

Response to Comments on Aromatic Extract Category Test Plan

General

ED felt that this category of chemicals was particularly data-rich for such a complex mixture and the matrix of SIDS elements versus available data in the Test Plan indicates that each of the required elements have been addressed for distillate aromatic extracts (DAE) and residual aromatic extracts (RAE) either by specific data or “read across”. The chemical/physical properties have been estimated to the extent possible and in summary ED found the submission as complete as could be expected for such a complex mixture of chemicals.

PCRM also found the chemical(s) in the Test Plan to be well characterized and the OECD SIDS data endpoints required by the program to be fulfilled using either existing data or by reading across the category, a strategy consistent with the aim of minimizing testing. They also felt that API used a rational toxicology approach, taking into account physicochemical data in order to fill gaps in knowledge of the two chemical subcategories.

EPA provided a number of comments concerning category justification and the overall adequacy of studies contained within the test plan and robust summary. The Testing Groups responses to EPA’s comments are outlined below:

Category Justification

EPA noted that no information was provided to show that testing on paraffinic DAEs would be representative of naphthenic DAEs. Thus, EPA recommends testing samples of both groups when there are data gaps.

The Testing Group agrees that no data were presented in either the test plan or the robust summaries to show that information on a paraffinic DAE provides read across data that could be used for a naphthenic DAE. The Testing Group also agrees to conduct testing where the Agency has identified data gaps on both types of substances.

While EPA agreed that the information on the “light” DAE streams could be used for “read across” to heavy streams, the Agency did not agree that data from heavy DAE could be used to “read across” to light DAE streams due to expectations that the “heavy” DAEs would be less bioavailable.

The Testing Group accepts EPA’s position that insufficient data were provided to demonstrate that information on a “heavy” DAE can be used to read across to a “light” DAE. The Testing Group will conduct testing on an appropriate light paraffinic DAE and a light naphthenic DAE to fill the identified data gaps.

EPA stated that no data were submitted for light or heavy naphthenic DAEs; the submitter needs to conduct acute, repeated-dose, genetic, and reproductive/developmental toxicity studies on light naphthenic DAEs and use the data to read across to heavy naphthenic DAEs.

The Testing Group proposes to test an appropriate light naphthenic DAE stream to address the repeated-dose, genetic (*in vivo*), and developmental endpoints. The Testing Group proposes to use modeling to address the genetic (*in vitro*) and reproductive endpoints for a naphthenic DAE. No acute toxicity testing is planned.

Since a major part of the submitter's rationale for grouping DAEs and RAEs into a single category is that their toxicity is proportional to their concentrations of DMSO extractable 3-7 ring PACs, EPA requested the submitter present data that demonstrate an association between PACs and mammalian toxicity, rather than referring the reader to other sources.

Since, the Test Group now proposes to conduct studies on a light paraffinic and a light naphthenic DAE stream to address the repeated-dose toxicity and developmental endpoints, the need to demonstrate a specific association between PACs and mammalian toxicity in this Test Plan is significantly diminished.

The Petroleum HPV Testing Group (HPV Group) originally indicated to EPA that the mammalian toxicity of aromatic extracts was primarily due to their polycyclic aromatic compounds PAC content. The original test plan also implied that the PAC content of these substances could be used to predict the toxicity of untested substances. The basis for these 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 HPV Group recognized that the underlying data for this assertion were limited and a new more sophisticated and robust analysis was needed. A report has been issued describing the results of this new evaluation of the association between PAC content and repeat-dose and developmental toxicity, and the subsequent development of predictive models of eleven (selected) endpoints of repeated-dose and developmental dermal toxicity in the rat (API, 2008). The report has been subject to a peer consultation process, the results of which are available to the public (TERA, 2008).

The HPV Group is in the process of investigating the relationship between PAC content and the potential genetic and reproductive toxicities of petroleum substances, and whether predictive models can also be used for these two endpoints. The results of these investigations will be reported separately.

Finally, the Testing Group has conducted additional compositional analysis on representative samples of eleven DAEs and ten RAEs obtained from US HPV refineries sponsoring this category. These data have not only been useful in identifying appropriate representative test samples but they have also helped to clarify and characterize compositional variability among category members.

Specific Comments on Test Plan and Robust Summaries

Physicochemical Properties

Generic Comments. EPA thought that the Testing Group should identify and characterize those individual PACs that would likely be responsible for inducing toxicity within the subcategories. EPA requested the PAC content of the test substances should be included in each robust summary.

Aromatic extracts are extremely complex mixtures of C15 to C54 petroleum hydrocarbons containing thousands of different chemical compounds (LOBS, 2003a&b). Therefore, it is difficult to identify or assign biologic activity to a single compound or individual PACs (Mackerer, et al., 2003). The Testing Group conducted additional compositional analyses on eleven DAEs and ten RAEs. The aromatic content and 1-7 ring PAC profile for each of these AEs have been included in the revised test plan.

Generic Comments. EPA noted the CAS number of one of the test materials in Section 1.1.1 of the Robust Summary was entered incorrectly.

The CAS number in Section 1.1.1 has been corrected.

Physicochemical Properties

Vapor Pressure. EPA did not think the open-range values presented in the Test Plan adequately characterized vapor pressure. The Agency requested the submitter to provide data on representative chemicals, as it did for partition coefficient and solubility in different media.

Vapor pressures of representative components of DAEs and RAE were added to the Robust Summary. The vapor pressure value of <0.01 in the Test Plan has been corrected to <0.1 hPa.

Environmental Fate

Fugacity. EPA did not think the Level I fugacity data included in the Test Plan are adequate. The Agency requested the submitter provide input values in Level III fugacity calculations.

After careful, in-depth review, including contacting outside experts, the Testing Group decided that the use of the EQC Level III model suggested by EPA for evaluating the transport and distribution behavior of petroleum mixtures is at this time, an inappropriate approach. The Testing Group continues to support the use of a Level I fugacity calculation. Expert modeling scientists from the Center for Environmental Modeling, Trent University, Toronto, Canada have stated that Level III fugacity predictions are inappropriate for complex mixtures. This is due to the lack of accurate emissions data and limitations of the algorithms which require input of chemical specific properties. Petroleum substances that are, with minimal exception, characterized as complex, heterogeneous mixtures consisting of chemicals from different alkyl and aryl hydrocarbon classes. Due to the variability in hydrocarbon number and hydrocarbon type

for petroleum constituents, representative hydrocarbons were selected to predict potential partitioning behavior using simple Level I multimedia modeling equations.

Ecological Effects

Acute Toxicity to Fish and Algae. EPA asked that the percent composition of the test substance in the Robust Summary needs to be provided.

Compositional analyses of the test substances used in the referenced aquatic toxicity tests were not provided in the data reports. However, the revised test plan now provides additional compositional data for representative distillate and residual aromatic extracts. Aromatic extracts are of such complexity that it is unlikely that compositional analyses would provide information to discern potential ecological effects, especially with the large body of data showing a lack of acute and chronic toxicity

Acute and Chronic Toxicity to Invertebrates. EPA asked that the percent composition of the test substance in the Robust Summary needs to be provided.

See the Testing Group's response to Acute Toxicity to Fish and Algae.

Health Effects

Acute Toxicity. EPA stated that the submitter needs to conduct an acute toxicity test on a representative light naphthenic DAE, preferably by the oral route. A read-across approach would be acceptable for the gap in data on heavy paraffinic and naphthenic DAEs and RAEs.

The Testing Group considers conducting an oral LD50 on a light naphthenic distillate extract to be unnecessary and would do little to strengthen the aromatic extract data set since acute studies on other aromatic extract streams show a low order of acute toxicity. Acute oral LD50s on a wide-range of refinery streams (including streams with high aromatic content) from a variety of refinery processes rarely show LD50s values < 5 gm/kg.

Acute Toxicity. EPA also noted that while on page 9 of the test plan, the submitter stated that oral LD50 values for light and heavy DAEs are > 5,000 mg/kg; this dose is not mentioned in the individual robust summaries.

LD50 values of greater than 5,000 mg/kg are given for a light and a heavy DAE in the robust summary (p.24/71). The description of the test substance has been modified to make this clearer.

Repeated Dose Toxicity. EPA considered that although the information submitted on the oral 13-week rat study on DAE was useful, a true dose-response was not possible since only two dose levels were used in the studies. The Agency also noted that the DAE used in the study was not identified.

The Testing Group does agree that the lack of a third dose level limits the usefulness of the 14 week study and the study by itself does not characterize the repeated dose toxicity of all DAEs. However, treatment related and dose-response effects were seen on mortality, body weight and in a number of histopathologic findings in the liver, kidney, adrenals and thymus. Additional specific pathologic information from the oral study was added to the robust summary to make the evaluation more complete.

The DAE used in the study was a heavy paraffinic DAE; the appropriate CAS # has now been added to the robust summary.

Repeated Dose Toxicity. EPA requested the submitter conduct new tests, following OECD TG 422, by the oral route on representative light paraffinic and light naphthenic DAEs, since they are expected to be more bioavailable. The EPA also expressed concern that dermal application of the test material might result in less material being bioavailable than if the test material had been administered orally.

The Testing Group disagrees with the Agency's position that new testing needs to be conducted by the oral route of administration. The dermal route is the primary route of human exposure. Moreover, conducting these studies via the dermal route will facilitate using the new data in human hazard and risk assessments. The fact that substantial systemic toxicity was observed in the existing heavy paraffinic DAE study, should address the Agency's concern that the test material was not being absorbed. Furthermore, two extra groups of rats received 125 or 500 mg/kg DAE via oral gavage for 13 weeks and similar toxicological effects were observed in treated animals from both the oral and dermal routes of exposure.

Repeated Dose Toxicity. EPA stated that the dermal data on a light paraffinic DAE included in the Test Plan and Robust Summary are inadequate because animals were treated 3 rather than 5 or 7 days/week

The Testing Group agrees that data submitted on the light paraffinic DAE was limited. The Testing Group proposes to conduct a repeated dose toxicity study on a light paraffinic DAE via the dermal route of exposure. This new study, along with the one proposed on a light naphthenic DAE, should characterize the repeated dose toxicity of all DAEs within the category.

Repeated-Dose Toxicity. The EPA stated that the robust summary for several of the repeat-dose studies does not indicate the method for the evaluation of sperm morphology and the magnitude of body weight changes.

A brief explanation of the method used to evaluate sperm morphology has been added to the appropriate sections of the robust summary. The robust summary has been modified to show the magnitude of the body weight changes.

Repeated-Dose Toxicity. The EPA stated that the robust summary for a 13-week rat dermal study using four RAEs does not include the number of hours that the test material remain on the application site.

Test material was only removed from the skin at the end of each week. The test material therefore, was in continuous contact with the skin for 5 days. This information was added to the robust summary.

Repeated-Dose Toxicity. The EPA stated that the robust summary for a 4-week rabbit dermal study states that the authors concluded that the high relative liver weights for the high dose males were not treatment-related since there was no supporting clinical pathology or histological data. It would be more correct to state that this effect was of unknown toxicological significance in view of the lack of histopathological evidence of toxicity.

A statement has been added to the robust summary stating that the biological significance of the increased relative liver weights observed in the high dose males is uncertain, due to the lack of confirmatory microscopic effects observed in the liver.

Genetic Toxicity. The EPA reserved judgment on the adequacy of the bacterial mutagenicity data set because the information came from review articles with insufficient detail. The Agency requested the submitter provide robust summaries for the original studies discussed in the review articles or conduct testing on a representative light naphthenic DAE.

Robust summaries of the original reports were prepared as appropriate.

Genetic Toxicity. The EPA reserved judgment on the mouse lymphoma assay on light paraffinic DAE because values reported in the Robust Summary were in conflicting units (nL/mL vs thousands of nL/mL).

The error in the robust summary concerning the units reported in the mouse lymphoma assay has been corrected. The Testing Group continues to believe the information is adequate.

Genetic Toxicity. The EPA reserved judgment on the adequacy of the DAE and RAE micronucleus assays until the submitter documents that an appropriate response was attained with positive controls. EPA will consider the data to be acceptable if the submitter documents that an appropriate response was attained with positive controls. The robust summary for the DAE study needs to include the CAS number or the composition of the test material.

No positive concurrent controls were employed in the study. Standard operating procedures in the laboratory at that time (1987-1988) did not employ positive controls for *in vivo* micronucleus assays. However, the laboratory that performed this study had performed two validation studies (Mobil, 1985 & 1986). These validation studies confirmed that personnel reading the slides could correctly identify and score the number of polychromatic erythrocytes (PCEs), normochromatic erythrocytes (NCEs), and

micronucleated erythrocytes. The CAS number and test material composition has been added to the robust summary for the DAE study.

Genetic Toxicity. EPA thought the robust summary for the mouse lymphoma assay needed to identify the strain of lymphoma cell used. A value reported as “150,00” should be corrected.

The robust summary has been modified to identify the strain of cells used in the mouse lymphoma assay. The reported value has been corrected.

Developmental Toxicity. The EPA stated that the dermal developmental studies done on samples of heavy paraffinic DAE and RAE were inadequate because the dermal bioavailability of these substances is limited.

As stated previously, the Testing Group proposes to conduct developmental toxicity studies on both a light paraffinic and a light naphthenic DAE stream to address this data gap and allay EPA’s concern about the dermal bioavailability of heavy DAE and RAE.

Reproductive Toxicity. ED and PCRM felt that the Test Plan adequately addressed the OECD SIDS endpoints for reproductive and developmental toxicity. EPA also agreed that submitter’s strategy to use reproductive histopathology and sperm morphology data generated in repeated-dose studies is reasonable, but judged the developmental toxicity data were inadequate. EPA felt that adequate repeated-dose testing was performed on a heavy paraffinic DAE and RAE. However, the Agency did not think these data could be “read across” to light DAEs since these test materials would be expected to be less bioavailable than other category members. EPA suggested conducting combined testing on light paraffinic and light naphthenic DAE, using OECD TG 422, should address data gaps for reproductive toxicity.

Since the PAC Analysis Task Group found that the developmental toxicity endpoints, including both *in utero* and postnatal development, were more sensitive than effects on the reproductive organs, the Testing Group did not feel that multiple reproductive toxicity studies were required on both a light paraffinic and a light naphthenic DAE in order to characterize the reproductive toxicity of all DAEs. The Testing Group proposes to conduct a reproductive toxicity study on a light paraffinic DAE. This study will be used to provide data to read across to the light naphthenic DAE stream.

Closing Remarks

The Testing Group appreciates the EPA, ED and PCRM’s comments and interest in the Aromatic Extracts testing program. The Testing Group believes that the new compositional analyses and proposed testing described in this response addresses all major comments of the reviewers. The revised test plan makes every effort to minimize the number of animals used in toxicity testing, meets the spirit of the EPA’s guidance on animal welfare, while at the same time allowing the sponsors to fulfill their commitment to the EPA HPV Challenge program.

References

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4. LOBS (Robust Summary of Information on Lubricating Oil Basestocks). Summary Prepared by American Petroleum Institute. March 24, 2003.
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