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Challenges in addressing phototoxicity and photodegradation in the environmental risk assessment of Polycyclic Aromatic Hydrocarbons in the aquatic compartment

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Challenges in addressing phototoxicity and photodegradation in the environmental risk assessment of Polycyclic Aromatic Hydrocarbons in the aquatic compartment

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ABSTRACT

This report summarises how light might interact with Polycyclic Aromatic Hydrocarbons (PAHs) to alter both their environmental fate and their potential to cause adverse effects. The quantification of these changes is complicated due to the difficulties in measuring these changes in the laboratory and subsequently, in relating these to how PAHs behave in the real environment. The consequences of attempting to account for these interactions within the framework of a generic environmental risk assessment are discussed and the conclusion reached that the impact of light on the behaviour of PAHs is best accounted for using a site-specific rather than a generic regional or generic local petroleum product risk assessment. The key elements of such an approach are described.

KEYWORDS

PAH, phototoxicity, photodegradation, environmental risk assessment

INTERNET

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SUMMARY

Polycyclic Aromatic Hydrocarbons (PAHs) are of interest because they may be present in some 'heavier' petroleum substances, and due to their chemical structure, certain PAHs will absorb energy in the UV waveband 280 to 400 nm, resulting in photo-induced toxicity (or photo-toxicity) and/or photodegradation.

There are two types of confounding factors to consider when assessing photodegradation of PAHs and how this may impact environmental fate. Firstly there are laboratory experimental design features. In the case of most petroleum substances containing PAHs standard test guidelines are not directly applicable due to their complex nature. In the case of individual PAHs the interpretation of historical laboratory studies maybe confounded by solvent effects, differences in light intensity and wavelength and the impact of dissolved oxygen. Secondly environmental factors including solar radiation fluctuation, sunlight filtering and attenuation caused by atmospheric absorption, attenuation of light intensity in water through absorption and scattering further complicate extrapolation of laboratory tests for single PAHs to the field. Thus the characterisation of photodegradation rates of PAHs in the aquatic environment for use in risk assessment is difficult. A logical simplification is to ignore photodegradation as a mechanism in generic risk assessments. In such cases the risk assessment approach considers only biotic degradation in exposure estimation, as a pragmatic, conservative way forward.

Two different mechanisms are considered to be responsible for photo-induced toxicity of PAHs, direct photolysis or photo-modification into new chemical species that will have different bioactivities compared to the parent compounds and photosensitisation which involves PAH accumulation into organism tissues and subsequent production of singlet oxygen, a reactive oxygen species that is highly damaging to biological molecules. These reactions are impacted by different environmental factors that are discussed in this review which again make the extrapolation of laboratory results to a generic risk assessment that is broadly applicable to the field very difficult.

Extrapolation of laboratory data to the environment for the purposes of a generic risk assessment will lead to high levels of uncertainty and potentially significant over-estimation of the impact of such effects. For example, past studies have often involved first exposing aquatic test organisms in the dark so that PAHs could accumulate in tissues followed by subsequent exposure to UV light to quantify photosensitisation effects. However, under field conditions, photodegradation of PAHs may preclude bioconcentration in tissues to levels where phototoxic effects are observed. One critical aspect of a sound risk evaluation is that any potential enhancement in hazard associated with phototoxicity must be judged relative to the decreased exposure associated with photodegradation processes. However, given that these processes are complex, site-specific and lack a standardized methodology to incorporate in a generic manner, they are processes that are best considered as part of a higher-tier site-specific risk assessment where PAH contamination is of particular concern. Consequently, assessing these processes for the large number of PAHs in a complex petroleum substance is currently not feasible and requires further research.

The use of OECD 316 (Photo-transformation of chemicals in water – direct photolysis) to generate a standard database of reliable and comparable photodegradation rates for specific PAHs, and accounting for a range of environmental factors, would be a useful action to be considered.

For the generic environmental risk assessment of petroleum substances the approach adopted is based on the assumption that blocks of hydrocarbons of similar structure will have similar physical-chemical properties and potentials to be partitioned and degraded in the environment. Using these “blocks” PEC values can be calculated for each environmental compartment and that PNECs are also estimated for the same individual components using the Hydrocarbon Block Method (HBM). However, in applying the HBM to PAHs two problems remain

- Firstly, individual PAHs, as described in the report, that may contribute to the same block vary considerably in their reactivity to light and hence cannot be assigned a single ‘generic’ profile of environmental characteristics. Consequently, it is currently not possible to develop blocks that adequately simulate the multitude of PAH reactions in a complex petroleum substance correctly;
- Secondly, the extent to which the photo-induced behaviour of the PAHs, is significantly affected by the environment within which these are present. Thus, a generic assessment is inappropriate.

Given the difficulties in extrapolating lab results to the field and the lack of an accepted methodology for quantitatively considering the photo-induced toxicity of PAHs, it is proposed to address this concern on a site-specific basis. For generic product risk assessments, CONCAWE recommends that PNECs for PAHs be developed using only toxicity data that represent the intrinsic toxicity of these and are not artificially enhanced by the presence of UV light, essentially as described in PETROTOX (HydroQual, 2010). PEC/PNEC ratios for PAH blocks can then be determined for local and regional scenarios in accordance with the REACH Technical Guidance Documents. In cases that a given scenario has the potential for raising concerns arising from the potential effect of photo-induced toxicity, the risk assessment should be refined in a tiered manner, if possible, by obtaining site-specific modelled or measured data.

Key components of a tiered site-specific approach could include:

- Determination of likelihood of combined UV-light and PAH exposure and the presence of potentially vulnerable species/life-stages at the site;
- Identification of the PAHs to be addressed, as not all PAHs are phototoxic or photodegradable, and such an identification allows for a site-specific approach to be targeted, e.g. Weinstein and Polk (2001);
- Characterisation of relevant site-specific conditions including light measurements and light attenuation (including Spectra assessment), and their fluctuations over time, see Diamond et al (2006);
- Measurements of site exposure concentrations of selected PAHs and/or in-situ toxicity data, see Rosen et al (2012);
- Development of toxicity data for PAHs of interest to site relevant organisms that can be used to account for differences in light intensity and attenuation in the field as well as specific site interactions. Examples include Scott et al (1996) and Mount et al (2001);
- Use of a model that allows for both hazard and exposure data, to be integrated into a risk assessment paradigm, see Mount et al (2001).

1. INTRODUCTION

Petroleum substances are typically complex mixtures of hydrocarbons with variable composition (UVCBs - substances of Unknown, Variable Composition or Biological Origin). The individual hydrocarbons comprising petroleum substances have different physical-chemical properties that influence their environmental effects and fate, including their rate of microbial biodegradation. To address this, the hydrocarbon block method has been developed, which groups hydrocarbons based on their physical-chemical and environmental fate and effect properties (ECHA, 2008). Polycyclic Aromatic Hydrocarbons (PAHs) are of interest because they may be present in some 'heavier' petroleum substances, and due to their chemical structure, certain PAHs will absorb energy in the UV waveband 280 to 400 nm, resulting in photo-induced toxicity (photo-modification or photosensitisation) and/or photodegradation. The extent to which the environmental effects and fate of these PAH may be impacted by the change in properties induced by this absorption poses challenges for the environmental risk assessment of petroleum substances containing PAHs.

Polycyclic Aromatic Hydrocarbons (PAHs) are ubiquitous, organic contaminants in freshwater and marine ecosystems. PAHs enter the aquatic environment from a wide range of both anthropogenic and natural sources mainly indirectly through the deposition and run-off from aerial emissions from the incomplete combustion of all types of fossil fuels, including wood, but also via organic wastes, certain industrial processes such as coke production and natural processes such as thermal geological reactions and natural fires.

This report addresses the mechanisms of photodegradation and photo-induced toxicity and how these processes are impacted by environmental factors. A pragmatic approach to the generic environmental risk characterization of PAH containing petroleum substances is then discussed. As such the report focuses on data for PAHs that are derived from studies of the individual PAHs, and does not address data generated on petroleum substances, which are complex substances. There are no guidelines that enable such complex petroleum substances to be addressed adequately.

2. OVERVIEW OF PAHS

Polycyclic Aromatic Hydrocarbons (PAHs) are ubiquitous, organic contaminants in freshwater and marine ecosystems. PAHs enter the aquatic environment from a wide range of both anthropogenic and natural sources (Jimmink, 2007) mainly indirectly through the deposition and run-off from aerial emissions from the incomplete combustion of all types of fossil fuels, including wood, but also via organic wastes, certain industrial processes such as coke production and natural processes such as thermal geological reactions and natural fires.

PAHs are produced by the incomplete combustion of organic matter, during the formation of fossil fuels or by biosynthesis. The distribution of PAHs is highly variable according to the different processes that form the PAHs. Three origins for PAHs can be distinguished:

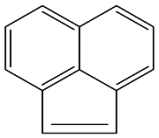
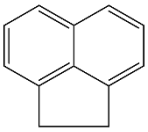
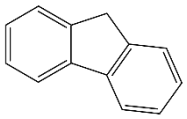
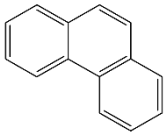
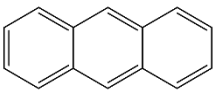
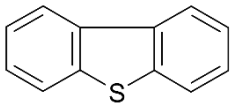
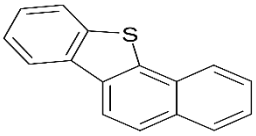
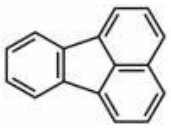
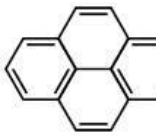
- pyrolytic processes, whereby PAHs are formed by the incomplete combustion of organic matter at high temperatures (>500°C), controlled by a kinetic process, e.g. burning wood in open fire places, wood and charcoal barbeques (Laflamme and Hites, 1978; Budzinski, 1993). These processes tend to produce 4 – 6 ring unsubstituted PAHs, especially at higher temperatures (e.g. 2000°C) (Laflamme and Hites, 1978). At lower temperatures (800°C) as in forest fires more alkylated compounds are formed (Laflamme and Hites, 1978).
- petrogenic processes, with PAHs resulting from the formation of fossil fuel, such as oil and coal, at high pressure and lower temperatures (<200°C), controlled by a thermodynamic process (Ho et al, 1974; Neff, 1979; Wakeham et al, 1980a & 1980b; Budzinski, 1993). These processes tend to generate a high proportion of alkylated compounds. Phenanthrene and thiophene derivatives are the main PAHs found in petroleum products because of their strong thermodynamic stability (Ho et al, 1974; Wakeham et al, 1980a; Wang et al, 2001).
- and diagenic processes, where PAHs are formed as a result of the synthesis of precursors by microorganisms (Laflamme and Hites, 1978; Neff, 1979; Hites et al, 1980; Budzinski, 1993; Hansen, 2003). The fingerprint of diagenic PAH contamination shows a high proportion of perylene together with retene and derivatives of phenanthrene and chrysene (Hites et al, 1980; Hansen, 2003).

2.1. STRUCTURE

PAHs are organic compounds made up of three or more fused, five and/or six membered aromatic rings, with molecular weights ranging from 136 to over 300. They have planar conformation. The structures of common ‘parent’ PAHs with the nomenclature adopted by the International Union of Pure and Applied Chemistry (Braslavsky, 2003) are presented in **Figure 1** and **Table 1**. While these parent structures are often the PAHs determined in many analytical schemes, they form the skeleton of over 10,000 possible structures (Neff, 1979) differing in that they contain heteroatoms (e.g. sulphur or nitrogen) and the extent and positioning of hydrocarbon side-chains, referred to as alkylated PAHs.

As a result of this, PAHs cover a large range of physical-chemical properties, persistence and toxicities.

Figure 1 Structure of PAHs

			
Acenaphthylene	Acenaphthene		
			
Fluorene	Phenanthrene	Anthracene	Dibenzothiophene
			
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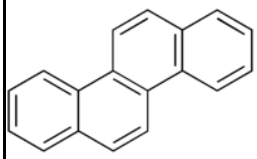
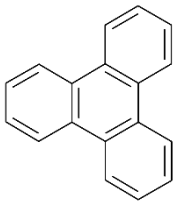
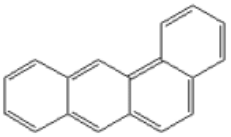
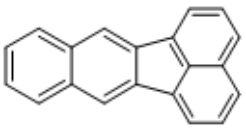
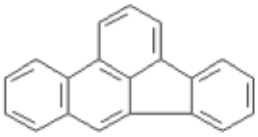
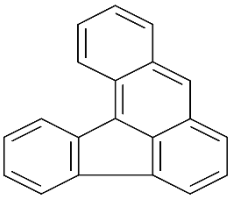
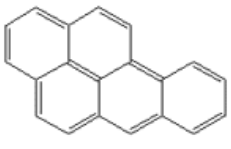
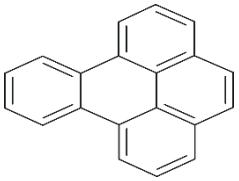
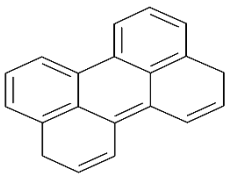
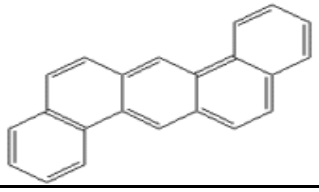
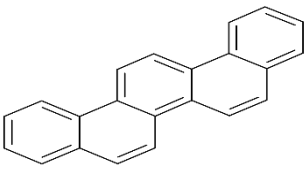
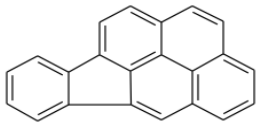
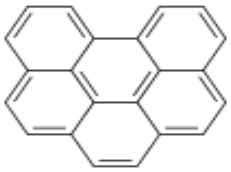
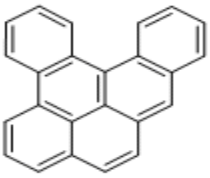
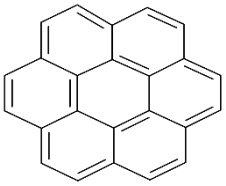
			
Chrysene	Triphenylene	Benzo(a)anthracene	Benzo(k)fluoranthene
			
Benzo(b)fluoranthene	Benzo(a)fluoranthene	Benzo(a)pyrene	Benzo(e)pyrene
			
Perylene	Dibenzo(a,h)Anthracene	Picene	

			
Indeno(1,2,3-cd)pyrene	Benzo(g,h,i)perylene	Dibenzo(a,l)pyrene	Coronene

2.2. PHYSICAL-CHEMICALS PROPERTIES

The commonly used physical-chemical properties used are shown in **Table 1**.

Table 1 Table of PAH physical-chemical properties (Mackay et al, 1992)

Substance	Molecular weight (g/mol)	Water solubility (µg/l)	Log Kow	Vapour pressure (Pa at 25 °C)	Henry's constant (Pa m^3/mol at 25 °C)
Acenaphthene	152.2	3910	4.00	3.3 10-1	14.3
Acenaphthylene	150.2	16100	3.62	4.8 10-1	11.5
Fluorene	166.2	1800	4.22	8.3 10-2	8.5
Anthracene	178.2	47	4.68	9.4 10-4	4.3
Phenanthrene	178.2	974	4.57	2.6 10-2	3.7
Fluoranthene	202.3	200	5.20	1.2 10-3	1.1
Pyrene	202.3	125	4.98	1.0 10-3	1.4
Benz[a]anthracene	228.3	10.2	5.9	7.6 10-6	0.81
Chrysene	228.3	1.65	5.81	5.7 10-7	0.079
Benzo[a]pyrene	252.3	1.54	6.13	7.3 10-7	0.034
Benzo[b]fluoranthene	252.3	1.28	6.12	3.3 10-6	0.051
Benzo[k]fluoranthene	252.3	0.93	6.11	1.3 10-7	0.043
Benzo[ghi]perylene	276.3	0.14	6.22	1.4 10-8	0.027
Dibenz[a,h]anthracene	278.4	0.82	6.50	3.7 10-10	1.3.10-4
Indeno[1,2,3-cd]pyrene	276.3	0.1	6.58	1.7 10-8	0.046

3. ENVIRONMENTAL RISK ASSESSMENT – A BRIEF OVERVIEW

Environmental risk assessment is based on the comparison of a predicted environmental concentration (PEC) with the predicted no-effect concentration (PNEC).

The PEC is derived from utilising information relating to emissions – how much and to which compartment, with distribution and fate properties of the substance also being considered. The emissions are thus partitioned and degraded, depending on the type of risk assessment, the scale of assessment etc. For the purposes of this report, the main issue is the extent to which the photodegradation behaviour of PAHs impacts on their environmental degradation as derived from other tests or observations. The normal degradation, abiotic and biotic, of substances is generally not amended for photo-reactivity effects unless photo-derived effects are included due to the nature of the studies conducted. The degradation of PAHs has been reviewed by Lampi et al (2010) and the reader is referred to this document if conducting a PAH risk assessment and degradation data are required. **Appendix 1** shows a table which includes the degradation data for specific PAHs (both predicted and measured). A further source of biodegradation data is available in CONCAWE (2012).

PECs are also required for the indirect exposure of organisms to substances via the aquatic food chain. The main property used in this assessment is the potential for bioaccumulation of the substance. The bioaccumulation of PAHs was reviewed by Lampi and Parkerton (2009) and this report should be considered if an indirect exposure assessment, for the aquatic compartment, is required. In this assessment. Laboratory (BMF) and field (TMF and BSAF) provide complementary information for assessing bioaccumulation properties of PAHs. These data clearly demonstrate that all the PAHs investigated, including phenanthrene, exhibit a low biomagnification potential. **Appendix 2A** shows the BCF data and **Appendix 2B** shows BMF data for PAHs as compiled by Lampi and Parkerton (2009).

When deriving a PNEC for the hydrocarbons in petroleum substances the Target Lipid Model, TLM has been used (Redman et al, 2012). The TLM accounts for acute toxic effects by a non-specific toxicity mode of action (e.g., narcosis) such as exhibited by hydrocarbons that are found in petroleum substances (Karickhoff et al, 1991; Shiu et al, 1998). Empirical Acute-to-Chronic-Ratios (ACR) are used for predicting chronic toxic effects regardless of the underlying mechanism (Hermens et al, 1984). Using this assumption, the model used to predict the toxicity of hydrocarbon substances is PETROTOX (Redman et al, 2012). The complications of adapting this approach with the complexities of photo-induced changes is discussed further in Section 6.

4. PHOTODEGRADATION

It is worthwhile bearing in mind, when reading the following section, that there are no standard protocols that enable the assessment of photodegradation of complex substances. The following discussion is therefore focused on single substances.

4.1. MECHANISTIC CONSIDERATIONS

PAH photodegradation can be either a direct or an indirect process:

- Direct photolysis involves direct absorption of light by PAHs followed by chemical reaction, and is also known as photo-modification;
- Indirect or "sensitised" photolysis is initiated via light absorption by natural substances which subsequently react with PAHs (e.g. reaction with OH-radicals formed by UV radiation (Atkinson, 1987) or by reaction between the PAHs and the formed "free" singlet oxygen $^1O_2$ ("self-sensitised" photolysis).

Self-sensitised photodegradation appears to be insignificant relative to direct photodegradation for most natural surface waters. This is due to the fact that $^1O_2$ is not stable long enough as it relaxes to the ground state rapidly in water (Turro, 1978) and this, along with the low concentrations at which PAHs occur (Kochany and Maguire, 1994; Fasnacht and Blough, 2003a, 2003b) suggests that the molecules would never encounter each other within sufficient time for a reaction to occur. Furthermore, the calculations of Zepp and Schlotzhauer (1979) also indicated that the photosensitised oxygenation of PAHs in natural waters was slow compared with direct photolysis and consequently, that singlet oxygen did not play a significant role in the photochemical transformation of PAHs in the aquatic environment.

As a result of their high hydrophobicity and very low aqueous solubility, early PAH photodegradation kinetic studies were commonly conducted in organic solvents. Interpreting such studies when considering the environmental fate of PAHs in aquatic systems is consequently confounded by the effect of the solvent. The "availability" and the photodegradation phenomena of PAHs is a function of the medium of dissolution (organic/aqueous; polar/apolar solvent; protonic or not). Hence, studies reporting results in organic solvents cannot be reliably related to photodegradation in the aquatic environment.

The rate and extent of photodegradation varies widely among PAHs due to the large differences between quantum yields and half-lives, and is a complex function of their structure especially on the number of condensed aromatic rings (Fasnacht and Blough, 2002; Kosian et al, 1998; Krylov et al, 1997). Generally, compounds with higher molecular weight and with more condensed aromatic rings photolyze faster (Kochany and Maguire, 1994; Jacobs et al, 2008). The kinetics of PAH photo-oxidation in water solutions generally follow a first-order equation, and the half-lives vary from a few minutes to a few days (with apparent rate constants varying from 10^{-3} to 10^{-4} s^{-1}) (Zepp and Schlotzhauer, 1979; Kochany and Maguire, 1994), strongly depending on the exposure design (e.g. solvent, PAH concentration and irradiance spectra) of the experiment. However, first-order kinetics for PAH loss have not always been observed in natural waters (Mill et al, 1981). Kochany and Maguire (1994) observed direct photo-oxidation half-lifes for different PAHs ranging from 2.2 min to 71 h, with the more rapid photodegradation occurring for the larger PAHs. It appears also that alkyl-substituted PAHs are more photo-labile than unsubstituted PAHs (Zepp and Scholtzhauer, 1979).

The impact of size and alkylation on photodegradation of crude oil was investigated by Garret et al (1998) and Prince et al (2003). They studied the photo-oxidation of an artificially weathered Alaskan North Slope crude oil exposed to the atmosphere in a shallow dish in the dark or exposed to a laboratory UV lamp. They monitored four groups of components (saturates, aromatics, resins and polars) together with selected PAHs and Dibenzothiophenes (DBTs). The saturated compounds were resistant, but the aromatic compounds were particularly sensitive to photo-oxidation. Greater size and increasing alkyl substitution increased the sensitivity of aromatic compounds to photochemical oxidation. The photo-oxidized products (70% of aromatic compounds) appeared in the resin and polar fractions. The four-ring chrysene was substantially more affected than the three-ring phenanthrene and dibenzothiophene, and in each family the extent of loss increased with increasing alkylation.

Photodegradation of PAHs results in the formation of products of higher polarity and solubility in water such as phenols, quinones, and acids (Stucki and Alexander, 1987; Beltran et al, 1996a and 1996b; Mallakin et al, 1999). A combination of increased solubility, bioavailability and reactivity was suggested to account for a large part of the higher toxicity of some photo-modified PAHs (Duxbury et al, 1997; Huang et al, 1997; McConkey et al, 1997; Lampi et al, 2005). Duxbury et al, 1997 observed experimentally that the assimilation by the duckweed Lemna gibba of photo-modified benzo(a)pyrene, delivered from artificially contaminated sand, was twice that of the parent (non-irradiated) compound. Additionally these by-products are more bioavailable for microorganisms with an increased overall natural biodegradation rate as a result (Lehto et al, 2000; Grote et al, 2005).

4.2. CONFOUNDING FACTORS

There are two types of confounding factors to consider when assessing photodegradation of individual PAHs and how this may impact environmental fate.

Firstly there are laboratory experimental design features;

1. The use of organic solvents due to the very low solubility of PAHs will lead to difficulties in interpreting the results and extrapolation to the field. Furthermore, the more polar the solvent, the faster the degradation process of PAHs (Lehto et al, 2000). Thus the choice of solvent will alter the experimental results.
2. The impact of oxygen (O_2) has been observed by some researchers (Mill et al, 1981; Sigman et al, 1996; Sigman et al, 1998; Miller and Olejnik, 2001) and a strong dependence of the photodegradation quantum yield on oxygen concentration. However, Zepp and Schlotzhauer (1979), who investigated the photodegradation of 12 PAHs in different solvents and in degassed or air-saturated solutions, reported that oxygen concentration had minor or inconsistent effects upon the photodegradation rates of PAHs, and suggested that the primary photochemical processes in water may not involve molecular oxygen.
3. Photolysis rates are strongly influenced by the intensity and spectral distribution of the light source (Zepp and Cline, 1977). As irradiation instruments are not standardized in PAH photodegradation studies, there is a potential source of discrepancy between monochromatic/polychromatic irradiation and choice of the lamp. Fluorescent, metal vapour and halide arc lamps have large differences in spectra and intensity irradiance.
4. Finally, PAH photodegradation mechanisms seem to be a concentration-dependent process. In dilute solutions, (<1 µM), photoionization probably

dominates (Sigman et al, 1996; Fasnacht and Blough, 2002); while at higher concentrations it is possible for PAHs to photo-degrade through reaction of the PAH with singlet oxygen (a self-sensitized process) (Stevens, 1973). This difference in mechanism can be explained by the short lifetime of $^1O_2$ in water and the low concentration of PAH present (Turro, 1978).

Secondly there are environmental factors to consider, which will impact on a generic approach to risk assessment;

1. Solar radiation fluctuates on daily, to seasonal, to millenary scale and varies constantly with the position of the Earth relative to the sun. Generally, the intensity decreases with decreasing angular height of the sun, and from the tropics to higher latitudes. Daily and annual fluctuations of solar radiation follow parabolic curves over time with maxima reached at solar noon and in the summer season (Karentz and Lutze, 1990; Boelen et al, 1999; Barron et al, 2000; Schubert et al, 2001; Diamond et al, 2006); in the study of Barron et al (2000) UV-A and UV-B maxima ranged from 3 to 12 times higher than minimal intensities during a day (California Coast, United States). Furthermore, the decrease in UV-B intensity with decreasing solar altitudes is much more pronounced than the decrease in visible or UV-A (320-400 nm) intensity.
2. As sunlight passes through the atmosphere, its intensity is decreased through absorption by atmospheric gases such as ozone or by cloud cover and through scattering by atmospheric particles/haze (Barron et al, 2000; Schubert et al, 2001). Barron et al (2000) observed a two-fold variation in visible and UV-A radiations and a 4-fold variation in UV-B in different atmospheric conditions (solar disk visibility, cloud, atmospheric haze) at a location on the California coast, United States.
3. The intensity of sunlight is also attenuated in natural waters through absorption and scattering (Zepp and Cline, 1977). Attenuation due to light scattering is less important than attenuation by absorption in most natural waters, especially in the ultraviolet region (Duntley, 1963). However, light scattering is likely to have an important effect in turbid lakes and rivers (Zepp and Cline, 1977). In the ocean, absorption is primarily due to water itself (Duntley, 1963), except where turbidity is evident in the vicinity of river estuaries.
4. Williamson et al (1996) reported an exponential increase in UV-B attenuation (attenuation depths of 1 to 15 m) with increasing dissolved organic carbon (DOC) below a 1-2 mg/l range; above 2 mg/l, the attenuation depths (1% of surface irradiance) were less than 1 m and were less influenced by DOC concentration changes. Although UV penetration in aquatic systems is clearly influenced by DOC concentration, its prediction and generalization is dependent on DOC origin and composition (Huovinen et al, 2003).

4.3. CONCLUSIONS

The characterisation of photodegradation rates of PAHs in the aquatic environment for use in risk assessment is clearly fraught with difficulties. It would seem prudent not to use photodegradation as a mechanism to demonstrate enhanced degradation rates in generic risk assessments. In such cases the risk assessment approach should focus on biotic degradation only, as this would appear to offer the most pragmatic way forward.

The OECD 316 guideline, Photo-transformation of Chemicals in Water – Direct Photolysis, was adopted in 2008. The guideline outlines how it is possible, using

standard approaches, to determine the direct photo-transformation rate constants of chemicals. Direct photolysis rate constants for chemicals can be determined in the laboratory using filtered xenon arc lamps or sunlight irradiation and extrapolated to natural water. The transformation pathway and the identities, concentrations, and rates of formation and decline of photo-transformation products resulting from direct photolysis can also be investigated. The guideline includes the option to measure the quantum yield and resulting estimated direct photolysis rate constants for chemicals for various types of water bodies.

5. PHOTO-INDUCED TOXICITY

5.1. MECHANISTIC CONSIDERATIONS

Two different mechanisms are considered to be responsible for photo-induced toxicity of PAHs:

- Direct photolysis or photo-modification into new chemical species that will have different bioactivities than the parent compounds (Huang et al, 1995, 1997; Ren et al, 1996; Krylov et al, 1997; McConkey et al, 1997) and
- photosensitisation (Newsted and Giesy, 1987; Hall and Oris, 1991; Huang et al, 1993; Mekenyan et al, 1994; Arfsten et al, 1996; Krylov et al, 1997; Huang et al, 1997)

Photo-modification of PAHs, often via oxidation reactions of the excited PAHs with dissolved oxygen may occur, resulting in photoproducts with different chemical properties and often altered bioactivities. Upon absorbing sunlight, a PAH can be rapidly transformed into a variety of compounds, most of which are oxidation products. The primary photo-oxidation pathway of PAHs proceeds via unstable endoperoxide and/or peroxide intermediates leading to quinones and diols (Zepp and Schlotzhauer, 1979; Katz et al, 1979; David & Boule, 1993).

In contrast, photosensitisation generally involves the production of singlet oxygen, a reactive oxygen species that is highly damaging to biological molecules. Such processes imply activation of the chemicals that have bioaccumulated in biological matrices following radiant energy absorption, generating reactive oxygen species that can disrupt membranes via lipid peroxidation. Photo-sensitization should not be confused with sensitized photolysis of PAHs (see Section 4). In photo-sensitisation, the triplet oxygen is photo-sensitized by the excited PAHs (leading to the formation of singlet oxygen) whereas in the latter process, PAHs are photosensitized by other primary photon receptors (singlet oxygen or other excited natural substances).

The impact of either of these mechanisms is that biological effects can be observed due to cell damage or photo-cytotoxicity (Schirmer et al, 1998), impacts on the cell membrane and genotoxicity. The impacts may lead to biological disorders relating to organism development, growth inhibition and/or morphological disorders.

In Schirmer et al (1998), the authors classified phototoxic PAHs into two groups: they distinguished PAHs that were photo-cytotoxic only at relatively high concentrations (with weakest/null potency of impact) i.e. above their limit of solubility in water, from those found to be photo-cytotoxic at concentrations below their theoretical solubility limit (fluoranthene, pyrene, anthracene, benzo(a)anthracene and benzo(g,h,i)perylene). For the second (most potent) group of PAHs, they took into account factors relating to PAH availability in aquatic systems (water solubility, stability and ability to persist in the main target tissues) in order to determine the potency of the impact. Benzo(a)pyrene had an EC50 value that was too close to its maximum water solubility to be likely to have an environmental impact. Based on these considerations, fluoranthene and pyrene appeared to be the PAHs with the most potential to impact on fish through phototoxicity. The relevance of these studies needs to be addressed, however, as they were conducted on rainbow trout gill cells.

In fish, the disruption of cell membranes has been proposed as the major effect of photo-induced toxicity of anthracene and fluoranthene (Oris and Giesy, 1985;

McCloskey and Oris, 1993). Weinstein et al (1997) studied the UV-induced toxic action of fluoranthene in the juvenile fathead minnow (*Pimephales promelas*) by an examination of ultra-structural pathologies. They exposed juveniles to fluoranthene (0.2 to 12 µg/l) and solar ultraviolet radiation (SUVR) (6 to 96 h) and observed mucosal cell hypertrophy and an inflammatory reaction with decreased oxygen capacity of the gills, which caused asphyxiation.

Utsumi and Elkind (1979) showed that the phototoxic effects of dimethylbenz(a)anthracene (DMBA) were particularly pronounced in cells undergoing DNA synthesis, indicating that DNA is a target of the phototoxicity of PAHs (Toyooka and Ibuki, 2007). This can be via DNA oxidation, single and/or double strand break or via photo-mutagenicity.

As previously mentioned there are thousands of parent and substituted parent PAHs that can exist in the environment. However, not all PAHs are phototoxic. Larger PAHs appear to have greater reactivity, and thus photo-degrade more readily than smaller PAHs, owing to their higher extinction coefficients at wavelengths present in sunlight (Kochany and Maguire, 1994; Bertilsson and Widenfalk, 2002; Jacobs et al, 2008). This may also be explained in terms of a better overlap of the absorption spectrum of these compounds with the solar spectrum.

Although there is a high variability among experimental conditions, published reports can be used to highlight critical data with respect to some PAHs in regard to photo-enhanced toxicity. Wernersson and Dave (1997) exposed Daphnia magna for 24 hours to individual PAHs, followed by a 2 hour exposure to UV; UV toxicity increase was more than 100% for fluoranthene, benzofluoranthene and dibenzoanthracene, and less than 10% for acridine, pyrene and benzo(a)pyrene. Wernersson, 2003, simultaneously compared the toxicity of more than 15 PAHs with and without UV co-exposure in the crustacean Daphnia magna. In contrast to the previous study, greater enhancement was observed for pyrene, with 750-times higher values under UV radiation. UV-EC50 values were under 10 µg/l for anthracene, benzo(a)anthracene, benzo(a)pyrene, dibenz(a,h)anthracene, fluoranthene and pyrene, and above 1,000 µg/l for acenaphthylene, acenaphthene, benzo(g,h,i,)perylene, fluorene, indeno(1,2,3-cd)pyrene. Using the fluoranthene equivalent factors (FEF) proposed by Schirmer et al (1998), PAHs were categorised according to their phototoxicity potential (expressed as a ratio relative to fluoranthene). Benzo(a)pyrene, pyrene, dibenz(a,h)anthracene, benz(a)anthracene and anthracene yielded equivalency factors of 4.3, 3.6, 2.8, 1.5 and 0.9, respectively. In the same way, Lampi et al (2007) assessed photo-enhanced toxicity of some PAHs in Daphnia magna. These authors found that UV co-exposure induced lower PAH toxicity increases than those observed by Wernersson et al (2003). These differences highlight the impact of exposure conditions on the photo-enhanced toxicity assessment of PAHs and particularly in relation to tested PAH concentrations, test duration and UV doses.

QSARs are available that relate the toxicity of phototoxic PAHs with physical chemical properties, such as HOMO-LUMO gap (H-L-G) and electron density shape features (Mekenyan et al, 1994; Mezey et al, 1998). Mekenyan et al (1994) and Veith et al (1995) have shown that the H-L-G is a useful predictor of the photo-induced toxicity of PAHs. A comparison of the observed toxicity to Daphnia magna and model prediction results as a function of H-L-G demonstrates that the PAHs that exhibited photo-induced toxicity were consistently within a H-L-G window of 7.2 +/- 0.4 eV (electron Volts). These authors also observed that alkyl and hydroxyl substituents do not significantly change the H-L-G of PAHs. Consequently, alkylated aromatics and phenols are likely to be phototoxic only if the parent aromatic structure is phototoxic. These authors also discuss the impact of spatial variation and changes to the energy

component that relate to the potential for photo-induced effects. This further complicates conducting a generic risk assessment as discussed in the next session.

5.2. CONFOUNDING FACTORS

The same type of confounding factors discussed in the section on photodegradation will impact on the phototoxicity behaviour of PAHs. There are however, also some additional factors to consider, which will be discussed in the following two sections. Firstly those environmental properties or interactions which are not apparent in laboratory studies and thus impact on the ability to extrapolate to the field. Secondly there are biological factors that may also vary in the field and give cause for concern when using data from laboratory studies for environmental risk assessment.

5.2.1. Environmental Factors

1. Dissolved Organic Matter (DOM) - While the role of DOM in the photo-induced toxicity of PAHs has yet to be established with certainty, DOM does play an important role in protecting aquatic organisms against the photo-induced toxicity of PAHs, by firstly reducing bioaccumulation and secondly by attenuating the active portion of solar UV radiation, thus decreasing the photodegradation/phototoxicity of PAHs (Oris et al, 1990). Dissolved humic materials have been shown to bind to PAHs (McCarthy et al, 1985; Chiou et al, 1986), and as a consequence, there is a decrease in the PAH bioavailability and bioaccumulation by aquatic organisms (McCarthy and Jimenez, 1985; Kukkonen and Oikari, 1991). Several authors have reported a significant reduction of the PAH phototoxicity in the presence of DOM to fathead minnows (*Pimephales promelas*) (Oris et al, 1990; Weinstein and Oris, 1999) and to *Daphnia magna* (Oris et al, 1990; Nikkilä et al, 1999). Particulate organic matter (POM) is likely to exhibit a similar effect in reducing light penetration and bioavailability. Since laboratory toxicity tests are typically performed in clean water where DOM and POM are often present at low concentrations which are not characteristic of natural waters, results from these studies may overstate toxicity in the field.
2. In addition to DOM/POM quantity and quality, UV radiation penetration depends other water characteristics such as particle density and water column depth.

5.2.2. Biological Factors

1. Burrowing behaviour - Some benthic organisms have natural burrowing behaviours responding to different needs such as protection from predators or to search for food. These behaviours provide protection against photo-enhanced toxicity for benthic organisms that live in shallow waters. During their water-only versus sediment condition experiments, Bell et al (2004) showed that sediment provides an effective protective barrier for midge *Chironomus tentans* larvae with respect to photo-induced toxicity; percent survival for fluoranthene/UV-exposure with sediment was more than 87%, similar to those from control (without UV exposure), whereas survival fell below 19% in water-only conditions. Other behavioural adaptations include avoidance of contaminated areas or seeking refuge in shade, e.g. leaves (Hatch and Burton, 1999)
2. Body design – For example, the presence of a carapace or shell will all lead to some level of light protection, resulting in crustaceans or bivalves usually being less impacted than other organisms.

3. Pigmentation - Pigmentation of the organism offers important shielding since UV radiation may be reflected or absorbed by these pigments (Karentz, 1994). This means of protection from the direct effect of UV light may thus serve as protection from photo-induced toxicity. Whereas many studies have demonstrated that petroleum products have great photo-enhanced toxicity to translucent organisms (Pelletier et al, 1997; Wernersson, 2003), Barron et al (2005) observed no photo-enhanced toxicity of crude oil on heavily pigmented juvenile pink salmon.

4. Elimination via metabolism will also significantly reduce exposure as does elimination via exuviae (the shedding of exoskeletons). In their study on the bioaccumulation and phototoxicity of fluoranthene in larval and adult stages of *Chironumus tentans* (midges whose complete egg development occurs in freshwater), Bell et al (2004) were the first to report that elimination can occur via the molted exuviae. They observed much lower fluoranthene body burden in adults than in larvae and relatively high proportions in exuviae. The authors thought that the chitinous components of the exoskeleton served as a partitioning phase for fluoranthene, which might result in decreased body burden during the transition from larvae to the adult form.

5.3. CONCLUSIONS

A review of the papers mentioned in the previous sections, highlights the variability in exposure conditions with regard to light characteristics (both intensity and wavelength), test duration and PAH concentrations used. Consequently, extrapolation of laboratory toxicity data to the environment for the purposes of a generic risk assessment will lead to high levels of uncertainty and possible over-estimation of the impact of such effects. For example, past studies have often involved unrealistically high light intensities or clean waters that are not representative of natural water where greater light attenuation is expected. In other cases, study designs may overstate the influence of photo-enhanced toxicity. For example, some toxicity studies have involved first exposing aquatic test organisms in the dark so that PAHs could accumulate in test organism tissues followed by subsequent exposure to UV light to quantify photo-sensitisation effects. However, under field conditions, photodegradation of PAHs is expected to reduce bioconcentration in tissues to levels where photo-toxic effects may not be observed

A local site-specific risk assessment using knowledge of the actual PAHs present, field concentrations, prevailing light exposure and the types of organisms that are likely vulnerable to potential phototoxicity would again seem to be the best approach.

6. CURRENT ENVIRONMENTAL RISK ASSESSMENT APPROACH FOR PETROLEUM PRODUCTS AND THE COMPLEXITIES OF PAHS

The compositional complexity of many petroleum hydrocarbon substances originates from the fact that they are derived from crude oil. This compositional complexity poses particular problems when environmental risk assessment is required. Difficulties in carrying out a risk assessment for petroleum substances arise because individual components of them have specific and different physical-chemical and ecotoxicological properties, and potentials to be partitioned between and degraded in various environmental compartments. This means that on release to the environment, each component will behave independently and reach a concentration in each environmental compartment. It follows, that a PEC for the whole petroleum substance does not exist. It would in theory, be possible to identify each individual component of a petroleum substance and then to determine a PEC for each constituent. In practice this approach demands a degree of analytical resolution that is not achievable for most petroleum substances and even if possible, handling such large quantities of data would be impractical. However, since hydrocarbons of similar structure will have similar physical-chemical properties and potentials to be degraded in the environment they will have similar distributions and fates within a given environmental compartment. It is therefore possible to group or "block" such hydrocarbons, so that components having similar properties may be considered together (it should be recognised that a "block" may consist of a single component or a large number of components with similar fate and distribution properties). Once the "blocks" for a substance have been established, PEC values can be calculated for each representative structure that is used to simulate the "block" for each environmental compartment. Given that PECs can only be obtained for single components, it follows that PNECs must also be estimated for the same individual components and that a further assumption is that these should show similar modes of action. This approach is described in further detail by (Redman et al, 2012 and in the PETRORISK Manual (HydroQual, 2010)).

From the above discussion it is clear that the PEC/PNEC ratio of the whole substance cannot be derived directly, as neither the PEC, nor the PNEC for the whole substance will be available. The PEC/PNEC ratio is therefore derived from the PEC/PNEC ratios of the of components that are used to simulate the blocks, based on the proportional contribution of each of the "blocks" to the composition of the whole substance, and assuming that effects will be concentration additive. This is referred to as the Hydrocarbon Block Method (HBM) (ECHA, 2008).

Even when the HBM is applied to PAHs two problems remain in trying to incorporate photo-induced behaviour into the assessment;

- Firstly, the individual PAHs, as has been noted in the previous sections, vary considerably in their reactivity and thus the impact of light. Consequently, it would not be possible to develop blocks based on PAHs that incorporated their photo-induced behaviour correctly;
- Secondly, as discussed in the previous sections, the extent to which the photo-induced behaviour of the PAHs occurs is significantly affected by the environment within which these are present. Thus a generic environmental risk assessment is inappropriate.

An example of the complexities of accounting for the potential impact of photo-induced fate and effects may be gained from the following study by Barron et al, 2000. In their study (Barron et al, 2000), UV-A was found to range from 460-1100 µW/cm² at noon at a 0.1 m depth in a variety of aquatic habits examined during the summer in California (corresponding in latitude to southern Europe). However, UV-A intensities at noon were found to drop below 40 µW/cm² at 0.3 to 0.5 m. Moreover, average light intensities over the daily photoperiod are approximately a factor of three lower than peak values at noon. Additionally, the presence of dissolved organic carbon present in natural waters can further mitigate photosensitization by either competing with the parent PAH for UV light absorption and/or reducing PAH bioavailability thereby limiting uptake by aquatic organisms (Valentine & Zepp, 1993; Gensemer et al, 1998; Nikkila et al, 1999).

Given the limited scale over which photo-toxicity concerns are expected, difficulties in extrapolating laboratory results to the field and the lack of an accepted methodology for quantitatively considering the photo-induced toxicity of PAHs in an EU risk assessment, it is proposed to address this concern qualitatively in a risk characterization.

For the generic risk assessment of a product, PNECs for PAHs should be developed using only toxicity data that is not confounded by the presence of UV light, essentially as described in PETROTOX. These PNECs should be determined based on the results of reliable chronic data for Daphnia sp. and algae and mode of action-based considerations, for example see HydroQual (2002). The PEC/PNEC ratios for the hydrocarbon blocks can then be determined for local and regional scenarios in accordance with the REACH Technical Guidance Documents.

In cases that a given scenario has the potential for raising concerns arising from the potential effect of photo-induced toxicity, it is recommended that the risk assessment should be refined in a tiered manner, if possible, by obtaining site-specific modelled or measured data.

Further discussions on potential methods for addressing PAHs in site-specific assessments are addressed in the next section.

7. POTENTIAL SITE-SPECIFIC RISK ASSESSMENT APPROACHES ADDRESSING PAHS

To address the potential impacts of PAHs in site-specific situations, information relating to simultaneous field exposure of light and dissolved PAHs in water and tissue as well as the ecotoxicity of the PAHs under differing light conditions needs to be considered. The following section discusses a number of options that address one or both of these information needs.

Ankley et al (1997) compared predictions of toxicity using a model described in an earlier paper, Ankley et al (1995), with that of measured toxicity of anthracene, pyrene, fluorene and fluoranthene to *Lumbriculus variegatus*. The results were able to demonstrate that the previously described SAR had utility in predicting the ecotoxicity of phototoxic PAHs.

An alternative approach to establishing the ecotoxicity information was described by Scott et al (1996), who measured the effect that PAHs had to *Ceriodaphnia dubia* under differing light conditions and with various site-specific derived samples, including wet-weather run-off. The authors describe what they called a “significant correlation” between in situ *C. dubia* survival and turbidity when organisms were exposed to sunlight.

In “A Review of the Anthracene Risk Assessment Document” (Hydroqual, 2002), two alternative approaches to carrying out site specific risk assessments were described. In the first, Mount et al (2001), developed a scenario for addressing the risks of PAHs, using the following assumptions;

- The most sensitive species are fish fry or oligochaetes
- UV exposure is constant and equal to that of a mid-summer day. Therefore a 14-hour LT50 is the effect threshold
- The dose-response is a function of the UV exposure and tissue residue
- The organism is at equilibrium with the water column
- No metabolism of PAHs occurs in the organism (most conservative case)
- PAH toxicity of mixtures is additive
- PAH potency is that described by the HOMO-LUMO QSAR (Figure 5 of HydroQual, 2002).

Finally, as described in HydroQual (2002) and by Mount et al (2001), site-specific PAH exposure measurements would also be required as model inputs.

The second example given was based on Weinstein and Polk (2001), who examined the phototoxicity of anthracene to mussels. By conducting toxicity tests with different UV intensities and measuring the BCF of anthracene in the species being addressed, it was possible to relate toxicity to light intensity and the concentration of anthracene. Then, using these BCFs and toxicity data, plus information on water concentrations at different water depths, predicted effects could be estimated.

An approach addressing a PAH contaminated sediment site was described by Rosen et al (2012), who used a comprehensive, weight-of-evidence based ecological risk assessment approach. In this study, laboratory and in situ bioaccumulation and toxicity testing was integrated with passive sampler devices, hydrological characterization tools, continuous water quality sensing, and multi-phase chemical analyses. Results suggested observed toxicity at one station may have been due to PAH photo-activation thereby explaining the differences between in situ and laboratory amphipod survival. These authors were also able to demonstrate that PAH concentrations on solid-phase micro-extraction (SPME) fibers were positively correlated with in situ PAH bioaccumulation in amphipods. This study indicates the utility of these analytical tools as biomimetic surrogates and demonstrate the linkage between PAH bioavailability and effects in the field.

As described by McDonald and Chapman (2002), laboratory studies describe hazard and do not address risk. The authors conclude that the ecological relevance of the observed phototoxicity behaviour of some PAHs has yet to be described and future work should address risk, not hazard. Field determinations of population impairment at PAH-contaminated sites should be integrated with appropriate investigative/manipulative studies involving ecologically relevant exposure scenarios (e.g. field re-colonisation experiments as described by West et al, 2001), including determination of causation.

Based on work summarized above, key components of a tiered site-specific approach could include:

- Determination of likelihood of combined UV-light and PAH exposure and the presence of potentially vulnerable species/life-stages at the site;
- Identification of the PAHs to be addressed, as not all PAHs are phototoxic or photodegradable. Such an identification allows for a site-specific approach to be targeted, e.g. Weinstein and Polk (2001). Characterisation of relevant site-specific conditions include light measurements and light attenuation (including spectra assessment), and their fluctuations or stabilities over time, see Diamond et al (2006);
- Development of toxicity data for PAHs of interest which can be used to account for differences in light intensity and attenuation in the field as well as specific site interactions. Examples include, Scott et al (1996) and Mount et al (2001);
- Use of a model that allows for both hazard and exposure data to be integrated into a risk assessment paradigm, see Mount et al (2001);
- Measurements of site exposure concentrations of selected PAHs and/or in-situ toxicity data, see Rosen et al (2012).

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9. GLOSSARY

ACR	Acute to Chronic Ratio
BCF	Bioconcentration Factor – the ratio of the concentration of a substance in an aquatic organism to that of the concentration of the same substance in water at steady state.
BioHCwin	A Computer program was developed specifically for the biodegradation half-life prediction of petroleum hydrocarbons.
BMF	Biomagnification Factor - the ratio of the concentration of a substance in an organism to that of the concentration of the same substance in its diet at steady state.
BSAF	Biota-Sediment Accumulation Factor - the ratio of the concentration of a substance in an organism to that of the concentration of the same substance in sediment at steady state.
DNA	Deoxyribonucleic acid
DBT	Dibenzothiophene is an organo-sulphur molecule consisting of two benzene rings fused to a central thiophene ring.
DMBA	Dimethylbenz(a)anthracene
DOC	Dissolved Organic Carbon
DOM	Dissolved Organic Matter
EC_{50}	The Effect Concentration affecting 50 5 of the tested population
ECHA	European Chemicals Agency
eV	Electron Volt
FEF	Fluoranthene Equivalent Factors
HBM	Hydrocarbon Block Method – an approach used for the environmental risk assessment of complex hydrocarbons that allows for the grouping of hydrocarbons of a similar structure and environmental behaviour.
H-L-G	HOMO-LUMO Gap
HOMO	Highest Occupied Molecular Orbital – a molecular descriptor used in QSAR modelling corresponding to the highest energy level containing electrons in a molecule.

LUMO	Lowest Unoccupied Molecular Orbital – a molecular descriptor used in QSAR modelling corresponding to the lowest energy level containing no electrons in a molecule.
OECD	The Organisation for Economic Co-operation and Development
PAHs	Polycyclic Aromatic Hydrocarbons – comprised of three or more fused five and/or six member-ring aromatic compounds.
PEC	Predicted Environmental Concentration.
PETRORISK	Excel based model used to implement the Hydrocarbon Block Method for environmental risk assessment approaches petroleum products.
PETROTOX	Model that assumes narcosis mode of action for the effect of hydrocarbons on aquatic organisms, and utilising compositional information predicts the toxicity of petroleum products.
PHOTODEGRADATION	Process by which a molecule is altered via interactions from light.
PNEC	Predicted No Effect Concentration.
POM	Particulate Organic Matter
QSAR	Quantitative Structure Activity Relationship – a modelling process that uses properties derived from a molecule (measured or calculated) to predict some other property, often an activity, for example toxicity to fish.
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals Regulation (EU 1907/2006)
SPME	Solid Phase Micro Extraction
SUVR	Solar Ultra-Violet Radiation
TLM	Target Lipid Model
TMF	Trophic Magnification Factor - the average increase in the ratio of concentration of a substance in the environment, going from one trophic level to another.
UV	Ultraviolet
UVCBs	Substances of Unknown, Variable Composition or Biological Origin

APPENDIX 1: PREDICTED AND MEASURED BIODEGRADATION HALF LIVES (LAMPI, ET AL 2010)

Hydrocarbon	Carbon nr	BioHCwin Predicted Half-Life (days)	Measured Seawater Half-Life (days)	Measured Freshwater Half-Life (days)
fluorene	13	15.1		2.5
methylfluorene	14	24.2		4.2
phenanthrene	14	15.0	5.0	2.6
1-methylphenanthrene	15	23.9	2.8	
2-methylphenanthrene	15	23.9	4.2	
3-methylphenanthrene	15	23.9	4.2	
9-methylanthracene	15	196.7	6.0	
9-methylphenanthrene	15	23.9	4.2	
fluoranthene	16	191.4	9.2	
pyrene	16	283.4	151	
1-methylpyrene	17	108.8	64.0	
benzo(b)fluorene	17	347.6	4.2	
1-methyl-7-(1methylethyl)-phenanthrene	18	56.0	20	
9-n-butylphenanthrene	18	22.2	72.0	
benzo[a]anthracene	18	343.8	> 182	
chrysene	18	343.8	> 182	
m-terphenyl	18	6.7	15	
o-terphenyl	18	6.7	> 182	
triphenylene	18	343.8	> 182	
7-methylbenz(a)anthracene	19	131.9	4.7	
benzo(k)fluoranthene	20	284.7	11.4	
benzo[a]pyrene	20	421.6	16.5	

APPENDIX 2A: FISH BIOCONCENTRATION FACTORS (BCF) (LAMPI AND PARKERTON, 2009)

PAH	Lipid-normalized BCF	BCF from reference	% Lipid	Exp. Dur.	Method	Reliability rank	Comment (reason for rank)	Reference
Acenaphthene	**979 (le) carp**	489-1000	3.8	56 d	FT	2	OECD; all details not reported (ie. variations in exposure concentrations)	(CITI 1992)
	1003 (he) carp	254-1270	3.8	56 d	FT	2	OECD; all details not reported (ie. variations in exposure concentrations)	(CITI 1992)
	275 turbot	165	3	21 d	FT	4	Unclear how BCF calculated (what concentration from the series was used).	(Baussant et al. 2001a)
Acenaphthylene	**579, 596 carp**	271, 279	2.33, 2.34	28 d	FT	1	OECD 305	(Yakata et al. 2006)
	507 (le) carp	225-545	3.8	56 d	FT	2	OECD; all details not reported (ie. variations in exposure concentrations)	(CITI 1992)
	488 (he) carp	237-505	3.8	56 d	FT	2	OECD; all details not reported (ie. variations in exposure conc)	(CITI 1992)
	67 rainbow trout	1 d ($t_{0.5}$)	8	5 d	D	2	No standard dietary test guideline available.	(Niimi and Dookhran 1989)
	2665 turbot	1599	3	21 d	FT	4	Unclear how BCF calculated (what concentration from the series was used).	(Baussant et al. 2001a)
Benz[a] anthracene	**977 rainbow trout**	0.7 d ($t_{0.5}$)	2.4	10 d	D	2	No standard dietary test guideline available.	(EMBSI 2005)
	90 rainbow trout	2 d ($t_{0.5}$)	8	48 d	D	2	No standard dietary test guideline available.	(Niimi and Palazzo 1986)
	260 fathead minnow	260	NR	5-336 h	FT	4	Only aqueous BCF study available; lipid content not provided.	(de Maagd et al. 1998)
Benzo[a]pyrene	**977 rainbow trout**	1.1 d ($t_{0.5}$)	2.4	10 d	D	2	No standard dietary test guideline available.	(EMBSI 2005)

PAH	Lipid-normalized BCF	BCF from reference	% Lipid	Exp. Dur.	Method	Reliability rank	Comment (reason for rank)	Reference
	172 rainbow trout	2 d ($t_{0.5}$)	8	48 d	D	2	No standard dietary test guideline available.	(Niimi and Palazzo 1986)
Benzo[b] fluoranthene	No data							
Benzo[k] fluoranthene	**327 rainbow trout**	0.6 d ($t_{0.5}$)	5.6	10	D	2	No standard dietary test guideline available.	(EMBSI 2007a)
	423 carp	0.6 d ($t_{0.5}$)	3.6	10	D	2	No standard dietary test guideline available.	(EMBSI 2007b)
Benzo[ghi] perylene	**303 rainbow trout**	0.6 d ($t_{0.5}$)	3.3	7 d	D	2	No standard dietary test guideline available.	(EMBSI 2008)
Chrysene	**153 rainbow trout**	435	1.27	28 d	SR	2	Not guideline study, but long-term, static renewal exposure	(EMBSI 2009)
	90 rainbow trout	2 d ($t_{0.5}$)	8	48 d	D	2	No standard dietary test guideline available.	(Niimi and Palazzo 1986)
	615	0.8 d ($t_{0.5}$)	3.6	7 d	D	2	No standard dietary test guideline available.	EMBSI (2008)
	2105 rainbow trout	2.1 d ($t_{0.5}$)	3.2	14 d	D	2	No standard dietary test guideline available.	(EMBSI 2006)
	90 turbot	54	3	21 d	FT	4	BCF calculated using time variable water concentrations that are not reported.	(Baussant et al. 2001b)
Dibenz[a,h] anthracene	**1466 rainbow trout**	0.9 d ($t_{0.5}$)	2.4	10 d	D	2	No standard dietary test guideline available.	(EMBSI 2005)
Fluoranthene	**435 rainbow trout**	435	1.27	28 d	SR	2	Not guideline study, but long-term, static renewal exposure	(EMBSI 2009)
	1931 fathead minnow	1700	4.4	28 d	F	2	BCF from lowest concentration.	(Carlson et al. 1979)
	218 rainbow trout	0.4 d ($t_{0.5}$)	5.6	10 d	D	2	No standard dietary test guideline available.	(EMBSI 2007a)

PAH	Lipid-normalized BCF	BCF from reference	% Lipid	Exp. Dur.	Method	Reliability rank	Comment (reason for rank)	Reference
	352 carp	2.1 d ($t_{0.5}$)	3.6	10 d	D	2	No standard dietary test guideline available.	(EMBSI 2007b)
	271 rainbow trout	6 d ($t_{0.5}$)	8	48 d	D	2	No standard dietary test guideline available.	(Niimi and Palazzo 1986)
	1358 fathead minnow	9054	NR	24 h	S	4	BCF in paper was dry wt., short duration, little concentration information.	(Weinstein and Oris 1999)
	N/A fathead minnow	14836	NR	24 h	FT	3	Ambiguous units; short exposure time (24 h); no lipid info presented; concentrations likely toxic.	(Cho et al. 2003)
	N/A fathead minnow	3388	NR	48 h	S	3	Short duration, decreasing concentrations, limited samples.	(de Maagd 1996)
Fluorene	**672 (le) carp**	219-830	3.9	56 d	FT	2	OECD; all details not reported (ie. variations in exposure conc).	(CITI 1992)
	780 (he) carp	396-821	3.9	56 d	FT	2	OECD; all details not reported (ie. variations in exposure conc).	(CITI 1992)
	1875 fathead minnow	1650	4.4	28 d	FT	2	Not guideline study, but long-term, flow-through exposure.	(Carlson et al. 1979) Experiment 3, Tank 3
	1146 fathead minnow	1100	4.8	28 d	FT	2	Not guideline study, but long-term, flow-through exposure.	(Carlson et al. 1979) Experiment 1, Tank 1
	316 rainbow trout	7 d ($t_{0.5}$)	8	48 d	D	2	No standard dietary test guideline available.	(Niimi and Palazzo 1986)
	270 bluegill	1800	NR	30 d	FT	4	No lipid info, dry wt used.	Finger et al 1985)(
	1655 turbot	993	3	21 d	FT	4	Unclear how BCF calculated (what concentration from the series was used).	(Baussant et al. 2001a)

PAH	Lipid-normalized BCF	BCF from reference	% Lipid	Exp. Dur.	Method	Reliability rank	Comment (reason for rank)	Reference
	2418 turbot	1495	3	21 d	FT	4	BCF calculated using time variable water concentrations that are not reported.	(Baussant et al. 2001b)
	1177 guppy	2120	9	2 d	S	4	n = 2; no feeding information; no water or fish concentration information	(de Voogt et al. 1991)
Indeno[1,2,3-cd] pyrene	**303 rainbow trout**	0.6 d ($t_{0.5}$)	3.3	7 d	D	2	No standard dietary test guideline available.	(EMBSI 2008)
Naphthalene	**515 (le) sheepshead minnow**	999	9.7	36 d	FT	1	Very similar to OECD 305: high quality, long-term, flow-through study.	(Jonsson et al. 2004)
	461 (he) sheepshead minnow	895	9.7	36 d	FT	1	Very similar to OECD 305: high quality, long-term, flow-through study.	(Jonsson et al. 2004)
	85 (le) carp	23-146	NR	56 d	FT	2	OECD; all details not reported (ie. variations in exposure conc).	(CITI 1992)
	102 (he) carp	36.5-168	NR	56 d	FT	2	OECD; all details not reported (ie. variations in exposure conc).	(CITI 1992)
	814 rainbow trout	0.4 d ($t_{0.5}$)	2.4	10 d	D	2	No standard dietary test guideline available.	(EMBSI 2005)
	702 turbot	421	3	21 d	FT	4	BCF calculated using time variable water concentrations that are not reported.	(Baussant et al. 2001b)
	N/A fathead minnow	302	NR	48 h	S	3	Short duration, decreasing concentrations, limited samples.	(de Maagd 1996)
Phenanthrene	**1149 (le) sheepshead minnow**	2229	9.7	36 d	FT	1	Very similar to OECD 305: high quality, long-term, flow-through study.	(Jonsson et al. 2004)

PAH	Lipid-normalized BCF	BCF from reference	% Lipid	Exp. Dur.	Method	Reliability rank	Comment (reason for rank)	Reference
	417 (he) sheepshead minnow	810	9.7	36 d	FT	1	Very similar to OECD 305: high quality, long-term, flow-through study.	(Jonsson et al. 2004)
	2329 fathead minnow	2050	4.4	28 d	FT	2	Not guideline study, but long-term, flow-through exposure.	(Carlson et al. 1979) Experiment 3, Tank 3
	3546 fathead minnow	3050	4.3	28d	FT	2	Not guideline study, but long-term, flow-through exposure.	(Carlson et al. 1979) Experiment 3, Tank 2
	2927 fathead minnow	2400	4.1	28 d	FT	2	Not guideline study, but long-term, flow-through exposure.	(Carlson et al. 1979) Experiment 3, Tank 1
	3684 fathead minnow	2800	3.8	7/10 d	FT	2	BCF averaged from last 2 time points (7+10 d) at steady-state. Higher, non-steady state BCFs observed after this time were accompanied by decreasing water PAH concentration.	(Carlson et al. 1979) Experiment 2, Tank 2
	2218 rainbow trout	1.7 d ($t_{0.5}$)	2.8	10 d	D	2	No standard dietary test guideline available.	(EMBSI 2001)
	407 rainbow trout	9 d ($t_{0.5}$)	8	48 d	D	2	No standard dietary test guideline available.	(Niimi and Palazzo 1986)
	515 turbot	309	3	21 d	FT	4	Unclear how BCF calculated (what concentration from the series was used).	(Baussant et al. 2001a)
	1560 turbot	936	3	21 d	FT	4	BCF calculated using time variable water concentrations that are not reported.	(Baussant et al. 2001b)
	2604 fathead minnow	2500	4.8	28d	FT	3	Decreasing concentrations.	(Carlson et al. 1979) Experiment 2, Tank 1

PAH	Lipid-normalized BCF	BCF from reference	% Lipid	Exp. Dur.	Method	Reliability rank	Comment (reason for rank)	Reference
	N/A fathead minnow	6760	NR	48 h	S	3	Short duration, decreasing concentrations, limited samples, water concentrations approaching LC50	(de Maagd 1996)
Pyrene	**75 (le) sheepshead minnow**	97	9.7	36 d	FT	1	Very similar to OECD 305: high quality, long-term, flow-through study.	(Jonsson et al. 2004)
	50 (he) sheepshead minnow	145	9.7	36 d	FT	1	Very similar to OECD 305: high quality, long-term, flow-through study.	(Jonsson et al. 2004)
	892 fathead minnow	785	4.4	28 d	FT	2	BCF from exposure at lowest concentration.	(Carlson et al. 1979)
	977 rainbow trout	0.7 d ($t_{0.5}$)	2.4	10 d	D	2	No standard dietary test guideline available.	(EMBSI 2005)
	90 rainbow trout	2 d ($t_{0.5}$)	8	48 d	D	2	No standard dietary test guideline available.	(Niimi and Palazzo 1986)
	1088 guppy	1959	9	2 d	S	4	n = 2; no feeding information; no water or fish concentration information.	(de Voogt et al. 1991)

Preferred values are in **bold**. Dashed line (**- - - -**) separates high quality data from lower quality data.

Abbreviations: he-high exposure concentration; le-low exposure concentration; NR-not reported; S-static; SR-static renewal; FT-flow through; D-dietary.

Reliability ranking: 1 – reliable without restrictions; 2 – reliable with restrictions; 3 – unreliable; 4 – not assignable.

APPENDIX 2B: LIPID NORMALIZED DIETARY BIOMAGNIFICATION FACTORS (BMF) (LAMPI AND PARKERTON, 2009)[1]

PAH	BMF	Reference
Acenaphthylene	0.005	(Niimi and Dookhran 1989)
Benz[a]anthracene	0.005	(EMBSI 2005)
	0.001	(Niimi and Palazzo 1986)
Benzo[a]pyrene	0.005	(EMBSI 2005)
Benzo[g,h,i]perylene	0.032	(EMBSI 2007c)
Benzo[k]fluoranthene	0.012	(EMBSI 2007a)
	0.009 (Carp)	(EMBSI 2007b)
Chrysene	0.016, 0.04	(EMBSI 2006, EMBSI 2008a)
Dibenz[a,h]anthracene	0.007	(EMBSI 2005)
Fluoranthene	0.032, 0.046	(EMBSI 2007a, EMBSI 2008b)
	0.006 (Carp)	(EMBSI 2007b)
Fluorene	0.03	(Niimi and Palazzo 1986)
Indeno[1,2,3-cd]pyrene	0.029	(EMBSI 2007c)
Naphthalene	0.005	(EMBSI 2005)
Phenanthrene	0.076	(EMBSI 2001)
	0.01	(Niimi and Palazzo 1986)
Pyrene	0.005	(EMBSI 2005)
Benzo[b]fluorene	0.041, 0.024	(EMBSI 2007a, EMBSI 2008b)
	0.01	(EMBSI 2007b)
Triphenylene	0.026	(EMBSI 2007a)
	0.018	(EMBSI 2007b)

[1] All values are for rainbow trout except where noted

APPENDIX 2C: 2A/2B REFERENCES

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