WEIGHT OF EVIDENCE APPROACH TO ASSESS THE ACUTE AQUATIC TOXICITY OF GTL SOLVENTS RELATIVE TO OTHER HYDROCARBON SOLVENTS

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INTRODUCTION

- GTL ("gas-to-liquids") solvents are a category of hydrocarbon solvents produced from natural gas using the Fischer-Tropsch process. They typically contain a complex mix of linear and simply branched paraffins, with low levels of naphthenics and virtually no aromatics.
- Aquatic toxicity of hydrocarbons occurs by a non-specific narcosis mode of action. This forms a basis on which aquatic toxicity data may be read-across from one hydrocarbon solvent to another. This read-across can be further justified by the use of screening techniques.
- Screening techniques include both experimental and computational methods, and can be used to inform decision-making, or to build a weight of evidence to assess a particular endpoint in the absence of guideline experimental data.
- A range of screening methods have been used to assess the acute aquatic toxicity of GTL solvents relative to other types of hydrocarbon solvents. Results were compared with compositional information and available data from OECD guideline tests.

MATERIALS AND METHODS

- A range of samples from each of the categories; GTL solvents, de-aromatised solvents, isoparaffins and white spirits were selected for evaluation of aquatic toxicity (Table 1).
- These substances are examples of complex UVCBs and are identified by their Hydrocarbon Solvents Producers Association (HSPA) chemical names.
- Screening methods included testing of water-accommodated fractions (WAFs) using solid-phase micro-extraction (SPME) combined with gas chromatography (GC) analysis, MICROTOX™ and DAPHTOXKIT F™ assays, and toxicity predictions using the PETROTOX model. Further details on screening methods are provided below.
- Available GLP experimental data from guideline toxicity tests on fish (OECD 203; *O. mykiss*), daphnia (OECD 202; *D. magna*) and algae (OECD 201; *P. subcapitata*) have been collated and compared to screening data. In all cases test solutions were prepared as WAFs and results expressed as nominal loading rates according to standard practice.

DETAILS OF SCREENING METHODS

SPME-GC

- 19.5 g of a 100 mg/L loading rate WAF was added to a Wheaton vial and the soluble hydrocarbon components extracted onto a SPME fibre (Supelco, 30 µm polydimethylsiloxane film coating) over a period of 10 minutes. The fibre was then analysed using GC-FID and the total peak area calculated.

Microtox™

- The Microtox™ system is a biosensor-based measurement system for determining the toxicity of a water sample using changes in light emission of *Vibrio fischeri*, a bioluminescent photobacteria that reduces or ceases light emission in the presence of toxic compounds. A 2.5 mL sample of 100 mg/L loading rate WAF was used and EC_{50} values calculated using the MicrotoxOmni software, based on four serial dilutions at 5 and 15 minute suspension times. The EC_{50} values represent the concentration of WAF, as a percentage, which produced a 50% inhibition in bacterial light emission.

DAPHTOXKIT F™

- DAPHTOXKIT F™ employs a static test system using the ephippia of *Daphnia magna* that can be hatched on demand without the requirement to maintain a culture. The system was adapted to use sealed glass test vessels to minimise any reduction in test concentration as a result of volatilisation and adsorption. Ten daphnia were added to each vessel containing 100 mL of test solution and a small amount of headspace. A series of four individual loading rates (1, 10, 100, 1000 mg/L) were tested. Each vessel was incubated for 48 hours in the dark, and immobilisation recorded at 24 and 48 hours.

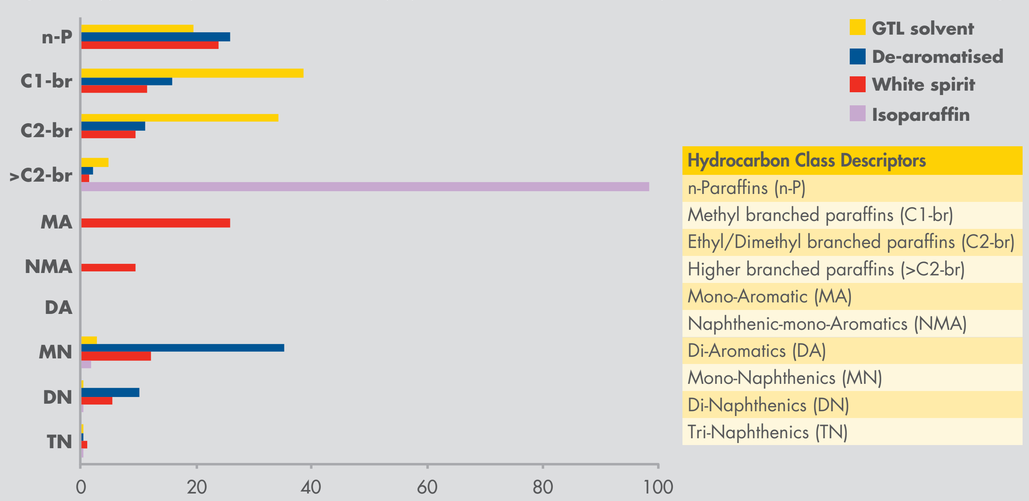
PETROTOX

- PETROTOX is a spreadsheet model for predicting the aquatic toxicity of complex petroleum substances based on substance compositional information (Redman, 2012). Acute EL50 values for *Oncorhynchus mykiss*, *Daphnia magna* and *Pseudokirchneriella subcapitata* were calculated using substance GCxGC compositional data and model default values for headspace and bioavailability correction.

Table 1: Overview of products tested

		HSPA name (as registered, if applicable)	Sample ID
CONVENTIONAL	WS	Hydrocarbons, C9-C12, n-alkanes, isoalkanes, cyclics, aromatics (2-25%)	A0912
		Hydrocarbons, C10-C13, n-alkanes, isoalkanes, cyclics, aromatics (2-25%)	A1013a
		Hydrocarbons, C10-C13, n-alkanes, isoalkanes, cyclics, aromatics (2-25%)	A1013b
	Iso.	Hydrocarbons, C10-C12, isoalkanes, <2% aromatics	I1012
		Hydrocarbons, C11-C12, isoalkanes, <2% aromatics	I1112
	De-aromatised	Hydrocarbons, C9-C10, n-alkanes, isoalkanes, cyclics, <2% aromatics	D0910
		Hydrocarbons, C9-C11, n-alkanes, isoalkanes, cyclics, <2% aromatics	D0911
		Hydrocarbons, C10-C13, n-alkanes, isoalkanes, cyclics, <2% aromatics	D1013
		Hydrocarbons, C11-C14, n-alkanes, isoalkanes, cyclics, <2% aromatics	D1114
		Hydrocarbons, C12-C15, n-alkanes, isoalkanes, cyclics, < 2% aromatics	D1215
GTL		Hydrocarbons, C8-C11, n-alkanes, isoalkanes, <2% aromatics	G0811
		Hydrocarbons, C9-C12, n-alkanes, isoalkanes, <2% aromatic	G0912
		Hydrocarbons, C10-C13, n-alkanes, isoalkanes, <2% aromatics	G1013
		Hydrocarbons, C12-C15, n-alkanes, isoalkanes, <2% aromatics	G1215
		Hydrocarbons, C14-C16, n-alkanes, isoalkanes, <2% aromatics	G1416
		Hydrocarbons, C15-C19, n-alkanes, isoalkanes, <2% aromatics	G1519
		Hydrocarbons, C18-C24, isoalkanes, <2% aromatics	G1824

Figure 1: Typical compositional breakdown by hydrocarbon class of different hydrocarbon solvents in the C10-C13 range



Hydrocarbon Class Descriptors
n-Paraffins (n-P)
Methyl branched paraffins (C1-br)
Ethyl/Dimethyl branched paraffins (C2-br)
Higher branched paraffins (>C2-br)
Mono-Aromatic (MA)
Naphthenic-mono-Aromatics (NMA)
Di-Aromatics (DA)
Mono-Naphthenics (MN)
Di-Naphthenics (DN)
Tri-Naphthenics (TN)

RESULTS AND DISCUSSION

- Data from the experimental screening approaches (SPME-GC, Microtox™ and DAPHTOXKIT F™) are presented in Table 2. Acute aquatic toxicity data from guideline studies as well as those predicted using the PETROTOX model are summarised in Table 3.
- There is reasonable agreement between the toxicity data from guideline studies and the screening approaches, with all screening approaches correctly predicting the white spirits to be the most toxic substances and indicating a lack of toxicity in substances with higher carbon chain lengths due to their very low water solubility. The white spirits exhibit greater toxicity due to their aromatic content, which contributes more to the overall toxicity than other hydrocarbon classes.
- SPME-GC data indicate a much greater response in the white spirits than in other substances, which is indicative of the higher concentration of hydrocarbons present in these WAFs. De-aromatised, isoparaffinic and GTL solvents have similar responses at comparable carbon ranges which gradually reduce to baseline levels at higher carbon number ranges, indicating that very little material is present in these solutions. This suggests that toxicity of these substances is more comparable.
- Microtox™ results are in agreement with SPME-GC data, but appear to be less sensitive. Apart from the white spirits, only the lightest de-aromatised solvent exhibited toxic effects, which is in line with the results from guideline tests.
- DAPHTOXKIT F™ showed a similar relationship with carbon number, although in some cases the results were more or less sensitive than in guideline studies. For example, effects were seen in the lightest GTL solvent grade which were not reproduced in the guideline test, and effects in the lightest de-aromatised solvent were much less severe than in the guideline test. In a previous sensitivity comparison with 30 substances, a strong correlation ($R^2 = 0.971$) was found between results of DAPHTOXKIT F™ and OECD 202 guideline tests (Ulm et al., 2000).
- PETROTOX calculations predicted a similar relationship with hydrocarbon chain length, although results were generally much more conservative than experimental data. Also toxicity was predicted to occur in higher chain length solvents than was observed experimentally.
- In the absence of guideline acute aquatic toxicity data for higher carbon chain GTL solvents, the data generated using screening approaches provide a robust weight of evidence of an overall lack of toxicity, and support read-across of data for non-aromatic containing solvents to support endpoint requirements such as those under REACH.

Table 2: Results of experimental screening methods

		Sample ID	SPME-GC	Microtox™ EC50	DAPHTOXKIT F™
			Total Peak Area[1]	15 mins	EL50 (mg/L)
CONVENTIONAL	WS	A0912	$3.6x10^7$	11.98%	10-100
		A1013a	$3.3x10^7$	12.62%	10-100
		A1013b	$1.5x10^7$	16.21%	N.D.
	Iso.	I1012	$1.3x10^6$	>100%	N.D.
		I1112	$9.2x10^5$	>100%	N.D.
	De-aromatised	D0910	$4.5x10^6$	34.26%	1000
		D0911	$3.5x10^6$	>100%	>1000
		D1013	$2.3x10^6$	>100%	N.D.
		D1114	$7.8x10^5$	>100%	N.D.
		D1215	$8.5x10^5$	>100%	N.D.
GTL		G0811	$4.3x10^6$	>100%	N.D.
		G0912	$2.5x10^6$	>100%	>1000
		G1013	$8.3x10^5$	>100%	N.D.
		G1215	$4.5x10^5$	>100%	N.D.
		G1416	$5.0x10^5$	>100%	N.D.
		G1519	$4.6x10^5$	>100%	N.D.
		G1824	$4.6x10^5$	>100%	N.D.

N.D. = Not determined [1] Distilled water blank: ~$4.0x10^5$

Figure 2: Comparison of SPME-GC total peak area and percentage toxicity obtained via Microtox™

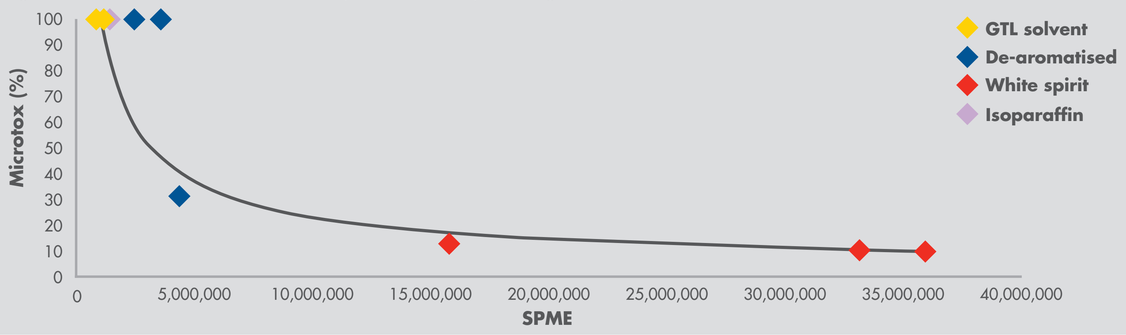


Table 3: Results from guideline experimental tests and PETROTOX acute toxicity predictions

		Sample ID	Experimental acute EL50 (mg/L)			PETROTOX acute EL50 (mg/L)		
			O. mykiss	*D. magna*	*P. subcapitata*	*O. mykiss*	*D. magna*	*P. subcapitata*
CONVENTIONAL	WS	A0912	10-30	10-22	4.6-10	0.58	1.01	0.80
		A1013a	10-100	100-200	10-100	0.56	3.91	1.43
		A1013b	10-100	100-200	10-100	0.53	3.42	1.50
	Iso.	I1012	>1000	>1000	>1000	>1000	>1000	>1000
		I1112	N.D.	N.D.	N.D.	>1000	>1000	>1000
	De-aromatised	D0910	10-30	22-46	>1000	1.03	1.79	0.99
		D0911	>1000	>1000	>1000	0.65	1.14	0.83
		D1013	>1000	>1000	>1000	0.66	>1000	>1000
		D1114	>1000	>1000	>1000	>1000	>1000	>1000
		D1215	N.D.	N.D.	N.D.	>1000	>1000	>1000
GTL		G0811	N.D.	>100	>100	0.94	1.64	1.36
		G0912	N.D.	>180	>391	0.83	>1000	17.72
		G1013	N.D.	N.D.	N.D.	>1000	>1000	>1000
		G1215	N.D.	N.D.	N.D.	>1000	>1000	>1000
		G1416	N.D.	N.D.	N.D.	>1000	>1000	>1000
		G1519	N.D.	N.D.	N.D.	>1000	>1000	>1000
		G1824	N.D.	N.D.	N.D.	>1000	>1000	>1000

N.D. = Not determined

CONCLUSIONS

- Screening approaches can form powerful and cost-effective tools to assess substance properties as part of an integrated testing strategy or weight of evidence approach.
- The techniques presented have been demonstrated to act as good indicators for aquatic toxicity of substances with a non-polar narcotic mode of action. Further work could be performed to validate their applicability for other modes of action.
- The data presented support that GTL solvents are of low acute toxicity to aquatic organisms and permit these endpoints to be assessed in a robust manner, without the need for further testing on animals.

- Redman AD, Parkerton TF, McGrath JA and DiToro DM (2012) "PETROTOX: an aquatic toxicity model for petroleum substances", Environ. Toxicol. Chem. 31(11), 2498-2506
- Ulm L, Vrzina J, Schiesl V, Puntaric D and Smit Z (2000) "Sensitivity comparison of the conventional acute *Daphnia magna* immobilization test with the Daphtoxkit F™ microbiotest for household products", Proceedings of the International Symposium on New Microbiotests for Routine Toxicity Screening and Biomonitoring, held June 1-3, 1998 in Brno, Czech Republic, published in 2000 by Kluwer Academic/Plenum Publishers, New York, ISBN: 0-306-46406-3