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Guidance for improving comparability and relevance of oil toxicity tests

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ABSTRACT

The complex nature and limited aqueous solubility of petroleum substances pose challenges for consistently characterizing exposures in aquatic life hazard assessments. This paper reviews important considerations for the design, conduct and interpretation of laboratory toxicity tests with physically and chemically dispersed oils based on an understanding of the behavior and toxicity of the hydrocarbons that comprise these substances. Guiding principles are provided that emphasize the critical need to understand and, when possible, characterize dissolved hydrocarbon exposures that dictate observed toxicity in these tests. These principles provide a consistent framework for interpreting toxicity studies performed using different substances and test methods by allowing varying dissolved exposures to be expressed in terms of a common metric based on toxic units (TUs). The use of passive sampling methods is also advocated since such analyses provide an analytical surrogate for TUs. The proposed guidance is translated into a series of questions that can be used in evaluating existing data and in guiding design of future studies. Application of these questions to a number of recent publications indicates such considerations are often ignored, thus perpetuating the difficulty of interpreting and comparing results between studies and limiting data use in objective hazard assessment. Greater attention to these principles will increase the comparability and utility of oil toxicity data in decision-making.

1. Introduction

The composition of petroleum substances is complex, variable and often characterized by generally limited aqueous solubility. These attributes pose significant challenges for evaluating the hazard of these substances to aquatic and marine life. Further, differences in the design of aquatic toxicity studies often compound the difficulty of comparing and correctly applying toxicity test data in decision-making (Landrum et al., 2011; Lewis and Pryor, 2013; Bejarano et al., 2014).

Toxicity testing of petroleum substances has varied objectives, which influence the choice of exposure system. In some cases open test systems are used to simulate the loss of more volatile hydrocarbon components that would occur during a spill to a water surface. In contrast, closed test systems may be used to for comparing hazard across petroleum substances. Chemical regulations require testing to support substance classification and labeling (UN, 2009; King et al., 2001), which relies on water accommodated fractions (WAF) using multiple substance loadings in closed test systems with standard test species and durations (Girling et al., 1992; OECD, 2002). Toxicity assessments of dispersed oils transported in commerce may be performed to identify comparative hazards in the event of a spill or evaluate potential spill impacts. Such hazard data may be generated by different methods including testing of variable oil loadings (Cohen et al., 2014) or WAF dilutions (Anderson, 1985), use of continuously diluted WAFs mimicking spill events in the field (Clark et al., 2001), or use of oiled substrates to mimic longer term exposures of weathered oil components (Carls et al., 1999; Heintz et al., 1999; Brannon et al., 2006). These latter two exposure systems, in particular, result in time-variable exposures. Such testing may also involve the application of chemical dispersants to evaluate if the dispersant modulates oil toxicity. Such data are used for guiding decisions on dispersant use in oil spill response (Hemmer et al., 2011; USEPA, 2015). The use of chemical dispersants can alter exposure by increasing the concentrations of both dissolved and particulate (i.e. oil droplets) hydrocarbons (Rhoton et al., 2001; Davies et al., 1998).

An essential element of hazard assessment is the establishment of reproducible concentration–response relationships. A recurring challenge in hazard evaluation of petroleum substances is that multiple exposure metrics are routinely used for establishing exposure-effect relationships and interpreting toxicity test results. For example, nominal loading, percent WAF dilution, total

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petroleum hydrocarbons (TPH) or total petroleum aromatic hydrocarbons (TPAH) are commonly reported in the analysis of toxicity datasets (Bejarano et al., 2014).

Following the tragic *Deepwater Horizon* blowout and subsequent release of oil into the Gulf of Mexico, over one billion dollars of research funding has been made available (GOMRI, 2010; Shen, 2013). These funds include support to further study the effects of physically and chemically dispersed oil on diverse marine life. Such initiatives afford an unprecedented opportunity to gain knowledge for preventing and responding to future oil spills, including building on our current mechanistic understanding of toxicity to improve hazard and risk assessment of petroleum substances. However, there is a growing recognition that such advancement will be predicated on the quality of ecotoxicity research that ensures adequate characterization of test substance exposures (Harris et al., 2014). Further, failure to adequately characterize exposure in oil toxicity studies can result in unsupported conclusions that can unintentionally misguide decision-making (Coelho et al., 2013; Prince and Parkerton, 2014).

The objectives of this paper are to review different experimental methods that have been applied in past studies for performing aquatic toxicity studies with petroleum substances. Examples of recently published studies are used to illustrate how different experimental approaches can confuse comparison and interpretation across studies. Our intent is not to advocate a specific testing approach but rather to promote an understanding of how study design can alter exposures and thereby influence test interpretation. We then outline recommendations for improving exposure characterization in future oil toxicity studies that will increase comparability and improve use of such data in hazard and risk assessments including net environmental benefit analysis for evaluating trade-offs of dispersant use in spill response (Bejarano et al., 2014.

2. Methods

Model predictions were performed using the PETROTOX model (Redman et al., 2012a), which assumes a closed WAF system at steady-state. Compositional information was taken from available sources for gasoline, gas oil (McGrath et al., 2005), heavy fuel oil (HFO) (Redman et al., 2014) and Endicott crude oil (Supplemental Information). This manuscript relies on the Target Lipid Model (TLM) and PETROTOX models (McGrath and Di Toro, 2009; Redman et al., 2012a) to predict the bioavailability and toxicity of petroleum substances. These are convenient models as they have been applied to a wide range of individual hydrocarbons and petroleum substances. PETROTOX models the equilibration of petroleum substances in closed WAF systems including both the dissolution into water and partitioning to headspace based physicochemical properties of representative hydrocarbons used to simulate substance composition.

3. Study design considerations

Typical study design considerations are given in Table 1. This list is not exhaustive but provides a general framework of key decisions that must be addressed when conducting aquatic toxicity testing with petroleum substances.

3.1. Substance selection

The oil composition is a key consideration since it influences the concentration of dissolved phase hydrocarbons (Redman et al., 2012a). The composition of an oil substance varies as a function of geographic source, refining (e.g., crude oil vs gasoline), and weathering (Speight, 1998). The composition of crude oils can include a range of constituents from lighter hydrocarbons, such as are found in gasoline, to much higher molecular weight compounds, such as are found in bitumen. Refined substances can be prepared through distillation or other separation methods to target certain structural moieties. As a result their composition ranges from substances with a well-defined composition of individual components like gasoline, to categories of substances which include a broad range of constituents from different hydrocarbon classes and carbon numbers like gas oils or HFO (CONCAWE, 2012).

The choice of test substance has implications for selection of subsequent elements of the study design. For example, substances with lighter constituents can volatilize thereby affecting the dissolved phase exposure. Additional analytical characterization of the oil and WAF may be needed to quantify exposure to lighter constituents. In contrast, heavier substances such as high boiling point distillate fuels (e.g., heavy fuel oils) or weathered oils may not contain these constituents so such analyses would provide little value. However, for many fresh crude oils, such as Alaska North Slope or South Louisiana Light, there can be sufficient concentrations of benzene, toluene, ethylbenzene, xylenes (BTEX), and other lighter components, which can contribute substantially to toxicity (Di Toro et al., 2007; Pelz et al., 2012).

3.2. Dosing method

Exposure to substances with lighter components is impacted by the nature and dimensions of exposure chambers since these components will partition to available headspace, or the overlying atmosphere in the case of open test chambers. Volatilization losses in both open and closed test systems will reduce the amount of constituents in the aqueous medium potentially resulting in reduced toxicity. This is particularly important for lighter refined petroleum substances containing $<C_{12}$ constituents such as naphtha streams and gasoline (McGrath et al., 2005).

Depending on the mixing energy, or water flow in case of oiled gravel tests, and/or the presence of chemical dispersants a substantial amount of entrained droplets can occur that are carried into the exposure chamber (Martin et al., 2014). Droplets have the potential to interact with test organisms (Hansen et al., 2012; Nordtug et al., 2011a) and can act as source of dissolved hydrocarbons when diluted into aqueous test media (Redman et al., 2014).

Dissolved exposures will depend on the substance loading in the WAF preparation vessels, or an effective loading in terms of an oiled substrate (Carls et al., 1999) or nozzle-delivered (Nordtug et al., 2011b) dosing method. Aquatic hazard classification and labeling of petroleum substances is determined using multiple oil loadings which accounts for substance-specific differences in solubility behavior of the underlying hydrocarbon constituents comprising the substance (King et al., 2001; UN, 2009). This approach results in treatments with different compositions of dissolved components. As a result, other investigators have often used a dilution series prepared at a single fixed nominal oil loading in an attempt to maintain similar exposure compositions in treatments. Fig. 1 illustrates the two most common WAF preparation methods for evaluating the aquatic toxicity of physically and chemically dispersed oils. WAF preparation using multiple loadings is depicted from top to bottom while serial dilutions are shown from left to right. The two WAF test systems pictured (top vs bottom test chambers) contrast the differences in oil droplet exposures that can result from different dosing procedures.

3.3. Exposure regime

Since hydrocarbons can degrade, volatilize and partition out of the aqueous phase the duration and nature of the exposure are