol were applied to the shaved backs of CD-1 mice and the DNA adducts were isolated and separated using multidimensional TLC and reverse-phase HPLC (Weyand et al. 1993b). Benzo[b]fluoranthene formed one major adduct and 4 minor adducts. The DNA adducts formed from 5-hydroxybenzo[b]fluoranthene-9, l0 diol had identical retention to the major and one of the minor adducts of benzo[b]fluoranthene. These two adducts accounted for 58% of the modified nucleotides produced by benzo[b]fluoranthene application to mouse skin.

The DNA binding of manufactured gas plant residue (MGP) components in male B6C3Fl mice was investigated following topical administration (Weyand and Wu 1994). For topical exposure, male mice were treated with 10 mg MGP residue in 200 μL acetone, and sacrificed 24 hours later. Two mixtures of MGP residue were used: Mix of 3 combining equal amounts of samples from three different MGP plant sites, and Mix of 7 combining equal amounts of samples from seven different MGP plant sites, including those used in the Mix of 3. The mixtures contained pyrene, benz[a]anthracene, chrysene, benzo[b]fluorene, benzo[k]fluorene, benzo[a]pyrene, indeno[l,2,3-cd]pyrene, dibenz[a,h]anthracene, benzo[g,h,i]perylene. Data were presented in terms of pyrene. Animals were sacrificed 24 hours after treatment, and skin and lung were excised and DNA isolated. Chemical-DNA adduct formation was evaluated. Topical application MGP residue in acetone resulted in similar levels of DNA adduct in the skin for both the Mix of 3 and the Mix of 7. The total level of adducts detected in the lung after topical administration was identical to the response after dietary exposure, i.e., the Mix of 3 (1.4 mg/kg/day pyrene) produced adduct levels that were two times greater than the levels induced by the Mix of 7 (1.2 mg/kg/day pyrene). Other genotoxicity studies are discussed in Section 2.4.

2.2.3.8 Cancer

No studies were located that gave evidence of a direct association between human dermal exposure to individual PAHs and cancer induction. However, reports of skin tumors among individuals exposed to mixtures containing PAHs lend some qualitative support to their potential for carcinogenicity in humans. The earliest of these is the report by Pott (1775) of scrotal cancer among chimney sweeps. More recently, skin cancer among those dermally exposed to shale oils has been reported (Purde and Etlin 1980). However, these reports provide only qualitative suggestions pertaining to the human carcinogenic potential of all of the 17 PAHs discussed in this profile, or at least the compounds found in chimneys and shale oils, such as benzo[a]pyrene, chrysene, dibenz[a,h]anthracene, benz[a]anthracene, and benzo[b]fluoranthene. Limitations in these reports include no quantification of exposure to individual PAHs and concurrent exposure to other putative carcinogens in the mixtures.

It has been suggested that an increase in the number of skin melanocytes correlates with the sebaceous gland suppression index, and that the short-term melanocyte-activation test is useful for the detection of skin carcinogens and promoters. Some chemical carcinogens have been shown to induce melanogenesis in melanoblasts in the skin. Anthracene, benzo[a]pyrene, chrysene, fluoranthene,

fluorene, and pyrene were examined for their ability to induce melanocyte activation by topical application to the backs of mice for 1 or 2 consecutive days. Benzo[a]pyrene, an animal skin carcinogen, was a potent melanocyte inducer at doses of 20-100 μg/mouse (0.005-0.025 mg/cm^2) as demonstrated by an increase of up to 19 times over controls in the number of dopa-positive cells, whereas no effects were seen at 4 μg/mouse (0.001 mg/cm^2). Chrysene, a weak skin carcinogen (in animals), increased the number of dopa-positive cells to four times that of controls following an application of 50 μg/mouse (0.0125 mg/cm^2), while larger doses did not cause further increases in the numbers of these cells. Other PAHs such as anthracene, fluoranthene, fluorene, and pyrene (PAHs that are considered to be noncarcinogenic) produced no increases in the number of active melanocytes when applied at a dose of 200 μg/mouse (0.05 mg/cm^2) (Iwata et al. 1981).

Complete Carcinogenesis Studies. Studies in laboratory animals have demonstrated the ability of benz[a]anthracene, benzo[b]fluoranthene, benzo[j]fluoranthene, benzo[a]pyrene, chrysene, dibenz[a,h]anthracene, and indeno[l,2,3-c,d]pyrene to induce skin tumors (i.e., they are complete carcinogens) following intermediate dermal exposure. Anthracene, fluoranthene, fluorene, phenanthrene, and pyrene do not act as complete carcinogens. The data supporting these conclusions are discussed below by chemical. Only those studies considered adequate and reliable with respect to study design and adequacy of reporting are presented in Table 2-3.

Anthracene. Skin painting experiments were conducted on groups of 20 male C3H/HeJ mice (Warshawsky et al. 1993). Anthracene dissolved in toluene was applied to shaved skin twice weekly for six months at a dose of 0.05 mg. Tumor incidence was determined at the end of the study. For anthracene, administration alone produced tumors in 0 of 14 animals. With coadministration of 0.05 mg benzo[a]pyrene, 1 of 13 (8%) had a papilloma, with a mean latency period of 85 weeks. Anthracene was negative as a complete carcinogen following chronic dermal exposure (Habs et al. 1980). Swiss mice receiving 10% anthracene in acetone topically applied to their backs three times a week throughout their lifetime did not develop any skin tumors after 20 months (Wynder and Hoffmann 1959a).

Benz[a]anthracene. Benz[a]anthracene has been shown to cause skin tumors in mice following intermediate-duration dermal application. Graded concentrations of benz[a]anthracene in toluene or *n*-dodecane applied to the backs of mice for 50 weeks resulted in dose-related increases in tumor incidence (Bingham and Falk 1969). This response was enhanced when *n*-dodecane was the solvent

compared with toluene. Malignant tumors were observed at dose levels of about 0.02% benz[a]anthracene (0.15 mg/kg/day) and above when toluene was the solvent; however, when n-dodecane was the solvent, tumors were observed.at much lower concentrations of 0.0002% benz[a]anthracene (0.0015 mg/kg/day). The dose-response relationship reported in this study is extremely shallow (sublinear) over two orders of magnitude. A possible explanation for this is that the tumorigenic potency of certain PAHs is tempered by their cytotoxicity. Thus, the cytotoxic effects to epithelial cells may self-limit their potency as tumorigens. No solvent controls were included for comparison (Bingham and Falk 1969).

Intermediate-duration topical application of benz[a]anthracene to the backs of mice for 30 weeks resulted in a slightly elevated (2.6%) (but not statistically significant) skin tumor incidence. No definitive conclusions can be drawn from this study since only one dose was employed and no statistical analysis was performed.

Benzo[b]fluoranthene. A dose-response relationship for the dermal carcinogenicity of benzo[b]fluoranthene has been demonstrated over a one order-of-magnitude dose range in Swiss mice receiving (0.0l-0.5%) benzo[b]fluoranthene throughout their lifetime. Survival was also dose related. Although this study was designed as a long-term (chronic) bioassay, malignant tumors (90% carcinomas) appeared as early as 4 months in the high-dose group. Papillomas and carcinomas (65% and 85%, respectively) also appeared after 5 months in the mid-dose group. As a result, this study provides evidence that benzo[b]fluoranthene is carcinogenic following intermediate-duration exposure. The lowest dose at which benzo[b]fluoranthene elicited malignant tumors was 0.l%, which is approximately equal to a dose of 2.9 mg/kg received three times weekly, or an average daily dose of 1.2 mg/kg (Wynder and Hoffmann 1959b, see Table 2-3). In another chronic dermal study, benzo[b]fluoranthene produced a significant carcinogenic response of approximately one-third the potency of benzo[a]pyrene. The lowest dose at which tumors appeared was 3.4 μg benzo[b]fluoranthene; however, no distinction was made between papillomas and carcinomas (Habs et al. 1980).

Benzo[j]fluoranthene. Benzofilfluoranthene (0.1% or 0.5%) applied to the skin of female Swiss mice thrice weekly for life induced skin papillomas in 70% and 95% of the animals, respectively, and skin carcinomas in 105% and 95% of the animals, respectively, after 9 months of treatment (Wynder and Hoffmann 1959b).

No statistically significant increase in the incidence of skin tumors was noted in female NMRI mice dermally administered 3.4, 5.6, or 9.2 μg benzo[j]fluoranthene in acetone twice weekly for life (Habs et al. 1980). A total of 4 benzo[j]fluoranthene-treated mice developed skin tumors (application site sarcoma, papillomas, and a carcinoma).

Benzo[k]fluoranthene. Chronic dermal application of benzo[k]fluoranthene to Swiss mice resulted in no tumors, but skin papillomas were observed in 10% of animals when the concentration of benzo[k]fluoranthene was increased. Statistical analyses were not performed (Wynder and Hoffmann 1959b). In another study, no significant increase in tumor incidence was observed in NMRI mice painted with up to 9.2 μg of benzo[k]fluoranthene twice a week for a lifetime; no effect on mortality was noted (Habs et al. 1980).

Benzo[a]pyrene. Benzo[a]pyrene is a potent experimental skin carcinogen, and it is often used as a positive control in bioassays of other agents. Mixtures of PAHs that include benzo[a]pyrene such as coal tar were shown to be dermal carcinogens in animals as early as 1918 (Yamagiwa and Ichikawa 1918). In its role as a positive control, benzo[a]pyrene is usually administered at a single dose level, and thus quantitative evaluation of dose-response relationships is not possible.

Intermediate (19-20 weeks) topical application of a benzo[a]pyrene solution to the backs of mice resulted in a dose-related development of skin papillomas and squamous cell carcinomas (Cavalieri et al. 1988b, see Table 2-3; Shubik and Porta 1957). Benzo[a]pyrene was applied once weekly to the skin of female ICR/Harlan mice (43-50/group) at doses of 16, 32, or 64 μg for 29 weeks (Albert et al. 1991ba). Cell kinetics, morphometrics, and tumor formation were evaluated. Skin tumors were first apparent 12-14 weeks after the start of exposure in the 32- and 64-μg groups and after 18 weeks in the 16-μg group. The overwhelming majority of these tumors were benign. The average time of progression from benign papillomas to malignant carcinomas was 8.1 ± 4.5 weeks. Because there was good correspondence between the dose-response patterns for epidermal damage and the occurrence of skin tumors, and because tumors that initially appear as benign can be the result of tumor promoting agents that increase cell proliferation rates, the authors proposed that the tumors seen after benzo[a]pyrene treatment were the result of promotion related to benzo[a]pyrene-induced tissue damage. However, benign tumors can be formed as a result of genetic damage as well. Because benzo[a]pyrene causes genetic damage in addition to increased rates of cell proliferation, it is likely that genetic damage also played a role. Following a similar protocol in mice (once weekly dermal

applications of benzo[a]pyrene in acetone at doses of 16, 32, and 64 μg/mouse for 34 weeks), tumor development was reported to be best described by a dose-squared response. Quantitative data for tumor incidence were not presented (Albert et al. 1991b).

Carcinogenicity experiments on mouse skin were conducted with groups of 20 female CD-l mice. Benzo[a]pyrene (0.05 mg) dissolved in 50 mL toluene was applied to shaved skin of mice twice weekly for 6 months (Warshawsky et al. 1993). Tumor incidence was determined at the end of the study. Benzo[a]pyrene produced no tumors.

In mice, the tumorigenic dose of benzo[a]pyrene is influenced by the solvent used for delivery. Graded concentrations of benzo[a]pyrene dissolved in decalin or a solution of n-dodecane and decalin were topically administered to the backs of mice for 50 weeks (Bingham and Falk 1969). Use of the n-dodecane and decalin solvent mixture significantly enhanced the potency of benzo[a]pyrene at lower doses in comparison with decalin alone. Malignant tumors appeared in 21% of the animals at 0.00002% (0.0054 mg/kg/day) benzo[a]pyrene in dodecane and decalin solvent. In contrast, a 42% skin tumor incidence was not observed until 0.02% (4.8 mg/kg/day) benzo[a]pyrene in decalin alone was applied. The method of application was not specified, sample sizes were small and no decalin solvent controls were included; however, decalin is not considered to be carcinogenic. In this same study, intermediate (50 weeks) dermal application of benzo[a]pyrene dissolved in the co-carcinogens 1-dodecanol or 1-phenyldodecane produced skin tumors in animals exposed to 0.05% benzo[a]pyrene in either solvent. The tumor incidence varied depending on the solvent concentration; however, the latency period was reduced only when 1-dodecanol was the solvent (Bingham and Falk 1969).

Mice receiving 0.00l-0.01% of benzo[a]pyrene dermally applied to their backs throughout their lifetimes exhibited a dose-response relationship for skin tumors (Wynder and Hoffmann 1959a). A dose of 0.001% benzo[a]pyrene produced skin carcinoma and papilloma incidences of 4% and 45%, respectively. In another study conducted by Wynder and Hoffmann (1959b), higher concentrations of benzo[a]pyrene produced an 85% incidence of combined papillomas and carcinomas. These studies had a number of weaknesses, including no statistical treatment and no solvent control group. Dose quantification is difficult because of the method of application (Wynder and Hoffmann 1959a, 1959b). NMRI mice topically administered 2 μg benzo[a]pyrene throughout their lifetime also developed skin papillomas and carcinomas (45%) (Habs et al. 1984, see Table 2-3). CH3 mice administered a higher dose of 12.5 μg benzo[a]pyrene for 99 weeks exhibited malignant skin tumors (94%) (Warshawsky

and Barkley 1987, see Table 2-3). Increasing malignant carcinoma incidences in these dermal application studies can be correlated to increasing benzo[a]pyrene concentrations.

In mice, the tumorigenic dose of benzo[a]pyrene is dependent on the strain. For example, Habs et al. (1980) tested 1.7-4.6 μg benzo[a]pyrene (0.016-0.04 mg/kg/day) in order to determine its dose-response relationship as a carcinogen when topically applied to the backs of NMRI mice throughout their lifetimes. A clear-cut dose-response relationship was seen for benzo[a]pyrene and the induction of tumors. The lowest dose at which skin tumors appeared was 1.7 μg (0.016 mg/kg/day). This strain of NMRI mice also has a high (70%) background incidence rate of systemic tumors, so an evaluation of the effects of benzo[a]pyrene on any organ other than the site of administration was not possible:

Chrysene. Skin painting experiments with intermediate (6 months) dermal exposure with chrysene were conducted on groups of 20 male C3H/HeJ mice (Warshawsky et al. 1993). Chrysene (0.05 mg) dissolved in toluene was applied to shaved skin twice weekly for 6 months. Tumor incidence was determined at the end of the study. For chrysene, administration alone produced papillomas in 1 of 15 animals (7%), with a mean latency period of 81 weeks. With coadministration of 0.0005 mg benzo[a]pyrene, 3 of 13 (23%) had tumors (papillomas and malignant), with a mean latency period of 70 weeks.

Chrysene has elicited skin tumors in mice following chronic (68-82 weeks) dermal exposure. Topical application of a chrysene solution in *n*-dodecane/decalin to the skin of mice produced a significant increase in the carcinogenic potency of chrysene compared with the use of decalin alone; 26% and 63% of mice exhibited papillomas and carcinomas, respectively, at 49 weeks (Horton and Christian 1974). Because only one dose level was employed, no dose-response relationship can be inferred, and no solvent control was included. However, in other experiments decalin and *n*-dodecane have been shown to be noncarcinogenic in mice (Bingham and Falk 1969). An average dose of 1.2 mg/kg/day is the lowest dose of chrysene that has been found to elicit malignant tumors in laboratory animals.

In another chronic study, a higher concentration of chrysene applied dermally to the backs of Swiss mice for a lifetime also resulted in increased papilloma and carcinoma incidences (48% and 42%, respectively) compared to controls (Wynder and Hoffmann 1959a). Since only one dose was employed, no dose-response information can be inferred from this study.

Dibenz[a,h]anthracene: Dibenz[a,h]anthracene has also demonstrated a dose-response relationship for skin tumors over a two-orders-of-magnitude dose range following chronic exposure. Swiss mice received concentrations of 0.00l-0.1% dibenz[a,h]anthracene applied to their backs throughout their lifetimes and exhibited dose-related papilloma and carcinoma incidences at the site of application at the two lowest doses. A decreased tumor rate at the highest dose tested probably reflects dibenz[a,h]anthracene's toxicity and the resulting decreased survival observed. The lowest concentration at which dibenz[a,h]anthracene elicited tumors was 0.001% (40% incidence of papillomas and 40% incidence of carcinomas), which is approximately equal to a dose of 0.029 mg/kg (0.012 mg/kg/day) (Wynder and Hoffmann 1959a). In another chronic derrnal study of dibenz[a,h]anthracene, a dose-related increase in skin carcinoma formation was observed, as well as decreased survival time and tumor latency period (Van Duuren et al. 1967).

Groups of 50 female NMRI mice received dermal applications of dibenz[a,h]anthracene in acetone (total doses = 0, 136, 448, or 1,358 nmol) three times a week for a total of 112 weeks (Platt et al. 1990). Papillomas were observed in 6%, 8%, and 32% of the treated animals, respectively.

Fluoranthene. Skin painting experiments with intermediate-duration (6 months) dermal exposure were conducted on groups of 20 male C3H/HeJ mice (Warshawsky et al. 1993). Fluoranthene was dissolved in toluene and applied to shaved skin twice weekly for 6 months. Tumor incidence was determined at the end of the study. For fluoranthene, administration alone produced tumors in 0 of 15 animals. With coadministration of 0.0005 mg benzo[a]pyrene, 1 of 12 (8%) had papillomas, with a mean latency period of 95 weeks.

Chronic dermal application of up to 1% fluoranthene to the backs of mice did not induce skin tumors following a lifetime of application (Hoffmann et al. 1972; Horton and Christian 1974; Wynder and Hoffmann 1959a).

Fluorene. Fluorene has been reported to be negative as a complete carcinogen (dose not specified) (Kennaway 1924). This information was obtained from an old, secondary source and therefore, its reliability is not known.

Indeno[1,2,3-c,d]pyrene. Indeno[1,2,3-c,d]pyrene was applied to the skin of female Swiss mice three times weekly for 12 months in concentrations of 0.5% (500 μg/application), 0.l%, 0.05%, and 0.01%

(20 mice per group) using acetone as the solvent. A tumor dose-response with 7 papilloma-bearing mice and 5 carcinoma-bearing mice for 0.5%, 6 papilloma-bearing and 3 carcinoma-bearing mice for 0.l%, and no skin tumors for 0.05% and 0.01% solutions was observed (Hoffmann and Wynder 1966). Chronic dermal application of indeno[1,2,3-c,d]pyrene in dioxane to mice did not produce an increased incidence of skin tumors. Similarly, chronic topical application of up to 9.2 μg of indeno[l,2,3-c,d]pyrene in acetone to the backs of mice for a lifetime resulted in no tumor induction (Habs et al. 1980).

Phenanthrene. Phenanthrene tested negative as a complete carcinogen in a mouse study inadequately reported in an old secondary source (Kennaway 1924). Skin painting experiments with intermediate-duration (6 months) dermal exposure were conducted on groups of 20 male C3H/HeJ mice (Warshawsky et al. 1993). Phenanthrene dissolved in toluene was applied to shaved skin twice weekly for 6 months. Tumor incidence was determined at the end of the study. For phenanthrene, administration alone produced papillomas in 1 of 12 animals (8%), with a mean latency period of 100 weeks. With coadministration of 0.0005 mg benzo[a]pyrene, 1 of 17 (6%) had malignant tumors, with a mean latency period of 53 weeks.

Pyrene. Skin painting experiments with intermediate-duration (6 months) exposure were conducted on groups of 20 male C3H/HeJ mice. Pyrene dissolved in toluene was applied to shaved skin twice weekly for 6 months. Tumor incidence was determined at the end of the study. For pyrene, administration alone produced papillomas in 1 of 13 animals (8%), with a mean latency period of 96 weeks. With coadministration of 0.0005 mg benzo[a]pyrene, 0 of 13 animals had tumors.

Mice chronically administered a 10% pyrene solution throughout their lifetimes did not develop skin tumors (Wynder and Hoffmann 1959a). However, prolonged dermal exposure of mice to 0.5% pyrene in decalin/n-dodecane solvent produced a slightly elevated (15%) skin carcinoma incidence; the level of statistical significance was not provided (Horton and Christian 1974).

Mixtures. Chronic dermal exposure of NMRI mice to a tar condensate that contained several PAHs (pyrene, fluoranthene, chrysene, benz[a]anthracene, benzo[a]pyrene, indeno[1,2,3-c,d]pyrene, benzo[g,h,i]perylene) in addition to other compounds produced a carcinogenic effect as evidenced by an increase in the incidence of skin papillomas and carcinomas (Habs et al. 1984). Because of the

presence of other compounds in the tar condensate, the carcinogenic effect cannot be definitely attributed to the PAHs present in the mixture.

Skin painting experiments of a mixture of anthracene, chrysene, fluoranthene, phenanthrene, and pyrene were conducted on groups of 20 male C3H/HeJ mice (Warshawsky et al. 1993). Compounds dissolved in toluene were applied to shaved skin twice weekly for 6 months. Tumor incidence was determined at the end of the study. Treatment included solutions of a mixture of the five noncarcinogenic PAHs at 0.05 mg, or the same compounds in solution with 0.0005 mg benzo[a]pyrene, a dose known to be noncarcinogenic in a similar study design. For the mixture of the 5 PAHs at 0.05 mg each, administration alone produced papillomas and malignant tumors in 3 of 13 animals (23%), with a mean latency period of 73 weeks. With coadministration of benzo[a]pyrene, 8 of 17 (47%) had tumors (papillomas and malignant), with a mean latency period of 66 weeks.

Initiation-Promotion Studies. Carcinogenesis has been demonstrated to be a multistage process in the cells of certain animal tissues, including skin, lung, liver, and bladder. This process is believed to occur in human tumorigenesis as well. The PAHs have been studied extensively for their ability to act as tumor initiators and/or promoters. Following is a brief discussion, by chemical, of the results of the initiation-promotion studies performed with 13 of the 17 PAHs discussed in this profile. Only those studies considered adequate and reliable are presented in Table 2-3.

The difficulty inherent in extrapolating initiation-promotion experiments to human exposure precludes their being used as the basis for human cancer effect levels. Since PAHs occur in complex mixtures of chemicals that may include tumor promoters, their activity as initiating agents is noteworthy. Thus, it is possible that humans dermally exposed to PAHs that are initiating agents, concomitantly with other chemicals that may be active as tumor promoters (including other PAHs) found at nearby hazardous waste sites, may have an increased risk of skin cancer.

Anthracene. Anthracene has been found to be inactive as an initiating agent under a dermal initiation/promotion protocol using tetradecanoyl phorbol acetate (TPA) as the promoter (LaVoie et al. 1983a).

Benz[a]anthracene. Benz[a]anthracene has been observed to be a tumor initiator in an intermediate-duration dermal study. CD-l mice topically administered 2.5 μmol (0.57 mg) benz[a]anthracene

followed by promotion with TPA (for 25 weeks) exhibited an increased skin tumor incidence (36%) as compared to controls (Levin et al. 1984, see Table 2-3).

Benzo[a]pyrene. Benzo[a]pyrene is active as a tumor initiator using initiation/promotion protocols. Topical application of a single initiating dose of benzo[a]pyrene to the backs of mice followed by promotion with TPA or croton oil resulted in an 80-92% incidence of skin papillomas (Cavalieri et al. 1988b, see Table 2-3). Ten doses of benzo[a]pyrene (0.1 mg/dose) topically applied to the backs of Swiss mice followed by promotion with croton oil (for 20 weeks) also resulted in the development of skin tumors (Hoffmann et al. 1972).

In a dermal initiation/promotion assay, groups of 24 female SENCAR mice were administered a single dermal application of benzo[a]pyrene at doses ranging from 4 to 300 nmol (initiating dose), followed 7 days later by twice weekly applications of the promoter TPA, for a total of 24 weeks. Benzo[a]pyrene was active as a skin tumor initiator; the number of tumors per tumor bearing mouse, the percentage of tumor bearing mice, and the number of tumors per mouse were all significantly greater than in acetone controls and increased in a dose-related manner at doses ≥20 nmol (Cavalieri et al. 1991).

In a similar experiment, 24 8-week-old female SENCAR mice were treated dermally with 0.0002 mg (1 nmol) of benzo[a]pyrene in acetone on a shaved portion of dorsal skin (Higginbotham et al. 1993). One week later, tumor promotion was begun with TPA twice weekly for 27 weeks. The number of skin tumors was charted weekly and the mice were killed after experimental weeks. Complete necropsies were performed and tissues were fixed in formalin. Benzo[a]pyrene-treated mice had no skin tumors.

Benzo[e]pyrene. Benzo[e]pyrene is inactive as a skin tumor initiator in mouse skin (Slaga et al. 1980a).

Benzo[b]fluoranthene. The ability of benzo[b]fluoranthene to initiate skin tumor formation has been demonstrated using a standard initiation/promotion protocol with either croton oil or phorbol myristate acetate as a tumor promoter (Amin et al. 1985a; LaVoie et al. 1982). In another study, dermal applications of initiation doses of benzo[b]fluoranthene (10-100 μg) followed by TPA (for 20 weeks)

to the backs of CD-l mice elicited a dose-related skin tumor incidence, predominantly consisting of squamous cell papillomas (LaVoie et al. 1982).

In a dermal initiation/promotion assay, groups of 20 female CD-l mice were administered 10 dermal applications of benzo[b]fluoranthene at total doses of 0, 1, and 4 umol (initiating dose), followed 10 days later by thrice weekly applications of the promoter TPA for a total of 20 weeks (Weyand et al. 1991). Benzo[b]fluoranthene was active as a skin tumor initiator; the number of tumors per tumor-bearing mouse (8.5) and the percentage of tumor-bearing mice (100%) were significantly greater than in acetone controls and were increased in a dose-related manner.

Tumor initiation experiments were conducted with groups of 20 female CD-1 mice. Benzo[b]fluoranthene was applied to the shaved backs of the mice every other day using a total of 10 subdoses (Weyand et al. 1993b). Total doses were 10.1, 30.3, and 100.9 μg in acetone. Negative control mice were treated with acetone. Ten days after the last dose, promotion was begun by applying 2.5 μg TPA thrice weekly for 20 weeks. Tumors were counted weekly. Benzo[b]fluoranthene produced a 35%, 90%, and 95% incidence of tumor-bearing mice with 0.45, 3.70, and 8.65 tumors per mouse for the low, mid and high doses, respectively. No distinction was made between papillomas and carcinomas (Weyand et al. 1993b). Groups of 20 female Crl:CD-1 mice were dermally exposed to a total of 0, 0.01, 0.03, or 0.1 mg of benzo[b]fluoranthene in acetone applied every other day for 20 days in 10 subdoses (LaVoie et al. 1993a). Negative control mice were treated with acetone only. Ten days after the last application of acetone or hydrocarbon, tumor promotion was begun by applying 2.5 μg TPA in acetone three times weekly for 20 weeks. Tumor incidence was recorded after 20 weeks of promotion. The study was repeated for the 0.03 and 0.1 mg doses. Of the mice receiving 0.01 mg benzo[b]fluoranthene, 35% had developed tumors, with an average of 0.45 tumors/mouse. For the 0.03 mg dose, 70-90% of the mice developed tumors with an average of 1.4-3.7 tumors per mouse, whereas the 0.1 mg dose caused 95% of the mice to develop and average of 7.1-8.6 tumors. Of the animals treated with acetone only, 5-15% developed skin tumors. In this study, tumors were not identified as papillomas or carcinomas.

Benzo[j]fluoranthene. Benzo[j]fluoranthene has also been demonstrated to be a tumor initiator in mice, although it is not as potent as benzo[b]fluoranthene. Benzo[j]fluoranthene, however, is more potent than benzo[k]fluoranthene. Mice receiving initiating doses of benzo[j]fluoranthene (30-1,000 μg) followed by TPA promotion exhibited a dose-related increase in tumor incidence

(LaVoie et al. 1982, 1993a; Weyand et al. 1992). Groups of 20 female Crl:CD-1 mice were dermally exposed to a total of 0, 0.006, 0.012, 0.025, or 0.25 mg of benzojjlfluoranthene in acetone applied every other day for 20 days in 10 subdoses (LaVoie et al. 1993b). Negative control mice were treated with acetone only. Ten days after the last application of acetone or hydrocarbon, tumor promotion was begun by applying 2.5 μg TPA in acetone three times weekly for 20 weeks. Tumor incidence was recorded after 20 weeks of promotion. Five percent of the mice receiving 0.006 mg benzo[j]fluoranthene had developed tumors, with an average of 0.4 tumors per mouse. For the 0.012 mg dose, 10% of the mice developed tumors with an average of 0.4 tumors per mouse; 0.025 mg PAH caused 45% of the mice to develop tumors at an average of 0.65 tumors per mouse, whereas the 0.25 mg dose caused 95% of the mice to develop an average of 8.7 tumors. The control animals developed no tumors.

Benzo[k]fluoranthene. Benzo[k]fluoranthene has also been demonstrated to be a tumor initiator in mice, although it too, is not as potent as benzo[b]fluoranthene. Mice receiving initiating doses of benzo[k]fluoranthene (30-1,000 μg) followed by TPA promotion exhibited a dose-related increase in ‘tumor incidence (LaVoie et al. 1982).

Benzo[g,h,i]perylene. Benzo[g,h,i]perylene has been shown to be inactive as an initiating agent when applied at a total dose of 0.25 mg/animal and negative as a complete carcinogen when a 1% solution was applied thrice weekly for 12 months (IARC 1983).

Chrysene. Chrysene is a tumor initiator in classic initiation/promotion bioassays on mouse skin using croton oil or phorbol myristate acetate as promoting agents (Slaga et al. 1980a; Wood et al. 1979a). Initiating doses of chrysene followed by promotion with TPA or croton resin induced a dose-related papilloma incidence in mice (Slaga et al. 1980a; Wood et al. 1979a).

Dibenz[a,h]anthracene. Dibenz[a,h]anthracene has also demonstrated tumor-initiating activity using a standard initiation/promotion protocol (Slaga et al. 1980a). Dibenz[a,h]anthracene has been reported to initiate skin development in a dose-response relationship at doses as low as 0.028 μg followed by promotion with TPA (for 25 weeks) (Buening et al. 1979a).

In a dermal initiation/promotion assay, groups of 50 female NMRI mice were administered a single dermal application of dibenz[a,h]anthracene at doses of 0, 300, or 600 nmol (initiating dose) followed

7 days later by twice weekly applications of the promoter, TPA, for a total of 24 weeks (Platt et al. 1990). Dibenz[a,h]anthracene was active as a skin tumor initiator only at the highest dose tested; 93% of the animals administered 600 nmol dibenz[a,h]anthracene developed skin tumors by 24 weeks.

Fluoranthene. Fluoranthene did not exhibit initiating activity in Swiss mice topically administered 10 doses followed by promotion with croton oil (for 20 weeks) (Hoffmann et al. 1972).

Indeno[1,2,3-c,d]pyrene. A pronounced dose-response relationship has been exhibited by indeno[l,2,3-c,d]pyrene in an initiation-promotion bioassay when TPA was employed as the promoting agent, although it was not as potent an initiator as benzo[b]fluoranthene (Rice et al. 1985a). In another study, 2.83 tumors/mouse were noted after a total initiating dose of 1.0 mg indeno[1,2,3-c,d]pyrene and promotion with TPA for 20 weeks (Rice et al. 1986).

The skin tumor initiating activity of indeno[1,2,3-c,d]pyrene and several of its metabolites generated *in* viva in mouse skin was tested in female Crl:CD/l mice (Rice et al. 1986). Initiating doses of indeno[1,2,3-c,d]pyrene or the metabolites were applied every other day to the shaved skin of groups of 25 mice for a total of 10 doses, which was followed 10 days later by thrice weekly applications of the tumor promotor, TPA, for 20 weeks. None of the metabolites were as active in inducing skin tumors as the parent compound (2.83 tumors/mouse as compared to 0.48-1.68 tumors/mouse at 20 weeks). These findings led the authors to conclude that the principal ultimate mutagenic metabolite, indeno[l,2,3-c,d]pyrene-1,2-oxide, is not the ultimate carcinogenic metabolite of indeno[l,2,3-c,d]pyrene.

Phenanthrene. Phenanthrene was ineffective as an initiator in various mouse strains (LaVoie et al. 1981b; Salaman and Roe 1956; Wood et al. 1979a). CD-1 mice topically administered a single dose of 10 μmol phenanthrene followed by a promoter were observed to have a papilloma incidence 2-4 times that of background; however, the incidences were not statistically significant in comparison to controls because of the small number of animals tested and the high spontaneous tumor incidence (Wood et al. 1979a).

Pyrene. Pyrene has been shown to be inactive as an initiating agent (Salaman and Roe 1956; Van Duuren and Goldschmidt 1976).

Mixtures. Co-administration of pyrene and benzo[a]pyrene to the backs of ICR/Ha mice has produced an enhancement of benzo[a]pyrene tumorigenicity (Van Duuren and Goldschmidt 1976). There is evidence that benzo[g,h,i]perylene is a co-carcinogen with benzo[a]pyrene when both are applied simultaneously to the skin of Swiss mice (Van Duuren et al. 1973). Dermal pretreatment with 100 μg pyrene substantially enhanced benzo[a]pyrene tumor initiation in CD-1 mice, while 100 μg fluoranthene produced a marginal enhancement (Slaga et al. 1979).

2.3 TOXICOKINETICS

Occupational studies provide evidence that inhaled PAHs are absorbed by humans. Animal studies also show that pulmonary absorption of benzo[a]pyrene occurs and may be influenced by carrier particles and solubility of the vehicle; however, the extent of absorption is not known. Absorption of benzo[a]pyrene following ingestion is low in humans, while oral absorption in animals varies among the PAH compounds depending on the lipophilicity. Oral absorption increases with more lipophilic compounds or in the presence of oils in the gastrointestinal tract. Percutaneous absorption of PAHs appears to be rapid for both humans and animals, but the extent of absorption is variable among these compounds and may be affected by the vehicle used for administration. Therefore, absorption of PAHs following inhalation, oral, or dermal exposure may be affected by vehicle of administration.

There was no information available on the distribution of PAHs in humans. PAHs appear to be widely distributed in tissues of animals following oral and inhalation exposure; peak tissue concentrations occurred earlier with higher exposure levels. Placental transfer of PAHs appears to be limited, and therefore, fetal levels are not as high as maternal levels.

Metabolism of PAHs occurs in all tissues and involves several possible pathways. Metabolism of PAHs has been studied extensively *in vitro* and *in vivo.* The metabolism products include epoxide intermediates, dihydrodiols, phenols, quinones, and their various combinations. The phenols, quinones, and dihydrodiols can all be conjugated to glucuronides and sulfate esters; the quinones also form glutathione conjugates.

Quantitative data on the excretion of PAHs in humans are lacking. In general, feces is the major elimination route of PAHs in animals following inhalation exposure. Excretion of benzo[a]pyrene appears to be high following low-level exposure in rats but low in dogs and monkeys. PAHs are

eliminated to a large extent within 2 days following low- and high-level oral exposure in rats. Following dermal exposure, elimination of PAHs occurs rapidly in the urine and feces of guinea pigs and rats.

Absorption of inhaled PAHs appears to occur through the mucous lining of bronchi, while ingested PAHs are taken up by the gastrointestinal tract in fat-soluble compounds. Percutaneous absorption is through passive diffusion. The mechanism of action of most PAHs involves covalent binding to DNA by PAH metabolites. The bay region diol epoxide intermediates of PAHs are currently considered to be the ultimate carcinogen for alternant PAHs. Once the reactive bay region epoxide is formed, it may covalently bind to DNA and other cellular macromolecules and presumably initiate mutagenesis and carcinogenesis.

2.3.1 Absorption

2.3.1.1 Inhalation Exposure

Absorption of PAHs in humans following inhalation exposure can be inferred from the presence of urinary metabolites of PAHs in workers exposed to these compounds in an aluminum plant (Becher and Bjorseth 1983). The high concentration of PAHs in the occupational setting did not correspond to the amount of PAHs deposited, metabolized, and excreted in the urine in this study. The authors suggested that PAHs adsorbed to airborne particulate matter may not be bioavailable and that the dose-uptake relationship may not be linear over the entire PAH concentration range.

Twelve workers from a coke plant participated in an intensive skin monitoring program combined with personal air sampling and biological monitoring during 5 consecutive 8-hour shifts (Van Rooij et al. 1993b). The mean concentration of total pyrene in the breathing zone air of the 12 workers ranged from 0.1 to 5.4 μg/m^3. The mean respiratory uptake of pyrene varied between 0.5 and 32.2 μg/day. Based on the estimates of the dermal and respiratory pyrene uptake, it is concluded that an average of 75% of the total absorbed amount of pyrene enters the body through the skin. The total excreted amount of urinary 1-hydroxypyrene as a result of exposure to PAHs during the five consecutive work shifts varied between 36 and 239 nmol. Analysis indicated that dermal absorption was most important in contributing to 1-hydroxypyrene excretion. Of the total dose absorbed by both routes combined,

13-49% is excreted as 1-hydroxypyrene. Variation in excretion is influenced by smoking habits, and consumption of alcohol (see Section 2.3.1.3).

Eleven healthy male smokers and 11 male smokers with lung cancer between the ages of 30-60 years, with a smoking history of 15-25 cigarettes per day for over 10 years were involved in a study (Likhachev et al. 1993). Urinary excretion of benzo[a]pyrene-7,8-diol and 3-hydroxybenzo[a]pyrene was determined. Both benzo[a]pyrene metabolites were detected in the urine, but quantities of 3-hydroxybenzo[a]pyrene were very low. The level of benzo[a]pyrene-7,8-diol in the urine varied considerably both in healthy smokers and smokers with lung cancer. However, the average value of this metabolite in the urine of healthy smokers was significantly higher than in the urine of lung cancer patients who smoked (1.06 mg/kg/day versus 0.56 mg/kg/day).

Animal studies on inhalation absorption of PAHs are limited to benzo[a]pyrene exposure. Rapid absorption was evident following inhalation exposure of low and high levels of benzo[a]pyrene to rats. Acute and intermediate-duration exposure to 4.8 mg/m^3 [^{14}C]-benzo[a]pyrene by nose-only inhalation in rats resulted in elevated levels of radioactivity in tissues and excreta within 3 hours of exposure (Wolff et al. 1989c). High levels of radioactivity were detected in the gastrointestinal tract, which may be due to biliary excretion or mucocilliary clearance of benzo[a]pyrene from the upper respiratory tract. Intratracheal administration of 0.001 mg/kg [^{3}H]-benzo[a]pyrene to rats also resulted in rapid absorption through the lungs. Radioactivity in the liver reached a maximum of 21% of the administered dose within 10 minutes of instillation (Weyand and Bevan 1986, 1988). Presence of radioactivity in other tissues and the bile was also indicative of its absorption in rats. Similar results were also seen in guinea pigs and hamsters following intratracheal exposure to benzo[a]pyrene (Weyand and Bevan 1986, 1987b, 1988).

Pregnant Wistar rats were exposed head-only to 200, 350, 500, 650, or 800 mg/m^3 of [^{14}C]-benzo[a]pyrene aerosol for 95 minutes on gestational day 17 (Withey et al. 1993a). Animals were killed immediately or 6 hours postexposure. Concentration of benzo[a]pyrene and metabolites in maternal blood sampled immediately after exposure were elevated 10-fold over the 4-fold increase in dose. At 6 hours postdosing, the increase was still approximately 10-fold, although the actual concentrations were 2-7-fold less than at 0 hours. Concentrations of benzo[a]pyrene and metabolites in fetal blood sampled immediately after exposure were elevated 5-fold over the 4-fold increase in exposure concentrations. Fetal tissues sampled 6 hours post-dosing had a 9-fold increase in

benzo[a]pyrene and metabolite concentration over the dose range, due to lower concentration in the 200-650 mg/m^3 dose groups at 6 hours compared to 0 hours. Fetal concentrations were 2-10-fold less than maternal concentrations. Benzo[a]pyrene concentrations in blood, lung, liver, and fetal tissues were significantly decreased from 0 to 6 hours postexposure while levels in fat tissue increased. For benzo[a]pyrene sampled immediately postdosing, lung > blood > liver > kidney > fat > fetus. For total metabolites sampled immediately postdosing, lung > blood > liver > kidney > fetus > fat. For benzo[a]pyrene sampled 6 hours postdosing, fat > lung > kidney > liver > blood > fetus. For total metabolites sampled 6 hours postdosing, lung = fat > kidney > liver = blood > fetus.

Twenty male Fisher 344 DuCrj rats were divided into two groups and exposed to diesel exhaust containing 0.151 mg/m^3 pyrene or HEPA-filtered air for 8 weeks, 5 days/week, 7 hours/day (Kanoh et al. 1993). At 2, 4, and 8 weeks during the exposure, the rats from each group were put into a metabolic cage and their urine was collected for 24 hours. Urinary levels of 1-hydroxypyrene in the rats of the exposure group increased remarkably over those of the control group, reaching 2.4 times as much by the end of the 2nd week, and 5.6 times by the 4th and 8th weeks.

Inhalation absorption of benzo[a]pyrene may be affected by the size of particles on which benzo[a]pyrene is adsorbed. The elimination of benzo[a]pyrene from the lungs was studied following intratracheal administration of pure benzo[a]pyrene crystals or benzo[a]pyrene coated on carbon particles in two size ranges (0.5-1.0 μm and 15-30 μm) (Creasia et al. 1976). Fifty percent of the pure benzo[a]pyrene crystals was cleared from the lungs within 1.5 hours and >95% cleared within 24 hours, while only 50% of the benzo[a]pyrene adsorbed to the small carbon particles cleared within 36 hours. Elution of benzo[a]pyrene was even slower with the larger particle size (approximately 4-5 days). These results indicate that the bioavailability of benzo[a]pyrene is altered by the particle size of the carrier. The initial lung deposition of [^{3}H]-benzo[a]pyrene adsorbed onto gallium oxide (Ga_2O_3) particles was 4.9 μg of which 3.1% remained after 30 minutes (Sun et al. 1982). A control study, conducted without the Ga_2O_3 particles at a concentration of 1 mg/m^3, found that 8.2 μg was inhaled, of which 0.9% remained in lungs after 30 minutes. The excretion of hydrocarbon was monitored for over 2 weeks at which time nearly all the initial lung burden was recovered in the excreta, indicating complete absorption of the instilled hydrocarbon. Significant differences in the clearance of benzo[a]pyrene coated with Ga_2O_3 and pure benzo[a]pyrene suggested that a substantial amount of benzo[a]pyrene/Ga_2O_3 particles was removed from the lungs by mucocilliary clearance and subsequent ingestion.

The above results corroborate findings in an *in vitro* experiment by Gerde and Scholander (1989) who developed a model of the bronchial lining layer. These investigators concluded that the release rate of PAHs from carrier particles is the rate-determining step in the transport of PAHs from these particles to the bronchial epithelium.

The absorption of benzo[a]pyrene may also be affected by the solubility of the vehicle used in administration. Approximately 70% of benzo[a]pyrene administered with triethylene glycol was excreted 6 hours following intratracheal instillation (Bevan and Ulman 1991). Excretion rates of benzo[a]pyrene were only 58.4% and 56.2% with ethyl laurate and tricaprylin, respectively, within a 6-hour period. The small volume of benzo[a]pyrene instilled is probably deposited in the bronchial region which allows more water-soluble materials (triethylene glycol) to pass the mucous layer lining than water-insoluble compounds (ethyl laurate and tricaprylin) (Bevan and Ulman 1991).

Nasal instillation of [^{3}H]-benzo[a]pyrene (0.13 mg/kg) to hamsters resulted in the metabolism of [^{3}H]-benzo[a]pyrene in the nasal cavity (Dahl et al. 1985). A large fraction of the metabolites was recovered from the epithelial surface, indicating that benzo[a]pyrene was first absorbed in the mucosa, metabolized, and returned to the mucus. Monkeys and dogs received nasal instillation of [^{14}C]-benzo[a]pyrene at doses of 0.16-0.21 mg/kg (Petridou-Fischer et al. 1988). Radiolabeled metabolites were detected in the nasal cavity, but little or no activity was detected in the blood and excreta of either species during the 48 hours after exposure. These results indicate that absorption of benzo[a]pyrene and/or its metabolites was poor or very slow following nasal instillation in monkeys and dogs.

2.3.1.2 Oral Exposure

There is evidence suggesting that benzopyrene is orally absorbed in humans (Buckley and Lioy 1992; Hecht et al. 1979). Following ingestion of diets containing very low levels of benzo[a]pyrene, the metabolite, 1-hydroxypyrene, was detected in the urine (Buckley and Lioy 1992). No quantitative data on the excretion of the benzo[a]pyrene were provided. The concentration of benzo[a]pyrene in human feces was examined in eight volunteers who ingested broiled meat that contained approximately 9 µg of benzo[a]pyrene (Hecht et al. 1979). The feces of these individuals did not contain detectable levels of benzo[a]pyrene (<0.1 µg/person), which is similar to what was seen following consumption of

control meat that contained undetectable amounts of benzo[a]pyrene by these same volunteers, suggesting that most of the ingested benzo[a]pyrene was absorbed.

Oral absorption of benzo[a]pyrene in rats is incomplete and may be influenced by the presence of oils and fat in the gastrointestinal tract. Oral absorption of benzo[a]pyrene was estimated to be 40%, with a bioavailability of 7.8-11.5%, in Sprague-Dawley rats infused intraduodenally to a total dose of approximately 0.0005 mg/kg for 90 minutes (Foth et al. 1988a). Nearly 80% of a gavage dose of 0.0527 mg/kg [^{14}C]benzo[a]pyrene in peanut oil was detected in the excreta of rats 48 hours after exposure; however, some of the recovered radioactivity may never have been absorbed by the alimentary tract of the rats, but may have passed into the excreta in the peanut oil (Hecht et al. 1979). Radioactivity found in the liver, lungs, kidneys, and testis following a low dose of [^{3}H]-benzo[a]pyrene to Sprague-Dawley rats provides supporting evidence of oral absorption (Yamazaki and Kakiuchi 1989; Yamazaki et al. 1987). The extent of oral absorption in rats is enhanced when benzo[a]pyrene is solubilized in a vehicle (triolein, soybean oils, high-fat diet) that is readily absorbed following low- and high-dose levels (Kawamura et al. 1988; O'Neill et al. 1991).

Oral absorption of benzo[a]pyrene was estimated to be 38-58% following dietary or gavage exposure to high levels in rats (Chang 1943). Anthracene was absorbed to a slightly higher extent (53-74%) than benzo[a]pyrene in rats while phenanthracene was poorly absorbed (4-7%) (Chang 1943). However, the data were limited because an inadequate number of rats was used and study details were lacking.

In general, the oral absorption of chrysene, dibenzanthracene, and pyrene was high following exposure to high doses in rats (Chang 1943; Grimmer et al. 1988; Withey et al. 1991). Following dietary or gavage administration of chrysene in rats, 64-87% of the dose was excreted in the feces (Chang 1943). Recovery of chrysene in excreta of Wistar rats was 74% four days after a single gavage dose of 22 mg/kg chrysene in corn oil (Grimmer et al. 1988). Administration of dibenz[a,h]anthracene in the diet (250 mg) or by stomach tube (200 mg) resulted in more than 90% of the dose being excreted in the feces of white rats (Chang 1943). As with chrysene, absorption of dibenz[a,h]anthracene could not be quantified. Male Wistar rats administered 2-15 mg/kg of [^{14}C]-pyrene recovered 68-92% of the dose in the excreta by 6 days postexposure (Withey et al. 1991). Bioavailability of pyrene and its metabolites was 65-84% over a period of 8 hours following administration.

Blood levels of fluoranthene, pyrene, and benz[a]anthracene after oral administration were examined in rats (Lipniak and Brandys 1993). Fluoranthene, pyrene, or benz[a]anthracene in Tween 80/isotonic saline, was administered orally to rats at a dose of 20 mg/kg. Blood levels after administration indicated that peak concentrations of the three compounds were reached at 1-2 hours after administration. The peak concentration of fluoranthene (≈30 mg/cm^3) was twice as high as that of pyrene, and 5 times higher than benz[a]anthracene.

The effect of diet matrix (gel or powder) on urinary excretion of 1-hydroxypyrene and hydrocarbon binding to DNA was investigated in mice (Wu et al. 1994). Female mice were fed a gel or powder diet containing manufactured gas plant (MGP) residue (coal tar) at 0.1% or 0.3% for 15 days, or the corresponding control diet. Two mixtures of MGP residue were used: Mix of 3 combining equal amounts of samples from three different MGP plant sites, and Mix of 7 combining equal amounts of samples from seven different MGP plant sites, including those used in the Mix of 3. The mixtures contained pyrene, benz[a]anthracene, chrysene, benzo[b]fluorene, benzo[k]fluorene, benzo[a]pyrene, indeno[1,2,3-cd]pyrene, dibenz[a,h]anthracene, benzo[g,h,i]perylene. Data were presented in terms of pyrene consumed and 1-hydroxypyrene excreted. Urine was collected on the first, seventh, and fourteenth day of diet administration. 1-Hydroxypyrene levels in the urine were determined using HPLC and fluorescence. Diet matrix had little effect on the bioavailability of the PAHs.

The intestinal absorption of PAHs is highly dependent on the presence of bile (Rahman et al. 1986). To study the role of bile in the intestinal absorption of PAHs, conscious rats with bile duct and duodenal catheters were given [^{3}H]-benzo[a]pyrene, phenanthrene, anthracene, 2,6-dimethylnaphthalene (DMN), and 7,12-dimethylbenz[a]anthracene (DMBA) with or without exogenous bile. The efficiency of PAH absorption was estimated from the cumulative recovery of radioactivity in the bile and urine over 24 hours. The efficiencies of absorption without bile (as a percentage of absorption with bile) were benzo[a]pyrene, 22.9%; phenanthrene, 96.7%; anthracene, 70.8%; DMN, 91.6%; DMBA, 43.4%. Absorption of the four- or five-membered rings (DMBA and benzo[a]pyrene) was strongly dependent on the presence of bile in the intestinal lumen. The absorption of the tricyclic PAHs (phenanthrene and anthracene) differed with respect to their dependency on bile for efficient absorption. This difference correlated with a difference in water solubility, with anthracene being 18 times less water-soluble than phenanthrene. Those products with low water solubility are dependent on the creation of an intermediate phase of the products of lipolysis and bile salts (Rahman et al. 1986). These reactions occur during the normal process of lipid digestion and absorption in the intestine.

2.3.1.3 Dermal Exposure

Application of 2% crude coal tar to the skin of humans for 8-hour periods on 2 consecutive days yielded evidence of PAH absorption (Storer et al. 1984). Phenanthrene, anthracene, pyrene, and fluoranthene were detected in the blood, but benzo[a]pyrene (which is present in coal tar) was not detected. This difference was attributed to differences in percutaneous absorption, rapid tissue deposition after absorption, or metabolic conjugation with rapid urine excretion. In another study, coal tar ointment was applied to skin of volunteers at various sites (Van Rooij et al. 1993a). The surface disappearance of PAH and the excretion of urinary 1-hydroxy-pyrene were used as parameters for dermal absorption. Surface disappearance measurements showed low but significant differences in dermal PAH absorption between anatomical sites: shoulder > forehead, forearm, groin > ankle, hand (palmar site). An *in vitro* study using human skin revealed that the extent of permeation across viable human skin after 24 hours was estimated to be 3% of the total applied radioactivity from [^{14}C]-benzo[a]pyrene (10 µg/cm^2) (Kao et al. 1985). Using human cadaver skin, it was shown that 23.7±9.7% of the applied benzo[a]pyrene penetrated into the skin (Wester et al. 1990). These results suggest that substantial metabolism and/or binding of benzo[a]pyrene takes place in viable human skin which limits the amount of PAH available to penetrate the skin into the systemic circulation.

Twelve workers from a coke plant participated in an intensive skin monitoring program combined with personal air sampling and biological monitoring during 5 consecutive 8-hour shifts (Van Rooij et al. 1993b). Measurements on exposure pads at six skin sites (jaw/neck, shoulder, upper arm, wrist, groin, ankle) showed that mean total skin contamination of the 12 workers ranged between 21 and 166 µg pyrene per day. The dermal uptake of pyrene ranged between 4 and 34 µg/day, which was about 20% of the pyrene contamination on the skin. The mean concentration of total pyrene in the breathing zone air of the 12 workers ranged from 0.1 to 5.4 µg/m^3. The mean respiratory uptake of pyrene varied between 0.5 and 32.2 µg/day. Based on the estimates of the dermal and respiratory pyrene uptake, it is concluded that an average of 75% of the total absorbed amount of pyrene enters the body through the skin. The total excreted amount of urinary 1-hydroxy-pyrene as a result of exposure to PAHs during the 5 consecutive work shifts varied between 36 and 239 nmol. Analysis indicated that dermal absorption was most important in contributing to 1-hydroxy-pyrene excretion. Of the total dose absorbed by both routes combined, 13-49% is excreted as 1-hydroxy-pyrene. Variation in excretion is influenced by smoking habits, and consumption of alcohol.

Percutaneous absorption of [^{14}C]-benzo[a]pyrene in mice, rats, monkeys, and guinea pigs is rapid and high (Ng et al. 1992; Sanders et al. 1986; Wester et al. 1990; Yang et al. 1989). A single dose of 7 μg/cm^2 [^{14}C]-benzo[a]pyrene in acetone was applied to a 4-cm^2 area of the dorsal skin of female hairless guinea pigs for 24 hours (Ng et al. 1992). Approximately 73% of the administered dose was absorbed dermally by 7 days postexposure; most of the dose was absorbed by day 3. The skin wash at 24 hours of exposure contained about 10.6% of dose (Ng et al. 1992). *In vitro* absorption of benzo[a]pyrene through guinea pig skin demonstrated similar results; 67% absorption in a 24-hour exposure (Ng et al. 1992). Seven days after exposure to 125 μg/cm^2 benzo[a]pyrene, 80% of the total recovered radioactivity was eliminated in the feces of mice (Sanders et al. 1986). The site of application still retained 7% of the recovered radioactivity after 7 days. However, the area of application was not covered to prevent animals from licking the test material which may have lead to ingestion of benzo[a]pyrene.

Groups of 12 male Wistar rats were dosed with 2, 6, or 15 mg/kg of [^{14}C]-pyrene applied to 4 cm^2 of a shaved area of the mid-back (Withey et al. 1993b). Three animals in each dose group were killed at 1,2,4, and 6 days postdosing, and the brain, lungs, heart, liver, spleen, kidneys, testes, muscle, and perirenal fat were removed and analyzed for pyrene and [^{14}C]-pyrene equivalents. Blood, urine, and feces, as well as the skin from the application site were also analyzed. The rate of uptake from the skin was rapid (t1/2 = 0.5-0.8 d) relative to rate processes for the other organs, and about 50% of the applied dose was excreted over the 6 days of the study. Levels of pyrene were highest in the liver, kidneys, and fat. Levels of metabolites were also high in the lung.

Dermal absorption of benzo[a]pyrene in rats and monkeys may be affected by the vehicle of administration (Wester et al. 1990; Yang et al. 1989). Following application of 10 ppm benzo[a]pyrene on the skin of rhesus monkeys, an average absorption of 51 ± 22% was reported with acetone vehicle and 13.2 ± .4% with soil; however, absorption data were based on radioactivity recovered in urine only, and not in feces (Wester et al. 1990). The great variation in the absorption with the acetone vehicle limits these results. This may be related, in part, to the dependence on monitoring radioactivity recovered in urine only as opposed to monitoring radioactivity recovered in urine and feces. Disappearance of the applied dose from the application site was 40% at 24 hours following administration (Wester et al. 1990). Sprague-Dawley rats absorbed 4-5 times more of a 1 ppm dose of benzo[a]pyrene when it was applied dermally alone, compared to a soil-sorbed crude oil mixture (Yang et al. 1989). The greater lipophilicity of the crude oil alone probably increased the

rate of dermal uptake of the test material, since the authors determined that absorption was dependent on the monolayer of soil in contact with the skin, which is comparable to the contact between the skin and oil. Therefore, the soil binding of the PAHs may have slowed absorption. However, no quantitative data were available.

Female Sprague-Dawley rats were exposed dermally to [^{3}H]-benzo[a]pyrene (1 ppm) containing petroleum crude oil alone or in fortified soil matrix for 4 days (Yang et al. 1989). Recovery of radioactivity was 35.3% of the dose applied in oil, as follows: urine (5.3% of dose), feces (27.5%), and tissues (2.5%) 96 hours after beginning of exposure. Recovery was 9.2% of applied dose with benzo[a]pyrene from petroleum crude-fortified soil; recoveries in urine, feces, and tissues were 1.9%, 5.8%, and 1.5%, respectively, at 96 hours. Benzo[a]pyrene (10 ppm) with acetone vehicle or in soil was applied to a 12 cm^{2} area of abdominal skin of female rhesus monkeys for 24 hours (Wester et al. 1990). Urine contained 5l ± 22% of the dose with acetone vehicle and 13.2 ± 3.4% with soil.

The percutaneous absorption of [^{14}C]-anthracene (9.3 µg/cm^{2}) was 52.3% in rats, estimated from radioactivity in urine, feces, and tissues over a 6-day period (Yang et al. 1986). Over time, the permeation of anthracene significantly decreased suggesting that anthracene was dermally absorbed in a dose-dependent manner. Diffusion of anthracene through the skin (stratum corneum) depended on the amount of anthracene on the skin's surface.

When 6.25 µg/cm^{2} [^{14}C]-phenanthrene and pyrene was applied to guinea pigs, dermal absorption was 80% and 94%, respectively (Ng et al. 1991, 1992). *In vitro* absorption of phenanthrene and pyrene in guinea pig skin was about 79-89% and 70%, respectively (Ng et al. 1991, 1992).

Monitoring the removal of compounds from the epidermis is indicative of measuring the compound's dermal absorption. The disappearance of radiolabeled benzo[a]pyrene and its metabolites from the epidermis was monophasic, following first order kinetics with a half-life of approximately 2 hours (Melikian et al. 1987). Recovery of the radiolabel was 99-100% throughout the period of the experiment (8 hours), indicating that volatilization of benzo[a]pyrene from the skin was not a confounding factor (Melikan et al. 1987). In contrast, removal of one of its metabolites, 7,8-dihydroxy-9,10-epoxy-7,8,9,l0-tetrahydrobenzo[a]pyrene (anti-BPDE), from the epidermis was biphasic. The second, slower phase of removal suggested that the stratum comeum, the outermost layer of skin which consists of several layers of inactive, keratinized cells surrounded by extracellular

lipids, may act as a reservoir that can retain and slowly release topically applied lipophilic substances such as benzo[a]pyrene but is penetrated rapidly by more polar compounds such as anti-BPDE.

2.3.2 Distribution

2.3.2.1 Inhalation Exposure

No studies were located regarding the distribution of PAHs in humans following inhalation exposure. In general, tissue distribution of benzo[a]pyrene following inhalation exposure is qualitatively similar for different species (Bevan and Weyand 1988; Weyand and Bevan 1986, 1987a, 1988; Wolff et al. 1989c). Highest radioactivity was distributed to the cecum, small intestine, trachea, kidneys, and stomach in rats following a 3-hour or 4-week inhalation exposure to 4.8 mg/m^3 [^{14}C]-benzo[a]pyrene (Wolff et al. 1989c). The lungs and liver of rats contained 2.7% and 4.6% of the recovered dose 6 hours after intratracheal administration of 0.001 mg/kg [^{3}H]-benzo[a]pyrene (Bevan and Weyand 1988). [^{3}H]-Benzo[a]pyrene intratracheally administered to rats demonstrated that the highest fractions were distributed to the lung, liver, kidney, gastrointestinal tract, and carcass (Weyand and Bevan 1986, 1987a, 1988). The concentration of benzo[a]pyrene and its metabolites in the intestine increased with time, suggesting the occurrence of biliary excretion and enterohepatic recirculation. Tissue distribution of radioactivity was qualitatively similar in guinea pigs and hamsters (Weyand and Bevan 1987b). Mice that received 2.5 mg/kg benzo[a]pyrene intratracheally also experienced a similar tissue distribution, but Schnizlein et al. (1987) noted that as the lung burden of benzo[a]pyrene diminished, radioactivity continued to increase in the lung-associated lymph nodes for 6 days. This accumulation may eventually affect humoral immunity.

The distribution pattern of benzo[a]pyrene was not significantly affected following aerosol exposure with or without Ga_2O_3 particles (Sun et al. 1982). However, significant differences in the levels of benzo[a]pyrene delivered to the different tissues did exist. Maximum levels were achieved in the liver, esophagus, small intestine, and blood at 30 minutes following exposure. At 12 hours, maximum levels were seen in the lower gastrointestinal tract. Higher tissue levels of hydrocarbon resulted from absorption of benzo[a]pyrene-Ga_2O_3 particles. Inhaled benzo[a]pyrene adsorbed on insoluble Ga_2O_3 particles was cleared predominantly by mucocilliary transport and ingestion. This latter mechanism of

absorption led to the increased levels and longer retention times of benzo[a]pyrene in the stomach, liver, and kidneys (Sun et al. 1982).

Pregnant Wistar rats inhaled head-only high levels of [^{14}C]-pyrene aerosol on gestational day 17 (Withey et al. 1992). Concentrations of pyrene and metabolites in maternal and fetal blood were elevated 8-fold with a fourfold increase in exposure concentrations. However, pyrene levels in fetal blood were about 10 times lower than maternal blood immediately after exposure. In general, radioactivity increased in fat but decreased in blood, lungs, and liver tissues from 0 to 6 hours postexposure. There was only a small increase in the concentration of radioactivity in fetuses over the whole exposure range compared to maternal levels, suggesting placental transfer of pyrene and its metabolites are limited or that metabolism in fetal tissues is limited.

In a similar study, pregnant Wistar rats were exposed head-only to 200, 350, 500, 650, or 800 mg/m^3 of [^{14}C]-benzo[a]pyrene aerosol for 95 minutes on gestational day 17 (Withey et al. 1993a). Animals were killed immediately or 6 hours postexposure. Concentration of benzo[a]pyrene and metabolites in maternal blood sampled immediately after exposure were elevated 10-fold over the 4-fold increase in dose. At 6 hours postdosing, the increase was still approximately 10-fold, although the actual concentrations were 2-7-fold less than at 0 hours. Concentrations of benzo[a]pyrene and metabolites in fetal blood sampled immediately after exposure were elevated 5-fold over the 4-fold increase in exposure concentrations. Fetal tissues sampled 6 hours post-dosing had a 9-fold increase in benzo[a]pyrene and metabolite concentration over the dose range, due to lower concentration in the 200-650 mg/m^3 dose groups at 6 hours compared to 0 hours. Fetal concentrations were 2-10-fold less than maternal concentrations. Benzo[a]pyrene concentrations in blood, lung, liver, and fetal tissues were significantly decreased from 0 to 6 hours postexposure while levels in fat tissue increased. For benzo[a]pyrene sampled immediately postdosing, lung > blood > liver > kidney > fat > fetus. For total metabolites sampled immediately postdosing, lung > blood > liver > kidney > fetus > fat. For benzo[a]pyrene sampled 6 hours postdosing, fat > lung > kidney > liver > blood > fetus. For total metabolites sampled 6 hours postdosing, lung = fat > kidney > liver = blood > fetus.

2.3.2.2 Oral Exposure

No studies were located regarding the distribution of PAHs in humans following oral exposure.

Tissue levels of benzo[a]pyrene in Sprague-Dawley rats were highest 2-8 days after initial exposure to multiple doses of 0.0005 mg [^{3}H]-benzo[a]pyrene (Yamazaki and Kakiuchi 1989). The highest radioactivities were found in the kidney and testis. [^{3}H]-Benzo[a]pyrene distributed to the protein fractions of the liver, lung, and kidney (Yamazaki et al. 1987). The radioactivity in the protein fractions of these tissues increased gradually over time. In contrast, the radioactivity in the lipid fractions of these tissues accounted for 70% of the administered dose at 3 hours but decreased rapidly with time. The nucleic acid fraction maintained approximately 10% of the total radioactivity throughout the experiment. The increase in protein binding of radioactivity associated with benzo[a]pyrene and its metabolites, and the persistence of the radioactivity associated with the protein fractions, suggests that protein binding may allow benzo[a]pyrene and its metabolites to accumulate in certain tissues, thus increasing the likelihood of cytotoxicity, mutagenicity, or carcinogenicity of benzo[a]pyrene and its metabolites in these organs. In addition, these organs have low metabolic activity while the liver has a high detoxification potential and can facilitate the excretion of these toxic products (Yamazaki et al. 1987).

Single oral doses of 12 mg/kg [^{14}C]-benzo[a]pyrene were administered by gavage to pregnant NMRI:Han mice on gestational days 11, 12, 13, or 18 (Neubert and Tapken 1988). Distribution of radioactivity was measured at 6, 24, and 48 hours after exposure. Maternal and embryo levels were highest with exposure on gestational day 11. The difference in radioactivity between maternal and embryo liver tissues increased when exposure occurred at later gestation. In another experiment, mice were exposed to 24 mg/kg benzo[a]pyrene for 3 consecutive days during early (gestational days 9-l1) or late gestation (days 15-17) (Neubert and Tapken 1988). Maternal tissue levels were not much different from those observed following the administration of single doses. After multiple dose administration, elimination appeared to be faster in maternal tissues, but slower in embryonic tissues. Placental levels were always higher than those in embryonic tissue. Results suggest that benzo[a]pyrene does not cross the placental barrier readily and, therefore, that levels in embryonic tissues of mice never reach levels found in maternal tissues.

In general, orally absorbed benz[a]anthracene, chrysene, and pyrene were rapidly and widely distributed in the rat (Bartosek et al. 1984; Withey et al. 1991). Maximum concentrations of benz[a]anthracene and chrysene in perfused tissues, like the liver, blood, and brain, were achieved within l-2 hours after administration of high doses (76 and 152 mg/kg) (Bartosek et al. 1984). Maximum levels in lesser perfused tissues, like adipose and mammary tissue, were reached in

3-4 hours. In male Wistar rats receiving a gavage dose of 2-15 mg/kg of [^{14}C]-pyrene, the fat had the highest tissue levels of radioactivity, followed by kidney, liver, and lungs (Withey et al. 1991).

Orally absorbed dibenz[a,h]anthracene in rats was also widely distributed to several tissues (Daniel et al. 1967). However, maximum tissue concentrations were not reached until 10 hours after administration. Highest tissue concentrations were in the liver and kidneys, followed by adrenal glands, ovaries, blood, and fat. Soon after administration, large quantities of dibenz[a,h]anthracene were found in the liver and kidneys. The elimination rate from these organs was rapid. At 3-4 days after administration, dibenz[a,h]anthracene was distributed only in the adrenal glands, ovaries, and fat.

The permeability of the placenta to dimethylbenz[a]anthracene, benzo[a]pyrene, and 3-methylcholanthrene (MC) was compared by Shendrikova and Aleksandrov (1974). Pregnant rats received the PAH orally in sunflower oil at a dose of 200 mg/kg on the 21st day of pregnancy. Within 30 minutes after administration of dimethylbenz[a]anthracene, trace amount of the compound could be detected in the fetus. Maximum levels (1.53-1.6 μg/g) were reached 2-3 hours after administration. Only trace amounts were detected in the fetus at 5 hours after administration. Concentration profiles in the liver and placenta were similar to those seen in the fetus. Benzo[a]pyrene was detected in the fetus at 2.77 μg/g. MC was only present in trace amounts. Concentration differences in the fetus among the various PAHs appeared to be highly dependent on the gastrointestinal absorption of the compound. The difference in fetal concentration of the PAHs did not reflect their ability to permeate the placenta.

2.3.2.3 Dermal Exposure

No studies were located regarding the distribution of PAHs in humans following dermal exposure. Evidence regarding the distribution of PAHs in animals following dermal exposure is limited. Although PAHs can readily penetrate the skin, there are few data on distribution to tissues. In one published study on this subject, only 1.3% of an applied dose of [^{14}C]-anthracene (9.3 μg/cm^2) was detected in tissues, primarily liver and kidneys, of rats 6 days after administration (Yang et al. 1986). Groups of 12 male Wistar rats were dosed with 2, 6, or 15 mg/kg of [^{14}C]-pyrene applied to 4 cm^2 of a shaved area of the mid back (Withey et al. 1993b). Three animals in each dose group were killed at

1, 2, 4, and 6 days postdosing, and the brain, lungs, heart, liver, spleen, kidneys, testes, muscle, and perirenal fat were removed and analyzed for pyrene and [^{14}C]-pyrene equivalents. Blood, urine, feces, as well as the skin from the application site were also analyzed. The rate of uptake from the skin was rapid (t1/2 = 0.5-0.8 d) relative to rate processes for the other organs, and about 50% of the applied dose was excreted over the 6 days of the study. Levels of pyrene were highest in the liver, kidneys, and fat. Levels of metabolites were also high in the lung.

2.3.3 Metabolism

The lipophilicity of PAHs enables them to readily penetrate cellular membranes and remain in the body indefinitely. However, the metabolism of PAHs renders them more water-soluble and more excretable. Metabolism of PAHs occurs in all tissues. The metabolic process involves several possible pathways with varying degrees of enzyme activities. The activities and affinities of the enzymes in a given tissue determine which metabolic route will prevail.

The metabolism of PAHs has been studied extensively *in vitro* and *in vivo.* The most commonly used system is the rat liver microsomal fraction, although other species are also used. Cells and cultured tissues from human and other animals have also significantly contributed to the elucidation of the PAH metabolic scheme.

The structural similarity of PAHs contributes to the similarities that exist in their biotransformation. Benzo[a]pyrene metabolism has been extensively reviewed and will be used as a model for PAH metabolism. In the many microsomal, cell, and cultured tissue preparations that have been examined, the metabolic profiles are qualitatively similar. However, there are differences in the relative levels and rates of formation of specific metabolites among tissues and cell preparations used from various animal species and strains. These differences are susceptible to change as a result of pretreatment of the animals with either inducers or inhibitors of particular enzymes. Furthermore, it is known that the metabolism of alternant PAHs (such as benzo[a]pyrene, benz[a]anthracene, chrysene, and dibenz[a,h]anthracene) differs from nonalternant PAHs (such as benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[j]fluoranthene, and indeno[1,2,3-c,d]pyrene) (see Section 2.2). Therefore, the metabolism of benzo[b]fluoranthene will also be discussed as a model for nonalternant PAH metabolism.

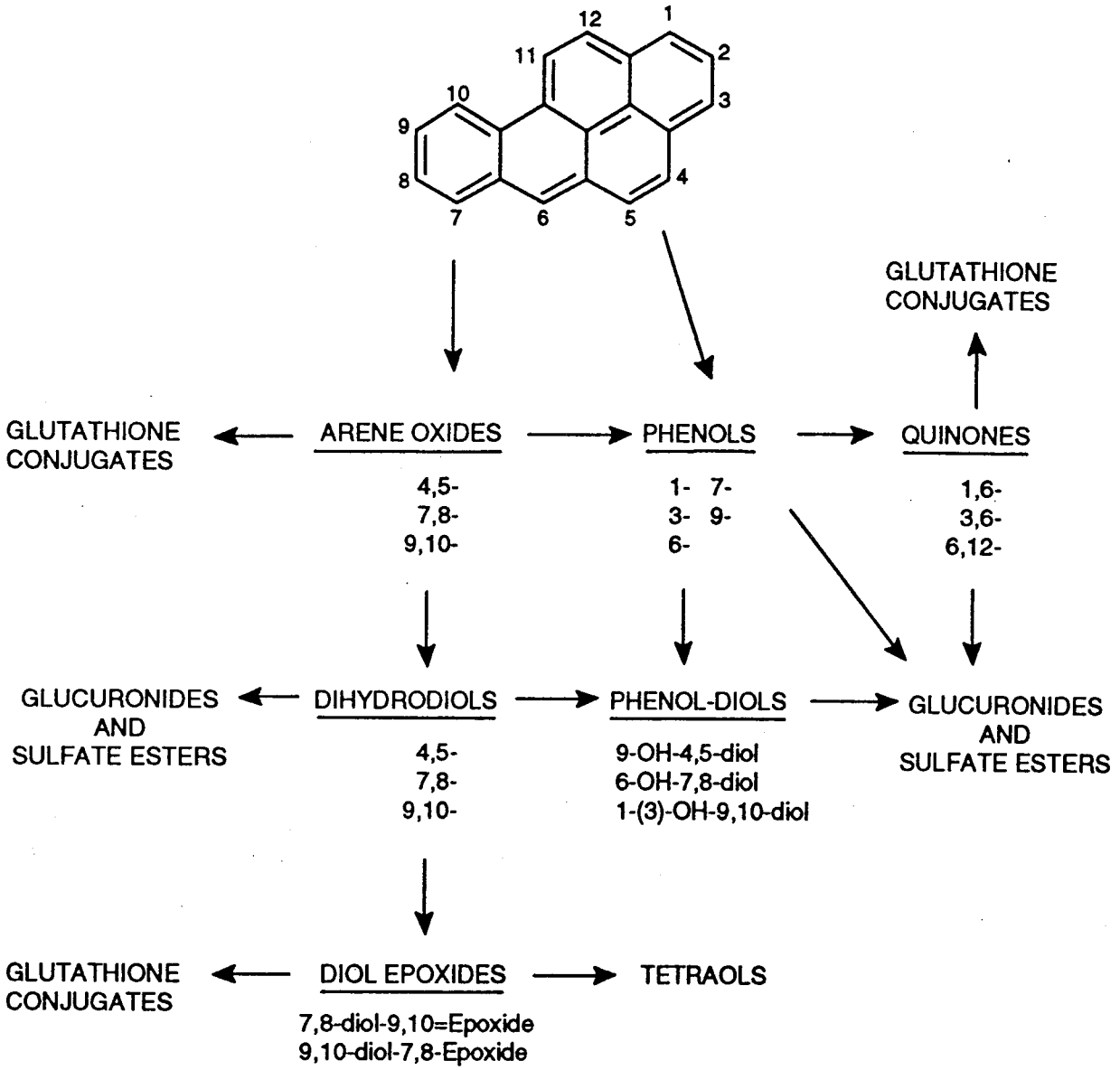
The metabolism of benzo[a]pyrene is summarized in Figure 2-3. Benzo[a]pyrene is metabolized initially by the microsomal cytochrome P-450 systems to several arene oxides. Once formed, these arene oxides may rearrange spontaneously to phenols, undergo hydration to the corresponding trans-dihydrodiols in a reaction catalyzed by microsomal epoxide hydrolase, or react covalently with glutathione, either spontaneously or in a reaction catalyzed by cytosolic glutathione-S-transferases (IARC 1983). Phenols may also be formed by the P-450 system by direct oxygen insertion, although unequivocal proof for this mechanism is lacking. 6-Hydroxybenzo[a]pyrene is further oxidized either spontaneously or metabolically to the 1,6-, 3,6-, or 6,12-quinones. This phenol is also a presumed intermediate in the oxidation of benzo[a]pyrene to the three quinones catalyzed by prostaglandin endoperoxide synthetase (Panthanickal and Marnett 1981). Evidence exists for the further oxidative metabolism to two additional phenols. 3-Hydroxybenzopyrene is metabolized to the 3,6-quinone and 9-hydroxy-benzo[a]pyrene is further oxidized to the K-region 4,5-oxide, which is hydrated to the corresponding 4,5-dihydrodiol (4,5,9-triol). The phenols, quinones, and dihydrodiols can all be conjugated to glucuronides and sulfate esters; the quinones also form glutathione conjugates (Agarwal et al. 1991; IARC 1983).

In addition to being conjugated, the dihydrodiols undergo further oxidative metabolism. The cytochrome P-450 system metabolizes benzo[a]pyrene-4,5-dihydrodiol to a number of uncharacterized metabolites, while the 9,10-dihydrodiol is metabolized predominantly to its l- and/or 3-phenol derivative with only minor quantities of a 9,10-diol-7,8-epoxide being formed. In contrast to the 9,10-diol, benzopyrene-7,8-diol is metabolized to a 7,8-dihydrodiol-9,10-epoxide, and phenol-diol formation is a relatively minor pathway. The diol epoxides can be conjugated with glutathione either spontaneously or by a glutathione-S-transferase catalyzed reaction. They may also hydrolyze spontaneously to tetrols (Hall and Grover 1988).

The route by which PAHs and other xenobiotics enter the body may determine their fate and organ specificity. For example, an inhaled compound may bypass the first-pass effect of the liver and reach peripheral tissues in concentrations higher than one would see after oral exposures. Enzyme activities among tissues are variable.

Benzo[a]pyrene was metabolized *in vitro* by human bronchial epithelial and lung tissue to the 9,10-dihydrodiol, 7,8-dihydrodiol, and small quantities of the 4,5-dihydrodiol and 3-hydroxybenzo[a]pyrene, all of which are extractable into ethyl acetate (Autrup et al. 1978; Cohen et

FIGURE 2-3. Proposed Metabolic Scheme for Benzo(a)pyrene.



al. 1976; Kiefer et al. 1988). These metabolites also conjugated with glutathione and sulfates, but none conjugated with glucuronide. The rate of formation of the dihydrodiols was greater in the bronchial epithelium than in the lung (Autrup et al. 1978; Cohen et al. 1976). This may render some areas of the respiratory tract more sensitive to the effects of carcinogens. One principal difference seen in human lung was the generation of a major ethyl acetate-soluble metabolite that was identified as the sulfate conjugate of 3-hydroxybenzo[a]pyrene, benzo[a]pyrene-3-yl-hydrogen sulfate. This sulfate is very lipid soluble and, thus, would not be readily excreted in the urine (Cohen et al. 1976). Activation of benzo[a]pyrene has also been detected in human fetal esophageal cell culture (Chakradeo et al. 1993).

Intratracheal instillation of benzo[a]pyrene to rats resulted in quinones constituting the highest concentration of metabolites in the lung and the liver within 5 minutes after instillation (Weyand and Bevan 1986, 1988). An *in vitro* study with rat lung demonstrated that the lung tissue has a high capacity to form quinones originating from oxidation at the six position of benzo[a]pyrene to form quinones and subsequently to water-soluble products. Ozone exposure resulted in an increase in the metabolism of benzo[a]pyrene metabolites with the greatest increase observed in the formation of metabolites generated by oxidation at the six position. The proposed retention of quinones following ozone exposure might lead to cytotoxicity associated with superoxide-anion generation by quinone-quinol redox-cycling. However, the high levels of benzo[a]pyrene used in this *in vitro* study may not relate to what occurs *in vivo*. Metabolism of benzo[a]pyrene at carbon six was higher at a lower dose than at the higher dose. Therefore, quinone production and detoxification may represent a major pathway of lung PAH detoxification *in vivo* (Basett et al. 1988).

Approximately 50% of the benzo[a]pyrene that was intratracheally instilled in hamsters was metabolized in the nose (Dahl et al. 1985). The metabolite produced in the hamster nose included tetrols, the 4,5-, 7,8-, and 9,10-dihydrodiol, quinones, and 3-and 9-hydroxybenzo[a]pyrene. Similar metabolites were detected in nasal and lung tissues of rats inhaling benzo[a]pyrene (Wolff et al. 1989b). The prevalence of quinone production was not seen in hamsters as it was in rats (Dahl et al. 1985; Weyand and Bevan 1987a, 1988). In monkeys and dogs, dihydrodiols, phenols, quinones, and tetrols were identified in the nasal mucus following nasal instillation of benzo[a]pyrene (Petridou-Fischer et al. 1988). *In vitro* metabolism of benzo[a]pyrene in the ethmoid turbinates of dogs resulted in a prevalence of phenols (Bond et al. 1988). However, small quantities of .quinones and dihydrodiols were also identified.

Rat lung microsomes facilitated the dissociation of small amounts of benzo[a]pyrene from diesel particles, but only a small fraction of the amount dissociated was metabolized (Leung et al. 1988). The ability to dissociate benzo[a]pyrene was related to the lipid content of the microsomal fraction. Microsomes are able to enhance the slow dissociation of a small amount of benzo[a]pyrene from diesel particles in a form that can be metabolized. Free benzo[a]pyrene was principally and extensively metabolized to the 9,10-dihydrodiol.

A human hepatoma cell line (HepG2) has high benzo[a]pyrene-metabolizing activity and converts benzo[a]pyrene to metabolites (Diamond et al. 1980). When [^{3}H]-benzo[a]pyrene was added to the incubate, a large fraction of the radioactivity was not extractable into chloroform. The extractable fraction contained 9,10-dihydrodiols, 7,8-dihydrodiols, quinones, 3-hydroxybenzo[a]pyrene, and the unchanged parent compound. The cell lysate also consisted of the same metabolites, but the proportions of 3-hydroxybenzo[a]pyrene and the parent compound were much higher than in the medium. Conversely, the proportion of water-soluble metabolites in the cell lysate was lower than in the medium. Treatment of the medium and cell lysate with β-glucuronidase converted only 5-7% of the water-soluble metabolites to chloroform-extractable material. Aryl sulfatase had no effect on radioactivity. These results suggested that this human liver tumor cell line does not extensively utilize the phenol detoxification pathway (Diamond et al. 1980).

Metabolism of benzo[a]pyrene in the primary culture of human hepatocytes primarily resulted in the formation of 3-hydroxybenzo[a]pyrene, 4,5-dihydrodiol, 9,10-dihydrodiol, and 7,8-dihydrodiol (Monteith et al. 1987). As the dose of benzo[a]pyrene increased, the amount of metabolites increased linearly. Binding to DNA was associated with the amount of unconjugated 7,8-dihydrodiol. DNA binding was linear up to a benzo[a]pyrene concentration of 100 μmol. At this concentration, binding increased 64-844 times over the extent of binding at 10 umol. As the concentration of benzo[a]pyrene increased, the ratio of dihydrodiol/phenolic metabolites also increased. Although the capacity to form dihydrodiols was not saturated at 100 umol benzo[a]pyrene, there was a change in the relative proportion of the dihydrodiol metabolites formed as the dose of benzo[a]pyrene increased. As benzo[a]pyrene concentration increased, the 9,10-dihydrodiol was the more prevalent metabolite, but levels of 7,8-dihydrodiol also increased (Monteith et al. 1987).

Epoxide hydrolase is a microsomal enzyme that converts alkene and arene oxides to dihydrodiols. Appreciable enzyme activity was observed in human livers. Comparison of epoxide hydrolase

activities with various substrates revealed that the human liver has a single epoxide hydrolase with broad substrate specificity (Kapitulnik et al. 1977). Epoxide hydrolase activity is also present in other tissues and increases the likelihood for carcinogenic effects in these organs. Ethyl acetate extracts of human and rat bladder cultures contained 9,10-dihydrodiol, 7,8-dihydrodiol, and 3-hydroxybenzo[a]pyrene. Covalent binding of [^{3}H]-benzo[a]pyrene with DNA occurring in both human and rat bladder cultures suggested that benzo[a]pyrene-7,8-diol-9, 10-epoxide is generated. The urothelium of the bladder clearly has the ability to generate the ultimate carcinogen (Moore et al. 1982).

Hepatic microsomes from rats induced with 3-methylcholanthrene convert benzo[a]pyrene to benzo[a]pyrene-7,8-diol-9,10-epoxide (BPDE) 10 times faster than untreated microsomes. The rate-limiting step in BPDE formation is the competition for P-450 between benzo[a]pyrene and the 7,8-dihydrodiol. Formation of BPDE is directly correlated with the 3-methylcholanthrene inducible form(s) of P-450 (Keller et al. 1987). Formation of the proximate carcinogen, 7,8-dihydrodiol, is stereoselective. Rabbit hepatic microsomes generated more of the 7R,8R enantiomer with an optical purity of >90% (Hall and Grover 1987). The major stereoisomer formed by rat liver microsomes is (+)-diol-epoxide-2 (R,S,S,R absolute conformation) (Jerina et al. 1976, 1980). This metabolite is highly tumorigenic (Levin et al. 1982) and gives rise to the major adduct formed upon reaction with DNA. The adduct is a diol epoxide-deoxyguanosine formed by alkylation at the exocyclic nitrogen (N-2) of deoxyguanosine. This diol epoxide-deoxyguanosine has been isolated from several animal species (Autrup and Seremet 1986; Horton et al. 1985) and human tissue preparations (Harris et al. 1979).

Studies using rat liver microsomes have shown that hydroxy metabolites of benzo[a]pyrene undergo glucuronidation (Mackenzie et al. 1993). Assays with three different DNA-expressed glucuronidases from human liver indicate preferential glucuronidation for the 2- and 5-hydroxy, 4- and 11-hydroxy, or l-, 2-, and 8-hydroxy derivatives of benzo[a]pyrene. There are differences in preferential activities for the glucuronidation of various benzo[a]pyrene metaboiites among the various DNA-expressed glucuronidases from human liver, with some glucuronidases being relatively or totally inactive toward this class of compounds (Jin et al. 1993). The results of this study suggest that the relative content of particular types of glucuronidases in a cell or tissue may be important for determining the extent to which a particular carcinogen is deactivated.

Several xenobiotics can induce enzymes to influence the rat liver microsomal metabolite profiles of various PAHs. For example, AHH, the cytochrome P-450 isoenzyme believed to be primarily responsible for the metabolism of benzo[a]pyrene and other PAHs, is subject to induction by PAHs. Treatment of pregnant and lactating rats with a single intraperitoneal dose of Aroclor 1245 increased the metabolism of benzo[a]pyrene by liver microsomes from pregnant and fetal rats 9-fold and 2-fold, respectively, and 2-fold in lactating rats (Borlakoglu et al. 1993). The pretreatment enhanced the formation of all metabolites, but the ratio of the 7,8-diol (the proximate carcinogen) was increased 3-fold in lactating rats and 5-fold in pregnant rats. Similar results were observed in rabbit lung microsomes (Ueng and Alvares 1993). Cigarette smoke exposure has been shown to increase PAH metabolism in human placental tissue (Sanyal et al. 1993), and in rat liver microsomes (Kawamoto et al. 1993). In studying benz[a]anthracene metabolism, some xenobiotics were found to be weak or moderate inducers, but even less efficient ones altered the benz[a]anthracene profile significantly. Thiophenes equally enhanced oxidation at the 5,6- and the 8,9-positions. Benzacridines favored K-region oxidation (5,6-oxidation) (Jacob et al. 1983b). Indeno[1,2,3-c,d]pyrene stimulated the bay region oxidation (3,4-oxidation) of benz[a]anthracene (Jacob et al. 1985). Similar xenobiotic effects were observed with chrysene as a substrate (Jacob et al. 1987). While some enzyme activities are being enhanced, alternate enzymatic pathways may be suppressed (Jacob et al. 1983a).

Rat liver microsomes also catalyzed benzo[a]pyrene metabolism in cumene hydroperoxide (CHP)-dependent reactions which ultimately produced 3-hydroxybenzo[a]pyrene and benzo[a]pyrene-quinones (Cavalieri et al. 1987). At low CHP concentrations, 3-hydroxybenzo[a]pyrene was the major metabolite. As CHP concentrations increased, levels of quinones increased and levels of 3-hydroxybenzo[a]pyrene decreased. This effect of varying CHP levels was reversed by preincubating with pyrene. Pyrene inhibited quinone production and increased 3-hydroxybenzo[a]pyrene production. Pretreatment with other PAHs like naphthalene, phenanthrene, and benz[a]anthracene nonspecifically inhibited the overall metabolism. The binding of benzo[a]pyrene to microsomal proteins correlated with quinone formation. This suggested that a reactive intermediate was a common precursor. The effects of pyrene on benzo[a]pyrene metabolism indicated that two distinct microsomal binding sites were responsible for the formation of 3-hydroxybenzo[a]pyrene and benzo[a]pyrene-quinone (Cavalieri et al. 1987).

Rat mammary epithelial cells (RMEC) have been shown to activate PAHs (Christou et al. 1987). Cytochrome-P-450 in RMEC is responsible for the monooxygenation of DMBA. Prior exposure’of

cultured cells to benz[a]anthracene induced DMBA metabolism. The metabolite profile following benz[a]anthracene-induction was significantly different from the profile obtained with purified P-450c, the predominant PAH-inducible enzyme in rat liver. The bay region 3,4-dihydrodiol, which was not formed with P-450c, was clearly detectable in RMEC. Low epoxide hydrolase activity in the benz[a]anthracene-induced RMEC limited the formation of all other DMBA dihydrodiols. The DMBA monooxygenase activity of benz[a]anfhracene-induced RMEC was inhibited by a-naphthaflavone. The study concluded that DMBA metabolism by RMEC depended on the induction of P-450c and at least one additional form of P-450 that is sensitive to α-naphthaflavone (Christou et al. 1987).

As expected from results of other studies, the perfused rat lung can release high quantities of benzo[a]pyrene metabolites and conjugates into the perfusate (Molliere et al. 1987). Addition of a liver to this perfusion system up gradient from the lungs reduces the concentration of parent compound and free metabolites to less than 20% of that seen in the liver's absence. The liver provides a protective effect on the lung to inhibit covalent binding of benzo[a]pyrene metabolites to pulmonary macromolecules.

The effects of various factors that can modify the hepatic clearance of PAHs, specifically benz[a]anthracene and chrysene, were studied by Fiume et al. (1983). The hepatic clearance and rate constants of these PAHs were significantly reduced in the perfused livers of fasted rats relative to those of fed rats. This reduction was attributed to a decrease in aryl hydrocarbon hydroxylase activity. Fasting also accelerated the depletion of cytochrome P-450 and other microsomal enzymes. In contrast, pretreatment of the rats with these PAHs resulted in increased clearance of both hydrocarbons from the perfusion medium when compared to control rats.

It was also noted by Fiume et al. (1983) that the livers of male rats demonstrated a significantly higher hepatic clearance of benz[a]anthracene than female rats, perhaps suggesting a sexual difference with aryl hydrocarbon hydroxylase activity. Similar findings regarding sexual differences in the metabolism of chrysene by rat livers were also reported by Jacob et al. (1985, 1987). Furthermore, Fiume et al. (1983) demonstrated that age can play a role in PAH metabolism. The hepatic clearance of PAHs in older rats (2 years) was significantly less than the hepatic clearance in younger rats (8 weeks). However, activation of benzo[a]pyrene to mutagenic derivatives, as measured by the *Salmonella typhimurium* test, with hepatic microsomes from male rats from 3 weeks to 18 months of age showed no age-dependent changes (Hilali et al. 1993).

A proposed metabolic scheme for the metabolism of the nonaltemant PAH, benzo[b]fluoranthene is presented in Figure 2-4. Nonalternant PAHs, in contrast to several alternant PAHs, do not appear to exert their genotoxic effect primarily through the metabolic formation of simple dihydrodiol epoxides. In the case of benzo[b]fluoranthene, there is evidence to suggest that metabolism to the dihydrodiol precursor to its bay-region dihydrodiol does occur. Rather than this metabolite being converted to its dihydrodiol epoxide; however, it appears to be extensively converted to its 5-hydroxy derivative. It is the further metabolism of this phenolic dihydrodiol to 5, 9, 10-trihydroxy- 11,12-epoxy-9,10,11,12-tetra-hydrobenzo[b]fluoranthene that has been linked to the genotoxic activity of benzo[b]fluoranthene in mouse skin (Weyand et al. 1993b). In the case of benzo[j]fluoranthene, two potentially genotoxic metabolites have been identified. These are the trans-4,5- and 9,10-dihydrodiols of benzo[j]fluoranthene. It is the conversion of trans-4,5-dihydro-4,5-dihydroxybenzo[j]fluoranthene to anti-4,5-dihydroxy-5,6a-epoxy-4,5,6,6a-tetrahydrobenzo[j]fluoranthene that is principally associated with DNA adduct formation in mouse skin (LaVoie et al. 1993b; Weyand et al. 1993a). Benzo[k]fluoranthene in rat microsomes was shown to result in the formation of 8,9-dihydrodiol. This dihydrodiol can form a dihydrodiol epoxide that is not within a bay region. This may represent an activation pathway of benzo[k]fluoranthene that may be associated, in part, with its genotoxic activity. In the case of nonalternant PAHs, reactive metabolites, that deviate from classical bay region dihydrodiol epoxides, have been linked to their tumorigenic activity.

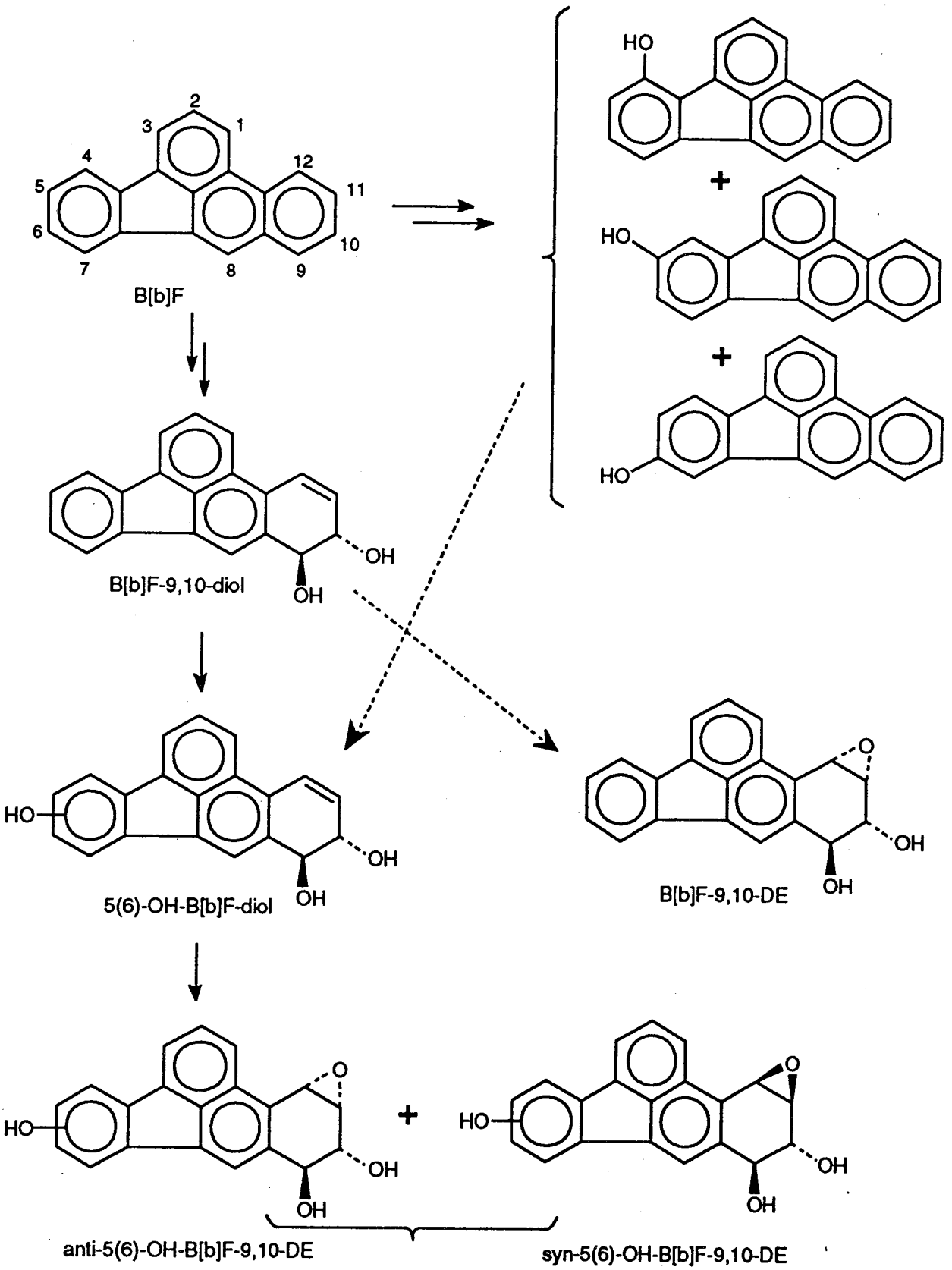
2.3.4 Excretion

2.3.4.1 Inhalation Exposure

Urinary metabolites of PAHs were detected in workers exposed to these compounds in an aluminum plant (Becher and Bjorseth 1983). The ambient levels of PAHs in the workplace (indicated by the authors to exceed 95 mg/m^3, although these data were not presented) did not correspond to the amount of PAHs deposited, metabolized, and excreted in the urine in this study. No quantitative inhalation data were available in humans regarding the excretion of PAHs.

Thirty-four workers in an electrode paste plant were monitored for response to exposure (Ovrebo et al. 1994). Exposure to benzo[a]pyrene was 0.9 µg/m^3; exposure to pyrene was 3.5 µg/m^3. 1-Hydroxypyrene was measured in the urine. Results from these workers were compared to two reference control groups, research and development workers and nickel refinery workers.

FIGURE 2-4. Proposed Metabolic Scheme for Benzo(b)fluoranthene.



Measurements of PAH levels were collected by personal sampling and at two stationary sites. PAH aerosols were collected on filters during the mixing of hot coal tar with carbon. The value of PAHs on the filters varied from 4.3 to 84.6 μg/m^3, with a mean of 14.4 μg/m^3. The PAH particulates were assayed for 8 of 11 PAHs classified as carcinogenic, including benz[a]anthracene, benz[g,h,i]perylene, benzo[a]pyrene, benzo[e]pyrene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[j]fluoranthene, chrysene, fluoranthene, and indeno[1,2,3-c,d]pyrene, and presented relative to the marker compound, pyrene. Content of these compounds ranged from 30 to 150% of the pyrene content on the filters, which was 1.6 μg/m^3. For example, the benzo[a]pyrene level was 0.8 μg/m^3. Urine analysis indicated a mean urinary value of 1-hydroxypyrene among electrode paste plant workers of 6.98 umol/mol creatinine, compared to 0.08 μmol/mol creatinine for the R&D workers, and 0.14 μmol/mol creatinine for the industrial worker group. Smokers had higher levels of 1-hydroxypyrene compared to non-smokers in all groups. The urinary 1-hydroxypyrene level in the electrode plant workers correlated inversely with age. No correlation was found between frequency of use of a protective mask and the urinary 1-hydroxypyrene concentration.

In an ongoing comprehensive evaluation of biological markers, workers in or near an iron foundry with varying exposures to PAHs were analyzed for response to exposure (Santella et al. 1993). Exposure to benzo[a]pyrene, determined by personal monitors, was 2-60 ng/m^3. 1-Hydroxypyrene was measured in the urine. Cigarette smoking, but not age or charbroiled food, influenced the level of 1-hydroxypyrene. When workers were classified into three categories (low, <.0005 mg/m^3; medium, 0.0005-0.0012 mg/m^3; high, >0.0012 mg/m^3), mean 1-hydroxypyrene levels were 2.7, 1.8, and 3.6 μmol/mol creatinine, respectively. There was a significant difference between the groups after controlling for smoking exposure, but there was no consistent trend. The authors indicate that this study evaluates biological markers of exposure at PAH levels that are very low, compared to other studies.

Workers employed in a graphite electrode producing plant (n=16) and a coke oven (n=33) were compared to a control population of maintenance workers in a blast furnace (n=54) (Van Hummelen et al. 1993). The concentration of PAHs in the environment was measured by personal air samplers, the concentration of hydroxypyrene in urine was measured, and smoking habits were evaluated. The mean age of the workers was 40, and did not differ significantly between the three plants. The proportion of smokers was not different among the three groups. The mean exposure for workers in the graphite electrode producing plant was 11.33 μg/m^3 (0.011 mg/m^3) and was correlated with a urinary

hydroxypyrene concentration of 3.18 µg/g creatinine prior to the shift and 6.25 µg/g creatinine after the shift. For the coke oven workers, airborne PAHs were measured at 23.7 µg/m^3 but this was not illustrative of true exposure, since there were a few very high exposures in the sample: 90.10 µg/m^3 for 7 workers compared to 5.57 µg/m^3 for 26 workers. In accordance with the predominantly low exposure, the urinary hydroxypyrene levels were 0.51 µg/g creatinine before the shift and 0.75 µg/g creatinine after the shift.

The excretion of benzo[a]pyrene following low-level inhalation exposure is rapid and high in rats (Bevan and Weyand 1988; Weyand and Bevan 1986; Wolff et al. 1989c); however, elimination is low in dogs and monkeys (Petridou-Fischer et al. 1988). After nose-only inhalation of 4.8 mg/m^3 [^{14}C]-benzo[a]pyrene for a single exposure or daily for 4 weeks, excretion of radioactivity in the feces of Fischer-344 rats was approximately 96% of the administered concentration (Wolff et al. 1989c). The excretion half-lives in feces and urine were 22 and 28 hours, respectively.

2.3.4.2 Oral Exposure

Five volunteers (21-41 years of age) ingested specially prepared diets high in PAHs, specifically benzo[a]pyrene (from grilled beef), for 2-3 days for an intake of approximately 0.007-0.02 mg/day (Buckley and Lioy 1992). A 100-250-fold increase in ingested benzo[a]pyrene in the high-PAH diet corresponded to a 6-12-fold increase in the elimination of 1-hydroxypyrene. However, benzo[a]pyrene and its other metabolites (not specified) were not measured in excreta, which prevented determination of the total excretion (and an estimate of oral absorption).

Male Sprague-Dawley rats received a low dose of [^{3}H]-benzo[a]pyrene (suspended in 10% ethanolsoybean oil) daily for 3, 5, or 7 days (Yamazaki and Kakiuchi 1989). Highest radioactivity was excreted 2-8 days after first exposure. Polar and phenol metabolites represented approximately 60% and 20% of the radioactivity detected in urine, respectively. The conjugated form accounted for 80% of these urinary metabolites. Only small amounts of unmetabolized benzo[a]pyrene were detected in the urine. Excretion into the feces was not studied.

Male Wistar rats eliminated a large amount of a single gavage dose of 0.22 mg/kg chrysene by 2 days postexposure (Grimmer et al. 1988). The unchanged parent compound represented 0.17% and 13.09% in the urine and feces, respectively. The recovery of the dose in excreta was 74% of dose after 4 days

of which about 61% was represented by hydroxychrysene compounds. The metabolite pattern was similar for urine and feces. The major metabolites identified were 1- and 3-hydroxychrysene, with about 100 times higher amounts in the feces (33.1% and 17.87%, respectively, of administered dose) than urine (0.79% and 0.35%, respectively). Approximately 79% of a large chrysene dose (1,217-2,717 mg/kg in the diet) was eliminated in the feces of rats; however, levels in the urine were not measured (Chang 1943). As the dose of chrysene in the diet increased, the percentage of excreted hydrocarbon also increased.

Male Wistar rats were given single oral doses of 2, 4, 6, 9, or 1.5 mg/kg of [^{14}C]-pyrene (Withey et al. 1991). Recovery of the dose in excreta was 82% for the two low-dose groups and 50-63% for the other groups 2 days postexposure. The urine and feces contained 34-45% and 21-50% of the dose, respectively, at 4 days postexposure. Recovery of radioactivity in the bile was approximately 10% of the dose after 6 hours.

2.3.4.3 Dermal Exposure

No studies were located regarding the excretion of PAHs in humans following dermal exposure to single PAHs. Excretion patterns of 1-hydroxypyrene in urine were studied in two psoriatic patients, each treated daily with coal tar pitch covering over 50% of their skin for 3 weeks (Hansen et al. 1993). The coal tar contained 1.43 mg/g pyrene. After 1 week of treatment, the urinary concentration of 1-hydroxypyrene increased approximately 100 times. However, the concentration after 3 weeks of treatment was decreased to that observed before treatment was initiated. The authors speculate that the healing of the psoriatic lesions may have rendered the skin less permeable to the PAHs after 3 weeks of treatment and healing.

The urinary excretion of 1-hydroxypyrene was evaluated in 65 automotive repair workers (automobile or diesel truck repair) whose skin was exposed to used mineral oils, and compared to values from non-professionally exposed control males on control diets (Granella and Clonfero 1993). Smoking exposure was equivalent in both groups. Pyrene concentrations were determined in the oily material taken from cloths used to clean various types of engines. Values of urinary 1-hydroxypyrene were determined on Friday at the end of the working week, and again on Monday morning prior to work. Smoking and PAH-rich diets were considered confounding factors. At both the beginning and the end of the working week, the values of urinary 1-hydroxypyrene were slightly higher in exposed subjects

(0.178 + 0.150 and 0.194 + 0.135 µmol creatinine on Monday and Friday, respectively) than in controls ((0.124 + 0.090 µmol/mol creatinine on Monday and Friday, respectively). The urinary 1-hydroxypyrene values were higher in both smoking and non-smoking subjects than controls. The highest values were found in urinary samples of smokers exposed to used mineral oils (0.259 + 0.090 µmol/mol creatinine). In non-smoking workers, post-shift 1-hydroxypyrene values were 0.154 + 0.105 µmol/mol creatinine, compared to 0.083 + 0.042 µmol/mol creatinine for the non-smoking controls. In automobile repair workers, there was no significant difference in the levels of 1-hydroxypyrene at the beginning and end of the work week. Tobacco smoking had more influence on the levels of 1-hydroxypyrene than did occupational exposure in this group. The influence of PAHs in the diet was only detectable when the subjects returned to work after the weekend. No explanation was given for this finding. This data suggests that exposure to PAHs through dermal contact with used engine oil is low.

The elimination of benzo[a]pyrene was rapid and high in mice and guinea pigs following low- and high-level dermal exposure (Ng et al. 1992; Sanders et al. 1986). The percentages of recovered radioactivity in urine and feces were 24.5%, 46.9%, and 25% for Swiss Webster mice dermally exposed to benzo[a]pyrene at 1.25, 12.5, and 125 µg/cm^2, respectively, for 7 days (Sanders et al. 1986). The feces in the high-dose animals had 35%, 58%, and 80% of the total recovered radioactivity after 24 hours, 48 hours, and 7 days, respectively. The amount of radioactivity excreted in urine was about 10% of amount excreted in feces. A elimination half-life of about 30 hours was estimated for benzo[a]pyrene. The data are limited because the exposed area of skin was not reportedly covered or collars were not employed to prevent ingestion of test compound by the animal. In guinea pigs, 73% of the dose was excreted 7 days after low-level (0.28 mg) exposure to benzo[a]pyrene (Ng et al. 1992).

The excretion of dermally absorbed phenanthrene and pyrene was rapid in guinea pigs (Ng et al. 1991, 1992). The presence of [^{14}C]-activity in the urine and feces of rats that received [^{14}C]-anthracene applied to the skin provides evidence of its absorption (Yang et al. 1986). Six days after administration of 9.3 µg/cm^2, the amounts detected in the urine and feces were 29.1% and 21.9% of the dose, respectively.

2.3.4.4 Other Routes of Exposure

No studies were located regarding excretion of PAHs in humans following other routes of exposure.

Three female Beagle dogs were given a bolus of aerosolized crystals of 7.7 mg/kg benzo[a]pyrene or 2.8 mg/kg phenanthrene in a single breath by intratracheal instillation (Gerde et al. 1993a). The blood borne clearance of the PAHs was monitored by repeatedly sampling blood through catheters in the ascending aorta and the right atrium of the dog. Half of the benzo[a]pyrene cleared within 2.4 minutes. Half of the phenanthrene cleared in 1.0 minute. Compared to clearance of phenanthrene, a less lipophilic PAH, the data indicate that the clearance of benzo[a]pyrene was limited by diffusion through the alveolar septa, while clearance of the moderately lipophilic phenanthrene was limited mostly by the rate of perfusion of the blood. The results indicate that inhaled PAHs of sufficient lipophilicity to limit diffusion through cells have a greater potential for toxicity to the lung than less lipophilic PAHs. Because of the thicker epithelia, bronchi should be at greater risk than the alveoli for PAH-induced toxicity exerted at the port of entry. Clearance of PAHs from the respiratory tract follows a biphasic pattern, with a rapid clearance of most of the PAH followed by a slow clearance of a small fraction. Previously published models predict that the rapid phase represents clearance through the thin epithelial barriers in the alveoli, the slow clearance is through the thicker epithelium of the airways, and the rate of clearance from either region will be slowed if the PAH has a high degree of lipophilicity. This study sought to validate model predictions for rates of alveolar clearance of PAHs of different lipophilicities. In a companion study, 3 female Beagle dogs were given doses of 0.00091 g/kg benzo[a]pyrene instilled on the surface of the conducting airways (Gerde et al. 1993b). Sequential lavage of the mucous-retained materials followed the instillations. Benzo[a]pyrene was retained within the mucous lining layer sufficiently to be transported with the mucocilliary escalator. Fractions of benzo[a]pyrene penetrating to the bronchial epithelium had a clearance half-time in the range of 1.4 hours. This long retention indicates a diffusion-limited uptake of benzo[a]pyrene by the airways. Physiological models have predicted that the lipophilicity of solutes such as PAHs will delay clearance from the respiratory tract. This clearance consists of a delayed penetration of the mucous lining layer, allowing mucocilliary clearance, followed by a slow penetration of PAHs through walls of the conducting airways.

Excretion of radioactivity in the urine of rats following intratracheal instillation only accounted for 2.2% of the administered benzo[a]pyrene at 6 hours (Bevan and Weyand 1988; Weyand and Bevan

1986). Amounts of radiolabel in the blood were also very small. However, levels could still be determined in order to derive toxicokinetic parameters. The data obtained by Weyand and Bevan (1986) fit best to a three-compartment model whereby the half-lives for the three phases were 4.3, 31.5, and 277 minutes.

Results obtained by Weyand and Bevan (1986, 1988) revealed that a large fraction of the administered dose was excreted in the bile of rats, therefore suggesting that fecal elimination is the major excretion route. After 6 hours, 53% of the 0.001 mg/kg dose was excreted into the intestine and intestinal contents of rats that were without a bile duct cannula, while cannulated rats excreted 74% in the bile over the same period (Weyand and Bevan 1986). These results indicate the occurrence of enterohepatic recirculation. Metabolites excreted in the bile included thioether conjugates (62.5%), glucuronide conjugates (22.8%), sulfate conjugates (7.4%), and free benzo[a]pyrene (9.8%) (Weyand and Bevan 1988). Radioactivity detected in the gastrointestinal tract of rats following pulmonary exposure to benzo[a]pyrene also suggests that biliary excretion occurs (Weyand and Bevan 1987a; Wolff et al. 1989c). The percentage of benzo[a]pyrene excretion into bile declined as intratracheal doses increased from 0.00016 to 0.35 mg in rats and guinea pigs (Weyand and Bevan 1987b). However, the biliary excretion of benzo[a]pyrene in hamsters remained the same after administration of either dose.

Female Wistar rats received low doses of chrysene (0.002 and 0.004 mg/kg) intratracheally (Grimmer et al. 1988). The major metabolite in the excreta was 1-hydroxychrysene. Hydroxychrysene compounds represented 31.26-48.9% of dose in the feces and about 3% in the urine. The unmetabolized parent compound was 17-19% of the administered dose in feces and only l-2% in urine.

Less than 10% of instilled radioactivity was excreted in the urine and feces of dogs and monkeys 48 hours after intratracheal administration of [^{3}H]-benzo[a]pyrene into the nostril (Petridou-Fischer et al. 1988).

Nineteen outbred male rats were dosed intraperitoneally once with 200 mg/kg benzo[a]pyrene in sunflower oil (Likhachev et al. 1993). Concentrations of benzo[a]pyrene-7,8-diol, a marker metabolite of bioactivation of benzo[a]pyrene, and 3-hydroxy-benzo[a]pyrene, a marker metabolite of deactivation, were measured daily in the urine and feces for 15 days. Levels of these metabolites were

correlated with tumor latency. Another group of- 10 rats was dosed intraperitoneally once with 15 mg/kg benzo[a]pyrene in sunflower oil and urine was collected for 3 days. Five rats were killed on day 3 and the other 5 were killed on day 8. Liver DNA concentrations of benzo[a]pyrene-7,8,9,10-tetrols were determined in animals killed on day 8. Considerable individual variation was observed in the levels of daily and total excretion of benzo[a]pyrene-7,8-diol and 3-hydroxy-benzo[a]pyrene in rats receiving 200 mg/kg benzo[a]pyrene. Both metabolites were excreted primarily in the feces. More than half of the total metabolites excreted were detected during the first five days, and peak concentrations were observed on the second day after benzo[a]pyrene administration. Peritoneal malignant fibrous histiocytomas developed in 10 of the 16 survivors at 15 days. Levels of urinary benzo[a]pyrene-7,8-diol correlated positively with tumor latency. In the animals exposed to 15 mg/kg benzo[a]pyrene, a high correlation was found between excretion of benzo[a]pyrene-7,8-diol and benzo[a]pyrene-DNA adducts in the liver.

[^{14}C]-Benzo[a]pyrene was administered to male germfree rats (Yang et al. 1994). Urine was collected 24 hours before and every 24 hours for 7 days after administration. Urinary metabolites, consisting of 9% of the administered radioactivity, were fractionated by lipophilic ion exchange chromatography, and characterized by reversed-phase HPLC, ultraviolet spectrometry, and gas chromatography/mass spectrometry. About 90% of the administered dose was excreted within 7 days; 80% in the feces and 9% in the urine. About 90% of the radioactivity in the urine was recovered in the methanol eluate. In this eluate, more than 80% of the urinary metabolites were conjugated, while neutral metabolites constituted 13-18%. The neutral metabolites consisted of 7,8,9,10-tetrols (trace), trans-11,12-dihydrodiol (major), trans-7,8-dihydrodiol (trace), three isomer trihydroxy-benzo[a]pyrenes (major), carboxylic methyl ester derivatives of benzo[a]pyrene quinones, and trioxo-benzo[a]pyrenes (major). Most of the urinary radioactivity was excreted within 72 hours of dosing, with a peak excretion of 24-48 hours. A similar time course was observed for excretion in feces (data not shown).

Six hours after intravenous administration of 0.08 mg/kg [^{14}C]-benzo[a]pyrene to rabbits, 30% and 12% of the dose was excreted in the bile and urine, respectively (Chipman et al. 1982). Excretion of activity into the bile was biphasic over a period of 30 hours with apparent half-lives of 0.27 and 4.623 hours for the rapid and slow phases, respectively. Treatment of the bile and urine with β-glucuronidase and aryl sulfatase increased the amount of activity in the bile and urine that was extractable into ethyl acetate indicating the presence of glucuronide and sulfate conjugates.

Intraduodenal administration of the bile resulted in 21% and 14% of the intraduodenal dose being excreted into the bile and urine, respectively. Since biliary metabolites undergo enterohepatic recirculation, the half-life for ^{14}C-activity is expected to be longer in animals without biliary fistulae (Chipman et al. 1982).

The overall elimination of [^{3}H]-benzo[a]pyrene following intravenous administration (0.001 mg/kg) best fits a triexponential model, as after inhalation exposure (Weyand and Bevan 1986). The half-lives for the three phases were 1.5, 22.4, and 178 minutes. These parameters were very similar to those derived from intratracheal instillation.

2.3.5 Mechanisms of Action

PAHs are absorbed through the lungs by transport across the mucus layer lining the bronchi (Bevan and Ulman 1991). In general, PAHs are lipophilic compounds that can cross the lungs through passive diffusion and partitioning into lipids and water of cells (Gerde et al. 1991, 1993a, 1993b). The rapid, blood-bound redistribution of hydrocarbons at low blood concentrations from lungs to other organs indicates that diffusion is the rate-determining step (Gerde et al. 1991). The absorption rates vary among the PAHs, probably depending on the octanol/water partition coefficient. Essentially all of gastrically instilled benzo[a]pyrene is absorbed via uptake of fat-soluble compounds (Busbee et al. 1990). Oral absorption of benzo[a]pyrene is enhanced by some oils (such as corn oil) in the gastrointestinal tract (Kawamura et al. 1988). The mechanism of dermal absorption of PAHs is most likely passive diffusion through the stratum comeum (Yang et al. 1986).

PAHs and their metabolites are distributed to tissues by transport through the blood. Therefore, PAHs reach more-perfused tissues rapidly following exposure and are eliminated more slowly from less-perfused tissues (Bartosek et al. 1984). A large fraction of orally absorbed benzo[a]pyrene is believedto be transported by lipoproteins from the gastrointestinal tract to the blood via the thoracic duct lymph flow (Busbee et al. 1990).

The carcinogenic mechanism of action of altemant PAHs is fairly well elucidated, but it is not as well described for nonaltemant PAHs. Furthermore, it is not known exactly how PAHs affect rapidly proliferating tissues. PAHs express their carcinogenic activity through biotransformation to chemically reactive intermediates that then covalently bind to cellular macromolecules (i.e., DNA) leading to

mutation and tumor initiation. The products of PAH metabolism include epoxide intermediates, dihydrodiols, phenols, quinones, and their various combinations. The bay region (e.g., the sterically hindered, cup-shaped area between carbons 10 and 11 of benzo[a]pyrene or 1 and 12 of benz[a]anthracene) diol epoxide intermediates of PAHs are considered to be the ultimate carcinogen for alternant PAHs (Jerina et al. 1980). These diol epoxides are easily converted into carbonium ions (carbocations) which are alkylating agents and thus mutagens and initiators of carcinogenesis. Therefore, the carcinogenic and toxic potential of PAHs relies on their metabolites. However, several of the tumorigenic PAHs (i.e., the nonaltemant PAHs) discussed in this profile do not have a bay region, or have been shown not to be similarly activated via a simple bay region epoxide (e.g., Amin et al. 1985a, 1985b). This observation has important implications regarding the expression of carcinogenicity for the nonalternant PAHs. If these chemicals are activated to carcinogens via a mechanism that differs from alternant PAHs, then they may also differ with respect to tumor site and species specificity.

A prerequisite for conversion of PAHs into these active bay region diol epoxides is the presence of cytochrome P-450 and associated enzymes.responsible for this conversion. These enzymes can be found primarily in the liver, but they are also present in the lung, intestinal mucosa, and other tissues. Thus, factors such as distribution to the target tissue(s), solubility, and intracellular localization proximate to these enzymes figure prominently in the expression of a PAH's carcinogenicity. In fact, in order to assess whether there was any correlation between carcinogenic potency and the ability to induce P-450 isoenzymes, several indices of P-450 isoenzyme activity (*O*-demethylation of ethoxyresorufin, metabolic activation of 2-amino-6-methyldipyrido [1,2-α:3',2'd]imadazol [Glu-P-I] to mutagens, and immunological detection of polyclonal antibodies against purified rat P-450 I) were measured in microsomal preparations incubated with benzo[a]pyrene and benzo[e]pyrene (Ayrton et al. 1990). While both PAHs increased several parameters of P-450-I activity, benzo[a]pyrene was markedly more potent than benzo[e]pyrene. Based on these results, the authors concluded that the carcinogenic potency of the PAHs tested could be predicted by the degree to which they induced these enzymes.

Changes in the cytochrome P-450 system can affect the carcinogenicity of the PAHs. This system is susceptible to induction by the PAHs themselves as well as other chemicals commonly found in the environment. The degree and specificity (i.e., which enzymes are affected) of induction depend on the tissue and species and strain. The induction of one enzyme particularly important to the metabolism

of PAHs, AHH, is also known to be under genetic control (see discussions in Section 2.2.2 on responsive versus nonresponsive mouse strains). Given the heterogeneity of human genotypes, it is likely that certain human subpopulations exist that are more susceptible to AHH induction and thus more susceptible to the induction of cancer (see Section 2.7).

Once the reactive bay region epoxide is formed, it may covalently bind to DNA and other cellular macromolecules and presumably initiate mutagenesis and carcinogenesis. Indeed, the level of DNA-adduct formation has been found to correlate with tumor induction activity for a number of PAHs in newborn rat liver and lung (Weyand and LaVoie 1988) and in mouse skin (Albert et al. 1991b; Alexandrov and Rojas-Moreno 1990). Furthermore, no benzo[a]pyrene-DNA-adducts were found in rat skin, which is known to be resistant to PAH-induced skin tumor formation (Alexandrov and Rojas-Moreno 1990). The types of adducts formed in various tissues may dictate target organ susceptibility to PAH-induced carcinogenicity. Various metabolites of benzo[a]pyrene were administered to rats intraperitoneally and DNA adducts from lung, liver, and lymphocytes were measured (Ross et al. 1991). The only metabolites that led to DNA binding were 2-, 9-, and 12-hydroxybenzo[a]pyrene and the truns-7,8-dihydrodiol of benzo[a]pyrene. The authors suggested that different DNA adducts resulting from the *in vivo* metabolism of benzo[a]pyrene in different tissues may be related to tissue specificity of benzo[a]pyrene-induced carcinogenicity.

Although the bulk of this work on PAH-induced carcinogenicity has been done in animal models and animal *in vitro* systems, work in human *in vitro* systems indicates that these same mechanisms of activation may be involved in humans. For example, induction of AHH and formation of the reactive intermediate, benzo[a]pyrene 7,8-dihydrodiol, has been observed in the epithelial tissue from human hair follicles (Merk et al. 1987). All the steps necessary for cellular transformation and cancer induction were demonstrated in cultured human skin fibroblasts: inducible AHH activity, altered cellular proliferation kinetics, and DNA damage (Milo et al. 1978). Thus, humans are likely to be susceptible to tumor induction by PAHs by these mechanisms.

Carcinogenic PAHs have been suggested to have an effect on immune function (Luster and Rosenthal 1993; Saboori and Newcombe 1992), thereby allowing the induction of carcinogenesis, while noncarcinogenic PAHs do not affect immune function (see Section 2.4). The effects of dermally applied benzo[a]pyrene alone or following dermal pretreatment with the prostaglandin synthetase inhibitor, indomethacin, on contact hypersensitivity (cell-mediated immunity), production of antibodies

to DNP (humoral immunity), and the induction of skin tumors was studied in male BALBc mice treated for 6 weeks to 6 months (Andrews et al. 1991b). A group of mice treated with acetone served as controls. Skin tumors were observed in the mice treated with benzo[a]pyrene beginning at week 18 of treatment. Pretreatment with indomethacin significantly increased (by 21%) the latency of tumor induction by benzo[a]pyrene and significantly reduced (by 46%) the weight of benzo[a]pyrene-induced skin tumors. Based on these findings, the authors suggested that benzo[a]pyrene-induced skin carcinogenesis may be mediated by a mechanism that involves prostaglandin suppression of cellular immunity. Undoubtedly, several other factors yet to be determined are involved in the ultimate expression of PAH-induced toxicity and carcinogenicity.

2.4 RELEVANCE TO PUBLIC HEALTH

PAHs occur ubiquitously in the environment from both synthetic and natural sources. PAHs occur in the atmosphere most commonly in the products of incomplete combustion. These products include fossil fuels; cigarette smoke; industrial processes (such as coke production and refinement of crude oil); and exhaust emissions from gasoline engines, oil-fired heating, and burnt coals. PAHs are present in groundwater, surface water, drinking water, waste water, and sludge. They are found in foods, particularly charbroiled, broiled, or pickled food items, and refined fats and oils. Individuals living in the vicinity of hazardous waste sites where PAHs have been detected at levels above background may experience exposure to these chemicals via inhalation of contaminated air or ingestion of contaminated food, soil, or water.

Evidence exists to indicate that certain PAHs are carcinogenic in humans and animals. The evidence in humans comes primarily from occupational studies of workers who were exposed to mixtures containing PAHs as a result of their involvement in such processes as coke production, roofing, oil refining, or coal gasification (e.g., coal tar, coke oven emissions, soot, shale, and crude oil). Cancer associated with exposure to PAH-containing mixtures in humans occurs predominantly in the lungs and skin following inhalation and dermal exposure, respectively. Some ingestion of PAHs is probably due to swallowing of particulates containing PAHs subsequent to mucocilliary clearance from the lung. Certain PAHs have also been shown to induce cancer in animals. The site of tumor induction is influenced by route of administration: stomach tumors are observed following ingestion, lung tumors following inhalation, and skin tumors following dermal exposure, although tumors can form at other locations (e.g., lung tumors after dermal exposure).

Noncancer adverse health effects associated with PAH exposure have been observed in animals but generally not in humans (with the exception of adverse hematological and dermal effects). Animal studies demonstrate that PAHs tend to affect proliferating tissues such as bone marrow, lymphoid organs, gonads, and intestinal epithelium.

Minimal Risk Levels (MRLs) for Polycyclic Aromatic Hydrocarbons

Inhalation MRLS

No inhalation MRLs have been derived for PAHs because no adequate dose-response data that identify threshold levels for noncancer health effects are available in humans or animals for any duration of exposure.

Oral MRL.s

No acute, or chronic oral MRLs were derived for PAHs because there are no adequate human or animal dose-response data available that identify threshold levels for appropriate noncancer health effects. Serious reproductive and developmental effects in animals associated with acute oral exposure to PAHs have been reported. These are not appropriate end points for the derivation of an MRL. Noncancer effects noted in longer term oral toxicity studies in animals include increased liver weight (generally not considered to be adverse) and aplastic anemia (a serious effect), neither of which is an appropriate end point for the derivation of an MRL. Intermediate-duration oral MRLs were derived for acenaphthene, anthracene, fluoranthene, and fluorene.

Acenaphthene

- An MRL of 0.6 mg/kg/day has been derived for intermediate-duration oral exposure (15-364 days) to acenaphthene.

The MRL was based on a minimal LOAEL of 175 mg/kg/day for liver weight (EPA 1989c). Four groups of CD-l mice (20/sex/group) were gavaged daily with 0, 175, 350, or 700 mg/kg/day acenaphthene for 90 days. The toxicological evaluations of this study included body weight changes, food consumption, mortality, clinical pathological evaluations (including hematology and clinical

chemistry), organ weights and histopathological evaluations of target organs. The results of this study indicated no treatment-related effects on survival, clinical signs, body weight changes, total food intake, and ophthalmological alterations. Liver weight changes accompanied by microscopic alterations (cellular hypertrophy) were noted in both the mid- and high-dose groups, and seemed to be dose-dependent. Additionally, high-dose males and mid- and high-dose females showed significant increases in cholesterol levels. Increased relative liver weights in males, and increased absolute and relative liver weight in females, without accompanying microscopic alterations or increased cholesterol levels were also observed at the low dose; in light of the effects seen at higher doses, this change was considered to be a minimum LOAEL. There was no NOAEL. The MRL was obtained by dividing the LOAEL value by 300 (3 for a minimum LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability) and rounding to one significant figure.

MRLs for acute-duration and chronic-duration have not been derived because suitable NOAEL and LOAEL values have not been identified in the available literature.

Fluoranthene.

- An MRL of 0.4 mg/kg/day has been derived for intermediate-duration oral exposure (15-364 days) to fluoranthene.

The MRL was based on a minimal LOAEL of 125 mg/kg/day for increased relative liver weight in male mice (EPA 1988e). Four groups of CD-1 mice (20/sex/group) were gavaged daily with 0, 125, 250, or 500 mg/kg/day fluoranthene for 90 days. The toxicological evaluations of this study included body weight changes, food consumption, mortality, clinical pathological evaluations (including hematology and clinical chemistry), organ weights, and histopathological evaluations of target organs. The results of this study indicated no treatment-related effects on survival, clinical signs, body weight changes, total food intake, or ophthalmological alterations. All treated mice exhibited nephropathy, increased salivation, and increased liver enzyme levels in a dose-dependent manner. However, these effects were either not significant, not dose-related, or not considered adverse at 125 mg/kg/day. Mice exposed to 500 mg/kg/day had increased food consumption throughout the study. Mice exposed to 250 mg/kg/day had statistically increased SGPT values and increased liver weight. Compound-related microscopic liver lesions (indicated by pigmentation) were observed in 65 and 87% of the mid- and high-dose mice, respectively. Male mice exposed to 125 mg/kg/day had increased relative liver

weight. The LOAEL is 125 mg/kg/day, based on-relative liver weight in males. There was no NOAEL. The MRL was obtained by dividing the LOAEL value by 300 (3 for a minimal LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability) and rounding to one significant figure.

Fluorene.

- An MRL of 0.4 mg/kg/day has been derived for intermediate-duration oral exposure (15-364 days) to fluorene.

The MRL was based on a minimal LOAEL of 125 mg/kg/day for relative liver weight (EPA 1989e). Four groups of CD-l mice (20/sex/group) were gavaged daily with 0, 125, 250, or 500 mg/kg/day fluorene for 90 days. The toxicological evaluations of this study included body weight changes, food consumption, mortality, clinical pathological evaluations (including hematology and clinical chemistry), organ weights and histopathological evaluations of target organs. The results of this study indicated no treatment-related effects on survival, body weight changes, total food intake, or ophthalmological alterations. All treated male mice exhibited increased salivation, hypoactivity, and urine-wet abdomens. A significant decrease in red blood cell count and packed cell volume was observed in females treated with 250 mg/kg/day and in males and females at 500 me/kg/day. Decreased hemoglobin concentration was also observed in the high-dose group. A dose-related increase in relative liver weight was observed in all treated mice, and in absolute liver weight at >250 mg/kg/day. A significant increase in absolute and relative spleen and kidney weight was observed at 250 mg/kg/day.. Increases in absolute and relative liver and spleen weights at the high dose were accompanied by histopathological increases in hemosiderin in the spleen and in the Kupffer cells of the liver. The LOAEL is 125 mg/kg/day based on increased relative liver weight. There was no NOAEL. The MRL was obtained by dividing the LOAEL value by 300 (3 for a minimal LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability) and rounding to one significant figure.

Anthracene.

- An MRL of 10 mg/kg/day has been derived for intermediate-duration oral exposure (15-364 days) to anthracene.

The MRL was based on a NOAEL of 1,000 mg/kg/day for liver effects (EPA 1989d). The objective of this study was to evaluate the toxicity of anthracene in a subchronic toxicity study. Four groups of male and female CD-1 mice (20/sex/group) were placed on study, and were dosed with 0, 250, 500, and 1,000 mg/kg/day fluorene in corn oil by gavage for 13 weeks. The mice were observed twice daily for clinical signs. Body weights and food consumption were reported weekly. Hematologic and serum chemistry evaluations were completed at final sacrifice. At final sacrifice, gross post-mortem examinations were completed, organ weights were taken, and histological examinations were subsequently done on the tissues collected from all organ systems. No treatment-related finding were noted in survival, clinical signs, mean body weights, food consumption, and ophthalmological examinations, hematology, clinical chemistry, organ weights , gross pathology, and histopathology. In summary, anthracene produced no discemable effects. This study was conducted under the same laboratory conditions as the 90-day study of acenaphthene (EPA 1989c), and under similar laboratory conditions as the 90-day studies of fluoranthene (EPA 1988e) and fluorene (EPA 1989e), from which intermediate-duration MRLs were derived, based on liver effects. In these studies (EPA 1988e, 1989c, 1989e), many other treatment-related and dose-related effects were observed, including renal, hematological, and splenic, that lent support to the derivation of the MRL for each compound. Thus, although no toxic effects were noted even at the highest dose tested in the study cited for anthracene (EPA 1989d), this free-standing NOAEL has considerable credibility, based on the assumption that toxic effects would have been observed if present, as was seen for the other compounds using the same study design. The NOAEL is 1,000 mg/kg/day based on the absence of liver effects, and any other effects in the organ systems studied. The NOAEL was the highest dose used in the study. The MRL was obtained by dividing the NOAEL value by 100 (10 for extrapolation from animals to humans, and 10 for human variability).

Dermal MRLs

No acute-, intermediate-, or chronic-duration MRLs were derived for the 17 PAHs because of the lack of appropriate methodology for the development of dermal MRLs.

Death. There have been no reports of death in humans following exposure to any of the PAHs. However, benzo[a]pyrene is fatal to mice following ingestion, and aeath in animals has been reported following parenteral exposure to a number of PAHs. The intraperitoneal LD_{50} values in mice for pyrene, anthracene, and benzo[a]pyrene are 514, >430, and 232 mg/kg, respectively (Salamone 1981).

Reduced survival time in "Ah-responsive" mice (those capable of producing increased levels of cytochrome P-450 enzymes) was observed following a single intraperitoneal dose of 500 mg/kg benzo[a]pyrene (Robinson et al. 1975). In contrast, oral exposure to 120 mg/kg/day benzo[a]pyrene results in reduced survival of "Ah-nonresponsive" mice (those whose P-450 enzymes are not induced by PAHs).

While the results in animal studies indicate that exposure to high doses of PAHs is lethal, the majority of the data are from parenteral exposure. This route is not applicable to exposure routes humans may expect to encounter, so the relevance of these findings to public health is not known. Parenteral administration bypasses the first-pass effect in the liver that occurs following oral exposure (PAHs may be expected to be ultimately biotransformed to inactive metabolites more quickly in the liver than in other tissues). However, because death has been observed in animals following oral exposure as well, it can be assumed that acute exposure to high enough doses of the PAHs can be lethal.

Systemic Effects.

Respiratory Effects. Adverse noncancer respiratory effects, including bloody vomit, breathing problems, chest pains, chest and throat irritation, and abnormalities in chest X-rays have been reported in humans exposed to PAHs and respirable particles in a rubber factory (Gupta et al. 1993). Inhalation is a significant route of exposure to PAHs in humans. *In vitro* studies using human lung tumor cells demonstrate that the benzo[a]pyrene-induced cytotoxicity (as measured by protein incorporation or cloning efficiency) observed was most likely due to formation of such reactive products as the 7,8-diol 9,10-epoxide metabolite of benzo[a]pyrene (Kiefer et al. 1988). Thus, human lung cells are capable of metabolizing PAHs to reactive intermediates. This implies that inhalation exposure to PAHs could result in toxicity in the respiratory tract.

Adverse effects on the respiratory tissue of laboratory animals have also been observed. The effects of benzo[e]pyrene, pyrene, anthracene, benz[a]anthracene, and benzo[a]pyrene on respiratory mucosa were studied in tracheal explants in rats (Topping et al. 1978). The PAHs were incorporated into beeswax pellets that were placed into tracheal grafts that had been transplanted subcutaneously into the subscapular region of isogenic host rats and the pellets remained in place for 4 weeks. Approximately 50-60% of the test substance was delivered to the tracheal tissue by the end of 4 weeks, in most instances. Benzo[e]pyrene induced only mild changes that included slight hyperplasia of the tracheal

epithelium. A more long-lasting epithelial hyperplasia was observed with pyrene, anthracene, and benz[a]anthracene, and tracheas implanted with pyrene also exhibited a more severe mucocilliary hyperplasia. In addition, undifferentiated epithelium and small areas of squamous metaplasia were also seen with these PAHs, effects that persisted at least 8 weeks after exposure. Severe and long-lasting hyperplasia and transitional hyperplasia as well as metaplasia were seen in tracheas exposed to benzo[a]pyrene, and after 8 weeks, 75% of the epithelium was still abnormal. Acute inflammation (edema and/or granulocyte infiltration), subacute inflammation (mononuclear infiltration and an increase in fibroblasts), and fibrosis and hyalinization in the second half of the experiment were seen with all PAHs. The authors concluded that all of the PAHs tested induced pathological changes in the respiratory mucosa of the transplanted tracheas. The effects were different for the noncarcinogenic PAHs (benzo[e]pyrene, pyrene, anthracene, benz[a]anthracene), as compared to the carcinogenic PAH (benzo[a]pyrene); the former induced changes that were short-lived while the latter produced more severe, long-lasting (metaplastic) changes.

Cultured fetal hamster tracheal explants were exposed to two concentrations each of benz[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[e]pyrene, and pyrene *in vitro* for 4 days, and the effects of these PAHs on the respiratory epithelium were evaluated by scanning electron microscopy (Richter-Reichhelm and Althoff 1986). Exposure to benzo[e]pyrene and pyrene, as well as the lower concentrations of benz[a]anthracene and benzo[b]fluoranthene, resulted in effects similar to those seen in the DMSO controls: up to a 10% incidence of focally slight inhibition of epithelial differentiation and/or metaplasia. When the concentrations of all of the PAHs except benzo[e]pyrene and pyrene were doubled, the frequency of these lesions increased to 50-l00%, and the incidence of dysplasia (including hyperplasia) 1 was also observed to occur in a dose-related manner in explants exposed to benz[a]anthracene, benzo[b]fluoranthene, and benzo[k]fluoranthene. These lesions were not seen in the cultures exposed to benzo[e]pyrene, pyrene, or DMSO. The authors note that these effects on respiratory epithelium seen *in vitro* are similar to the preneoplastic changes seen *in vivo* following exposure to PAHs, and thus, this system may serve as a good screen for assessing risk to the respiratory tract.

These observations, coupled with the fact that the respiratory system appears to be a target for PAH-induced cancer in humans, suggest that the respiratory system may be a target organ for PAH-induced noncancer adverse effects in humans as well.

Cardiovascular Effects. PAHs are contained in cigarette smoke, and smoking is a well-established risk factor in the development of atherosclerosis. Arterial smooth muscle cell proliferation, collagen synthesis, lipid accumulation, and cellular necrosis are all involved in the pathogenesis of the atherosclerotic plaque. *In vitro* studies conducted using bovine, rabbit, and human smooth muscle cells from arteries demonstrated that benzopyrene affects some of the aforementioned processes. Cell proliferation was not affected by benzo[a]pyrene, but a decrease in collagen secretion and an increase in cellular toxicity were noted in both the animal and human cell cultures (Stavenow and Pessah-Rasmussen 1988).

Male White Leghorn chickens (six/group) were given weekly intramuscular injections of benzo[a]pyrene, benzo[e]pyrene, anthracene, and dibenz[a,h]anthracene for 16 weeks prior to removal of the abdominal aorta to investigate the effects of benzo[a]pyrene on the development of arteriosclerotic plaques (Penn and Snyder 1988). Animals injected with DMSO (the vehicle) served as controls. Microscopic plaques were found in the aortas of all treated and control animals. However, the plaque volume index (PVI), which is a measure of both plaque cross-sectional area and plaque length, was nine times larger in the benzo[a]pyrene animals than the controls. Benzo[e]pyrene and dibenz[a,h]anthracene also caused an increase in plaque volume as compared to controls. However, the plaque sizes in the animals treated with anthracene were no different than controls. Therefore, the authors concluded that benzo[a]pyrene, benzo[e]pyrene, and dibenz[a,h]anthracene “promoted” the development of preexisting atherosclerotic plaques in male chickens as opposed to initiating the development of new plaques. The ability to promote plaque development was not correlated with the mutagenicity or carcinogenicity of the PAH tested. Similarly, administration of benzo[a]pyrene or benzo[e]pyrene into atherosclerosis-susceptible or atherosclerosis-resistant pigeons for 3-5 months of treatment indicated that benzo[a]pyrene, but not benzo[e]pyrene, enhanced the formation of arterial lesions in female, but not male, birds (Hough et al. 1993). Female pigeons were also infertile, and showed ovarian abnormalities.

These results, therefore, suggest that PAHs may contribute to the pathogenesis of atherosclerosis in humans. This is a particularly relevant health risk for those individuals who are exposed to high levels of PAHs in the environment and who also smoke cigarettes.

Gastrointestinal Effects. Anthracene has been associated with gastrointestinal toxicity in humans. Humans that consumed laxatives that contained anthracene (anthracene concentration not specified) for

prolonged periods were found to have an increased incidence (73.4%) of melanosis of the colon and rectum as compared to those who did not consume anthracene-containing laxatives (26.6%) (Badiali et al. 1985). The authors suggested that the melanosis observed may be attributed to the consumption of anthracene laxatives and not to intestinal stasis. This study is severely limited because of confounding factors such as the existence of other predisposing factors for melanosis and lack of follow-up.

Several PAHs discussed in this profile have been shown to alter enzyme activity in the intestinal mucosa of animals following oral administration, which could conceivably lead to increased production of reactive intermediates and tissue injury. Given the selectivity of PAHs for rapidly proliferating tissues such as gastrointestinal mucosa and the results discussed above, exposure to PAHs (particularly oral) by humans could lead to adverse gastrointestinal effects.

Hematological Effects. Adverse hematological effects have been observed in animals following exposure to PAHs. For example, administration of a single intraperitoneal dose of benzo[a]pyrene to mice resulted in a small spleen, marked cellular depletion, prominent hemosiderosis, and follicles with large lymphocytes. These pathological lesions were associated with death (Shubik and Porta 1957). Death due to adverse hematological effects (e.g., aplastic anemia and pancytopenia resulting in hemorrhage) has also been observed in mice following intermediate-duration oral exposure to benzo[a]pyrene (Robinson et al. 1975). Fluoranthene and fluorene administered by gavage to male and female mice for 13 weeks caused hematological effects including decreased packed cell volume and decreased hemoglobin content (EPA 1988e, 1989e). In addition, it has been shown that benzo[a]pyrene is toxic to cultured bone marrow cells when applied directly (Legraverend et al. 1983)

PAHs appear to affect other blood elements, as well. The influence of several PAHs on calcium ionophore-induced activation of isolated rabbit platelets was studied (Yamazaki et al. 1990). The activation of the platelets was assessed by measuring thromboxane B_2 synthesis in response to stimulation by the calcium ionophore, A-23 187. The authors reported that thromboxane B_2 synthesis was inhibited by incubation of the stimulated platelets with benz[a]anthracene, chrysene, benzo[a]pyrene, and benzo[g,h,i]perylene, and stimulated by incubation with anthracene and pyrene. However, no statistical analysis was performed on these data, and the changes reported are generally within ±10% of control values. In addition, the effects of the PAHs on thromboxane B_2 synthesis are bidirectional, and in many instances, the same compound induced both inhibition and stimulation at different concentrations.

As discussed above, PAHs tend to exert their adverse effects on rapidly proliferating tissues, such as the bone marrow blood forming elements. It is likely that PAH-induced toxicity in this tissue is due to a specific attack on DNA of cells in the S or synthetic phase of mitosis (EPA 1988a).

Although the human data available on PAH-induced hematological toxicity are flawed by confounding factors, they, together with the animal data and the propensity for PAHs to attack rapidly proliferating tissues, indicate that humans exposed to PAHs may be at risk for developing hematological toxicity.

Hepatic Effects. No adverse hepatic effects have been reported in humans following exposure to PAHs. However, hepatic effects have been observed in animals following acute oral, intraperitoneal, or subcutaneous administration of various PAHs. These effects include the induction of preneoplastic hepatocytes, known as y-glutamyl transpeptidase foci, induction of carboxylesterase and aldehyde dehydrogenase activity, an increase in liver weight, and stimulation of hepatic regeneration (an indication of a proliferative effect) (Danz et al. 1991; Gershbein 1975; Kemena et al. 1988; Robinson et al. 1975; Shubik and Porta 1957; Torronen et al. 1981; Tsuda and Farber 1980). These hepatic changes are not considered serious adverse effects, but their incidence and severity have been shown to correlate with the carcinogenic potency of particular PAHs. Thus, monitoring of liver function and tissue integrity may prove useful in the evaluation of PAH exposure.

More serious effects indicative of hepatic injury have been observed in animals. For example, an acute intraperitoneal injection of phenanthrene to rats resulted in liver congestion with a distinct lobular pattern, and an increase in serum aspartate aminotransferase, gamma-GT, and creatinine (Yoshikawa et al. 1987). Similarly, a single intraperitoneal injection of pyrene resulted in minimal swelling of the liver but no significant alterations in serum chemistry. Longer-term administration of PAHs has also been reported to result in adverse hepatic effects in animals. For example, increased absolute and relative liver weight correlated with hepatocellular hypertrophy was seen in male and female mice given 350 mg/kg/day acenaphthene by gavage for 13 weeks (EPA 1989c). Increased liver weight and dose-related centrilobular pigmentation accompanied by an increase in liver enzymes was observed in both male and female mice receiving 250 mg/kg/day fluoranthene by gavage for 13 weeks (EPA 1988e).

Renal Effects. Adverse renal effects associated with PAHs have not been reported in humans. A single injection of anthracene or fluorene had no adverse effect on the kidneys of mice (Shubik and

Porta 1957). Dilated tubules were observed in the kidneys of mice administered pyrene in the diet for 25 days (Rigdon and Giannukos 1964); the toxicological significance of this effect is not known. Renal tubular regeneration, and interstitial lymphocytic infiltrates and/or fibrosis were observed after 13-week oral administration of fluoranthene to female mice at 250 mg/kg/day, and to male mice at 500 mg/kg/day (EPA 1988e). Given the lack of renal toxicity in humans and the limited value of the observations made in animals, the risk to humans for renal toxicity following exposure to PAHs is not known.

Endocrine Effects. There is suggestive evidence that PAHs may adversely affect endocrine function as well. The number of thymic glucocorticoid receptors in 6-week-old rats treated once with 2 mg/kg benzo[a]pyrene was measured (Csaba et al. 1991). It is assumed that the route of exposure was by oral gavage, but this was never explicitly stated. The number of these receptors was decreased by 40% in females and was unaffected in males relative to the vehicle control animals. The statistical significance of these effects was not indicated, nor was the adversity of a decrease in receptor number assessed by examination of functional parameters.

Dermal Effects. The skin is susceptible to PAH-induced toxicity in both humans and animals. Regressive verrucae were reported following intermediate-duration application of benzo[a]pyrene to human skin (Cottini and Mazzone 1939). Although reversible and apparently benign, these changes were thought to represent neoplastic proliferation. Benzo[a]pyrene application also apparently exacerbated skin lesions in patients with pre-existing skin conditions (pemphigus vulgaris and xeroderma pigmentosum) (Cottini and Mazzone 1939). Workers exposed to substances that contain PAHs (e.g., coal tar) experienced chronic dermatitis and hyperkeratosis (EPA 1988a). Coal tar preparations containing PAHs are used in the therapeutic treatment of some skin disorders. Adverse reactions have been noted in these patients, also.

Adverse dermatological effects have also been noted in animals in conjunction with acute and intermediate-duration dermal exposure to PAHs. These effects include destruction of sebaceous glands, skin ulcerations, hyperplasia, and hyperkeratosis (Bock and Mund 1958), and alterations in epidermal cell growth (Albert et al. 1991b; Elgjo 1968).

The observation that PAHs adversely affected the skin in both humans and animals is not surprising. The skin undergoes rapid cell turnover and is thus a likely target for PAH attack on DNA synthesis.

Given the information discussed above, the ubiquitous nature of PAHs in the environment, and the susceptibility of the skin to PAH-induced toxicity, adverse skin effects may occur in individuals exposed to these chemicals by the dermal route.

Immunological and Lymphoreticular Effects. Humoral immunity was depressed in male iron foundry workers exposed to benzo[a]pyrene (Szczeklik et al. 1994). IgG, and IgA were depressed in those workers exposed to high levels. There are reports in the literature concerning the immunotoxicity of PAHs following dermal and parenteral exposure in animals. The carcinogenic PAHs as a group have an immunosuppressive effect. There are limited data that suggest that the degree of immunosuppression correlates with the carcinogenic potency. For example, using spleen cell cultures from C3H/Anf mice, suppression of humoral immunity (as measured by the plaque-forming cell [PFC] response to sheep red blood cells) and cell-mediated immunity (as measured by the one-way mixed lymphocyte response) were observed following incubation with 10^{-5}-10^{-7} mol benzo[a]pyrene (Urso et al. 1986). There was no loss in cell viability at these concentrations. These immunological responses were unaffected by treatment with equivalent concentrations of benzo[e]pyrene. These findings led the authors to speculate that carcinogenic PAHs alter immune function, thereby allowing the induction of carcinogenesis while noncarcinogenic PAHs do not affect immune function. In addition, benzo[a]pyrene, but not benzo[e]pyrene, in the presence of S9 metabolic activation mix, has been shown to inhibit interferon induction by viruses by 60-70% in cultured LLC-MK_2 cells (Hahon and Booth 1986).

Benzo[a]pyrene has been shown to markedly inhibit the immune system, especially T-cell dependent antibody production by lymphocytes exposed either *in vivo* or *in vitro* (Blanton et al. 1986; Lyte and Bick 1985; White and Holsapple 1984). These effects are generally seen at high dose relative to those that can induce cancer in animals.

The effects of benzo[a]pyrene on several parameters of cell-mediated immune function in isolated and T-cell enriched mononuclear cell populations from three strains of mice (C57, C3H, and DBA) given a single intraperitoneal injection of 10-50 mg/kg benzo[a]pyrene or benzo[e]pyrene following stimulation with phytohemagglutinin (PHA) were studied (Wojdani and Alfred 1984). Neither benzo[a]pyrene nor benzo[e]pyrene had an inhibitory effect on lymphocyte blastogenesis induced by PHA; blastogenesis was slightly stimulated at 2.5 and 10 mg/kg of either PAH. Dose-related suppression of cell-mediated cytotoxicity of allosensitized lymphocytes was observed in all strains of

mice treated with benzo[a]pyrene, but no effect on this parameter was observed following treatment with benzo[e]pyrene. The percentage and adherence of macrophages from benzo[a]pyrene-treated mice were increased. The authors suggest that benzo[a]pyrene, but not benzo[e]pyrene, causes alterations in cell-mediated immune function that could compromise the animal's immune function allowing the development of PAH-induced tumors. A major limitation of this study was the lack of statistical analysis, thereby making it difficult to determine the validity of the changes seen. As mentioned previously, relatively high doses of benzo[a]pyrene were employed in these studies.

Benzo[a]pyrene-induced immune suppression was reported in male $B6C3F_1$ mice (Lyte and Bick 1985) and in the offspring of C3H/Anf mice treated intraperitoneally with benzo[a]pyrene (Urso and Gengozian 1980). Cell-mediated and humoral immune function of the liver, thymus, and spleen were evaluated in both maternal animals and the offspring of C3H mice administered one intraperitoneal dose of benzo[a]pyrene (150 mg/kg) during "mid-pregnancy" (Urso et al. 1992). The offspring were evaluated at 1 week and 18 months of age. Suppression of these various aspects of the immune system was observed in both the mothers and the offspring at these relatively high doses. However, the study lacked sufficient detail to adequately assess either the protocol or the results.

Groups of four $B6C3F_1$ female mice were administered single injections of 0, 50, or 200 mg/kg benzo[a]pyrene in corn oil to study the correlation between DNA adduct formation (as measured by ^{32}P-postlabelling analysis) and the suppression of polyclonal immune responses (^{3}H-TdR incorporation following stimulation by *Escherichia coli* lipopolysaccharide [LPS] and concanavalin A [Con A] and IgM secretion) and decreased cell viability in splenic lymphocytes harvested from the treated mice (Ginsberg et al. 1989). Spleen weight was significantly decreased (18%, $p<0.05$) at 50 mg/kg. The polyclonal response to LPS and Con A was suppressed by 30-45%, and this suppression was statistically significant at 200 mg/kg. IgM secretion was also significantly depressed (42%) at 200 mg/kg. These immunosuppressive effects were accompanied by high levels of benzo[a]pyrene/DNA adducts. The authors speculated that the immunosuppressive effects of benzo[a]pyrene were due to a cytotoxic mechanism (as supported by *in vitro* experiments) that in turn resulted partially from the genotoxic effects of benzo[a]pyrene (i.e., the formation of benzo[a]pyrene/DNA adducts).

Benzo[a]pyrene exerts its inhibitory effects on antibody production through alterations on the normal functioning of macrophages, T cells, and B cells (Blanton et al. 1988; Zhao et al. 1990). In contrast,

benzo[a]pyrene has no effect on most cellular immune responses before the appearance of tumors (Dean et al. 1983b), although benzo[a]pyrene exposure does inhibit IL-2-dependent proliferation (Myers et al. 1988).

Benzo[a]pyrene may also induce autoimmune responses. Groups of eight female Sprague-Dawley rats were administered a single subcutaneous injection of 2 mg benzo[a]pyrene or benzo[e]pyrene (11.1 mg/kg) in sesame oil in the right thigh (Faiderbe et al. 1992). The animals were observed for up to 150 days and blood samples were taken at regular intervals to measure anti-phosphatidylinositol (PtdIns) antibodies. Serum levels of anti-PtdIns in animals treated with benzo[a]pyrene exceeded those of the oil-injected controls after day 10, and the difference became statistically significant ($p<0.05$) after day 40. The levels reached a peak at day 60 after which time they reached a plateau. The anti-PtdIns were of the IgG type and specific to phosphatidylinositol. Malignant sarcomas developed at the injection site in the animals treated with benzo[a]pyrene within 100-120 days. Serum levels of anti-PtdIns in animals treated with benzo[e]pyrene did not differ from those of the oil-injected controls. No malignant sarcomas developed at the injection site in 100% of the animals administered benzo[e]pyrene within 100-120 days. The authors speculated that constant stimulation of lymphocytes reactive for PtdIns by an endogenous antigen, of which PtdIns could be a part, was responsible for the increased serum levels of anti-PtdIns. The authors suggested that PtdIns metabolism is altered in rapidly proliferating malignant cells (the neoplasia being stimulated by benzo[a]pyrene), resulting in the synthesis of the PtdIns-containing antigen. The lack of an autoimmune response to benzo[e]pyrene was due to the fact that benzo[e]pyrene was not carcinogenic; there was no neoplastic transformation occurring that could result in the production of PtdIns-containing antigens such as was seen with benzo[a]pyrene. Therefore, this study provides evidence that benzo[a]pyrene-induced neoplasia may cause an alteration in the metabolism of endogenous substances, resulting in the production of autoimmune antibodies to those substances.

The immunotoxic effects of benzo[a]pyrene have been noted *in vitro* as well (e.g., Ladies et al. 1991), and these studies provide some insight into the mechanism of action of benzo[a]pyrene-induced immunological effects. Splenic lymphocytes from $B6C3F_1$ mice were incubated with various concentrations of benzo[a]pyrene for either 2 hours or the entire culture period (Ginsberg et al. 1989). A dose- and duration-related decrease in splenic lymphocyte viability (as measured by ^{3}H-TdR incorporation) and immune response (as measured by IgM secretion) was observed in the absence of S9 activation. Addition of S9 enhanced this effect after acute-duration exposure. However, there was

very little formation of benzo[a]pyrene/DNA adducts at benzo[a]pyrene concentrations of l-200 µmol in the splenic lymphocytes; this lack of effect was accompanied by a very low level of benzo[a]pyrene metabolism to DNA-adducting metabolites. Benzo[a]pyrene/DNA adducts were measured in liver and lung; however, in the *in vivo* experiment. This led the authors to suggest that benzo[a]pyrene-induced immunotoxicity as expressed by splenic lymphocytes was the result of a cytotoxic effect that was mediated, in part, by a genotoxic mechanism involving the formation of benzo[a]pyrene/DNA adducts remote from the spleen and a direct cytotoxic effect not requiring activation of benzo[a]pyrene to the reactive intermediate.

Incubation of human lymphocytes with 0.1-01.0 µg/mL benzo[a]pyrene resulted in a suppression of lymphokine-activated killer cell (LAK) activity against tumor targets after 3 and 7 days (Lindemann and Park 1989). LAK DNA synthesis was also inhibited after 3 or 7 days of incubation with benzo[a]pyrene. However, benzo[a]pyrene had no effect on LAK binding with tumor targets, and benzo[a]pyrene did not interfere with the cytotoxic effect of natural killer cells added to the incubation medium. Based on these results, the authors concluded that benzo[a]pyrene interferes with the development of the immunological defense killer cells.

Benzo[a]pyrene has also been shown to affect immune responses to viral infection. Benzo[a]pyrene can reversibly inhibit the induction of viral interferon in 32 different mammalian cell lines but only in the presence of S9 metabolic activation (Hahon and Booth 1988). This inhibition must occur at an early level and not affect viral interferon interactions because the activity of exogenous interferon was unaffected. In addition, influenza virus multiplication was also inhibited by activated benzo[a]pyrene. Benzo[e]pyrene had no effect on interferon induction. The authors suggest that benzo[a]pyrene's inhibition of interferon induction may be an early step in compromising the host's immune function, thereby allowing the induction of carcinogenesis.

There is evidence to suggest that PAHs may alter the levels of brain neurotransmitters, which in turn affects the function of the immune system. The levels of two catecholamines, dopamine and norepinephrine, were determined in discrete brain areas in mice in which fibrosarcomas had been induced following a single subcutaneous injection of benzo[a]pyrene (Dasgupta and Lahiri 1992). Both dopamine and norepinephrine levels were significantly decreased in some brain regions (e.g., the corpus striatum and the hypothalamus), and these decreases were evident in both early and late tumor development. The authors state that since immunological function is compromised during

carcinogenesis and certain alterations in brain catecholamines impair immune function, the mechanism by which carcinogens such as benzo[a]pyrene cause immunosuppression and subsequent carcinogenesis may be via depression of brain catecholamines.

Very little information is available on the immunological effects of other PAHs. Mice treated with high doses of dibenz[a,h]anthracene exhibited a reduced serum antibody level in response to antigenic challenge by comparison to controls (Malmgren et al. 1952). The immunosuppressive effects of dibenz[a,h]anthracene were studied in AHH-inducible mice (C57BL/6) and AHH-noninducible mice (DBA/2N) by intraperitoneal and oral administration (Lubet et al. 1984). Immunosuppression occurred in both strains and was more pronounced in the C57BL/6 mice than in the DBA/2N mice, following intraperitoneal administration. However, the DBA/2N mice were more susceptible to immunosuppression following oral administration. These results suggest that PAHs are rapidly metabolized and excreted following oral administration in AHH-inducible mice, whereas in AHH-noninducible mice, the PAHs are absorbed and distributed to target organs. Based on these results, the authors concluded that AHH inducibility plays an important role in the immunosuppressive activity of PAHs.

B-cell lymphopoiesis in mouse bone marrow has been shown to be inhibited by incubation with fluoranthene *in vitro* at concentrations of ≥5 µg/mL (25 µmol). This effect on B-cell precursors may be mediated in part by a stimulation of programmed cell death; as demonstrated by the increase in DNA fragmentation induced by fluoranthene 15-17 hours after addition to the incubation medium. Furthermore, fluoranthene-induced DNA fragmentation always preceded fluoranthene-induced B-cell precursor death. Another mechanism for fluoranthene-induced inhibition of B-cell lymphopoiesis may be alterations in cell growth rates (fluoranthene was shown to slow the rate of B-cell precursor growth at concentrations <5 µg/mL) and/or altered cell survival (Hinoshita et al. 1992).

The lymphoid system, because of its rapidly proliferating tissues, is susceptible to PAH-induced toxicity. The mechanism of action for this effect is most likely inhibition of DNA synthesis. No adverse effects on this system associated with PAH exposure have been reported in humans, but several accounts of lymphoid toxicity in animals are available. A single intraperitoneal injection of benzo[a]pyrene to mice resulted in a small spleen with marked cellular depletion, prominent and edematous trabeculae, and large lymphocytes. These lesions resulted in death (Shubik and Porta 1957). The Shubik and Porta (1957) study was severely limited by the following: the benzo[a]pyrene

was only partly in solution, only one dose was employed, there was a small sample size, the purity of benzo[a]pyrene was not specified, only one sex was tested, and the presence of benzo[a]pyrene in the peritoneal cavity indicates inadequate absorption. No other similar studies were found in the literature.

Even though these effects have not been noted in humans, and the data in animals are contained in only one study, the rapidly proliferating nature of this tissue suggest that humans exposed to PAHs may be a risk for the development of adverse effects on the lymphoid system.

Given the high potential for exposure to PAHs in the vicinity of hazardous waste sites, the evidence from animal studies, and the heterogeneity of human genotypes with regard to enzyme induction capabilities, it would be prudent to consider that PAHs may pose an immunotoxic risk to humans living in areas surrounding hazardous waste sites.

Neurological Effects. No information is available on the short- or long-term neurotoxic effects of exposure to PAHs in humans and animals. Acute-, intermediate-, or chronic-duration studies conducted in animals do not indicate that any of the PAHs tested showed evidence of neurotoxicity, although these tests were not designed to detect subtle neurological changes.

However, there is evidence to suggest that PAHs may alter the levels of brain neurotransmitters. The levels of two catecholamines, dopamine and norepinephrine, were determined in discreet brain areas in mice in which fibrosarcomas had been induced following a single subcutaneous injection of benzo[a]pyrene (Dasgupta and Lahiri 1992). The mice were divided into two groups: early tumor development and late tumor development 3-4 months after administration of the benzo[a]pyrene. Both dopamine and norepinephrine levels were significantly decreased in some brain regions (e.g., the corpus striatum and the hypothalamus), and these decreases were evident in both early and late tumor development. The authors state that because immunological function is compromised during carcinogenesis and because certain alterations in brain catecholamines impair immune function, the mechanism by which carcinogens such as benzo[a]pyrene cause immunosuppression and subsequent carcinogenesis may be via depression of brain catecholamines.

Reproductive Effects. In both prospective and retrospective studies, a decrease in fecundity was observed in women who were exposed prenatally to cigarette smoke (i.e., their mothers smoked when pregnant) (Weinberg et al. 1989; Wilcox et al. 1989). This association was apparent even after

adjustment for age, frequency of intercourse, current smoking status, age at menarche, childhood exposure to cigarette smoking, educational level, reproductive history, body weight, and consumption of alcohol and caffeine. On the other hand, an increase in fecundity was observed in a retrospective study of women who were exposed to cigarette smoke in early childhood. These apparently opposite effects may be partially explained by the fact that when a woman smokes during pregnancy, her fetus is exposed to the components of the cigarette smoke that cross the placenta as well as changes in fetal and placental oxygenation and metabolism that are secondary to changes in maternal metabolism resulting from smoking. However, childhood exposure involves direct inhalation of cigarette smoke. The authors could offer no explanation as to why fecundity should be *increased* as a result of childhood exposure to cigarette smoke (Weinberg et al. 1989; Wilcox et al. 1989).

The testes and ovaries contain rapidly proliferating cells and therefore should be considered susceptible to damage by PAHs. The reproductive toxicity data in animals for the PAHs are limited. The available animal studies exclusively discuss the reproductive effects of benzo[a]pyrene. Adverse effects such as decreased fertility and total sterility in F_1 progeny of CD-1 mice (Mackenzie and Angevine 1981) and decreased incidence of pregnant female rats at parturition (Rigdon and Rennels 1964) were reported following oral exposure to benzo[a]pyrene. However, no adverse reproductive effects were observed in Swiss mice fed benzo[a]pyrene in their diet (Rigdon and Neal 1965). The metabolic differences and method of benzo[a]pyrene administration could account for the differential response to benzo[a]pyrene induced toxicity in these studies. A single intraperitoneal injection of benzo[a]pyrene to female C57BL/6N mice decreased the number of corpora lutea (Swartz and Mattison 1985). The, antiestrogenic effects causing decreased uterine weights in pseudopregnant Sprague-Dawley rats were reported following daily subcutaneous injections of benzo[a]pyrene during days 6-11 of pseudopregnancy (Bui et al. 1986). Similar treatment to pregnant rats during gestation caused resorptions, reduced percentage of viable litters, and decreased uterine weights (Bui et al. 1986; Cervello et al. 1992). Female mice exhibited decreased ovary weights after 13 weeks oral exposure to 700 mg/kg/day acenaphthene (EPA 1989c). The studies conducted on the reproductive effects of benzo[a]pyrene via parenteral routes are briefly discussed below.

Single intraperitoneal injection of benzo[a]pyrene to female C57BL/6N mice at doses as high as 500 mg/kg body weight produced a dose- and time-dependent decrease in the number of corpora lutea (Swartz and Mattison 1985). The NOAEL in this study was 1 mg/kg/day. Groups of 20 C57BL/6N mice were given single intraperitoneal injections of 0-500 mg/kg benzo[a]pyrene (Miller et al. 1992)

and were killed at various intervals after injection. Total ovarian volume, total corpora lutea volume, and total number of corpora lutea per ovary were significantly reduced by doses of benzo[a]pyrene >5 mg/kg in a dose-related manner. These effects resolved in a dose- and time-dependent fashion, so that after 4 weeks, most changes were only evident in the animals treated with the 100 or 500 mg/kg benzo[a]pyrene. Individual corpora lutea volume actually increased in the treated animals, indicating that compensatory hypertrophy was probably occurring. The authors concluded that based on these findings and previous findings, benzo[a]pyrene impairs corpora lutea formation by destroying follicles. In another study, DBA/2N (D2), C57BL/6N (B6), and (DBA/2N x C57BL/6N)F_1 (F_1) mice (7-8/group) were injected with 10 µg of either benzo[a]pyrene or one of three different metabolites of benzo[a]pyrene ([+]7,8-oxide, [-]7,8-dihydrodiol, or [+]-diol-epoxide-2) into the right ovary (Mattison et al. 1989). The left ovary served as a control, and an additional control group injected with the vehicle (DMSO) also served as controls. Ovarian volume, wet weight, and small, growing, and large follicle number were measured in both the treated and contralateral control ovaries. Benzo[a]pyrene and one or more of its'metabolites caused decreases in the treated ovarian weight, the ovarian volume, and the small, growing, and large follicles in one or more strains. In most instances, the contralateral untreated ovary exhibited a compensatory response; ovarian weight and volume increased as compared to the DMSO controls. This study shows that benzo[a]pyrene and some of its metabolites are toxic to the ovaries of mice, and that the ovary is capable of metabolizing benzo[a]pyrene into reactive metabolites. Similarly, administration of benzo[a]pyrene or benzo[e]pyrene into atherosclerosis-susceptible or atherosclerosis-resistant pigeons for 3-5 months of treatment indicated that benzo[a]pyrene, but not benzo[e]pyrene, rendered female pigeons infertile, with ovarian abnormalities (Hough et al. 1993). Cumulatively, these results demonstrate the sensitivity of integrated hypothalamic-pituitary-ovarian function to adverse effects of benzo[a]pyrene.

Daily subcutaneous injection of benzo[a]pyrene beginning on day 6 of gestation for 6 days as opposed to 3 days significantly increased the number of resorptions, and decreased the fetal survival and uterine weights in Sprague-Dawley rats (Bui et al. 1986). In pseudopregnant (i.e., condition occurring following sterile matings in which anatomical and physiological changes occur similar to those of pregnancy) rats, similar benzo[a]pyrene treatment during days 6-l1 of pseudopregnancy significantly decreased the cyclic nucleotide levels and uterine weights suggesting an antiestrogenic effect (Bui et al. 1986). Use of a single dosage level precluded the assessment of dose response in these studies.

Pregnant Sprague-Dawley rats were administered subcutaneous injections of benzo[a]pyrene (in DMSO and corn oil) or the vehicle alone on gestation days 7, 9, 11, 13, and 15 (Cervello et al. 1992). The animals were sacrificed on gestation day 16. There were no maternal deaths or signs of maternal toxicity. However, the number of fetuses per litter and number of live fetuses per litter were significantly decreased in the animals treated with benzo[a]pyrene, and the number of resorptions was significantly increased. In addition, uterine weight, and whole uterine gravid weight were significantly decreased and increased, respectively. These results demonstrate the reproductive toxicity of benzo[a]pyrene, but a dose-response relationship could not be established because only one dose was tested.

These results suggest that the potential for adverse reproductive effects may be increased in humans exposed to benzo[a]pyrene in the workplace or at hazardous waste sites.

Developmental Effects. The developmental toxicity data for PAHs are mostly limited to *in utero* exposure of pregnant animals to benzo[a]pyrene via various routes of exposure. The placental transfer of benzo[a]pyrene has been shown in mice following oral and intravenous exposure of dams (Shendrikova and Aleksandrov 1974) and in rats after intratracheal administration (Srivastava et al. 1986). The available data from oral studies in animals indicate that exposure of pregnant dams to benzo[a]pyrene produced resorptions and malformations in fetuses (Legraverend et al. 1984) and sterility in F_1 mouse progeny (Mackenzie and Angevine 1981). Investigations by Legraverend et al. (1984) suggest that benzo[a]pyrene metabolites generated in the fetus rather than in the maternal tissues are responsible for these adverse effects. Also, the genetic differences observed in this study using the oral route were contrary to those induced by intraperitoneal administration of benzo[a]pyrene (Hoshino et al. 1981; Shum et al. 1979), thus emphasizing the importance of route of administration in benzo[a]pyrene metabolism and resulting toxicity.

The developmental effects of benzo[a]pyrene have also been investigated in animals using the parenteral route of administration. Intraperitoneal injection of benzo[a]pyrene to pregnant mice produced stillbirths, resorptions and malformations at a greater incidence in Ah-responsive mice than in Ah-nonresponsive mice (Shum et al. 1979); testicular changes including atrophy of seminiferous tubules with lack of spermatids and spermatozoa; interstitial cell tumors (Payne 1958); immunosuppression (Urso and Gengozian 1980); and tumor induction (Bulay and Wattenberg 1971; Soyka 1980). Adverse effects observed following subcutaneous injection of benzo[a]pyrene include

increased fetal resorptio'ns in rats (Wolfe and Bryan 1939) and lung tumor induction in mice (Nikonova 1977). Decreased fetal survival (Wolfe and Bryan 1939) and lung tumor development (Rossi et al. 1983) were reported in Swiss mice following direct intra-embryonal injection of benzo[a]pyrene.

Results of *in vitro* studies suggest that benzo[a]pyrene may affect a number of enzyme and hormone activities in the human placenta. The effects of benzo[a]pyrene on the binding of epidermal growth factor (EGF) and receptor autophosphorylation were studied in human placental cell cultures from early and late gestation placentas (Guyda et al. 1990). In a subsequent study, the effects of benzo[a]pyrene on the uptake of aminoisobutyric acid (AIB) by early and late gestational human placental cells was also studied (Guyda 1991). Benzo[a]pyrene decreased binding of EGF (37-60%) to the early gestation placental cells, but not the late gestation placental cells. The decrease in binding was due to a decrease in the number of high-affinity EGF binding sites. This effect was specific for EGF receptor sites and not due to a nonspecific effect of benzo[a]pyrene on the membranes because benzo[a]pyrene had no effect on the binding of ^{125}I-labeled insulin and insulin-like growth factors. The authors concluded that the effects of benzo[a]pyrene on EGF binding were specific and related to gestational age. Benzo[a]pyrene stimulated AIB uptake by both early and late gestational cells and enhanced EGF-stimulated AIB uptake in spite of a decrease in the number of EGF receptors. The implications of these finding are that benzo[a]pyrene could alter EGF-induced secretion of human chorionic gonadotrophin and human placental lactogen secretion as well as metabolic functions, thereby affecting the regulation of cell growth and differentiation in human placentas.

The activity of quinone reductase, a major protective enzyme, was increased 2-3-fold in first trimester human placental extracts *in vitro* when incubated for 6 hours with benz[a]anthracene, dibenz[a,h]anthracene, and chrysene at a concentration of 50 µmol (Avigdor et al. 1992). Based on these results, it can be postulated that the early placenta is capable of metabolizing certain toxic xenobiotics such as PAH quinone metabolites to inactive intermediates thereby protecting the developing embryo.

Benzo[a]pyrene (50 µmol) has been shown to stimulate human gonadotropin release by first trimester human placental explants *in vitro* (Bamea and Shurtz-Swirski 1992). This stimulation was evident following static exposure for 24 hours and also in cultures that were superfused, meaning the benzo[a]pyrene had a delayed effect and did not need to be present for this effect to be expressed.

The implication of these findings is that benzo[a]pyrene can alter human placental endocrine function early in pregnancy.

Results of animal and *in vitro* studies suggest that benzo[a]pyrene may produce adverse effects in the offspring of women exposed during pregnancy. Furthermore, the results of genetic studies conducted via oral and intraperitoneal routes emphasize the importance of route of administration in benzo[a]pyrene metabolism and resulting toxicity and the severity of the effect may vary depending upon the genotype of the individual exposed (see Section 2.7, Populations That Are Unusually Susceptible). Based on these observations, it is therefore prudent to consider that the genetically heterogeneous human population may show variation in response to *in utero* exposure to benzo[a]pyrene.

Other PAHs such as anthracene, benzanthracene, chrysene, and dibenz[a,h]anthracene have also been tested for developmental effects via parenteral routes. Of these compounds, dibenz[a,h]anthracene produced fetolethal effects in rats (Wolfe and Bryan 1939), while chrysene produced liver tumors in the mouse progeny (Buening et al. 1979a; Grover et al. 1975)

Genotoxic Effects. As the results presented in Tables 2-4 and 2-5 indicate, benzo[a]pyrene has been thoroughly studied in genetic toxicology test systems. It induces genetic damage in prokaryotes, eukaryotes, and mammalian cells *in vitro* and produces a wide range of genotoxic effects (gene mutations in somatic cells, chromosome damage in germinal and somatic cells, DNA adduct formation, UDS, sister chromatid exchange, and neoplastic cell transformation). In cultured human cells, benzo[a]pyrene binds to DNA and causes gene mutations, chromosome aberrations, sister chromatid exchange, and UDS.

The results of *in vivo* studies indicate that many of the same types of adverse effects observed *in vitro* were seen in mice, rats, and hamsters exposed to benzo[a]pyrene via the oral, dermal, or intraperitoneal routes. The available data also indicate that benzo[a]pyrene is genotoxic in both somatic and germinal cells of intact animals (Table 2-4). The only study that was found regarding genotoxic effects in humans following exposure to benzo[a]pyrene reported no correlation between aluminum plant workers' exposure to PAHs, including benzo[a]pyrene, and sister chromatid exchange frequency (Becher et al. 1984). The findings from assays using human cells as the target, in

TABLE 2-4. Genotoxicity of Polycyclic Aromatic Hydrocarbons *In Vivo*

Species (test system)	End point		Results	Reference
	ANTHRACENE			
Mammalian systems:				
Chinese hamster/bone marrow	Chromosome aberrations	–		Roszinsky-Kocher et al. 1979
Chinese hamster/bone marrow	Sister chromatid exchange		–	Roszinsky-Kocher et al. 1979
Mouse/bone marrow	Micronuclei		–	Salamone et al. 1981
Mouse	Sperm abnormalities	–		Topham 1980
Host-mediated systems:				
Salmonella typhimurium/mouse host-mediated	Gene mutation		(+)[a]	Simmon et al. 1979
Saccharomyces cerevisiae/mouse host-mediated	Gene mutation	–		Simmon et al. 1979
Chinese hamster V79 cells/mouse host-mediated	Sister chromatid exchange	–		Sirianni and Huang 1978
	BENZ(*a*)ANTHRACENE			
Mammalian systems:				
Chinese hamster/bone marrow	Chromosome aberrations	–		Roszinsky-Kocher et al. 1979
Chinese hamster/bone marrow	Sister chromatid exchange		+	Roszinsky-Kocher et al. 1979
Insect systems:				
Drosophila melanogaster/sex-linked recessives	Gene mutation		–	Zijlstra and Vogel 1984
D. melanogaster/somatic mutation	Gene mutation	–		Fahmy and Fahmy 1980
Host-mediated systems:				
S. typhimurium/mouse host-mediated	Gene mutation		+	Simmon et al. 1979
S. cerevisiae/mouse host-mediated	Gene mutation	–		Simmon et al. 1979
	BENZO(*b*)FLUORANTHENE			
Mammalian systems:				
Chinese hamster/bone marrow	Chromosome aberrations	–		Roszinsky-Kocher et al. 1979
Chinese hamster/bone marrow	Sister chromatid exchange		–	Roszinsky-Kocher et al. 1979
Mouse skin	DNA binding		+	Weyand et al. 1987, 1992a

TABLE 2-4. Genotoxicity of Polycyclic Aromatic Hydrocarbons *In Vivo* (continued)

Species (test system)	End point	Results	Reference
Rat/lung, liver, peripheral blood lymphocytes	DNA binding	+	Ross et al. 1992
Rat peripheral blood lymphocytes	Sister chromatid exchange	+	Ross et al. 1992
New born mice/lung, liver	DNA binding	+	Weyand et al. 1993b
	BENZO(*j*)FLUORANTHENE		
Mammalian systems:			
Mouse skin	DNA binding	+	La Voie et al. 1991a; Weyand et al. 1987, 1993a
	BENZO(*k*)FLUORANTHENE		
Mammalian systems:			
Mouse skin	DNA binding	+	Weyand et al. 1987
	BENZO(*g,h,i*)PERYLENE		
Host-mediated systems:			
Hamster embryos/transplacental exposure	Transformation	–	Quarles et al. 1979
	BENZO(*a*)PYRENE		
Mammalian systems:			
Mouse/dominant lethals	Gene mutation	+	Epstein 1968; Generoso et al. 1982; Russell 1977
Mouse/spot test	Gene mutation	+	Davidson and Dawson 1976, 1977
Rat hepatocytes/unscheduled DNA synthesis	DNA damage	–	Miralis et al. 1982
Rat hepatocytes/unscheduled DNA synthesis	DNA damage	–	Mullaart et al. 1989
Mouse germ cells/unscheduled DNA synthesis	DNA damage	–	Sega 1979
Mouse skin/lung	DNA binding	+	Mukhtar et al. 1986
Mouse skin	DNA binding	+	Morse et al. 1985; Rice et al. 1984; Weyand and Bevan 1987a

TABLE 2-4. Genotoxicity of Polycyclic Aromatic Hydrocarbons *In Vivo* (continued)

Species (test system)	End point	Results	Reference
Mouse/bone marrow	DNA binding	+	Wielgosz et al. 1991
Mouse/spleen cells	DNA binding	+	Wielgosz et al.1991
Rat liver parenchymal cells	DNA single strand breaks	+	Mullaart et al. 1989
Rat liver nonparenchymal cells	DNA single strand breaks	–	Mullaart et al. 1989
Rat intestinal cells	DNA single strand breaks	+	Mullaart et al. 1989
Mouse/bone marrow	Chromosome aberrations	+	Adler and Ingwersen 1989
Mouse/bone marrow	Chromosome aberrations	+	Adler et al. 1989
Mouse/embryos	Chromosome aberrations	+	Adler et al. 1989
Chinese hamster/bone marrow	Chromosome aberrations	+	Roszinsky-Kocher et al. 1979
Chinese hamster/bone marrow	Chromosome aberrations	(+)	Bayer 1978
Mouse/heritable translocation	Chromosome aberrations	–	Generoso et al. 1982
Mouse/bone marrow	Chromosome aberrations	+	Wielgosz et al. 1991
Mouse/spleen cells	Chromosome aberrations	+	Wielgosz et al. 1991
Chinese hamster/bone marrow	Sister chromatid exchange	+	Bayer 1978; Roszinsky-Kocher et al. 1979
Mouse/bone marrow	Micronuclei	+	Salamone et al. 1981
Mouse/bone marrow	Micronuclei	–	Bruce and Heddle 1979
Mouse/fetal liver	Micronuclei	+	Harper et al. 1989
Mouse/bone marrow	Micronuclei	+	Harper et al. 1989
Mouse/bone marrow	Micronuclei	+	Awogi and Sato 1989
Mouse/keratinocytes	Micronuclei	+	He and Baker 1991
Mouse/bone marrow	Micronuclei	+	Shimada et al. 1990

TABLE 2-4. Genotoxicity of Polycyclic Aromatic Hydrocarbons *In Vivo* (continued)

Species (test system)	End point	Results	Reference
Mouse/peripheral blood reticulocytes	Micronuclei	+	Shimada et al. 1992
Rat/peripheral blood reticulocytes	Micronuclei	+	Shimada et al. 1992
Chinese hamster/bone marrow	Micronuclei	–	Bayer 1978
Mouse	Sperm abnormalities	+	Bruce and Heddle 1979; Topham 1980
Mouse	Sperm abnormalities	(+)	Salamone et al. 1988
Mouse	Sperm abnormalities	+	Salamone and Logan 1988
Mouse/bone marrow	Micronuclei	+	Balansky et al. 1994
Mouse/bone marrow	Micronuclei	+	Koratkar et al. 1993
Mouse papilloma cells	Gene mutation	+	Colapietro et al. 1993
Human lung	DNA damage	+	Weston et al. 1993a
Mouse papilloma cells	Gene mutation	+	DiGiovanni et al. 1993
Insect systems:			
D. melanogaster/sex-linked recessive	Gene mutation	(+)	Vogel et al. 1983
D. melanogaster/sex-linked recessive	Gene mutation	–	Valencia and Houtchens 1981; Zijlstra and Vogel 1984
D. melanogaster/somatic mutation	Gene mutation	+	Fahmy and Fahmy 1980
D. melanogaster	Chromosome aberration	(+)	Vogel et al. 1983
Host-mediated systems:			
S. typhimurium/mouse host-mediated	Gene mutation	–	Glatt et al. 1985; Simmon et al. 1979
S. cerevisiae/mouse host-mediated	Gene mutation	–	Simmon et al. 1979
Chinese hamster V79/mouse host-mediated	Sister chromatid exchange	+	Sirianni and Huang 1978
Hamster embryos/transplacental exposure	Transformation	+	Quarles et al. 1979

TABLE 2-4. Genotoxicity of Polycyclic Aromatic Hydrocarbons *In Vivo* (continued)

Species (test system)	End point	Results	Reference
	BENZO(*e*)PYRENE		
Mammalian systems:			
Chinese hamster/bone marrow	Sister chromatid exchange	(+)	Roszinsky-Kocher et al. 1979
	CHRYSENE		
Mammalian systems:			
Chinese hamster/bone marrow	Chromosome aberrations	–	Roszinsky-Kocher et al. 1979
Chinese hamster/bone marrow	Sister chromatid exchange	+	Roszinsky-Kocher et al. 1979
Host-mediated systems:			
S. typhimurium/mouse host-mediated	Gene mutation	–	Simmon et al. 1979
S. cerevisiae/mouse host-mediated	Gene mutation	–	Simmon et al. 1979
	DIBENZ(*a,h*)ANTHRACENE		
Mammalian systems:			
Chinese hamster/bone marrow	Chromosome aberrations	–	Roszinsky-Kocher et al. 1979
Chinese hamster/bone marrow	Sister chromatid exchange	+	Roszinsky-Kocher et al. 1979
	FLUORANTHENE		
Mammalian systems:			
Mouse/bone marrow	Sister chromatid exchange	–	Palitti et al. 1986
	INDENO(1,2,3-*c,d*)PYRENE		
Mammalian systems:			
Mouse skin	DNA binding	+	Weyand et al. 1987
	PHENANTHRENE		
Mammalian systems:			
Chinese hamster/bone marrow	Chromosome aberrations	–	Bayer 1978; Roszinsky-Kocher et al. 1979

TABLE 2-4. Genotoxicity of Polycyclic Aromatic Hydrocarbons *In Vivo* (continued)

Species (test system)	End point	Results	Reference
Chinese hamster/bone marrow	Sister chromatid exchange	(+)	Bayer 1978; Roszinsky-Kocher et al. 1979
Chinese hamster/bone marrow	Micronuclei	–	Bayer 1978
Host-mediated systems: *S. typhimurium*/mouse host-mediated	Gene mutation	–	Simmon et al. 1979
S. cerevisiae/mouse host-mediated	Gene mutation	–	Simmon et al. 1979
Hamster embryos/transplacental exposure	Transformation	–	Quarles et al. 1979
	PYRENE		
Mammalian systems: Mouse/bone marrow	Micronuclei	–	Salamone et al. 1981
Mouse	Sperm abnormalities	–	Salamone et al. 1988
Mouse	Sperm abnormalities	–	Salamone and Logan 1988
Insect systems: *D. melanogaster*/sex-linked recessive	Gene mutation	–	Valencia and Houtchens 1981
Host-mediated systems Chinese hamster V79/mouse host-mediated	Sister chromatid exchange	–	Sirianni and Huang 1978

[a]A positive result was obtained in one experiment; this result was not reproduced in the same laboratory or in a second laboratory.

DNA = deoxyribonucleic acid; – = negative result; + = positive result; (+) = weakly positive result

TABLE 2-5. Genotoxicity of Polycyclic Aromatic Hydrocarbons *In Vitro*

Species (test system)	End point	Result		Reference
		With activation	Without activation	
		ACENAPHTHENE		
Prokaryotic organisms:				
Salmonella typhimurium	Gene mutation	–	No data	Pahlman and Pelkonen 1987
Escherichia coli SOS chromotest	Gene mutation	–	No data	Mersch-Sundermann et al. 1992b
		ACENAPHTHYLENE		
Prokaryotic organisms:				
S. typhimurium	Gene mutation	–	No data	Bos et al. 1988
		ANTHRACENE		
Prokaryotic organisms:				
S. typhimurium	Gene mutation	+	–	Sakai et al. 1985
S. typhimurium	Gene mutation	+	No data	Carver et al. 1986
S. typhimurium	Gene mutation	–	–	LaVoie et al. 1983b; 1985; Rosenkranz and Poirier 1979; Simmon 1979a
S. typhimurium	Gene mutation	–	No data	Bos et al. 1988; LaVoie et al. 1979; Pahlman and Pelkonen 1987
Escherichia coli/Pol A	DNA damage	–	–	Rosenkranz and Poirier 1979
E. coli WP2-WP100/rec-assay	DNA damage	–	No data	Mamber et al. 1983
E. coli/differential killing	DNA damage	–	–	Tweats 1981
E. coli SOS chromotest	DNA damage	–	No data	Mersch-Sundermann et al. 1992b
E. coli SOS chromotest	DNA damage	–	–	Mersch-Sundermann et al. 1992a
Eukaryotic organisms:				
Fungi: *Saccharomyces cerevisiae D3*	Miotic recombination	–	–	Simmon 1979b
S. cerevisiae D4-RDII	Gene conversion	No data	–	Siebert et al. 1981

TABLE 2-5. Genotoxicity of Polycyclic Aromatic Hydrocarbons *In Vitro (continued)*

Species (test system)	End point	Result		Reference
		With activation	Without activation	
Mammalian cells:				
Fischer rat embryo cells	Gene mutation	No data	–	Mishra et al. 1978
Mouse lymphoma L5178Y/TK$^{+/-}$	Gene mutation	(+)	No data	Amacher and Turner 1980
Mouse lymphoma L5178/TK$^{+/-}$	Gene mutation	–	No data	Amacher et al. 1980
Human lymphoblasts TK6	Gene mutation	–	No data	Barfknecht et al. 1982
Human epithelial cells EUE	Gene mutation	No data	–	Rochhi et al. 1980
HeLa cells/unscheduled DNA synthesis	DNA damage	–	–	Martin et al. 1978
Human skin fibroblasts	DNA damage	No data	–	Milo et al. 1978
Rat liver cells RL1	Chromosome aberrations	No data	–	Dean 1981
Rat liver cells ARL18	Sister chromatid exchange	No data	–	Tong et al. 1981
Hamster BHK21 clone 13	Transformation	–	No data	Greb et al. 1980
Syrian hamster embryo cells	Transformation	No data	–	Dunkel et al. 1981
Mouse C3H/10T1/2 clone 8	Transformation	No data	–	Dunkel et al. 1981
Mouse Balb/3T3 cells	Transformation	No data	–	Lubet et al. 1983b; Peterson et al. 1981
Fischer rat embryo cells	Transformation	No data	–	Mishra et al. 1978
Fischer rat embryo cells/leukemia virus transformation	Transformation	No data	–	Dunkel et al. 1981
	BENZ[a]ANTHRACENE			
Prokaryotic organisms:				
S. typhimurium	Gene mutation	+	–	Norpoth et al. 1984; Simmon 1979a

TABLE 2-5. Genotoxicity of Polycyclic Aromatic Hydrocarbons *In Vitro (continued)*

Species (test system)	End point	Result		Reference
		With activation	Without activation	
S. typhimurium	Gene mutation	+	No data	Bos et al. 1988; Carver et al. 1986; Coombs et al. 1976; Hermann 1981; Pahlman and Pelkonen 1987
S. typhimurium	Gene mutation	+[a]	No data	Phillipson and Ioannides 1989
S. typhimurium	Gene mutation	–[b]	No data	Phillipson and Ioannides 1989
S. typhimurium	Gene mutation	–	–	Rosenkranz and Poirier 1979
E. coli/Pol A	DNA damage	–	–	Rosenkranz and Poirier 1979
E. coli SOS chromotest	DNA damage	+	–	Mersch-Sundermann 1992a
E. coli SOS chromotest	DNA damage	+	No data	Mersch-Sundermann 1992a
Eukaryotic organisms: Fungi: *S. cerevisiae* D3	Mitotic recombination	–	–	Simon 1979b
Mammalian cells:				
Mouse lymphoma L5178Y/TK$^{+/-}$	Gene mutation	+	No data	Amacher et al. 1980
Mouse lymphoma L5178Y/TK$^{+/-}$	Gene mutation	+	–	Amacher and Turner 1980; Amacher and Paillet 1982
Mouse lymphoma L5178Y/TK$^{+/-}$	Gene mutation	–	–	Amacher and Paillet 1983
Chinese hamster V79	Gene mutation	–	–	Huberman 1975
Chinese hamster V79	Gene mutation	–	No data	Huberman 1975
Human lymphoblasts TK6	Gene mutation	+	No data	Barfknecht et al. 1982
Human epithelial cells EUE	Gene mutation	No data	–	Rochhi et al. 1980
Human keratinocytes	Gene mutation	No data	–	Allen-Hoffmann and Rheinwald 1984
HeLa cells/unshceduled DNA synthesis	DNA damage	+	No data	Martin et al. 1978

TABLE 2-5. Genotoxicity of Polycyclic Aromatic Hydrocarbons *In Vitro (continued)*

Species (test system)	End point	Result		Reference
		With activation	Without activation	
Rat liver cells ARL18	Sister chromatid exchange	No data	(+)	Tong et al. 1981
Hamster BHK21 clone 13	Transformation	+	No data	Greb et al. 1980
Hamster embryo cells	Transformation	No data	(+)	DiPaolo et al. 1969, 1971
Hamster embryo cells	Transformation	–	No data	Grover et al. 1971
Syrian hamster embryo cells	Transformation	No data	+	Dunkel et al. 1981
Syrian hamster lung cells FSHL	Transformation	No data	+	Emura et al. 1980
Mouse ventral prostate C3H clone G23	Transformation	No data	–	Grover et al. 1971; Marquardt et al. 1972
Mouse Balb/3T3 cells	Transformation	No data	(+)	Dunkel et al. 1981
Fischer rat embryo cells/leukemia virus transformation	Transformation	No data	+	Dunkel et al. 1981
	BENZO[b]FLUORANTHENE			
Prokaryotic organisms:				
S. typhimurium	Gene mutation	+	No data	Amin et al. 1984; Hermann 1981; LaVoie et al. 1979
S. typhimurium	Gene mutation	–	No data	Mossanda et al. 1979
S. typhimurium/fluctuation test	Gene mutation	–	No data	Mossanda et al. 1979
E. coli PQ37 SOS chromotest	DNA damage	+	–	Mersch-Sundermann et al. 1992b
Mammalian cells:				
Chinese hamster V79	Gene mutation	–	–	Huberman 1975
Hamster BHK21 clone 13	Transformation	+	No data	Greb et al. 1980
Syrian hamster lung cells FSHL	Transformation	No data	+	Emura et al. 1980

TABLE 2-5. Genotoxicity of Polycyclic Aromatic Hydrocarbons *In Vitro (continued)*

Species (test system)	End point	Result		Reference
		With activation	Without activation	
BENZO[j]FLUORANTHENE				
Prokaryotic organisms:				
S. typhimurium	Gene mutation	+	No data	LaVoie et al. 1979
S. typhimurium/fluctuation test	DNA binding	+	No data	Marshall et al. 1992; Weyand et al. 1992
S. typhimurium/fluctuation test	Gene mutation	+	+	Marshall et al. 1993
E. coli SOS chromotest	DNA damage	+	No data	Mersch-Sundermann et al. 1992b
BENZO[k]FLUORANTHENE				
Prokaryotic organisms:				
S. typhimurium	Gene mutation	+	No data	Amin et al. 1985b; LaVoie et al. 1979; LaVoie et al. 1980; Weyand et al. 1988
Mammalian cells:				
Syrian hamster lung cells FSHL	Transformation	No data	–	Emura et al. 1980
BENZO[g,h,i]PERYLENE				
Prokaryotic organisms:				
S. typhimurium	Gene mutation	+	–	Andrews et al. 1978; Sakai et al. 1985
S. typhimurium	Gene mutation	+	No data	Carver et al. 1986; LaVoie et al. 1979; Mossanda et al. 1979
S. typhimurium/fluctuation test	Gene mutation	(+)	No data	Mossanda et al. 1979
E. coli SOS chromotest	DNA damage	+	–	Mersch-Sundermann et al. 1992a
E. coli SOS chromotest	DNA damage	+	No data	Mersch-Sundermann et al. 1992b
BENZO[a]PYRENE				

TABLE 2-5. Genotoxicity of Polycyclic Aromatic Hydrocarbons *In Vitro (continued)*

Species (test system)	End point	Result		Reference
		With activation	Without activation	
Prokaryotic organisms:				
S. typhimurium	Gene mutation	+	–	Glatt et al. 1987; Grolier et al. 1989; Prasanna et al. 1987; Rosenkranz and Poirier 1979; Sakai et al. 1985; Simmon 1979a
S. typhimurium	Gene mutation	+	No data	Alfheim and Randahl 1984; Alzieu et al. 1987; Ampy et al. 1988; Andrews et al. 1989; Antignac et al. 1990; Bos et al. 1988; Bruce and Heddle 1979; Carver et al. 1986; Hermann 1981; LaVoie et al. 1979; Lee and Lin 1988; Marino 1987; Norpoth et al. 1984; Pahlman and Pelkonen 1987
S. typhimurium	Gene mutation	+	No data	Phillipson and Ioannides 1989a
S. typhimurium	Gene mutation	–	No data	Gao et al. 1991
S. typhimurium	Gene mutation	–	No data	Phillipson and Ioannides 1989b
S. typhimurium TM677	Gene mutation	+	–	Rastetter et al. 1982
S. typhimurium TM677	Gene mutation	+	No data	Babson et al. 1986
S. typhimurium	Gene mutation	+	No data	Balansky et al. 1994
E. coli WP2-WP100/rec-assay	DNA damage	+	No data	Mamber et al. 1983
E. coli/PoLA		+	–	Rosenkranz and Poirier 1979
E. coli/differential killing	DNA damage	+	–	Tweats 1981
E. coli/SOS chromotest	DNA damage	+	–	Mersch-Sundermann et al. 1992b
Eukaryotic organisms:				
Fungi: *S. cerevisiae* D3	Mitotic recombination	–	–	Simmon 1979b
S. cerevisiae D4-RDII	Mitotic recombination	No data	–	Siebert et al. 1981

TABLE 2-5. Genotoxicity of Polycyclic Aromatic Hydrocarbons *In Vitro (continued)*

Species (test system)	End point	Result		Reference
		With activation	Without activation	
Animal systems:				
Chinese hamster V79	Gene mutation	+	No data	Arce et al. 1987; Diamond et al. 1980; Huberman 1975
Chinese hamster V79	Gene mutation	+	–	Huberman 1975
Chinese hamster CHO	Gene mutation	+	(+)	Gupta and Singh 1982
Fischer rat embryo cells/OUA®	Gene mutation	No data	+	Mishra et al. 1978
Mouse lymphoma L5178Y/TK$^{+/-}$	Gene mutation	+	–	Amacher and Paillet 1983; Clive et al. 1979
Mouse lymphoma L5178Y/TK$^{+/-}$	Gene mutation	+	No data	Amacher et al. 1980; Amacher and Turner 1980; Arce et al. 1987
Mouse lymphoma L5178Y/HGPRT	Gene mutation	(+)	No data	Clive et al. 1979
Human lymphoblasts AHH	Gene mutation	No data	+	Crespi et al. 1985
Human lymphoblasts TK6	Gene mutation	(+)	No data	Crespi et al. 1985
Human lymphoblasts	Gene mutation	+	No data	Danheiser 1989
Human epithelial cells EUE	Gene mutation	No data	+	Barfknecht et al. 1982; Rocchi et al. 1980
Human fibroblasts HSC172	Gene mutation	+	–	Gupta and Goldstein 1981
Human keratinocytes	Gene mutation	No data	+	Allen-Hoffmann and Rheinwald 1984
Rat hepatocytes/DNA repair	DNA damage	No data	+	Williams et al. 1982
Rat tracheal epithelial cells	DNA damage	No data	+	Cosma and Marchock 1988; Cosma et al. 1988
Mouse C3H/10T1/2 clone 8	DNA damage	No data	(+)	Lubet et al. 1983b
HeLa cells/unscheduled DNA synthesis	DNA damage	+	NR	Martin et al. 1978
Human skin fibroblasts	DNA damage	No data	+	Milo et al. 1978
Human mammary cells	DNA damage	No data	+	Leadon et al. 1988

TABLE 2-5. Genotoxicity of Polycyclic Aromatic Hydrocarbons *In Vitro (continued)*

Species (test system)	End point	Result		Reference
		With activation	Without activation	
Human fibroblasts/unscheduled DNA synthesis	DNA damage	+	No data	Agrelo and Amos 1981
Human fibroblasts WI-38/unscheduled DNA synthesis	DNA damage	+	–	Robinson and Mitchell 1981
Human lymphocyte	DNA damage	No data	+	Wienke et al. 1990
Calf thymus DNA	DNA binding	+	No data	Cavalieri et al. 1988a
Chick embryo fibroblasts	DNA binding	No data	+	Liotti et al. 1988
Chick embryo hepatocytes	DNA binding	No data	+	Liotti et al. 1988
Chinese hamster V79	DNA binding	No data	No data	Arce et al. 1987
Mouse lymphoma L5178Y/TK$^{+/-}$	DNA binding	+	No data	Arce et al. 1987
Mouse C3H/10T1/2	DNA binding	+	No data	Arce et al. 1987
Syrian hamster embryo SHE	DNA binding	No data	+	Arce et al. 1987
Rat bladder epithelial cells	DNA binding	No data	+	Moore et al. 1982
Rat mammary epithelial cells	DNA binding	No data	+	Moore et al. 1987
Human liver HepG2 cells	DNA binding	No data	+	Diamond et al. 1980
Human mammary cells	DNA binding	No data	+	Leadon et al. 1988; Moore et al. 1987
Human bladder cells	DNA binding	No data	+	Moore et al. 1982
Human endometrial cells	DNA binding	No data	+	Dorman et al. 1981
Human bronchus cells	DNA binding	No data	+	Harris et al. 1984
Human colon cells	DNA binding	No data	+	Harris et al. 1984
Human lymphocytes	DNA binding	No data	+	Pavanello and Levis 1992

TABLE 2-5. Genotoxicity of Polycyclic Aromatic Hydrocarbons *In Vitro (continued)*

Species (test system)	End point	Result		Reference
		With activation	Without activation	
Chinese hamster V79-4	Chromosome aberrations	–	–	Popescu et al. 1977
Chinese hamster CHL	Chromosome aberrations	+	–	Matsuoka et al. 1979
Mouse lymphoma L5178Y/TK$^{+/-}$	Chromosome aberrations	+	No data	Arce et al. 1987
Rat liver cells RL1	Chromosome aberrations	No data	+	Dean 1981
Human fibroblasts WI-38	Chromosome aberrations	+	–	Weinstein et al. 1977
Chinese hamster V79	Sister chromatid exchange	+	No data	Arce et al. 1987; Mane et al. 1990
Chinese hamster V79	Sister chromatid exchange	+	–	Popescu et al. 1977; Wojciechowski et al. 1981
Chinese hamster bone marrow	Sister chromatid exchange	No data	+	Roszinsky-Kocher et al. 1979
Chinese hamster Don-6	Sister chromatid exchange	No data	+	Abe et al. 1983b
Chinese hamster CHO	Sister chromatid exchange	+	–	Husgafvel-Pursiainen et al. 1986
Rat pleural mesothelial cells	Sister chromatid exchange	No data	+	Achard et al. 1987
Rat liver cells ARL18	Sister chromatid exchange	No data	+	Tong et al. 1981
Rat hepatoma Reuber H4-II-E	Sister chromatid exchange	No data	+	Dean et al. 1983a
Rat esophageal tumor R1	Sister chromatid exchange	No data	+	Abe et al. 1983b
Rat ascites hepatoma AH66-B	Sister chromatid exchange	No data	+	Abe et al. 1983b
Human fibroblasts TIG-II	Sister chromatid exchange	+	(+)	Huh et al. 1982
Human hepatoma C-HC-4	Sister chromatid exchange	No data	+	Abe et al. 1983a, 1983b
Human hepatoma C-HC-20	Sister chromatid exchange	No data	+	Abe et al. 1983a, 1983b
Human lymphocyte	Sister chromatid exchange	+	–	Lo Jacono et al. 1992
Human lymphocyte	Sister chromatid exchange	No data	+	Wienke et al. 1990

TABLE 2-5. Genotoxicity of Polycyclic Aromatic Hydrocarbons *In Vitro (continued)*

Species (test system)	End point	Result		Reference
		With activation	Without activation	
Golden hamster embryo cells	Transformation	+	No data	Mager et al. 1977
Hamster BHK21 clone 13	Transformation	+	–	Greb et al. 1980
Hamster embryo cells/SA7 virus transformation	Transformation	No data	+	Casto et al. 1977
Syrian hamster embryo cells	Transformation	No data	+	DiPaolo et al. 1969, 1971; Dunkel et al. 1981
Syrian hamster embryo cells/focus assay	Transformation	No data	+	Casto et al. 1977
Syrian hamster lung FSHL	Transformation	No data	+	Emura et al. 1980, 1987
Syrian hamster SHE/SA7 virus transformation	Transformation	No data	+	Arce et al. 1987
Mouse C3H/10T1/2	Transformation	No data	+	Arce et al. 1987; Lubet et al. 1983b; Peterson et al. 1981
Mouse Balb/3T3	Transformation	No data	+	Dunkel et al. 1981
Mouse Balb/3T3 clone A31-1-1	Transformation	No data	+	Little and Vetroys 1988
Fischer rat embryo cells	Transformation	No data	+	Mishra et al. 1978
Rat embryo cells/SA7 virus transformation	Transformation	No data	+	DiPaolo and Casto 1976
Fischer rat embryo cells/leukemia virus transformation	Transformation	No data	+	Dunkel et al. 1981
Human breast cancer cells	Gene expression	+	No data	Moore et al. 1994
Human breast epithelial cells	Transformation	No data	+	Calaf et al. 1993
Rat heptocyte	Sister chromatid exchange	+	No data	Kulka et al. 1993
Chinese hamster cells	Sister chromatid exchange	No data	+	Kulka et al. 1993

TABLE 2-5. Genotoxicity of Polycyclic Aromatic Hydrocarbons *In Vitro (continued)*

Species (test system)	End point	Result		Reference
		With activation	Without activation	
Mouse Balb/c-3T3 cells	Transformation	No data	+	Matthews 1994
Hamster tracheal cells	DNA damage	No data	+	Roggeband et al. 1994
Rat tracheal cells	DNA damage	No data	+	Roggeband et all. 1994
	BENZO[e]PYRENE			
Prokaryotic organisms:				
S. typhimurium	Gene mutation	+	No data	Andrews et al. 1978
S. typhimurium	Gene mutation	(+)	No data	Wood et al. 1979b
S. typhimurium	Gene mutation	+[c]	No data	Wood et al. 1979b
S. typhimurium	Gene mutation	+	No data	LaVoie et al. 1979b
E. coli SOS chromotest	DNA damage	+	No data	Mersch-Sundermann et al. 1992b
E. coli SOS chromotest	DNA damage	(+)	–	Mersch-Sundermann et al. 1992a
Mammalian cells:				
Chinese hamster V79 ovary cells	Sister chromatid exchange	–	No data	Mane et al. 1990
Prokaryotic organisms:				
S. typhimurium	Gene mutation	+	–	Sakai et al. 1985
S. typhimurium	Gene mutation	+	No data	Bos et al. 1988; Carver et al. 1986; Hermann 1981; LaVoie et al. 1979; Pahlman and Pelkonen 1987
S. typhimurium	Gene mutation	(+)	No data	Wood et al. 1977
S. typhimurium	Gene mutation	–	–	Rosenkranz and Poirier 1979; Simmon 1979a
E. coli/PolA	DNA damage	–	–	Rosenkranz and Poirier 1979
E. coli SOS chromotest	DNA damage	+	–	Mersch-Sundermann et al. 1992a
E. coli SOS chromotest	DNA damage	+	No data	Mersch-Sundermann et al. 1992b

TABLE 2-5. Genotoxicity of Polycyclic Aromatic Hydrocarbons *In Vitro (continued)*

Species (test system)	End point	Result		Reference
		With activation	Without activation	
Eukaryotic organisms:				
Fungi:				
S. cerevisiae D3	Mitotic recombination	–	–	Simmon 1979b
S. cerevisiae D4-RDII	Mitotic recombination	No data	–	Siebert et al. 1981
Mammalian cells:				
Chinese hamster V79	Gene mutation	–	No data	Huberman 1975
Human lymphoblasts TK6	Gene mutation	+	No data	Barfknecht et al. 1982
Human ephithelial cells EUE	Gene mutation	No data	–	Rocchi et al. 1980
Hamster BHK21 clone 13	Tranformation	+	No data	Greb et al. 1980
Mouse ventral prostrate C3H clone G23	Transformation	No data	–	Marquardt et al. 1972
	DIBENZ[a,h]ANTHRACENE			
Prokaryotic organisms:				
S. typhimurium	Gene mutation	+	No data	Andrews et al. 1978; Carver et al. 1986; Hermann 1981; Lecoq et al. 1991b; Pahlman and Pelkonen 1987; Wood et al. 1978
S. typhimurium	Gene mutation	–[a,b]	No data	Phillipson and Ioannides 1989
E. coli SOS chromotest	DNA damage	+	–	Mersch-Sundermann et al. 1992a
E. coli SOS chromotest	DNA damage	+	No data	Mersch-Sundermann et al. 1992b
Eukaryotic organisms:				
Fungi:				
Neurospora crassa	Gene mutation	No data	+	Barrat and Tatam 1958
S. cerevisiae D4-RDII	Mitotic recombination	No data	–	Siebert et al. 1981
Mammalian cells:				
Chinese hamster V79	Gene mutation	(+)	No data	Huberman 1975
Human epithelial cells EUE	Gene mutation	No data	(+)	Rocchi et al. 1980

TABLE 2-5. Genotoxicity of Polycyclic Aromatic Hydrocarbons *In Vitro (continued)*

Species (test system)	End point	Result		Reference
		With activation	Without activation	
HeLa cells/unscheduled DNA synthesis	DNA damage	+	NR	Martin et al. 1978
Human bronchus cells	DNA binding	No data	+	Harris et al. 1984
Hamster embryo cells	Transformation	–	No data	Grover et al. 1971
Hamster embryo cells/SA7 virus transformation	Transformation	No data	+	Casto 1973; Casto et al. 1977
Syrian hamster embryo cells	Transformation	No data	+	DiPaolo et al. 1969
Syrian hamster embryo cells/focus assay	Transformation	No data	+	Casto et al. 1977
Hamster BHK21 clone 13	Transformation	+	–	Greb et al. 1980
Mouse ventral prostate C3H clone G23	Transformation	No data	–	Marquardt et al. 1972
Mouse C3H/10T$^{1/2}$ clone 8	Transformation	No data	(+)	Lubet et al. 1983b
Rat embryo cells/SA7 virus transformation	Transformation	No data	+	DiPaolo and Casto 1976
Prokaryotic organisms:				
S. typhimurium	Gene mutation	+	No data	Bos et al. 1988; Carver et al. 1986; Hermann 1981; LaVoie et al. 1979
S. typhimurium	Gene mutation	(+)	No data	Bos et al. 1987
S. typhimurium	Gene mutation	–	No data	Mossanda et al. 1979
S. typhimurium/fluctuation test	Gene mutation	+	No data	Mossanda et al. 1979
S. typhimurium/fluctuation test	Gene mutation	+	–	Bhatia et al. 1987
S. typhimurium/taped plate assay	Gene mutation	+	–	Bos et al. 1987
S. typhimurium TM 677	Gene mutation	+	–	Rastetter et al. 1982

TABLE 2-5. Genotoxicity of Polycyclic Aromatic Hydrocarbons *In Vitro (continued)*

Species (test system)	End point	Result		Reference
		With activation	Without activation	
S. typhimurium TM 677	Gene mutation	+	No data	Babson et al. 1986
E. coli SOS chromotest	DNA damage	+	–	Mersch-Sundermann et al. 1992a
E. coli SOS chromotest	DNA damage	+	No data	Mersch-Sundermann et al. 1992b
Mammalian cells:				
Human lymphoblasts AHH1	Gene mutation	No data	–	Crespi et al. 1985
Human lymphoblasts TK6	Gene mutation	+	No data	Barfknecht et al. 1982
Chinese hamster CHO-1	Sister chromatid exchange	+	–	Palitti et al. 1986
Prokaryotic organisms:				
S. typhimurium	Gene mutation	–	–	Sakai et al. 1985
S. typhimurium	Gene mutation	–	No data	Bos et al. 1988; Hermann 1981; LaVoie et al. 1979; Pahlman and Pelkonen 1987
E. coli WP2-WP100/rec-assay	DNA damage	–	No data	Mamber et al. 1983
E. coli SOS chromotest	DNA damage	–	–	Mersch-Sundermann et al. 1992a
E. coli SOS chromotest	DNA damage	–	No data	Mersch-Sundermann et al. 1992b
Mammalian cells:				
Chinese hamster lung cell	Chromosome abberrations	+	–	Matsuoka et al. 1991
IDENO[1,2,3-c,d]PYRENE				
Prokaryotic organisms:				
S. typhimurium	Gene mutation	+	–	Rice et al. 1985b
S. typhimurium	Gene mutation	+	No data	LaVoie et al. 1979
E. coli SOS chromotest	DNA damage	+	–	Mersch-Sundermann et al. 1992a
E. coli SOS chromotest	DNA damage	+	No data	Mersch-Sundermann et al. 1992b

TABLE 2-5. Genotoxicity of Polycyclic Aromatic Hydrocarbons *In Vitro (continued)*

Species (test system)	End point	Result		Reference
		With activation	Without activation	
Mammalian cells:				
Syrian hamster lung cells FSHL	Transformation	No data	+	Emura et al. 1980
		PHENANTHRENE		
Prokaryotic organisms:				
S. typhimurium	Gene mutation	+	–	Sakai et al. 1985
S. typhimurium	Gene mutation	+	No data	Carver et al. 1986; Oesch et al. 1981
S. typhimurium	Gene mutation	(+)	No data	Bos et al. 1988
S. typhimurium	Gene mutation	–	–	LaVoie et al. 1981b; Rosenkranz and Poirier 1979; Simmon 1979a
S. typhimurium	Gene mutation	–	No data	Hermann 1981; LaVoie et al. 1979; Pahlman and Pelkonen 1987
E. coli/PolA	DNA damage	–	–	Rosenkranz and Poirier 1979
E. coli SOS chromotest	DNA damage	+	–	Mersch-Sundermann et al. 1992a
E. coli SOS chromotest	DNA damage	+	No data	Mersch-Sundermann et al. 1992b
Eukaryotic organisms:				
Fungi: *S. cerevisiae* D3	Mitotic recombination	–	–	Simmon 1979b
S. cerevisiae D4-RDII	Mitotic recombination	No data	–	Siebert et al. 1981
Mammalian cells:				
Fischer rat embryo cells	Gene mutation	No data	–	Mishra et al. 1978
Chinese hamster V79	Gene mutation	–	No data	Huberman 1975
Human lymphoblasts TK6	Gene mutation	(+)	No data	Barfknecht et al. 1982
Human skin fibroblasts	DNA damage	No data	–	Milo et al. 1978
Chinese hamster V79-4	Chromosome aberrations	–	–	Popescu et al. 1977

TABLE 2-5. Genotoxicity of Polycyclic Aromatic Hydrocarbons *In Vitro (continued)*

Species (test system)	End point	Result		Reference
		With activation	Without activation	
Chinese hamster Don	Chromosome aberrations	No data	–	Abe and Sasaki 1977
Chinese hamster CHL	Chromosome aberrations	No data	–	Ishidate and Odashima 1977
Chinese hamster CHL	Chromosome aberrations	–	–	Matsuoka et al. 1979
Chinese hamster V79-4	Sister chromatid exchange	–	–	Popescu et al. 1977
Chinese hamster Don	Sister chromatid exchange	No data	–	Abe and Sasaki 1977
Syrian hamster embryo cells	Transformation	No data	–	DiPaolo et al. 1969; Dunkel et al. 1981
Hamster BHK21 clone 13	Transformation	–	No data	Greb et al. 1980
Hamster embryo cells/SA7 virus transformation	Transformation	No data	–	Casto et al. 1977
Mouse ventral prostate C3H clone G23	Transformation	No data	–	Marquardt et al. 1972
Mouse C3H/10T$^{1/2}$	Transformation	No data	–	Lubet et al. 1983b; Peterson et al. 1981
Mouse Balb/3T3 cells	Transformation	No data	–	Dunkel et al. 1981
Fischer rat embryo cells	Transformation	No data	–	Mishra et al. 1978
Fischer rat embryo cells/leukemia virus transformation	Transformation	No data	–	Dunkel et al. 1981
Prokaryotic organisms:				
S. typhimurium	Gene mutation	+	–	Sakai et al. 1985
S. typhimurium	Gene mutation	+	No data	Bos et al. 1988
S. typhimurium	Gene mutation	–	No data	Carver et al. 1986; Hermann 1981; LaVoie et al. 1979
S. typhimurium/fluctuation test	Gene mutation	+	+	Bhatia et al. 1987
S. typhimurium/taped plate	Gene mutation	+	No data	Bos et al. 1988
E. coli WP2-WP100/rec-assay	DNA damage	–	No data	Mamber et al. 1983

TABLE 2-5. Genotoxicity of Polycyclic Aromatic Hydrocarbons *In Vitro (continued)*

Species (test system)	End point	Result		Reference
		With activation	Without activation	
E. coli/differential killing	DNA damage	–	–	Tweats 1981
E. coli/SOS chromotest	DNA damage	–	–	Mersch-Sundermann et al. 1992a
E. coli/SOS chromotest	DNA damage	–	No data	Mersch-Sundermann et al. 1992b
Mammalian cells:				
Fisher rat embryo cells	Gene mutation	No data	+	Mishra et al. 1978
Chinese hamster V79	Gene mutation	–	–	Huberman 1975
Chinese hamster V79	Gene mutation	–	No data	Huberman 1975
Mouse lymphoma L5178Y/TK$^{+/-}$	Gene mutation	–	No data	Amacher et al. 1980
Human lymphoblasts TK6	Gene mutation	–	No data	Barfknecht et al. 1982
HeLa cells/unscheduled DNA synthesis	DNA damage	–	–	Martin et al. 1978
Rat hepatocytes/DNA repair	DNA damage	No data	–	Williams et al. 1982
Human skin fibroblasts	DNA damage	No data	–	Milo et al. 1978
Human skin fibroblasts	DNA damage	–	No data	Agrelo and Amos 1981
Human fibroblasts WI-38 unscheduled DNA synthesis	DNA damage	+	–	Robinson and Mitchell 1981
Chinese hamster V79-4	Chromosome aberrations	+	–	Popescu et al. 1977
Human fibroblasts WI-38	Chromosome aberrations	–	–	Weinstein et al. 1977
Rat liver cells RL1	Chromosome aberrations	No data	–	Dean 1981
Chinese hamster V79-4	Sister chromatid exchange	–	–	Popescu et al. 1977
Rat liver cells ARL18	Sister chromatid exchange	No data	–	Tong et al. 1981
Syrian hamster embryo cells	Transformation	No data	–	DiPaolo et al. 1969
Fischer rat embryo cells	Transformation	No data	–	Mishra et al. 1978

TABLE 2-5. Genotoxicity of Polycyclic Aromatic Hydrocarbons *In Vitro (continued)*

Species (test system)	End point	Result		Reference
		With activation	Without activation	
Hamster embryo cells/SA7 virus transformation	Transformation	No data	–	Casto et al. 1977
		Chrysene		
Prokaryotic organisms: *S. typhimurium*	Gene mutation	+	No data	Glatt et al. 1993
S. typhimurium	Gene mutation	+	No data	Cheung et al. 1993

[a]Noninduced hamster S9
[b]Noninduced mouse, rat, pig and human S9
[c]Synthetically prepared diol epoxide was strongly mutagenic

AHH = aromatic hydrocarbon hydroxylase; CHO = Chinese hamster ovary; DNA = deoxyribonucleic acid; FSHL = female sex hormone lutenizing; NR = Not reported; PAHs = polycyclic hydrocarbons; SOS = DNA repair assay; – = negative result; + = positive result; (+) = weakly positive result

conjunction with the data from whole animal experiments, suggest that benzo[a]pyrene would probably have similar deleterious effects on human genetic material.

Because the genotoxic activity of benzo[a]pyrene is well established, it is frequently used as a positive control to demonstrate the sensitivity of various test systems to detect the genotoxic action of unknown compounds. It also serves as the model compound for PAHs, and the available information on the formation of metabolites and structure of benzo[a]pyrene can theoretically be used to predict potential genotoxicity/carcinogenicity of other PAHs that have not been as extensively studied.

Benzo[a]pyrene is generally considered to be biologically inert but can be metabolized by enzyme systems into at least 27 identified metabolites; however, only a few of these metabolites are reactive species that can damage DNA (De Bruin 1976). Benzo[a]pyrene 7,8-diol-9,10-epoxide is thought to be the ultimate mutagenic/carcinogenic metabolite. The primary metabolic pathway leading to the formation of the genotoxic/carcinogenic diol epoxides is assumed to be cytochrome P-450-dependent mixed-function oxidases (MFO), which in the case of PAHs are called AHHs. AHH is an ubiquitous enzyme system and has been found in a variety of tissues including liver, lung, and gastrointestinal tract of rats, mice, hamsters, and monkeys. AHH has also been detected in human liver, lung, placenta, lymphocytes, monocytes, and alveolar macrophages (Singer and Grunberger 1983). The evidence indicating that a variety of human tissues including human lymphocytes (GAO 1991; Wiencke et al. 1990), human lymphoblasts (Danheiser et al. 1989), and human mammary epithelial cells (Mane et al. 1990) can serve as a source of exogenous metabolic activation tends to support the role of AHH systems in initiating the conversion of benzo[a]pyrene to genotoxic forms. However, human erythrocytes, which do not contain an effective cytochrome P-450 system, were more efficient than induced rat liver fractions in converting benzo[a]pyrene to a genotoxin as indicated by higher sister chromatid exchange and micronuclei frequencies observed in human lymphocytes co-cultivated with human erythrocytes (Lo Jacono et al. 1992). The findings, while unconfirmed, suggest that enzymatic systems other than AHH may yield reactive intermediates. Similar evidence that uninduced lung, kidney, or spleen from Sprague-Dawley rats or BALB/c mice did not convert benzo[a]pyrene to a mutagen in *S. typhimurium* was reported by Ampy et al. (1988) who concluded that these tissues may, therefore, not be at risk from exposure. Superficially, the data from the studies conducted by Phillipson and Ioannides (1989) (indicating that neither benzo[a]pyrene nor benz[a]anthracene were mutagenic in *S. typhimurium* TA100 in the presence of noninduced hepatic fractions from rats, mice, pigs, or humans) would tend to support the data reported by Lo Jacono et al. (1992) and Ampy et al.

(1988) (suggesting either that other enzyme systems are involved in the bioactivation of these compounds or that certain tissues are not at risk). However, it is important to note, as pointed out by Phillipson and Ioannides (1989), that the level of P-450 isoenzyme proteins in unexposed animals is relatively low. Support for this statement was provided by the data demonstrating that uninduced hamster liver fractions, which contain high cytochrome P-448 levels, converted both benzo[a]pyrene and benz[a]anthracene to mutagens. By inference, it can reasonably be assumed that repeated exposures are required to induce the requisite enzyme systems to metabolize these promutagens to ultimate mutagenic/carcinogenic forms. It can further be assumed that the tissues of any species, including humans, that contain the appropriate inducible enzyme system are at risk.

The function of other enzyme systems in the biotransformation of benzo[a]pyrene should not be ruled out. However, the evidence that cytochrome P-448 plays a major role in this process was further substantiated by the observation that rat liver enzymes induced by PAHs such as 3-MC or dibenz[a,h]anthracene were more efficient in metabolizing benzo[a]pyrene, dibenz[a,h]anthracene, and benz[a]anthracene to mutagenic metabolites for *S. typhimurium* than was phenobarbital (Teranishi et al. 1975). This finding is consistent with the well-documented observation that various inducing agents such as phenobarbital and 3-MC cause the preferential synthesis of specific forms of cytochrome P-450. In the case of 3-MC, cytochrome P-448 is the principal form of induced cytochrome (Singer and Grunberger 1983).

Epoxidation is thought to be the major pathway for benzo[a]pyrene metabolism pertinent to macromolecular interaction. The metabolic attack consists of the cytochrome P-450/P-448-dependent MFO system converting the benzo[a]pyrene molecule into an epoxide; the epoxide is acted upon by epoxide hydrolase to form a dihydrodiol, and a second cytochrome MFO reaction gives rise to the ultimate mutagenic/carcinogenic form, benzo[a]pyrene 7,8-diol-9,10-epoxide. One of the unique structural features of the diol epoxide is that it appears to form in the area of the PAH molecule referred to as the bay region (i.e., a deep-pocketed area formed when a single benzo ring is joined to the remainder of the multiple ring system to form a phenanthrene nucleus). The location of the bay region(s) for the various PAHs in this profile is depicted in Chapter 3 (Table 3-l).

An additional feature of bay region diol epoxides is the ease of carbonium ion formation, which renders the PAH molecule highly reactive and susceptible to attack by nucleophiles (Jerina 1980; Singer and Grunberger 1983). The carbonium ion is more likely to form in structures where the

epoxide is part of the bay region of a saturated terminal angular ring than in an area where the diol epoxide is not associated with a bay region. Further enhancement of bay region epoxides can occur by the formation of an intramolecular hydrogen bond between the oxygen molecule of the epoxide and an associated hydroxyl group. These metabolites are also more resistant to enzymatic detoxification by epoxide hydrolase and glutathione transferase. The increased reactivity conferred by intramolecular hydrogen bonding and the decreased rate of further metabolism favor the interaction with DNA.

Analysis of the bay region diol epoxides and their contribution to the DNA binding, genotoxicity, and carcinogenicity of various PAHs has provided the basis for the bay region hypothesis (Wood et al. 1979a). For example, DNA adducts formed with non-bay region diol epoxides of benzo[a]pyrene have low mutagenic potential (MacLeod et al. 1994). The hypothesis further predicts that structures with more reactive bay regions would probably be more genotoxic and more carcinogenic. The body of evidence on the mutagenic and tumorigenic activity of the PAHs that form bay region diol epoxides (benzo[a]pyrene, benz[a]anthracene, chrysene, dibenz[a,h]anthracene; benzo[b]fluoranthene, benzo[j]fluoranthene, benzo[k]fluoranthene, and indeno[1,2,3-c,d]pyrene) supports this hypothesis.

Based on these considerations, the available genetic toxicology results from studies conducted with the other PAHs in this profile are discussed relative to the bay region hypothesis. It is cautioned, however, that while the use of structural relationships to predict potentially reactive compounds is a powerful tool, it is not infallible, nor does it replace *in vitro* or *in vivo* testing. The formation of bay region epoxides is not an absolute requirement for carcinogenic activity because several PAHs that cannot form bay region epoxides are known to be carcinogens. It can, nevertheless, serve as a warning system to alert regulatory agencies to a potential health hazard and to enable investigators to establish priority lists for testing PAHs.

There is no convincing evidence that the PAHs lacking a bay region structure (acenaphthene, acenaphthylene, and fluorene) are genotoxic; the results for acenaphthene and acenaphthylene are consistently negative. The induction of chromosome aberrations only, at a single dose in Chinese hamster lung cells exposed to fluorene (Matsuoka et al. 1991), is not sufficient to conclude that fluorene is a clastogen. However, none of these compounds have been extensively studied in *in vitro* assays, and they have not been tested *in vivo*.

The majority of the data for anthracene and pyrene were negative. Although isolated positive results were obtained, particularly in microbial systems, neither compound produced consistent genotoxic effects in mammalian cells *in vitro*, and both were negative in the limited *in vivo* studies that have been performed.

The results obtained with fluoranthene, the remaining PAH without a bay region configuration, illustrate the need to apply the bay region hypothesis judiciously. There is ample evidence indicating that fluoranthene induced gene mutations in bacteria and human lymphoblasts and sister chromatid exchange in Chinese hamster ovary cells. Based on the evidence of a powerful response in the *Escherichia coli* PQ37 SOS DNA repair assay (SOS chromotest), Mersch-Sundermann et al. (1992a) predicted that fluoranthene has a relatively high probability of being genotoxic (80%). However, fluoranthene did not induce sister chromatid exchanges in mouse bone marrow cells (Palitti et al. 1986). The work of Busby et al. (1984) in newborn mice, suggests that fluoranthene should be classified as a carcinogen.

The occurrence of a bay region structure on the phenanthrene molecule suggests that this compound is genotoxic. However, the overall findings from the genetic toxicology studies do not support such a prediction. Similarly, the reported observation that the intraperitoneal injection of phenanthrene resulted in sister chromatid exchange induction in Chinese hamster bone marrow cells was not convincing (Bayer 1979; Roszinsky-Kocher et al. 1979). In both studies, the sister chromatid exchange increase over background was less than 1.5 fold and comparable doses did not cause chromosome aberrations. As stated earlier, the occurrence of a bay region on the molecule in conjunction with the reactivity of the bay region appear to be the determinants of genotoxic/carcinogenic activity. It is, therefore, probable that the bay region on phenanthrene is not very reactive, which would account for the lack of genotoxicity and for the low carcinogenicity index (<2) assigned to this compound (Arcos et al. 1968). Similarly, quantum mechanical calculations indicate a low probability of carbonation formation for the bay region diol epoxide of phenanthrene (Jerina 1980).

The lack of genotoxicity for phenanthrene is thought to be related to the metabolism of this substance to its 9,10-dihydrodiol. However, specific methylated phenanthrenes, which direct the metabolic fate of this tricyclic hydrocarbon towards the formation of a classical bay region dihydrodiol epoxide, have exhibited significant genotoxicity (LaVoie et al. 1983a). It was demonstrated that the presence of a

methyl group at or adjacent to the K-region of phenanthrene can inhibit the formation of the 9,10-dihydrodiol and produce powerful mutagens in *Salmonella.* Additionally, the presence of a halogen at the K-region site produced similar results, which further support the association between inhibition of 9,10-dihydrodiol formation and mutagenic potency of substituted phenanthrenes. The study authors concluded that derivatives of phenanthrene that can inhibit metabolism at this site have a greater probability of exerting genotoxic effects. Additionally, methylated derivatives of phenanthrene may act as tumor initiators, as shown on mouse skin (LaVoie et al. 1981b).

The weight of evidence from the *in vitro* and *in vivo* studies conducted with benz[a]anthracene, dibenz[a,h]anthracene, and chrysene indicates that these three agents are genotoxic and that they exert their genotoxic effects through the binding of bay region diol epoxides to cellular DNA. Similarly, there is a substantive body of evidence that confirms the hypothesis implicating the formation of bay region diol epoxides as the major mechanism of action for both the genotoxicity and carcinogenicity induced by these PAHs (Cheung et al. 1993; Fuchs et al. 1993a, 1993b; Glatt et al. 1993; Lecoq et al. 1989, 1991a; Wood 1979). It is also of note that these three compounds, as well as benzo[a]pyrene, induced neoplastic cell transformation in at least one cell line (see Table 2-5).

Both benzo[b]fluoranthene and indeno[1,2,3-c,d]pyrene are known to exhibit mutagenic activity in *S. typhimurium* TA100 in the presence of rat liver homogenate (Amin et al. 1984; Hermann 1981; LaVoie et al. 1979; Rice et al. 1985b). Both of these agents were positive for *in vitro* cell transformation (Emura et al. 1980; Greb et al. 1980) and were shown to bind to mouse skin DNA *in vivo* (Hughes et al. 1993; Weyand 1989; Weyand et al. 1987). In addition, benzo[b]fluoranthene formed DNA adducts in the lungs and livers of adult rats (Ross et al. 1992) and newborn mice (Weyand et al. 1993b) and formed DNA adducts as well as induced sister chromatid exchange in peripheral blood lymphocytes of treated rats (Ross et al. 1992). These data are consistent with reports on the tumorigenic activity of these PAHs in rodents and their potential to act as carcinogens in humans.

Studies on the mutagenic activity of benzofilfluoranthene and benzo[k]fluoranthene have also indicated that these nonalternant PAHs are mutagenic in *S. typhimurium* TAl00 (Amin et al. 1985b; LaVoie et al. 1979, 1980a; Weyand et al. 1988, 1992). There is also evidence that application of either of these PAHs to mouse skin results in DNA adduct formation (Hughes et al. 1993; LaVoie et al. 1991a, 1991b; Weyand et al. 1987, 1993a). The relative extent of binding to mouse skin DNA (i.e.,

benzo[j]fluoranthene > benzo[k]fluoranthene) parallels the relative tumorigenic potency of these hydrocarbons on mouse skin (i.e., benzo[j]fluoranthene is more potent than benzo[k]fluoranthene as a tumor initiator) (LaVoie et al. 1982).

Benzo[g,h,i]perylene has been reported to be mutagenic in *S. typhimurium* and to cause DNA damage in *E. coli*. Benzo[g,h,i]perylene has been shown to be responsible for the formation of DNA adducts isolated after topical application of pharmaceutical grade coal tar to the skin of mice (Hughes et al. 1993). However, the few studies that were found were insufficient to draw meaningful conclusions.

The final compound in this group, benzo[e]pyrene, contains two equivalent bay regions. *In vivo,* benzo[e]pyrene induced a marginal increase in sister chromatid exchanges but did not cause structural chromosome aberrations in bone marrow cells harvested from Chinese hamsters receiving two daily intraperitoneal doses of 450 mg/kg (Roszinsky-Kocher et al. 1979). Similarly, sister chromatid exchange frequencies were not increased in V-79 cells co-cultivated with rat mammary epithelial cells as the source of exogenous metabolic activation (Mane et al. 1990). Benzo[e]pyrene was, however, reported to be weakly mutagenic in *S. typhimurium* (Andrews et al. 1978) and weakly genotoxic in the *E. coli* SOS chromotest (Mersch-Sundermann et al. 1992a, 1992b).

The weak genotoxicity and the very weak carcinogenicity of benzo[e]pyrene appear to contradict the bay region diol epoxide hypothesis. Quantum mechanical analysis of the ringed structure strongly suggests the likelihood of carbonium ion formation and an associated chemical reactivity equivalent to the bay region diol epoxide of dibenz[a,h]anthracene (Wood et al. 1979a). Similarly, synthetically prepared bay region tetrahydro-epoxides of benzo[e]pyrene were found to be highly mutagenic in bacteria and mammalian cells, suggesting that bay region diol epoxide(s), if formed, would also be mutagenic. However, the parent compound was not metabolized to a reactive state by Aroclor 1254 S9 or by purified cytochrome P-450 derived from rat livers induced with Aroclor 1254. From these results, Wood et al. (1979a) concluded that the lack of mutagenicity for benzo[e]pyrene may be associated with the failure of the cytochrome P-450-dependent monooxygenase system to catalyze the critical oxidations necessary to form the bay region diol. Specifically, there was very little formation of the bay region 9,10-dihydrodiol and low conversion of authentic 9,10-dihydrodiol to the bay region diol epoxide. Subsequent studies with authentic bay region diol epoxides of benzo[e]pyrene showed that they had relatively low mutagenic and tumorigenic activity as a result of the diaxial conformation of the diol. The diaxial conformation of the 9,10-dihydrodiol also provides an explanation for the low

formation of the bay region diol epoxide since metabolism is shifted away from the adjacent isolated double bond (Chang et al. 1981; Wood et al. 1980). Since structural-activity analysis suggests that the bay region diol epoxides would have biological activity, Wood et al. (1979a) caution that extrapolation of these findings to *in vivo* metabolic events in species other than rats should be approached with caution. It is conceivable that benzo[e]pyrene would be genotoxic in species capable of carrying out the appropriate enzymatic steps.

In summary, several general conclusions can be reached for the unsubstituted PAHs evaluated in this profile. The formation of diol epoxides that covalently bind to DNA appears to be the primary mechanism of action for both genotoxicity and carcinogenicity of several of the unsubstituted PAHs that are genotoxins (benzo[a]pyrene, benz[a]anthracene, dibenz[a,h]anthracene, chrysene, benzo[b]fluoranthene, benzo[j]fluoranthene). There was insufficient evidence to draw meaningful conclusions regarding the genotoxic potential of benzo[g,h,i]perylene, although some evidence does exist.

With regard to the unsubstituted PAHs that either lack a bay region configuration (acenaphthene, acenaphthylene, anthracene, fluorene, and pyrene) or appear to have a weakly reactive bay region (phenanthrene), there is no compelling evidence to suggest that they interact with or damage DNA. The five PAHs that appear to be exceptions to the bay region diol epoxide hypothesis are fluoranthene, benzo[k]fluoranthene, benzo[j]fluoranthene, and indeno[1,2,3-cd]pyrene (no bay region), and benzo[e]pyrene (two bay regions). The evidence does suggest, however, that fluoranthene possesses genotoxic properties while benzo[e]pyrene is either weakly mutagenic or nonmutagenic.

Cancer. Evidence exists to indicate that mixtures of PAHs are carcinogenic in humans. The evidence in humans comes primarily from occupational studies of workers exposed to mixtures containing PAHs as a result of their involvement in such processes as coke production, roofing, oil refining, or coal gasification (e.g., coal tar, roofing tar, soot, coke oven emissions, soot, crude oil) (Hammond et al. 1976; Lloyd 1971; Maclure and MacMahon 1980; Mazumdar et al. 1975; Redmond et al. 1976; Wynder and Hoffmann 1967). PAHs, however, have not been clearly identified as the causative agent. Cancer associated with exposure to PAH-containing mixtures in humans occurs predominantly in the lung and skin following inhalation and dermal exposure, respectively. Some ingestion of PAHs is likely because of swallowing of particles containing PAHs subsequent to mucocilliary clearance of these particulates from the lung.

Certain PAHs are carcinogenic to animals by the oral route (e.g., benz[a]anthracene, benzo[a]pyrene, and dibenz[a,h]anthracene) (Berenblum and Haran 1955; Chu and Malmgren 1965; Klein 1963; McCormick et al. 1981; Neal and Rigdon 1967; Rigdon and Neal 1966; Snell and Stewart 1963; Spamins et al. 1986; Wattenberg and Leong 1970). The results of dermal studies indicate that benz[a]anthracene, benzo[a]pyrene, benzo[b]fluoranthene, benzo[j]fluoranthene, benzo[k]fluoranthene, chrysene, dibenz[a,h]anthracene, and indeno[l,2,3-c,d]pyrene are tumorigenic in mice following dermal exposure (Albert et al. 1991b; Cavalieri 1988b; Habs et al. 1984; Levin et al. 1984; Warshawsky and Barkley 1987; Wilson and Holland 1988; Wynder and Hoffmann 1959b). The sensitivity of mouse skin to PAH tumorigenesis forms the basis for the extensive studies performed using dermal administration. This tumorigenicity can be enhanced or modified with concomitant exposure to more than one PAH, long straight-chain hydrocarbons (i.e., dodecane), or similar organic compounds commonly found at hazardous waste sites. Thus, humans exposed to PAHs in combination with these substances could be at risk for developing skin cancer.

For many of the carcinogenic PAHs discussed in this profile, it appears that the site of tumor induction is influenced by the route of administration and site of absorption, i.e., forestomach tumors are observed following ingestion, lung tumors following inhalation, and skin tumors following dermal exposure. However, the observations (discussed below) that (1) mammary tumors are induced following intravenous injection in Sprague-Dawley rats, (2) the susceptibility to tumor development on the skin after dermal application is not similar in rats and mice, and (3) oral cavity tumors are not observed when benzo[a]pyrene is administered in the diet, suggest that the point of first contact may not always be the site of PAH-induced tumors. The results of carcinogenicity studies conducted with the 17 PAHs discussed in this profile by parenteral routes of exposure are summarized in Table 2-6.

TABLE 2-6. Summary of Carcinogenicity Studies with Polycyclic Aromatic Hydrocarbons Using Parenteral Routes of Exposure

Chemical	Species	Route	Duration	Result/Site	Reference
Anthracene	Rat	Lung implantation	1 dose, 55-week observation	–	Stanton et al. 1972
	Newborn mouse	Subcutaneous	Single dose	–/lung	Platt et al. 1990
	Rat	Intrapulmonary	Single dose	+/lung	Deutsch-Wenzel et al. 1983
Benz[a]anthracene	Mouse	Subcutaneous	10 weeks	+/injection site	Boyland and Sims 1967
		Intramuscular	No data	+/fibrosarcomas, hemangioendotheliomas	Klein 1952
	Newborn mouse	Intraperitoneal	3 days	+/lung	Levin et al. 1984
	Hamster	Intratracheal	30 weeks	–	Sellakumar and Shubik 1974
Benzo[a]pyrene	Mouse	Subcutaneous	Single dose	+/injection site sarcomas	Pfeiffer 1977
	Newborn mouse	Intraperitoneal	Single dose	+/liver	LeVoie et al. 1987
	Newborn mouse	Intraperitoneal	No data	+/lung	Busby et al. 1984
	Rat	Intramammary	Single dose	+/mammary gland	Cavalieri et al. 1988b, 1988c
	Mouse	Intravaginal	5 months	+/cervix	Naslund et al. 1987
	Rat	Intrapulmonary	Single dose	+/lung	Deutsch-Wenzel et al. 1983
	Hamster	Intratracheal	Chronic	–	Kunstler 1983
	Rat	Tracheal implant	Intermediate	+/tracheal	Nettesheim et al. 1977
	Rat	Intrapulmonary	Single dose	+/lung	Iwagawa et al. 1989
	Newborn mouse	Subcutaneous	15 days	+/lung	Busby et al. 1989
	Rat	Intramammary	Single dose	+/mammary gland	Cavalieri et al. 1991
	Mouse	*In utero*	2 days	+/lung	Turusov et al. 1990
	Rat	Tracheal explant	Single dose	+/lung	Topping et al. 1981
	Rat	Intrapulmonary	Single dose	+/lung	Wenzel-Hartung et al. 1990
	Mouse	Intraperitoneal	Single dose	+/lung	Mass et all. 1993
	Hamster	Silastic implant	170 d	+/lung	Hammond and Benfield 1993
	Hamster	Intratracheal	1 time, for 6 weeks	+/lung	Kimizuka et al. 1993
Benzo[b]fluoranthene	Newborn mouse	Intraperitoneal	Single dose	+/liver	LaVoie et al. 1987
	Rat	Intrapulmonary	Single dose	+/lung	Deutsch-Wenzel et al. 1983
	Hamster	Intratracheal	30 weeks	–	Sellakumar and Shubik 1974
Benzo[e]pyrene	Rat	Tracheal explant	Single dose	–/trachea	Topping et al. 1981
	Rat	Intrapulmonary	Single dose	–/lung	Deutsch-Wenzel et al. 1983
Benzo[j]fluoranthene	Rat	Intrapulmonary	Single dose	+/lung	Deutsch-Wenzel et al. 1983
Benzo[g,h,i]perylene	Mouse	Subcutaneous	Single dose	–	IARC 1983
	Rat	Intrapulmonary	Single dose	+	Deutsch-Wenzel et al. 1983
Benzo[k]fluoranthene	Newborn mouse	Intraperitoneal	Single dose	–	LaVoie et al. 1987
	Rat	Intrapulmonary	Single dose	+/lung	Deutsch-Wenzel et al. 1983

TABLE 2-6. Summary of Carcinogenicity Studies with Polycyclic Aromatic Hydrocarbons Using Parenteral Routes of Exposure (continued)

Chemical	Species	Route	Duration	Result/Site	Reference
Anthracene	Rat	Lung implantation	1 dose, 55-week observation	–	Stanton et al. 1972
Chrysene	Newborn mouse	Intraperitoneal	No data	–	Buening et al. 1979a
	Newborn mouse	Intraperitoneal	Single dose, 70-week, week observation	+/liver	Grover et al. 1975
	Mouse	Subcutaneous	10 weeks	+/injection site tumors	Boyland and Sims 1967
	Rat	Intrapulmonary	Single dose	+/lung	Wenzel-Hartung et al. 1990
Dibenz[a,h]anthracene	Newborn mouse	Intraperitoneal	No data	+/lung	Buening et al. 1979b
	Mouse	Subcutaneous	10 weeks	+/injection site tumors	Boyland and Sims 1967
	Hamster	Intratracheal	30 weeks	–	Sellakumar and Shubik 1974
	Newborn mouse	Subcutaneous	Single dose	+/lung	Platt et al. 1990
	Newborn mouse	Subcutaneous	Single dose	+/injection site sarcoma	O'Gara et al. 1965
	Rat	Intrapulmonary	Single dose	+/lung	Wenzel-Hartung et al. 1990
Fluoranthene	Mouse	Intraperitoneal	15 days	+/lung	Busby et al. 1984
	Mouse	Intraperitoneal	3 doses in 15 days, 9-month observation	+/lung	Wang and Busby 1993
Fluorene	Mouse	Subcutaneous	Single dose	–	Roe 1962; Shear and Luter 1941; Steiner 1955
Ideno[1,2,3-c,d]pyrene	Newborn mouse	Intraperitoneal	Single dose	–	LaVoie et al. 1987
	Rat	Intrapulmonary	Single dose	+/lung	Deutsch-Wenzel et al. 1983
Phenanthrene	Newborn mouse	Intraperitoneal	No data	–	Buening et al. 1979a
	Rat	Intrapulmonary	Single dose	–/lung	Wenzel-Hartung et al. 1990
Pyrene	Hamster	Intratracheal	30 weeks	–	Sellakumar and Shubik 1974
	Newborn mouse	Subcutaneous	Single dose	–/lung	Busby et al. 1989

+ = increased incidence of tumors; – = no increase in tumor incidence

EPA has performed weight-of-evidence evaluations of several of the PAHs discussed in this profile. The carcinogenicity classifications currently verified by EPA's Carcinogenicity Risk Assessment Verification Endeavor Work Group (EPA 1994) are listed below:

PAH	EPA Classification
Acenaphthylene	D (not classifiable as to human carcinogenicity)
Anthracene	D
Benz[a]anthracene	B2 (probable human carcinogen)
Benzo[b]fluoranthene	B2
Benzo[k]fluoranthene	B2
Benzo[g,h,i]perylene	D
Benzo[a]pyrene	B2
Chrysene	B2
Dibenz[a,h]anthracene	B2
Fluoranthene	D
Fluorene	D
Indeno[1,2,3-c,d]pyrene	B2
Phenanthrene	D
Pyrene	D

A quantitative cancer risk estimate (i.e., cancer potency factor) has thus far been developed for benzo[a]pyrene only (EPA 1992). This cancer potency factor (q_1^*) is 7.3 per (mg/kg)/day and is based on the geometric mean of risk estimates calculated from the Neal and Rigdon (1967) and Brune et al. (1981) studies.

EPA and others have developed a relative potency estimate approach for the PAHs (EPA 1993a; Nisbet and LaGoy 1992). By using this approach, the cancer potency of the other carcinogenic PAHs can be estimated based on their relative potency to benzo[a]pyrene. Following are the toxicity equivalence factors (based on carcinogenicity) calculated for PAHs discussed in this profile considered by the authors of one of these approaches to be of most concern at hazardous waste sites (Nisbet and LaGoy 1992):

Compound	Toxicity Equivalency Factor (TEF)
Dibenz[a,h]anthracene	5
Benzo[a]pyrene	1
Benz[a]anthracene	0.1
Benzo[b]fluoranthene	0.1
Benzo[k]fluoranthene	0.1
Indeno[1,2,3-c,d]pyrene	0.1
Anthracene	0.01
Benzo[g,h,i]perylene	0.01
Chrysene	0.01
Acenaphthene	0.001
Acenaphthylene	0.001
Fluoranthene	0.001
Fluorene	0.001
Phenanthrene	0.001
Pyrene	0.001

EPA (1993) has derived the following relative potency estimates based on mouse skin carcinogenesis:

Compound	Relative Potency[a]
Benzo[a]pyrene	1.0
Benz[a]anthracene	0.145
Benzo[b]fluoranthene	0.167
Benzo[k]fluoranthene	0.020
Chrysene	0.0044
Dibenz[a,h]anthracene	1.11
Indeno[1,2,3-c,d]pyrene	0.055[b]

[a]Model was $P(d)=1-\exp[-a(1+bd)^2]$ for all but indeno[1,2,3-c,d]pyrene

[b]Simple mean of relative potencies (0.021 and 0.089); the latter derived using the one-hit model

2.5 BIOMARKERS OF EXPOSURE AND EFFECT

Biomarkers are broadly defined as indicators signaling events in biologic systems or samples. They have been classified as markers of exposure, markers of effect, and markers of susceptibility (NAS/NRC 1989).

A biomarker of exposure is a xenobiotic substance or its metabolite(s), or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NRC 1989). The preferred biomarkers of exposure are generally the substance itself or substance-specific metabolites in readily obtainable body fluid(s) or excreta. However, several factors can confound the use and interpretation of biomarkers of exposure. The body burden of a substance may be the result of exposures from more than one source. The substance being measured may be a metabolite of another xenobiotic substance (e.g., high urinary levels of phenol can result from exposure to several different aromatic compounds). Depending on the properties of the substance (e.g., biologic half-life) and environmental conditions (e.g., duration and route of exposure), the substance and all of its metabolites may have left the body by the time samples can be taken. It may be difficult to identify individuals exposed to hazardous substances that are commonly found in body tissues and fluids (e.g., essential mineral nutrients such as copper, zinc, and selenium). Biomarkers of exposure to PAHs are discussed in Section 2.5.1.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that, depending on magnitude, can be recognized as an established or potential health impairment or disease (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are not often substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effects caused by PAHs are discussed in Section 2.5.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism’s ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed

dose, a decrease in the biologically effective dose;or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 2.7, Populations That Are Unusually Susceptible.

2.5.1 Biomarkers Used to Identify or Quantify Exposure to Polycyclic Aromatic Hydrocarbons

PAHs and their metabolites can be measured in the urine of exposed individuals. In workers exposed to PAHs and dermatology patients treated with coal tar, the PAH metabolite 1-hydroxypyrene has been detected in the urine at concentrations of 0-40 µg/g creatinine or 290 ng/g creatinine, respectively (Jongeneelen et al. 1985). The amount of 1-hydroxypyrene detected in urine samples taken during the weekend was less than that detected during the weekdays, when the exposure was presumably higher than on the weekends. No correlation was found between occupational exposure levels and urine levels, so it is not known whether urinary metabolites could be detected following exposure to low levels of PAHs (as might be expected to occur in individuals living in the vicinity of hazardous waste sites). The presence of 1-hydroxypyrene in urine has also been demonstrated in workers exposed to PAHs in several different environments (creosote-impregnating plant, road workers laying asphalt, and workers exposed to diesel exhaust fumes) (Jongeneelen et al. 1988). In another study, the levels of urinary 1-hydroxypyrene significantly correlated with the environmental levels of pyrene and benzo[a]pyrene in coke plants, steel plants, and several Chinese cities where coal burning occurs (Zhao et al. 1990). The usefulness of monitoring urinary 1-hydroxypyrene concentration by liquid chromatography in occupationally exposed individuals as a biomarker for exposure to environmental PAHs was assessed. Postshift 1-hydroxypyrene urinary levels were significantly increased over preshift 1-hydroxypyrene levels in exposed workers as compared to nonexposed controls (the net mean change was 17-fold higher in the exposed workers as opposed to the nonexposed controls), and smoking status did not affect this result. In addition, in this work setting (an aluminum production plant), environmental levels of pyrene were strongly correlated with the environmental levels of total PAHs, indicating that pyrene is an appropriate environmental PAH marker in this situation. Thus, 1-Hydroxypyrene levels in urine may be used as a biomarker of exposure to PAHs in certain situations (Tolos et al. 1990). Additional studies have evaluated the usefulness of determining PAH or metabolite levels in human urine as a measure of exposure in industrial and environmental exposure settings (Granella and Clonfero 1993; Hansen et al. 1993; Herikstad et al. 1993; Kanoh et al. 1993; Likhachev et al. 1993; Ovrebo et al. 1994; Santella et al. 1993; Strickland et al. 1994; Van Hummelen et al. 1993; Van Rooij et al. 1993a, 1993b; Viau et al. 1993). Based on these results, the identification

of PAH metabolites in the urine could serve as a method of biological monitoring of exposed workers, and possibly individuals living in the vicinity of hazardous waste sites where PAHs have been detected although it would be very difficult to distinguish exposures resulting from hazardous waste sites form those resulting from normal human activities.

Autopsies performed on cancer-free patients found PAH levels of 1l-2,700 ppt in fat samples. Several PAHs were detected, including anthracene, pyrene, benzo[e]pyrene, benzo[k]fluoranthene, benzo[a]pyrene, and benzo[g,h,i]perylene, with pyrene being detected in the highest concentrations (Obana et al. 1981). A similar study done on livers from cancer-free patients found levels of 6-500 ppt of all of the same PAHs except benzo[e]pyrene, which was not detected in the liver. As in the fat samples studies, pyrene appeared in the highest concentrations in the liver, but the overall levels were less than in fat (Obana et al. 1981). However, because of the ubiquitous nature of PAHs in the environment, detection of PAH metabolites in the body tissues or fluids is not specific for exposure to PAHs from hazardous waste sites. In addition, it is impossible to determine from these biological media whether exposure was to high or low levels of PAHs or if the exposure duration was acute, intermediate, or chronic. Benzo[a]pyrene and 1-nitropyrene were determined in excised lung samples from Chinese and Japanese cancer patients (Tokiwa et al. 1993). Exposure to the two marker compounds was from burning coal (Chinese, benzo[a]pyrene) or oil (Japanese, 1-nitropyrene). Compound levels in the lung correlated with individual exposure history. Using a large sample population, total PAH levels in lung tissue has also been shown to correlate to cancer incidence (Seto et al. 1993).

PAHs form DNA adducts that can be measured in body tissues or blood in both humans and laboratory animals following exposure to PAHs or mixtures containing PAHs (e.g., Assennato et al. 1993; Bjelogrlic et al. 1994; Chou et al. 1993; Culp and Beland 1994; Day et al. 1990; Fuchs et al. 1993a, 1993b; Garg et al. 1993; Garner et al. 1988; Gallagher et al. 1993; Herberg et al. 1990; Hughes and Phillips 1990; Hughes et al. 1993; Jones et al. 1993; Khanduja and Majid 1993; Lee et al. 1993; Lewtas et al. 1993; Likhachev et al. 1993; Lu et al. 1993; Mass et al. 1993; Mumford et al. 1993; Newman et al. 1988, 1990; Nowak et al. 1992; Oueslati et al. 1992; Roggeband et al. 1994; Ross et al. 1990, 1991; Shamshuddin and Gan 1988; Van Schooten et al. 1991, 1992; Weston et al. 1988, 1993a; Weyand et al. 1993a, 1993b). PAHs also form adducts with other cellular macromolecules, such as hemoglobin, globin, and other large serum proteins (e.g., Bechtold et al. 1991; Sherson et al. 1990; Weston et al. 1988). Again, these PAH-DNA and PAH-protein adducts are not specific for any

particular source of PAHs, and the adducts measured could have been from exposure to other sources of PAHs, such as complex mixtures that contain PAHs (e.g., crude oils, various high-boiling point distillates, complex petroleum products, coal tars, creosote, and the products of coal liquification processes), as well as vehicle exhausts, wild fires, agricultural burning, tobacco smoke, smoke from home heating of wood, cereals, grains, flour, bread, vegetables, fruits, meat, processed or pickled foods, beverages, and grilled meats. It is impossible to determine from these adducts whether exposure was to high or low levels of PAHs or if the exposure duration was short or long.

In another study, an evaluation of mutations in peripheral lymphocytes was conducted in workers in or near an iron foundry; data were examined for correlations to benzo[a]pyrene exposure determined by personal monitors. Exposure levels for benzo[a]pyrene were 2-60 ng/m^3, which are the lowest levels yet analyzed in foundry workers. Mutations at the hypoxanthine guanine phosphoribosyl transferase (HPRT) and glycophorin A (GPA) loci, which are measures of molecular effects in lymphocytes and erythrocytes, respectively, were assessed to demonstrate their relationship to external exposure at these low levels. The rate of mutation was also compared to PAH-DNA adducts in the blood (Santella et al. 1993). Workers were classified into three exposure categories, low (<5), medium (5-12), and high (>12). HPRT mutant frequencies for these groups were 1.04, 1.13, and 1 .82x10^{-6} cells, respectively, and demonstrated an upward trend that was marginally significant. In contrast, HPRT mutations were highly correlated with PAH-DNA adducts (Santella et al. 1993). GPA variants were not correlated with PAH exposure. These results support the use of both biomonitoring and personal environmental monitoring in the determination of exposure.

Three methods were evaluated for their usefulness as biomarkers of exposure to benzo[a]pyrene in Wistar rats administered a single dose of l-200 mg/kg (Willems et al. 1991). These three methods were mutagenicity observed in urine and fecal extracts, chromosome aberrations and sister chromatid exchanges in peripheral blood lymphocytes, and DNA adduct formation in peripheral blood lymphocytes and liver. Mutagens were measured in urine and feces at levels of 10 and 1 mg/kg, respectively. DNA adduct formation (measured by ^{32}P-postlabelling) could be detected at doses of ≥10 mg in lymphocytes and ≥100 mg in liver, and the levels were twice as high in the lymphocytes as in the liver. Only a slight increase in sister chromatid exchanges and no enhanced frequency of chromosomal aberrations were seen. These results indicate that mutagenicity observed in excreta and DNA adducts in lymphocytes are both useful biomarkers of exposure in the rat, with mutagenic activity in feces being the more sensitive.

The ability of phenanthrene, fluoranthene, pyrene, benz[a]anthracene, chrysene, benzo[a]pyrene, benzo[b]fluoranthene, benzo[j]fluoranthene, indeno[l,2,3-c,d]pyrene, and dibenz[a]anthracene to bind to mouse hemoglobin and serum proteins after tail vein injection was investigated (Singh and Weyand 1994). Urinary excretion of these compounds was also investigated. A direct correlation between urinary excretion and hydrocarbon molecular weight was observed. Binding to both globin and serum proteins was detectable, with binding to serum proteins 10-fold higher than to globin. These results provide an assessment of the potential usefulness of various PAHs as biomarkers of exposure to complex mixtures.

2.5.2 Biomarkers Used to Characterize Effects Caused by Polycyclic Aromatic Hydrocarbons

The available genotoxicity data indicate that several of the 17 PAHs discussed in this profile are genotoxic in both nonmammalian and mammalian systems and are indirect mutagens (i.e., requiring the presence of an exogenous mammalian metabolic system). There were no tests reported for humans exposed to benzo[a]pyrene (the most widely tested PAH) *in vivo*, but several types of cultured human tissue cells demonstrated positive results for benzo[a]pyrene-induced genotoxicity (as evidenced by the induction of chromosomal aberrations, sister chromatid exchange) and binding of benzo[a]pyrene to DNA. The measurement of DNA adduct formation as well as the induction of sister chromatid exchange in human lymphocytes has been proposed as a biomarker of benzo[a]pyrene-induced effects for human monitoring programs (Wiencke et al. 1990). It is probable, however, that the analysis of DNA adducts would be the more sensitive diagnostic tool since hundreds of benzo[a]pyrene-DNA adducts per nucleus would be required to yield a detectable increase in the sister chromatid exchange frequency for an exposed population. Although these results are exclusively from *in vitro* tests and the limited genotoxicity tests conducted on urine obtained from humans exposed to creosote (a complex mixture containing PAHs) have been negative, the genotoxic effects observed in human tissue cells, particularly DNA adduct formation, may serve as a biomarker of effects for at least one of the PAHs, benzo[a]pyrene. It would not be possible to identify the source.of the benzo[a]pyrene, however.

PAHs have been shown to cause noncancer adverse effects on rapidly proliferating tissues such as the hematopoietic system, the lymphoid system, and the skin in both humans and animals. The skin is susceptible to PAH-induced toxicity in both humans and animals. Regressive verrucae were reported following intermediate-duration application of benzo[a]pyrene to human skin (Cottini and Mazzone

1939). Although reversible and apparently benign, these changes were thought to represent neoplastic proliferation. Benzo[a]pyrene application also apparently exacerbated skin lesions in patients with pre-existing skin conditions (pemphigus vulgaris and xeroderma pigmentosum) (Cottini and Mazzone 1939). Workers exposed to substances that contain PAHs (e.g., coal tar) experienced chronic dermatitis and hyperkeratosis (EPA 1988a). However, none of these end points is specific to PAHs, and all can be seen with other agents. No other biomarkers of effect (specific or otherwise) have been identified following exposure to PAHs.

For more information on biomarkers for renal and hepatic effects of chemicals see ATSDR/CDC Subcommittee Report on Biological Indicators of Organ Damage (1990) and for information on biomarkers for neurological effects see OTA (1990). Additional information can be found in a series of reports on biomarkers issued by the National Research Council (NRC 1989, 1992).

2.6 INTERACTIONS WITH OTHER SUBSTANCES

Because humans are usually exposed to PAHs in complex mixtures rather than to individual PAHs, it is important to understand the potential interactions between the PAHs and other components of the mixture. Interactions may occur among chemicals in a mixture prior to exposure, or may occur after exposure as a result of differing effects of the mixture components on the body. Synergistic and/or antagonistic interactions with regard to the development of health effects, particularly carcinogenesis, may occur.

The extent of human exposures to PAH mixtures in occupational settings is generally not known in quantitative terms. However, exposures to complex chemical mixtures that include PAHs, such as use of tobacco products and exposure to roofing tar emissions, coke oven emissions, coal tar, and shale oils, have been associated with adverse health effects in humans. The biological consequences of human exposure to complex mixtures of PAHs depend on the interaction of the various strongly carcinogenic, weakly carcinogenic, or noncarcinogenic PAHs. For example, there is evidence to suggest that PAHs in cigarette smoke require other components in the smoke in order to exert their tumorigenic effect (Akin et al. 1976).

The interaction between noncarcinogenic and carcinogenic PAHs has been extensively examined in animals. Noncarcinogenic PAHs exhibit co-carcinogenic potential and tumor-initiating and promoting

activity when applied with benzo[a]pyrene to the skin of mice. Simultaneous administration of weakly carcinogenic or noncarcinogenic PAHs including benzo[e]pyrene, benzo[g,h,i]perylene, fluoranthene, or pyrene significantly elevated the benzo[a]pyrene-induced tumor incidence. Benzo[e]pyrene, fluoranthene, and pyrene were more potent co-carcinogens than benzo[g,h,i]perylene (Van, Duuren and Goldschmidt 1976; Van Duuren et al. 1973). Benzo[e]pyrene, fluoranthene, and pyrene have also demonstrated weak tumor-promoting activity following initiation with benzo[a]pyrene, and these compounds increased benzo[a]pyrene-DNA adduct formation (Di Giovanni et al. 1982; Lau and Baird 1992; Rice et al. 1984, 1988; Slaga et al. 1979; Smolarek et al. 1987; Van Duuren and Goldschmidt 1976; Van Duuren et al. 1973).

Interactions between selected noncarcinogenic PAHs and carcinogenic benzo[a]pyrene have also been documented to reduce the carcinogenic potential of benzo[a]pyrene in animals. Benzo[a]fluoranthene, benzo[k]fluoranthene, chrysene, perylene, and a mixture of anthracene, phenanthracene, and pyrene significantly inhibited benzo[a]pyrene-induced injection-site sarcomas. However, other PAHs including anthracene, benzo[g,h,i]perylene, fluorene, and indeno[1,2,3-c,d]pyrene had no antagonistic effects (Falk et al. 1964). Coexposure of tracheal explants to benzo[e]pyrene and benzo[a]pyrene resulted in an increased incidence of tracheal epithelial sarcomas over that seen with either PAH alone (Topping et al. 1981). Phenanthrene administration with benzo[a]pyrene decreased the DNA adduct formation in mice (Rice et al. 1984).

There is evidence to suggest that benz[a]anthracene may serve as an anticarcinogen when administered with benzo[a]pyrene. Coadministration of benz[a]anthracene and benzo[a]pyrene decreased benzo[a]pyrene metabolism, benzo[a]pyrene-DNA adduct formation, and reduced the mutagenic activity of benzo[a]pyrene on hamster embryo cells. It has been postulated that the antimutagenic effect of benz[a]anthracene results from competition with benzo[a]pyrene for MFO enzymes, rather than the induction of detoxifying enzymes (Smolarek et al. 1986).

The synergistic effect of individual PAHs on the mutagenicity of benzo[a]pyrene has also been demonstrated. Anthracene and benzo[e]pyrene enhanced the mutagenicity of benzo[a]pyrene, the maximal increase being obtained with anthracene. Benzo[e]pyrene (at a ratio of 2:l) had no effect on benzo[a]pyrene-induced mutation frequencies in V79 cells, but at a ratio of 15:1, benzo[e]pyrene inhibited the benzo[a]pyrene-induced mutations by approximately 10-fold. Benzo[e]pyrene inhibited the metabolism of benzo[a]pyrene by cultured hamster embryo only at high doses, but at both low and

high doses, the proportion of metabolites formed was altered by benzo[e]pyrene (Baird et al. 1984). The percentage of water-soluble metabolites was decreased, whereas the percentage of diols was increased. The authors postulated that benzo[e]pyrene alters the activity of other PAHs by inhibiting the conversion of the proximate carcinogenic diol of a particular PAH to a diol epoxide.

Benzo[a]pyrene and dibenz[a,h]anthracene in combination with 10 noncarcinogenic PAHs were less potent tumor-inducers than was dibenz[a,h]anthracene alone or in combination with benzo[a]pyrene. The noncarcinogenic or weakly carcinogenic PAHs include benzo[e]pyrene, phenanthrene, anthracene, pyrene, fluoranthene, chrysene, perylene, benzo[g,h,i]pyrene, and coronene. Dose-response relationships for tumor incidences were observed for benzo[a]pyrene and dibenz[a,h]anthracene either alone or in combination with the 10 noncarcinogenic PAHs; however, no treatment-related sarcoma incidences were observed for any of the 10 noncarcinogenic PAHs (Pfeiffer 1977).

Phenanthrene, a noncarcinogenic PAH, demonstrated a dose-related inhibition of dibenz[a,h]anthracene-induced carcinogenicity in mice. Phenanthrene significantly reduced the incidence of injection-site sarcomas elicited by dibenz[a,h]anthracene, especially at low doses. However, when triethylene glycol was the vehicle administered in combination with phenanthrene and dibenz[a,h]anthracene, a substantial increase (50%) in the rate of tumor induction was observed (Falk et al. 1964).

Several experiments have shown that most PAH mixtures are considerably less potent than individual PAHs. Various combustion emissions and benzo[a]pyrene have been examined for carcinogenic potency and tumor initiation activity on mouse skin. In all cases, PAH mixtures were much less potent than benzo[a]pyrene. The authors calculated relative potency estimates that ranged from 0.007 for coke oven emissions extract to less than 0.002 for diesel engine exhaust extract, using papillomas per mouse per milligram of the mixture as the end point (Slaga et al. 1980b). Another study demonstrated that the relative tumorigenicities, as compared to benzo[a]pyrene, of automobile exhaust .condensate (AEC), diesel emission condensate, and a representative mixture of carcinogenic PAHs were 0.0053, 0.00011, and 0.36, respectively, following chronic application to mouse skin (Misfeld 1980). AEC has also exhibited an antagonistic influence on benzo[a]pyrene carcinogenicity when subcutaneously administered to mice; this effect was particularly augmented at higher benzo[a]pyrene concentrations (Pott et al. 1977).

Carcinogenic and noncarcinogenic PAHs, comprising a quantitative fraction of automobile exhaust gas condensate, were selected for carcinogenicity testing via dermal exposure of female NMRI mice. The purpose was to identify interactions between mixtures of the carcinogenic and noncarcinogenic PAHs (Schmahl et al. 1977). The carcinogenic PAHs were benzo[a]pyrene, dibenz[a,h]anthracene, benz[a]anthracene, benzo[b]fluoranthene; and the noncarcinogenic PAHs were phenanthrene, anthracene, fluoranthene, pyrene, chrysene, benzo[e]pyrene, and benzo[g,h,i]perylene. Treatment was carried out twice a week, for the natural lifetime of the animals. Although the carcinogenic action observed could be attributed almost entirely to the action of the carcinogenic PAHs, in relatively small doses, addition of the noncarcinogenic PAHs did not inhibit carcinogenesis, but had an additive effect.

Predicting the toxicity of a complex mixture on the basis of one of several of its components may be misleading, because the interactions among the components may modify toxicity. Since PAHs require metabolic activation by monoxygenases to elicit carcinogenic effects, any alteration in these metabolic pathways will influence the observed toxicity. There are two primary mechanisms by which chemicals interact with PAHs to influence toxicity. A compound may compete for the same metabolic activating enzymes and thereby reduce the toxicity of carcinogenic PAHs, or it may induce the metabolizing enzyme levels to result in a more rapid detoxification of the carcinogenic PAHs (Levin et al. 1982). Chaloupka et al. (1993) showed that a mixture of PAHs, produced as by-products from a manufactured gas plant, was 706 times more potent than expected, based on its benzo[a]pyrene content (0.17%) at inducing mouse hepatic microsomal ethoxyresorufin O-deethylase. Alternatively, compounds may compete for a deactivating pathway, thereby increasing the toxicity of PAHs (Furman et al. 1991). Many monooxygenase inducers are ubiquitous in the environment, and they may have an effect on the toxicity of PAHs. For example, environmental contaminants such as tetrachlorodibenzo-p-dioxin (TCDD), polychlorinated biphenyls (PCBs) and 3,3’,4,4’-tetrachlorobiphenyl (TCBP) can increase microsomal enzyme activity and consequently affect PAH toxicity (Jacob et al. 1987; Kouri et al. 1978). The dermal absorption of benzo[a]pyrene was measured in the presence or absence of complex organic mixtures derived from coal liquefaction processes (Dankovic et al. 1989). The dermal half-life of benzo[a]pyrene was 3.0 hours when applied alone, 6.7 hours when measured as a component of a mixture, and ranged from 7.8 to 29.7 hours in the presence of different mixtures. The authors proposed that these mixtures inhibit the dermal absorption of benzo[a]pyrene by inhibiting the metabolism of benzo[a]pyrene at the application site. Interactions. can thus play important modulatory roles in the expression of PAH toxicity that may not be adequately reflected based on the toxicity of a single PAH.

The majority of human exposures to PAHs occur in the presence of particles that affect the pharmacokinetics of PAHs in a manner that can enhance their carcinogenicity. Coadministration of benzo[a]pyrene and particulate material, such as hematite (Fe_2O_3) and arsenic trioxide (As_2O_3), greatly increases respiratory tract tumor yields in laboratory animals following intratracheal instillation (Pershagen et al. 1984; Saffiotti et al. 1972; Stenback and Rowland 1979; Stenback et al. 1976). The effects of particles on the potential human carcinogenicity of PAHs are likely to be similar. When benzo[a]pyrene is particle-bound, clearance from hamster lungs is slower than that of pure benzo[a]pyrene aerosol, increasing the length of time the lungs are exposed and increasing the dose to the gastrointestinal tract as a result of mucocilliary clearance. Respirable benzo[a]pyrene-containing particulates such as diesel exhaust, when coated with the phospholipid component of a pulmonary surfactant, are genotoxic (Wallace et al. 1987). Dusts can increase the rates of pulmonary cell proliferation (Harris et al. 1971; Stenback and Rowland 1979; Stenback et al. 1976), which in turn increases the cells' susceptibility to an initiation event in the presence of a carcinogen.

Environmental exposure to PAHs can also occur along with exposure to other environmental pollutants. The effects of exposure to SO_2 (either by inhalation or systemically with endogenous sulfite/bisulfite anions that accumulated as a result of induced sulfite oxidase deficiency) on benzo[a]pyrene-induced lung tumors were studied in male Sprague-Dawley rats (Gunnison et al. 1988). The animals, were administered benzo[a]pyrene (1 mg) by weekly intratracheal instillation for 15 weeks during which time they were exposed daily by inhalation to 30 or 60 ppm SO_2 or were maintained on a high tungsten to molybdenum diet. There were no statistically significant differences between the benzo[a]pyrene only and the benzo[a]pyrene + SO_2 or benzo[a]pyrene + sulfite/bisulfite groups with respect to the incidence of squamous cell carcinomas of the lung, latency for tumor development, or rate of appearance. Although benzo[a]pyrene alone induced almost 100% tumor incidence leaving little room for an SO_2-induced enhanced response, a shortened latency or increased rate of appearance would have suggested that SO_2 potentiates the carcinogenicity of benzo[a]pyrene, and this did not occur. Therefore, the authors concluded that SO_2 does not potentiate the carcinogenicity of benzo[a]pyrene in the lung.

Concomitant exposure to solvents may also occur, particularly in an occupational setting. It has been demonstrated that pretreatment of rats with toluene (1 g/kg intraperitoneally) inhibits the total cytochrome P-450 content in microsomes isolated from the lungs (Furman et al. 1991). In addition, formation of 3-hydroxybenzo[a]pyrene (a nontoxic metabolite) was inhibited by 36% by the

microsomes *in vitro* whereas the formation of several diols (reactive intermediates) was unaffected by toluene pretreatment. These results indicate that toluene alters the balance between toxification and detoxification of benzo[a]pyrene by cytochrome P-450 in the lung, favoring the formation of reactive genotoxic/carcinogenic intermediates. Therefore, the authors suggested that concomitant exposure to solvents, such as toluene, and PAHs may result in an increased risk for lung cancer.

Asbestos exerts a synergistic influence on cigarette smoke (which contains several PAHs) in the development of bronchopulmonary cancers. This has important implications for workers occupationally exposed to asbestos, who also smoke. The interaction between cigarette smoke and asbestos may be explained partly by differences in the kinetics of PAH cell uptake when PAHs are preadsorbed on asbestos (Foumier and Pezerat 1986). Plutonium oxide (PuO_2) has also been shown to enhance benzo[a]pyrene-induced lung carcinogenesis following simultaneous inhalation of both compounds (Metivier et al. 1984).

Another component of cigarette smoke, nicotine, may also affect the toxicokinetics of PAHs. When introduced in the perfusion medium with benzo[a]pyrene, nicotine inhibited the elimination of benzo[a]pyrene from the lung (Foth et al. 1988a).

Naturally occurring compounds have been found to induce the enzymes that metabolize PAHs, leading to either increased or decreased toxicity. Compounds that exert a protective effect against the carcinogenicity of PAHs and are enzyme inducers include plant flavonoids, plant phenols, antioxidants, retinoids (vitamin A), garlic oil, selenium, molybdenum, turmeric extracts, nitrates, soy sauce, and Chinese herbs. Plant flavonoids can induce microsomal monooxygenases and reduce the carcinogenicity of benzo[a]pyrene (Weibel 1980). Flavones administered orally or dermally increased benzo[a]pyrene hydroxylase activity in the small intestine and skin, respectively, and prevented the formation of pulmonary adenomas and forestomach and skin tumors initiated by benzo[a]pyrene (Rahimtula et al. 1977; Wattenberg and Leong 1970). A series of flavonoids and isoflavonoids, compounds that are found in fruits and vegetables, were tested for their ability to inhibit metabolism of benzo[a]pyrene in cultured hamster embryo cells (Chae et al. 1992). The results indicated that the flavonoids are generally more active than the isoflavonoids, and that two hydroxyl, two methoxyl, or methyl and hydroxyl substituents at the 5- and 7-positions and a 2,3-double bond are the structural characteristics required for inhibition of benzo[a]pyrene metabolism to reactive intermediates. Two of these compounds, acacetin and kaempferide also inhibited benzo[a]pyrene-induced mutation in Chinese

hamster V79 cells. Therefore, the authors proposed that the protective effect of these compounds may be due to their ability to inhibit metabolism of benzo[a]pyrene to reactive intermediates. Similar results have been obtained with pine cone extracts, tested in the *Salmonella typhimurium* assay (Lee et al. 1993). These results suggest that these compounds may be useful as potential chemopreventive agents in individuals exposed to genotoxic/carcinogenic PAHs.

Dietary plant phenols, such as tannic acid, quercetin, myricetin, and anthraflavic acid exhibit a protective effect against the tumorigenicity of benzo[a]pyrene and other PAHs by altering the metabolic pathways that detoxify and activate PAHs to their ultimate carcinogenic metabolites, thus suppressing PAH metabolism and subsequent PAH-DNA adduct formation. It has been suggested that the possible mechanism for the anticarcinogenic effect of these plant phenols may be an inhibitory effect on the binding of the ultimate carcinogen to the target tissue DNA (Mukhtar et al. 1988). Oral administration of these compounds has been associated with a decrease in tumorigenesis induced by benzo[a]pyrene in mouse forestomach (Katiyar et al. 1993a, 1993b; Zheng et al. 1993). Antioxidants also affect benzo[a]pyrene hydroxylation by rat liver microsomal MFOs, and reduce the bacterial mutagenicity of benzo[a]pyrene in the presence of rat liver microsomes and cofactors (Rahimtula et al. 1977). Antioxidants such as BHA, BHT, phenothiazine, phenothiazine methosulfate, and ethoxyquin all can reduce the quantitative yield of benzo[a]pyrene metabolites in incubations with rat liver microsomes (Sullivan et al. 1978).

Retinoids, of which vitamin A is a member, have demonstrated an antagonistic effect on benzo[a]pyrene-induced carcinogenicity. (Vitamin A has been shown to prevent and/or reverse the genetic damage caused by benzo[a]pyrene.) Similarly, the ability of benzo[a]pyrene (75 mg/kg, oral administration) to induce micronuclei *in vivo* was completely inhibited in Swiss mice orally administered doses of vitamin A ranging from 750 to 1,500 mg/kg 1 hour prior to benzo[a]pyrene treatment (Rao and Nandan 1990). Although the protective mechanism has not been fully elucidated, it has been suggested that vitamin A interferes with the activation of benzo[a]pyrene to its reactive metabolites, thus reducing the amount of benzo[a]pyrene-DNA binding in rat liver and target tissues (i.e., stomach) to prevent the mutagenic action of benzo[a]pyrene. It has also been suggested that vitamin A can enhance DNA repair (McCarthy et al. 1987; Rao et al. 1986). Conversely, vitamin A deficiency enhances the mutagenicity and carcinogenic effect of cigarette smoke and benzo[a]pyrene. This activity is related to a decreased level of free radical scavengers like ascorbic acid and glutathione in the liver (Alzieu et al. 1987). Another study observed that vitamin A deficient animals exposed to

cigarette smoke via inhalation exhibited enhanced benzo[a]pyrene-DNA adduct formation (Gupta et al. 1987, 1990). This has important implications for humans who smoke and consume diets deficient in vitamin A. Mammary tumor incidence was reduced by 27% (from 67% to 40%) in female rats receiving retinyl acetate before, during, and after the administration of either a single dose or eight weekly doses of benzo[a]pyrene. These results demonstrate that retinyl acetate is capable of inhibiting benzo[a]pyrene-induced mammary tumor formation in rats when given before, during, and after carcinogen treatment (McCormick et al. 1981).

The oral gavage administration of 25 mg/kg *n*-acetylcysteine (NAC) prevented the formation of benzo[a]pyrene-diol-epoxide-DNA adducts in rats receiving benzo[a]pyrene by intratracheal instillation for 3 consecutive days (25 mg/kg in 2% Tween 80) (De Flora et al. 1991). Inhibition of DNA adduct formation was more efficient in the liver than in the lungs. Similarly, micronuclei induction in the benzo[a]pyrene-treated rats was completely reversed by NAC. These results suggest that NAC, which is a glutathione precursor, may be effective in preventing or reversing the binding of the reactive intermediates of PAHs to cellular macromolecules and, therefore, may prevent the subsequent toxic effects of PAHs.

Coumarin, also known to be anticarcinogenic, inhibited benzo[a]pyrene-induced micronuclei in male ICR mice pretreated with 65 or 130 mg/kg/day coumarin for 6 days prior to the intraperitoneal administration of 150 mg/kg benzo[a]pyrene (Morris and Ward 1992). However, pretreatment with either dose of coumarin did not alter the genotoxicity of benzo[a]pyrene when females were included in the study.

Garlic oil also exhibits an antagonistic effect on benzo[a]pyrene by inhibiting benzo[a]pyrene-induced skin carcinogenesis in Swiss mice during the initiation phase (Sadhana et al. 1988). A primary constituent of garlic oil, allyl methyl trisulfide (ATM), has also demonstrated an inhibitory effect on benzo[a]pyrene induced neoplasia of the forestomach in mice (Spamins et al. 1986).

Selenium has been shown to reduce the mutagenicity of benzo[a]pyrene as well as AHH activity (Lee and Lin 1988). Selenium also inhibits the metabolism of benzo[a]pyrene *in vitro* (Bompart and Clements 1990). Several different salts of molybdenum inhibited the formation of certain metabolites of benzo[a]pyrene by lung and liver microsomes *in vitro* obtained from rats pretreated with 3-methylcholanthrene (Bompart et al. 1989). In a later study, it was demonstrated that benzo[a]pyrene

metabolism *in vitro* by lung and liver microsomes isolated from rats that were exposed to 40 or 80 mg/kg of ammonium heptamolybdate for 8 weeks was inhibited (Bompart 1990). These results suggest that molybdenum interferes with the cytochrome P-450 enzymes responsible for the activation of benzo[a]pyrene, and thus may have a protective effect against benzo[a]pyrene-induced toxicity/carcinogenicity.

Ferric oxide has been shown to increase the metabolism of benzo[a]pyrene by hamster alveolar macrophages (Greife and Warshawsky 1993). Alveolar macrophages, the primary lung defense cell, have been shown to metabolize benzo[a]pyrene to a more biologically active form, and then release the metabolites. Concurrent exposure of hamster alveolar macrophages to benzo[a]pyrene-coated ferric oxide resulted in a significant increase in the amount of benzo[a]pyrene metabolites and superoxide anions, which have been shown to produce localized lipid peroxidation and edema *in vivo.*

Lindane, an isomer of hexachlorocyclohexane, is an organochlorine pesticide which is extensively used in agricultural and public health programs in developing countries (Khan et al. 1993). Pretreatment of rat lungs with lindane by intratracheal injection inhibited benzo[a]pyrene hydoxlase activity in the lungs. Reduced elimination of intravenously administered benzo[a]pyrene from the lungs of rats after lindane pretreatment was also observed, suggesting that lindane may alter the clearance of benzo[a]pyrene from the lungs.

Prostacyclin has been shown to significantly reduce genetic damage caused by benzo[a]pyrene to mouse bone marrow cells, using the micronucleus test (Koratkar et al. 1993).

Chinese herbs commonly used in anti-cancer drugs have also been demonstrated to inh.ibit the mutagenicity of benzo[a]pyrene (Lee and Lin 1988).

Aqueous extracts of turmeric, curcumin-free aqueous turmeric extract, and curcumin were tested for their ability to inhibit benzo[a]pyrene-induced mutagenicity in the *S. typhimurium* assay and the bone marrow micronucleus test in Swiss mice (Azuine et al. 1992). A dose-dependent inhibition of benzo[a]pyrene-induced mutagenicity was observed in two strains of *Salmonella* in the presence of Aroclor-1254-induced rat liver homogenate, and 3 mg/kg of these three extracts also significantly inhibited benzo[a]pyrene-induced bone marrow micronuclei formation by 43%, 76%, and 65%. Female Swiss mice were treated with either aqueous turmeric extract (3 mg/day), curcumin-free

aqueous tumeric extract (1 mg/day), or curcumin (1 mg/day) 5 days/week for 2 weeks prior to receiving twice weekly gavage administrations of 20 mg/kg benzo[a]pyrene for 4 weeks. The treatment with the turmeric extracts continued for another 2 weeks after the cessation of benzopyrene treatment, and the animals were observed until they were 180 days old. A group receiving just benzo[a]pyrene served as a control. The benzo[a]pyrene-only animals exhibited 100% tumor incidence with 9.1±0.6 papillomas/mouse. All three extracts significantly ($p<0.00l$) inhibited the formation of forestomach papillomas by benzo[a]pyrene by 53%, and the average numbers of papillomas per mouse was also significantly decreased. The authors suggested that turmeric extracts may be useful as chemopreventive agents, but that there are probably several mechanisms of action for these inhibitory effects.

Pumark, a mixture of solvent extracts of tumeric, betel leaf, and catechu, was tested for its chemopreventative activity against benzo[a]pyrene-induced DNA damage (Ghaisas and Bhide 1994) Sister chromatid exchange and micronuclei formation in human lymphocyte culture were used as markers to assess the protective effect of Pumark. Pumark gave 50-60% protection against benzo[a]pyrene-induced chromosomal damage.

Other environmentally ubiquitous substances, such as nitrites and nitrates, have been shown to interact with PAHs. Pyrene is not mutagenic in the *Salmonella typhimurium* assay. However, when injected intraperitoneally into mice at doses of 10-200 mg/kg in combination with inhalation exposure to 50 or 100 ppm nitrous oxide (NO_2) mutagenic metabolites of pyrene were recovered from the urine (Kanoh et al. 1990). In addition, 1-hydroxypyrene (the major urinary metabolite of pyrene) administration in combination with NO_2 exposure also produced mutagenic metabolites. These results suggest that combined exposure to pyrene, a prevalent environmental PAH, and nitrogen compounds could result in the formation of nitrogenated mutagenic metabolites of pyrene. The effects of nitrite (in drinking water) and/or soy sauce (in food) on the forestomach tumors induced by twice weekly gavage administration of eight total doses of benzo[a]pyrene were studied in mice (Benjamin et al. 1988). The combination of nitrite and soy sauce resulted primarily in a significant reduction in the number of tumors per animal induced by benzo[a]pyrene, and also a reduction in tumor incidence. Neither substance alone had much effect on the carcinogenicity of benzo[a]pyrene alone. The mechanism for this protective effect of the combination of nitrite and soy sauce is not known.

The fungicide (prochloraz), the topical antifungal agent (miconazole) and 7,8-benzoflavone (a strong inhibitor of cytochrome P-450) limited the conversion of benzo[a]pyrene to a mutagen in S. *typhimurium.* The order of inhibitory action was 7,8-benzoflavone ≥ prochloraz ≥ miconazole (Antignac et al. 1990).

Diet (i.e., dietary fat levels) can also have an effect on the disposition and toxicity of PAHs. The metabolism of benzo[a]pyrene in hepatocytes *in vitro* from rats fed high-fat (as corn oil) diets was decreased (Zaleski et al. 1991). This effect was not due to a decrease in the activity of AHH. The authors postulated that the high-fat diets allowed benzo[a]pyrene, which is highly lipophilic, to become sequestered in lipid droplets and, therefore, become inaccessible to the P-450 enzymes. Another study suggests that caloric restriction, per se, can reduce the metabolic activation capacity of the liver, thereby reducing the production of mutagenic metabolites from PAHs (Xiao et al. 1993).

Organosulphur compounds, e.g., S-methyl cysteine sulphoxide (SMCSO) and its metabolite methyl methane thiosulphinate, both naturally occurring compounds present in Brassica vegetables (broccoli, cabbage) were found to inhibit benzo[a]pyrene-induced micronucleus formation in mouse bone marrow by 31 and 33%, respectively, after oral administration (Marks et al. 1993).

o-Cresol, often found in conjunction with PAHs in industrial waste from coking, oil processing, shale processing, and other industries, was found to protect mice from benzo[a]pyrene-induced forestomach tumors after oral administration, when o-cresol was administered before or after benzo[a]pyrene (Yanysheva et al. 1993).

Topical application of 1% croton oil twice weekly at 4 weeks of age had an inhibitory effect on tumor formation in offspring of ICF/Ha female mice treated with intraperitoneal injections of benzo[a]pyrene in sesame oil on the llth, 13th and 15th days of pregnancy (Bulay and Wattenberg 1971).

Administration of a diet containing 3% myo-inositol to mice beginning one week after oral benzo[a]pyrene administration reduced the number of pulmonary adenomas by 40% but did not prevent forestomach tumors (Estensen and Wattenberg 1993). Under the same conditions, administration of 0.5% dexamethasone in the diet inhibited pulmonary adenomas by 57% and also inhibited forestomach tumor formation to a similar degree. A combination of the two compounds resulted in additive chemoprevention.

The ubiquitous nature of PAHs in the environment, particularly as constituents of complex mixtures such as automobile emissions, coal tar, coke oven emissions, and combustion products of tobacco, increases the likelihood that the type of interactions discussed will occur. Thus, interactions may play a decisive role in the expression of toxicity and the development of cancer in exposed populations.

2.7 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to PAHs than will most persons exposed to the same level of PAHs in the environment. Reasons include genetic make-up, developmental stage, age, health and nutritional status (including dietary habits that may increase susceptibility, such as inconsistent diets or nutritional deficiencies), and substance exposure history (including smoking). These parameters result in decreased function of the detoxification and excretory processes (mainly hepatic, renal, and respiratory) or the pre-existing compromised function of target organs (including effects or clearance rates and any resulting end-product metabolites). For these reasons we expect the elderly with declining organ function and the youngest of the population with immature and developing organs will generally be more vulnerable to toxic substances than healthy adults. Populations who are at greater risk due to their unusually high exposure are discussed in Section 5.6, Populations With Potentially High Exposure.

Data suggest that specific subsections of the population may be susceptible to the toxic effects produced by exposure to PAHs. These include people with various conditions, such as aryl hydrocarbon hydroxylase (AHH) that is particularly susceptible to induction, nutritional deficiencies, genetic diseases that influence the efficiency of DNA repair, and imrnunodeficiency due to age or disease. Other subsections of the population that may be susceptible to the toxic effects of PAHs are people who smoke, people with a history of excessive sun exposure, people with liver and skin diseases, and women, especially of child-bearing age. Human fetuses may also be particularly susceptible to the toxic effects produced by exposure to PAHs. Data also indicate that the general population may be at increased risk of developing lung cancer following prolonged inhalation of PAH-contaminated air, and skin cancer following concurrent dermal exposure to PAHs and sunlight. There is some limited evidence that indicates that all people could be sensitive at some point to the toxic effects of environmental contaminants, such as PAHs, as a result of stress and/or circadian rhythms.

Pre- and post-natal exposure to PAHs could produce adverse reproductive and developmental effects in human fetuses. Most PAHs and their metabolites cross the placenta because of their lipid solubility (Calabrese 1978; Shendrikova and Aleksandrov 1974). Fetuses are susceptible to the toxic effects produced by maternal exposure to PAHs, such as benzo[a]pyrene, because of an increased permeability of the embryonic and fetal blood-brain barrier and a decreased liver-enzyme conjugating function (Calabrese 1978; Shendrikova and Aleksandrov 1974). Because of PAH exposure, higher incidences of embryo- and fetolethality, stillbirths, resorptions, and malformations of the kidney and bladder have been observed in animals (Legraverend et al. 1984; Urso and Gengozian 1980; Urso and Johnson 1987). Delayed effects have been observed in the progeny of mothers exposed to PAHs, such as benzo[a]pyrene (Urso and Gengozian 1980). These .delayed effects include sterility of progeny, immune suppression, possible alteration of endocrine function, and cancer in rodents (Csaba and Inczefi-Gonda 1992; Csaba et al. 1991; Legraverend et al. 1984; Mackenzie and Angevine 1981). Tobacco smoke contains both PAHs and particulate matter. These could interact synergistically in pregnant women who smoke to produce decreased birth weight, increased perinatal morbidity and mortality, and other diseases of the newborn (NRC 1983). PAHs in cigarette smoke, such as benzo[a]pyrene, have been associated with the induction of AHH activity in human placental tissue and a decrease in placental size (NRC 1983). Results of *in vitro* studies indicate that benzo[a]pyrene alters human placental endocrine and metabolic function (Avigdor et al. 1992; Bamea and Shurtz-Swirski 1992; Guyda 1991; Guyda et al. 1990).

People with AHH that is particularly susceptible to induction may also be susceptible to the possible carcinogenic effect of exposure to PAHs. This enzyme is mixed function oxidase (MFO) that is involved in the metabolism of PAHs to certain reactive intermediates that can cause cell transformation, mutagenicity, and cytotoxicity. The incidence of this inducible genetic trait is low in 53%, intermediate in 37%, and high in 10% of the white population in the United States (Calabrese 1978). It has been proposed that genetically expressed AHH inducibility is related to the development of bronchogenic carcinoma in persons exposed to PAHs contained in tobacco smoke. On the other hand, individuals with a greater ability to induce AHH may be able to rapidly detoxify PAHs and eliminate them, thus making them less susceptible to the toxic effects of PAHs. Based on the population frequency of genetically controlled AHH induction, some scientists predict that approximately 45% of the general population are considered to be at high risk, and 9% of the 45% are considered to be at very high risk, of developing bronchogenic carcinoma following exposure to PAHs (Calabrese 1978).

Certain nutritional deficiencies have been associated with an increased cancer incidence in PAH-exposed animals. These include deficiencies in vitamins A and C, iron, and riboflavin (Calabrese 1978). It is estimated that at least 25% of all children between the ages of 7 and 12 years and all children of low-income families consume less than the recommended dietary allowance (RDA) of vitamin A. It has also been estimated that between 10% and 30% of the infants, children, and adults of low-income groups consume less than the RDA for vitamin C, 98% of all children consume less than the RDA for iron, and 30% of women and 10% of men between the ages of 30 and 60 years consume less than two-thirds of the RDA for riboflavin (Calabrese 1978). Other nutrients such as vitamin D, selenium, and protein can also influence the cancer incidence in animals exposed to PAHs (NRC 1983; Prasanna et al. 1987). Several studies have been conducted to investigate the interaction between nutrition and PAH exposure by administering benzo[a]pyrene to laboratory animals. The nutritional factors listed above either reduced the amount of benzo[a]pyrene binding to DNA in rat liver or forestomach tissue (McCarthy et al. 1987), prevented or reversed genetic damage (Rao et al. 1986), or reduced the activity of AHH (Prasanna et al. 1987). It has also been observed that fasted rats showed altered toxicokinetics of PAHs resulting from benz[a]anthracene and chrysene exposure. This altered toxicokinetics included reduced hepatic clearance because of decreased AHH activity and the acceleration of the depletion of cytochrome P-450 and other microsomal enzymes required to metabolize PAHs (Fiume et al. 1983).

Individuals who undergo a rapid reduction in body fat may be at risk from increased toxicity because of the systemic release and activation of PAHs that had been stored in fat. The metabolism of benzo[a]pyrene in hepatocytes *in vitro* from rats fed high-fat (as corn oil) diets was decreased (Zaleski et al. 1991). This effect was not due to a decrease in the activity of AHH. The authors postulated that the high-fat diets allowed benzo[a]pyrene, which is highly lipophilic, to become sequestered in lipid droplets and, therefore, become inaccessible to the P-450 enzymes.

People exposed to PAHs in conjunction with particulates from tobacco smoke, fossil-fuel combustion, coal fly ash, and asbestos fibers are at increased risk of developing toxic effects, primarily cancer. Even people not susceptible to the toxic effects of PAHs may become affected when exposure occurs in conjunction with exposure to particulates (NRC 1983). This enhanced effect results from the adsorption of PAHs onto the particulates. They are vacuolized into cells, and distributed differently in tissues depending on the size and type of particulate matter. This increased PAH uptake may result in more efficient induction of AHH activity at low PAH concentrations. This activity also increases the

dose to the gastrointestinal tract as a result of mucocilliary clearance (NRC 1983). This synergistic action between PAHs and particulate matter in air pollution has been associated with the occurrence of stomach cancer in humans (Fraumeni 1975).

Immunocompetence is an important factor in decreasing or preventing human susceptibility to toxicity and disease after exposure to environmental contaminants. Small children have an immature immune system, and the elderly may have a deficient immune system due to age, genetic factors, or disease (Calabrese 1978; NRC 1983). It is possible that individuals whose immune systems are compromised could be at an increased risk of carcinogenesis, including that produced by PAHs. Some genetic diseases that may predispose a person to immune deficiency include ataxia telangiectasia, Wiskott-Aldrich syndrome, Bloom's syndrome, common variable immunodeficiency, selective IgA deficiency, Bruton's agammaglobulinemia, severe combined immunodeficiency, selective IgM deficiency, AIDS, and immunodeficiency with normal or increased immunoglobulins (NRC 1983).

Genetic diseases that reduce DNA-repair capabilities also increase an individual's susceptibility to PAH-related malignancy by reducing the efficiency of DNA repair. In fact, the level of benzo[a]pyrene/DNA adducts in peripheral lymphocytes was slightly but significantly higher in 22 lung cancer patients who had at least one "first-degree" relative with lung cancer than in 30 healthy controls (Nowak et al. 1992). This finding led the authors to speculate that altered metabolic activation and deactivation and increased formation of adducts may indicate a genetic predisposition for lung cancer. Some of the diseases that reduce DNA repair capability are also associated with an abnormality of the immune system (NRC 1983). Diseases that may be associated with DNA-repair deficiencies are classical and variant xeroderma pigmentosum, ataxia telangiectasia, Bloom's syndrome, Fanconi's anemia, familial retinoblastoma, D-deletion retinoblastoma, progeria (Hutchinson-Gilford) syndrome, Down's syndrome, dyskeratosis congenita, Cockayne's syndrome, actinia keratosis, and cutaneous malignant melanoma (NRC 1983).

Women may be at increased risk of reproductive dysfunction following exposure to high levels of PAHs. Data from animal studies indicate that oocyte and follicle destruction occurs following dosing with PAHs (Mattison et al. 1989; Miller et al. 1992; Urso and Gengozian 1980; Urso and Johnson 1987). Benzo[a]pyrene exposure may reduce fertility and the ability to bear children (Mackenzie and Angevine 1981; Rigdon and Rennels 1964). Exposure may also reduce fertility of exposed women by

causing ovarian dysfunction (Swartz and Mattison 1985). However, the doses that produced the effects discussed above are high relative to expected environmental exposures to PAHs.

Subsections of the population that suffer from liver and skin diseases may be at increased risk of developing adverse effects from exposure to PAHs. People with pre-existing skin conditions, such as pemphigus vulgaris and xeroderma pigmentosum, and those with normal skin may be at increased risk of developing adverse dermal effects ranging from rashes to cancer following exposure to some PAHs, such as benzo[a]pyrene, anthracene, benz[a]anthracene, and pyrene (Bingham and Falk 1969; Cavalieri et al. 1977, 1988b; Cottini and Mazzone 1939; Forbes et al. 1976; Habs et al. 1980; Shubik and Porta 1957). Exposure to more than one PAH may enhance or reduce tumor development (Slaga et al. 1979; Van Duuren and Goldschmidt 1976; Van Duuren et al. 1973). Skin cancer induction in laboratory animals has been associated with exposure to benz[a]anthracene, benzo[a]pyrene, benzo[b]fluoranthene, benzohlfluoranthene, benzo[k]fluoranthene, chrysene, dibenz[a,h]anthracene, and indeno[1,2,3-c,d]pyrene.

People with significant exposure to ultraviolet radiation, such as from sunlight, may also be at increased risk of developing skin cancer due to PAH exposure. Ultraviolet radiation has a synergistic influence on PAH-induced skin cancer following dermal exposure. It enhances benzo[a]pyreneinduced skin carcinogenesis in the mouse, which is dependent on the dose of benzo[a]pyrene (Gensler 1988). Combined exposure to anthracene and sunlight could produce mutagenic lesions (Blackbum and Taussig 1975; Forbes et al. 1976). Laboratory animals exposed concurrently to chronic ultraviolet irradiation and to PAHs were at a higher risk of skin tumor induction (Mukhtar et al. 1986).

2.8 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to PAHs. However, because some of the treatments discussed may be experimental and unproven, this section should not be used as a guide for treatment of exposures to PAHs. When specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice.

2.8.1 Reducing Peak Absorption Following Exposure

General recommendations to reduce absorption follo.wing acute exposure to PAHs include removing the individual from the source of exposure, removing contaminated clothing, and decontaminating exposed areas of the body. It has been suggested that contaminated skin should be washed with soap and water, and eyes exposed to PAHs should be flushed with water or saline (Stutz and Janusz 1988). Administration of activated charcoal following ingestion of PAHs is recommended; however, it has not been proven to reduce absorption of PAHs in the gastrointestinal system (Stutz and Janusz 1988). The use of emetics as a means of gastrointestinal decontamination of PAHs is not recommended (Bronstein and Currance 1988). There is a risk of causing chemical pneumonitis in the patient by the aspiration of the PAHs.

2.8.2 Reducing Body Burden

There are no known methods currently available for reducing the body burden of PAHs. Evidence from acute-duration studies in experimental animals indicates that PAHs are rapidly metabolized and conjugated to form water-soluble metabolites that are essentially completely eliminated in the urine and feces within a matter of days (see Sections 2.3.3 and 2.3.4). No data are available on the kinetics of PAHs following chronic exposure, so it is not known if PAHs or their metabolites bioaccumulate in these exposure situations. Given the relatively rapid and complete excretion observed following short-term exposures, it is not likely that PAHs bioaccumulate to an appreciable degree. However, PAHs are lipophilic, so it is conceivable that unmetabolized parent compound could accumulate in tissue fat stores. In fact, diet (i.e., dietary fat levels) may have an effect on the disposition and toxicity of PAHs. The metabolism of benzo[a]pyrene in hepatocytes *in vitro* from rats fed high-fat (as corn oil) diets was decreased (Zaleski et al. 1991). This effect was not due to a decrease in the activity of AHH. The authors postulated that the high-fat diets allowed benzo[a]pyrene, which is highly lipophilic, to become sequestered in lipid droplets and, therefore, become inaccessible to the P-450 enzymes. Therefore, high-fat diets may favor the accumulation of parent PAHs in lipids so that they are not metabolized to reactive intermediates or water-soluble conjugates. Alternatively, rapid fat loss may result in the release of sequestered parent PAHs, making them available to the P-450 enzymes to be metabolized to reactive intermediates as well as water-soluble conjugates that can be easily excreted. Thus, modulating body fat content may reduce body burden of PAHs by hastening

their metabolism to water-soluble conjugates. However, the result may also be an increase in toxicity due to increased metabolism to reactive intermediates.

2.8.3 Interfering with the Mechanism of Action for Toxic Effects

As discussed in Sections 2.3.5 and 2.4, it is currently believed that the toxic and carcinogenic effects of PAHs are mediated by reactive diol-epoxide intermediates that interact directly with DNA and RNA, producing adducts. The formation of these adducts leads to neoplastic transformation as well as interfering with the normal functioning of rapidly proliferating tissues. As discussed above, these reactive intermediates are formed when PAHs are biotransformed by the P-450 enzymes. Interference with these metabolic pathways, by inactivation of the activated diol epoxides, reduction in tissue levels of cytochrome P-450, and direct inhibition of the cytochrome P-450 enzymes responsible for the formation of the reactive intermediates, could reduce the toxic and carcinogenic effects of PAHs. A number of drugs, such as cobaltous chloride, SKF-525-A, and 6-nitro- 1,2,3-benzothioadiazole, have been reported to inhibit P-450 enzymes. In addition, as discussed in Section 2.7, other compounds that exert a protective effect against the carcinogenicity of PAHs by interfering with cytochrome P-450 enzymes include plant flavonoids, plant phenols, antioxidants (such as BHA, BHT, phenothiazine, phenothiazine methosulfate, and ethoxyquin), retinoids (vitamin A), garlic oil, selenium, organosulphur compounds, o-cresol, myo-inositol, lindane, and molybdenum (Bompart 1990; Bompart et al. 1989; Chae et al. 1992; Estensen and Wattenberg 1993; Ghaisas and Bhide 1994; Katiyar et al. 1993a, 1993b; Khan et al. 1993; Lee and Lin 1988; Lee et al. 1993; Marks et al. 1993; Mukhtar et al. 1988; Rahimtula et al. 1977; Rao and Nandan 1990; Sullivan et al. 1978; Weibel 1980, Yanysheva et al. 1994; Zheng et .al. 1993). Reduced caloric intake has also been shown to cause decreased metabolism of PAHs by liver microsomes, thus protecting against genotoxic effects (Xiao et al. 1993). P-450 metabolism also results in products that can be more readily eliminated than can the parent compound. Hence, any side products of the drugs or substances listed above, along with their potential to increase the biological half-life of the PAHs, would also need to be considered in any protocol. Further research to determine which cytochrome P-450 isozymes are involved in the metabolism to the reactive intermediates, as well as which isozymes are involved in enhancing the elimination of PAHs, could lead to the development of strategies to selectively inhibit specific isozymes and, thus, reduce the toxic effects of PAHs.

It has also been suggested that some of these compounds may act at other points in the activation, macromolecular binding sequence described above. For instance, vitamin A can also enhance DNA repair (McCarthy et al. 1987; Rao et al. 1986).

Because PAHs are detoxified by conjugation with substances such as glutathione (see Sections 2.3.3 and 2.3.4), sufficient glutathione stores in the body may reduce the chances of toxic effects following acute exposure to PAHs. For example, the oral gavage administration of NAC prevented the formation of benzo[a]pyrene-diol-epoxide-DNA adducts in rats receiving benzo[a]pyrene (De Flora et al. 1991). Inhibition of DNA adduct formation was more efficient in the liver than in the lungs. Similarly, micronuclei induction in the benzo[a]pyrene-treated rats was completely reversed by NAC. These results suggest that NAC, which is a glutathione precursor, may be effective in preventing or reversing the binding of the reactive intermediates of PAHs to cellular macromolecules and, therefore, may prevent the subsequent toxic effects of PAHs.

2.9 ADEQUACY OF THE DATABASE

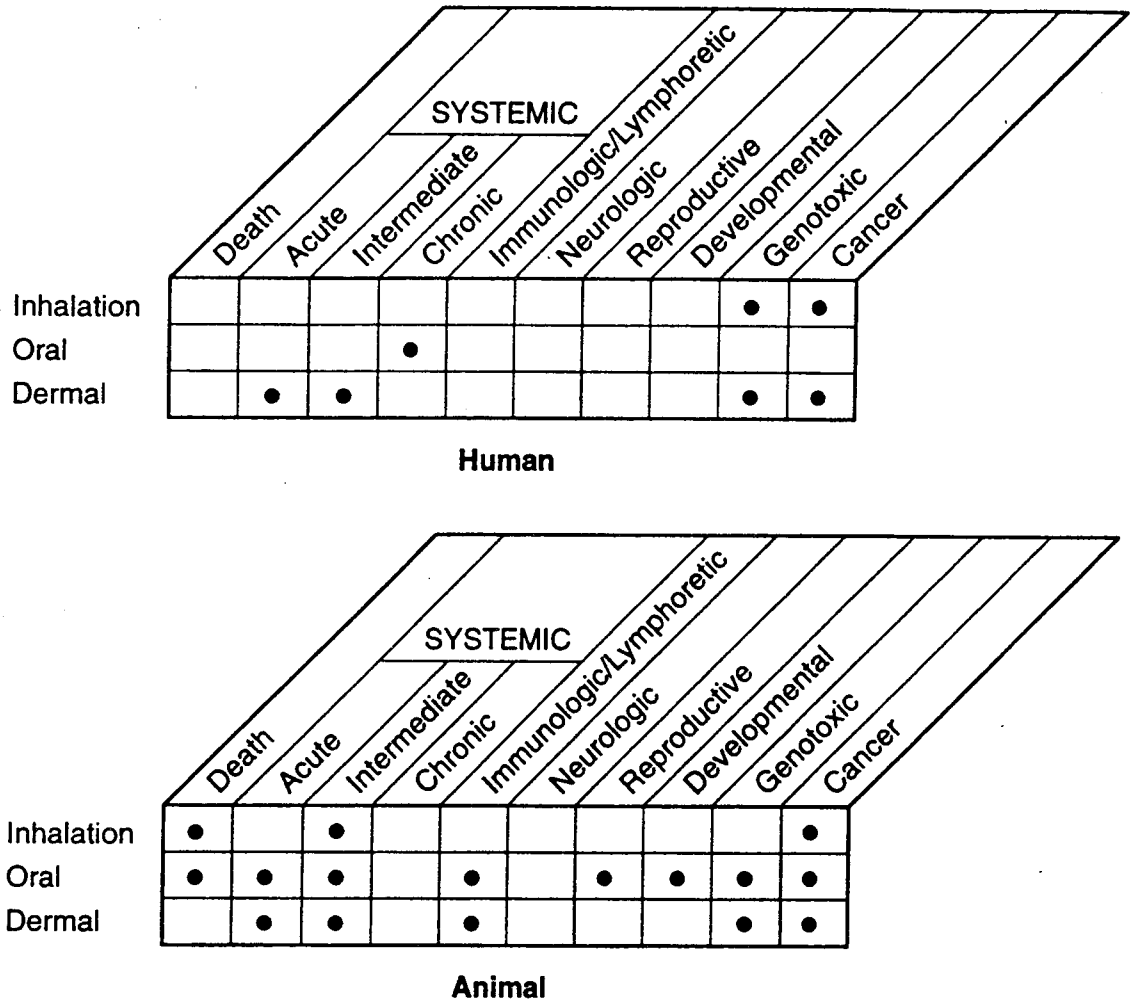
Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of PAHs is available. Where adequate information is not available, ATSDR, in conjunction with the National Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of PAHs.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

2.9.1 Existing Information on Health Effects of Polycyclic Aromatic Hydrocarbons

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to PAHs are summarized in Figure 2-5. The purpose of this figure is to illustrate the existing

FIGURE 2-5. Existing Information on Health Effects of Polycyclic Aromatic Hydrocarbons



● Existing Studies

information concerning the health effects of PAHs. Each dot in the figure indicates that one or more studies provide information associated with that particular effect. The dot does not imply anything about the quality of the study or studies. Gaps in this figure should not be interpreted as "data needs." A data need, as defined in ATSDR's Decision Guide for Identifying Substance-Specific Data Needs Related to Toxicological Profiles (ATSDR 1989), is substance-specific information necessary to conduct comprehensive public health assessments. Generally, ATSDR defines a data gap more broadly as any substance-specific information missing from the scientific literature.

The vast majority of literature reviewed concerning the health effects of PAHs in humans described case reports and chronic-duration studies in workers linking the occurrence of lung and skin cancer and adverse noncancer skin effects with exposure to PAH-containing mixtures such as coke oven emissions, roofing-tar emissions, shale oils, and soot, and exposure to cigarette smoke. The predominant routes of exposure in the studies are inhalation and dermal, but the possibility of some degree of oral exposure cannot be ruled out, especially in light of muco-cilliary clearance and ingestion following inhalation exposure. Because of the lack of quantitative exposure information and the presence of other potentially carcinogenic substances in these mixtures, it is impossible to evaluate the contribution of an individual PAH or even the PAHs as a class to the effects observed.

The database for the health effects of PAHs in experimental animals consists primarily of older animal studies that would be considered inadequate by current standards, and two-stage dermal carcinogenesis studies. As can be seen in Figure 2-5, very little information is available on the effects of inhalation exposure to PAHs in animals. However, oral and dermal exposures to relatively high doses of PAHs have been shown in numerous studies to induce skin, lung, and forestomach tumors in animals, and noncancer adverse effects in rapidly proliferating tissues such as bone marrow, lymphoid organs, gonads, and intestinal epithelium. Benzo[a]pyrene is by far the most extensively studied of the PAHs; therefore, the adverse effects of other less-studied PAHs must generally be inferred from the results obtained with benzo[a]pyrene. This may over- or underestimate the health risk associated with the various PAHs.

2.9.2 Identification of Data Needs

Acute-Duration Exposure. Little is known regarding the adverse health effects associated with acute-duration inhalation exposure to any of the PAHs in either humans or animals. Limited

information is available on the effects of acute-duration oral and dermal exposures to PAHs in animals; the skin and the liver have been identified as target organs of PAH toxicity in animals (Iwata et al. 1981; Nousiainen et al. 1984). Available information is insufficient to derive an acute inhalation or oral MRL. Identification of target organs (other than the developing fetus) from acute-duration animal studies following inhalation and oral exposures would be helpful in order to assess the risk associated with the acute inhalation of contaminated air or ingestion of PAH-contaminated water or soils by humans living in areas surrounding hazardous waste sites. Additional inhalation and oral studies in animals involving a range of exposure concentrations and employing sensitive histological and biochemical measurements of injury to a comprehensive set of end points would be useful for establishing dose-response relationships and identifying thresholds for these effects. This information would be useful for determining levels of significant exposure to PAHs that are associated with adverse health effects. Both routes are considered significant for individuals living in the vicinity of hazardous waste sites because exposures to particulate PAHs in air and PAHs bound to soil particles, sediments in water, and contaminated food are possible in such areas. Furthermore, the pharmacokinetic data on PAHs are insufficient to determine whether similar effects may be expected to occur across different routes of exposure. Additional studies should be conducted on the effects of PAHs after acute-duration dermal exposure, since dermal exposure may be important to populations around hazardous waste sites. It is known that acute-duration dermal exposure to PAHs results in adverse dermal effects. Further studies determining the relative importance of exposure by this route, with regard to subsequent toxicity, would be useful. Studies describing dermal and oral absorption of PAHs from complex mixtures, including soil and other mixtures that may actually be the vehicles of human exposure, would be useful in furthering understanding of the toxicity of these compounds. The studies should be conducted with benzo[a]pyrene, because this PAH has been found at the highest number of NPL sites and it is a representative altemant PAH; studies should also be conducted with a representative nonaltemant PAH such as fluoranthene, benzo[b]fluoranthene, or benzo[j]fluoranthene. In addition, since PAHs rarely occur alone, but are found in the environment in combinations and mixtures, studies, particularly feeding studies, addressing mixtures of PAHs should be conducted. In all cases, research should be cOnducted to determine the most appropriate animal species for extrapolation to humans.

Intermediate-Duration Exposure. Little is known regarding the adverse health effects associated with intermediate-duration inhalation exposure to any of the PAHs in either humans or animals. One inhalation study in rats failed to establish an effect level (Wolff et al. 1989c). Information is available

on the effects of intermediate-duration exposures to some of the PAHs in humans (dermal) and in animals (oral and dermal). Regressive verrucae and other epidermal changes were noted in the skin of human volunteers treated with topically applied benzo[a]pyrene (Cottini and Mazzone 1939). Intermediate-duration dermal exposure to benzo[a]pyrene in patients with preexisting dermal conditions of pemphigus vulgaris and xeroderma pigmentosum was associated with an exacerbation of the abnormal skin lesions (Cottini and Mazzone 1939). Target organs identified in animal studies with some of the PAHs were the skin, the liver, and the hemolymphatic system (Legraverend et al. 1983; Old et al. 1963; Robinson et al. 1975). The available information is insufficient to derive an intermediate inhalation MRL for PAHs because no intermediate-duration inhalation animal studies exist that adequately describe the effects of inhalation exposure to PAHs. Intermediate duration MRLs have been derived for acenaphthene, fluoranthene, fluorene, and anthracene, based on 90-day gavage studies in mice (EPA 1988e; 1989c; 1989d, 1989e). For acenaphthene, fluoranthene, and fluorene, liver effects, supported by effects in other organ systems were identified as the target toxicity. For anthracene, no effect was seen in the liver or any other organ system, even at the highest dose of 1,000 mg/kg/day. The PAHs in these studies were administered by gavage, a route that does not mimic the potential exposure of people living near hazardous waste sites. Identification of target organs from intermediate-duration animal studies following inhalation and oral (drinking water) exposures would be useful in order to assess the risk associated with the intermediate-duration inhalation of contaminated air or ingestion of PAH-contaminated water or soils by humans living in areas surrounding hazardous waste sites. Ninety-day studies in animals by the inhalation and oral (drinking water) routes would be helpful to establish dose-response relationships and to identify other possible target organs or systems in individuals living around hazardous waste sites who can be exposed to low levels of PAHs for an intermediate-duration period of time. Both routes are considered important for individuals living in the vicinity of hazardous waste sites because exposure to particulate PAHs in air and PAHs bound to soil particles, sediments in water, and contaminated food are significant routes of exposure for individuals living in the vicinity of hazardous waste sites. Furthermore, the pharmacokinetic data on PAHs are insufficient to determine whether similar effects may be expected to occur across different routes of exposure. Additional studies should be conducted on the effects of PAHs after intermediate-duration dermal exposure, since dermal exposure may be important to populations around hazardous waste sites. It is known that acute-duration dermal exposure to PAHs results in adverse dermal effects. Further studies determining the relative importance of exposure by this route, with regard to subsequent toxicity, would be useful. Studies describing dermal and oral absorption of PAHs from complex mixtures, including soil and other

mixtures that may actually be the vehicles of human exposure, would be useful in furthering the understanding of the toxicity of these compounds. The studies should be conducted with benzo[a]pyrene, because it has been found at the hi.ghest number of NPL sites and it is a representative altemant PAH, and with a representative nonaltemant PAH such as fluoranthene, benzo[b]fluoranthene, or benzofilfluoranthene. In addition, since PAHs rarely occur alone, but are found in the environment in combinations and mixtures, studies, particularly feeding studies, addressing mixtures of PAHs should be conducted. In all cases, research should be conducted to determine the most appropriate animal species for extrapolation to humans.

Chronic-Duration Exposure and Cancer. Few controlled epidemiological studies have been reported in humans on the effects of exposure to PAHs or PAH-containing mixtures; such studies would be difficult to conduct because of the presence of too many confounding factors. However, information is available on the effects of chronic-duration dermal exposures to PAH-containing mixtures in humans. Workers exposed to substances that contain PAHs (e.g., coal tar) experience chronic dermatitis and hyperkeratosis (EPA 1988a). Several chronic ingestion, intratracheal installation, and skin-painting studies have been conducted in animals using various PAHs, but none identified adverse effects other than cancer. Therefore, threshold levels for chronic-duration inhalation and oral exposure have not been thoroughly investigated, and no MRLs have been developed from this database. Although the existing animal studies are inadequate to establish threshold levels and dose-response relationships for toxic effects resulting from chronic exposure to PAHs, the data from 90-day studies recommended above should be evaluated before chronic studies are conducted. Inhalation and ingestion are probably the most significant routes of exposure for individuals living in the vicinity of hazardous waste sites contaminated with PAHs. Low dose chronic studies are needed to mimic these exposures.

Human data on the carcinogenicity of PAHs are available only for mixtures containing PAHs. Animal carcinogenicity data are available for only benzo[a]pyrene following inhalation exposure, for a limited number of PAHs following ingestion, and for almost all of the 17 PAHs following dermal exposure. A large database on carcinogenicity exists on complex mixtures that contain PAHs (such as crude oils, various high boiling point distillates, complex petroleum products, coal tars, creosote, and the products of coal liquification processes). It is difficult to ascertain the carcinogenicity of the component PAHs in these mixtures because of the potential interactions that could occur and the presence of other carcinogenic substances in the mixtures. Furthermore, the levels of PAHs were not quantified in any

of these reports. However, most of the available information on the carcinogenicity of PAHs in humans must be inferred from studies that reported the effects of exposure to complex mixtures that contain PAHs. Epidemiologic studies have shown increased mortality due to lung cancer in humans exposed to coke oven emissions (Lloyd 1971; Mazumdar et al. 1975; Redmond et al. 1976), roofing-tar emissions (Hammond et al. 1976), and cigarette smoke (Maclure and MacMahon 1980; Wynder and Hoffmann 1967). Despite the limitations inherent in these studies, reports of this nature provide qualitative evidence of the potential for mixtures containing PAHs to cause cancer in humans, and more definitive studies in humans on individual PAHs are not recommended at this time.

Inhalation exposure to benzo[a]pyrene has been shown to induce respiratory tract tumors in hamsters (Thyssen et al. 1981). Certain PAHs are carcinogenic to animals by the oral route (e.g., benz[a]anthracene, benzo[a]pyrene, and dibenz[a,h]anthracene), and tumors have been noted in the liver, mammary gland, and respiratory and gastrointestinal tracts following oral administration of these compounds (Neal and Rigdon 1967; Rigdon and Neal 1969). However, only a few PAHs have been assayed by the oral route. The results of dermal studies indicate that benz[a]anthracene, benzo[a]pyrene, benzo[b]fluoranthene, benzo[j]fluoranthene, benzo[k]fluoranthene, chrysene, dibenz[a,h]anthracene, and indeno[l,2,3-c,d]pyrene are tumorigenic in rats and mice following dermal exposure. Although many of these studies would be considered inadequate by current standards, the results nevertheless indicate that these PAHs can induce skin tumors as well as act as tumor initiators and promoters (Habs et al. 1984; Warshawsky and Barkley 1987; Wynder and Hoffmann 1959b). Therefore, additional studies on the carcinogenicity of PAHs in animals are probably not necessary at this time.

Genotoxicity. The genotoxic potential of several of the PAHs (both altemant and nonaltemant) has been extensively investigated using both *in vivo* and *in vitro* assays. All but three of the PAHs (acenaphthene, acenaphthylene, and fluorene) were reported to be mutagenic in at least one *in vitro* assay with the bacteria *S. typhimurium*. No further genotoxicity data are considered necessary at this time.

Reproductive Toxicity. No data were located regarding reproductive effects of PAHs in humans, and the available information regarding reproductive effects of PAHs in animals is limited; data exist on only one of the PAHs (benzo[a]pyrene), and these data are conflicting. Adverse effects such as decreased fertility and total sterility in F1 progeny of CD-1 mice (Mackenzie and Angevine 1981) and

decreased incidence of pregnant female rats at parturition (Rigdon and Rennels 1964) were reported following oral exposure to benzo[a]pyrene. However, no adverse reproductive effects were observed in Swiss mice fed benzo[a]pyrene in their diet (Rigdon and Neal 1965). The metabolic differences and method of benzo[a]pyrene administration could account for the differential response to benzo[a]pyrene-induced toxicity in these studies. Parenteral studies in animals have also demonstrated adverse reproductive effects (Bui et al. 1986; Cervello et al. 1992; Mattison et al. 1992; Miller et al. 1992; Swartz and Mattison 1985). The limited animal data suggest that PAHs may be reproductive toxicants, but these data are not extensive enough to draw firm conclusions. Furthermore, the testes and ovaries contain rapidly proliferating cells and therefore should be considered susceptible to damage by PAHs. The 90-day studies identified above should be conducted with special emphasis on reproductive organ pathology. If reproductive effects are observed in these studies, multigeneration animal studies could then be conducted to evaluate properly the relevance of this end point. In addition, since PAHs rarely occur alone, but are found in the environment in combinations and mixtures, studies, particularly feeding studies, addressing mixtures of PAHs should be conducted. In all cases, research should be conducted to determine the most appropriate animal species for extrapolation to humans. Future epidemiological studies should give special emphasis to evaluation of reproductive toxicity.

Developmental Toxicity. No studies were located regarding developmental effects in humans exposed to PAHs by any route. However, results of *in vitro* studies suggest that human placental endocrine and hormonal function may be affected by exposure to benzo[a]pyrene (Avigdor et al. 1992; Bamea and Shurtz-Swirski 1992; Guyda 1991). Only limited data are available in animals on a few PAHs (mostly benzo[a]pyrene). These data indicate that ingested or parenterally administered PAHs have a potential to induce adverse developmental effects such as resorptions and malformations (Legraverend et al. 1984; Shum et al. 1979), sterility in F_1 progeny (Mackenzie and Angevine 1981), testicular changes including atrophy of seminiferous tubules with lack of spermatids and spermatozoa, interstitial cell tumors (Payne 1958), immunosuppression (Urso and Gengozian 1980), and tumor induction (Bulay and Wattenberg 1971; Soyka 1980). However, another study found no developmental effects when benzo[a]pyrene was administered orally to mice (Rigdon and Neal 1965). The available animal data suggest that PAHs may be developmental toxicants. However, most of the data are from parenteral routes of exposure, and there are no inhalation data. The oral data are limited because of conflicting results across studies, the use of inconsistent protocols (e.g., varying numbers of animals, administration of the test compound during different times of gestation), the use of only one

dose, lack of study details, and the fact that data are available only on benzo[a]pyrene. Furthermore, some studies have shown that the toxic manifestations of benzo[a]pyrene are dependent on the route of exposure. Therefore, a two-species developmental toxicity study would be helpful to assess fully the potential for PAHs to affect development in humans. The route of exposure should be determined following evaluation of the reproductive organs in the 90-day studies to see if any particular route of exposure has a greater effect. The pharmacokinetic data on PAHs are insufficient,to determine whether similar effects may be expected to occur across different routes of exposure. Developmental toxicity should also be assessed in future animal reproductive toxicology testing and human epidemiological studies. In addition, since PAHs rarely occur alone, but are found in the environment in combinations and mixtures, studies, particularly feeding studies, addressing mixtures of PAHs should be conducted. In all cases, research should be conducted to determine the most appropriate animal species for extrapolation to humans.

Immunotoxicity.No studies were located regarding immunological effects in humans after exposure to PAHs by any route, or in animals following inhalation exposure. In the one oral exposure study in animals that was located, a single dose of fluorene failed to affect thymus or spleen weight (Danz and Brauer 1988). However, there is information available in animals on the immunotoxicity of several PAHs following dermal exposure (contact hypersensitivity) (Old et al. 1963) and intraperitoneal or subcutaneous administration (suppression of both humoral and cellular immunity) (Blanton et al. 1986, 1988; Lubet et al. 1984; Lyte and Bick 1985; White and Holsapple 1984). In general, the degree of immunosuppression correlates with the individual PAH's carcinogenic potency. Because of the information in animals that suggests that PAHs may affect the immune system, Tier I testing to assess PAH-induced immunotoxicity, as recently defined by the NTP (Luster et al. 1988) is recommended. The parameters that should be measured include immunopathology, humoral-mediated immunity, cell-mediated immunity, and nonspecific immunity. Although relatively high doses of PAHs must be used to obtain immunotoxicity in animals, much information could be gained from these studies. In addition, since PAHs rarely occur alone, but are found in the environment in combinations and mixtures, studies, particularly feeding studies, addressing mixtures of PAHs should be conducted. In all cases, research should be conducted to determine the most appropriate animal species for extrapolation to humans. Future epidemiologic studies should also place emphasis on evaluation of this end point.

Neurotoxicity. The potential for short- or long-term neurotoxic effects following exposure to PAHs by any route has not been specifically studied in humans or animals. Although acute-, intermediate-, and chronic-duration studies conducted in animals do not indicate that any of the PAHs tested showed gross evidence of neurotoxicity, these tests were not designed to detect subtle neurological changes. It is recommended that neurobehavioral as well as neuropathological end points be included in future 90-day toxicity testing of PAHs. If these preliminary data indicate that any of the PAHs are neurotoxicants, then a more comprehensive neurotoxicity battery, using sensitive functional and neuropathological tests, could be conducted to characterize further the neurotoxic potential of these PAHs. In addition, since PAHs rarely occur alone, but are found in the environment in combinations and mixtures, studies, particularly feeding studies, addressing mixtures of PAHs should be conducted. In all cases, research should be conducted to determine the most appropriate animal species for extrapolation to humans.

Epidemiological and Human Dosimetry Studies. There are no epidemiological studies available that have investigated the effects of single PAHs by any route of exposure. Most of the available information on the effects of PAHs in humans comes from reports of occupational exposures to PAH-containing mixtures. For example, epidemiologic studies have shown increased mortality due to lung cancer in humans exposed to coke oven emissions (Lloyd 1971; Mazumdar et al. 1975; Redmond et al. 1976), roofing-tar emissions (Hammond et al. 1976), and cigarette smoke (Maclure and MacMahon 1980; Wynder and Hoffmann 1967). Each of these mixtures contains benzo[a]pyrene, chrysene, benz[a]anthracene, benzo[b]fluoranthene, and dibenz[a,h]anthracene as well as other potentially carcinogenic PAHs and other carcinogenic and potentially carcinogenic chemicals, tumor promoters, initiators, and co-carcinogens such as nitrosamines, coal tar pitch, and creosote. Limitations inherent in these studies include unquantified exposure concentrations and durations, as well as concomitant exposure to other potentially toxic substances. Despite their inadequacies, studies in humans suggest that PAH-containing mixtures are dermal irritants and carcinogens following inhalation and/or derrnal exposure. If either worker or general populations with appropriate exposure can be identified, epidemiologic studies should be undertaken with special emphasis placed upon evaluation of cancer (of the skin and other organs) and other adverse skin effects, reproductive/ developmental toxicity, and immunotoxicity. However, such studies would be difficult to conduct. With a group of chemicals that are as ubiquitous as PAHs, it would be extremely difficult to distinguish between exposed and nonexposed populations. The more these groups overlap, the higher the chance for misclassification bias. In addition, the statistical power of an epidemiological study

depends partially on the variance of the exposure measurements. If there is enormous variation in the exposure levels among the exposed and nonexposed groups, then the population size needed to obtain statistical significance in the study would be unmanageable and would most likely not be found in any one occupational setting or hazardous waste site. Furthermore, because of the size of the population needed, it would be very difficult to control for confounding factors such as smoking, geographical location, lifestyle.

Biomarkers of Exposure and Effect.

Exposure. Sensitive analytical methods are available to quantify PAH exposure in humans. Although PAHs can be detected in the body fluids and tissues, because of the ubiquitous nature of PAHs in the environment, these biomarkers are not specific for any particular source of PAH exposure. PAHs and their metabolites (e.g., 1-hydroxypyrene) can be measured in the urine of exposed individuals. PAHs form DNA adducts that can be measured in body tissues or blood following exposure to PAHs and mixtures that contain PAHs. Studies attempting to identify suitable and reliable biomarkers from phenanthrene, chrysene, and fluoranthene have been conducted (e.g., Grimmer et al. 1988). However, no other biomarkers (specific or otherwise) that have practical utility have been identified following exposure to PAHs. Further work on developing biomarkers that enable exposure to be quantified would be useful to ascertain whether individuals have been exposed to potentially toxic levels of PAHs. In addition, since PAHs rarely occur alone, but are found in the environment in combinations and mixtures, studies addressing mixtures of PAHs should be conducted.

Effect. The available biomarkers of effect for PAHs are not specific for effects induced by PAHs. The available genotoxicity data indicate that several of the 17 PAHs considered here are genotoxic in both nonmammalian and mammalian systems and are indirect mutagens (i.e., requiring the presence of an exogenous mammalian metabolic system). There were no tests reported for humans exposed to benzo[a]pyrene (the most widely tested PAH) *in vivo*, but several types of cultured human tissue cells demonstrated positive results for benzo[a]pyrene-induced genotoxicity (as evidenced by the induction of chromosomal aberrations, sister chromatid exchange) and binding of benzo[a]pyrene to DNA. Thus, although these results are exclusively from *in vitro* tests and the limited genotoxicity tests conducted on urine obtained from humans exposed to PAHs have been negative, these genotoxic effects observed in human tissue cells may serve as a biomarker of effects for at least one of the PAHs, benzo[a]pyrene. The formation of benzo[a]pyrene-DNA adducts has been demonstrated, and

this may serve as a biomarker of PAH-induced carcinogenicity. Additional studies on the relative sensitivity of DNA adducts and sister chromatid exchanges to identify threshold levels of exposure that could be detected in human populations would be useful. In addition, since PAHs rarely occur alone, but are found in the environment in combinations and mixtures, studies addressing mixtures of PAHs should be conducted.

Absorption, Distribution, Metabolism, and Excretion. The quantitative data on the toxicokinetics of PAHs are based, to a large extent, on short-term exposure to benzo[a]pyrene in animals. Occupational exposure to PAHs generally occurs as a mixture. Therefore, inhalation, oral, and dermal studies exploring how PAHs interact with each other to affect their disposition would be more representative of exposures in humans.

The presence of PAHs and their metabolites in human urine and blood following inhalation, oral, and dermal exposures indicates that PAH absorption occurs in humans (Becher and Bjorseth 1983; Buckley and Lioy 1992; Hecht et al. 1979). However, there was no quantitative information on the extent and rate of PAH absorption in humans. Most of the information regarding the pulmonary and oral absorption and distribution of PAHs in animals is based on acute-duration exposures (Chang 1943; Hecht et al. 1979; Weyand and Bevan 1986, 1987b, 1988; Withey et al. 1991; Wolff et al. 1989c). PAHs appear to be widely distributed in tissues of animals following oral and inhalation exposure; peak tissue concentrations occurred earlier with higher exposure levels. Studies on the absorption and distribution of PAHs following long-term exposures would indicate whether the kinetics are similar to acute-duration exposures. The dermal study conducted by Storer et al. (1984) revealed that several PAHs in a crude coal tar mixture were absorbed, but that benzo[a]pyrene was not. In contrast, animal studies indicate that benzo[a]pyrene was dermally absorbed (Ng et al. 1992; Wester et al. 1990; Yang et al. 1989); however, tissue distribution was not discussed.

PAH metabolism has been extensively reviewed in human and animal tissue homogenates, cultures, and perfused systems (Autrup et al. 1978; Cavalieri et al. 1987; Cohen et al. 1976; Kiefer et al. 1988; Leung et al. 1988). However, these studies are limited to the biotransformation of individual compounds. Since most metabolic pathways have been identified or can be predicted for the individual PAHs, it is now important to understand how these metabolic pathways are affected when the PAHs compete. The carcinogenic and toxic potential of PAHs is associated with their metabolites. Alterations in rates of metabolism and metabolite profiles may affect the toxic consequences of PAHs.

Available data on several of the nonaltemant tumorigenic PAHs discussed in this profile indicate that they exert their adverse effects by mechanisms that differ from those that have been more recently elucidated for altemant PAHs (Amin et al. 1982, 1985b; Rice et al. 1987b). The mechanisms by which benzo[b]fluoranthene and benzo[j]fluoranthene are metabolically activated to genotoxic agents have been elucidated (LaVoie et al. 1993b; Marshall et al. 1993; Weyand et al. 1993a, 1993b). Additional studies designed to assess the potential toxic effects of these reactive metabolites in various species and at various organ sites would be useful.

No studies were located that monitored the rate and extent of PAH excretion in humans. Most studies in animals concentrated on the extent of PAH excretion and the distribution of the compound and its metabolites in urine, feces, and bile following short-term exposures (Bevan and Weyand 1988; Grimmer et al. 1988; Petridou-Fischer et al. 1988; Weyand and Bevan 1986; Wolff et al. 1989c; Yamazaki and Kakiuchi 1989). Data regarding the excretion pattern and rate following long-term exposure to PAHs would be useful to determine if bioaccumulation occurs.

In addition, since PAHs rarely occur alone, but are found in the environment in combinations and mixtures, studies, particularly feeding studies, addressing mixtures of PAHs should be conducted. In all cases, research should be conducted to determine the most appropriate animal species for extrapolation to humans.

Comparative Toxicokinetics. Occupational studies provide evidence that inhaled PAHs are absorbed. Animal studies also show that pulmonary absorption of benzo[a]pyrene occurs, but the extent of absorption is not known. Ingestion of benzo[a]pyrene is low in humans while oral absorption in animals varies among the PAH compounds depending on the lipophilicity. The absorption and distribution of PAHs in various species would be expected to be similar based on the lipophilicity of the compounds. In general, percutaneous absorption of PAHs in several animal species appears to be rapid and high (Ng et al. 1992; Sanders et al. 1986; Wester et al. 1990; Yang et al. 1989). This suggests that dermal absorption in humans may also occur rapidly; however, the extent of absorption may vary depending on the vehicle.

There was no information available on the distribution of PAHs in humans. In general, tissue distribution of benzo[a]pyrene following inhalation exposure is qualitatively similar for different species (Bevan and Weyand 1988; Weyand and Bevan 1986, 1987a, 1988; Wolff et al. 1989c). In

general, orally absorbed PAHs were rapidly and widely distributed in the rat (Bartosek et al. 1984; Withey et al. 1991; Yamazaki and Kakiuchi 1989). Qualitative similarities in distribution among species suggest that distribution in humans would also be similar. Placental transfer of PAHs in mice and rats appears to be limited (Neubert and Tapken 1988; Withey et al. 1992); therefore, human fetuses may be exposed to PAHs, but levels would not be as high as maternal levels.

Qualitatively, metabolism and excretion would be relatively similar in humans and animals, but variability in specific activities of enzymes will alter the metabolic profiles among the species. Knowledge of these differences in enzyme activity in various species would assist in predicting which pathways and metabolites would prevail. For instance, AHH activity is not induced by PAHs in some strains of mice. Therefore, it would be useful to examine the metabolism of those less-well-studied PAHs in several species (i.e., rodent and nonrodent) so that the carcinogenic potential of PAHs in various species could be predicted. The feces (via the bile) appears to be the major excretion route, but the extent of elimination of PAHs varies among species (Bevan and Weyand 1988; Grimmer et al. 1988; Ng et al. 1992; Petridou-Fischer et al. 1988; Sanders et al. 1986; Weyand and Bevan 1986; Wolff et al. 1989c). Further comparative studies on excretion would be useful because differences in human and animal excretion rates are not known. In addition, many of the toxicity tests have used mice, while a larger proportion of toxicokinetic studies have used rats. Thus, more kinetic studies should be conducted in mice to provide data to correspond to the toxicity data. Further, since PAHs rarely occur alone, but are found in the environment in combinations and mixtures, studies, particularly feeding studies, addressing mixtures of PAHs should be conducted. In all cases, research should be conducted to determine the most appropriate animal species for extrapolation to humans.

Methods for Reducing Toxic Effects. Efforts are currently underway to develop ways to mitigate the adverse effects of PAHs, especially with regard to natural products. Efforts to reduce or eliminate cigarette smoking in the general population also contribute toward reducing exposure to and toxic effects of PAHs. The target organs of PAHs have been identified (i.e., the skin and rapidly proliferating tissue such as the hematopoietic and lymphoid systems). Furthermore, several PAHs are considered to be carcinogenic. The mechanism of action for altemant PAH-induced carcinogenicity is fairly well understood. However, additional information would be useful to understand the mechanism of nonaltemant PAH-induced carcinogenicity, how PAHs exert their adverse effects on rapidly proliferating tissue, and how various interactions between PAHs can affect their toxicity and carcinogenicity.

2.9.3 Ongoing Studies

Ongoing research on the health effects and toxicokinetics of PAHs is summarized in Table 2-7.

TABLE 2-7. Ongoing Studies on Polycyclic Aromatic Hydrocarbons[a]

Investigator	Affiliation	Research description	Sponsor
E.J. La Voie	Rutgers State University, New Brunswick, NJ	Environmental polyclics—Metabolism and activation (mice, humans)	National Institute of Environmental Health Sciences
S.P. Mudzinski	Albany Medical College of Union University, Albany, NY	Immunotoxicologic screening of chemical carcinogens (mice)	National Institute of Environmental Health Sciences
W.F. Busby	Massachusetts Institute of Technology, Cambridge, MA	Core—Tumorigenicity testing (mice)	National Institute of Environmental Health Sciences
D.R. Bevan	Virginia Polytechnic Institute, Blacksburg, VA	Disposition of benzo(*a*)pyrene *in vivo*	U.S. Department of Agriculture
R.L. Hill	Florida State University, Tallahassee, FL	The impact of energy-related pollutants on chromosome structure	Not specified
N. Hahon	NIOSH DRDS, Morgantown, WV	Polycyclic aromatic hydrocarbons, particulates and defense mechanisms	National Institute of Occupational Safety and Health
M.K. Sanyal	Yale University, New Haven, CT	Abnormal fetal development during to toxic exposure (humans, rats, mice)	National Institute of Environmental Health Sciences
M. Koreeda	University of Michigan, Ann Arbor, MI	Synthesis and reactions of polycyclic aromatic hydrocarbon metabolites	National Institute of Environmental Health Sciences
L.M. Anderson	National Institutes of Health, Bethesda, MD	Metabolic and pharmacological determinants in perinatal carcinogenesis	National Institutes of Health
K. Frenkel	New York University Medical Center, New York, NY	Tumor promoters affecting base modification in DNA (mice)	National Institutes of Health
M.E. Knuckles	Meharry Medical College, Nashville, TN	Acute and subchronic inhalation and oral toxicity testing of benzo(a)pyrene and fluoranthene	Agency for Toxic Substances and Disease Registry

[a]Information obtained from Federal Research in Progress (October, November 1992) and CRISP (October 1992) databases