Available online at www.sciencedirect.com
ScienceDirect
Chemosphere 68 (2007) 518–527
CHEMOSPHERE
www.elsevier.com/locate/chemosphere

Detection of naphthenic acids in fish exposed to commercial naphthenic acids and oil sands process-affected water

R.F. Young, E.A. Orr, G.G. Goss, P.M. Fedorak *

Department of Biological Sciences, University of Alberta, Edmonton, AB, Canada T6G 2E9

Received 13 September 2006; received in revised form 12 December 2006; accepted 15 December 2006
Available online 6 February 2007

Abstract

Naphthenic acids are a complex mixture of carboxylic acids that occur naturally in petroleum. During the extraction of bitumen from the oil sands in northeastern Alberta, Canada, naphthenic acids are released into the aqueous phase and these acids become the most toxic components in the process-affected water. Although previous studies have exposed fish to naphthenic acids or oil sands process-affected waters, there has been no analytical method to specifically detect naphthenic acids in fish. Here, we describe a qualitative method to specifically detect these acids.

In 96-h static renewal tests, rainbow trout (*Oncorhynchus mykiss*) fingerlings were exposed to three different treatments: (1) fed pellets that contained commercial naphthenic acids (1.5 mg g^{-1} of food), (2) kept in tap water that contained commercial naphthenic acids (3 mg l^{-1}) and (3) kept in an oil sands process-affected water that contained 15 mg naphthenic acids l^{-1}. Five-gram samples of fish were homogenized and extracted, then the mixture of free fatty acids and naphthenic acids was isolated from the extract using strong anion exchange chromatography. The mixture was derivatized and analyzed by gas chromatography–mass spectrometry. Reconstructed ion chromatograms ($m/z = 267$) selectively detected naphthenic acids. These acids were present in each fish that was exposed to naphthenic acids, but absent in fish that were not exposed to naphthenic acids. The minimum detectable concentration was about 1 μg naphthenic acids g^{-1} of fish.

Keywords: Naphthenic acids; Gas chromatography–mass spectrometry; Reconstructed ion chromatogram; Tainting; Rainbow trout

1. Introduction

Naphthenic acids are a complex mixture of compounds that occur naturally in petroleum. They are acyclic and cycloaliphatic carboxylic acids with the general chemical formula $C_nH_{2n+Z}O_2$ (Brient et al., 1995), where n indicates the carbon number and Z is zero or a negative, even integer whose absolute value divided by 2 gives the number of rings, fused or bridged, in the compound. Unlike natural fatty acids, which also fit the formula for naphthenic acids for $Z = 0$, the acyclic components of naphthenic acids can be highly branched (Rudzinski et al., 2002).

The biodegradation of petroleum in the Athabasca oil sands near Fort McMurray, Alberta, Canada produced naphthenic acids in the deposits (Tissot and Welte, 1978). Some of these deposits are being surface mined and the bitumen is extracted and upgraded to produce synthetic fuels. The alkaline pH of the hot water extraction process used to separate bitumen from the oil sands dissolves the naphthenic acids and concentrates them in an aqueous slurry. The slurry, containing water, sand, clay, naphthenic acids, residual bitumen and inorganic and organic constituents, is known as oil sands tailings, and these tailings are stored on site in large settling ponds. As the solids separate from the tailings, water is released forming a surface water layer with low solids. The so-called ''process-affected water'' is recycled back to the extraction plant.

Process-affected waters can contain 20–120 mg naphthenic acids l^{-1} (Holowenko et al., 2002) and these acids

* Corresponding author. Tel.: +1 780 492 3670; fax: +1 780 492 9234.
E-mail address: phil.fedorak@ualberta.ca (P.M. Fedorak).

0045-6535/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.chemosphere.2006.12.063

are the most toxic components to aquatic organisms (MacKinnon and Boeger, 1986; Verbeek et al., 1994). Because of their toxicity, process-affected waters are not intentionally released to any receiving waters, and the oil sands companies are accumulating and storing vast volumes of these waters. Although some of the process-affected waters are not acutely toxic to fish, there are sublethal effects for fish exposed to oil sands waters containing naphthenic acids (Nero et al., 2006).

Oil sands process-affected waters contain countless different compounds, residual bitumen, heavy metals, polycyclic aromatic hydrocarbons, benzothiophenes, dibenzothiophenes, and naphthenic acids (MacKinnon, 1989; Madill et al., 2001), which may contribute to fish toxicity (MacKinnon and Boeger, 1986; Nix and Martin, 1992) or fish tainting (Koning and Hrudey, 1992). Fish tainting, an unnatural flavor or aroma in the fish, can be studied using sensory panels, but this method can be subjective, inaccurate, and expensive. Jardine and Hrudey (1988) determined the threshold concentration of a number of compounds associated with oil sands process-affected waters that can impair the flavor of fish. They found that naphthalene, benzothiophene, and 2,5-dimethylphenol caused strong tainting in walleye fish. However, the role of naphthenic acids in fish tainting is unknown (Griffiths et al., 2006).

Several investigations have exposed fish (Dokholyan and Magomedov, 1984; Nix and Martin, 1992; Koning and Hrudey, 1992; Van den Heuvel et al., 1999a,b, 2000; Siwik et al., 2000; Nero et al., 2006), or rats (Rogers et al., 2002) to naphthenic acids or oil sands process-affected waters. Some of these studies have reported changes in various tissues and organs in the animals (Van den Heuvel et al., 2000; Rogers et al., 2002; Nero et al., 2006). However, we are aware of only one study in which attempts to detect naphthenic acids were made in the test organism. In that study, Kamaluddin and Zwiazek (2002) measured the uptake of naphthenic acids by aspen seedlings.

The oil sands industry standard procedure for measuring naphthenic acids involves extraction of these acids from a sample, and measuring the absorbance by the carboxylic acid portion of the naphthenic acids using Fourier transform infrared (FTIR) spectroscopy (Jivraj et al., 1995; Holowenko et al., 2001). This method has been used to examine naphthenic acids in laboratory-exposed plants (Kamaluddin and Zwiazek, 2002), but it lacks specificity because naturally occurring fatty acids in plants or animals are also measured as naphthenic acids.

Merlin et al. (2007) describe a method to specifically detect naphthenic acids in waters from a variety of sources. After extraction, cleanup, derivatization, and analysis by gas chromatography–mass spectrometry (GC–MS), reconstructed ion chromatograms (RICs, Murray et al., 2006) for nominal mass $m/z = 267$ were obtained which specifically indicated the presence of naphthenic acids in the waters (Merlin et al., 2007).

The objective of this study was to develop a method to extract naphthenic acids from fish and to apply the GC–MS method of Merlin et al. (2007) to specifically detect naphthenic acids in the extracts. Initially, fish muscle samples were spiked with naphthenic acids to work out the procedures. Subsequently, one group of rainbow trout fingerlings was fed pellets containing naphthenic acids, a second group of fingerlings was exposed to a solution of commercial naphthenic acids, and a third group was exposed to oil sands process-affected water. The newly developed method detected naphthenic acids in the fingerlings, regardless of the exposure route.

2. Materials and methods

2.1. Naphthenic acids and oil sands process-affected waters

Refined Merichem naphthenic acids were a gift from Merichem Chemicals and Refinery Services LLC (Houston, TX). Naphthenic acids originating from one of the oil sands tailings ponds (West In Pit, WIP) operated by Syncrude Canada Ltd. were used to spike fish tissue and evaluate our analytical method. The acids from WIP were collected using the method of Holowenko et al. (2001). Briefly, a WIP water sample was acidified with H_2SO_4 to pH 2–3 and the acids were left to settle for 1 week. The water was siphoned off and the remaining material was centrifuged at 25000*g* for 15 min. The supernatant was discarded and the pellet was washed with 0.1 M NaOH to ionize the naphthenic acids, shaken for 15 min and centrifuged to obtain a brownish supernatant that was decanted and stored at 4 °C. The concentration of naphthenic acids in the WIP extract was 1400 mg l^{-1}, based on analysis by high performance liquid chromatography (HPLC, Yen et al., 2004). The WIP extract (10 ml) was subsequently extracted at pH 11 with three 2-ml portions of dichloromethane. Then the remaining aqueous fraction was acidified to pH < 2 with HCl and extracted with three 2-ml portions of dichloromethane, dried under nitrogen and redissolved in dichloromethane before addition to the fish samples.

Water was obtained (May 1, 2006) from Syncrude Pond 9 to use in fish exposure experiments. This 4-ha pond was constructed in 1993 when it was filled with 50000 m^3 of tailings process water (Siwik et al., 2000). No fresh process water has been added to Pond 9 since it was established.

2.2. Fish extraction for naphthenic acids analysis

Our extraction method was adapted from the procedures reported by Bernárdez et al. (2005) for the analysis of free fatty acids from fish. All glassware used for fish extraction was thoroughly cleaned and rinsed with dichloromethane prior to use. To 5 g of rainbow trout fillet (purchased from a local supermarket) in a 250-ml Erlenmeyer flask, 50 μg of Merichem naphthenic acids was added, to give a concentration of 10 μg g^{-1} of fish fillet. Then