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Contribution of Nonpolar Organic Compounds to the Toxicity of a Chemical Works Effluent

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The U.S. Environmental Protection Agency (EPA) has developed a set of methods to identify toxic pollutants in complex effluent (Norberg-King et al. 1991; Durhan et al. 1993; Mount et al. 1993). Effluent can contain thousands of chemicals, but usually only a few chemicals are responsible for any observed toxicity. The goal of EPA's toxicity-based method is to separate the toxicant from the nontoxic components by using the response of an aquatic organism along with fractionation techniques. These toxicity identification evaluation procedures have been successfully applied in numerous studies concerning the separation and identification of toxic compounds from complex mixtures of chemicals. This method enables direct relationships to be more easily established between toxicants and measured analytical data, thereby avoiding the problems inherent with chemical-specific approaches to limiting toxicity. It is quite useful for identifying causes of a water pollution accident.

The objectives of the present study were (a) to perform a phase I TIE experiment on the Chemical Works effluent (b) to simplify the phase II TIE experiment based on the historical monitoring data (c) to confirm the key toxicants with the deletion approach of TIE. The result is very important in regard to effluent discharge and treatment.

MATERIALS AND METHODS

The effluent sample was collected from mother liquor of a chemical works near the Yangzi River, China on July 25, 1996, using a 10-L glass container. The main product of the chemical works is a class of naphthalenesulfonic acids. The collected sample was stored at 4°C. Effluent characteristics were dissolved oxygen 7.52 mg/L, conductivity 700 μs/ cm, pH 7.65.

Upon arrival, 24-h and 48-h toxicity tests described in the EPA TIE method were conducted on the sample using ≤ 24-h-old *Daphnia magna.* Test chambers were 30-ml glass beakers. *Daphnia* test volumes were 10 ml. Five animals were exposed in each chamber. All toxicity test were conducted at 20±2°C with one or

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two replicates, depending on the TIE phase, and a photoperiod of 16:8-h 1ight:dark.

These toxicity identification evaluation procedures are manipulations designed to characterize classes of toxicants (phase I), identify specific toxic compounds within these classes (phase II) and confirm that these actually are the true toxicants (phase III) (Giesy et al. 1988).

The objective of phase I TIE procedures is to characterize classes of compounds causing toxicity in an aqueous sample. Tests that were done included EDTA addition (which indicates the presence of acutely toxic cationic metals). Control tests for pH adjustment/aeration, and pH adjustment/filtration were conducted and consisted of sample aliquots at pHi (initial pH), pH 3 and pH 11 that were allowed to stand for periods of time required to complete aeration and filtration. Sample pHs were adjusted to pH 3 and 11 by the addition of reagent-grade HCl and NaOH, respectively. After sample manipulations (aeration, filtration) at altered pH, the samples were readjusted to pHi by the addition of NaOH and HCl, respectively, prior to testing.

Solid-phase extraction (SPE), with a C_{18} column of the effluent at pHi, pH 3 and pH 11 also was performed in phase I to detect the presence of nonpolar organic compounds causing toxicity. Two aliquots of sample (one after passage of 25 ml and one corresponding to the final 150 ml of sample) were tested for toxicity to determine whether the column's removal capacity had been exceeded (Durhan et al. 1993). After passage of the effluent over the C_{18} column, attempts were made to recover toxicity with a 100% methanol elution. The methanol fraction also was tested for toxicity to *D. magna* (Norberg-King et al. 1991).

The final phase I manipulation was the graduated pH test at pHs of 6, 7 and 8. This test indicates the presence of pH-dependent toxicants such as ammonia or metals (Norberg-King et al. 1991). The test pHs were altered through the addition of HCl and NaOH.

The objective of phase II TIE procedures is to identify specific compounds causing toxicity within the classes of compounds characterized in phase I. Thus, phase II toxicity identification procedures were dictated by the results of the phase I analyses. In this study, phase II of the EPA TIE procedures was simplified. Based on the previous monitoring data, the effluent included mainly naphthalenesulfonic acid isomers. Therefore, C_{18} SPE columns of phase I were eluted with 100% methanol. The fractions were then analyzed directly by HPLC. The procedures of HPLC fractionation and GC/MS analysis described in the EPA TIE method could be omitted.

In terms of the results of phase I and II, the deletion approach in the phase III was used to confirm the contribution of various compounds to the effluent toxicity. The details of this method is described elsewhere (Mount et al. 1993).

Median lethal concentrations were calculated for all aqueous toxicity test, including those in phase I, II and III of TIE, using the Trimmed Spearman-Karber method (U.S. Environmental Protection Agency 1990). For certain among-sample comparisons, LC50s were converted to TU (toxicity unit) by dividing 100% by the LC50 (% effluent). In the case of individual compound toxicity, TU were calculated by dividing the compound concentration in the sample by its LC50 to *Daphnia,* as obtained from the literature or laboratory toxicity tests.

RESULTS AND DISCUSSION

Toxicity of the effluent sample to *Daphnia magna* was reasonably consistent through the storing period at 4°C ranging from 4.12% to 4.22% (Table 1). This meant that the effluent was quite stable.

Table 1. Acute 24-h LC50 for *Daphnia magna*

Test date	07/26/96	08/23/96	11/05/96
LC50 (% effluent)	4.12	4.12	4.22

Phase I TIE results are depicted in Table 2. Phase I toxicity characterization results revealed that effluent toxicity could be obviously reduced by the C_{18} SPE treatment. Other phase I treatment did not alter the toxicity of the effluent sample.

Table 2. Phase I test results for the July 26, 1996, sample

Test type		24-h LC50	24-h TUs
baseline		4.12 (3.03 - 5.60)	24.27
pH 6		3.92 (2.84 - 5.50)	25.51
pH 7		4.22 (3.94 - 7.80)	23.70
pH 8		4.61 (NC)[a]	21.69
pH 3 adjustment/aeration		4.42 (2.87 - 6.79)	22.62
pHi adjustment/aeration		5.07 (3.30 - 7.80)	19.72
pH 11 adjustment/aeration		5.07 (3.30 - 7.80)	19.72
pH 3 adjustment/filtration		5.83 (4.30 - 7.90)	17.15
pHi adjustment/filtration		5.83 (3.94 - 8.63)	17.15
pH 11 adjustment/filtration		3.84 (3.00 - 4.93)	26.04
pH 3 adjustment/post-C_{18} SPE	1[b]	7.69 (4.30 - 13.76)	13.00
	2	6.69 (3.84 - 11.66)	14.95
pHi adjustment/post-C_{18} SPE	1	13.39 (7.29 - 24.59)	7.47
	2	10.15 (5.53 - 18.63)	9.85
pH 11 adjustment/post-C_{18} SPE	1	15.39 (9.09 - 26.04)	6.50
	2	13.39 (7.69 - 23.32)	7.47
EDTA		the same as baseline	24.27

LC50 values are in percent effluent, with 95% C.I. in parentheses.
[a]NC, 95% C.I. could not be calculated due to lack of partial mortality in test chambers.
[b]Post-C_{18} column samples: No. 1 taken after passage of 25 ml, No. 2 taken during passage of last 150 ml of sample.

Solid phase extraction with a C_{18} column has been recommended for removal and recovery of nonpolar organic toxicity (Durhan et al. 1993). A toxicity loss occurred in the post-C_{18} samples, indicating that chemical(s) causing the acute toxicity was retained on the column. This toxicant was suspected to be as a nonpolar organic compound.

In phase II, we eluted the C_{18} SPE column of phase I using 100% methanol and then analyzed the column run-off solution by HPLC. Results of the HPLC analysis showed that there were mainly four isomers such as 1-nitro-5-naphthalenesulfonic acid, 1-nitro-6-naphthalenesulfonic acid, 1-nitro-7-naphthalenesulfonic acid and 1-nitro-8-naphthalenesulfonic acid in this effluent (Table 3).

Table 3. Results of the HPLC analysis of the elution solution from phase I C_{18} column with 100% methanol

Chemical[a]	Retention Time (min)	Concentration[b] (mg/L)	24-h LC50[c] (mg/L)	24-h TUs
No.1	3.25	1.80	0.25	7.20
No.2	5.33	0.57	0.18	3.17
No.3	10.12	0.46	0.30	1.53
No.4	15.28	2.28	0.21	10.86

[a]No.1: 1-nitro-5-naphthalenesulfonic acid, No.2: 1-nitro-6-naphthalenesulfonic acid, No.3: 1-nitro-7-naphthalenesulfonic acid, No.4: 1-nitro-8-naphthalenesulfonic acid
[b]Concentration of naphthalenesulfonic acid isomers in the effluent.
[c]Determined values from our laboratory.

The contribution rate of these chemicals to the toxicity were reported in Table 4.

Table 4. The contribution rate of chemicals to the toxicity for *Daphnia magna*

sample	pure effluent	No. 1	No.2	No.3	No.4
24-h TUs	24.27	7.20	3.17	1.53	10.86
contribution rate (%)		29.67	13.06	6.30	44.75
total contribution rate of four chemicals (%)			93.78		

Based on the historical toxicity data and the total contribution rate to the toxicity, the four naphthalenesulfonic acid isomers were selected as the most likely

candidates causing acute toxicity. Although naphthalenesulfonic acid concentrations accounted for most of the toxicity, it appears that other nonpolar organic compounds were sometimes present at low and occasionally toxic amounts.

In order to confirm that naphthalenesulfonic acid isomers were major toxicants, we adopted the deletion approach in phase III (Mount et al. 1993). 5 mL pure effluent was directly placed through C_{18} SPE column, not treated by any manipulation. The toxicity of 5 mL postcolumn sample was also tested at 4×, 2×, 1× and o.5× the 24-h baseline LC50. All *daphnia* in each diluted postcolumn sample were survival. This meant the toxicants were removed by the C_{18} SPE column. Then the 100% methanol elute was again analyzed with HPLC. The results of HPLC analysis still showed that the major organic compounds were four naphthalenesulfonic acid isomers. The result was the same as that of phase II. This confirmation was consistent with the selection of naphthasulfonic acids as being major toxicants present in the sample.

The simplified Toxicity Identification Evaluation procedures used in the present study was especially useful in the evaluation of the potentially toxic components responsible for acute toxicity in the simple component effluent, which save our cost and time because the procedures of HPLC fractionation and GC/MS analysis were omitted. Toxicity of the effluent was attributed to nonpolar organic compounds concerning 1-nitro-5-naphthalenesulfonic acid, 1-nitro-6-naphthalenesulfonic acid, 1-nitro-7-naphthalenesulfonic acid and 1-nitro-8-naphthalenesulfonic acid. These results assists us with finding out what chemical is the key to a water pollution accident.

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