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Aquatic toxicity structure-activity relationships for the zwitterionic surfactant alkyl dimethyl amine oxide to several aquatic species and a resulting species sensitivity distribution

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ABSTRACT

Amine oxide (AO) is a cationically charged surfactant at environmental pH and has previously been assessed in the OECD (Organization for Economic Cooperation and Development) High Production Volume (HPV) chemicals program. Typical of cationic chemicals, AO is highly aquatically toxic. In this study we vastly improve the knowledge of AO toxicity by developing acute Quantitative Structure Activity Relationships (QSARs) for an alga (*Desmodesmus subspicatus*), an invertebrate (*Daphnia magna*) and a fish (*Danio rerio*) using the appropriate array of OECD Test Guidelines. A chronic toxicity QSAR was also determined for the most sensitive taxon, *Desmodesmus*. Pure AO spanning the chain lengths of C8 to C16 were tested individually with trace analytical confirmation of exposures in all tests. The QSARs were all of high quality (R^2 0.92–0.98) with slopes ranging from −0.338 to −0.484. QSARs were then used to normalize toxicity outcomes for a larger, previously published data set used in HPV, European REACH (Registration, Evaluation, and Authorization of Chemicals), and peer reviewed publications. Two additional species, *Lemna gibba* (macrophyte) and *Ankistrodesmus falcatus* (alga) were studied in exposures to dodecyl (C12) AO to provide sufficient taxonomic diversity to conduct a Species Sensitivity Distribution (SSD) analysis. The SSD 5th percentile hazardous concentration (HC5) to C12 AO was found to be 0.052 mg/L which is similar to an existing AO 28-d, 3-community periphyton community bioassay normalized to C12 AO (No-observed-effect-concentration or NOEC=0.152 mg/L). The statistical properties of the SSD was probed suggesting that new studies of additional taxa would be required that were at least 10-fold more sensitive than the most sensitive taxon to move the HC5 lower by a factor of 3. The overall AO hazard assessment suggests a large margin of safety relative to published environmental exposure data.

1. Introduction

Amine oxides (AO) are a group of amphoteric surfactants that change from net cationic to zwitterionic to nonionic as pH goes from low (5) to high (9) pH values. At typical environmental pH AO are cationic. The majority of AO are used in consumer and personal care cleaning contexts. Amine oxides were the subject of an OECD (Organization for Economic Cooperation and Development) HPV (High Production Volume) assessment in 2006 and estimated usage volumes were 26,000, 16,000, and 6800 metric tons in the United States, Europe and Japan respectively (OECD, 2006b). Currently (2015), amine oxide usage in the United States, Europe and Japan is 44,000, 25,000, and 3200 metric tons, respectively (Janshekar et al., 2016). The category was subjected to environmental risk assessment as summarized by Sanderson et al. (2009). An EU REACH dossier was compiled on the amine oxide category (http://echa.europa.eu/web/guest/information-on-chemicals/registered-substances and use the search term amine oxide) conveying information similar to that of Sanderson et al. (2009).

The aquatic toxicity of AO has been studied in detail primarily using a variety of technical mixtures. Algae appear to be the most sensitive group of aquatic organisms versus invertebrates and fish. The green algae *Desmodesmus* (formerly *Scenedesmus*) *subspicatus*, *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*) and *Chlorella vulgaris*, the diatoms *Diatoma elongatum* and *Navicula seminulum*, and the blue green cyanobacterium *Anabaena*

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flos-aquae had chronic toxicity values (No-observed-effect concentration [NOECs] or Effective Concentrations that adversely impact the population at 10% of the control [EC10s]) that ranged from 0.007 to 1.5 mg/L (Sanderson et al., 2009). *Desmosdemsus* and *Pseudokirchneriella* green algae were the most sensitive taxa tested with species geometric means of 0.024 and 0.019 mg/L, respectively. The 21 d NOEC for *Daphnia magna* reproduction was 0.354–0.7 mg/L in two different studies and a fish full life cycle (302-d) NOEC of 0.5 mg/L based on hatchability has also been described (Maki, 1979). The apparent sensitivity of algae to AO led a research team to investigate algal population and community dynamics in a 28 d periphyton microcosm study during exposure to C12.7 AO (Belanger et al., 1996). In this study, 3 distinct algal communities (two different river systems used as a colonization source, one river which had two different substrates colonized by periphyton) were simultaneously exposed in a flow through system at 5 different treatment levels of AO. Population and community metrics in replicated chambers were assessed weekly. Over one hundred algal species and 7 Divisions were present in the test system. A 28 d NOEC of 0.067 mg/L was concluded for all three communities (Belanger et al., 1996).

PNECs (predicted no-effect concentrations for ecosystems) associated with amine oxide are difficult to determine. AO aquatic toxicity studies have been conducted on a wide range of test materials that differ with respect to chain length. Fifteen different amine oxide CAS registry numbers are included in the HPV assessments for example (OECD, 2006b). It is well accepted that a key determinant of the toxicity of any particular amine oxide is the length of the alkyl hydrophobe. The holistic determination of the environmental hazard of numerous surfactant categories utilizes the concept of toxicity normalization (van de Plassche et al., 1999). Homologous structures that have been empirically tested for their aquatic toxicity are “normalized” to a set or targeted chain length using an appropriate QSAR, or Quantitative Structure Activity Relationship. QSARs relate physical-chemical property descriptors of a compound to a biological property (Roberts, 1991). The octanol-water partition coefficient (K_{ow}) is the most frequent property descriptor employed for describing surfactants as toxicity has long been considered to be driven by hydrophobicity (Roberts, 1992). In this manner, the aquatic hazard of anionic and nonionic surfactants have been assessed including linear alkylbenzene sulfonates (Belanger et al., 2016b; van de Plassche et al., 1999), alcohol sulfates (Dyer et al., 1997; van de Plassche et al., 1999); alcohol ethoxysulfates (Dyer et al., 2000; van de Plassche et al., 1999), alcohol ethoxylates (Belanger et al., 2006; Boeije et al., 2006; van de Plassche et al., 1999), and long chain alcohols (Belanger et al., 2009). Normalization is a valuable concept in that the use of a surfactant technical mixture in a product application, once disposed in wastewater, results in a very different distribution of related homologues entering river water receiving systems. For example, the predominant mixture of LAS used in the US is approximately 11.9 carbons long, however, the average leaving wastewater treatment is 11.26 (McDonough et al., 2016). The shift is due to preferential sorption and biodegradation of the somewhat longer alkyl chains. This has profound toxicological consequences as C11.26 LAS is about ½ the toxicity of C11.9 LAS to fish (Belanger et al., 2016b). Thus normalization is a means to link diverse empirical ecotoxicity data to complex wastewater treatment plant effluent scenarios where the mixture entering the environment is related, but not the same as that tested. Further, normalization provides a mechanism to compare technical mixtures by the same algorithms. Normalization QSARs can either be acute or chronic. Chronic QSARs are preferred, but the inference of acute QSARs having a highly similar slope to chronic QSARs when assessing closely related homologous structures appears to largely hold true (Boeije et al., 2006; Dyer et al., 1997, 2000). This is, in fact, an implicit assumption when extrapolating from acute to chronic and chronic to ecosystem in hazard assessment.

PNECs for AO have been established using both Species Sensitivity Distributions (SSD) and extrapolation of the periphyton microcosm results. For SSDs, the data set is marginal in that 8 taxa have been tested. In REACH (EC 2008), for example, a minimum of ten taxa are desirable. Additional application factors are usually applied for less robust data sets. The SSD for AO, normalized to C12.9, provided an HC5 (5th percentile of the hazardous concentration) of 0.023 mg/L (Sanderson et al., 2009). Similarly, the periphyton microcosm was normalized to C12.9 from a test material of C12.6 with a resulting NOEC of 0.050 mg/L. In both instances, the normalization algorithm utilized a QSAR derived from Fendinger et al. (Fendinger et al., 1994) for anionic surfactants because an amine oxide-specific QSAR was not available.

This paper summarizes a suite of experiments to develop a more robust SSD by (1) expanding the number of tested species to ten (an additional two species) and (2) by developing AO-specific QSARs for algae, invertebrates and fish. In this particular instance, acute QSARs are developed for each group and a chronic QSAR is developed for algae (OECD, 2006c) because algal chronic toxicity is a straightforward re-interpretation of the same data using a different statistic. Lastly, in order to minimize the use of fish in this endeavor, the fish embryo test (FET, OECD Test Guideline 236) (OECD, 2013), was used as an acute toxicity replacement assay for the standard fish acute toxicity test (OECD Test Guideline 203) (OECD, 1992). The FET has proven to be an exceptionally good predictor of surfactant acute toxicity while minimizes the use of protected life stages of fish (Belanger et al., 2013; Busquet et al., 2014; Vaughan and van Egmond, 2010).

Table 1
Test material descriptions for the linear alkyl dimethyl amine oxides used in aquatic toxicity investigations.

Compound	C8	C10	C12	C14	C16
CASNO	2605–78–9	2605–79–0	1643–20–5	3332–27–2	7128–91–8
MW (g/mol)	173.3	201.35	229.41	257.46	285.52
Percent active[a]	29.4	99.9	99.6	99.2	26.3
Physical state	colorless liquid	white powder	white powder	white powder	opaque white paste
Solubility	$>10^3$ mg/L	$\sim10^3$ mg/L	$\sim10^3$ mg/L	10–20 mg/L	~0.8 mg/L
Typical stock solution concentrations (mg/L)	5000 – FET 1000 – algae 1500 – *Daphnia*	1000 – FET 100 – algae, *Daphnia*	1000 – FET 100 – algae, *Daphnia*	10 – FET 10 – algae 5 – *Daphnia*	1 – FET 0.8 – algae 4 – *Daphnia*
Purity of deuterated internal standard (%)[b]	80.6	98	97.6	99.2	96

[a] Note that percent active above in Table 1 is lower for both C16 and C8 compounds as these were initially in a liquid state and that the balance of the solution was water.
[b] Impurities in deuterated standards were confirmed to not be unlabeled amine oxide.