

U.S. Army Center for Health
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**Wildlife Toxicity Assessment for
Benzo(a)pyrene**

**FINAL REPORT
DECEMBER 2005**

**Prepared by
Health Effects Research Program
Environmental Health Risk Assessment Program**

**USACHPPM Document No: 39-EJ-1138-01P
Approved for public release; distribution unlimited.**

Readiness Thru Health

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Acknowledgements

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Wildlife Toxicity Assessment for Benzo(a)pyrene

CAS No. 50-32-8

December 2005

1. INTRODUCTION

Benzo(a)pyrene is one of a large number of polycyclic aromatic hydrocarbons (PAHs) that are formed during the incomplete combustion of organic matter. Though structurally related, the compounds display considerable heterogeneity in the number and spatial arrangement of their fused aromatic rings. Mixtures of PAHs are ubiquitous in the air as a result of stack emissions, smoke from household wood and coal fires, cigarettes, barbecue grills, automobile exhausts, etc. Fossil fuels are also a major source of PAHs, and this is of relevance to military sites. The compounds may be secondarily dispersed to other environmental media. Seventeen PAHs including benzo(a)pyrene have been identified as being especially important for environmental monitoring and compliance purposes (ATSDR 1995). These can be further identified by whether or not they have been demonstrated or are thought likely to induce or promote tumor formation in experimental animals. Benzo(a)pyrene is the most well-studied PAH, a cancer-inducing substance for which toxicological data serve as quantitative benchmarks for the entire carcinogenic sub-group. Thus, although toxicological data on the carcinogenicity of other PAHs are poor, quantitative estimates of their carcinogenic potency can be expressed relative to that of benzo(a)pyrene through the use of toxicity equivalency factors (TEFs). (USEPA 1993). The carcinogenic potency of benzo(a)pyrene (increased risk per(mg/kg/day/)) was first estimated, actually the geometric mean of determinations in several systems. This was estimated as 3.1 / (mg/kg/day). The cancer potency for a number of related PAHs was estimated and then each was rounded off to a simple multiple (1.0, 0.1, 0.01, or 0.001) of the benzo(a)pyrene value. PAHs always occur in complex mixtures, and the TEF approach may be used to provide an aggregate assessment of risk, rather than focusing on B(a)P alone.

Much of the experimental data on the toxicity of benzo(a)pyrene is irrelevant to the development of wildlife toxicity reference values (TRVs) for the compound, since their primary focus is on tumor formation. In general, studies on non-cancer effects, of which there is a paucity for benzo(a)pyrene, TRVs presented are intended to serve as benchmarks for screening-level ecological risk assessments. The protocol for the development of are more appropriate for TRV development. This Wildlife Toxicity Assessment summarizes available information on the likely effects of benzo(a)pyrene on wildlife. The

TRVs is documented in the U.S. Army Center for Health Promotion and Preventive Medicine Technical Guide 254, *Standard Practice for Wildlife Toxicity Reference Values* (USACHPPM 2000).

2. TOXICITY PROFILE

2.1 Literature Review

Relevant biomedical, toxicological, and ecological databases were electronically searched June 21, 2001, using the literature search strategy provided in Appendix A to identify primary reports of studies and reviews on the toxicology of benzo(a)pyrene. Separate searches were carried out linking the compound to laboratory mammals, birds, reptiles and amphibians (combined), or wild mammals. In general, a two-tiered approach was used in which all citations were first evaluated as titles and “key words in context.” All available abstracts of those articles selected in the first tier as possibly relevant to TRV development were then evaluated for relevancy and retention for evaluation in the second tier. For benzo(a)pyrene, 61 articles were marked for retrieval from 1276 initial hits. Details of the search strategies and the results of each are documented in Appendix A. Secondary references and sources of information on benzo(a)pyrene included the National Library of Medicine’s Hazardous Substances Databank (HSDB 2001), an Agency for Toxic Substances and Disease Registry (ATSDR) Toxicological Profile for polycyclic aromatic hydrocarbons (ATSDR 1995), An International Agency for Research on Cancer (IARC) monograph on benzo(a)pyrene (IARC 1983), and the U.S. EPA's Integrated Risk Information System (IRIS) (U.S. EPA 2001).

2.2 Environmental Fate and Transport

There is an extensive bibliography on the dispersion of PAHs such as benzo(a)pyrene in environmental media, with HSDB (2001) and ATSDR (1995) providing summaries of the available information. The fact that benzo(a)pyrene has been detected in all environmental media and in foodstuffs (HSDB 2001) attests to the compound's ability to become widely dispersed when released to the atmosphere, and to the comparative ease with which it can become taken up into the food chain.

ATSDR (1995) discusses the potential for benzo(a)pyrene and other PAHs to become dispersed throughout environmental media following their release to the atmosphere in combustion emissions. Natural sources of PAH emissions include forest fires and volcanoes, while anthropogenic sources include the residential burning of wood; generation of industrial power, incineration of waste, production of coal tar, coke and asphalt, and exhaust emissions from use of fossil fuels, as in automobiles, among many others. Typically, a complex mixture of PAHs will be released by these processes of which benzo(a)pyrene is but a single, and not necessarily the most heavily represented, component.

Airborne PAHs undergo widespread dispersion in the atmosphere but will ultimately become deposited in surface water or soil as a result of wet or dry deposition. In surface water, the compounds can volatilize, photolyze, oxidize, biodegrade, become bound to suspended particles or sediments and/or bioaccumulate in aquatic organisms. However, the combined low water-solubility and vapor pressure, and high octanol-water partition coefficient (K_{ow}) of benzo(a)pyrene suggest that the compound will partition to the soil compartment primarily, with sediment secondary, and air, water and biota much less heavily favored.

The capacity for PAHs to undergo abiotic degradation in the atmosphere, for example, by photooxidation, is governed to a large extent by the physical nature of the compound. ATSDR (1995) reports that those compounds absorbed to soot are more resistant to photochemical reactions than pure compounds. However, photolysis may be an important process by which benzo(a)pyrene molecules in water can be degraded.

A number of microbial genera are able to break down PAHs to related but not necessarily simplified structures. In addition, some algae and fungi are effective, with biodegradation being an important process by which PAHs can be removed from soils and sediments.

Physical-chemical characteristics of benzo(a)pyrene relevant to its environmental fate and transport are summarized in Table 1.

Table 1. Summary of Physical-chemical Properties of Benzo(a)pyrene

CAS No.	50-32-8
Molecular weight	252.32
Color	Pale yellow
Physical state	Monoclinic crystals/plates
Melting point	179°C
Boiling point	310–312°C (at 10mm Hg)
Odor	Faint aromatic
Solubility in water	1.6–2.3 µg/L at 25 °C: miscible with hydrocarbon solvents
Partition coefficients:	
Log K_{ow}	5.97–6.06
Log K_{oc}	6.74
Vapor pressure at 25 °C	$5.5\text{--}5.6 \times 10^{-9}$ mm Hg
Henry's Law constant at 25 °C	4.9×10^{-7} atm.m ³ /mole
Conversion factors	1 ppm = 10.32 mg/m ³ 1 mg/m ³ = 0.097 ppm

Sources: HSDB (2001), ATSDR (1995)

2.3 Summary of Mammalian Toxicity

2.3.1 Mammalian Oral Toxicity

2.3.1.1 Mammalian Oral Toxicity - Acute

There are no listed values for the median lethal dose (LD₅₀) of benzo(a)pyrene via the oral route in such secondary references as IARC (1983), ATSDR (1995), Lewis (1992), and HSDB (2001). However, a number of studies have explored the acute toxicity of benzo(a)pyrene to experimental animals. For example, Reddy et al. (1991) studied the induction of nuclear anomalies in the gastrointestinal tract of B6C3F1 mice gavaged with a single dose of benzo(a)pyrene (and other PAHs) in dimethyl sulfoxide (DMSO). All experimental animals were sacrificed 24–48 hours after dosing, and the incidence of nuclear anomalies was monitored in the forestomach epithelium by quantitative histopathology/cytology. By using a range of PAHs of differing carcinogenic potential, the authors concluded that the induction of nuclear anomalies by the compounds being tested was broadly in accordance with their relative carcinogenic potency in the gastrointestinal tract. In general, cancer studies are not considered as very relevant to a wildlife toxicity assessment.

Knuckles et al. (2001) evaluated the acute toxicity of benzo(a)pyrene to F-344 rats. An equal number of rats/sex/group were given one dose each, via gavage of 0, 100, 600 or 1000 mg benzo(a)pyrene/kg in peanut oil. Fourteen days after dosing, the rats were sacrificed and blood and organ samples were taken. The ovaries, liver, stomach, kidney and testes were weighed and evaluated for gross and histopathological changes. Blood samples were used to quantify a number of hematological parameters. Data obtained from hematological analyses and evaluations of the organs were analyzed using a number of statistical tests including analysis of variance (ANOVA), Fisher's Exact Test and the Cochran-Armitage test.

Knuckles et al. (2001) found that the liver to body weight ratio was significantly lower compared to the controls at 100 mg/kg and 600 mg/kg for males and females, respectively. Also, the white blood cell counts and mean cell hemoglobin concentration were significantly lower than the controls at 600 mg/kg in the males only.

The study by Reddy et al. (1991) illustrated the propensity of PAHs such as benzo(a)pyrene to cause profound site-of-impact lesions when administered to experimental animals (at doses from 48 to 192 mg/kg/day), for which the development of skin papilloma in the well-known nude-mouse skin-painting experimental protocol is the best documented example. However, there is a large body of evidence that at least a portion of the applied dose can cross the absorption barrier and be transported to remote sites. For example, Kliesch et al. (1982) studied the induction of chromosome damage in mouse bone marrow by benzo(a)pyrene administered to (101×C3H) F₁ mice in single oral doses ranging from 0–250 mg/kg.

Chromosomal analysis of bone marrow was carried out 12, 30, or 54 hours after dosing, and the incidence of micronuclei was monitored in a total of 2000 polychromatic erythrocytes. Both responses showed dose-dependent increases caused by treatment. Benzo(a)pyrene can cause both site-of-impact lesions and effects in distant organs or tissues resulting from migration from the port of entry. Benzo(a)pyrene is known to bind to the aryl hydrocarbon hydroxylase receptor (AHH), and this can lead, as with the dioxins, to various toxic metabolites. At the same time, this leads to the breakdown of the PAHs, thereby limiting bioaccumulation and decreasing the overall toxic load.

2.3.1.2 Mammalian Oral Toxicity – Subacute

Anselstetter and Heimpel (1986) studied the subacute hematotoxicity of benzo(a)pyrene in DBA/2 mice and BDF1 mice. Severe bone marrow depression with almost complete destruction of pluripotent haematopoietic stem cells was seen in female DBA/2 mice after administration of oral B(a)P (125 mg/kg body weight/day) for 13 days. Extreme resistance to bone marrow toxicity was observed in BDF1 mice fed for 19 days (Anselstetter & Heimpel, 1986).

2.3.1.3 Mammalian Oral Toxicity – Subchronic

Ramesh et al. (2000) carried out a subchronic study in F-344 rats exposed to benzo(a)pyrene in the diet at concentrations equivalent to doses of 0, 5, 50, or 100 mg/kg for 90 days. Five animals/sex/group were sacrificed after 30, 60, and 90 days, and liver microsomal preparations were monitored for aryl hydrocarbon hydroxylase (AHH) activity. Both dose- and time-dependent increases in AHH activity were obtained, potentially contributing to the formation of toxic reactive metabolites and disease symptoms in target organs such as the liver.

Knuckles et al. (2001) evaluated the subchronic toxicity of benzo(a)pyrene to F-344 rats for 90 days. Forty rats/sex/group were given 0, 5, 50, and 100 mg/kg/day of benzo(a)pyrene administered via feed. The actual doses received (consumed) by the animals were probably about ten (10) percent of the nominal doses, i.e., 0.5, 5, and 10 mg/kg bw/day, respectively. The researchers evaluated the effects of benzo(a)pyrene to body weight, blood, and organs. The diet was a mixture of lab meal from Purina Ralston Company and the respective benzo(a)pyrene concentration. The liver, kidney, stomach, prostate, testes, and ovaries were weighed and evaluated for gross and histopathological changes. Blood samples were used to quantify a number of hematological parameters. Data obtained from hematological analyses and evaluations of the organs were analyzed using two-way ANOVA, Fisher's Exact Test and the Cochran-Armitage test.

At 90 days, Knuckles et al. (2001) found that the red blood cells (RBC) and hematocrit levels had significantly decreased at 50 mg/kg/day for males and 100 mg/kg/day in females. Hemoglobin levels had significantly decreased for males and females at 100 mg/kg/day.

Wolford et al. (1986) established reference blood parameter data for mice and rats. The study presented typical blood parameter values observed in mice and rats. Although Knuckles et al. (2001) states that RBC, hematocrit and hemoglobin levels had significantly decreased at particular doses at 90 days, these values fall within the normal range (Wolford et al. 1986) indicating that the findings are not biologically significant.

No significant differences between the control and experimental groups regarding body weight were reported. Tubular casts in the kidney tissues were reported to be abnormal from the controls in males at 50 and 100 mg/kg/day after 90 days. Eighty percent of the males were affected at the 50 mg/kg/day level and 100% of the males were affected at 100 mg/kg/day. Ten percent of the females at 50 mg/kg and 100 mg/kg were found to have abnormalities in the tubular casts in the kidneys (Knuckles et al. 2001).

DeJong et al. (1999) studied the immunotoxicological effects of benzo(a)pyrene to male Wistar SPF rats. Eight rats/ group were dosed via gavage with 3, 10, 30, or 90 mg benzo(a)pyrene/kg bw in soybean oil. After 35 days of exposure, the rats were necropsied and body weight, blood and organs were evaluated. The immunotoxicological parameters studied were bone marrow, thymus, spleen, and lymph nodes. The adrenals, brain, bone marrow, colon, caecum, jejunum, heart, kidney, liver, lung, lymph nodes, esophagus, pituitary, spleen, stomach, testis, and thymus were evaluated for gross and histopathological changes. All organs were weighed with the exception of the bone marrow, pituitary, and GI tract.

The immunotoxicological parameters and organ weights were assessed using one-way ANOVA. The critical values in the blood data were determined by using the protocol per Statistical Tables and Biometry and The Principles and Practice of Statistics in Biological Research (Rohlf and Sokal 1981; Sokal and Rohlf, 1981). The histopathological data were evaluated using Fisher's exact test.

The results in the study stated that there was a significant difference in body weight between the control and the 3 mg/kg dose groups. This was true in the 90 mg group but not in the intermediate 10 and 30 mg groups. There was a significant difference in RBC, hemoglobin, and hematocrit between the control and the 10 mg/kg dose groups. Although all values in the DeJong et al. (1999) study are statistically significant, the findings are not biologically significant. The RBC levels fall within the range of normalcy per Wolford et al. (1994). The hemoglobin levels in the DeJong et al. (1999) study are slightly higher than the values stated in Wolford et al. (1994) yet is not considered biologically significant since the increase was not that much higher compared to the reference value. The hematocrit value is higher than the reference value in Wolford et al. (1994) yet are not considered biologically significant since the other blood parameters, RBC and hemoglobin levels, fall within the range of normalcy and it is likely that because there was an increase in RBC and hemoglobin levels, the hematocrit levels had also increased.

2.3.1.4 Mammalian Oral Toxicity – Chronic

No studies on the chronic toxicity of benzo(a)pyrene were located.

2.3.1.5 Mammalian Oral Toxicity – Other

MacKenzie and Angevine (1981) conducted an experiment to examine the reproductive and developmental effects of oral benzo(a)pyrene exposure. The study assessed the effects of benzo(a)pyrene to CD-1 mice upon exposure during the fetal stage. Thirty to 60 pregnant female mice/group were gavaged with 0, 10, 40, or 160 mg/kg bw in 0.2 mL corn oil on gestation days (GDs) 7–16, then allowed to deliver and nurse their young. After treatment and giving birth to pups, the litter size, pup weight, and percentage of viable litters were recorded. Litters (F₁) were allowed to remain with their mothers until postnatal day 20, at which point the dams were removed. At 7 to 8 weeks old, each F₁ male was housed with two untreated virgin females every 5 days for 25 days. Pregnant females from these matings were sacrificed on GD 19, and the number of implants, fetuses and resorptions was recorded. The F₂ young were examined for gross abnormalities. At 7 to 8 weeks old, each F₁ female was housed with untreated proven breeder males for periods of up to 6 months. All resulting F₂ young were counted and examined for gross abnormalities on day 1 of life, then sexed and weighed at 4 days of age.

The data on litter size for F₁ and F₂ populations was analyzed using the least significant difference method. The data on pup weight and the fertility index in the F₁ males and females was analyzed using nonparametric statistics. There was no significant difference between the control and all experimental dose groups regarding the mean litter size and percentage of viable litters in the F₁ population. There was a statistically significant decrease in mean pup weight in all benzo(a)pyrene exposed groups (F₁ population). A significant decrease in fertility index (females pregnant/females exposed to males x 100) had resulted in the F₁ males and females at 10, 40 and 160 mg benzo(a)pyrene /kg-day. F₂ mean litter size by F₁ females was significantly lower at 10 mg/kg-day compared to controls. The results indicate that the effects of benzo(a)pyrene exposure during development had manifested in adult reproduction for the F₁ population. These results indicate that a prenatal exposure of 10 mg/kg-day is an appropriate Lowest Observable Adverse Effect Level (LOAEL).

Kristensen et al. (1995) reported that benzo(a)pyrene may suppress development of primordial oocytes during fetal life. BaP was given to female NMRI mice, 10 mg/kg/body weight by oral intubation on days 7-16 of gestation (F₀ generation). The female pups (F₁ generation), thus exposed prenatally to BaP, showed markedly reduced fertility with few ovarian follicles compared to controls.

2.3.1.6 Studies Relevant for Mammalian TRV Development for Ingestion Exposures

Although the majority of studies on benzo(a)pyrene focus on carcinogenic effects, there are several studies that are potentially suitable for deriving a mammalian TRV. Ramesh et al. (2000) found that there was a dose-dependent and time-dependent increase in AHH activity. However, due to the lack of histopathological analyses to determine if increased levels of AHH had an adverse effect on the liver, this study will not be used to derive a mammalian TRV. Knuckles et al. (2001) and DeJong et al. (1999) assessed the toxic effects of benzo(a)pyrene during subchronic exposures. Knuckles et al. (2001) found that there was no significant effect of benzo(a)pyrene on body weight whereas DeJong et al. (1999) did find a significant decrease in body weight in benzo(a)pyrene exposed rats. The blood parameter data in both studies were not biologically significant as determined by comparisons with reference data in Wolford et al. (1994). The only endpoint suitable for TRV development in the above mentioned studies was the altered growth of benzo(a)pyrene exposed rats (at the lowest level, 3 mg, and at the highest level, 90 mg/kg, but not at the 10 mg or 30 mg levels.), as seen by DeJong et al. (1999). This study, however, was not chosen for TRV development because of the biological relevance of the statistical 3 mg/kg LOAEL finding and because the MacKenzie and Angevine (1981) study was considered more suitable because it was a multigenerational reproductive study with clear effect-based endpoints identified.

MacKenzie and Angevine (1981) conducted a developmental/reproductive study showing significant reproductive effects of benzo(a)pyrene in mice. The study assessed the effects of benzo(a)pyrene on the development of F₁ pups exposed during gestation, and when they matured, on the reproduction of the F₁ population. Importantly, although this study is not chronic in the classic sense (i.e. study duration), the exposure occurred during a sensitive life stage (development), so it was considered equivalent in weight of a chronic exposure regime (USACHPPM 2000). Pregnant dams exposed to 10 mg benzo(a)pyrene/kg-day from GD 7 to 16 had given birth to F₁ pups with significantly lower birth weights. Subsequently, when the F₁ mice (exposed to 10 mg/kg-day during development only) reached adulthood, females produced significantly smaller, or no (at higher doses) litters and males and females had a significantly lower fertility index indicating that the effects of prenatal exposure to benzo(a)pyrene were evident even in adulthood at the lowest experimental dose (10 mg/kg-day). As a result, the data regarding fertility index will be used to derive the mammalian TRVs. This result, with regard to female mice of the NMRI strain, is supported by the findings of Kristensen et al. (1995), in which a LOAEL of 10 mg/kg/day was established for reduction in litters and litter size and in ovarian follicles. As outlined in TG254, endpoints used to derive a TRV should have a clear link to an ecologically relevant effect. For benzo(a)pyrene, the reduction in reproductive output and the apparent long-lasting effects of prenatal exposure shown by MacKenzie and Angevine (1981) has clear implications for the sustainability of exposed mammalian populations.

Table 2. Summary of Relevant Mammalian Data for TRV Derivation

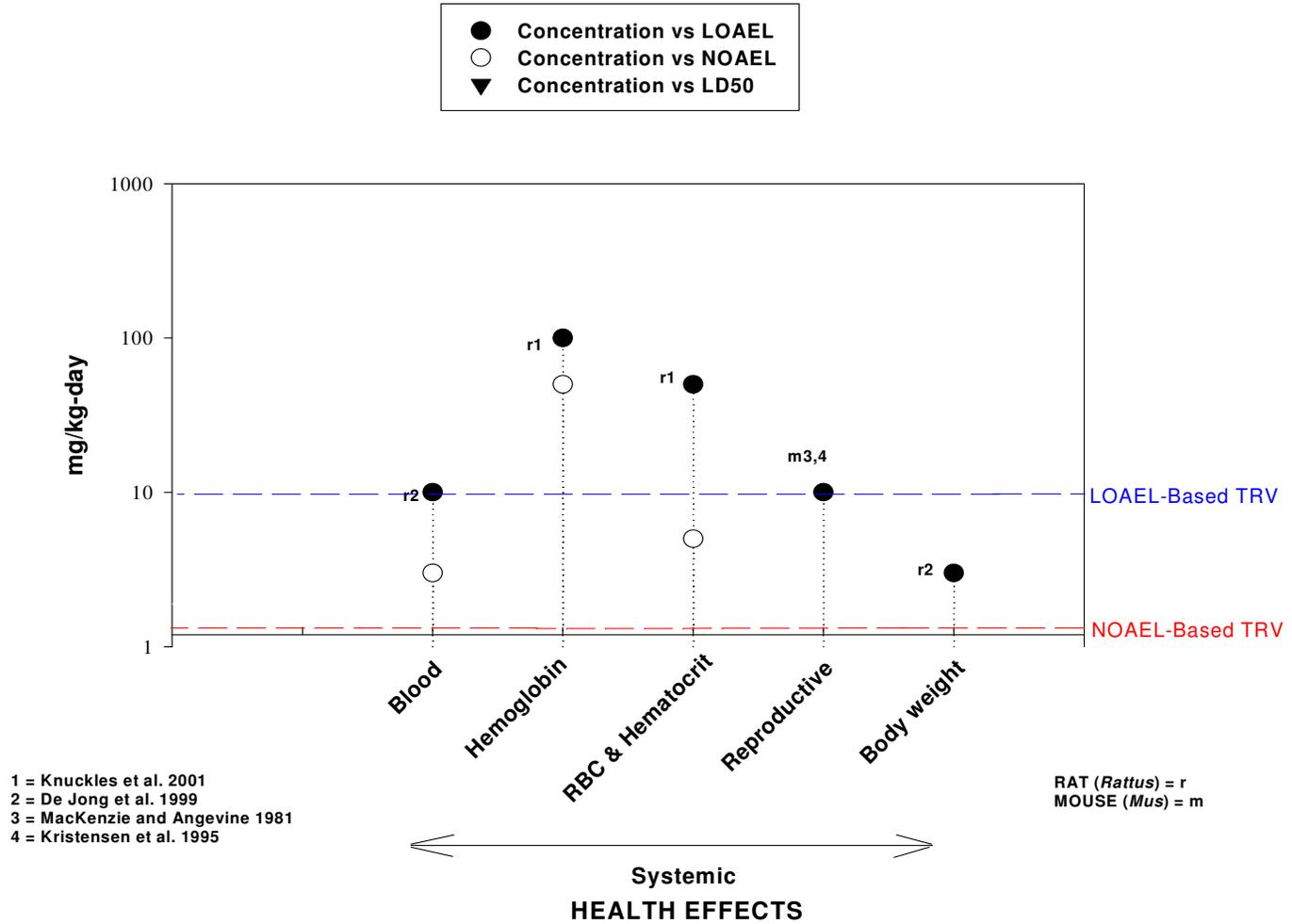
Study	Test Organism	Test Duration	Test Results		
			NOAEL (mg/kg/d)	LOAEL (mg/kg/d)	Effects Observed at the LOAEL
MacKenzie and Angevine (1981)	Mice (CD-1)	GD 7 to 16	ND	10	Reproductive effects (fertility, decreased birth weight)
Kristensen et al. (1995)	Mice (NMRI)	GD 7 to 16	ND	10	Decreased ovarian follicles, litters, litter size
DeJong et al. (1999)	Rats (Wistar SPF; males)	35 days	ND	3	Decreased body weight, decreased growth
			3	10	Increased RBC, hematocrit, & hemoglobin
Knuckles et al. (2001)	Rats (F-344)	90 days	5	50	Increased RBC and hematocrit levels (males)
			50	100	Increased hemoglobin levels

GD= gestation day

ND = Not determined

Figure 1

BENZO(a)PYRENE: HEALTH EFFECTS TO MAMMALS



2.3.2 Mammalian Inhalation Toxicity

As summarized in ATSDR (1995), several inhalation studies exist which addressed the carcinogenicity of benzo(a)pyrene via the inhalation route, especially those resulting in site-of-impact lesions to the respiratory tract. Greenwood et al. (2000) conducted an acute inhalation study pertaining to non-carcinogenic effects of benzo(a)pyrene emphasizing the neurotoxicological effects at the molecular level in Sprague Dawley rats. The maternal rats were exposed to a dose of benzo(a)pyrene:carbon black aerosol (100 ug/m³) via inhalation on GD 15 for four hours. An Sp1 transcription factor consensus sequence was examined by electrophoretic mobility shift analysis of nuclear extracts from various brain regions in pups on post-natal day (PND) 3, 5, 7, 10, 15. The results showed changes in the developmental expression of Sp1 abundance. The data obtained on the temporal and spatial regulation of gene expression in the brain indicate that an effect of the transplacental deposition of desorbed benzo(a)pyrene to the fetus is in-utero neurotoxicity.

2.3.3 Mammalian Dermal Toxicity

No studies were found.

2.4 Summary of Avian Toxicology

Hoffman and Gay (1981) found that benzo(a)pyrene causes embryotoxicity in avian species. Hoffman and Gay's (1981) study design consisted of surface applications of a mixture containing benzo(a)pyrene to mallard duck eggs during a critical period of development (72 hours of development). Proportions of benzo(a)pyrene (0.02%, 0.10%, or 0.50%) was combined with a petroleum hydrocarbon mixture and this mixture was applied to the egg surface. The benzo(a)pyrene mixture of 0.02% resulted in a significant decrease in embryonic growth and increased occurrence of abnormal survivors. Levels of 0.1% benzo(a)pyrene resulted in a significant decrease in survival and more abnormal survivors than the 0.05% benzo(a)pyrene group. Levels of 0.5% benzo(a)pyrene resulted in 100% mortality. Abnormal survivors were characterized as having bill defects, gastroschisis, incomplete ossification, stunted embryos with incomplete feather formation, eye and brain defects, anophthalmia and exencephaly.

Chen et al. (1994) assessed the mean weight change, food intake, egg production, eggshell thickness, liver weight, and hepatic microsomal proteins in white leghorn hens after treatment with 5 mg of benzo(a)pyrene for five days. Benzo(a)pyrene was dissolved in 0.25 mL corn oil and placed in gelatin capsules. The hens were orally administered benzo(a)pyrene capsules. Chen et al. (1994) found no significant differences in the measured attributes.

A portion of Brunstrom et al. (1990) study tested benzo(a)pyrene for embryotoxicity in white leghorn hen embryos. The study design consisted of an egg injection of 2.0 mg/kg benzo(a)pyrene. Benzo(a)pyrene was injected into the yolk of eggs preincubated for four days and embryonic mortality

was measured two weeks later. The vehicle was peanut oil, lecithin, and water. Thirty percent embryonic mortality had resulted, a significant difference from the control.

None of the data in the avian studies will be used to derive avian TRVs. Chen et al. (1994) did not find any significant differences, and the egg injection study by Brunstrom et al. (1990) and egg surface application study by Hoffman and Gay (1981) do not accurately represent real-life exposures for birds.

2.5 Amphibian Toxicology

Studies designed to obtain more information on the carcinogenic effects of benzo(a)pyrene have been conducted (Sadinski et al. 1995, Fernandez and Jaylet 1987, Fernanadez et al. 1989, Marty et al. 1989, Morse et al. 1996). To date, no amphibian studies have evaluated the noncarcinogenic health effects of benzo(a)pyrene.

2.6 Reptilian Toxicology

No data are available.

3. RECOMMENDED TOXICITY REFERENCE VALUES

3.1 Toxicity Reference Values for Mammals

3.1.1 TRVs for Ingestion Exposures for the Class Mammalia

Results from the chronic study on mice conducted by MacKenzie and Angevine (1981) showed significant effects of benzo(a)pyrene on fertility index in male and female F₁ mice and on mean litter size in female F₁. These reproductive effects have clear implications for the sustainability of exposed populations; thus, this study is ideal for the derivation of a mammalian TRV. The LOAEL for effects on the fertility index was 10 mg/kg-day, which was the lowest dose tested. The MacKenzie and Angevine (1981) study was suitably designed and executed, was equivalent to chronic data (since exposure occurred during a sensitive life stage, USACHPPM 2000). Because the dose-response data does not include a No-observable-adverse-effect-level (NOAEL), the TRV was derived using the NOAEL-LOAEL approach and the results are presented in Table 3. An uncertainty factor of 10 was used to derive the NOAEL-Based TRV from the LOAEL (USACHPPM 2000). The TRVs are the same for both males and females since effects on fertility were similar. A medium confidence rating is applied to these TRVs since the study was appropriately designed and was representative of a chronic study. Too few mammalian orders were represented, precluding a high confidence TRV.

Table 3. Selected Ingestion TRVs for the Class Mammalia

TRV	Dose (mg/kg-d)	Confidence
NOAEL-Based	1.0	Medium
LOAEL-Based	10	Medium

3.1.2 TRVs for Inhalation Exposures for the Class Mammalia

Not available at this time.

3.2 Toxicity Reference Values for Birds

Not available at this time.

3.3 Toxicity Reference Values for Amphibians

Not available at this time.

4. IMPORTANT RESEARCH NEEDS

The limited availability of data on the toxicity of benzo(a)pyrene to wildlife species precludes the development of a high-confidence TRV. Hence, more studies on the toxicity of benzo(a)pyrene to wildlife species are needed. Particularly warranted are long-term, chronic toxicity studies on mammals and additional studies on non-mammalian wildlife such as birds, reptiles and amphibians. Although there are a number of studies on the effects of benzo(a)pyrene on mammalian species, many of these are of poor quality and cannot be used to generate TRVs. Further experimentation, with close attention to experimental design, would greatly increase confidence in benzo(a)pyrene TRVs.

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APPENDIX A

LITERATURE REVIEW

The following files were searched in DIALOG:

File 155 MEDLINE; File 156, TOXLINE, File 5 BIOSIS, File 35 Dissertation Abstracts, File 76 Life Sciences Collection, and File 185 Zoological Record.

The search strategy for **Amphibians & Reptiles**:

- ◆ The expression benzo(a)pyrene and its CAS number.
- ◆ AND (amphibi? or frog or frogs or salamander? or newt or newts or toad? or reptil? or crocodil? or alligator? or caiman? snake? or lizard? or turtle? or tortoise? or terrapin?)
- ◆ AND (reproduc? or dietary or systemic or development or histolog? or growth or neurological or behav? or mortal? or lethal? or surviv? or (drinking()water))
- ◆ RD (reduce duplicates)

The search strategy for **Birds**:

- ◆ The expression benzo(a)pyrene and its CAS number.
- ◆ And chicken? or duck or duckling? or ducks or mallard? or quail? or (japanese()quail?) or coturnix or (gallus()domesticus) or platyrhyn? or anas or aves or avian or bird? or (song()bird?) or bobwhite? or (water()bird) or (water()fowl)
- ◆ AND (reproduc? or dietary or systemic or development or histolog? or growth or neurological or behav? or mortal? or lethal? or surviv? or (drinking()water))
- ◆ RD (reduce duplicates)

The search strategy for **Wild Mammals**:

- ◆ The expression benzo(a)pyrene and its CAS number.
- ◆ AND (didelphidae or opossum? or soricidae or shrew? Or talpidae or armadillo? or dasypodidae or ochotonidae or leporidae) or canidae or ursidae or procyonidae or mustelidae or felidae or cat or cats or dog or dogs or bear or bears or weasel? or skunk? or marten or martens or badger? or ferret? or mink? Or aplodontidae or beaver? or sciuridae or geomyidae or heteromyidae or castoridae or equidae or suidae or dicotylidae or cervidae or antilocapridae or bovidae arvicolinae or myocastoridae or dipodidae or erethizontidae or sigmodon? or (harvest()mice) or (harvest()mouse) or microtus or peromyscus or reithrodontomys or onychomys or vole or voles or lemming?
- ◆ AND (reproduc? or diet or dietary or systemic or development? or histolog? or growth or neurological or behav? or mortal? or lethal? or surviv? or (drinking()water))
- ◆ RD (reduce duplicates)

- ◆ NOT (human? or culture? or subcutaneous or vitro or gene or inject? or tumor? or inhalation or carcin? or cancer?)/ti,de
- ◆ NOT (patient? or cohort? or worker? or child? or infant? or women or men or occupational)

The search strategy for **Laboratory Mammals**:

- ◆ The expression benzo(a)pyrene and its CAS number.
- ◆ AND (rat or rats or mice or mouse or hamster? or (guinea()pig?) or rabbit? or monkey?)
- ◆ AND (reproduc? or diet or dietary or systemic or development? or histolog? or growth or neurological or behav? or mortal? or lethal? or surviv? or (drinking()water))
- ◆ NOT (human? or culture? or subcutaneous or vitro or gene or inject? or tumor? or inhalation or carcin? or cancer?)/ti,de
- ◆ NOT (patient? or cohort? or worker? or child? or infant? or women or men or occupational)
- ◆ RD (reduce duplicates)
- ◆ NOT (skin or skin()painting or nude or sencar or papilloma or dermal or initiat? or promot?)
- ◆ NOT ((meeting()poster) or (meeting()abstract))
- ◆ NOT (LA=Russian)

The strategy outlined above yielded 31 hits for benzo(a)pyrene with reptiles/amphibians, 107 articles with birds, 45 with wild mammals and 1093 articles with laboratory mammals.

All abstracts from the DIALOG search were reviewed and encoded in ProCite. When the search retrieved an appreciable number of hits, *keywords in context* were reviewed to minimize costs before any abstracts were downloaded (Tier 1). However, when only a limited number of studies were identified by the search, the abstracts were downloaded at the time of the search (Tier 2).

As noted above and in Section 2.1, 1276 hits on benzo(a)pyrene were obtained in the initial search, of which 61 were selected (Tier 2) as being relevant to this survey of the impacts of benzo(a)pyrene in wildlife.