Response to EPA's Hazard Characterization of the Waxes and Related Materials Category
The American Petroleum Institute Petroleum HPV Testing Group
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The following comments are in response to EPA's Hazard Characterization (HC) for the Waxes and Related Materials Category (WRM) (U.S. EPA, 2011). This Category was sponsored by the American Petroleum Institute (API) Petroleum HPV Testing Group (Testing Group) as part of EPA's HPV Chemical Challenge Program (www.petroleumhpv.org).

Below is EPA's generic table of content for all the HPV Hazard Characterizations they have prepared, including WRM. The Testing Group's comments are found on the page numbers indicated below.

Summary

The key reason for the data "gaps" identified by EPA for this Category is the organization of the 8 substances into subcategories. EPA treated subcategories as barriers that don't allow read-across of mammalian data between them. The Testing Group believes the WRM Category is better described as a continuum of similar substances and the primary mammalian hazard of this category are associated with the potential presence of polycyclic aromatic compounds (PACs). The environmental fate and effects are related to the carbon number range for the substances in the Category.

Wax is primarily composed of normal paraffins which can aggregate and solidify depending on their carbon chain length and temperature. Wax is isolated from lubricating oils by processes which do not change their chemical structure. The process of solvent de-waxing lubricating oils may leave some residual oil in the wax material (called slack wax, it can have 5 to 20% oil in it). Depending on the previous process steps and process conditions, PACs are possibly present in that oil fraction. The potential mammalian toxicity of WRM substances decreases as they are refined from slack wax (high oil content) to FDA compliant refined wax and petrolatum. This knowledge, existing test data, and long-term human experience with WRM makes additional acute, repeat-dose, repro/developmental, and gentox studies unnecessary.

The Testing Group described a modeling approach for assessing the repeat-dose, developmental, and gentox endpoints of substances in the WRM Category. However, EPA did not acknowledge the utility of the statistical models developed by the Testing Group to evaluate untested samples of WRM and other high-boiling petroleum substances. In the original Test Plan for this category, a relationship between mammalian toxicity and the polycyclic aromatic compound (PAC) content of the substances in that category was asserted or implied. To study this relationship further, toxicology studies and analytical reports on high-boiling petroleum substances (HBPS) were collected from the Testing Group's member companies and analyzed in order to address two key questions: 1) Are there quantitative relationships between PAC content of petroleum substances and their critical effects as identified in repeat-dose, developmental, bacterial genotoxicity, and reproductive toxicity studies, and 2) can the critical effects/levels of untested petroleum substances be predicted from their PAC content?

The assessment by the Testing Group showed (a) that the toxicological effects of high boiling petroleum-derived substances (i.e., final boiling points > 650 ^{o}F) were associated with PAC content, (b) that subchronic effects associated with PAC content included liver enlargement, thymic weight reductions, reduced hematological parameters, and developmental effects including reduced live-births and birth-weight, and (c) that the effects of these high boiling petroleum-derived substances could be predicted from PAC contents using predictive statistical models for several repeat-dose, mutagenic, and developmental toxicity endpoints. The models used the weight percent of each of the aromatic ring classes (the "PAC profile") as the independent variable. The effects found to be associated with the PAC profile are consistent with those reported for a number of individual PAHs and PAC-containing materials. A predictive model for bacterial mutagenesis was also developed. The Testing Group had the results of its model building exercise reviewed through an expert peer consultation process (TERA, 2008). The Testing Group has followed up the peer consultation with additional testing and analysis and has several detailed manuscripts in-press (Murray et al., 2013; Nicolich et al., 2013; Roth et al., 2013; Mckee et al., 2013).

Category Justification

1. The EPA hazard characterization for several Petroleum HPV Categories including WRM, refers to the category members as complex mixtures when in fact they are Class 2 UVCB substances (HC pages 8 and 12).

Substances on the US TSCA Inventory are divided into two classes for ease of identification (EPA 1995). Class 1 substances are those single compounds composed of molecules with particular atoms arranged in a definite, known structure. However, many commercial substances that are subject to TSCA are not Class 1 substances, because they have unknown or variable compositions or are composed of a complex combination of different molecules. These are designated Class 2 substances. Class 2 includes substances that have no definite molecular formula representation and either partial structural diagrams or no structural diagrams. These are the "UVCB" substances (Unknown or Variable compositions, Complex reaction products and Biological materials). An example of this kind of substance is given below.

CAS Number: 64742-51-4
CAS Name: Paraffin wax (petroleum), hydrotreated
CAS Definition: A complex combination of hydrocarbons obtained by treating a petroleum wax with hydrogen in the presence of a catalyst. It consists predominantly of straight chain paraffinic hydrocarbons having carbon numbers predominantly in the range of about C20 through C50.

Petroleum substances are subject to nomenclature rules developed jointly by the U.S. EPA and the American Petroleum Institute (EPA, 1995b). In that guidance document, EPA adopts the definitions of petroleum process stream terms provided in API's published reference document Petroleum Stream Terms Included in the Chemical Substance Inventory under TSCA (1983, reprinted in 1985). The Stream Terms definitions include the CAS definition and registry number, the source of the substance and process (i.e., last refining step), short name, indication of carbon number, and indication of distillation range (or other appropriate characteristic). Therefore all members of the Waxes and Related Materials Category are UVCB substances, not mixtures, under EPA's nomenclature guidance.

Justification for Supporting Chemicals

EPA cites 1-tetradecene (CASRN 1120-36-1) as a model hydrocarbon that can be used to represent the aquatic toxicity of the WRM Category. Yet the studies supporting the aquatic toxicity of 1-tetradecene employed the same testing methods that EPA criticized in their review of other studies submitted by the Testing Group.

EPA wrote that the Testing Group included environmental effects data for several lubricating base oils to supplement a technical discussion to satisfy the environmental effects endpoints in the WRM Category. The supporting chemicals included solvent refined light naphthenic distillate (CASRN 64741-97-5), solvent-refined light paraffinic distillate (CASRN 64741-89-5), solvent-refined heavy paraffinic distillate (CASRN 64741-88-4), solvent refined residual oil (CASRN 64741-01-4) and hydrotreated light naphthenic distillate (CASRN 64742-53-6). Lubricant base oils contain similar hydrocarbon ranges and structures common to materials in the WRM and related materials category. EPA initially agreed with the use of these chemicals to support the technical discussion and fulfill the environmental effects endpoints for the WRM category. Upon further review, EPA found the data for CASRNS 64742-04-7, 64741-89-5, 64742-01-4, and

64741-88-4 to be inadequate to address the toxicity to aquatic organisms because these data were tested above the water solubility limit for the respective compounds. In addition, these studies are unacceptable due to the fact that they are derived from WAF (water accommodated fraction) preparation methods without the analytical monitoring to accompany the values for loading rates, which makes calculating an LC50 or EC50 value impossible. Therefore, for the ecotoxicity endpoints, EPA determined that the measured data from 1-tetradecene (CASRN 1120-36-1) is appropriate to support the WRM category based on similar physico-chemical properties, environmental fate and mode of toxic action (narcosis). The supporting chemical, 1-tetradecene (CASRN 1120-36-1: SIAM 11), has been assessed in the OECD HPV program as a member of the alpha olefins category (http://www.chem.unep.ch/irptc/sids/OECDSIDS/AOalfaolefins.pdf).

In response to EPA's rejection of data from studies using WAF methodology and their use of data on 1-tetradecene as a surrogate, the Testing Group has these comments;

1. EPA used 1-tetradecene (CASRN 1120-36-1) to represent the aquatic toxicity of the WRM Category. Yet the studies supporting the aquatic toxicity of 1-tetradecene employed the same testing methods that EPA criticized in their review of the studies submitted by the Testing Group. The supporting data for 1-tetradecene was submitted as part of the SIDS Initial Assessment Report for Alpha Olefins (11th SIAM, January 2001). The robust summary for the fish test is included after the References on page 9. This summary shows that exposure solutions were prepared as WAFs, at concentration well above the solubility limit of 1-tetradecene (calculated solubility of 0.004 mg/L by WSKOW V1.41, EPI-Suite^{TM} V4.0) without analytical data to accompany the values for loading rates.

EPA's use of these surrogate data, although redundant, supports the Testing Group's use of lethal loading based on WAF preparations. Therefore, the Testing Group interprets EPA's use of the surrogate data as accepting studies run employing WAF preparations.

2. The Testing Group agrees with EPA's conclusion that substances in the WRM Category show no aquatic toxicity at their water saturation limit. However, the Testing Group believes that results for petroleum UVCBs (multi-constituent, poorly soluble hydrocarbons) should be expressed as lethal loadings (LL) rather than lethal/effect concentrations (LC, EC). Lethal loading rates are a more effective means of comparing two substances to each other because the hydrocarbon composition in the WAF varies with composition of these streams. Loading is a reflection of the composition and chemistry of the substance and implicitly accounts for multi-constituent dissolution and volatilization.

Aquatic toxicity of petroleum streams is attributed to the neutral organic hydrocarbon constituents whose toxic mode of action is non-polar narcosis. Hydrocarbons are equitoxic in tissues where the toxic mechanism of short-term toxicity for these chemicals is disruption of biological membrane function (van Wezel and Opperhuizen, 1995). The differences between toxicities (i.e., LC/LL5O, EC/EL50) can be explained by the differences between the target tissue-partitioning behaviors of the individual chemicals (Verbruggen et al., 2000). The existing fish toxicity database for hydrophobic neutral chemicals supports a critical body residue (CBR, the internal concentration that causes mortality) of approximately 2-8 mmol/kg fish (wet weight) (McGrath and Di Toro, 2009). When normalized to lipid content the CBR is approximately 50 µmol/g of lipid for most organisms (Di Toro et al., 2000).

When compared on the basis of standard test methods and exposure solution preparation procedures, lubricating oil basestocks are expected to produce a similar range of toxicity for the three trophic level species. Results expressed as measured concentrations of the fraction of the substance in solution are of little value since it will be virtually impossible to extrapolate to spill situations where the only relevant measures of concentration will be the amount of product spilled and the volume of the receiving environment (i.e., the loading rates). Loading rates provide a unifying concept for expressing the results of a toxicity test with poorly-soluble substances (European Eco-Labeling Criteria; ASTM 2009; GESAMP; OECD 2006; ECHA). Preparation of independent WAFs based on test substance loading rates is the appropriate procedure for products in this category because these products are multi-constituent hydrocarbons whose constituent hydrocarbons vary in water solubility. The dissolution thermodynamics of a multi-constituent hydrocarbon in an aqueous medium limit the likelihood of consistent proportional concentrations of the constituent hydrocarbons at various test substance loading rates. For this reason,

- exposure solutions are not prepared from dilutions of a stock solution (the relative proportion of hydrocarbon constituents in the dilutions would not accurately reflect the relative concentration of those constituent chemicals in individually prepared, successively lower exposure solutions of the test material), and
- separate exposure solutions are prepared at each exposure loading for products that are multi-constituent hydrocarbons.

3. EPA is critical of the Testing Group's submitted data and claim they are inadequate because the test substances were tested above their solubility limit. When properly prepared, WAFs represent the equilibrium condition of maximally dissolved test substance for its respective loading rate. Any excess test substance is separated from the solutions used in testing, allowing the use of only dissolved constituents or those that create stable dispersions.

3. Human Health Hazards

Acute Toxicity (Slack Wax)

Acute toxicity data are not specifically available for slack wax but data are available on the acute toxicity of the raw vacuum distillate from which both lubricating oil basestocks and slack waxes are derived (API, 1986). Acute toxicity data are also available on the lubricating oil basestocks that contain the same types of saturated hydrocarbons with similar carbon numbers found in slack wax and are derived from the same vacuum distillates. Both the raw vacuum distillate precursor stream and the lubricating base oils have oral LD50s in the rat > 5 g/kg (CONCAWE, 1997). Therefore, both the waxy and oil portions of slack wax have oral LD50s > 5 g/kg. The Testing Group believes that additional acute toxicity testing on Slack Wax is unnecessary.

Repeated-Dose Toxicity (Slack Wax)

The Testing Group proposed to conduct a repeated dose reproductive/developmental screening study (OECD 422) in the 2002 WRM test plan. This testing was proposed because based on process history, Slack Wax may contain biologically significant PAC concentrations and elicit target organ effects. The Testing Group requested Slack Wax samples from member companies; all samples received had no detectable PAC content (this is not surprising for reasons described in the January 21, 2011 Category Assessment Document or CAD). The Testing Group chose not to conduct the repeat-dose tests on these samples, as this most certainly would have produced misleading, 'false-negative' results inappropriate for determining potential hazards of less-refined Slack Wax. Use of the statistical models developed by the

Testing Group for repeat-dose endpoints is a better way to satisfy the requirements of the HPV Challenge.

The CAD describes lower melting point WRM and lower viscosity white mineral oils caused some inflammatory changes in F344 rats but only minimal changes in other rat strains and species. Comparative toxicity, pharmacokinetic and pathology studies indicate that the response seen in the F344 rat is not applicable to human health assessment.

Slack Wax has already been registered in the E.U. for compliance with REACH and CLP regulatory requirements. Slack Wax is classified as a repeated dose toxicant, a developmental toxicant and carcinogenic *unless the full process history is known and it can be shown that the substance from which it was produced is not a carcinogen (CONCAWE, 2012)* (emphasis added). In other words, in the absence of data Slack Wax will be conservatively classified as a repeat-dose toxicant.

Genetic Toxicity (all WRM subcategories)
Slack Wax represents a 'worst-case' test substance for WRM, as it is the least processed of the materials, contains the broadest spectrum of chemical components and highest concentration of potentially toxic components. The samples of Slack Wax obtained from USA manufacturers for HPV evaluation contained no detectable PAC content. Optimized Ames tests (ASTM E 1687) on those samples were negative as expected.

In vivo genetic toxicity studies have not been reported for WRM but have been reported for other petroleum samples expected to contain high PAC content. Results have led to the conclusion that PAC-containing petroleum substances do not produce chromosomal effects when tested in SIDS-level assays under *in vivo* conditions (McKee et al, 2013). Poorly refined Slack Waxes that are classified as carcinogenic are expected to be positive in *in vitro* Optimized Ames testing. Otherwise, WRM substances in this category are expected to be non-mutagenic in both *in vitro* and *in vivo* tests.

Developmental and Reproductive Toxicity (all WRM subcategories)

Developmental Toxicity
As noted above, Slack Wax samples obtained from USA manufacturers had no detectable PAC content. Developmental toxicity testing was not conducted as this would likely have resulted in a misleading negative finding. Use of the statistical models developed by the Testing Group for developmental endpoints is a better way to satisfy the requirements of the HPV Challenge.

Slack Wax is classified in the E.U. as a developmental toxicant unless the refining history is known and it is not carcinogenic. These are the same criteria as applicable for repeated-dose toxicity and for practical purposes means this classification is based on PAC content as determined by method IP346. This also means that in the absence of this information or data, a Slack Wax substance is conservatively classified as a developmental toxicant. Effect levels may be determined by the PAC models, or be reasonably estimated by read-across from poorly refined vacuum distillates.

WRM substances other than Slack Wax are not expected to contain significant PAC concentrations and would not be classified as developmental toxicants. Their potential for developmental toxicity can be accessed from two developmental toxicity studies and a

reproductive/developmental toxicity study conducted on lubricating oil basestocks in which no developmental effects were observed; the NOAEL was > 1000 mg/kg/day.

Reproductive Toxicity
All the samples of WRM collected from Testing Group members contained no detectable PAC, hence their potential for reproductive toxicity are predicted to be low without further testing. Again, use of the statistical models developed by the Testing Group for repeat-dose and developmental effects would be a better way to satisfy the requirements of the HPV Challenge Program. Because of their low oral and dermal toxicity, refined/finished waxes and petrolatum have been safely used for decades in food, cosmetic and pharmaceutical applications.
Also, no reproductive effects would be predicted based on read-across from a reproductive/developmental screening study conducted on a lubricating base oil control with a NOAEL of approximately 1000 mg/kg/d (WIL, 1995).

References cited in this response to EPA's HC for the WRM Category

API (1986). Acute oral toxicity study in rats Acute dermal toxicity study in rabbits, Primary dermal irritation study in rabbits, Primary eye irritation study in rabbits, Dermal sensitization study in Guinea pigs, API 84-01, Light paraffinic distillate CAS 64741-50-0. Study conducted by Hazleton Laboratories Inc. API Med. Res. Publ.: 33-30595 Washington DC: American Petroleum Institute.

ASTM. 2009. ASTM D6081 – 98 (2009) Standard Practice for Aquatic Toxicity Testing of Lubricants: Sample Preparation and Results Interpretation.

CONCAWE (1997). Lubricating Oil Basestocks. Product Dossier No. 97/108. Brussels: CONCAWE.

CONCAWE (2012). Hazard classification and labelling of petroleum substances in the European Economic Area – 2012. Report No. 8/12. CONCAWE, Brussels.

Di Toro DM, McGrath JA, Hansen DJ. (2000). Technical basis for narcotic chemicals and polycyclic aromatic hydrocarbon criteria. I. Water and tissue. Environ Toxicol Chem. 19:1951-1970.

ECHA Guidance on information requirements and chemical safety assessment. Chapter R.7b: Endpoint http://echa.europa.eu/documents/10162/13632/information_requirements_r7b_en.pdf

European eco-lubricant labeling criteria:
http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2005:118:0026:0034:EN:PDF

GESAMP: The Revised GESAMP Hazard Evaluation Procedure for Chemical Substances Carried by Ships
http://www.gesamp.org/publications/publicationdisplaypages/rs64

McGrath JA, Di Toro DM. (2009). Validation of the target limpid model for toxicity assessment of residual petroleum constituents: moncyclic and polycyclic aromatic hydrocarbons. Environ Toxicol Chem 28:1130-1148.

McKee, R.H., Schreiner, C., Nicolich, M.J., and Gray, T. (2013) Genetic toxicity of HPV petroleum streams containing polycyclic aromatic compounds. Regulatory Toxicology and Pharmacology. Accepted for publication.

Murray J, Roth R, Nicolich M, Gray T, Simpson B. (2013). The relationship between developmental toxicity and aromatic ring class content of high boiling petroleum substances. Regulatory Toxicology and Pharmacology. Accepted for publication.

Nicolich M, Simpson B, Murray J, Roth R, Gray T. (2013). The development of statistical models to determine the relationship between the aromatic ring class content and repeat-dose and developmental toxicities of high boiling petroleum substances. Regulatory Toxicology and Pharmacology. Accepted for publication.

OECD: Guidance for Testing of difficult substances and mixtures: http://search.oecd.org/officialdocuments/displaydocumentpdf/?cote=env/jm/mono(2000)6&doclanguage=en

Roth R, Simpson B, Nicolich M, Murray R, Gray T. (2013). The relationship between repeat dose toxicity and the aromatic ring class content of high boiling petroleum substances. Regulatory Toxicology and Pharmacology. Accepted for publication.

Toxic Substances Control Act Inventory Representation for Chemical Substances of Unknown or Variable Composition, Complex Reaction Products and Biological Materials: UVCB Substances (March 29, 1995a); available from http://www.epa.gov/oppt/newchems/pubs/uvcb.txt

Toxic Substances Control Act Inventory Representation for Certain Chemical Substances containing Varying Carbon Chain Lengths (Alkyl Ranges Using the Cx-y Notation) (March 29, 1995b); available from: http://www.epa.gov/oppt/newchems/pubs/alkyl-rg.txt

TERA (2008) "Peer Consultation on Relationship Between PAC Profile and Toxicity of Petroleum Substances" (API Report), http://www.tera.org/peer/API/APIWelcome.htm, accessed 28 Oct 2009 and "Report of the Peer Consultation on Relationship between PAC Profile and Toxicity of Petroleum Substances Volume I" (TERA peer review) http://www.tera.org/peer/API/PAC MEETING REPORT Final.pdf, accessed 28 Oct 2009

United Nations (2011). Globally Harmonized System of Classification and Labelling of Chemicals (GHS). Fourth revised edition. http://www.unece.org/trans/danger/publi/ghs/ghs_rev04/04files_e.html

U. S. EPA (2011). Screening Level Hazard Characterization of High Production Volume Chemicals; Waxes and Related Materials Category. http://www.epa.gov/chemrtk/hpvis/hazchar/Category_Waxes%20and%20Related%20Materials_September_2011.pdf

van Wezel AP, Opperhuizen A. (1995). Narcosis due to environmental pollutants in aquatic organisms: residue-based toxicity, mechanisms, and membrane burdens. Critical Rev Toxicol. 25(3):255-279.

Verbruggen EMJ, Vaes WHJ, Parkerton TH, and Hermens JLM. (2000). Polyacrylate-coated SPME fibers as a tool to simulate body residues and target concentrations of complex organic mixtures for estimation of baseline toxicity. Environ Sci Technol. 34:324-331.

WIL (WIL Research Laboratories Inc.). 1995. An oral reproduction/developmental toxicity screening study of **** in finished oil in rats. WIL Laboratory Study No. WIL-187007. Ashland, Ohio.

Excerpt from Alpha Olefins SIAR, for 11th SIAM, January 2001

4.1 Acute/Prolonged Toxicity to Fish

Test substance: Blend of three suppliers' 1-tetradecene, 99% purity

Type:	Semistatic
Species:	Oncorhynchus mykiss
Exposure Period:	96 hour
Analyt. Monitoring:	No
Method:	OECD Guideline 203
GLP:	Yes
Test Results:	LC50 >1000 mg/L (author assigned)
	LL0 = 1000 mg/L (EPA reviewed)

Comment: Water-accommodated fractions (WAFs) were prepared by adding the appropriate amount of 1-tetradecene to dilution water on a weight-volume basis. The WAFs were mixed for 24 hour inside a covered glass vessel using a magnetic stirrer. After the mixing period, the mixture was allowed to settle for one hour before the water phase containing the WAF was siphoned off to use. Test solutions were renewed daily using freshly prepared WAFs.

The range finding test used test concentrations of WAFs from 10, 100, and 1000 mg test article per liter, and five fish per chamber. No deaths were seen during the range finding test.

A definitive limit test was then conducted using 7 fish per chamber and two replicates each in the control and treatment (WAF from 1000 mg/L) groups. No deaths or abnormal signs were noted at any time point in the control or treated groups. The 96-hour LC50 was thus greater than WAF from 1000 mg test article/liter.

LL0 = lethal loading based on the WAF testing procedure, no mortality observed at the highest loading indicated.

Reference:

Drottar, L.R., and Swigert, J.P., "1-Tetradecene: A Water-Accommodated Fraction 96-hour Semistatic Acute Toxicity Test with the Rainbow Trout (Oncorhyncus mykiss)". Wildlife International Ltd., Easton, Maryland 1995b. Chemical Manufacturers Association, Alpha Olefins Panel, Sponsor