Oklahoma Fish Kill Study: Looking for a Toxic Needle in an Environmental Haystack

Introduction

Since December 2011 the U.S. Environmental Protection Agency's Office of Research and Development, National Exposure Research Laboratory-Environmental Sciences Division (EPA/ORD-NERL-ESD) has assisted EPA Region 6 and the State of Oklahoma Department of Environmental Quality (OKDEQ) in identifying unknown contaminant(s) that were present during four fish kills in the Red River watershed. These environmental samples were unique in that they were collected during the active phase of the fish kills along the Red River (Oklahoma, United States) in 2011, 2012 and 2013. Using liquid chromatography-time-of-flight high-resolution mass spectrometry (LC-TOFMS), LC-Fourier transform mass spectrometry (LC-FTMS) and/or liquid chromatography-ion trap mass spectrometry (LC-ITMS), the conditional assignments of the molecular weights and chemical formulas of the significant unknown contaminants were determined.

Methods

Environmental water samples were extracted using a solid phase extraction (SPE) method. Sediment samples were extracted using a modified sonication liquid extraction method. All extracts were screened and analyzed by LC-ITMS, LC-TOFMS and/or LC-FTMS.

Subsequently the extracts were then re-analyzed using collision induced dissociation (CID) (either in the ion trap, or in-source CID for TOFMS and FTMS) for product ion formation to elucidate chemical structural components, and re-analyzed by LC-TOFMS and LC-FTMS for accurate mass assignments.

Preliminary data

All extracts were screened using LC-ITMS for any chromatographic peaks that were significantly above baseline. Many chromatographic peaks were present, but most could be attributable to ambient background contamination, e.g., surfactants and phthalates. From the screening analyses of the samples, two major unknowns were discovered in three of the four fish kills, detected at masses *m/z* 624.3 Da and *m/z* 639.3 Da. The LC-FTMS gave the following accurate masses: *m/z* 639.31735 $(M+H)^+$, with the chemical formula, $C_{37}H_{43}N_4O_6$; and *m/z* 624.31794 $(M+H)^+$, with the chemical formula, $C_{36}H_{42}N_5O_5$. The unknown at mass *m/z* 639.3 Da has been unequivocally identified as a porphyrin, specifically chlorin e6 trimethyl ester, mw 638.310425 Da, $C_{37}H_{42}N_4O_6$. A standard of chlorin e6 trimethyl ester was obtained from Frontier Scientific (Logan, Utah). The other unknown present at *m/z* 624.4 Da $(M+H)^+$, has a core chemical structure similar to chlorin e6 trimethyl ester, except that two of the functional groups are different. A tentative identification was assigned to the mass as an amide-containing porphyrin by comparing the CID spectra from the LC-ITMS data, the LC-TOFMS data,

and the LC-FTMS data. The chemical formula, as calculated by LC-FTMS, is $C_{36}H_{42}N_5O_5$, *m/z* 624.31794 $(M+H)^+$. No commercial chemical standard is available for confirmation.

Another significant unknown was detected in only one sample from the 2013 fish kill. This unknown eluted earlier than the porphyrin series, and was assigned the chemical formula: $C_{46}H_{94}N_6O_6$, with an accurate mass of *m/z* 826.72275 $(M^{+\cdot})$ [doubly charged ion detected at: *m/z* 413.36039 (M^{+2})]. This chemical has been tentatively identified as belonging to the chemical class of diquaternary ammonium compounds. The accurate mass was *m/z* 826.72275 $(M^{+\cdot})$, and has been tentatively identified as N,N,N,N',N',N'-Hexamethyl-4,20,27,43-tetraoxo-3,44-dioxa-6,19,28,41-tetraazahexatetracontane-1,46-diaminium; with a theoretical monoisotopic mass of, 826.722412 Da. No commercial chemical standard is available for confirmation.

Novel Aspect

A new emerging contaminant, chlorin e6 trimethyl ester, was detected during on-going fish kills.