

Challenges in The Regulatory Use of Water Accommodated Fractions for Assessing Complex Substances

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Commentary

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Abstract

The use of the Water Accommodated Fraction (WAF) approach for the preparation of exposure systems of complex substances such as petroleum products has been a standard way to perform aquatic toxicity tests on these substances for over 30 years. In this Commentary we briefly describe the historical development, rationale, and guidance for the use and reporting of the WAF approach to assess complex substances. We then discuss two case studies, with coal tar pitch and kerosene/jet fuel, which illustrate challenges from regulatory authorities in Europe and the United States when using the WAF approach. We describe how the WAF approach is the only currently known method for testing the toxicity of the whole of a complex substance, even when some of its constituents remain unknown; it accounts for differences in the solubility of the constituents within a complex substance; and use of loading rates to describe any toxic effects is a unifying concept that allows direct comparison with releases of readily soluble substances in hazard assessment and chemical classification.

Background

The use of the Water Accommodated Fraction (WAF) approach for the preparation of exposure systems of complex substances such as petroleum products has been a standard way to perform aquatic toxicity tests on these substances for over 30 years (Girling et al. 1992). However, there have been recurrent misinterpretations by various regulatory bodies of the data generated for these types of tests on substances of "Unknown or Variable Composition, Complex reaction products and Biological materials" (UVCBs). In the past, the United States Environmental Protection Agency (USEPA) has also questioned the use of WAF data in the High Production Volume (HPV) Challenge Program (USEPA 2000).

In this Commentary we briefly describe the historical development, rationale, and guidance for the use and reporting of the WAF approach to assess UVCBs. We then discuss two cases in which reported WAF results have been challenged by regulatory agencies: a recently concluded legal case in which European regulatory authorities were found to have misinterpreted results from WAF testing of coal tar, and a separate case in which the USEPA rejected WAF data in their assessment of kerosene/jet fuel. Clarifying the WAF methodology and data interpretation is important because of the very large number of substances that have been classified as UVCBs in both Europe and the United States. For example, Sauer et al. (2019) report that approximately 20% of substances registered under REACH have been declared as UVCBs (ECHA 2017), and approximately 25% of substances listed on the USEPA Toxic Substances Control Act Inventory are identified as UVCBs (USEPA 2015, 2020). Therefore, the proper performance of WAF studies, consistent application of the dosing metric, and appropriate reporting and use of these data are critically important for clear and transparent regulatory processes.

What Is A Water-accommodated Fraction (Waf)?

Girling et al. (1992), CONCAWE (1993), and ECETOC (1996) extensively explain why common procedures followed by standard test methods for measuring aquatic toxicity are difficult to apply to UVCBs such as petroleum products, attributing these challenges mainly to their relative insolubility, high volatility, and multi-constituent nature. These methodological issues have been addressed by the OECD Guidance Document on aquatic toxicity testing of difficult substances and mixtures (OECD GD 23; 2019) by recommending testing approaches which involve assessment of only their "water-soluble fraction" (WSF) or their WAF. A WAF is prepared by gently stirring the test medium with a known loading of the test substance for an amount of time pre-determined to allow saturation (Figure 1). After ceasing stirring and allowing the phases to settle, the droplet-free water fraction is then siphoned or otherwise removed for use as the testing medium. The key references cited above argue that the results of such tests should be expressed as a "loading rate", which is the ratio of test substance to aqueous medium used in preparation of the aquatic toxicity test medium. The loading rate is then expressed as either a Lethal Loading (LL) or an Effective Loading (EL) when reporting toxicity test results for, respectively, lethal or sublethal effects in acute tests. Typically, the No observable effect loading rate (NOELR) and Lowest observable effect loading rate (LOELR) are used for long term chronic studies.

Special preparation techniques are important because the solubility of UVCB substances (or rather the constituents therein) is a complex issue (Birch et al. 2019). Which constituents are dissolved in the water phase, and the proportions of each of these, depends not only on the initial loading but also on interactions amongst the constituents which may affect their solubilities. Therefore, a whole host of processes involving solid-liquid interactions, co-solvent effects, volatility, and degradation require an environmentally relevant and integrated description. In addition, there is the inescapable fact that the composition of UVCBs is variable and often not fully known, so standard approaches to exposure characterisation cannot be reliably applied.

As a result of this, in WAF tests the exposure expression is based on the nominal concentration used to prepare the WAF and not on any measure of the dissolved material that the test organisms are exposed to in the test system. This concept is of particular significance for UVCBs as the exposure expression represents the whole substance and not just the bioavailable fraction that is presented to the test organism. Such an approach is standard in regulatory aquatic toxicity testing (with the highest test concentration being determined by the test substance's solubility in test media, or by a limit level) in order to avoid non-chemical or physical effects of particles that may otherwise overestimate toxicity. The procedure is also supported by the fact that effects that are not inherent to the investigated chemical toxicity are not considered relevant to likely realistic exposures in the environment.

Figure 2 illustrates the process for good practise when preparing WAF test media for aquatic toxicity tests:

1. A) A preparation trial is performed to demonstrate the mixing time required to achieve equilibrium of the dissolved phase (which determines the duration of the stir period). Sealed vessels should be considered to minimise losses if some constituents are known to be volatile. Each treatment level is prepared as an individual WAF and is not serially diluted from a higher loaded WAF. This is because there is no direct correlation between the aqueous partitioning to the test substance: water loading ratio due to the wide range of physicochemical properties of the constituents (Singer et al. 2000). However, serial dilution of one WAF loading rate may be necessary if the WAF is highly toxic and the accuracy to measure small amounts of test material (i.e. lower than 1 mg/L) introduces greater error than serial dilution (OECD GD 23 2019). Efforts are made to standardise mixing energies across individual WAFs, for example by measuring vortex depth. The above steps are intended to maximise the dissolution of components into test media and ensure consistency of

preparation. Chemical analysis (or a surrogate measure such as Total Organic Carbon) is important to determine if acceptable loading of the WAF has been achieved (OECD GD 23; 2019), and for assuring that the ideal preparation time to reach equilibrium is employed in the definitive study.

2. B) The resulting WAF mixture is left to settle for a suitable period to ensure separation of the aqueous phases and/or sedimentation of particles. The test exposure medium is then taken via siphon or other means to exclude the non-dissolved fraction. In some cases, it may be necessary to perform a further separation step (e.g. filtration or centrifugation) to remove any suspended undissolved emulsified components – the resulting solution is then referred to as a Water Soluble Fraction (WSF). In either case, WAF or WSF undissolved material/constituents are removed so that the medium tested is of a different composition to that of the 'whole test substance'.
3. C) Therefore, the exposure concentration in a subsequent aquatic toxicity test represents only the bioavailable dissolved fraction of the test material.

ECETOC (1996) notes that UVCB WAF preparation often produces test solutions with a composition that is quite different to that of the substance itself. This is because when the solubility of different constituents varies the composition of the test medium will depend on the method of preparation. This is for the reasons described above and has more recently been demonstrated in *in vitro* systems in which DMSO extraction of petroleum substances was shown to select for certain polycyclic aromatic compounds, with different dilutions leading to different chemical profiles and bioavailabilities (Luo et al. 2020). Chemical analysis of WAF solutions during toxicity testing can therefore mechanistically aid interpretation of the data. Chemical analysis may also help to i) determine the relationship between the toxic end point and the actual exposure concentrations, ii) assess the stability of the exposure over the test period and, in some cases, iii) identify the active constituent. This is in addition to the importance of analysis to demonstrate that the duration of mixing during preparation of the WAF was sufficient to maximise the concentration of dissolved constituents (see above). However, no current guidance recommends that these measurements of individual constituent concentrations should be used to report toxicity results.

The recommended approach to report the *entire* WAF loading rate rather than analytically determined constituents is supported by guidance for the environmental hazard classification of chemical substances. For example, the United Nations Globally Harmonized System of Classification and Labelling of Chemicals (UNECE 2020) as well as OECD guidance documents on the classification of chemicals which are Hazardous for the Aquatic Environment (OECD GD 27, 2001 and OECD GD 23, 2019) state that the loading level may be used directly in the classification criteria. Under REACH guidance (Chapter R.7b: Endpoint specific guidance (version 4.0; ECHA 2017) test data obtained with WAFs apply to the multi-constituent substance as an entity. The exposure is generally expressed as the 'loading rate' (mass to volume ratio of the substance to medium) used to prepare the WAF, although the measured concentration of test substance in the WAF can also be used. Use of either of these types of measurement in chemical risk assessment is not supported because their comparison with a Predicted Environmental Concentration is for a different entity entirely. This comparison is therefore meaningless except in certain specific applications such as oil spill risk assessment. Instead, constituent-based approaches are more meaningful for chemical risk assessment.

There are alternatives to WAF approaches. In 2000 the Organisation for Economic Cooperation and Development (OECD) first published Guidance Document 23 on aqueous-phase aquatic toxicity testing of difficult to test chemicals (now revised and updated as OECD 2019). OECD GD 23 (2019) outlines several alternative dosing techniques for sparingly soluble substances that could be applied to UVCBs. These include generator systems, saturator columns and passive dosing. Generator and saturator systems may not be ideal for UVCBs since elution of the various constituents may vary over time. Therefore, the eluent will not be a constant reflection of the soluble portion of the test item over time. Passive dosing systems apply a biocompatible polymer pre-loaded with the test substance which acts as a partitioning donor that controls exposure concentrations throughout the test. However, in a section of the Guidance Document on multi-constituent substances (which now includes UVCBs in the updated version) the use of WAF and loading rates is still recommended, and this guidance is also referred to in Guidance Document 27 on classification of chemicals (OECD 2001). OECD (2019) recommends the following process for aquatic toxicity testing of UVCBs:

1. Analytically determine the composition of the UVCB so that its identity can be confirmed and its constituents tracked throughout testing.
2. If a UVCB is only partially soluble in water then prepare a WAF for each individual loading rate, according to a set of described standard methods.
3. Report the results as a median lethal loading (LL50), median effective loading (EL50), or No Observable Effect Loading Rate (NOELR), which are determined following the same statistical methods as would be used to determine LC50, EC50, and NOEC values, respectively.

The simplicity and wide applicability of WAFs and loading rates means they have been used extensively in aquatic toxicity testing, especially for petroleum products, since the seminal Girling et al. (1992) paper. For example WAF testing has been performed on algae (e.g. Chao et al. 2012, Jiang et al. 2010), crustaceans (e.g. Blinova et al. 2016, Asadi 2017), gastropod and bivalve molluscs (e.g. MacFarlane et al. 2004, Solé et al. 2007), and fish (e.g. Blenkinsopp et al. 1997, Perrichon et al. 2016), in both freshwater (e.g. Schein et al. 2009) and salt waters (e.g. Brakstad and Faksness 2000). Singer et al. (2000) describe the extensive work undertaken by the Chemical Response to Oil Spills Ecological Research Forum (CROSERF) to develop standardised approaches for performing aquatic toxicity tests with WAFs, including analytical verification of test concentrations, sealing test vessels to minimise volatilisation, and the need to prepare individual treatment loading rates instead of serially diluting from a stock solution (as above).

In summary, WAF testing of UVCBs and the expression of test results as loading rates has the following advantages:

1. It is the only currently known method for testing the toxicity of the whole of a complex substance, even when some of its constituents remain unknown;
2. It accounts for differences in the solubility of the constituents within a complex substance; and
3. Use of loading rates to describe any toxic effects is a unifying concept that allows direct comparison with releases of readily soluble substances in hazard assessment and classification.

However, despite this understanding of the theoretical advantages, and successful practical use, of WAF testing over three decades, the approach has recently been challenged and frequently dismissed by regulatory authorities. We provide two examples of this below: one for coal tar regulation in Europe and one for kerosene jet fuel regulation in the United States.

EXAMPLE 1: COAL TAR PITCH, HIGH TEMPERATURE (CTPHT)

Coal tar pitch, high temperature (CTPHT; CAS # 65996-93-2, EC number 266-028-2) is a UVCB substance containing polycyclic aromatic hydrocarbons (PAHs). It is mainly used to produce refractories and electrode binders for the aluminium and steel industry, with minor uses as clay targets, coating for corrosion protection, kerosene-resistant airfield applications, road construction, roofing and briquetting (InfoCuria Case Law 2015). CTPHT is produced or imported into the EU at a volume of more than 100 thousand tonnes per year.

Several WAF tests have been performed with CTPHT, as summarised in ECHA's Committee for Risk Assessment (RAC) Opinion (2011). These include different WAF test solution preparation methods (direct addition without filtration, direct addition with supernatant after the solid material was siphoned out of solution, and dilution of the saturated stock) in the absence of ultraviolet irradiation in acute toxicity tests of fish and invertebrates. The direct addition method (with and without filtration) produced CTPHT LC50 values between 100 and 1000 mg/L for all species. The dilution method produced an LC50 of > 1000 mg/L for the fish *Oryzias latipes* (other species were not tested).

WAF toxicity studies with algae (*Desmodesmus subspicatus*) and daphnids (*Daphnia magna*) were also performed (ground material, slow stirred, and settled) in the absence of ultraviolet radiation and with no analytical determination of test media. Both tests produced EC50 values >100 mg/L.

A semi-static chronic study with *D. magna* (using crushed and filtered material pumped through a column at a nominal loading of 100 mg/L). The test solution was analysed for individual PAH concentrations. No significant effects on daphnid mortality and reproduction were observed in this test.

Additional information is available in the opinion paper (2011) on the solubility of pulverised CTPHT. This indicated that the water-soluble fraction of single PAHs was measured at concentrations far below individual water solubilities and was consistent with analytical measurements within the *D. magna* chronic study.

Despite the WAF results described above, in September 2010, a proposal was submitted to the European Chemicals Agency (ECHA) for a classification for CTPHT as Aquatic Acute 1 (H400), and Aquatic Chronic 1 (H410) under the CLP Regulation. This classification was upheld by ECHA's Risk Assessment Committee (RAC 2011). In its consideration of environmental hazards the RAC agreed that CTPHT is a UVCB substance and therefore i) is very difficult to classify on the basis of its individual constituents; ii) not all the constituents can be analysed when diluted in water; iii) different CTPHT constituents influence each other's solubility in the water phase and so the composition in water will not be the same at different loadings. The RAC concluded that the WAF approach is most appropriate for classifying CTPHT, citing OECD guidance described earlier in this Commentary.

However, the RAC also pointed out that all the toxicity data for CTPHT from the WAF studies was obtained in the absence of ultraviolet irradiation, although several PAHs are known to be phototoxic. In addition, they were concerned that chemical analytical data were only measured at a loading of 100 mg/L in a chronic *Daphnia* study and that this prevented comparison between the dissolved PAH concentrations at different loadings and the toxicity data obtained for the individual PAHs. As a result of these concerns an alternative approach for the environmental classification of CTPHT was taken by the Netherlands competent authority and the RAC, in which CTPHT was considered a "mixture", with classification based on the summation of a subset of constituents. The lowest available EC50 or LC50 values for 16 individual polyaromatic hydrocarbons (PAHs) that are also CTPHT constituents were collated and combined with degradability and bioaccumulation data to produce an individual classification for each. Multiplying (M) factors were then used according to the EU CLP (2008) to multiply the weight-based percentage of each constituent in CTPHT according to its toxicity. The contributions of all classifiable Aquatic Acute 1 (H400) substances and Aquatic Chronic 1 (H410) substances were then summed to produce an overall estimate of the contribution of these substances' toxicity to the overall acute or chronic toxicity of CTPHT.

The results of this process for both acute and chronic toxicity are shown in Table 1. The estimated contribution of the individual PAHs to the overall acute toxicity of CTPHT is 14521% and the contribution to overall chronic toxicity is 2169%. These values are considerably higher than the 25% limit in the CLP regulation so RAC (2011) proposed to classify CTPHT as Aquatic Acute 1 and Chronic 1, and this classification was adopted by the European Commission in October 2013.

There are two main issues with the process followed by the European Commission to classify CTPHT as Aquatic Acute 1 and Chronic 1:

1. It was inconsistent with the provisions of REACH and EU CLP (EC 2017) guidance documents regarding WAF test results interpretation and use in support of classification of UVCBs, respectively; and
2. It was inconsistent with the European Commission's own technical guidance on dealing with UVCBs. Indeed, even if the Commission had considered the substance to be a "mixture" and not a UVCB, the tiered classification approach for mixtures would first consider test results for the mixture itself rather than of its constituents.

After 18 companies decided to appeal against the classification of CTPHT as toxic for the aquatic environment the European General Court ruled in October 2015 (InfoCuria Case Law 2015) that the Commission had committed a manifest error of assessment. The Court ruling stated that "*neither the RAC's opinion on CTPHT nor the background document contains any reasoning which demonstrates that, in assuming that all of the PAHs present in that substance dissolve in the water phase and are available to aquatic organisms, account was taken of the low water solubility of CTPHT*" and "*by assuming that all of those PAHs dissolve in water, the Commission therefore, in essence, based the classification in question on the assumption that 9.2% of CTPHT could dissolve in water. However, ... such a value is not realistic, given that the maximum rate is 0.0014%*".

The European Commission immediately appealed this decision but lost that appeal in a judgement in November 2017 (InfoCuria Case Law 2017b) based on legal opinion provided by the Advocate General in September 2017 (InfoCuria Case Law 2017a). Costs were awarded against the Commission at a level finally agreed by the Court in April 2020 (InfoCuria Case Law 2020).

CTPHT has now been classified for the environment in the EU as Chronic 4 (the lowest, “safety net” classification available), which is more consistent with WAF data on its solubility and aquatic toxicity.

Even though regulators acknowledge the applicability of WAF data, there was a reluctance to use the data. This may be related to the complexity of UVCB risk assessment in general. WAF testing of this UVCB was always both the most technically appropriate and regulatorily-compliant method, subject only to the relatively minor technical question of whether such testing should be conducted with or without UV radiation.

EXAMPLE 2: KEROSENE/JET FUEL

Kerosene and jet fuels are complex petroleum-derived UVCBs that are used as blending constituents for finished fuels such as aviation turbine fuels (jet fuels), No. 1-K kerosene (for heating and illumination), and No. 1 grades of fuel oil, diesel fuel, and gas turbine oil. In September 2010 the American Petroleum Institute submitted a kerosene/jet fuel Category Assessment Document to USEPA as part of the High Production Volume (HPV) Challenge Program (API 2010).

The HPV Challenge Program is a voluntary initiative to develop and make publicly available screening-level health and environmental effects information on chemicals manufactured in or imported into the United States in quantities greater than one million pounds (454 tonnes) per year. In the Challenge Program, producers and importers of HPV chemicals voluntarily sponsor chemicals and identify and assess the adequacy of existing toxicity data, conduct new testing if adequate data do not exist, and make both new and existing data and information available to the public.

Several WAF tests were reported in the submission to the HPV Challenge Program for kerosene/jet fuel (API 2010).

Two sets of aquatic acute toxicity studies (fish, invertebrate, and algae) were reported for hydrodesulfurized kerosene (CAS # 64742-81-0), sweetened kerosene (CAS # 91770-15-9), and hydrocracked naphtha (CAS # 101316-80-7). Fish tests with rainbow trout produced similar LL50 values of 10 mg/L and 100 mg/L. Tests with aquatic invertebrates resulted in some of the lowest toxicity endpoints for the three species but also some of the most variable, with values between 1.4 to 89 mg/L for the three substances. The toxicity to algae of these substances was reported as 5.0 and 30 mg/L.

The API Testing Group also commissioned a 21-day reproduction test with *D. magna* exposed to hydrodesulfurized kerosene. The study used independent WAF preparations for the exposure solutions, and the total dissolved hydrocarbons in each WAF treatment were measured by gas chromatography. When expressed as WAF loading rate, kerosene produced EL50 values of 0.89 mg/L for reproduction and 0.81 mg/L for immobilization, with LOELR and NOELR values of 1.2 mg/L and 0.48 mg/L, respectively for all endpoints. When expressed as mean measured concentrations of dissolved hydrocarbons the EC50 values were 0.16 mg/L for reproduction and 0.15 mg/L for immobilization and the LOELR and NOELR were 0.23 mg/L and 0.092 mg/L, respectively for all endpoints.

Despite submission of the WAF results described above, USEPA declared in their Hazard Characterization Document (USEPA 2011) that: “For aquatic toxicity, data submitted for the category member, hydrodesulfurized kerosene (petroleum) (CASRN 64742-81-0), were considered inadequate because the results were reported based on nominal loading rates, not measured concentrations. Similarly, data submitted for CASRN 91770-15-9 and 101316-80-7 are not adequate.” As a result of this, USEPA proceeded to characterise kerosene/jet fuel aquatic hazard based on read across from measured concentrations of selected hydrocarbons and not on the basis of loading rates from WAF tests for either hydrodesulfurized kerosene or the two supporting substances (sweetened kerosene and hydrocracked naphtha).

In their response to this USEPA decision, the API (2013) reiterated the view that results for UVCBs should be expressed as lethal loadings rather than lethal/effect concentrations. This is because the loading rate reflects the composition and chemistry of the substance and implicitly accounts for dissolution and volatilization of individual hydrocarbon constituents. In contrast to this, they viewed results expressed as measured concentrations of the fraction of the substance in solution as of little value because it prevents extrapolation 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 rate).

The two case studies presented in this Commentary demonstrate a continuing unease within some regulatory authorities to use WAF loadings directly for hazard assessment. Indeed, this is reflected in more recent examples experienced by authors of this Commentary in which authorities have increasingly requested that study results are expressed in terms of mean measured concentrations rather than nominal WAF loadings. Depending on the substance this can have ramifications for classification and labelling of substances, leading to increased transport costs or further restrictions that are not truly commensurate with the intrinsic hazard of the whole substance. This is not an issue affecting only a small number of substances: 16968 of 68091 substances currently listed on the non-confidential TSCA Inventory are UVCBs (USEPA 2020), and in early 2017 around 21 % of the substances registered under REACH, amounting to more than 2500 chemicals, were UVCBs (ECHA 2017).

Conclusions

The use of the WAF is the most technically coherent approach to evaluate the toxicological properties of UVCB substances for classification and risk assessment purposes. This is enshrined in guidance documents published by the OECD and ECHA in support of the implementation of REACH and CLP legislations. However, the examples provided above and frequent recent requests to industry for aquatic toxicity endpoints to be expressed in terms of measured values suggest that some regulatory authorities do not appear to agree with the current state of toxicological evaluation of UVCBs and have questioned the validity of the WAF approach for the expression of toxicity values. This contradicts established international guidance and ECHA legislation in which *the substance as a whole*, i.e. the UVCB itself and not its individual constituents, is the entity that requires evaluation regarding classification purposes.

There has been clarity about the use of WAF loading rates for hazard/risk assessment and CLP for almost 30 years, since Girling et al. (1992) stated the following:

"A major benefit which accrues from expressing [WAF] results in terms of loading rates is that the data are of real value when carrying out a hazard assessment. Results expressed simply in terms of a measured concentration of the fraction of the product in solution are of little value since it will be virtually impossible to extrapolate to a field situation in which the only relevant measures of concentration will be the amount of product spilled and the volume of the receiving environment i.e. the loading rate.

For the purposes of classification and labelling, loading rates allow products which are poorly soluble mixtures and those that are readily soluble pure substances or mixtures to be compared on an equal basis. Criteria for classifying substances as 'dangerous for the environment'...are related to potential concentrations of materials in the environment. Clearly the concentration (or loading rate) of a material in the environment will not only be a function of its relative water solubility but also the quantity entering the environment and the dispersive mechanisms that operate. Consequently, a water soluble product found to be non-toxic at a concentration of 100 mg/l in a laboratory toxicity test should receive the same classification as a poorly soluble product found to be non-toxic at a loading rate of 100 mg/l in a similar test."

Use of WAF is also one of the only ways to test and report on the "unknown" fraction of UVCBs that either are not, or cannot, be analytically measured. The European Chemicals Agency (ECHA 2012) summarises how the WAF approach, as described in OECD (2000), might be used under REACH for UVCBs that are highly insoluble in water. Their advice is that test data obtained with WAFs apply to the multi-constituent substance as a *whole entity* and can be used to provide a justification that aquatic toxicity is unlikely to occur, based on an assessment that includes all constituents that are dissolved or present as a stable dispersion or emulsion.

We hope this commentary highlights the practical and regulatory issues associated with the testing of UVCBs and underscores the importance of WAF approaches for appropriate environmental hazard evaluation and classification purposes.

List Of Abbreviations

API American Petroleum Institute

CAS Chemical Abstracts Service

CLP Classification, Labelling, and Packaging

CONCAWE Conservation of Clean Air and Water in Europe

CROSERF Chemical Response to Oil Spills Research Forum

CTPHT Coal Tar Pitch, High Temperature

ECETOC European Centre for Ecotoxicology and Toxicology of Chemicals

EC European Commission

ECHA European Chemicals Agency

EC50 Median Effective Concentration

EL Effective Loading

EL50 Median Effective Loading

EU European Union

GD Guidance Document

HPV High Production Volume

LC50 Median Lethal Concentration

LL Lethal Loading

LL50 Median Lethal Loading

LOELR Lowest Observable Effect Loading Rate

LOQ Limit of Quantitation

M Multiplying Factor

NC Not Classified

ND No Data

NOEC No Observed Effect Concentration

NOELR No Observable Effect Loading Rate

OECD Organisation for Economic Cooperation and Development

PAH Polyaromatic Hydrocarbon

RAC Risk Assessment Committee

UNECE United Nations Economic Commission for Europe

USEPA United States Environmental Protection Agency

UV Ultraviolet

UVCB Unknown or Variable Composition, Complex reaction products, and Biological Materials

WAF Water Accommodated Fraction

WS Water Solubility

WSF Water Soluble Fraction

Declarations

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Consent for publication: Not applicable

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Tables

Table 1
Acute and chronic aquatic toxicity data and calculations used for CTPHT classification by the summation method (RAC 2011)

PAH	Measured mean concentration (ng/L) in WAF test (Noack et al. 2009) at CTPHT loading rate of 100 mg/L	Acute classification					Chronic classification				
		Lowest aquatic E/LC50 (mg/L)	Content in CTPHT (% w/w)	Multiplying factor (M)	M x content in CTPHT (% w/w)	Concentration limit (%)	Content in CTPHT/concentration limit	Content in CTPHT (% w/w)/E/LC50	Lowest aquatic NOEC or EC10 (mg/L)	M	M x content in CTPHT (% w/w)/E/LC50
Naphthalene	<LOQ	1.0	0	1	0	25	0	0	0.020	1	0
Acenaphthylene	<LOQ	1.8	0	-	-	-	-	0	0.064	1	0
Acenaphthene	63.3	0.58	0.043	1	0.043	25	0.00172	0.074	0.038	1	0.043
Fluorene	53.9	0.025	0.047	10	0.47	2.5	0.0188	1.88	0.025	1	0.047
Anthracene	56	0.001	0.13	1000	130	0.025	5.2	130	0.0015	10	1.3
Phenanthrene	252	0.051	0.63	10	6.3	2.5	0.252	12.4	0.01	10	6.3
Pyrene	240	0.00023	0.95	1000	950	0.025	38	4130	0.0005	100	95
Chrysene	80.1	0.0007*	0.81	1000	810	0.025	32.4	1157	ND	1000 ⁺	810
Benz[a]anthracene	71.9	0.0018	0.77	100	77	0.25	3.08	428	0.0012	10	7.7
Fluoranthene	318	0.0001	1.1	10000	11000	0.0025	440	11000	0.0006	100	110
Benzo[a]pyrene	35.4	0.0012*	1.0	100	100	0.25	4	833	0.0005	100	100
Benzo[b]fluoranthene	33.2	>WS	1.2	-	-	-	-	-	ND	NC	NC
Benzo[k]fluoranthene	<LOQ	0.00065	0.61	1000	610	0.025	24.4	938	0.00017	100	61
Dibenz[a,h]anthracene	<LOQ	0.0018	0.17	100	17	0.25	0.68	94.4	0.00014	100	17
Benzo[ghi]perylene	<LOQ	>WS	0.87	-	-	-	-	-	0.000082	1000	870
Indeno[1,2,3-cd]pyrene	<LOQ	0.00027	0.91	1000	910	0.025	36.4	3370	0.00027	100	91
Total	1204 (0.0012%)		9.2		14521		581	21280			2169

LOQ = Limit of Quantitation; WS = water solubility; * = value derived in presence of UV light; ND = no data; ⁺ based on acute data; NC = not classified.

Figures



Figure 1

Typical WAF test medium preparation set-up

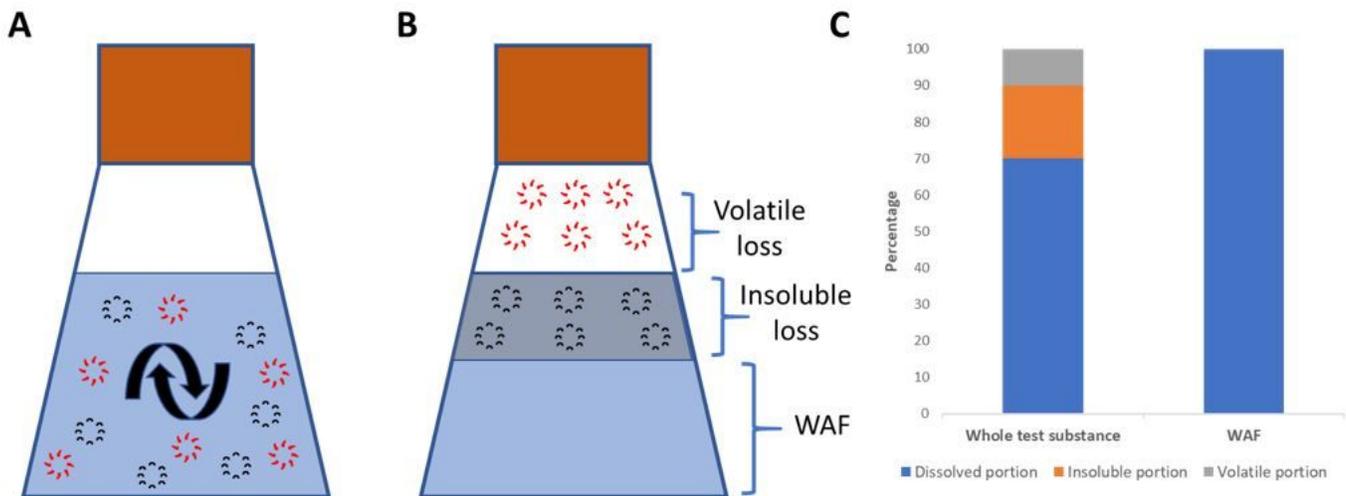


Figure 2

WAF test medium preparation (see text for details)