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Aquatic toxicity of petroleum substances: Extending the validation of the biomimetic extraction (BE) method for use in hazard assessments

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Aquatic toxicity of petroleum substances : Extending the validation of the biomimetic extraction (BE) method for use in hazard assessments.

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ABSTRACT

Analysis of Water Accommodated Fractions (WAFs) of petroleum product samples using Biomimetic Extraction (BE) with solid phase microextraction (SPME) predicts accumulation and baseline toxicity of chemicals with a narcotic mode of action. Recent BE-SPME screening of WAFs of a large set of petroleum product samples (e.g. naphthas, kerosines, heavy fuel oils, cracked gas oils and other gas oils), supported by high quality compositional data, has been carried out. Compositional data have been used to predict toxicity values in toxic units (TU) and accumulation in target lipid (C_{TL}) from the TU data. These data confirm that BE-SPME data correlate to accumulation in target lipid (C_{TL}) and as such provide additional support to the use of BE-SPME as a practical screening tool for assessing aquatic toxicity of petroleum substances and addressing the influence of variation in substance composition on aquatic toxicity within petroleum product categories.

KEYWORDS

SPME, petroleum hydrocarbons, UVCB, ecotoxicity, screening

INTERNET

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SUMMARY

Earlier studies have employed a biomimetic extraction procedure based on solid phase microextraction (BE-SPME) as an analytical screening method to estimate the aquatic toxicity of complex petroleum substances. This procedure has the potential to simplify aquatic hazard assessments of petroleum substances since the total moles of all hydrocarbons adsorbed to the SPME fibre can be related to toxic thresholds in target lipid of aquatic organisms. BE-SPME data collected on aqueous media dosed with a wide range of petroleum substances or from toxicity studies have been shown to be highly correlated to toxic units (TU) derived using the PETROTOX model. This study formed the technical basis for using BE-SPME to assess bioavailability and predict toxicity of petroleum substances.

A further study has been carried out by Concawe on BE-SPME screening of test media (i.e. water accommodated fractions) of a large set of petroleum product samples from their 2015 Analytical Programme. These more recent BE-SPME data are distinctly different from the previous data sets and are supported by high quality compositional data on the products (either DHA-GC (for naphthas) or two-dimensional gas chromatography (for the other substances)). Only those samples that had been evaluated using both BE-SPME and PETROTOX (based on specific analysis of each sample) were used in the correlations. Compositional data have been used to derive toxicity values in toxic units (TU) based on a product loading of 50 mg/L water, which was equivalent to the loading used for the BE-SPME screening study, and accumulation in target lipid (C_{TL}) based on TU data. These data confirm that BE-SPME data correlate both to Toxic Units and accumulation in target lipid (C_{TL}) and as such provides additional support to the use of BE-SPME as a more practical characterisation tool for addressing the influence of variation in substance composition on aquatic toxicity within petroleum product categories. BE-SPME is therefore shown to be a cost-effective approach to toxicity screening for petroleum substances, thereby offering an alternative method to enhance the current available ecotoxicity data sets by applying read-across within a category to fill data gaps and where appropriate reduce unnecessary testing, particularly on animal species.

1. INTRODUCTION

In earlier studies a biomimetic extraction procedure employing solid phase microextraction (BE-SPME) has been employed as an analytical screening method to estimate the aquatic toxicity of complex petroleum substances [1-3].The solid phase microextraction fibres coated with polydimethylsiloxane (PDMS) provide a convenient sampling format to characterise the bioavailability of petroleum substances.

A recent extensive study of petroleum substances [4] has detailed the technical basis for using passive sampling as a biomimetic extraction procedure to assess bioavailability and predict toxicity of petroleum substances. Hydrocarbons absorb onto the PDMS coating of the SPME fibres in proportion to both freely dissolved concentrations and partitioning properties of the individual constituents, which parallels the mechanistic basis used to predict aquatic toxicity in the PETROTOX model [5]. When deployed in a non-depletive manner, combining SPME with thermal desorption and quantification using gas chromatography with flame ionisation detection creates a biomimetic extraction procedure (BE-SPME) that has the potential to simplify aquatic hazard assessments of petroleum substances since the total moles of all hydrocarbons absorbed to the fibre can be related to toxic thresholds in target lipid of aquatic organisms. BE-SPME data collected on samples of aquaeous media dosed with a wide range of petroleum substances were highly correlated to predicted toxic units derived using the PETROTOX model.

A previous study by Concawe of ecotoxicity screening and predictions together with experimental and tests was performed on a limited number of representative samples of petroleum substances (PS), i.e. gas oil, bitumen and residual aromatic extract (RAE) samples [6]. A biomimetic extraction technique employing solid phase micro-extraction (BE-SPME) and the PETROTOX model based on two-dimensional chromatography (GCxGC) analysis of the hydrocarbon products were applied in this study, as well as OECD test methods using a water accommodated fraction (WAF) approach in sealed vessels. PETROTOX predictions were used to provide information to support revised category justification documents and enable the selection of 'worst case' products in each category for ecotoxicity testing. In addition, analysis of water accommodated fractions (WAFs) of these product samples using BE-SPME fibres was used to confirm that the SPME data correlates to toxic units predicted by the PETROTOX model from GCxGC data, thereby strengthening the linkage between composition, SPME data and aquatic toxicity. Consequently, BE-SPME can be used in a more practical characterisation tool for addressing the influence of variation in substance composition on aquatic toxicity within petroleum product categories. Comparison of predicted toxicity ranking showed that SPME results complement PETROTOX predictions (i.e. highly bioavailable samples show high predicted toxicity in *Daphnia magna*), thereby supporting SPME as a mechanistic surrogate measure of bioavailability. Predicted PETROTOX EL50 values also showed a positive correlation with experimental data and were comparatively more conservative than experimental equivalents, this supporting the model's applicability as a predictive tool for petroleum based UVCB hazard assessment.

A further, much more extensive, study has recently been carried out by Concawe on BE-SPME screening of a further larger set of petroleum product samples. Based on the compositional data for the various products determined by either VOC analysis (for naphthas) or two-dimensional gas chromatography (for the other substances), the toxicity values in toxic units (TU) have been calculated for these products at a product loading of 50 mg/L water, which was equivalent to the loading used for the BE-SPME screening study. Correlations between respective BE-SPME data and accumulation in target lipid (C_{TL}) data for these samples have been undertaken and are provided in this report.

2. EXPERIMENTAL

The bioavailable petroleum hydrocarbons in the water accommodated fractions (WAFs) of many of these test substances were measured using a biomimetic extraction technique employing solid-phase microextraction (BE-SPME) (see **Section 2.2.2**). The compositional data for the substances were used to compute TU and C_{TL} values for comparison with previously generated BE-SPME data (see **Section 2.2.3**).

2.1. SAMPLE COLLECTION AND ANALYSIS

Analytical Programme

Following discussions with the European Chemicals Agency (ECHA), Concawe carried out an Analytical Programme (AP) in 2013 which involved the chemical characterisation of 30 Petroleum Substances (PS) from 5 substance categories (e.g. straight run gas oils (SRGO), other gas oils (OGO), vacuum hydrocracked gas oils (VHGO), residual aromatic extracts (RAE) and bitumens) for which testing proposals were being submitted.

Following on from the 2013 AP, Concawe decided to extend the chemical characterisation of substances in the SRGO, OGO, VHGO, RAE and Bitumen categories to the remaining 15 PS categories. For completeness, 19 sub-samples of SRGO, OGO, VHGO and RAE samples from the 2013 AP were transferred to the 2015 AP to provide a comprehensive sample set covering all active registered PS in the 20 substance categories. Originally large samples (20 litres) of these substances were taken and to avoid sending the whole volume, sub-samples were taken.

As with the 2013 AP, samples from the 2015 AP were analysed using both the standard industry methods which Concawe recommended to member companies for characterising their PS [7-8] and by a range of other procedures to provide supplementary information on the substances. The suite of analytical procedures employed is summarised below:

First Phase

- **SIMDIS-GC**- carried out on all substances to provide information on the boiling and carbon number ranges of the components present.
- **Physical distillation** - carried out on LBPNs and Kerosines to provide information on the boiling range of these substances.

Second Phase

- **GCxGC** - carried out on most substances other than LBPNs to provide quantitative information on the types of chemical functionalities present for each carbon number. GCxGC is a powerful technique for the detailed characterization of complex hydrocarbon mixtures. Individual constituents are separated based on both their relative volatility (~25 individual hydrocarbon numbers) and polarity (15 different chemical functionalities). GCxGC can provide accurate quantitative information on >300 hydrocarbon groups and individual constituents present in middle distillate fuels with a detection limit of 0.01% (m/m). Although GCxGC is best suited to the analysis of middle distillate substances (i.e. C8-C30 range) this technique has been applied to a wide range of PS in the 2015 AP including CGO, HFO, Kerosine, LBO, MK1 diesel fuel, Paraffin and Hydrocarbon wax, UATO, TDAE, UDAE, Foots Oil, Petrolatum, Slack wax, HRBO, SRGO, OGO, VHGO, Oxidized asphalt and some heavy LBPN samples.

- **DHA-GC** (Detailed hydrocarbon analysis gas chromatography) - carried out on most LBPNs to provide qualitative and quantitative information on the individual components present.
- **PIONA-GC** (Paraffin, Iso-paraffin, Olefin, Naphthene, Aromatic gas chromatography) - carried out on most LBPNs to provide quantitative information on these chemical functionalities present for each carbon number.
- **PAH** (Detailed poly-aromatic hydrocarbon analysis by high resolution gas chromatography-mass spectrometry) - carried out on all substances other than LBPNs (except for some heavy LBPNs) to provide information on the quantities of EPA and Grimmer PAHs present.
- **PAC-2** (Poly-cyclic aromatic carbon analysis by DMSO extraction and gas chromatography-mass spectrometry) - carried out on all substances other than LBPNs (except for some heavy LBPNs) to provide information on the total quantities of 1, 2, 3, 4, 5, 6, 7+ ring PACs present.
- **Elemental analysis** (C, H, N, O, S, As, Cd, Co, Cu, Fe, Mo, Ni, P, Pb, V, Zn, Cl, Hg, F) - carried out on all substances to provide information on the major elements and specific minor elements present.
- **FIMS** (Field ionisation mass spectrometry) - carried out on LBOs and HRBO to provide quantitative information on the types of chemical functionalities present for each carbon number.
- **Spectroscopic analysis** - carried out on all substances to provide UV, IR, ^{1}H-NMR and ^{13}C-NMR spectra.
- **PCA** (Poly-cyclic aromatics analysis by DMSO extraction and gravimetric determination) - carried out on LBOs and HRBO to provide quantitative information on the total quantity of 3+ ring PCAs present.
- **HPLC** - carried out on SRGOs, OGOs, VHGOs, CGOs, Kerosines, some heavy LBPNs, some light LBOs and MK1 Diesel Fuel to provide quantitative information on the total quantities of mono-, di- and tri+ aromatics present.
- **TLC-FID** - carried out on Bitumens, Oxidised Asphalt, HFOs, Paraffin and Hydrocarbon Waxes, Foots Oils, Petrolatums, Slack Waxes, UATOs, TDAE, UDAEs, RAEs and VHGO to provide quantitative information on the basic chemical functionalities (saturates, aromatics, resins, asphaltenes) present.
- **LCC** (Liquid column chromatography) - carried out on Bitumen, Oxidised Asphalt, HFOs, Paraffin and Hydrocarbon Waxes, Foots Oils, LBOs, HRBO, Petrolatums, Slack Waxes, UATOs, TDAE, UDAEs, RAEs, OGOs and VHGO to provide quantitative information on the basic chemical functionalities (saturates, aromatics, polars) present.
- **Viscosity measurement** - carried out on all substances (LBOs, UATOs, HFOs, VHGOs) for which a viscosity statement is included in the formal substance description.

Further details of the analytical methodologies applied and the results obtained on samples from the 2015 Analytical Programme are reported elsewhere [(9)].

2.2. TEST METHODS

2.2.1. Test media generation

Poorly water soluble, complex chemical mixtures such as UVCB petrochemical products pose specific challenges when preparing aqueous solutions for toxicity testing. With soluble chemicals, the amount of chemical dissolved in water is varied in increments to produce a range of toxicities, from which a “dose-response” relationship and associated median lethal/effective concentration (L(C)50) may be derived. With poorly soluble complex chemicals, un-dissolved material appears as

soon as the least soluble component reaches water saturation. Thereafter, the relative composition of the water phase varies in a non-linear fashion from the “neat” substance composition [10]. This is in contrast to pure substances, where concentration will (if sufficient time is provided) equal the solubility limit when added in excess, regardless of the amount of excess. For poorly water soluble, complex chemicals, it has become standard practice to test toxicity at substances additions far in excess of the amount that will dissolve, resulting in a two-phase system.

There are many procedures for establishing and maintaining equilibrium between water and un-dissolved substance [11]. One recognised guideline [12] involves stirring various amounts (loadings) of poorly water-soluble test substance and water for a sufficient time to reach equilibrium, followed by separation of the water phase (“water accommodated fraction” or “WAF”). Toxicity testing of WAFs generated by this procedure allows determination of the amount of substance equilibrated with water that will cause 50% mortality/effect. This endpoint is termed L(C)L50 (lethal/effective loading) to distinguish it from L(E)C50 [13] and is also the approach specified by MARPOL for marine pollution testing of poorly soluble mixtures [14] and the OECD for aquatic toxicity testing of difficult substances and mixtures [15].

Testing hydrocarbon liquids is further complicated by their volatility, particularly from aqueous solution. Although environmentally unrealistic, it is necessary to prevent volatilisation of the substance to maintain constant concentrations and thereby determine its inherent toxicity. This necessitates using closed test systems. In preparing WAFs, some headspace in the test vessel is required to allow adequate interfacial area and mixing. In each test, measured amounts of test substance are added to measured volumes of the appropriate test medium. The vessels containing the medium and test substance are then sealed, leaving only a small headspace, and stirred for a period of time sufficient for the aqueous and test substance phases to equilibrate. After stirring, vessel contents are left to stand for one hour to facilitate phase separation. The aqueous phases – the “WAFs” – are then drawn off for use in tests via a stopcock at the bottom of the vessel for siphoning off the WAF without contaminating this with the undissolved surface layer, and system stirring is resumed once sampling has been completed. Control media undergo the same regime, but do not contain the test substance. It is important that mixing is sufficient to ensure the aqueous phase is in equilibrium with the un-dissolved hydrocarbon phase. Mixing must be slow enough not to cause dispersion or emulsification of the undissolved hydrocarbon, yet vigorous and long enough to attain equilibrium. In current studies, mixing was performed with magnetic stirring bars set to develop a vortex ≤10% of the static water depth.

WAFs were prepared for each test substance at a single loading of 50 mg/L by adding the appropriate amount of the test substance to the dilution water in a 4L aspirator bottle [16]. The vessels were sealed with Teflon© screw plugs. The solutions were mixed with Teflon©-coated stir bars on magnetic stir plates. Control systems were also prepared in parallel, which consisted of moderately hard water with no test substance added. The vortex was set at ≤10% of the static liquid depth. The solutions were mixed for 48 ± 2 hours at a target room temperature of 22°C ± 2°C. At the end of mixing, the solutions were allowed to settle for 1 hour ± 30 minutes. Following the settling period, aliquots of the solutions were removed from the mixing vessels through the outlet at the bottom of the vessels. Replicates were collected in ca. 20 mL clear glass vials with no headspace and sealed with Teflon© septa caps. Sufficient replicates were collected from each test system to permit duplicate analysis from each WAF and retain additional samples. Samples not analysed on the day of collection were refrigerated. Room temperature was monitored using a laboratory monitoring system and these ranged from 18.0°C to 21.9°C. It is considered unlikely that this slight deviation in room temperature from the target range would have any significant impact on the hydrocarbon solubility and hence the analytical results.

In this study, the measurement of bioavailable petroleum hydrocarbons in water accommodated fractions (WAFs) of the test substances was carried out using a biomimetic extraction technique employing solid phase microextraction (BE-SPME).

2.2.2. Screening studies using biomimetic extraction

The bioavailable petroleum hydrocarbons in the water accommodated fractions (WAFs) of many of these test substances were measured using a biomimetic extraction technique employing solid-phase microextraction (BE-SPME) [16].

Automated BE-SPME analysis was performed on a Perkin Elmer Autosystem gas chromatograph with flame ionisation detector (GC-FID). The GC was equipped with a 15 m x 0.53 mm id capillary column with 1.5 µm Rtx-1 stationary phase (Restek) or equivalent and a LEAP Technologies (CTC Analytics) Combi PAL autosampler configured for automated SPME injections. Individual WAF samples taken in ca. 20 mL vials were extracted with a 30 µm PDMS (0.132 µL PDMS) SPME fibre (Supelco) for 100 minutes at 30°C with orbital agitation at 250 rpm prior to injection and thermal desorption of the fibre. Duplicate samples were analysed from each test sample WAF for each of the test substances.

The BE method was calibrated by making 0.5 µL liquid (solvent) injections of a series of aromatic hydrocarbon standard solutions. The average molar response factor of 2,3-dimethylnaphthalene was used for converting the measured GC-FID response (total integrated area) to nanomoles of organic constituents on the PDMS fibre. BE-SPME results were normalised to the volume of PDMS and reported as micromoles (µmol) as 2,3-dimethylnaphthalene/millilitre (mL) PDMS.

Where necessary, SPME sample chromatograms were digitally background corrected by subtraction with a blank GC chromatographic run, to account for column bleed. Chromatograms were acquired and processed using Perkin Elmer TotalChrom chromatographic software. Integration parameters were optimised specifically for each sample type to integrate the area under the curve attributable to the SPME extracted sample.

2.2.3. PETROTOX toxicity modelling

Concawe has developed a general purpose spreadsheet-based model (PETROTOX) to predict the ecotoxicity of the Petroleum Substances under different test conditions [5]. The model provides a computational tool that relates the composition of complex petroleum substances to toxicity prediction and provides comparable predictions with experimental toxicity results from standardised tests. Detailed quantitative compositional information on hydrocarbon groups and components obtained from GCxGC is normally used to define and populate the hydrocarbon blocks. A three phase model is used to simulate the distribution of each hydrocarbon structure among the water-, air-, and oil-phase liquid in a laboratory test system. Toxicity is then computed based on the predicted aqueous concentrations and aquatic toxicity of each structure using the target lipid model[17]. The toxicity of the complex substance is computed assuming additivity of the contribution of the individually assigned hydrocarbons.

In this study PETROTOX has been used in a high resolution model (v3.06) based on a number of hydrocarbon classes and carbon blocks. The reported mass distribution

is mapped to library compounds, (i.e. individual representative hydrocarbons in a Concawe database) which are assigned physical-chemical properties from SPARC. Mass weighted structures are then used in solubility and toxicity calculations.

Compositional data for naphthas were provided by DHA-GC of VOCs and composition data for other substances were determined by two-dimensional gas chromatography.

The structuring requirement for PETROTOX was:

(1) % weight composition of the MonoN was distributed equally between n-CC5, n-CC6 and i-N by carbon number, and

(2) % weight composition of the TriAr, NTriAr were added to the % weight of the PolyAr class by carbon number

For the PETROTOX calculations [18], the GCxGC compositional data did not add up to 100% composition for several HFOs and naphtha substances. This was resolved with the PETROTOX model. For the HFOs, the unresolved mass was added to the non-toxic C30 paraffin, whereas for the naphthas, the unresolved mass was scaled up to 100%.

The volume of headspace in the model was set to 10%. The cut-off used for log K_{ow} was kept at the default value of 6. Calculations were performed for *Daphnia magna* using a critical target lipid body burden (CTLBB) of 116 µmol/g lipid and the bioavailability correction for POC, at a concentration of 0.05 mg POC/L. The PETROTOX model was run twice for each substance:

(1) Dose response calculation – to calculate the toxic unit for a product loading of 50 mg/l water (equivalent to the loading used for the BE-SPME screening)

(2) End point calculation – to calculate the corresponding acute medial lethal loading (LL50) endpoint of the substance.

The comprehensive sample set used covers all active registered PS in the 20 substance categories. This sample set, identified by the individual Concawe sample numbers, was used to prepare WAFs for each test substance at a single loading of 50 mg/L for use in the BE-SPME screening study. For many of these samples individual analytical data (DHA-GC for naphthas or GCxGC for other test substances) were used to compute Toxic Units or LL50 data based on the PETROTOX model. For some samples in a substance category (e.g. bitumen, HFO, LBO, paraffin wax, RAE) that were not analysed by GCxGC, the TU calculation was based on an average GCxGC profile for substances in that category. For this report, only those samples that had been evaluated using both biomimetic extraction (BE-SPME) and PETROTOX based on specific analysis of each sample were used in the correlations. TU data based on an average GCxGC profile were excluded from any of the correlations.

2.2.4. Evaluation of BE test data

The BE-SPME test data were evaluated by initially comparing BE-SPME measurements on the range of substances at a 50 mg/L loading to the predicted toxicity as toxic units (TU) using PETROTOX [5].

The PETROTOX model first calculates the profile of dissolved constituents based on the detailed substance composition and tested loading. The predicted dissolved concentrations are then converted to toxic units by normalization to the inherent effect concentrations (e.g., LC50) for each constituent, *i* (Eqn 1).

$$TU = \sum C_{W,i} / LC50_i \quad (1)$$

Effects are predicted using the TLM for acute endpoints (e.g., LC50) and chronic effects (e.g., EC10) using the median of compiled acute to chronic ratios [18].

$$LC50_i = k_{TL,i} * C^*_{TL} \quad (2)$$

where accumulation in target lipid (e.g., the assumed site of action) is modelled using the lipid-water partition coefficient (k_{TL}) for a given constituent, *i*. The TUs scale according to the critical target lipid body burden (µmol/g lipid, C^*_{TL}), which represents the sensitivity of the test species. The sum of the individual TUs represents the overall toxicity for a given exposure (i.e. loading) to a given substance. Assuming strict additive toxicity of hydrocarbons, the loading that is predicted to cause a 50% response (i.e. LL50 or EL50) corresponds to a predicted total TU=1.

TUs are a reflection of the collective accumulation of different petroleum hydrocarbons in the target lipid. The BE measurements and TUs reflect the dissolved phase exposure and are expected to correlate. In order to maintain a common basis for comparison with BE data, the predicted TUs were based on a critical target lipid body burden (CTLBB) of a median sensitivity organism, 116 µmol/g lipid [4]. The resulting TUs were converted to target lipid concentrations by combining Eqn 1 and 2, i.e. multiplying the sum TU by the median C^*_{TL}. This step was performed to facilitate comparison between PETROTOX predictions and passive sampling measurements since predicted target lipid concentrations are intuitively more directly comparable to BE-based PDMS concentrations than TUs. The more realistic evaluation was a comparison of the BE-SPME data with the predicted target lipid concentrations C^*_{TL}.

2.3. DATA AVAILABILITY

Individual BE-SPME screening data and TU (and subsequently C_{TL}) calculations using the PETROTOX model based on analytical characterisation data for the 13 categories of samples tested from the 2015 Analytical Programme have been reported [16,18]. Only those samples (n = 103) that had been evaluated using both BE-SPME and PETROTOX (based on specific analysis of each sample) were used in the correlations (see **Table 1** for summary). TU/C_{TL} data that were based on an average GCxGC profile or had no positive reading (i.e. 0) were excluded from the correlations. Similarly, BE-SPME data that were below the detection limit of 0.5 µmol/mL were excluded from the correlations.

Table 1 – Availability of BE-SPME and PETROTOX derived TU and C_{TL} data for samples within each category

Category	Total number of data points (for each category)	Number of quantifiable data points (for each category)
Naphthas	51	51
HFO	22	19
LBO	19	2
GO (includes OGO, SRGO and VHGO)	13	13
CGO	8	8
Kerosines	7	7
UDAE	3	1
UATO	2	1
TDAE	1	0
MK1	1	1
Total	127	103

3. RESULTS

3.1. SUMMARY OF BE-SPME SCREENING DATA AND PETROTOX (C_{TL}) PREDICTIONS FOR CONCAWE SAMPLES

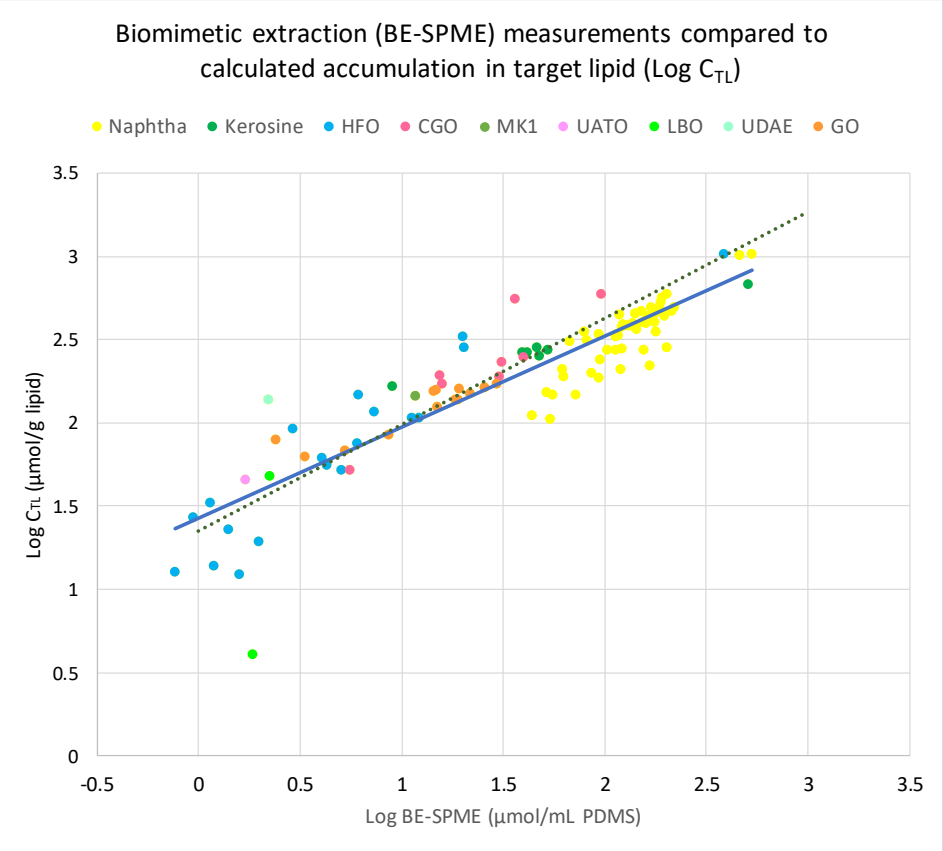
The individual BE-SPME screening data and TU (and also C_{TL}) predictions using PETROTOX based on analytical characterisation data for the 13 categories of samples tested from the 2015 Analytical Programme have been reported [16,18]. A compilation of these data is shown in Appendix (**Table S1**). Only those samples that had been evaluated using both BE-SPME and PETROTOX (based on specific analysis of each sample) were used in the correlations. TU (and hence C_{TL}) data based on an average GCxGC profile or had no positive reading (i.e. 0) were excluded from any of the correlations. Similarly, BE-SPME data that were below the detection limit of 0.5 µmol/mL were excluded from the correlations.

3.2. CORRELATIONS

The $\log_{10}$BE-SPME v $\log_{10}$ C_{TL} plot based on all the PS sample data detailed in **Table S1,** shows that there is a reasonably linear correlation between the two sets of data apart from at low (i.e. ND) BE-SPME values (**Figure 1**). The quantitation limit of the BE-SPME method is 0.5 µmol/mL PDMS. Comparison of BE-SPME measurements and predicted C_{TL} includes 127 data points consisting of 104 quantifiable measurements (from 9 categories) and 23 measurements below the detection limit. The slope (0.548) and intercept (1.426) of the correlation calculated in this study agreed with a comparable study by Redman et al., (2018) [4] (**Figure 1** and **Table 2**) indicating that the method is both robust and reproducible.

The colour coding of data points in **Figure 1** illustrates the results of the two approaches for the different substance categories. As can be seen, there is reasonable clustering of data points for individual categories, with categories representing lighter petroleum substances having higher BE-SPME and predicted C_{TL} than heavier categories. An exception to the clustering of categories appears to be the heavy fuel oils (HFO), which are known to be a particularly broad category owing to the nature of how they are produced and used. The gas oils (GO) show reasonable overlap with the cracked gas oils (CGO), but with the CGO category showing greater toxicity overall. This is in line with expectations and experimental aquatic toxicity data for these categories.

Figure 1 Log_{10}BE-SPME v $log_{10}C_{TL}$ correlation for all PS samples. The solid line indicates regression calculated by this study and dotted line indicates regression calculated from study by Redman et al. (2018) [4]. Colour coding indicates the substance category.



Interestingly, the data for the naphtha category appears to deviate below the overall correlation slope (**Figure 1**). This appears to be due to differential partitioning of hydrocarbons between PDMS and lipid, as described in Redman et al., (2018) [4]. The propensity to partition to lipid or PDMS is driven by hydrocarbon class and carbon number (**Figure S1**, **Appendix 1**), with preferential partitioning into lipid being driven by hydrocarbons with higher carbon number and greater aromaticity. As naphthas are generally lighter in carbon number and containing predominantly aliphatic and mono-aromatic constituents, compared with heavier categories composed of greater numbers of di- and tri-aromatic constituents, this reasonably explains this observed phenomenon. Naphthas were not included in the dataset of Redman et al., (2018) [4] which may explain the slightly greater slope of the regression in that study. However, the difference is not significant, and the present study provides further evidence to support the use of BE-SPME to assess aquatic toxicity of petroleum substances across a broad range of substances and categories.

Table 2 Correlation data for the BE-SPME v C_{TL} plot for petroleum products (this work) compared with previous published work by Redman et al. (2018) [4].

Category	Number of quantifiable data points	Regression line	Root Mean Squared Error (RMSE)	r^2	Slope Confidence Intervals (95%)	Intercept Confidence Intervals (95%)
This work	103	Log C_{TL} = 0.548 log BE-SPME + 1.426	0.82	0.81	0.5 – 0.6	1.3 – 1.5
Redman et al., 2018	280	Log C_{TL} = 0.64 log BE-SPME + 1.35	0.2	ND	0.6 – 0.7	1.3 - 1.4

ND = Not determined

3.3. CONCLUSIONS

Test media (i.e. WAFs) of petroleum substance test samples taken from the Concawe 2013 and 2015 Analytical Programmes have been analysed using Biomimetic Extraction (BE) with solid phase microextraction (SPME) fibres. These data have been used to confirm that BE-SPME data correlate both to Toxic Units and accumulation in target lipid (C_{TL}) as calculated by the PETROTOX model. This provides additional support to the use of BE-SPME as a more practical characterisation tool for addressing the influence of variation in substance composition on aquatic toxicity within petroleum product categories. BE-SPME thereby offers an alternative method to enhance the current available ecotoxicity data sets [19] by applying read-across within a category to fill data gaps and where appropriate reduce unnecessary testing, particularly on vertebrate species.

4. GLOSSARY

AP	Analytical Programme
BE	Biomimetic Extraction
CGO	Cracked Gas Oils
CTLBB	Critical Target Lipid Body Burden
DHA-GC	Detailed Hydrocarbon Analysis Gas Chromatography
ECHA	European Chemicals Agency
EL50	Loading Rate of Test Substance (in dilution water) which causes adverse effects in 50% of the exposed population
FIMS	Field Ionisation Mass Spectrometry
GCxGC	Two-dimensional gas chromatography
GO	Gas Oils
HFO	Heavy Fuel Oils
HPLC	High performance Liquid Chromatography
HRBO	Highly Refined Base Oils
i-N	Iso-naphthenics
LBO	Lubricant Base Oils
LBPN	Low Boiling Point Naphthas
LCC	Liquid Column Chromatography
LL50	Loading Rate of Test Substance (in dilution water) which causes lethality in 50% of the exposed population
MARPOL	Maritime Pollution
Mg/L	Milligrammes per Litre
MonoN	Mono Naphthenics
n-CC5	Normal C5 cycloalkanes
n-CC6	Normal C6 cycloalkanes
NTriAr	Naphthenic Tri-Aromatics
OECD	Organisation for Economic Co-operation and Development
OGO	Other Gas Oils

PAC-2	Poly Cyclic Aromatic carbon analysis by DMSO extraction and gas chromatography-mass spectrometry
PCA	Poly Cyclic Aromatics
PAH	Polycyclic Aromatic Hydrocarbons
PDMS	Poly Di-Methyl Siloxane
PIONA-GC	Paraffins, Isoparaffins, Olefins, Naphthenes and Aromatics by Gas Chromatography
POC	Particulate Organic Matter
PolyAr	Poly-Aromatics
RAE	Residual Aromatic Extracts
SIMDIS-GC	Simulated Distillation Gas Chromatography
SPME	Solid Phase Micro-Extraction
SRGO	Straight Run Gas Oils
TDAE	Treated Distillate Aromatic Extracts
TLC-FID	Thin Layer Chromatography with Flame Ionisation Detection
TLM	Target Lipid Model
TriAr	Tri-Aromatics
TU	Toxic Unit
UATO	Unrefined Acid Treated Oils
UDAE	Untreated Distillate Aromatic Extracts
UVCB	Substance of Unknown or Variable Composition, Complex Reaction Products and Biological Materials
VHGO	Vacuum gas oils, hydrocracked gas oils and distillate fuels
VOC	Volatile Organic Chemical
WAF	Water Accommodated Fraction

5. APPENDIX 1

Table S1 Compilation of biomimetic screening data and PETROTOX TU and C_{TL} predictions for Concawe samples

Concawe Sample Number	Product Substance Category	BE-SPME (µmol/mL) at 50 mg/L loading	Toxic Units (TU) at 50 mg/L loading	C_{TL} (µmol/g lipid)
1	Naphtha	201	2.469	283.9
3	CGO	31.1	1.99	228.9
6	HFO	1.59	0.107	13.31
8	HFO	19.9	2.831	325.6
9	Naphtha	>529	8.853	1018
11	Kerosine	8.98	1.444	165.6
12	CGO	39.4	2.155	248.4
13	Naphtha	160	3.407	391.8
14	Naphtha	113	2.369	272.1
18	HFO	0.25	0.084	9.66
19	Naphtha	137	3.42	393.3
20	HFO	5.02	0.454	52.21
21	HFO	2.9	0.794	91.31
22	Naphtha	174	3.929	451.8
23	Naphtha	154	2.36	271.4
25	HFO	1.39	0.197	22.66
26	Naphtha	43.8	0.959	110.3
27	Naphtha	67	2.686	308.9
28	HFO	0.944	0.234	26.91
29	Naphtha	122	3.355	385.8
30	Naphtha	92.6	1.614	185.6
31	HFO	1.19	0.119	13.69
33	Naphtha	164	3.796	436.5
34	HFO	6.07	1.285	147.8
35	Naphtha	121	2.41	277.2
37	Naphtha	102	2.375	273.1
39	Naphtha	152	3.511	403.8
41	CGO	5.52	0.445	51.75
42	Naphtha	62.3	1.631	187.6
43	Kerosine	39.2	2.291	263.4
45	Naphtha	53.5	0.91	104.7
46	Naphtha	134	3.301	379.6
47	Naphtha	94.8	2.063	237.2
48	Naphtha	211	4.07	468.1
49	Kerosine	52.3	2.383	274.0
52	Naphtha	61.5	1.818	209.1
53	Naphtha	141	3.897	448.2
54	Naphtha	202	5.165	594.0
55	Naphtha	116	2.914	335.1
56	Naphtha	51.1	1.316	151.3
57	Naphtha	135	3.399	390.9
58	HFO	11.1	0.93	107.0
59	MK1	11.7	1.251	143.9
64	UATO	0.25	0.056	6.44
66	LBO	0.25	0.009	1.035

Concawe Sample Number	Product Substance Category	BE-SPME (µmol/mL) at 50 mg/L loading	Toxic Units (TU) at 50 mg/L loading	C_{TL} (µmol/g lipid)
68	Naphtha	129	3.333	383.3
69	TDAE	0.25	0.001	0.115
70	CGO	15.4	1.671	192.1
71	HFO	1.14	0.286	32.89
72	LBO	0.25	0.001	0.115
74	LBO	0.25	0.25	133.2
75	LBO	0.25	0.063	7.245
76	Naphtha	114	2.958	340.2
77	Naphtha	178	3.06	351.9
78	HFO	4.27	0.482	55.43
79	HFO	1.97	0.167	19.21
80	HFO	12.1	0.934	107.4
81	LBO	0.25	0.003	0.345
82R	UATO	1.69	0.396	2.43
85A	LBO	0.25	0.003	0.345
85B	LBO	0.587	0	0.000
85C	LBO	0.25	0	0.000
86	Kerosine	47.6	2.171	249.6
87	Kerosine	46.2	2.467	284.1
89	UDAE	2.2	1.19	136.9
91	HFO	6.01	0.656	75.44
92	LBO	0.25	0.004	0.000
94	Naphtha	174	3.464	398.4
95	Naphtha	196	3.792	436.1
96B	UDAE	0.25	0.005	0.575
97	HFO	4.04	0.537	61.76
99	Naphtha	93.6	2.948	339.0
100	Naphtha	71.3	1.281	147.3
101	Naphtha	>461	8.833	1016
102	CGO	15.7	1.494	171.4
103	Naphtha	160	3.63	417.5
104R	Kerosine	>508	5.911	679.7
106	CGO	35.9	4.77	548.6
110	Naphtha	119	1.818	209.1
115	LBO	0.573	0	0.000
117	LBO	2.23	0.411	47.27
118	LBO	1.85	0.035	4.025
119	LBO	0.25	0.013	1.495
120	Naphtha	189	4.879	561.1
121	CGO	30.1	1.649	189.8
122	Naphtha	169	4.238	487.4
125	Naphtha	55.1	1.273	146.4
126	Naphtha	217	4.279	492.1
128	Kerosine	41	2.286	263.4
129	HFO	7.26	1.011	116.3
130	CGO	95.8	5.161	593.4
132	Naphtha	186	4.243	488.0
133	Naphtha	80.6	2.737	314.2
134	HFO	20.1	2.438	280.4
141	Naphtha	142	3.148	362.0

Concawe Sample Number	Product Substance Category	BE-SPME (µmol/mL) at 50 mg/L loading	Toxic Units (TU) at 50 mg/L loading	C_{TL} (µmol/g lipid)
142	Naphtha	113	2.833	325.8
143	Naphtha	118	3.879	446.1
144	Naphtha	150	4.055	466.3
146	Naphtha	188	4.583	527.0
147	LBO	0.25	0.009	1.035
148	LBO	0.25	0.007	0.805
150	LBO	0.25	0.004	0.460
151	LBO	0.25	0.008	0.920
153	LBO	0.25	0	0.000
154	LBO	0.926	0	0.000
155	HFO	383	8.862	1019
156	Naphtha	78.7	3.066	352.6
157	Naphtha	85.6	1.718	197.6
158	Naphtha	166	1.908	219.4
163R	Naphtha	120	3.242	372.8
164	HFO	0.25	0.208	23.92
166	HFO	0.763	0.11	12.65
168	GO	8.61	0.73	83.95
169	GO	19	1.38	158.7
171	GO	3.32	0.54	62.10
173	GO	25.4	1.4	161.0
174	GO	21.5	1.29	148.4
175	GO	14.7	1.37	157.6
176	GO	2.38	0.68	78.20
177	GO	18.9	1.2	138.0
178	GO	18.2	1.2	138.0
179	GO	29.1	1.49	171.4
181	GO	14.9	1.08	124.2
182	GO	5.22	0.59	67.85
188	GO	14.2	1.33	153.0
195	UDAE	0.25	0.66	75.90
197	HFO	0.25	0.25	28.75

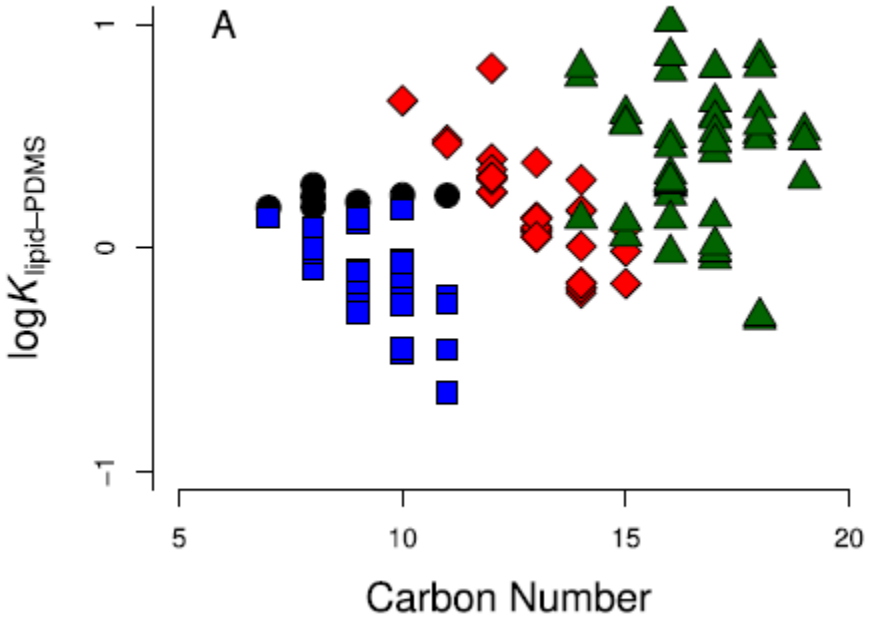


Figure S1 The predicted lipid-fibre partition coefficients by chemical class against carbon number taken from Redman et al (2018) [4]. Blue squares are monoaromatics; black circles are aliphatic; red diamonds are diaromatics; and black triangles are triaromatics.

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