Assessing the mammalian toxicity of high-boiling petroleum substances under the rubric of the HPV program

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1. Introduction

The purpose of this paper is to provide a background and perspective to the other papers in this supplement.

In 1998, the US EPA announced the HPV Challenge Program, a voluntary chemical data collection effort. The Petroleum HPV Testing Group (PHPVTG1) volunteered to provide data on approximately 110 high-boiling petroleum substances (HBPS), i.e. substances with final boiling points greater than 650 °F (343 °C). These HBPS are substances of unknown and variable composition (UVCBs) that are composed of numerous individual constituents. Toxicity studies have shown that some HBPS can produce systemic (repeat-dose) and developmental effects, and some are mutagenic under in vitro conditions. The papers in this supplement show that these effects are related to the profiles of aromatic constituents in these substances. Further, it is shown that the effects on selected repeat-dose and developmental toxicity endpoints and mutagenic activity in bacterial assays can be predicted from compositional information using models based on the aromatic-ring class profile, “ARC profile” as defined by gas chromatographic separation of the DMSO-soluble fraction of the starting materials. This chromatographic method and the predictive models provide an efficient means of characterizing for screening purposes the potential for repeat-dose, developmental effects and bacterial mutagenicity of HBPS and can reduce the number of animal tests that would be required if these tests were conducted on all 110 HBPS.

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and Development (OECD) as the minimum required to screen HPV chemical substances for toxicity (OECD, 2012). This internationally agreed-upon test battery, known as the “Screening Information Data Set” (SIDS), includes the following endpoints:

- Physical/chemical properties – melting point, boiling point, vapor pressure, n-octanol/water partition coefficient, and water solubility.
- Environmental fate – photolysis, hydrolysis, transport/distribution, and biodegradation.
- Ecotoxicity – studies in fish, invertebrates, and algae.
- Mammalian toxicity – acute toxicity; repeat dose toxicity; developmental and reproductive toxicity; mutagenicity (gene mutation and chromosomal aberration/damage assays).

As conceived by the OECD, the SIDS battery of tests is not intended to completely characterize a substance, but rather is intended to provide sufficient data to (1) allow an initial screening assessment, and (2) identify substances in need of more in-depth testing and assessment (US EPA, 2000; OECD, 2012).

At the start of the HPV Challenge Program, sponsors of chemicals submitted to EPA (1) a set of robust study summaries that summarized representative existing studies/data for each sponsored substance and (2) test plans that either justified an assessment that available data fulfilled the SIDS data requirements or identified data gaps and provided a means for obtaining the needed information. Sponsors had several options to fill data gaps:

- to read across available data from similar chemicals to untested chemicals;
- to conduct testing using OECD or other standard guidelines; or
- to use accepted predictive models.

Accordingly, within the petroleum industry HPV program, read across of available data from substances considered “similar” was used to characterize untested substances. When judged necessary, the industry conducted toxicity tests, following established guidelines. Predictive models were also developed and applied to characterize the repeat-dose, developmental toxicity, and in vitro mutagenicity endpoints that are among the SIDS data requirements. The development of these models, and suggested uses are described in papers included in this supplement.

2. Early focus on carcinogenicity and mutagenicity

Historically, the characterization of the toxicity of HBPS has focused on the dermal carcinogenic hazards of these substances. The focus on carcinogenicity was triggered by results from epidemiological studies that indicated there was an excess of skin cancer among workers using unrefined lubricating oils (Leitch, 1924). After extensive animal studies, it was found that the constituents of concern with regard to the dermal carcinogenic potential of HBPS were poly cyclic aromatic compounds (PAC) (Gilman and Vesselinovitch, 1955; Twort and Fulton, 1929; Twort and Lyth, 1939; Twort and Twort, 1930, 1931, 1933).

In the first half of the 20th century “cracking” processes were developed by which high molecular weight hydrocarbons were broken down either by heating (thermal cracking or coking) or in the presence of a catalyst (catalytic cracking) into lower molecular weight constituents that were more suitable for fuels blending. As refineries began adding cracking capacity after the Second World War, it was recognized that the products from these units included relatively high-boiling, more aromatic streams. This led to further studies to characterize the dermal carcinogenic hazards of these newer process streams and to also assess the effectiveness of refining processes in removal or conversion of the carcinogenic constituents to minimize the dermal carcinogenic risks to the extent possible (e.g., Dietz et al., 1952; Smith et al., 1950, 1951).

One outcome of this work was a series of publications that summarized work to investigate the relationship of physical/chemical properties of petroleum-derived materials and their dermal carcinogenic properties (King et al., 1984; Lewis et al., 1984) and to then assess the effectiveness of refining practices in reducing dermal carcinogenic potential (Halder et al., 1984; Kane et al., 1984). Another was the development of screening tools that could be used to determine whether certain types of petroleum products were likely to be dermal carcinogens (Mackerer et al., 2003; Roy et al., 1988a,b). One of these is the ISP46 test that measures the weight of the DMSO-soluble fraction of lubricant base oils and related materials (IP, 1993). It was shown that oils in which the DMSO-soluble material comprises >3 wt% of the original material are potential dermal carcinogens (CONCAWE, 1994), and this relationship was adopted in the EU to separate refined lubricant base oils from unrefined oils and aromatic extracts for classification and labelling purposes (EU, 1994). As an alternative approach, the Ames Salmonella test was optimized to measure mutagenic potential in lubricant base oils and related materials (Blackburn et al., 1984, 1986). Based on a correlation between the mutagenic index (MI), a parameter calculated from the initial slope of the dose–response curve and dermal carcinogenic potential, it was proposed to consider any lubricant base oil with an MI > 1 as a potential dermal carcinogen. The optimized Salmonella test was established as a standard method for use in assessing the dermal carcinogenic potential of mineral oils used in lubricant formulation (ASTM, 1995). Concurrent with the development of these two assays was the development of a GC-FID assay measuring 3–7-ring PAC content in a DMSO extract of oil, expressed as a percentage of the oil.

3. Characterization of the toxicity of HBPS – existing studies

As previously discussed, the HPV program required a characterization of the acute, repeat-dose, developmental and reproductive toxicities and the mutagenic potential of each sponsored substance. Since the available data on a variety of HBPS provided sufficient evidence that they were not acutely toxic, the Petroleum High Production Volume Testing Group (PHPVGT) focused on the potential for non-cancer hazards associated with repeated exposure.

The first published study that systematically assessed the potential for HBPS to produce effects unrelated to cancer summarized the results of 13 repeated dose and 11 developmental toxicity studies of gas oils, heavy fuel oil components and distillate aromatic extracts (Feuston et al., 1994). It was reported that repeated dermal application of these substances produced systemic effects including increased liver weight, decreased thymus weight, and reductions in the levels of certain hematological parameters. In developmental toxicity studies, the principal findings were increased resorption frequency and decreased fetal weight. The authors hypothesized that the aromatic constituents of these substances were the causative factors, and this was supported by evidence that the Lowest Observed Effect Levels (LOELs) in these studies were correlated (Spearman rank correlation) with concentrations of PACs. Although this initial paper provided presumptive evidence that PACs were the constituents of HBPS that caused these effects, the results could not be used prospectively as a means of assessing the toxicity of other, untested substances.

To extend these observations, the current authors obtained copies of the laboratory reports for these and other related studies. As described elsewhere in this supplement (Mckee et al., 2013; Murray et al., 2013a; Nicolich et al., 2013; Roth et al., 2013), a total
of 115 mammalian toxicity studies and 242 bacterial mutagenicity studies were reviewed by the authors, resulting in a compilation of the information on repeat-dose and developmental effects and the doses at which they were produced as well as information on the potential for in vitro mutagenic effects. This database became the source for the information on the effects of HBPS that was used to develop the predictive models described in other papers in this supplement.

The papers included in this supplement indicate that data from gas chromatographic separation of the components of DMSO extracts of HBPS, the Aromatic Ring Class (ARC) profiles, are correlated with the endpoints of repeat-dose and developmental toxicity described above as well as the in vitro mutagenic potential of these substances. The papers also provide details on the development of a set of statistically-based Quantitative Composition–activity relationship (QCAR) models on selected repeat-dose and developmental toxicity, and bacterial mutagenicity endpoints. The papers also provide examples of how these models, using as input information their ARC profiles as determined by a specified analytical method, can be used for screening purposes to characterize the potential repeat-dose, developmental toxicity and in vitro mutagenic potential of HBPS.

The other critical element in this program was the identification of an analytical method to characterize the substances. After considering a number of possibilities, the authors determined that the method best suited for use was “Method II” (Feuston et al., 1994) in which the samples are extracted with DMSO to isolate the condensed ring aromatic constituents that are then resolved by ring number by gas chromatography. The ARC profiles summarize the outcome of the chromatographic separation in terms of fraction of starting material with the specified number of aromatic rings. A complete description of “Method II” is provided as an appendix (Appendix A) to this paper.

Throughout this supplement, the following terms are used with regard to the aromatic content of HBPS:

Polycyclic aromatic hydrocarbons (PAHs): compounds of two or more fused-aromatic rings consisting of only carbon and hydrogen atoms.

Polycyclic aromatic compound (PAC): a comprehensive term that includes PAHs and molecules in which one or more atoms of nitrogen, oxygen, or sulfur replace one of the carbon atoms in the ring system.

PAC content: when used in this manuscript, refers to the PAC content of the DMSO-extract of a petroleum stream determined using the Method II chemical characterization procedure (see Appendix A). Although technically 1-ring structures do not fit the definition of polycyclic compounds, they were included in this evaluation to account for the possibility that 1-ring structures may play some role in defining the toxicity of high-boiling petroleum streams.

Aromatic Ring Class (ARC) Profile: the weight percent of each class of the DMSO soluble 1–7 and larger aromatic-ring compounds present in a petroleum substance as determined by the Method II chemical characterization procedure (See Section 2.4), e.g. the ARC 3 value would be the weight percent of the DMSO-soluble 3-ring aromatic compounds within the petroleum substance.

There are some additional considerations that should be kept in mind. No attempts were made to identify individual aromatic constituents when the test substances were analytically characterized, so it was not possible in this project to relate specific constituents to the reported biological effects of the test substances; rather the available ARC profiles were used in the relationship characterization and model building phases of the project. The Method II “ARC profile” used for building the final models is a function of DMSO extraction and gas chromatography and thus is an operational descriptor of the HBPS aromatic constituents. When used in both phases it provided good results. Note the ARC profile is not equivalent to other aromatic compound characterizations included in the documents submitted to EPA by the PHPVTG under the HPV program.

4. Chemical complexity and variability of HBPS

When filling SIDS data gaps on HBPS a major challenge to assessing the toxicity of an untested HBPS i.e., those with final boiling points > 650°F (343 °C), that are the subject of this supplement, is that the chemical composition of the HBPS is complex and variable. Among these substances, the compositions cannot be fully resolved into individual chemical entities and must rather be assessed in generic terms. In fact, all HBPS are TSCA Class II substances, i.e. chemical substances of Unknown or Variable composition, Complex reaction products and Biological materials (UVCBs). These chemical substances cannot be represented by unique structures and molecular formulas (US EPA, 1995).

The complexity and variability of HBPS arises as a consequence of the inherent complexity of the starting material (crude oil), which is compounded further by the processes used to refine the intermediate streams to manufacture end products. A further reason for the variability and complexity is that most of the HBPS are used to make fuels that are manufactured to performance specifications rather than in accordance with strict compositional requirements.

Another complicating issue is that the Chemical Abstract Services (CAS) registry numbers for these substances are relatively broad descriptors which are assigned based on the starting material and the last step in the refining process. The descriptions do not provide detailed compositional information. Consequently, compositionally similar products or refinery streams may have different CAS numbers. And conversely, this means that there may be substances which are described by the same CAS numbers but which have compositional differences and hence different toxicological properties.

However, there are ways to deal with this complexity. As presented in this supplement, it has been shown that effects on endpoints of repeat-dose and developmental toxicity and bacterial mutagenicity are related to the aromatic profile of an HBPS. The predictive models described in this supplement allow assessments to be made despite the complexity and variability of HBPS and do not require a complete understanding of their compositions.

5. Other papers in the supplement

The paper by Nicolich et al. (2013) describes the collection and evaluation of data that were made available to the authors including information from the literature and data from unpublished sources. It provides the statistical details of the development and testing of statistical models to characterize the relationships between the ARC profile and repeat-dose and developmental toxicities of HBPS, and describes how to apply these models to predict selected effects of similar untested substances.

The papers by Murray et al. (2013a) and Roth et al. (2013) describe the details of the biological basis of the development of the models for developmental toxicity and repeat-dose toxicity endpoints respectively. They cover the selection of endpoints, the preliminary modeling, and examples of how to use the models to make individual predictions and to characterize the toxicity of the substances through a newly developed measure (PDRf). The
papers also provide empirical evidence for the accuracy and consistency of the estimates. Simpson et al. (2012) describe how the results of modeling described in this supplement may be used in a practical sense to assist industry and regulators such as the United States Environmental Protection Agency (US EPA) and the European Union (EU). They describe, in detail with examples, the application of these models to a series of untested HBPS to characterize their potential repeat-dose and developmental toxicities. They also compare the modeled results to experimental values for some previously tested materials.

The paper by McKee et al. (2013) discusses an approach to fulfilling the requirements for mutagenicity information based on an “optimized” Salmonella test. This paper also summarizes the information on in vivo mutagenic effects, particularly the results of micronucleus tests in rat bone marrow and concludes that few HBPS, even those with high aromatic contents, produce in vivo mutagenic effects. The paper also includes a model that can be used to predict the in vitro mutagenic potential from HBPS based on their ARC profiles. It should be noted that this model differs from the others in this supplement in that the predictions are qualitative (i.e., mutagenic vs. non-mutagenic) whereas the others make quantitative predictions (i.e., numerical responses and PDR10 values).

Murray et al. (2013b) discuss the approach used to address the potential for the HBPS to produce reproductive toxicity. Within the HPV program the requirement to assess reproductive toxicity can be fulfilled in several different ways, one of which is by providing data from studies of developmental toxicity as well as information on reproductive organs from repeated dose toxicity studies. The Murray et al. (2013b) paper evaluates the hypothesis that effects in developmental and/or repeat-dose toxicity studies of HBPS would occur at doses lower than those that might affect fertility in rat one-generation reproductive studies. And finally, it assesses the possibility that, when adequate developmental and repeat-dose toxicity studies are available, a reproductive toxicity study of HBPS is unnecessary.

The paper by Patterson et al. (2013) describes the peer consultation process conducted by Toxicology Excellence for Risk Assessment (TERA) at the request of the PHPVTG. As an independent nonprofit organization, TERA was asked to ensure independent expert input on two key questions. One, are there quantitative relationships between PAC content of petroleum substances and their critical effects as identified in repeat-dose, developmental, and reproductive toxicity studies? And two, can the critical effects/levels of untested petroleum substances as would be identified in an OECD 422 study be predicted using their PAC content? This paper describes the results of the peer consultation process, the ensuing panel discussions, key recommendations, and their resolution.

6. Conclusions

The end product of this effort included the development of a series of statistical models by which the repeat-dose and/or developmental toxicity and bacterial mutagenic potential of HBPS can be predicted from compositional information. It was also shown how this information could be used, along with results of existing studies of HBPS, as a means of satisfying the HPV requirements for the approximately 110 HBPS substances that are sponsored by the PHPVTG.

The knowledge gained and new tools developed complement the previously existing knowledge on the hazards of HBPS. As discussed, it was previously known that the dermal carcinogenic properties of these substances were associated with their aromatic constituents, and this knowledge had been used to develop methods to distinguish dermally carcinogenic from non-carcinogenic substances. A similar approach taken to characterize non-carcinogenic effects of repeated dermal exposure to HBPS also led to the conclusion that these effects were related to aromatic constituents, specifically the ARC profiles. This led to the development of models by which selected non-carcinogenic toxicological properties of the HBPS could be predicted from their ARC profiles. The knowledge that the repeat-dose, developmental toxicities and bacterial mutagenic potential of HBPS can be estimated through the use of the ARC profiles provides a pragmatic basis for characterizing the SIDS level repeat dose and developmental toxicities and in vitro mutagenic potential of these substances.

Conflict of interest

One coauthor (RHM) is an employee of a company that manufactures petroleum products. Five of the co-authors (RNR, BJS, MJN, FJM, and AWV) are paid consultants to the Petroleum High Production Volume Testing Group and four (RNR, BJS, MJN, AWV) are former employees of companies that manufacture petroleum products. One co-author (TMG) is employed by the American Petroleum Institute.

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Appendix A. Method of extraction and analysis of the aromatic fraction of high-boiling (>650 °F/344 °C) petroleum substances

The analytical method that proved to be the most useful in the investigations reported in this supplement was the Method II analytical procedure. As this method is an integral part of the predictive models that are described in detail in this supplement, it seemed appropriate to also provide a detailed description of the method and the basis for its selection for use by others who may be interested in furthering these results. There have been references to this method since at least 1988 (Roy et al., 1988a), but it has not previously been described in detail. Further, there have been several modifications to the procedure since it was first described. Feuston et al. (1994) summarized the results of repeat dose and developmental toxicity studies of a number of HBPS and compared the biological effects with aromatic content determined by two analytical methods, Method I and Method II. In an expansion of the Feuston investigation, a recent evaluation reported in this supplement (Nicolich et al., 2013) found a strong correlation between aromatic profile, determined by either the Method I or Method II procedures, and the outcomes of repeat dose and developmental toxicity studies of HBPS. Of the two methods, only Method II is still
being performed. Consequently the work reported in this supplement relies on PAC concentrations determined using Method II.

Method II isolates, detects and measures dimethyl sulfoxide-soluble (DMSO-soluble) non-heterocyclic and heterocyclic aromatic compounds within the 1 to 7 ring compound distributions. This information is reported as the Aromatic Ring Class (ARC) Profile, the weight percent of each class of the 1–7 ring aromatic compounds present in a petroleum substance, e.g. the ARC 3 content would be the weight percent of the 3 ring aromatic compounds within the petroleum substance as determined by the Method II analytical procedure. The ring groups (1 to 7 rings) are defined with standard polycyclic aromatic hydrocarbon makers and published methods. While PACs are defined as compounds with 2 or more aromatic rings, those 1-ring structures that are found in the potentially PAH-rich DMSO extract are quantified in Method II.

Method II was developed for routine isolation, classification and quantitation of complex PAC present in petroleum fractions with boiling points ranging from >300°F (149 °C) to >1000°F (600 °C). The soluble aromatic and polycyclic aromatic compounds are first extracted into cyclohexane and then extracted with DMSO. This is then back-extracted into fresh pentane or cyclohexane by addition of water or saline to the DMSO.2 The extraction uses a 4 g sample dissolved with 6–10 mL pentane or cyclohexane, followed by extraction twice with 10–40 mL of DMSO. The DMSO fractions are diluted with twice the volume of distilled water or a 4% NaCl solution (40–400 mL). The diluted DMSO fraction is back extracted sequentially with 20 mL then 10 mL pentane or cyclohexane. For the higher molecular weight more viscous samples with initial boiling points of >1000°F, such as heavy vacuum distillates, residual aromatic extracts and other streams derived from the vacuum residuum, the cyclohexane back extraction was replaced with sequential extractions using 25 mL and then 10 mL of methylene chloride or carbon tetrachloride. The extract is then washed with the solvent used for back extraction, followed sequentially by 5 mL of 4% NaCl solution.

The solution is filtered through a folded filter paper containing 2 gm dried anhydrous sodium sulfate. The extract is then concentrated, first by rotary evaporation at 35–50°C to near dryness, then at 80°C for an additional 30 min to create a final sample volume of 1–3 mL for gravimetric analysis. This residue is then re-dissolved with the original solvent for instrumental analysis.

The extracts are initially analyzed by gas chromatography with mass spectrometry (GC–MS) or flame ionization detection (FID), with naphthalene, phenanthrene, pyrene, benz[a]pyrene, benzol[ghi]perylene and corenene standards to define the boundaries of retention times for PACs containing two- through seven-rings. After establishing the retention time markers for two through seven and greater PAC ring classes, the additional extracts are analyzed by gas chromatography with flame ionization detection (GC–FID). The methodology was adapted from a report by Naughts and Tompkins (1978) in which it was reported that aromatics with 2 or more rings are efficiently extracted into DMSO. However, it was also noted that alkylated aromatics are less selectively extracted. This may have implications because the aromatic constituents that are constitutively present in crude oil are highly alkylated (Speight, 2007), and these are also the types of molecules that would be present after refining processes such as distillation and/or solvent extraction that separate constituents according to their physical/chemical properties but do not change them chemically. In contrast, processes such as “cracking” by catalytic or thermal processes tend to remove or shorten alkyl side chains producing less alkylated aromatics. Petroleum substances from cracking processes typically contain the highest amounts of DMSO extractable material found in any refinery streams.

It should be noted that (1) the values given for the percentages of ring structures are based on a convention for reporting GC data, so strictly speaking they represent a “fraction of material that behaves like aromatics of the stipulated ring number”, and (2) that the method is not selective for low molecular weight aromatics so there may be large differences between the percentage of 1-ring structures reported in the ARC profiles and those actually present in the respective petroleum streams. The “ARC profiles” may differ from data on levels of aromatic constituents in the test samples as determined by other analytical methods. Consequently, the ARC profile data presented in this supplement is not equivalent to the detailed analytical characterization of aromatics included in the documents submitted to EPA by the PHPVTG under the HPV program.

An advantage of Method II is that the results are relatively unaffected by variations in solvent or solvent volumes, and final analytical method. Another advantage is that Method II can be performed quickly (Naughts and Tompkins, 1978).

The Method II procedure is an extension of the Institute of Petroleum’s, now known as the Energy Institute, method 346 (IP-346) (Energy Institute, 1992). The IP 346 assay involves a DMSO extraction of the petroleum substance to isolate and quantify the DMSO-soluble aromatic fraction by wt% (Roy et al., 1988a). However, Method II has an important advantage over IP 346, because it also provides information on ring distributions.

References


