Appendix 11: Commentary on Concordance/Lack of Concordance between Endpoints Selected for Modeling and Data from Other Reviews of Toxicology of PAH

A11.1. Repeat-Dose Studies

The biological endpoints selected for modeling in the present work are generally consistent (not inconsistent) with primary toxicity changes observed with administration of individual PAHs. This consistency lends support to the biological plausibility of the models that were developed and to the hypothesis that PACs are responsible for the toxicity observed with the petroleum streams reviewed here.

There is a limited database available for comparison (SCF, 2002; ATSDR, 1995; IPCS, 1998; IRIS 2007; RAIS, 2007). The link between PAHs and dermal carcinogenesis has been known for many years. Consequently, most of the research on pure PAHs has been on their carcinogenic properties and not on systemic, repeat-dose toxicity. The limited number of repeat-dose studies of pure PAHs most commonly used oral routes of administration, and/or were conducted in mice. The TG found references to only two dermal repeat-dose studies, and these were conducted in mice. Thus, there are no studies on pure PAHs that are equivalent in design to the studies utilized in the current project, i.e. no studies of similar duration, route of dosing, or species. Despite these differences, summaries of the animal studies on pure PAHs show that the effects most commonly reported are similar in nature to those identified in this project, i.e. increased liver weight, hematologic effects, and effects on lymphatic organs. A table summarizing these studies and their findings is shown in **Table A11-1.** below:

Table A11-1. Summary of reports of effects of PAH

Compound	Route	Species	Duration	Effect	Reference[a]
Acenaphthene	Oral	Rat	32 days	• peripheral blood changes • liver weight; hepatocellular hypertrophy; increased serum cholesterol • pulmonary effect	Knobloch et al., 1969 (Cited in 3,4)
Acenaphthene	Oral	Mouse	90 days	• liver weight; hepatocellular hypertrophy • increased serum cholesterol	EPA 1989 (cited in 1,2,4)
Acenaphthene	Inhal.	Rat	5 months	• blood effects (no details) • glandular tissues (no details)	Reshetyuk et al., 1970 (Cited in 3)
Anthracene	SC	Mouse	40 wks	• adverse lymphoid effects; increases in reticulum cells, accumulation of iron, decreased lymphoid cells, and dilated lymph sinuses	Hoch-Ligeti, 1941 (Cited in 3)
Anthracene	Diet	Rat	550 days	• no observed effects	Schmahl, 1955 (Cited in 3)
Anthracene	Oral	Rat	Not specified	• decrease in hemoglobin, reticulocytosis, leukopenia, and increase in residual blood nitrogen	Volkova, 1983 (Cited in 3)
Anthracene	IP	Human	9 wks	• hematologic toxicity - myelosuppression, characterized by leukopenia and thrombocytopenia	Falkson et al., 1985 (Cited in 3)
Anthracene	IP	Mouse	14 days	• no observed effects on immune response	White et al., 1985 (Cited in 3)
Anthracene	Oral	Mouse	13 wks	• no observed effects	EPA, 1989

					(cited in 1,2,3)
BaP	Oral	Mouse	180 days	• hepatic effects • hematological effects (e.g., aplastic anemia and pancytopenia resulting in hemorrhage) • in a "nonresponsive" strain (those not highly responsive to producing increased levels of cytochrome P-450 mediated enzymes)	Robinson et al. 1975 (cited in 1,3)
BaP	IP	Mouse	14 days	• immunosuppression - suppression of antibody response (more pronounced effects apparent in "nonresponsive" mice)	White et al., 1985 (Cited in 3)
BaP	IP	Mouse	14 days	• immunosuppression -depression of the T-cell-dependent antibody response (more pronounced effects apparent in "nonresponsive" mice).	Blanton et al., 1986 (Cited in 3)
BaP	IP	Mouse	5 days	• reduced thymic cellularity, alteration of thymocyte differentiation • reduced cellularity of the bone marrow	Holladay and Smith, 1995 (cited in 2)
BaP	Oral	Rat	35 days	• immunotoxic effects - decreased thymus and lymph nodes wts, decreased absolute and relative B cell numbers in the spleen and decreased numbers of red and white blood cells • decreased serum IgM and IgA levels	De Jong *et al.,* 1999 (cited in 2)
BaP	Oral	Rat	90 days	• liver weight	Kroese et al. 2001 (cited in 2)
Benz[a]anthracene	SubQ	Mouse	40 wks	• dilated lymph sinuses & decreased lymphoid cells • lymph glands contained increased numbers of reticulum (stem) cells & accumulation of iron • decreased spleen weights	Hoch-Ligeti, 1941, (Cited in 3)
Benz[a]anthracene	SubQ	Rats	Several wks	• extravascular red blood cells in lymph spaces & presence of large pigmented cells	Lasnitzki and Woodhouse 1944 (Cited in 3)
Benz[a]anthracene	IP	Mouse	12 hr	• severe degeneration of the thymus • reduced spleen & mesenteric lymph nodes weights • degeneration of bone marrow cells • retardation of thyroid gland development	Yasuhira, 1964 (Cited in 3)
Benz[a]anthracene	Oral	Rat	?	• injury to the intestinal epithelium • progressive anemia	Philips et al., 1973 (Cited in 3)
Benz[a]anthracene	?	?	?	• progressive anemia as well as agranulocytosis	Robinson, et al., 1975 (Cited in 3)
Dibenz[a,h]anth	SubQ	Mouse	40 wks	• lymphoid tissue changes	Hoch-Ligeti,

racene				• decreased spleen weights • liver and kidney lesions • increased number of lymph gland stem cells	1941 (Cited in 3)
Dibenz[a,h]anthracene	SubQ	Rats	Several wks	• pathological changes in the lymphoid tissues - extravascular red blood cells in lymph spaces & presence of abnormally large pigmented cells	Lasnitzki and Woodhouse, 1944 (Cited in 3)
Dibenz[a,h]anthracene	SubQ	Mouse	12 days	• serum antibody levels	Malmgren et al.,1952 (Cited in 3)
Dibenz[a,h]anthracene	SubQ	Mouse	14 days	• depressed immune responses • reduced absolute thymus weight	White et al., 1985 (Cited in3)
Fluoranthene	Oral	Mouse	13 wks	• liver weight, centrilobular pigmentation, increased SGPT values • hematological effects including decreased packed cell volume and hemoglobin content; • kidney toxicity	EPA 1988 (cited in 1,2,4)
Fluoranthene	?	Rats	Subchron	• nephropathy increased liver weights & enzyme levels	U.S. EPA, 1988 (Cited in 3)
Fluorene	Oral	Mouse	13 wks	• liver weight, increased hemosiderin • increased SGPT levels • hematological effects including decreased packed cell volume and hemoglobin content/concentration, red blood cell count • spleen weight, increased hemosiderin • kidney pathology	EPA 1989 (cited in 1,2,3,4)
1-Methylnaphthalene	Diet	Mouse	81 wks	• pulmonary alveolar proteinosis	Murata et al. 1993 (cited in 4)
2-Methylnaphthalene	Dermal	Mouse	61 wks	• lipid pneumonia	Emi and Konishi 1985 (cited in 4)
2-Methylnaphthalene	Diet	Mouse	81-wks	• pulmonary alveolar proteinosis • brain & kidney wts increased • decreased differential counts of stab (young) & segmented (adult) neutrophils • increased lymphocyte counts	Murata et al. 1997 (cited in 4)
2-Methylnaphthalene	Diet	Mouse	13 wks	• no observable effects	Murata et al. (1997) (cited in 4)
2-Methylnaphthalene/1-Methylnaphthalene Mixture	Dermal	Mouse	30 wks	• pulmonary alveolar proteinosis	Murata et al. 1992 (cited in 4)
Naphthalene	Derm	Human	Unknown	• hemolytic anemia	Schafer, 1951

	al	(infant)			(Cited in 3)
Naphthalene	Oral	Rat	600 days	• no observed effects	Schmahl 1955 (Cited in 3)
Naphthalene	Oral	Rat	11 wks	• liver toxicity • kidney toxicity	Kawai, 1979 (cited in 2)
Naphthalene	Oral	Rats	13 wks	• increased mortality • clinical signs of toxicity • kidney lesions (focal cortical lymphocyte infiltration and tubular degeneration) • lymphoid depletion of thymus • signs of anemia	NTP, 1980 (Cited in 3,4)
Naphthalene	Oral	Mouse	13 wks	• transient clinical signs of toxicity (lethargy, rough hair coats, decreased food consumption)	NTP, 1980 (Cited in 3)
Naphthalene	Oral	Rat	13 wks	• body weight • nephropathy	BCL, 1980 (cited in 2)
Naphthalene	Unknown	Rat	10 days	• increased liver weights • increased aniline hydroxylase and lipid peroxidase activity	Rao and Pandya 1981 (Cited in 3)
Naphthalene	Oral	Mouse	90 days	• decreased spleen weights • decreased hepatic hydrocarbon hydroxylase activity	Shopp et al., 1984 (Cited in 3,4)
Naphthalene	Oral	Mouse	90 days	• no discernable effects	Shopp, et al. 1984 (cited in 2)
Naphthalene	Oral/Inhalation	Human	Acute	• most common effects - hemolytic anemia associated with decreased hemoglobin and hematocrit values, increased reticulocyte counts, presence of Heinz bodies, & increased serum bilirubin levels	ATSDR, 1990 (Cited in 3)
Naphthalene	Inhal.	Mouse	103 wks	• Respiratory tract inflammatory and regenerative	NTP1992; (Cited 4)
Pyrene	Oral	Rats	40 days	• enlarged fatty livers in rats	White and White, 1939 (Cited in 3,4)
Pyrene	Oral	Mouse	13 wks	• kidney toxicity • increased liver weights • slight hematological changes (decreased RBC count, packed cell volume (PCV), and hemoglobin levels (HGB).)	EPA, 1989 (cited in 2,3,4)

[a] "cited in" refers to which general review(s) the study was noted in:
1 = ATSDR, 1995
2 = SCF, 2002
3 = RAIS, 2007
4 = IRIS, 2007

A.11.2. Developmental Toxicity Studies

Human Observations
Epidemiological studies by researchers at Columbia University have reported an association between exposure to PAH and various endpoints of developmental toxicity, including decreased fetal growth (Choi et al., 2006; Jedrychowski et al., 2003; Perera et al., 2005a, 2006b, 2006). However, these are environmental studies involving exposure to multiple PAHs, as well as many other chemicals and factors. For example, studies were conducted of women exposed to PAH from New York City air or from World Trade Center dust. It is not possible to evaluate the potential impact of individual PAH in these studies since subjects were exposed to multiple chemicals, including multiple PAH. Importantly, it is difficult to draw any conclusions about the effects of PAH on fetal development from these studies because it is difficult to identify and control for confounding factors. While these studies are valuable for hypothesis generation, they are inadequate for drawing definitive conclusions about whether PAH causes developmental effects in humans.

Toxicological Studies
A developmental toxicity study was reported in which two PAHs were administered dermally to pregnant rats on gestation days 0-20 (Dutson et al., 1997). Pregnant rats were given dermal doses of 2.5, 25, or 250 mg/kg/day of carbazole or benzo[a]carbazole. Carbazole produced no signs of maternal toxicity or developmental toxicity at doses up to 250 mg/kg/day. In comparison, 250 mg/kg/day of benzo[a]carbazole produced maternal toxicity (i.e., decreased body weight gain and food consumption) and developmental toxicity. The developmental toxicity consisted of decreased number of total and live pups on the day of parturition and decreased pup body weight gain (postnatal day 0 and 4). The signs of maternal and developmental toxicity were consistent with those observed in the developmental toxicity studies evaluated by the TG.

With respect to benzo[a]pyrene, the Scientific Committee on Foods (SCF, 2002) stated that "[T]here is clear evidence for developmental toxicity of benzo[a]pyrene in mice from oral and intraperitoneal administration in the form of embryonic and fetal death, reduced fetal weight, and malformations." However, the scientific evidence to support this statement is somewhat limited. In an early study, female rats were given diets containing 0 or 0.1% benzo[a]pyrene prior to mating, during mating, and during pregnancy (Rigdon and Rennels, 1964). According to the study authors, benzo[a]pyrene did not appear to affect fertility; however, there was suggestive evidence that benzo[a]pyrene had a deleterious effect on the survival and growth of the fetal offspring. Importantly, this study had many weaknesses and deficiencies, including small group sizes (3-5 pregnant females per group), and no reliable conclusions can be drawn. However, although this study is limited, the results are consistent with those of the studies evaluated by the TG and are supportive of the endpoints selected for evaluation.

The SCF summary also stated that "[I]n the rat, subcutaneous administration of benzo[a]pyrene caused fetal deaths and reduced fetal weight … (SCF, 2002). This statement appears to be based on a study by Bui et al. (1986). According to the authors, subcutaneous administration of 50 mg/kg of benzo[a]pyrene at different stages of gestation "affected the reproductive performance of pregnant rats by significantly increasing the number of resorptions and fetal wastage, and by decreasing the fetal weight."

A dose-related decrease in fetal survival was reported among the offspring of pregnant rats exposed to benzo[a]pyrene by inhalation on gestation days 11-20 (Archibong et al., 2002). The pregnant rats were exposed to 0, 25, 75 and 100 micrograms per cubic meter of benzo[a]pyrene for four hours per day.

Although there are limited data and some inconsistencies, the results of the developmental studies of benzo[a]pyrene, carbazole, and benzo[a]carbazole seem reasonably consistent with those available for review by the TG. Thus, it does seem plausible that the signs of developmental toxicity (i.e., decreased fetal survival and growth) associated with the PAC profile

are, in fact, due to systemic doses of PAHs (and presumably also the sulfur and nitrogen PACs, which could not be assessed directly because no toxicological data on these substances was found). A quantitative comparison of results is unlikely to provide much additional information because, in addition to the small number of studies, those conducted on pure PAH most commonly used the oral, intraperitoneal, and subcutaneous routes of administration; only one dermal developmental toxicity study was found. In addition, most of these studies were conducted in mice, not rats.

A.11.3. Reproductive Toxicity (laboratory animals)

Mackenzie and Angevine (1981) reported infertility in mice exposed *in utero* to benzo[a]pyrene. The study authors reported that oral administration of benzo[a]pyrene at levels up to 160 mg/kg/day significantly reduced fertility, number of live litters, and offspring weight gain. The authors also reported testicular atrophy and atrophic seminiferous tubules in male offspring of treated dams, as well as reduced fertility among the offspring of treated dams.

Disruption of testicular steroidogenesis and epididymal function was reported in male rats exposed to benzo[a]pyrene by inhalation for 10 days (Inyang et al., 2003). Sperm progressive motility and plasma testosterone were significantly decreased compared to controls at exposure concentrations of 75 micrograms per cubic meter and greater.

Inhibition of ovulation was observed in mice given a single intraperitoneal injection of benzo[a]pyrene (Swartz and Mattison, 1985). An effect on the number of corpora lutea was most pronounced at one week after treatment at dose levels above 1 mg/kg. According to the authors, the study results indicate that acute exposure to benzo[a]pyrene may have a transient adverse effect on follicle growth, ovulation, or formation of corpora lutea. The effect on the number of corpora lutea was not reversible over the course of this experiment at doses of 100 mg/kg/day or greater.

In a more recent study in female mice, Kristenson et al. (1995) suggested that benzo[a]pyrene suppressed the development of primordial oocytes during fetal development. These investigators examined the effect of benzo[a]pyrene given alone and in combination with lead. Benzo[a]pyrene was administered to pregnant mice by gavage on gestation days 7-16. According to the authors, the offspring of these dams showed markedly reduced fertility with few ovarian follicles compared to controls when treated with benzo[a]pyrene alone or in combination with lead.

Borman et al. (2000) compared the ovotoxicity of three PAHs (BaP, DMBA, and 3-MC) doses in rats and mice given repeated intraperitoneal doses. All three PAHs produced ovotoxicity; however, DMBA was the most potent ovarian toxicant.

Others have reported that *in utero* exposure to pure PAH results in long lasting immune suppression in the offspring (Urso and Gengozian, 1982, 1984; Rodriguez et al. 1999).

The design of the developmental toxicity studies available to the TG did not assess testicular development, ovarian toxicity, or immune system effects, so a direct comparison based on these endpoints is not possible. However, there was little evidence of toxicity to reproductive organs observed in the repeat dose studies evaluated by the TG. Thus, while there is evidence that large doses of individual PAHs (e.g., benzo[a]pyrene) in pure form may affect male and female reproductive toxicity, it appears that reproductive toxicity is not a sensitive endpoint for the petroleum substances evaluated by the TG.

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