Response to EPA's Hazard Characterization of the Naphtha Hydrocarbon Wastes from Petroleum Refining Category

The American Petroleum Institute Petroleum HPV Testing Group June 17, 2013

The following comments are in response to EPA's Hazard Characterization (HC) for the Naphtha Hydrocarbon Wastes from Petroleum Refining (U.S. EPA, 2011). This Category (Naphtha Wastes) 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 (HCs) they have prepared, including Naphtha Hydrocarbon Wastes. The Testing Group's comments are found on the page numbers indicated below.

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Summary

1. The EPA hazard characterization for several Petroleum HPV Categories including Naphtha Wastes, refers to the category members as complex mixtures when in fact they are Class 2 UVCB substances. (HC pages 4, 5, 7, and Tables 3, 4)

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: 68956-48-9

CAS Name: Fuel oil, residual, wastewater skimmings

<u>CAS Definition</u>: The recovered oil obtained by skimming all plant oil-bearing water streams. It consists predominantly of hydrocarbons having carbon numbers predominantly in the range of C7 through C10.

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 Naphtha Hydrocarbon Wastes Category are UVCB substances, not mixtures, under EPA's nomenclature guidance.

Justification for Supporting Chemicals

EPA appropriately includes data on "gasoline" to support the HC for the Naphtha Wastes Category. (HC pages 1, 4, 5, 22, 23, 24, 25, 26, 29, 30, 31, 33 and Table 2) However, EPA mis-identifies this "gasoline" as CAS 8006-61-9 which is "Gasoline, natural, which is actually; A complex combination of hydrocarbons separated from natural gas by processes such as refrigeration or absorption. It consists predominantly of saturated aliphatic hydrocarbons having carbon numbers predominantly in the range of C4 through C8 and boiling in the range of approximately minus 20.degree.C to 120.degree.C (-4.degree.F to 248.degree.F). While the substance "Gasoline, natural" could be used to blend commercial automotive gasoline, it is will not meet the EPA specifications for automotive gasoline by itself. In the USA, automotive gasoline is a mixture without a CAS identification number. In Europe, automotive gasoline is considered a substance with the CAS identification number, 86290-81-5.

Carcinogenicity

EPA cites the results of a chronic inhalation study with wholly-vaporized gasoline (PS-6) in rats as positive, causing increases incidence of renal carcinomas, sarcomas and adenomas in males. (HC page 31) But EPA does not point out that several years ago EPA found that these

kidney lesions were species and sex specific and not relevant to humans (EPA, 1991). Furthermore, real-world inhalation exposure to gasoline is weighted towards the more volatile constituents making it markedly different from the wholly-vaporized material.

Neurotoxicity

EPA points out the lack of neurological effects found in most animal studies done by inhalation exposure and the Testing Group agrees with that assessment. The one animal study EPA considered "positive" for neurotoxicity was a 13-week study on Naphtha (petroleum), light catalytic reformed (LCRN), conducted at doses up to 27.8 mg/l. (HC page 32) However, the increased motor activity was only observed at the high-dose and was not accompanied by changes in functional operational battery tests or neuropathology. For perspective, the Globally Harmonized System for Hazard Classification and Labelling of Chemicals (United Nations, 2011) requires target organ labeling for repeat-dose effects only when they occur at vapor concentrations below 1 mg/l. The high dose in the LCRN study (27.8 mg/l) represents 75% of the lower-explosive-limit, a concentration well above most human exposures, and far above accepted cut-offs for hazard classification.

EPA says that the neurotoxicity of gasoline is well-established in humans and that information on the human neurotoxicity of gasoline may be found in the ATSDR Toxicological Profile for Automotive Gasoline. (HC page 32) However, that ATSDR document says, "The majority of the data on the neurological effects of gasoline have come from case reports describing patients, usually adolescents, who were chronic gasoline sniffers (Owens et al. 1985). In most instances, the exposure concentrations could not be determined and the lead content in the gasoline was not specified." The Testing Group agrees that chronic gasoline sniffing is harmful to several target organs including the nervous system, but EPA's HC significantly overstates the neurotoxic hazard of gasoline and gasoline blending streams and therefore naphtha hydrocarbon wastes as well.

4. Hazard to the Environment

The Testing Group disagrees with the approach that EPA used to evaluate the ecotoxicity hazards of the Naphtha Hydrocarbon Wastes Category (also see our comments on the Gasoline Blending Streams Category). The endpoint values cited by EPA for read across to all category members were calculated based on measured concentrations of selected hydrocarbons used to verify the presence of hydrocarbons in the water accommodated fractions (WAFs). The Testing Group maintains that toxicity endpoints are more accurately expressed as 'loading rates'. Below is a summary of the EPA findings (HC page 4).

EPA states, "Based on the category member CASRN 64741-66-8, the 96-h LC50 for fish is 0.31 mg/L (lowest value) and the 48-h EC50 value to aquatic invertebrates is 0.56 mg/L (lowest value). Based on the category member CASRN 64741-46-4, the 96-h EC50 for algae is 0.26 mg/L (lowest value) for biomass. Based on the category member CASRN 64741-66-8, the lowest values for the chronic 21-d EC50 are 1.9 mg/L (based on survival), and 0.14 mg/L (based on reproduction), and the lowest 21-d chronic NOEC values are 0.23 mg/L (based on survival), and 0.03 mg/L (based on reproduction)."

In Section 4, Hazard to the Environment, EPA cites the following tests and endpoints in their HC, all calculated based on measured concentrations of selected hydrocarbons (HC page 36).

Acute Toxicity to Fish (fathead minnow: Pimephales promelas)

Paraffinic Naphthas		
Naphtha (petroleum), light alkylate (CASRN 64741-66-8) 96-h LC50 = 0.305 mg/L		
Naphtha (petroleum), light alkylate (CASRN 64741-66-8) 14-d LC50 = 0.15 mg/L		
Olefinic Naphthas		
Naphtha (petroleum), light catalytic cracked (CASRN 64741-55-5) 96-h LC50 = 4.1 mg/L		
Naphtha (petroleum), light catalytic cracked (CASRN 64741-55-5) 14-d LC50 = 1.5 mg/L		
Naphthenic Naphthas		
Naphtha (petroleum), light straight-run (CASRN 64741-46-4) 96-h LC50 = 0.689 mg/L		
Aromatic Naphthas		
Naphtha (petroleum), light catalytic reformed (CASRN 64741-63-5) 96-h LC50 = 11 mg/L		
Naphtha (petroleum), light catalytic reformed (CASRN 64741-63-5) 14-d LC50 = 0.67		
, , , , , , , , , , , , , , , , , , , ,		
mg/L		
Acuta Tavisituta Asuatia lavartahyataa (Olada aanan Dankuia maana)		
Acute Toxicity to Aquatic Invertebrates (Cladoceran: Daphnia magna)		
Paraffinic Naphthas		
Naphtha (petroleum), light alkylate (CASRN 64741-66-8) 48-h LC50 = 0.556 mg/L		
Olefinic Naphthas		
Naphtha (petroleum), light catalytic cracked (CASRN 64741-55-5) 48-h LC50 = 1.4 mg/L		
Naphthenic Naphthas		
Naphtha (petroleum), light straight-run (CASRN 64741-46-4) 48-h LC50 = 0.65 mg/L		
Aromatic Naphthas		
Naphtha (petroleum), light catalytic reformed (CASRN 64741-63-5) 48-h LC50 = 2.6 mg/L		
Toxicity to Aquatic Plants (Alga: Pseudokirchneriella subcapitata)		
Paraffinic Naphthas		
Naphtha (petroleum), light alkylate (CASRN 64741-66-8) 96-h LC50 = 0.741 mg/L		
Olefinic Naphthas		
Naphtha (petroleum), light catalytic cracked (CASRN 64741-55-5) 96-h LC50 = 4.6 mg/L		
Naphthenic Naphthas		
Naphtha (petroleum), light straight-run (CASRN 64741-46-4) 96-h LC50 = 0.26 mg/L		
Aromatic Naphthas		
Naphtha (petroleum), light catalytic reformed (CASRN 64741-63-5) 96-h LC50 =1.76		
mg/L		
111g/ L		
Chronic Toxicity to Aquatic Invertebrates (Cladoceran: Daphnia magna)		
Paraffinic Naphthas		
Naphtha (petroleum), light alkylate (CASRN 64741-66-8) 21-d EC50 (survival) = >0.46 mg/L		
21-d NOEC (survival) = 0.23 mg/L		
21-d EC50 (reproduction) = 0.14 mg/L		
21-d NOEC (reproduction) = 0.032 mg/L		
Olefinic Naphthas		
Naphtha (petroleum), light catalytic cracked (CASRN 64741-55-5) 21-d EC50 (survival) =1.9		
mg/L		
21-d NOEC (survival) = 0.68 mg/L		
21-d EC50 (reproduction) = 0.55 mg/L		
21-d NOEC (reproduction) = 0.11 mg/L		
Aromatic Naphthas		
Naphtha (petroleum), light catalytic reformed (CASRN 64741-63-5) 21-d EC50 (survival)		
= 7.5 mg/L		

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21-d NOEC (survival) = 3.8 mg/L
21-d EC50 (reproduction) = 3.2 mg/L
21-d NOEC (reproduction) = <0.069 mg/L
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As stated earlier, the endpoint values cited by EPA for read across to all category members were calculated based on measured concentrations of selected hydrocarbons used to verify the presence of hydrocarbons in the water accommodated fractions (WAFs). The Testing Group maintains that when toxicity endpoints are more accurately expressed as 'loading rates', the products in the Naphtha Hydrocarbon Wastes Category are expected to exhibit aquatic toxicity in the range from 1 to 200 mg/L for the three trophic levels. Loading is a reflection of the composition and chemistry of the substance and implicitly accounts for dissolution and volatilization of individual hydrocarbon constituents. Loading is a more effective means of comparing two substances to each other because the composition of WAFs varies with composition of the test substance.

Naphtha Hydrocarbon Wastes and Gasoline Blending Streams show a relatively narrow range of aquatic toxicity because the constituent chemicals of those products are neutral organic hydrocarbons 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 behavior 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 between 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).

Petroleum UVCB substances with a range of carbon numbers and water solubility as those in this category are expected to exhibit lower toxicity compared to the most toxic constituent alone. This occurs because the aqueous concentration of the constituent is a function of the partitioning of the constituents between the bulk hydrocarbon and water. Within the carbon number range of products in this category, a C9 hydrocarbon alone would be expected to exhibit the greatest toxicity based on the relationship of Kow with aquatic toxicity. However, products in this category are not composed of a single chemical and because two different products with a similar carbon number range can contain varying proportions of those carbon numbers, it is possible that different toxicities are expressed for the same organism. Thus, two products representing low or high carbon number ranges in this category can show different toxicities.

The Testing Group believes it is inappropriate for EPA to use these concentration values when they represent only an undetermined fraction of the dissolved hydrocarbons in the test solutions. Therefore, these 'toxic' concentrations represent lower acute toxicity values than what would have been calculated based on a complete analysis of the dissolved hydrocarbon composition. Additionally, the specific compounds of interest were different depending on the test substance, so the compounds measured in the light alkylate naphtha WAF differ from those measured in the light catalytic cracked naphtha WAF, as was true for each test substance. The following example In Table 1 shows the analyses of hydrocarbon constituents in the light straight-run naphtha (CASRN 64741-46-4) and the components selected for concentration determination of

the WAFs. This illustrates how the endpoints of the toxicity tests based on measured data are artificially low.

Table 1. List of hydrocarbon constituents in CASRN 64741-46-4.

Compound	Weight % in Test Substance
butane	5.6
n-pentane	16.3
n-heptane	2.0
n-hexane	6.3
n-octane	0.8
n-nonane	0.1
isopentane	22.5
2,3 dimethyl butane	1.1
2 methyl pentane	7.0
3 methyl pentane	3.7
2,3 dimethyl pentane	0.8
2 methyl hexane	0.8
3 methyl hexane	1.4
2 methyl heptane	1.0
3 methyl heptane	0.5
4 methyl heptane	0.2
cyclopentane	1.7
methyl cyclopentane	4.6
dimethyl cyclopentane isomers	4.1
ethyl cyclopentane	0.5
trimethylcyclopentane isomers	2.4
methyl ethyl cyclopentane (3 iso	
cyclohexane	1.7
methyl cyclohexane	2.5
dimethylcyclohexane isomers	1.6
trimethylcyclohexane isomers	0.9
benzene	0.8
toluene	1.1
m-xylene	0.3
p-xylene	0.1
o-xylene	0.2
ethylbenzene	0.3

The yellow highlighted compounds were those measured in the WAFs.



Sum of individual measurements (yellow highlight) does not reflect the concentration of all possible hydrocarbons dissolved in the WAFs.

Many of the most abundant constituents in the straight run naphtha shown in the example were not included in the analysis of the WAFs (e.g.,

n-pentane, iso-pentane, and others). These would be expected to contribute to the total dissolved hydrocarbons in the WAF because many have significant water solubilities and exist in significant proportions in the naphtha stream. Consequently the measured hydrocarbons cited in these studies do not represent the total hydrocarbon components to which the organisms were exposed, and the toxicological endpoints calculated on the basis of those measurements are artificially low.

The more appropriate and realistic evaluation of petroleum mixture aquatic toxicity is determined using composition of the petroleum product and QSAR based models, such as Petrotox (Redman et al. 2012). Toxicity QSARs may be used to provide LL50 estimates for hydrocarbons or blocks where acute toxicity data are not available, since these are well established for hydrocarbons (details are included in the EU Technical Guidance Document (TGD) which recommends procedures for risk assessment). Furthermore, the use of QSAR allows for extrapolation of LL50 values to hydrocarbons or blocks that are beyond the solubility 'cut-off' and have no measured LC50 value. This provides a conservative approach for assessing the partial contribution of hydrocarbons or blocks that are individually not expected to exert toxicity.

The Petrotox spreadsheet model predicts the aquatic toxicity of complex petroleum substances from petroleum substance composition. Substance composition is characterized by specifying mass fractions in constituent hydrocarbon blocks (HBs), based on available analytical information. The HBs are defined by their mass fractions within a defined carbon number range or boiling point interval. Physicochemical properties of the HBs are approximated by assigning representative hydrocarbons from a database of individual hydrocarbons with associated physicochemical properties. A three-phase fate model is used to simulate the distribution of each structure among the water-, air-, and oil-phase liquid in the laboratory test system. Toxicity is then computed based on the predicted aqueous concentrations and aquatic toxicity of each structure and the target lipid model. The toxicity of the complex substance is computed assuming additivity of the contribution of the individual assigned hydrocarbons. This is valid since hydrocarbons are equitoxic in tissues.

There are two situations when it may be necessary to estimate the toxicity of a petroleum-substance viz., to validate test results and to predict toxicity when data are lacking. This approach requires that the chemical composition of the petroleum substance should be known. In this procedure, the dissolved concentrations of individual hydrocarbons from a petroleum substance are estimated for a given loading rate and then normalized by their acute toxicity to yield Toxic Units (TU) which can be summed to predict the toxicity of the parent material (see below). As previously described, the quantity of any particular component of a petroleum substance detected in the water phase is related to the loading rate. Theoretically, using closed test systems brought to equilibrium, simple equilibrium partitioning and mass balance calculations may be used to estimate the concentration of each hydrocarbon constituent in water. The hydrocarbon/water partition coefficient (Kp) for each of the components is an essential part of the calculation. The details of this calculation approach have been published (Peterson 1994).

Further simplification is obtained by combining the concentration calculations for isomers of particular hydrocarbon species (e.g. iso-hexanes), since all of the isomers have essentially the same values of log Kow and Kp. This procedure is the equivalent of the "hydrocarbon block method" used in the risk assessment of petroleum substances (CONCAWE 1996; Hermens et al. 1985). Experimental Kp values simply related to Kow (Peterson 1994; Cline et al. 1991), for individual hydrocarbons are available in the published literature. In order to calculate the joint toxic action of a mixture of hydrocarbons dissolved in water, the concentrations cannot be added directly. Since each component will have a different toxicity, the concentration of each component must be scaled to its toxicity. This is done by division of the concentration by the toxicity (by the LL50 in the case of acute toxicity). The resulting values express the concentrations in equivalent "toxic units."

Thus, the sum of TUs for the components of a mixture will equal one at the LL50 of the mixture. Considerable experimental support for this conceptual framework has been developed, which confirms that mixtures of substances exerting toxicity via a common mechanism, are additive and further, that hydrocarbons act through a common mechanism of non-polar narcosis (Hermens et al. 1985; Deneer et al. 1988).

Given the compositional analysis (together with consideration of the variability of composition of the particular petroleum substance), acute toxicity can be calculated. This toxicity calculation is conservative in that it assumes that each component is maximally dissolved (completely equilibrated with un-dissolved phase) and that there are no losses from solution (due to adsorption to surfaces, absorption to test organisms or volatilization, etc.). While the model is built on these assumptions, variability in test methods and laboratory procedures will result in discrepancies between empirical and modeled endpoints. For gasoline blending streams, tests were conducted in sealed test vessels with zero headspace. In practice, WAF preparations cannot totally meet the expectations of the model. Thus, headspace above the vortex in the WAF preparation vessel and the method of distribution of the WAFs to the test vessels would allow some volatile loss.

Using the composition for CAS 64741-46-4 reported above, daphnid toxicity was calculated and results are shown below. The calculated lethal loading of 15 mg/L is similar to the actual LL50 determined for this stream as reported in the Testing Group's robust summary. However, the total dissolved concentration of hydrocarbons in the water phase (Figure 2) is approximately 5.08 mg/L, based on partitioning behavior and test conditions, which is significantly higher concentration than the 0.65 mg/L reported by EPA. See Appendix A for additional Petrotox analyses which compares predicted dissolved hydrocarbon concentration corresponding to the empirical EL/LL 50 values. Finally, EC50/LC50 values will not remain constant for untested streams identified by the same CAS number unless the new stream compositions are identical to those already evaluated. Since the composition of these petroleum streams may vary due to crude oil source and specific site refinery processes even for the same CAS number, it is unlikely that the hydrocarbon constituent's concentration will remain constant for process streams.

In summary, the rationale presented in the above response supports the Testing Group's position that when toxicity endpoints are presented on the basis of loading rates, the products in the Naphtha Hydrocarbon Wastes Category are expected to exhibit acute aquatic toxicity in the range from 1 to 200 mg/L for the three trophic levels. Loading is a reflection of the composition and chemistry of the substance and is the more accurate manner in which to present the aquatic toxicity of petroleum substances.

Consortium #1100997

Figure 1 PETROTOX Input Product Name: CAS 64741-46-4 naphtha, light straight-run
Date: 8/28/2012
PROTECTION CONCAWE Target S :Total Number of Loadings 0 **Target Species Selection Menu** Product Effect POC or Loading Data Daphnia magna $\overline{}$ INTERCEPT Test Conditions (mq/L water) (%) Volume of Water (L): Volume of Air (L): ID 15 SPECIES mol/goctanol) Daphnia magna Total Sustem Volume Calculate End Bioavailability Mode Point No bioavailability correction. Calculate Dose Set Input Resolution 28 :Total Number of Hydrocarbon Blocks Total Weight Percent: 88.99 Ending Starting Carbon Number n-P i-P n-CC5 n-CC6 Di-N n-Olefinsi-Olefins Poly-N NMAr i-N AIS MoAr DiAr **NDiAr** | Weight X| (weight X) 6.00 7.00 8.00 9.00 7.00 8.00 9.00 10.00

Figure 2 PETROTOX Output Product Name: CAS 64741-46-4 naphtha, light straight-run Report of Individual Loads Effect Data: Date: 28-Aug-12 Product Loading Model TU 0.99 No free product at this loading. Species: Daphnia magna Acute TUs Load (mg/L water): Toxic Units: 15.00 0.99 Volume of free product (µI): Test Condition Volume of water (L): Volume of headspace (L): Copy Input/Output Avg PNEC Avg PNEC Avg PNEC Mono-Avg PNEC Hydrocarbo Average log Cooled Molecular Henry's Law Product Water n,i-Paraffins Naphthenic Di-

Phase

0.0E+00 0.0E+00

0.0E+00

 Log(unitless)
 (mg/L product) ng/L headspace
 (ug/L water)

 0.94
 0.0E+00
 6.5E+01
 2.0E+03

 0.77
 0.0E+00
 3.8E+01
 2.0E+03

 1.36
 0.0E+00
 1.8E+01
 8.1E+02

Air Phase

1.8E+01 1.0E+01

Phase

(ug/L water) 0.0E+00

0.0E+00

0.0E+00 0.0E+00

0.0E+00

0.0E+00 0.0E+00

0.0E+00 0.0E+00

(ug/L water) 0.0E+00

2.1E+03 7.7E+02

(ug/L water) 1.0E+03

3.3E+02

1.1E+02 3.6E+01

Constant

1.36 1.60

Weight

83.49

98.41 112.68

128.26

(mg/L) 1.81E+02

9.20E+01

1.42E+01 3.31E+00

3.07

3.81 4.39

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Also in Section 4, Hazard to the Environment, EPA cites studies employing dilutions of water soluble fractions. (HC pages 128, 120). EPA cites the following two studies (one fish and one invertebrate) that used dilutions of a water soluble fraction as exposure solutions.

Solvent naphtha (petroleum), light arom. (CASRN 64742-95-6) 96-h LC50 = 1.03 mg/L

Solvent naphtha (petroleum), light arom. (CASRN 64742-95-6) 48-h EC50 = 1.2 mg/L

Results for petroleum UVCBs (multi-constituent, poorly soluble hydrocarbons) are expressed as lethal loadings (LL) rather than lethal concentrations (LC). Additionally, an inherent limitation of the dilution method is that the concentration of dissolved hydrocarbons in each successive dilution cannot be related back to a known amount of petroleum substance required to produce the observed effect. This is because the amount and composition of hydrocarbons in the dissolved phase is dependent upon the composition of the petroleum substance, temperature, and principally on the ratio of the volume of water to oil that comes in contact (Shiu et al. 1990). These factors led to the concept of 'loading rate' that is used to describe the total amount of petroleum product per unit volume of water used to prepare the WAFs. 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 substances in this category because they are petroleum UVCBs (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);
- separate exposure solutions are prepared at each exposure loading for products that are multi- constituent hydrocarbons; and

When compared on the basis of standard test methods and exposure solution preparation procedures, these gasoline blending streams 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).

References used in this response to EPA's HC of the Naphtha Hydrocarbon Wastes Category

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

Benson, J; Gigliotti, A; March, T; Barr, E; Tibbetts, B; Skipper, B; Clark, C; and Twerdok, L. (2011). Chronic Carcinogenicity Study of Gasoline Vapor Condensate (GVC) and GVC Containing Methyl Tertiary-Butyl Ether in F344 Rats. Journal of Toxicology and Environmental Health Part A, Volume 74, Number 10, pp. 638-657(20) DOI: http://dx.doi.org/10.1080/15287394.2011.538837

Cline PV, et al. (1991). Partitioning of aromatic constituents into water from gasoline and other complex solvent mixtures. Environ Sci Technol 25:914-920.

CONCAWE. (1996). Environmental risk assessment of petroleum substances: the hydrocarbon block method. Report No. 96/52. Brussels, Belgium.

Deneer JW, et al. (1988). The joint toxicity to *Daphnia magna* of industrial organic chemicals at low concentrations. Aquat Toxicol 12:33-38.

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

Hemptinne et al. (1998). Aqueous solubility of hydrocarbon mixtures. Revue de L'Institut Français du Petrole. 53(4):409-419.

Hermens JLM, et al. (1985). Quantitative structure-activity relationships in aquatic toxicity studies of chemicals and complex mixtures. Environ Toxicol Chem 4:273-279.

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.

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

Peterson DR. (1994). Calculating the aquatic toxicity of hydrocarbon mixtures. Chemosphere 29:2493-2506.

Redman AD, Parkerton TF, McGrath JA, Di Toro DM. (2012). PETROTOX: An aquatic toxicity model for petroleum substances. Environ Toxicol Chem. Aug 7. doi: 10.1002/etc.1982.)

Shiu, WY, Bobra M, Bobra AM, Maijanen A, Suntio L, and Mackay D. (1990). The water solubility of crude oils and petroleum products. Oil Chem Pollut. 7:57-84.

U.S. Environmental Protection Agency (2011). Screening level hazard characterization: Gasoline blending streams category. December 2011.

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

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. Environmental Protection Agency (1991). Alpha 2 microglobulin: association with chemically induced renal toxicity and neoplasia in the male rat. In Risk Assessment Forum. US Government Printing Office, Washington, DC: EPA: 85.

U.S. EPA (2011). Screening Level Hazard Characterization of High Production Volume Chemicals; Reclaimed Petroleum Hydrocarbons: Naphtha Hydrocarbon Wastes from Petroleum Refining Category.

http://www.epa.gov/chemrtk/hpvis/hazchar/Category Naphtha%20Hydrocarbon%20Wastes March 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.