Science of the Total Environment 409 (2011) 4125–4131

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Short Communication

Compositional heterogeneity may limit the usefulness of some commercial naphthenic acids for toxicity assays

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ARTICLE INFO

Article history:
Received 12 March 2011
Received in revised form 25 May 2011
Accepted 28 May 2011
Available online 6 July 2011

Keywords:
Naphthenic acids
Microtox
Toxicity
Alkylphenols
GC×GC–MS

ABSTRACT

Naphthenic acids are considered variously as monocarboxylic acids fitting the formula $C_nH_{2n+z}O_2$ (where z is a zero or negative even integer), as only alicyclic (*i.e.* non-aromatic) monocarboxylic acids fitting this formula ($z \leq 0$), or simply as those carboxylic acids occurring in petroleum products or crude oils that have been formed through biodegradation of hydrocarbons. Such acids are known constituents of the process-affected water associated with some expanding oil sands industries, of some immature and biodegraded crude oils, of produced water discharges from oil production platforms and are used as biocides and as components in the manufacture of steel radial tyres.

As a result of these potential vectors of the acids into the environment, various naphthenic acid mixtures which are available commercially have been used for a range of toxicity studies. However, as some manufacturers make clear, but which is not often emphasised in the toxicity studies, a range of different quality naphthenic acids is produced commercially. It has been suggested previously, and we showed recently and elucidate further herein, that such commercial mixtures therefore sometimes contain toxic components other than carboxylic acids. For example, we identify herein by two-dimensional comprehensive gas chromatography–mass spectrometry, a range of C_{0-6} alkylphenols in a batch of commercial naphthenic acids. Since these compounds are known toxicants, the contribution of such non-carboxylic acids, if any, to the toxicity attributed previously to the acids, should also be considered. This will be reflected in the concentrations and effective toxicities of such components. In order to establish the toxicity of the acids *per se*, assays of pure synthetic carboxylic acids of the type now known to be present in naphthenic acids from petroleum or oil sands may be more appropriate than tests of the toxicity of largely unknown, heterogeneous, mixtures.

1. Introduction

Complex mixtures of alkyl substituted, mainly alicyclic, carboxylic acids fitting the general formula $C_nH_{2n+z}O_2$, where z is zero or a negative even integer which denotes the hydrogen deficiency resulting from ring formation, known as naphthenic acids (reviewed by Headley et al., 2009), are reported to be among the components of oils sands process-affected water (OSPW). The OSPW is considered to be toxic to mammals, fish and zooplankton, although actually the toxicities vary considerably depending on the assay used and the particular oil sands acid mixtures tested (*e.g.* Frank et al., 2008 and references therein). Naphthenic acids are also known constituents of immature and biodegraded crude oils, of produced water discharges from oil production platforms, and are used as biocides and as components in the manufacture of steel radial tyres (reviewed by Headley et al., 2009).

As a result of these potential vectors of acids into the environment, various naphthenic acids preparations which are more readily available commercially in larger quantities than environmental samples, have, perhaps understandably, been used for a range of toxicity studies and assumed to be somewhat representative of oil sands or petroleum-derived naphthenic acids.

For example, Zhang et al. (2011) assessed the toxicity of a technical mixture of naphthenic acids purchased from Sigma Aldrich (lot #70340) using a microbial genome wide live cell reporter array system. They identified effects such as the up-regulation of genes in the pentose phosphate pathway and down-regulation of the ATP-binding cassette transporter complex at response concentrations of 10 to as high as 1000 mg L^{-1} (1 g L^{-1}), whereas, Thomas et al. (2009) showed that commercial naphthenic acids from Fluka and Acros produced androgen receptor (AR) antagonist potencies greater than that of a flutamide standard by 72 or 290 times. By comparison, some synthetic alicyclic and mono-aromatic carboxylic acids made by ourselves (*cf* Smith et al., 2008) were 100 times less potent that flutamide in the same assay (Thomas et al., 2009). Peters et al. (2007) exposed eggs of yellow perch (*Perca flavescens*) and embryos of Japanese medaka (*Orizias latipes*) to

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doi:10.1016/j.scitotenv.2011.05.061

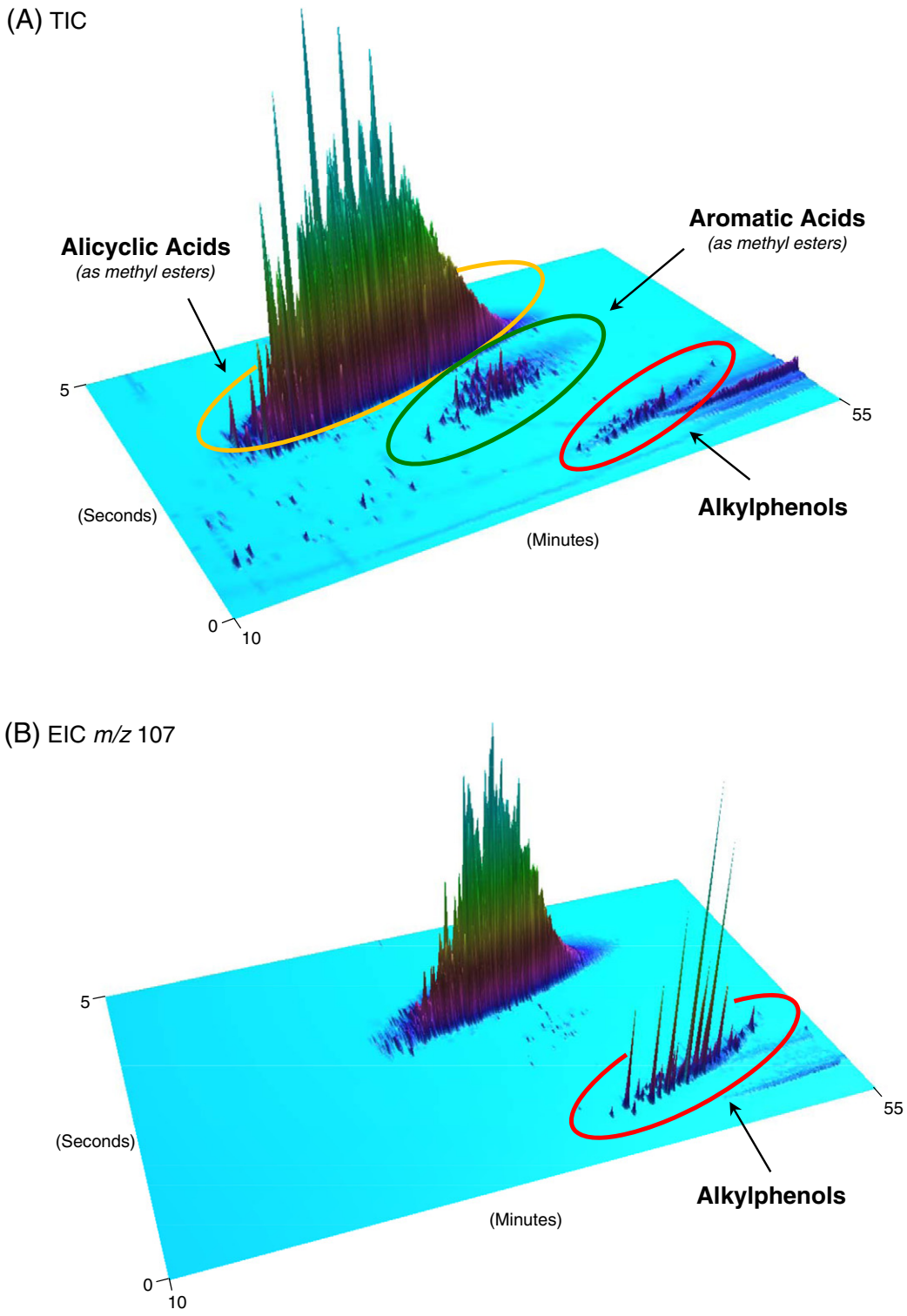

Fig. 1. GC × GC–MS (A) total ion current chromatogram and (B) extracted ion mass chromatogram (*m/z* 107) of methyl esters of commercial NA using a polar stationary phase in the first GC dimension, as described in Methods.

1.25 to 20 mg L^{-1} aqueous solutions of sodium salts of a further sample of commercial naphthenic acids, in this case supplied by Pfaltz-Bauer Inc. and observed increases in deformities and decreases in hatch length. These authors did note however, that the commercial acids possibly contained 'impurities' that were embryo-toxic, though they did not suggest what these might be.

The difficulties of attributing such biological effects directly to the carboxylic acids in commercial naphthenic acids, lies in the more or less, uncharacterised nature of the preparations. Methods of obtaining such technical mixtures may be variable and relatively non-specific, as may the source oils from which the naphthenic acids are isolated, leading to heterogeneous and variable products, perhaps even between different lots from the same supplier. Some suppliers indicate this by providing additional information. Certainly the broad profiles of naphthenic acids from different suppliers, as monitored by gas chromatography–mass spectrometry of derivatised acids, are often quite different (*e.g.* Clemente