THE HPV CHALLENGE PROGRAM AND PETROLEUM SUBSTANCES

Volume 1

A Description of the U.S. High Production Volume Chemicals Program and The Petroleum HPV Testing Group's Proposed Strategy for Closure of Those Categories of Petroleum Substances Containing Polycyclic Aromatic Compounds

Report of the PAC Analysis Task Group

Sponsored by the Petroleum HPV Testing Group

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1. Introduction

High Production Volume (HPV) chemicals are those which are manufactured in, or imported into, the United States in amounts equal to or greater than one million pounds per year. United States Environmental Protection Agency's (USEPA's) HPV Chemical Challenge Program is a collaborative partnership with industry and the goal of the program is to ensure that the American public has access to the type of information that would allow it to actively participate in environmental decision making at all levels of government - federal, state, and local.

As reported by Environmental Protection Agency (EPA), since 1998 manufacturers and importers have participated in the Program by sponsoring over 2,200 chemicals. Approximately 400 of the sponsored materials are petroleum substances and approximately 110 of those are potentially impacted by the analysis described in this report. HPV sponsorship involves a commitment to develop data summaries of relevant existing information and to conduct testing to fill essential data gaps. The resulting collection of screening-level hazard data, referred to as the Screening Information Data Set – SIDS (OECD, 2004), will provide the public with basic information about the chemicals that are produced in the largest quantities.

The Petroleum HPV Testing Group is an unincorporated group of manufacturers affiliated by contractual obligation to fund a voluntary data disclosure and toxicity testing program on certain petroleum-related chemical substances in response to EPA's HPV Challenge Program. The American Petroleum Institute (API) manages the Group's activities.

Due to the large number of chemicals on the HPV list, practical considerations for test data development allow consolidation of closely related chemicals into a category, rather than consider them as individual chemicals. In the category approach, not every chemical needs to be tested for every SIDS endpoint. However, the test data finally compiled for the category must prove adequate to support a screening-level hazard assessment of the category and its members. That is, the final data set must allow the assessment of the untested endpoints, ideally by interpolation between and among the category members. The use of categories is encouraged in EPA's Challenge Program as this strategy maximizes the usefulness of available hazard data and minimizes animal testing.

Sponsors of HPV chemicals must submit a Test Plan to EPA that summarizes the existing data, identifies data gaps (if any) that will be filled, and in some cases justifies the use of a category approach. The Petroleum HPV Testing Group originally submitted thirteen Test Plans for evaluation and comment by EPA and members of the public. All thirteen of these Test Plans utilize the category approach. These test Plans can be found on EPA's website, http://www.epa.gov/chemrtk/pubs/hpvrstp.htm, and include the categories; Petroleum Gases, Gasoline, Kerosene/Jet Fuel, Gas Oils, Heavy Fuel Oils, Lubricating Oil Base Stocks, Waxes and Related Materials, Aromatic Extracts, Asphalts, Crude Oil, Petroleum Coke, Reclaimed Refinery Substances, and Grease Thickeners.

In some of the Petroleum HPV Testing Group's Test Plans, it was either stated or implied that the repeated dose toxicity, genotoxicity, developmental toxicity and reproductive toxicity are associated with polycyclic aromatic compounds (PAC[1]) content. It was also implied that the PAC content of petroleum substances could be used to predict the toxicity of untested petroleum substances. The claims were made for Aromatic Extracts, Crude Oils, Gas Oils, Heavy Fuel Oils, Lubricating Oil Base Stocks, and Waxes and Related Materials. The basis for the claims was a publication by Feuston et al. (1994) that examined the correlation between the weight percentage of various chemical classes of compounds in thirteen refinery streams and the magnitude of various effects produced in rats treated dermally with these substances in repeated dose and developmental toxicity studies. The authors concluded:

[1] Polycyclic Aromatic Hydrocarbons (PAH) refers to compounds of two or more fused-aromatic rings consisting of carbon and hydrogen only. Polycyclic Aromatic Compounds (PACs) is a more inclusive term than PAH since in addition to the PAHs it also includes molecules in which one or more atoms of nitrogen, oxygen or sulfur (a heteroatom) replaces one of the carbon atoms in a ring system. See Appendix 1 of Volume 2 of this report series for additional nomenclature details.

> "*In general, toxicity was correlated with concentrations of polycyclic aromatic compounds (PAC) composed of 3, 4, 5, 6, and/or 7 rings (decreased thymus weight, increased liver weight, aberrant hematology and serum chemistry, increased incidence of resorptions, decreased fetal body weight), PAC containing nonbasic nitrogen heteroatoms (increased mortality, decreased body weight, decreased thymus weight, increased liver weight, decreased hemoglobin content, hematocrit level, decreased fetal body weight), and/or PAC containing sulphur heteroatoms (decreased red blood cell and platelet counts, increased sorbitol dehydrogenase). A relationship between 2- ring PAC and skin irritation was demonstrated. Severity of effect was ranked against concentration of component class and statistical significance determined by the rank order correlation of Spearman. For the 13 streams tested, the presence and severity of systemic and developmental toxicity were dependent upon the levels of PAC and nonbasic nitrogen PAC.*
>
> *It is reasonable to assume that refinery streams rich in 3- to 7-ring PAC, S-PAC, and nonbasic N-PAC (e.g., carbazole derivatives) would be toxic, not only to the adult animal, but to the fetus as well."*

The Petroleum HPV Testing Group recognized that the underlying data for the publication by Feuston et al (1994) were limited and a more sophisticated and robust analysis was needed to assess the possible relationship between the PAC content and SIDS mammalian toxicity endpoints of petroleum substances. Consequently, a Task Group (TG) comprised of experts in the fields of petroleum chemistry, toxicology, and biostatistics was commissioned. The objectives of the TG were:

1. Identify, obtain, and evaluate available information that could be used to assess the possible relationship between the PAC content and toxicity of petroleum substances for the mammalian toxicity endpoints required in the HPV Challenge.
2. Identify and characterize relationships between the PAC content and Screening Information Data Set (SIDS) mammalian toxicity endpoints of petroleum substances.
3. For any identified relationships, determine if they could be used to predict the toxicity of untested petroleum substances.

The results of the TG's evaluation are presented in three volumes. The topics covered in the three volumes in the series are:

Volume 1: A Description of the U.S. High Production Volume Chemicals Program and the Petroleum HPV Testing Group's Proposed Strategy for Closure of Those Categories of Petroleum Substances Containing Polycyclic Aromatic Compounds

Volume 2: An Investigation into the Relationship between the Polycyclic Aromatic Compound Content and Acute, Repeat-Dose, Developmental, and Reproductive Toxicity of Petroleum Substances

Volume 3: An Investigation into the Relationship between the Polycyclic Aromatic Compound Content and Genetic Toxicity of Petroleum Substances

2. The HPV Challenge Program

A collaborative partnership with industry, the goal of EPA's HPV Challenge Program is to ensure that the American public has access to the type of information that would allow it to actively participate in environmental decision making at all levels of government - federal, state, and local.

The HPV Challenge program requires that there be a screening information data set (SIDS) on all HPV substances. Sponsors of HPV chemicals must submit a Test Plan to EPA that summarizes the existing data, identifies data gaps (i.e. SIDS endpoints for which there are no data) that will be filled, and in some cases justifies the use of a category approach.

The elements of the SIDS, agreed on by the Organization for Economic Cooperation and Development (OECD) member states provides basic screening information needed for an initial

assessment of the physicochemical properties, environmental fate, and human and environmental effects of chemicals (EPA, 1999a).

With regard to mammalian toxicity, the SIDS endpoints include:

- acute toxicity,
- repeated dose toxicity,
- developmental and reproductive toxicity, and
- mutagenicity (assays for gene mutation and chromosomal aberration/damage) (EPA, 2000).

For each of the SIDS endpoints, studies that have been conducted as specified in appropriate OECD test guidelines or comparable EPA test guidelines (such as the OPPTS Harmonized Guidelines (http://www.epa.gov/opptsfrs/home/guidelin.htm), and appropriate Good Laboratory Practice Standards (GLPS) (e.g., see the TSCA GLPS at 40 CFR part 792) consistently generate data adequate to fulfill the HPV Initiative needs. Data from studies that did not follow these procedures, however, may not be adequate (EPA, 2000).

2.1. Use of Categories in Fulfilling HPV Requirements

Due to the large number of chemicals on the HPV list, EPA encourages sponsors to group materials that are related in some regular fashion into categories (EPA, 1999a). The use of categories maximizes the usefulness of available hazard data and minimizes animal testing. In the category approach, not every chemical in the category needs to be tested for every SIDS endpoint. However, a completed data matrix for a category will provide "…the full data set for all category members for all relevant endpoints (EPA, 2007)."

As the final step in fulfilling their responsibilities under the HPV program, for each category it has sponsored, a sponsor needs to perform a final category analysis that will in turn be reviewed by U.S. EPA. Upon EPA's review, the sponsors will be notified whether the category analysis is satisfactory or whether further work or clarification is needed. EPA has provided an evolving set of guidance as to the form and contents of this final category analysis that it strongly urges sponsors to consider when submitting their final category analysis (EPA, 2007).

EPA considers the final category analysis to have three basic elements:

1. **Definition of the Category**: A statement of the definition and justification for the category that reflects all available data, including any new test data or estimated values developed through execution of the test plan.
2. **Documentation for Data Derivation**: A thorough explanation should be provided for the method(s) of data derivation (i.e., extrapolation/interpolation) and rationale used by the sponsor to provide a "value" for each relevant endpoint for each untested category member.
3. **Data Matrix**: A completed data matrix that provides the full data set for all category members for all relevant endpoints. This matrix should include assignment of an appropriate value to each cell of the matrix. Each value in the final data matrix should be accompanied by an indication of how it was derived (i.e., measured data, estimate derived from applying a SAR/QSAR, read-across, etc.).

    a. For category members not tested directly, the format/form (including units as applicable) in which the interpolated/extrapolated/estimated values for a given endpoint are expressed should match the format/form of the measured values for that same endpoint for the tested category members. The appropriate format/form will vary with endpoint and method of interpolation/extrapolation/ estimation, but may include a single quantitative value, a maximum value (e.g., <X), a minimum value (e.g., >Y), a range (e.g., between X and Y), or, where appropriate, a qualitative descriptor (e.g., readily biodegradable, positive/negative). If a quantitative value or range can be provided, it should be, barring a compelling justification as to why it should not be.

b. The interpolated/extrapolated values provided should be "stand-alone," and should not require accessing analogous data for other category members in order to be understood or interpreted. For example, use of a term such as "similarly toxic" should be avoided; rather, the quantitative value or range of values for the tested category member(s) serving as the basis for the interpolated/extrapolated value should be what is inserted into the matrix cell for the untested category member.
c. Complex mixture and/or Class 2 substance (i.e., substances of unknown or variable composition) categories may warrant case-by-case consultation between the sponsor and OPPT in order to derive an acceptable category data matrix (EPA, 2007).

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Class 2 denotes a chemical that occurs as a complex mixture of different individual substances rather than existing as a single chemical species with a well-defined molecular structure (e.g., petroleum or fuel oil no. 2). Class 2 compounds also include unknown or variable composition complex reaction products, biological materials (UVCB). UVCB substances can for example be described by structural features (e.g. acid chlorides, alkaline earth compounds, polyoxyalkylenes), a significant precursor (e.g. Castor Oil or Tallow) or by a more general description (e.g. Resins or Waxes) (EPA, 1998; OECD, 2003b).

2.2. Completing a Category Data Matrix

As noted above, EPA requires a completed data matrix to "ultimately provide a complete set of screening-level hazard values for each endpoint for each member of the proposed category… EPA, 2007)."

Each cell within a category data matrix should contain information that falls into one of two groups:

1. those for which test (measured/empirical) data exists, and
2. those for which no test (measured/empirical) data exists.

The approach for completing the data matrix for each of these cell types differs.

For those cells for which test data exists, the data need to be reviewed to ensure that the tests are adequate and if the data is appropriate of the toxicity for all samples that could be placed in the cell [i.e., across the range of composition found in all samples with the same Chemical Abstracts Registry Number (CASRN)]. As part of the assessment of a test's adequacy, a minimum criteria is if the test was performed according to/or in a manner equivalent to the recommended EPA guidelines (see discussion of individual endpoints in subsequent sections).

For those cells for which no test data exists, data gaps can be filled by read-across, extrapolation or interpolation. This is specific to each category and therefore no definitive guidance can be provided for the moment. Available options for filling data gaps include:

1. Qualitative: it is concluded that all the members of the (sub)category do or do not possess a particular property e.g. in-vitro mutagenicity
2. Quantitative: it is concluded that all the members of the category possess a particular property with a similar potency or evolving according to a regular pattern. Data gaps can be filled, e.g.:
   a. by using the value from the closest analogue in the category;
   b. by using a worst-case approach i.e. using the value from the most hazardous substance in the category, or in case of interpolation, the value from the most hazardous of the two closest analogues;
   c. by estimating quantitatively the potency of the property from the potency of the two closest analogues or from the regular evolution of this potency over the different category members.

As OECD notes, "There is currently only limited experience with quantitative data gap filling for toxicological endpoints. It should be applied with caution and the guidance will be revised as soon as more experience is available (OECD, 2005)."

3. Route of Administration of Test Material

In general, EPA recommends that the oral route of administration should be used for mammalian toxicological studies conducted for the HPV Challenge Program (EPA, 2000). However, dependent upon the most important route of human exposure and physical-chemical properties of the substance, the dermal or the inhalation route could also be considered (OECD, 2006).

For petroleum substances, dermal and inhalation exposures are the major and most likely route of exposure during production and use of the materials. Consequently, most of the toxicity studies that have been conducted on petroleum substances are by either the inhalation or dermal routes. In the studies that were reviewed by the Petroleum HPV Testing Group (TG), systemic effects had been observed after dermal exposure, thus providing evidence that absorption of the test materials had occurred.

4. Petroleum Substances

4.1. Substances and Categories

A petroleum substance is defined as a material that is isolated from petroleum (crude oil) in a refining process and usually exists as a complex mixture of individual chemicals. There are obvious exceptions to this such as propane, butane, etc., but all of the materials considered in this project are TSCA Class II substances (UVCBs). A petroleum substance is typically defined by the process conditions that created it (EPA, 1998; OECD, 2003b). Each substance has a name and usually a definition. For example:

Distillates[1] (petroleum[2]), heavy catalytic cracked[3]
A complex combination of hydrocarbons produced by the distillation of products from a catalytic cracking process. It consists of hydrocarbons having carbon numbers predominantly in the range of C15 through C35 [4] and boiling in the range of approximately 260 °C to 500°C (500°F to 932°F) [5]. This stream is likely to contain 5 wt. % or more of 4- to 6-membered condensed ring aromatic hydrocarbons.

The substance name and definition usually contains the following information:

1. The primary hydrocarbon fraction such as Gases, Naphtha, Distillate/Gas Oil, or Residuum. Other nomenclature such as Extracts, Wax, etc. may be used for specific refinery processes.
2. The hydrocarbon source. The hydrocarbon source for all the substances included in this investigation is “petroleum” as opposed to ‘shale” or “coal”.
3. The last refining step.
4. The carbon number range
5. The boiling point range.

This particular definition includes some information on specific components in the substance, although this is typically not the case.

The six categories of petroleum substances dealt with in this project are extremely complex mixtures of thousands to trillions of individual hydrocarbons and the precise composition of any given substances is, therefore, not known. This is illustrated in Figure 1 (Appendix A) where just the potential number of paraffin isomers can reach an enormous number very quickly (Altgelt and Boduszynski, 1994). Similar enormous numbers of isomers can exist for the other primary classes of hydrocarbons, e.g., cycloparaffins, aromatics and olefins.

As has been described, a category approach has been used to organize the petroleum HPV substances. Test Plans for thirteen categories have been submitted to EPA and the public. The categories are mostly arranged around familiar petroleum products, and include:

- Petroleum Gases (LPG)
- Gasoline Blending Streams
- Kerosene/Jet Fuel

- **Gas Oils (Diesel #2 and Heating Fuel #2)**
- **Heavy Fuel Oils**
- **Lubricating Oil Basestocks**
- **Waxes and Related Substances**
- **Aromatic Extracts**
- **Crude Oil**
- Asphalt
- Petroleum Coke
- Reclaimed Refinery Substances
- Petroleum Grease Thickeners

The six categories bolded above are the subject of this investigation. The individual HPV substances in each of the six categories are listed in Table 1 (Appendix A). The approximate boiling ranges of the categories are also shown in Figure 1 (Appendix A). How boiling point can effect the composition of the substances can be seen in Figure 2. The polycyclic aromatics (2+ rings) start appearing at very low concentrations at the end of the gasoline boiling range (400 °F) and increase in concentration as the boiling temperature goes up. Polycyclic aromatic compounds are present in substances found in the Gasoline, Kerosene/Jet Fuel, Asphalt, and Petroleum Coke categories, however other approaches have been used to satisfy the requirements of the HPV Challenge for those categories.

4.2. Polycyclic Aromatic Compounds

Polycyclic Aromatic Hydrocarbons (PAHs) refers to compounds of two or more fused-aromatic rings consisting of carbon and hydrogen only. Polycyclic Aromatic Compounds (PACs) are a broader group of compounds than PAHs since in addition to the PAHs it also includes heteroatomic compounds in which one or more of the carbon atoms in the PAH ring system are replaced by nitrogen, oxygen, or sulfur atoms. See Table A.1.1 (Appendix A) for additional nomenclature details.

PACs are formed in nature by three types of processes. Diagenic, petrogenic, and pyrogenic processes all transform organic material into PAC (API, 2002). The types of PAC found in petroleum (crude oil) are petrogenic because they are formed when organic matter is converted into petroleum under elevated pressure and moderate temperatures (130 – 150 °C). The nature of the processes which convert organic matter into petroleum involves semi-random chemical processes. This can result in hundreds to thousands of individual PACs produced during the processes that form petroleum. The types of PACs formed in petroleum includes a complex variety of parent (i.e., unsubstituted) and alkylated structures. The alkyl-substitutions are usually one to four carbons long and can include non-carbon compounds such as sulfur. Multiple alkyl and cycloparaffin substitutions of the parent structure are also common, especially in higher boiling fractions of petroleum. The relative abundance of the alkylated polycyclic aromatics (C_{1-4}) in petroleum far exceeds the abundance of the parent compound (C_0) (Speight, 2007). A detailed chemical analysis of three materials has been included in Appendix A to illustrate this point (EPA, 2003). Figures 10.2 - 13.2 and Tables 10.18 – 13.18 show the distribution of parent and alkyl-substituted PAC, plus other PAHs, in a sample of fresh and weathered crude oil (West Texas Intermediate), fuel oil no. 2 (Diesel), and heavy fuel oil (HFO). The fact that the concentration of alkylated polycyclic aromatics is much greater than the parent polycyclic aromatics is the main feature of the petrogenic PACs found in petroleum.

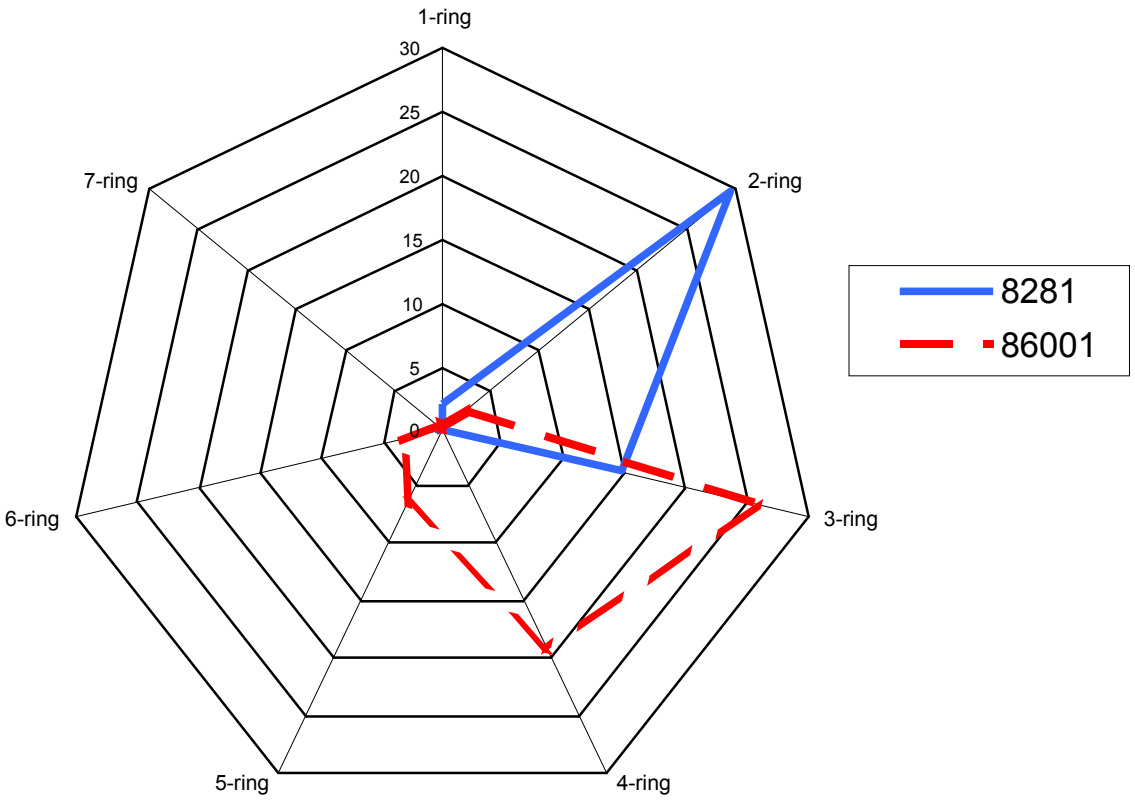
In addition to the concentration of PACs in a substance, the concept of “PAC profile” must be discussed to facilitate understanding of the analysis presented in Volume 2, “An Investigation into the Relationship between the Polycyclic Aromatic Compound Content and Acute, Repeat-Dose, Developmental, and Reproductive Toxicity of Petroleum Substances.”

The predictive models discuss in Volume 2 were developed using the weight percent of each of the 1- through 7-ring compounds in the test substance, referred to as the “PAC profile”. The analytical method used to determine the PAC profile includes extraction of the sample with DMSO to provide an un-alkylated and low- to moderately-alkylated aromatics-rich fraction, and

the subsequent separation by gas chromatography and determination by flame ionization or mass spectrometry of the concentration of ring-classes 1 through 7. The predictive models developed in this project use the concentration of each ring-class rather than the total weight percent of PAC or any subset of ring classes, e.g., 4-6 or 3-7-ring PACs. This approach was found to be essential as many substances with similar total weight percent of DMSO-extractable PAC were found to have significantly different toxicities. Two PAC profiles that were quantified using that analytical method are shown below.

	PAC rings (wt. %)							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**Total**
Samples								
CAS 64741-57-7 (Heavy Fuel oil, sample 85244)	0.0	0.06	2.5	1.9	1.2	0.5	0.0	6.16
CAS 8002-05-9 (Crude oil, sample 89645)	0.0	6.4	1.6	0.4	0.0	0.0	0.0	8.4

A graphical representation of two other PAC profiles, with 46.7 and 57.8 wt. % PAC (8281 and 86001 respectively) is shown below. Substances can have similar DSMO-extractable PAC concentrations, but their PAC profiles may be significantly different.



4.3. Refining Processes and PAC Profile

Petroleum refining is the process of transforming petroleum into finished products which meet required specifications (King 1988). US petroleum refineries produce only a small number of finished products (i.e., gasoline, jet fuel, diesel fuel). A diagram of a complex refinery and a list of major refining processes are included in Appendix A (OSHA 1996). Not all refineries use all of the processes in the diagram. Petroleum can go through several refining and blending steps before it meets the required product specifications, with each of the 149 USA refineries having its own approach to optimize production. Numerous intermediate substances can be created and then re-processed resulting in the plethora of different substances found in the primary categories (e.g., twenty-nine substances in the gas oil category).

Refining processes can increase, decrease, or leave unchanged the PAC concentration and PAC profile of the feedstock put into the process. For example,

- The initial refining step of atmospheric distillation simply separates the crude oil into different boiling fractions. As a consequence PACs will only be found in fractions that boil above 400 °F. Furthermore the concentrations of PACs in those fractions containing them will be greater than the starting crude oil.
- Solvent extraction processes separate a feedstock into a PAC-rich substance (soluble in the solvent) and a PAC-depleted substance (insoluble in the solvent).
- Hydroprocessing can be carried out under different conditions which could destroy PACs (severe hydrotreating or hydrocracking) or leave PACs relatively unchanged (mild hydrotreating).
- Cracking processes can remove or reduce the length of the alkyl-side chains, increasing the amount of DMSO-extractable PAC over the feedstock.

The refinery processes are influenced by such variables as the quality of the feedstock, temperature, space-velocity, catalyst efficiency, and product mix desired. Therefore for any given substance the composition may vary, within general limits, from refinery to refinery and also within a single refinery. Since the toxicity of a petroleum substance is dependent on its composition, it follows that its toxicity may also vary within general limits. This can present a challenge when attempting to describe the toxicity of a given substance because no single value may be appropriate due to the nature and concentration of its components. For some petroleum substances, such as those with high concentrations of PACs, a range of values may be needed to best-describe the range of toxicities that might be expected.

The TG has developed mathematical models to predict the critical adverse effects for SIDS-level mammalian toxicity endpoints (see Volumes 2 and 3 of this report). The TG believes these models can be used with the strategy described below (Section 5.) to meet the data requirements of untested substances contained in the TG's Gas Oils, Heavy Fuel Oils, Lubricating Oil Basestocks, Waxes and Related Substances, Aromatic Extracts, and Crude Oil Categories**.**

5. TG Strategies for Satisfying the Mammalian SIDS Endpoints Requirements

5.1. Acute toxicity

5.1.1 EPA expectations:

LD_{50} or LC_{50} value (range of values) for each category member (in every cell of matrix)

5.1.2 EPA recommended test(s) for developing toxicity values:

- Acute Oral Toxicity Test (rat)(OECD 425) or
- Acute Inhalation Toxicity Test (OECD 403)

For all other chemicals (i.e., those that are either liquids or solids at room temperature), acute toxicity testing should be conducted via oral administration (EPA, 2000).

5.1.3 Strategy for completing data matrices:

Cells for which data exists

- The adequacy of existing data will be assessed by determining if it was derived from EPA recommended/or equivalent test(s)
- Determine if existing data is representative of the CASRN on which it is derived
- Derive LD_{50} (or range) from existing data, if it is adequate and appropriate

Cells for which no data exists
- No new acute toxicity testing will be conducted
- Quantitative read-across from existing tests will be used to assign LD_{50} values of 5 g/kg and 2 g/kg for oral and dermal exposures, respectively

5.1.4 Rationale:

The reported oral LD_{50} values for aromatic extracts, crude oil, gas oils, heavy fuel oils, lubricating oil base stocks and waxes are all high, i.e., generally greater than the maximum doses tested, typically 5 g/kg and 2 g/kg for oral and dermal exposures, respectively (API 2001, 2002, 2003a, b, c & d, 2004). The Petroleum HPV Testing Group thinks these values are representative of the low acute toxicities of these categories of materials, and consequently can be used to characterize the acute toxicity of individual substances. While the EPA does not require dermal LD_{50} values, the Petroleum HPV Testing Group thinks, given the dermal route is a common route of exposure to these materials, a dermal value is desirable and preferable.

5.2. Repeat-dose toxicity

5.2.1 EPA expectations:

NOAEL or LOAEL value (or range of values) for each category member (every cell of matrix)

5.2.2 EPA recommended test(s) for developing toxicity values:

- Combined Repeated Dose Toxicity Study with the Reproduction/ Developmental Toxicity Screening Test (OECD 422), or,
- Repeated Dose 28-Day Oral Toxicity Screen (OECD 407)

If adequate data are available for reproductive and developmental toxicity but not for repeated dose toxicity, EPA recommends that the 28-day repeated dose toxicity test (OECD 407) be used by sponsors to meet the repeated dose data need for the voluntary HPV Challenge Program (EPA, 2000).

Although the EPA recommended tests are 28-day studies, EPA recognizes that adequately run 90-day studies would also be acceptable to meet the repeated dose SIDS endpoint (EPA, 1999b).

5.2.3 Strategy for completing data matrices:

Cells for which data exists
- The adequacy of existing data will be assessed by determining if it was derived from EPA recommended/or equivalent test(s)
- Determine if existing data is representative of the CASRN on which it is derived
- Derive NOAEL/LOAEL (or range) from existing data if it is adequate and representative

Cells for which no data exists
- For appropriate sample(s) of each CASRN, use Petroleum HPV Testing Group repeated dose Quantitative Compositional Activity Relationship (QCAR) models to predict dose-response curves for absolute thymus weight, hemoglobin concentration and platelet count.
- From the modeled dose-response curves, identify the predicted dose (PDx) that causes a defined effect, where “x” indicates the degree of change from the control value
- For predicted values that are interpolations, consider the lowest predicted PDx value(s) (or range of values) as LOAEL(s)

- Complete the data matrix using these LOAEL(s) (or ranges of LOAELs)
- If compositional data show that the prediction would be an extrapolation, consider performing toxicity studies according to recognized guidelines

5.2.4 Rationale:

The Petroleum HPV Testing Group has developed a series of mathematical models that utilize a petroleum substance's PAC profile to predict the dose-response curves of selected repeated dose toxicity endpoints. The endpoints include absolute thymus weight, hemoglobin concentration and platelet count. The predicted dose-response curves that can be generated permit the prediction of either 1) the effect at a given dose, or 2) the dose that causes a given effect. With regard to the second use, a degree of change from the control value can be established that would be considered indicative of an adverse effect or appropriate point of departure value (LOAEL) for each of the endpoints for which models have been developed. Because the Petroleum HPV Testing Group thinks it is reasonable to apply the models developed in this project to untested substances falling within the appropriate model domains, they could be used to predict LOAELs for untested samples.

For a complete discussion of these models, see Volume 2 of this series, "An Investigation into the Relationship between the Polycyclic Aromatic Compound Content and Acute, Repeat-Dose, Developmental, and Reproductive Toxicity of Petroleum Substances"

5.3 Developmental toxicity

5.3.1 EPA expectations:

NOAEL or LOAEL value (or range of values) for each category member (every cell of matrix)

5.3.2 EPA recommended test(s) for developing toxicity values:

- Combined Repeated Dose Toxicity Study with the Reproduction/ Developmental Toxicity Screening Test (OECD 422), or
- Reproduction/Developmental Toxicity Screening Test (OECD 421)

EPA recommends that the combined reproductive and developmental toxicity guideline (OECD 421) be used in lieu of separate testing for reproductive toxicity (OECD 415) and/or developmental toxicity (OECD 414) unless there is information indicating that separate testing may be advisable (EPA, 2000).

5.3.3 Strategy for completing data matrices:

<u>Cells for which data exists</u>

- The adequacy of existing data will be assessed by determining if it was derived from EPA recommended/or equivalent test(s)
- Determine if existing data is representative of the CASRN on which it is derived
- Derive NOAEL/LOAEL (or range) from existing data if it is adequate and appropriate

<u>Cells for which no data exists</u>

- For appropriate sample(s) of each CASRN, use the Petroleum HPV Testing Group's developmental QCAR models to predict dose response curves for maternal absolute thymus weight, fetal body weight, number of live fetuses per litter, percent resorptions, pup body weight, total pups per litter and live pups per litter
- From the modeled dose-response curves, identify the predicted dose that causes a defined effect, the "Predicted Dose x (PDx)", where "x" indicates the degree of change from the control value
- For predicted values that are interpolations, consider the lowest predicted PDx value(s) (or range of values) as LOAEL(s)
- Complete the data matrix using these LOAEL(s) (or ranges of LOAELs)
- If compositional data show that the prediction would be an extrapolation, consider performing toxicity studies according to recognized guidelines

5.3.4 Rationale:

The Petroleum HPV Testing Group has developed a series of models that utilize an untested petroleum substance's PAC profile to predict the dose-response curves of selected developmental toxicity endpoints. The endpoints included maternal absolute thymus weight, fetal body weight, number of live fetuses/litter and percent resorptions in the prenatal studies (studies in which the pups were delivered by Caesarean section) and pup body weight, total pups per litter and number of live pups per litter in the postnatal studies (in which the pregnant females delivered their young). The predicted dose-response curves that can be generated permit the prediction of either 1) the effect at a given dose, or 2) the dose that causes a given effect. With regard to the second use, a degree of change from the control value can be established that would be considered indicative of an adverse effect or appropriate point of departure value (LOAEL) for each of the endpoints for which models have been developed. Because the Petroleum HPV Testing Group thinks it is reasonable to apply the models developed in this project to untested substances falling within the appropriate model domains, they could be used to predict LOAELs for untested samples.

For a complete discussion of these models, see Volume 2 of this series "An Investigation into the Relationship between the Polycyclic Aromatic Compound Content and Acute, Repeat-Dose, Developmental, and Reproductive Toxicity of Petroleum Substances"

5.4 Reproductive toxicity

5.4.1 EPA expectations

NOAEL or LOAEL value (or range of values) for each category member (every cell of matrix)

5.4.2 EPA recommended test(s) for developing toxicity values:

- Combined Repeated Dose Toxicity Study with the Reproduction/ Developmental Toxicity Screening Test (OECD 422), or
- Reproduction/Developmental Toxicity Screening Test (OECD 421)

EPA recommends that the combined reproductive and developmental toxicity guideline (OECD 421) be used in lieu of separate testing for reproductive toxicity (OECD 415) and/or developmental toxicity (OECD 414) unless there is information indicating that separate testing may be advisable (EPA, 2000).

EPA also recognizes that the OECD HPV SIDS Program allows for other approaches that can be used to meet the reproductive toxicity endpoint needs covered by the combined protocol. EPA can also envision circumstances where other such approaches might make sense.

Examples given by OECD of other approaches to satisfying the SIDS requirements for reproductive toxicity include:

- Requirements are met if existing data on the chemical include a developmental toxicity study *(e.g. OECD Test Guideline 414)* and a 90-day (or longer) repeated dose study that sufficiently documents that reproductive organs were microscopically examined and indicate no effects. If results from a developmental toxicity study are not available then such a study is required (e.g. OECD Test Guideline 414).
- When either a ≥ 90-day (with no evaluation of reproductive organs) or a 28-day repeated dose study is the only repeated dose study available, it is recommended that at least a reproduction/developmental toxicity screening test (e.g. OECD Test Guideline 421) be carried out, in order to satisfy the requirements for the reproductive/ developmental toxicity endpoint.
- If reliable tests results from well performed tests according to OECD Test Guidelines 415 or 416 (one or two generation reproductive toxicity) are available, the SIDS requirements for reproductive/developmental toxicity are met (OECD, 2003a)

5.4.3 Strategy for completing data matrices:

Cells for which data exists

- The adequacy of existing data will be assessed by determining if it was derived from EPA recommended/or equivalent test(s)
- Determine if existing data is representative of CASRN on which it is derived
- Derive NOAEL/LOAEL (or range) from existing data if it is adequate and appropriate

Cells for which no data exists

For selected category members

- Perform testing (per EPA recommended tests) on appropriate samples
- Derive NOAEL/LOAEL (or range) from test results
- Use test results to validate the Petroleum HPV Testing Group's QCAR models for developmental and, where possible, repeated dose toxicity

For remaining untested category members

- Quantitative read-across from developmental toxicity study results on the same (CASRN) will be used to assign a PDx value (range of values)
- Consider the lowest predicted PDx value(s) (or range of values) as point(s) of departure (i.e. LOAEL(s)
- Complete data matrix using these LOAEL(s) (or range of LOAELs

5.4.4 Rationale:

The two reproductive studies provided to the TG did not comprise a sufficient number of studies on which to assess possible PAC content – toxicity relationships. However, the two reproductive toxicity studies indicated that reproductive toxicity is a less sensitive endpoint than developmental toxicity for a test material (carbon black oil) that has a high PAC content. Data from 13-week repeated dose studies provides valuable information on reproductive organs and, to a limited extent, on reproductive function. For example, the testes, accessory sex organs, and epididymides can be weighed in males; sperm can be examined for concentration, mobility and morphological abnormalities. There was little evidence of reproductive toxicity in the repeated dose studies of petroleum substances evaluated herein. Based on the results of a large number of repeated dose studies and two reproductive toxicity screening studies of petroleum substances, sensitive endpoints are more likely to be associated with developmental toxicity studies than with reproductive toxicity studies.

As noted in "EPA recommended test(s) for developing toxicity values", EPA has indicated a reproductive toxicity study may not be required if there is (1) a 90-day repeated dose study that sufficiently documents that reproductive organs were microscopically examined and indicate no effects and (2) a developmental toxicity study. Based on this guidance, the TG thinks reproductive toxicity studies may not be necessary for the classes of petroleum substances included in this project.

Since no significant adverse effects on reproductive organs were observed in the 90-day repeated dose studies of petroleum substances, the most sensitive endpoints of reproductive toxicity of petroleum substances are likely to be the sensitive endpoints observed in the developmental toxicity studies. Therefore, the PDx for developmental toxicity is likely to be a reasonably good predictor of the PDx for reproductive toxicity, as well. A more detailed explanation of the basis for this conclusion can be found in Volume 2, Appendix 8 of this series of reports (An Investigation into the Relationship between the Polycyclic Aromatic Compound Content and Acute, Repeat-Dose, Developmental, and Reproductive Toxicity of Petroleum Substances).

5.5 Genetic toxicity (assays for gene mutation and chromosomal aberration/damage)

The TG is in the process of investigating the relationship between PAC content and the genetic toxicity of petroleum substances. The results will be reported in Volume 3 of this series "An

Investigation into the Relationship between the Polycyclic Aromatic Compound Content and Genetic Toxicity of Petroleum Substances."

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