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Aquatic hazard assessment of a commercial sample of naphthenic acids

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HIGHLIGHTS

- The 1-, 2-, 3-ring, and acyclic NAs accounted for 84% of the test sample.
- Toxicity endpoints ranged 9.0–46 mg L^{-1} (loading rates) for four species.
- Fish (*Pimephales promelas*) was the most sensitive of the test species.
- Biomimetic extraction is consistent with a nonpolar narcosis mode of toxic action.

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ABSTRACT

This paper presents chemical composition and aquatic toxicity characteristics of a commercial sample of naphthenic acids (NAs). Naphthenic acids are derived from the refining of petroleum middle distillates and can contribute to refinery effluent toxicity. NAs are also present in oil sands process-affected water (OSPW), but differences in the NAs compositions from these sources precludes using a common aquatic toxicity dataset to represent the aquatic hazards of NAs from both origins. Our chemical characterization of a commercial sample of NAs showed it to contain in order of abundance, 1-ring > 2-ring > acyclic > 3-ring acids (~84%). Also present were monoaromatic acids (7%) and non-acids (9%, polyaromatic hydrocarbons and sulfur heterocyclic compounds). While the acyclic acids were only the third most abundant group, the five most abundant individual compounds were identified as C_{10-14} *n*-acids (*n*-decanoic acid to *n*-tetradecanoic acid). Aquatic toxicity testing of fish (*Pimephales promelas*), invertebrate (*Daphnia magna*), algae (*Pseudokirchneriella subcapitata*), and bacteria (*Vibrio fischeri*) showed *P. promelas* to be the most sensitive species with 96-h LL_{50} = 9.0 mg L^{-1} (LC_{50} = 5.6 mg L^{-1}). Acute EL_{50} values for the other species ranged 24–46 mg L^{-1} (EC_{50} values ranged 20–30 mg L^{-1}). Biomimetic extraction via solid-phase-microextraction (BE–SPME) suggested a nonpolar narcosis mode of toxic action for *D. magna*, *P. subcapitata*, and *V. fischeri*. The BE analysis under-predicted fish toxicity, which indicates that a specific mode of action, besides narcosis, may be a factor for fishes.

1. Introduction

Naphthenic acids (NAs) are constituents in crude oil usually present at 0–3% (Brient et al., 1995; CEATAG, 1998). They are complex and composed predominantly of alkyl-substituted cycloaliphatic carboxylic and acyclic (paraffinic) acids. Rings may be fused or bridged, and the acyclic carboxylic acids may be straight-chain or highly branched (Rowland et al., 2011a,b,c). NAs are assigned the general formula $C_nH_{2n+z}O_2$ (Clemente and Fedorak, 2005), where the value n represents the number of carbon atoms, and Z may be zero or a negative even integer, reflecting the hydrogen deficiency resulting from ring formation (Clemente and Fedorak, 2005).

NAs derived from petroleum refining have boiling points that fall within the range of middle distillate petroleum streams (e.g., kerosene, diesel) (Brient et al., 1995). They cause corrosion problems in distillation units (Brient et al., 1995; CEATAG, 1998) and can contribute to the aquatic toxicity of refinery wastewater (Dorn, 1992). Owing to their corrosive nature, NAs are removed

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from middle distillates to improve burning qualities, storage stability, and odor of the finished fuels (Brient et al., 1995; Suarez, 1996). This removal process provides a source of crude NAs that can be further refined for use in emulsifying agents, wetting agents, anti-fungal agents, gelling agents, antioxidants, and dryers in oil-based paints (Brient et al., 1995; CEATAG, 1998). The extraction of bitumen from oil sands deposits generates oil sands process-affected water (OSPW), which can also contain 20–120 mg NAs L^{-1} (Holowenko et al., 2002; Headley and McMartin, 2004; NRC, 2010). To our knowledge no commercial NA preparations are derived from OSPW.

NAs are considered the key constituents responsible for the aquatic toxicity of OSPW and some refinery effluents (MacKinnon and Boerger, 1986; Dorn, 1992; Verbeek et al., 1994). Due to their ready availability, commercial NAs have at times been used as a surrogate for evaluating NAs from OSPW extracts. However, significant compositional variability exists in NAs from different sources and the use of commercial NAs as surrogates for OSPW NAs has been questioned (West et al., 2011). These include differences in (i) the ranges of molecular weights of constituent NAs (Clemente et al., 2003; Lo et al., 2003), (ii) principal ring structure groups (Clemente et al., 2004; Grewer et al., 2010; Rowland et al., 2011a,b,c), and (iii) the presence of oxy-NAs in OSPW extracts (Grewer et al., 2010). Commercial NAs and OSPW extracts also demonstrated differences in aquatic toxicity. Nero et al. (2006) reported that at equal NAs concentrations, commercial NAs were more toxic to yellow perch than OSPW extracts, and Peters et al. (2007) demonstrated in two fish species that commercial NAs showed lower thresholds for body deformities and growth reductions than OSPW extracts.

Since commercial NAs are transported throughout the world, an accurate hazard assessment of these substances and their potential effects on the aquatic environment is important. Early studies of the aquatic hazard of commercial NAs have limited value due to incomplete compositional and exposure concentration analysis. Additionally, compositional differences between OSPW extracts and commercial NAs may prevent a single aquatic hazard dataset from representing NAs from all sources (West et al., 2011). Therefore, the Petroleum HPV Testing Group conducted testing under the US EPA (1996) High Production Volume (HPV) Challenge Program to evaluate aquatic hazard of a commercial sample of NAs with a comprehensive compositional analysis. The present study provides aquatic toxicity data for a commercial NAs sample to four species representing fish, invertebrates, algae, and bacteria. Various tools were used to assess the composition of the commercial NAs sample and to investigate a potential mode of toxic action of these NA substances. A detailed chemical analysis of the NAs test substance helped progress the knowledge of specific NA structures in commercial NAs samples. Biomimetic extraction–solid phase microextraction (BE–SPME) analyses were used to evaluate mode of action, and those measurements served as a surrogate for critical body burdens of these substances. These new data are anticipated to provide an aquatic hazard assessment that may be used in a regulatory context for risk assessment purposes.

2. Methods and materials

2.1. Test substance

The sample of commercial naphthenic acids (CAS# 1338-24-5) was supplied by Merichem Company (Houston, TX). Manufacturer's specification data for the test sample provided the following: acid number, 235 mg KOH gm^{-1}; total unsaponifiables, 4.9%; viscosity at 40 °C, 32 cst; specific gravity at 20 °C, 0.969 g mL^{-1}; color (Garner), 4.5 GI; water content, 0.07%; phenolic content (acid), 0.31%; total sulfur, 0.34%; CP – flash point (COC), 343 °F.

The test substance was used to prepare all test solutions, matrix spiking solutions and analytical standards. Quantification of the carbon numbers (C-num) and Z-family groups were performed by the Department of Biological Sciences, University of Alberta (Alberta, Saskatchewan) using published methods (Holowenko et al., 2002).

2.2. Test organisms and test design

Toxicity tests were conducted using a freshwater fish (fathead minnow, *Pimephales promelas*), an invertebrate (Cladoceran, *Daphnia magna*), an alga (*Pseudokirchneriella subcapitata*), and a bacterium, (*Vibrio fischeri*). Fish, invertebrate, and alga tests were performed following standard regulatory guidelines (US EPA, 1996, guidelines 850.1010, 850.1075, 850.5400; OECD, 2009, guidelines 201, 202, 203) and additional guidance (OECD, 2000) for testing mixtures using water accommodated fractions (WAF). The term WAF refers to the fraction of multicomponent substances that is dissolved and/or present in the water phase as a stable dispersion (OECD, 2000). To create each WAF, an amount of test substance was added to dilution medium on a weight/volume basis. Solutions were stirred using a Teflon-coated magnetic stir bar for approximately 24 h at a speed sufficient to create a vortex extending 30–50% of the solution depth. Solutions were allowed to settle for approximately 1 h after stirring, and the WAF was collected from the bottom of the stir vessel to avoid any un-dissolved test substance. Nominal concentrations were referred to as the loading rate of test substance from which the WAFs were created. A discussion and rationale for use of the term loading rate has been previously presented (Girling et al., 1992; OECD, 2000). The Microtox bacteria toxicity test was based on the general principles described by ISO (2007). Bacterial luminescence readings were measured using a Microtox Model 500 Analyzer (Modern Water Inc.). A summary of the test conditions are shown in Table 1. Fish, invertebrates, and algae were obtained from in-house cultures maintained by the testing facility. Freeze-dried *V. fischeri* were purchased (Modern Water, Inc). Dilution water used in the fish and *Daphnia* tests was laboratory freshwater prepared to yield a total hardness of 130–160 mg L^{-1} as CaCO3. Water pH ranged 8.0–8.4, 6.8–8.9, and 7.5–8.6 in the fish, algae, and *Daphnia* tests, respectively (Table 1). Dilution water used in the alga test was freshwater algal nutrient medium (ASTM, 1997), and Microtox diluent was used for the bacterial toxicity test.

2.3. Analytical characterization of naphthenic acids test substance

The test substance was characterized for Z-family and carbon number distributions using the gas chromatography–mass spectrometry (GC–MS) method described by Young et al. (2008). An aliquot of the test substance was derivatized with N-methyl-N-(t-butyldimethylsilyl)-trifluoroacetamide (MTBSTFA) to create t-butyldimethylsilyl esters of the constituent NAs. The extracted ion current chromatograms of selected M-57 ions for different carbon number (n) and Z number NAs were integrated. Those data were put into a Microsoft Excel spreadsheet (Holowenko et al., 2002) to create a table of the relative abundances of each NA group corresponding to the general formula, $C_nH_{2n+z}O_2$. This defined, approximately, the relative proportions of acyclic, 1-, 2-, 3-ring, etc. and aromatic NA classes (Fig. 1). It is known that this method may overestimate some NAs classes if oxy-NAs (e.g. hydroxy 'O_3'acids (Bataineh et al., 2006)) or 'O_4' diacids (Lengger et al., 2013), which may yield bis-derivatives, are present, as is common in OSPW NAs. However the proportions of such O_3 and O_4 species