

IDENTIFICATION OF ORGANIC TOXICANTS IN TREATED REFINERY EFFLUENTS

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Identification of Organic Toxicants in Treated Refinery Effluents

Health and Environmental Sciences Department

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ABSTRACT

In this study, effluents from five oil refineries were examined for the presence of nonpolar, organic chronic toxicity following suggested U.S. EPA guidelines for Phase I Toxicity Characterization procedures. The refinery effluent containing the most toxicity from nonpolar organic toxicants was selected for more detailed analyses and identification of these toxicants using Phase II procedures. Extraction and elution conditions were modified to increase chronic toxicity recovery and also reduce the complexity of the nonpolar organic effluent fraction containing toxicity.

Results showed that simple modifications of U.S. EPA guidance for C_{18} solid phase extraction (SPE) procedures combined with proper toxicity testing conditions successfully tracked and, to an acceptable degree, isolated toxicity in an effluent fraction amenable for identification of suspected nonpolar organic toxicants. Toxicity was observed only in 100% effluent concentrations, not in dilutions of the effluents. Further chronic toxicity was not consistently observed in the effluent fractions.

Findings from this study indicated that sources of refinery effluent toxicants were a phenol associated with a jet fuel additive and two brominated organics believed to be reaction products of cooling tower water treatment chemicals, rather than from crude oil constituents.

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EXECUTIVE SUMMARY

Prior to the passage of the Federal Water Pollution Control Act (PL 92-500; *Clean Water Act*) in 1972, refinery wastewater treatment systems were diverse in design and treatment effectiveness. Engineering- and technology-based treatment standards, initially developed under the Act to achieve prescribed effluent concentrations resulted in treatment system upgrades and improved wastewater quality. A subsequent EPA initiative to implement water-quality-based effluent limitations (49 Federal Register 9016), as measured by effluent and receiving water aquatic toxicity tests, substantially expanded and enhanced aquatic toxicity testing capabilities. During this same period, advancements in analytical chemistry and toxicity identification procedures helped identify refinery wastewater constituents and treatment processes which were responsible for observed toxicity. Treatment system upgrades designed to achieve these water-quality-based objectives further improved effluent quality.

This investigation represents the next level of sophistication in effluent quality assessments and similarly reflects a substantial change in the nature and magnitude of refinery effluent toxicity. The focus of this study was the isolation and identification of nonpolar, organic wastewater constituents causing measurable, chronic toxicity in treated refinery effluent. Nonpolar organic toxicants were operationally defined as those adsorbed by C₁₈ solid phase extraction (SPE) columns. Effluents from five refineries were selected for screening-level toxicity assessments.

Isolation and identification of the organic compounds responsible for the observed toxicity were accomplished after modifications were made to existing toxicity characterization and identification guidance. Specifically, effluent extraction and elution conditions were modified to reduce the complexity of the organic fraction and to increase recovery efficiency of the chronically toxic fraction. One avenue examined was adjustment of effluent pH before extraction using C₁₈ columns. Another avenue was modification of the standard Phase II C₁₈ column elution scheme suggested by the U.S. EPA guidance for performing Toxicity Identification Evaluations (TIEs).

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The toxicants were neither derived from crude oil or refined product nor were they conventional pollutants associated with refinery wastewater treatment systems. The identified toxicants were a phenol associated with a jet fuel additive and two aromatic brominated organics, believed to be reaction products of cooling tower water treatment chemicals. These compounds exhibited variable, intermittent, and low concentration toxicity and their identification required enhanced fractionation procedures.

None of the effluents tested had sufficient concentrations of total dissolved solids, ammonia, or hydrogen sulfide to be of concern for causing chronic toxicity or interfering with examination of the contribution by nonpolar compounds. Only one of five refinery effluents exhibited organic toxicity of sufficient magnitude to allow subsequent attempts at toxicant isolation and identification. Additionally, levels of chronic toxicity were generally found to be low. These results constitute a broader demonstration of the significant progress during the last 20+ years in refinery wastewater treatment as well as effluent toxicity characterization and identification.

Improvements in refinery effluent quality have been accomplished through treatment enhancements and through better housekeeping practices. Substances such as total dissolved solids, ammonia and hydrogen sulfide, formerly recognized as causing toxicity in refinery effluents, have been largely brought under control. Thus, acute toxicity in refinery effluents is often absent. Chronic toxicity often occurs only at higher effluent concentrations, as demonstrated in this study. Which levels of toxicity are considered of importance in the receiving water depends on the amount and rate of dilution that occurs in the receiving stream. Dilution allowance in the receiving water is usually recognized by regulatory authorities. The type and amount of toxicity identified in this study would be of concern only where available dilution was very low.

Section 1

INTRODUCTION

BACKGROUND

The convergent evolution of aquatic toxicity testing, analytical chemistry, and refinery wastewater engineering has progressed through several levels over the years. Whole effluent aquatic toxicity tests conducted early in this evolutionary process found acute toxicity at relatively low effluent concentrations. These discharges were, and continue to be, complex in chemical composition, and the nature and extent of their toxicity are variable. Prominent inorganic and organic constituents previously identified as responsible for acute and/or chronic toxicity were ammonia, total dissolved solids (TDS), and naphthenic acids. Combinations of test species selection and test conditions, treatment system operations and refinery wastewater stream characteristics all played roles in affecting effluent quality. As influences of these conditions were more clearly understood and appropriate enhancements made, the incidence and extent of acute effluent toxicity have generally declined.

More sensitive subacute tests were developed to identify effluent toxicity, which was usually observed at higher effluent concentrations. Treatment system design and operation were also improved to reduce or eliminate sporadic toxicity (e.g., ammonia excursions). Experience illustrated that attention to treatment system operational details and wastewater stream quality (i.e., refinery unit operation) could reduce whole effluent toxicity.

Even after the many improvements that have been made, the more sensitive toxicity tests (largely chronic tests) sometimes reveal measurable chronic toxicity at higher effluent concentrations. The importance of this toxicity to natural receiving systems depends on the degree of dilution occurring. In most situations, sufficient dilution is available in the receiving water. When dilution in the receiving water is very low, regulatory authorities may insist on further toxicity reduction. It was anticipated that nonpolar organic compounds from refinery processes were in the final effluent and would be frequently contributing to observations of chronic toxicity. Information about such toxicants was desired to provide a

better understanding of their contribution to refinery effluent toxicity and to direct efforts towards control and/or reduction. The EPA TIE procedures were used and modified to identify the small amounts of toxicity caused by nonpolar organic compounds in refinery effluents.

OBJECTIVES AND SCOPE

Toxicity characterization procedures with either larval fathead minnow (*Pimephales promelas*) or (*Mysidopsis bahia*) were performed with effluents from five refineries to identify nonpolar organics responsible for chronic toxicity. Test species selected for this study are also commonly used for determining compliance with effluent discharge toxicity limits. Any toxicity caused by more easily recognized substances, such as ammonia, was not of concern.

Characteristics of selected refinery effluents were initially examined to determine suitable effluents for identification of nonpolar organic toxicants. Nonpolar organic toxicants were operationally defined as those adsorbed by C₁₈ SPE columns. Desirable effluent characteristics were: 1) consistent presence of measurable chronic toxicity due to nonpolar organic compounds; and 2) a lack of toxicity from compounds other than nonpolar organics. Samples with these characteristics were preferred to minimize difficulties in tracking effluent toxicity through sample manipulations and to reduce the possibility of artifacts from the multiple treatments required to address toxicants belonging to more than one class of compounds.

Historically, several common difficulties have been encountered during identification of nonpolar organic toxicants in refinery effluents. Past problems included: 1) poor recovery of toxicity from C₁₈ solid phase extraction (SPE) columns, 2) poor resolution of toxicity during separative steps, 3) failure to recover toxicity following high performance liquid chromatography (HPLC) separation, and 4) inability to adequately simplify effluent fractions containing the nonpolar organic toxicants. Procedures were employed to: 1) simplify the toxic nonpolar organic effluent fraction, 2) achieve sufficient toxicant concentration to allow analytical measurement, and 3) remove water from the fraction to allow analysis by gas

chromatography/mass spectroscopy (GC/MS). GC analyses are often not definitive because of the hydrocarbon content of refinery effluents. To overcome these difficulties, modifications of the U.S. EPA's suggested guidance for Phase II Toxicity Identification Evaluation (TIE) procedures (U.S. EPA 1993) for nonpolar organic compounds were developed and tried.

Section 2

METHODS

GENERAL APPROACH

The initial approach used to screen five refinery effluents for nonpolar chronic toxicity was the U.S. EPA Phase I procedures (U.S. EPA 1991a). The effluent with the most nonpolar chronic toxicity (and the least toxicity from toxicants other than nonpolar toxicants) was selected for detailed toxicant identification using Phase II U.S. EPA procedures (U.S. EPA 1993). Modifications to resolve past TIE performance problems with refinery effluents were made. The modifications are described here and in the Results Section. If an organic compound seemed likely to be a contributor to observed toxicity, additional information was gathered by literature searches, single chemical toxicity exposures, and location of possible sources of the suspect toxicant within the refinery.

INITIAL TOXICITY SCREENS

Initial toxicity screens were performed immediately following sample receipt with each effluent sample using only one of the selected TIE species - either mysids, *Mysidopsis bahia*, or fathead minnows, *Pimphales promelas*. The screening methods for both species are described below. The presence of acute toxicity indicated that the sample was suitable for continued Phase I TIE testing. Generally, test concentrations were 25%, 50%, and 100% effluent, and a control. If the toxicity was sufficient, Phase I TIE procedures were completed for one species.

Fathead Minnow Tests

Dilution water for larval fathead minnow tests was moderately hard reconstituted water (MHRW) prepared following the standard U.S. EPA formula (U.S. EPA 1989). Dilutions were made with the smallest appropriate 14 sized graduated cylinders. Test chambers were 120-ml plastic cups (Plastics Inc., St. Paul, MN). Organisms were obtained from the AScI Corporation/ASCI-Duluth Environmental Testing Division's (ASCI-DETD) fathead minnow culture or from Environmental Consulting & Testing Inc. (Superior, WI). Organism age at test initiation was either < 24 hours or 24- to 48-hours old. Only organisms from one age bracket were used within any one test. Two concentration replicates each containing ten

fathead minnows were tested. Organisms were fed newly hatched artemia (Biomarine Inc., Hawthorne, CA) two or three times daily. Test solutions were renewed daily for seven days. After seven days, the fathead minnows were euthanized, dried 20-24 hours at 100°C, and weighed.

Mysid Tests

The dilution water for mysids was prepared by dissolving Instant Ocean® or hW-Marinemix® sea salt mixture in Millipore® water to obtain a test salinity of 25 ppt. The dilution water was aerated for at least 24 hours before use. Effluent salinity was also adjusted before testing to 25 ppt with Instant Ocean® or hW-Marinemix® sea salt addition. A minimum of three effluent concentrations (25%, 50%, and 100%) and a control were tested during each screening. Fresh test solutions were prepared each day with the appropriate size graduated cylinders. New test chambers were used each day. Test chambers were 30-ml or 120-ml plastic cups. When the 30-ml cups were used, 20 replicates with one organism in each were tested. When the 120-ml cups were used, two replicates each containing five organisms were tested. Organisms were supplied by Aquatic Research Organisms Inc. (Hampton, NH). The mysids were 2 to 6 days old at test initiation. Organisms were liberally fed newly hatched artemia two or three times daily. At the end of seven days, the mysids were euthanized, dried at 100°C for 20-24 hours, and weighed.

PHASE I METHODS

The methods used for characterization of chronic toxicity are described in U.S. EPA (1991b). One effluent sample each from four of five selected refineries was subjected to a Phase I test battery. The results of the toxicity characterization procedures were used to select the refinery effluent most appropriate for Phase II Toxicity Identification procedures.

PHASE II C₁₈ SPE METHODS

Nonpolar organic toxicity was tracked through various separation and concentration steps to ensure the cause of effluent toxicity was present in the fraction subjected to GC/MS analysis. In addition to following EPA Phase II procedures, some modifications were made to the column elution sequence to improve toxicity resolution and tracking. The basic approaches are described below and the rationale for selected changes are presented in the Results Section.

C₁₈ SPE Sorption and Elution

Filtered effluent or back-diluted, toxic Phase I methanol eluate was pumped at a rate of 5 ml per minute over a C₁₈ SPE column having a capacity sufficient to accommodate the volume of sample treated. Column volume capacity followed manufacturer's suggested guidelines. The standard Phase II elution series (25, 50, 75, 80, 85, 90, 95, and 100% methanol/water) was used to sequentially elute the loaded C₁₈ column. Subsamples of the fractions were then diluted and tested for toxicity. The test solutions were prepared to limit concentrations of methanol to less than 1.5% (v/v).

To transfer the effluent toxicity into the methanol phase, whole effluent samples containing measurable chronic toxicity were filtered through a standard glass-fiber filter (Gelman Sciences, Inc., Ann Arbor, MI) and then pumped through a high capacity C₁₈ SPE column (Analytichem International, Harbor City, CA) containing 10 g of sorbent. The loaded column was eluted with a large volume of methanol (20-80 ml). The eluate was then concentrated under a nitrogen stream to attain an appropriate concentration factor for use in testing. With the nonpolar toxicants concentrated in methanol, additional manipulations were done to further isolate the toxicant(s) from nontoxic effluent components. To ensure the toxicity in the methanol phase was the same as the toxicity in the whole effluent, the methanol phase was subjected to Phase I TIE procedures. Those findings were compared to whole effluent toxicity characteristics. If the toxicity in both the methanol and the effluent gave similar results, assurance was gained that the toxicant was the same.

Several modifications of the standard elution series were implemented to improve recovery of toxicity, or to increase separation of effluent components eluted in near proximity to the toxicant(s). In several cases, all of the eight methanol/water solutions were not used for column elution to avoid gradual bleeding of toxicity into multiple eluates. Additionally, multiple 100% methanol eluates were collected at the end of the series to increase recovery of highly nonpolar compounds from the column.

Concentration of Toxic Phase II Fractions

The concentration step is necessary to increase the concentration of analytes to detectable levels and to remove water from the fraction. Depending on eluate complexity, concentrated

toxic Phase II fractions with few components were directly analyzed by GC/MS. Fractions with many components were further separated by HPLC before analysis.

Toxic Phase II fractions were diluted 1:10 with Millipore® water and pulled by vacuum through a 1-ml C₁₈ SPE column. The column was then purged with nitrogen to remove any residual water. The dried column was eluted with multiple 100 µl aliquots of methanol. The elution volume was measured with a Hamilton® microsyringe. Generally, the column was eluted with 300 µl of methanol. However, when color was still present in the column additional methanol was pulled through until the column was clear. This eluate was then tested at the highest nominal effluent concentration possible while limiting the concentration of methanol in the solution to 1.5% (v/v).

HPLC Separation Techniques

Toxic, concentrated eluates were further separated by HPLC to decrease the number of compounds in each fraction associated with any observed toxicity. A Hewlett-Packard 1050 HPLC including quaternary solvent delivery pump, variable wavelength detector, automatic liquid sampler and HPLC(2D) Chemstation with a Spherisorb® 4.6 mm X 250 mm C₁₈ column (5 µm particle size) was used.

Chromatographic conditions for HPLC fractionation were as follows:

Injection volume:	50-450 µl.
Wavelength:	230 nm.
Flow rate:	1.0 ml/minute.
Mobile phase:	Methanol/water.
Solvent gradient:	30% methanol composition at injection linearly increased to 100% at the end of 20 minutes and isocratic for 5 minutes at 100% methanol.

Typically, 25 discrete fractions were collected at 1-minute intervals. The fractions were then tested at the highest nominal effluent concentrations possible while limiting the test solution methanol concentrations to 1.5%. Any of the HPLC fractions discovered to be toxic were concentrated as described above (procedure for concentrating Phase II fractions).