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Technical Memorandum

To: Brian A. Rakvica, P.E.
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From: Teri Copeland, M.S., DABT and Joanne Otani Fehling, R.N., M.S.N., P.H.N

Date: February 6, 2006

Re: Selection of Pyrene as a Noncarcinogenic Toxicological Surrogate for PAHs

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This technical memorandum provides a toxicity-based assessment to support the selection of a surrogate chemical for purposes of the assessment of potential noncancer toxicity associated with oral exposure to polycyclic aromatic hydrocarbons (PAHs) that have not been assigned a noncancer oral toxicity criterion by USEPA. These PAHs include:

PAHs Classified by USEPA as Carcinogens

Benzo(a)anthracene
Benzo(a)pyrene
Benzo(b)fluoranthene
Benzo(k)fluoranthene
Chrysene
Dibenz(a,h)anthracene
Indeno(1,2,3-c,d)pyrene

PAHs Classified by USEPA as Noncarcinogens

Acenaphthylene
Benzo(g,h,i)perylene
Phenanthrene

I. Methodology and Analysis

A toxicological surrogate was selected from a list of candidate chemicals (i.e., PAHs for which noncancer oral toxicity criteria have been assigned by USEPA) based on consideration of the following:

- Availability of relevant toxicity data;
- Target organs;
- Dose-response information; and
- Structure-activity considerations.

Additionally, physical-chemical properties were considered, although they relate more to environmental fate and transport than to toxicity. Many of the physical-chemical properties for the carcinogenic PAHs are similar to the selected toxicological surrogate, pyrene (within an order magnitude), with the exception of the vapor pressures and Henry's Law Constants.

A summary of information relied upon in the selection of the toxicological surrogate is presented in Tables 1 and 2.

Availability of Relevant Toxicity Data

Relevant oral toxicity data were reported by USEPA for six noncarcinogenic PAHs (acenaphthene, anthracene, fluoranthene, fluorene, naphthalene, and pyrene). Information was taken from the USEPA Integrated Risk Information System (IRIS, USEPA, 2006), the Toxicological Profile for Polycyclic Aromatic Hydrocarbons (ATSDR, 1990; 1995), and the Toxicity Summary for Pyrene (ORNL, 1993) and supporting documentation contained in those data sources. The primary toxicity studies for all six PAHs were oral gavage, subchronic (90-day) rodent studies. Relevant summaries are provided in Table 1.

Target Organs

For the six candidate PAHs,, the most sensitive target organs for noncancer toxicity were identified as liver, kidney, and blood (hematological effects) (see Table 1).

For the seven carcinogenic PAHs, target organs for noncancer toxicity include liver, kidney, and blood. Relevant data provided by USEPA (2006), ATSDR, 1995, and ORNL (1993) include:

- A single intragastric administration of benzo(a)pyrene (200 mg/kg-day), benzo(a)anthracene (200 mg/kg-day), or dibenz(a,h)anthracene (180 mg/kg-day) resulted in the induction of foci of altered hepatocytes[1] (Tsuda and Farber, 1980, cited in ATSDR, 1995);

- Benzo(a)pyrene, benzo(a)anthracene, and chrysene (as well as anthracene and phenanthrene) induced the liver enzyme aldehyde dehydrogenase following short-term intragastric administration of 100 mg/kg-day (Torronen et al., 1981, cited in ATSDR, 1995);

- Oral exposure to benzo(a)pyrene and benzo(a)anthracene increased relative liver weights in rats by 27% and 19%, respectively[2] (Torronen et al., 1981, cited in ATSDR, 1995);

- Benzo(a)pyrene, benzo(a)anthracene, and chrysene were moderate inducers of the enzyme carboxylase in the liver of rats that were intragastrically administered doses of 50 - 150 mg/kg-day (Nousiainen et al., 1984, cited in ATSDR, 1995);

- Mice that were orally administered benzo(a)pyrene at 120 mg/kg-day in the diet for 180 days exhibited a 13% increase in relative liver weights (Robinson et al., 1975, cited in ATSDR, 1995);

[1] Liver cells.

[2] Doses were not given in the ATSDR summary of this study.

- Benzo(a)pyrene induced the enzyme carboxylase in the kidney of rats that were intragastrically administered doses of 50 - 150 mg/kg-day (Nousiainen et al., 1984, cited in ATSDR, 1995);

- In a 13-week oral study, mice were administered pyrene in corn oil at doses of 0, 75, 125, or 250 mg/kg-day. Dose-related nephropathy[3] was observed in females; nephropathy was seen in males at the high dose (250 mg/kg-day) only. Absolute and relative kidney weights were reduced in the two highest dose groups. Relative liver weights were increased in males exposed to 250 mg/kg-day and in females exposed to 125 or 250 mg/kg-day. Treated males exhibited minor hematological changes that consisted of decreased red blood cell count, packed cell volume, and hemoglobin levels (Toxicity Research Laboratories, 1989, cited in ORNL, 1993);

- Adverse hematopoietic[4] effects were reported in mice following oral exposure to 120 mg/kg-day benzo(a)pyrene for 180 days (Robinson et al., 1975, cited in ATSDR, 1995).

Dose-Response Information

The no-observed-adverse-effect-level (NOAEL) for the six candidate PAHs ranged from 71 mg/kg-day (naphthalene) to 1,000 mg/kg-day (anthracene) (USEPA, 2006). The lowest-observed-adverse-effect-level (LOAEL) for the six candidate PAHs ranged from 125 mg/kg-day (pyrene) to 350 mg/kg-day (acenaphthalene). A LOAEL was not identified for anthracene, as no adverse effects were observed in the studies reviewed by USEPA (2006), however it is reasonable to conclude that a LOAEL for anthracene would be greater than the NOAEL of 1,000 mg/kg-day. For the carcinogenic PAHs, reported toxicological thresholds for noncancer effects are generally in the range of those for the noncancer PAHs (ATSDR, 1995).

The USEPA oral reference dose (RfD_o)[5] ranges from 0.3 mg/kg-day (anthracene) to 0.02 mg/kg-day (naphthalene). The RfD_o for naphthalene is based on a decrease in body weight rather than a specific organ effect.

Structure-Activity Considerations

PAHs are a class of structurally similar compounds characterized by the presence of fused aromatic rings (see Tables 1 and 2 for structures). As shown in Table 1, the six candidate noncarcinogenic PAHs consist of two to four rings. The seven carcinogenic PAHs consist of four to six rings. Based on the noncarcinogenic toxicity endpoints and ranges of noncarcinogenic threshold effect levels (for noncarcinogenic as well as carcinogenic PAHs) reported by USEPA (2006), ATSDR (1995), and ORNL (1993), it can be inferred that there is some degree of structure-activity relationship for noncancer toxicity[6], although the data are not adequate for quantitative assessment of structure-activity relationships.

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[3] Any disease of the kidney.

[4] Pertaining to or related to the formation of blood cells.

[5] In general, the RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime (USEPA, 2006).

[6] The structure-activity relationship for the carcinogenic PAHs is well documented (USEPA, 1993, 2006; ATSDR, 1995). ATSDR (1995) contends that PAH toxicity generally increases with the number of rings, however some exceptions are found in the available toxicity data.

II. Selection of Noncancer Toxicological Surrogate for Oral Exposure Routes

Pyrene was selected as the toxicological surrogate for purposes of characterizing potential noncancer risks (i.e., hazard quotients) for PAHs not assigned an oral reference dose by USEPA. This selection is supported by the following rationale:

Availability of Relevant Toxicity Data

Relevant oral toxicity data were reported by USEPA for acenaphthene, anthracene, fluoranthene, fluorene, naphthalene, and pyrene.

Target Organs

For the seven carcinogenic PAHs, target organs for noncancer toxicity include liver, kidney, and blood. For the six candidate PAHs, the most sensitive target organs for noncancer toxicity were identified as follows:

- None – Anthracene
- Kidney, liver, and hematological – Fluoranthene and fluorene
- Kidney and hematological – Pyrene
- Liver – Acenaphthalene
- Body weight (decreased) and possible hematological – Naphthalene

The RfD_o for naphthalene is based on a decrease in terminal body weight. It should be noted that hematological effects have also been reported, although USEPA questions the toxicological significance of those reported effects. According to the USEPA, the decrease in terminal body weight was considered the most significant and sensitive endpoint (USEPA, 1996).

Based on target organ information, flouranthene, fluorene, naphthalene, and pyrene are considered to be the best surrogate candidates.

Dose-Response Information

For the carcinogenic PAHs, reported toxicological thresholds for noncancer effects are generally in the range of those for the noncancer PAHs (ATSDR, 1995). The lowest LOAELs for the six candidate PAHs were reported at 125 mg/kg-day for pyrene and 142 mg/kg-day for naphthalene (USEPA, 2006). The lowest (most stringent) RfD_os assigned by USEPA are 0.02 mg/kg-day for naphthalene and 0.03 mg/kg-day for pyrene.

Based on dose-response information, naphthalene and pyrene are considered to be the best surrogate candidates.

Structure-Activity Considerations

The two surrogate candidates that exhibit the lowest thresholds for noncancer effects are naphthalene and pyrene. The pyrene structure is more closely associated with the structures of the carcinogenic PAHs, which contain four to six rings. ATSDR (1995) contends that PAH toxicity generally increases with the number of rings. However, for noncancer toxicity, there has been no formal analysis to support a broad application of that position.

Based on structure-activity considerations, pyrene is considered to be the best surrogate candidate.

Summary

Fluoranthene, flourene, naphthalene, and pyrene represent acceptable surrogate choices based on toxicity considerations (e.g., dose-response relationships, effect levels, and USEPA RfD_o). Of these four candidates, naphthalene and pyrene exhibit slightly higher noncancer toxicity compared with fluoranthene and fluorene. Pyrene was ultimately selected over naphthalene due to (1) noncancer toxicity endpoints are more consistent with those for carcinogenic PAHs and (2) the greater number of rings in the pyrene chemical structure.

III. References Cited

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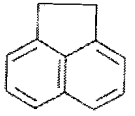
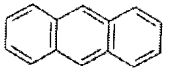
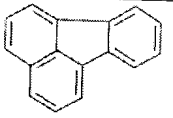
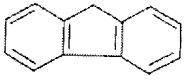
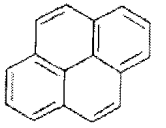
Table 1: Summary of Chemical-Physical Properties and Oral Noncancer Toxicity Information for Noncarcinogenic Polycyclic Aromatic Hydrocarbons*

Parameters	Acenaphthene	Anthracene	Fluoranthene	Fluorene	Naphthalene	Pyrene
Structure						
CAS No.	83-29-9	120-12-7	206-44-0	86-73-7	91-20-3	129-00-00
Formula	$C_{12}H_{10}$	$C_{14}H_{10}$	$C_{16}H_{10}$	$C_{13}H_{10}$	$C_{10}H_8$	$C_{16}H_{10}$
MW (g/mole)	154.21	178.2	202.26	166.2	128.19	202.3
MP/BP (°C)	95 /96.2	218 /340, 342	11 /375	116-117 / 295	80.5/ 218	156 /393, 404
H_20 Solubility (mg/L)	1.93	0.076	0.20-0.26	1.68-1.98	31.7	0.077
Log Kow/Koc	3.98/3.66	4.45/4.15	4.90/4.58	4.18/3.86	3.29/ 2.97	4.88/4.58
VP (mmHg)	4.47E-03	1.7E-05 @25 °C	5.0E-06 @25 °C	3.2E-04 @20 °C	8.7E-02 @25 °C	2.5E-06 @25 °C
HLC (atm-m^3/mol)	7.91E-05	1.77E-05	6.5E-06	1.0E-04	4.6E-04	1.14E-05
Oral RfD (mg/kg-day)	6E-02	3E-01	4E-02	4E-02	2E-02	3E-02
Study Length	90 days	90 days	13 weeks	13 weeks	13 weeks	13 weeks
Species/Route	Mouse/gavage	Mouse/gavage	Mouse/gavage	Mouse/gavage	Rat/gavage	Mouse/gavage
Organ	Liver	None	Kidney/Liver/Hematological	Kidney/Liver/Hematological	Body weight/Hematological	Kidney/Hematological
Toxicity Endpoint(s)	⇑ liver wt, dose-dependent cellular hypertrophy, sign. ⇑ cholesterol @ mid-high doses (350, 750 mg/kg-day) (USEPA, 1989a as cited in IRIS)	None observed at doses of 0, 250, 500, and 1,000 mg/kg-day (USEPA, 1989b as cited in IRIS)	Dose-dependent ⇑ liver enzymes, nephropathy, and salivation; ⇑ liver wt and hematological effects at 250 and 500 mg/kg-day (USEPA, 1988 as cited in IRIS)	⇓ RBC packed cell volume and Hemoglobin (both males/females at 250 mg/kg-day); also observed sign. ⇑ liver wt at 250/500; ⇑ kidney/spleen wts in high dose males/females (USEPA, 1989c as cited in IRIS)	⇓ mean terminal body wt in males (30% and 12% at high and mid-dose levels, respectively) considered most sensitive endpoint. ⇓ liver, brain, spleen wts in high dose females (133 mg/kg-day) (Shopp et al., 1984 as cited in USEPA 1998). Limited hematological effects at high dose (400 mg/kg-day)[1]. No dose-dependent changes in kidneys. (BCL, 1980 as cited in IRIS)	Renal tubular pathology characterized by multiple foci tubular regeneration often accompanied by interstitial lymphocytic infiltrates +/- interstitial fibrosis (dose-dependent in females); ⇓ kidney wt. ⇑ liver wt in males (250 mg/kg/day) & females (≥125 mg/kg/day). Slight ⇓ hematological parameters in males. (USEPA, 1989d as cited in IRIS; ORNL, 1993; ATSDR, 1995).
NOAEL LOAEL	175 mg/kg-day 350 mg/kg-day	1,000 mg/kg-day None	125 mg/kg-day 250 mg/kg-day	125 mg/kg-day 250 mg/kg-day	71 mg/kg-day 142 mg/kg-day	75 mg/kg-day 125 mg/kg-day
UF/MF	3000/1	3000/1	3000/1	3000/1	3000/1	3000/1
RfD Confidence (Study/Database/RfD)	Study – Low Database – Low RfD - Low	Study – Low Database – Low RfD - Low	Study – Medium Database – Low RfD - Low	Study – Medium Database – Low RfD - Low	Study – High Database – Low RfD – Low	Study – Medium Database – Low RfD – Low

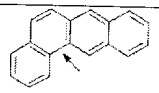
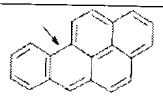
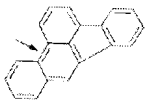
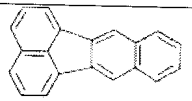
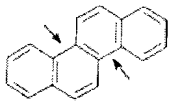
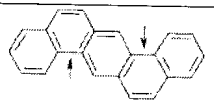
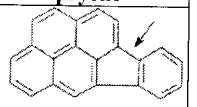
*No toxicity data are available for acenaphthylene, benzo(ghi)perylene, and phenanthrene.

MW – Molecular weight; MP/BP – Melting point/Boiling point; Kow – Octanol water partition coefficient; Koc – Octanol carbon partition coefficient; VP – Vapor pressure; HLC – Henry's Law Constant; NOAEL/LOAEL – No observable adverse effect level/ Lowest observable adverse effect level; RfD – reference dose; UF/MF – uncertainty factor/ modifying factor; sign – statistically significant; wt – weight; ⇑ - Increase value; ⇓ - Decrease value.

References for Physical/Chemical Properties: ATSDR, 1990 (naphthalene only); ATSDR, 2005 (all other PAHs).

[1]

Table 2: Summary of Chemical-Physical Properties for Carcinogenic Polycyclic Aromatic Hydrocarbons

Parameters	Benzo(a)anthracene	Benzo(a)pyrene	Benzo(b)fluoranthene	Benzo(k)fluoranthene	Chrysene	Dibenz(a,h)anthracene	Indeno(1,2,3-cd) pryene
Structure							
CAS No.	56-55-3	50-32-8	205-99-2	207-08-9	218-01-9	53-70-3	193-39-5
Formula	$C_{18}H_{12}$	$C_{20}H_{12}$	$C_{20}H_{12}$	$C_{20}H_{12}$	$C_{18}H_{12}$	$C_{22}H_{14}$	$C_{22}H_{12}$
MW (g/mole)	228.29	252.3	252.3	252.3	228.3	278.35	276.3
MP/BP (°C)	158-159; 162	179-179.3	168.3	215.7	255-256	262	163.6
H_20 Solubility (mg/L)	1.0E-02	2.3E-03	1.2E-03	7.6E-04	2.8E-03	5E-04	6.2E-02
Log Kow/Koc	5.61/5.30	6.06/6.74	6.04/5.74	6.06/5.74	5.16/5.30	6.84/6.52	6.58/6.20
VP (mmHg)	2.2E-08 @ 20 °C	5.6E-09	5.0E-07 @ 20-25 °C	9.59E-11	6.3E-07	1E-10	~10E-11 to 10E-06
HLC (atm-m^3/mol)	1.0E-06	4.9E-07	1.22E-05	3.87E-05	1.05E-06	7.3E-08	6.95E-08

↑ - Bay region. Active component of molecule for carcinogenicity (ATSDR, 1995).

MW – Molecular weight; MP/BP – Melting point/Boiling point; Kow – Octanol water partition coefficient; Koc – Octanol carbon partition coefficient; VP – Vapor pressure; HLC – Henry's Law Constant

References: ATSDR, 2005.