

Identification of Ammonia and Volatile Phenols as Primary Toxicants in a Coal Gasification Effluent

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In response to the increasing use of toxicity limits for discharges monitored under the National Pollutant Discharge Elimination System (NPDES), a series of toxicity-based procedures to isolate and identify toxic compounds in complex effluents have been developed in the United States (U.S.EPA 1991, 1993a, 1993b). These toxicity identification evaluation (TIE) procedures are manipulations designed to characterize classes of toxicants (Phase I), identify specific toxic compounds within these classes (Phase II) and confirm that these compounds actually are the true toxicants (Phase III) (Burkhard and Ankley 1989). The TIE approach has been applied successfully to a variety of toxic aqueous samples including effluents, sediment fractions, leachates and ambient water (Ankley and Burkhard 1992).

In recent years, the impacts of complex effluents to aquatic ecosystems have been getting much more serious with the development of industry in China, especially in the coastal southeast. The effluent discharge limits in China now emphasize the general physico-chemical parameters as well as a few priority toxic pollutants among thousands of chemicals. Toxic release cannot be effectively controlled, and some treated effluents, which were considered to have met the requirements of the national discharge limits, were still highly toxic to aquatic organisms based on bioassay results (Jin et al. 1994). Therefore, the TIE approach is useful for identifying the real toxicants causing whole effluent toxicity in China. It is also helpful to develop cost-effective effluent treatment and resource recovery technology for the toxicity reduction.

A fish kill incident was caused by the effluent discharged from the Coal Gasification Plant in Nanjing, China. In this study, TIE procedures using *Daphnia magna* were used in an attempt to identify the major toxicants causing the whole effluent toxicity.

MATERIALS AND METHODS

Three grab samples were collected at different dates from the Coal Gasification Plant in Nanjing, China and shipped to the laboratory in polyethylene or glass containers within half an hour. Samples were stored in the shipping containers at approximately 4°C during the course of TIE analyses.

Control/dilution water used for all tests was tap water which was dechlorinated with activated carbon. The general characteristics of water were as follows: pH 7.2-8.5, DO 6.9-8.0 mg/L, COD_{Mn} 1.8 mg/L, temperature 20±2°C, conductivity 294 µs/cm, and hardness 129 mg/L as CaCO₃.

All toxicity tests in three phases of TIE were performed as described in detail elsewhere (U.S.EPA 1991). Static 24h acute toxicity tests were conducted with no more than 24h old *Daphnia magna* at 20±2% in 10 mL test volume. The test end point was death. To establish LC₅₀ values, all tests were set up using 50% serial dilutions. LC₅₀ values for toxicity tests were calculated with the Trimmed Spearman-Kärber (TSK) Program Version 1.5 (U.S.EPA 1990). For certain comparisons, effluent toxicity was converted to toxic units (TU) by dividing 100% by the LC₅₀ of effluent. For specific chemicals, TU was calculated by dividing the concentration of the chemical in the sample by its LC₅₀ to *D. magna* which was cited from relevant literature or gained from the laboratory toxicity test.

Phase I toxicity characterization procedures including initial toxicity test, baseline toxicity test, pH adjustment test, pH adjustment/aeration test, pH adjustment/filtration test, pH adjustment/C₁₈ solid-phase extraction (SPE) test, EDTA chelation test, oxidant reduction test and graduated pH test were conducted with effluent sample 1 (U.S.EPA 1991). The purpose of initial toxicity test was to determine the initial toxicity of effluent and to set desired test concentrations for the subsequent tests. Baseline toxicity tests were performed on successive days with each set of manipulation tests, and used to detect any change in whole effluent toxicity from initial toxicity and provide baseline toxicity value for comparison to the manipulated effluent. By observing the alteration of effluent toxicity, the classes of chemicals causing the whole effluent toxicity could be identified.

In Phase II toxicity identification procedures, zeolite test was performed with effluent sample 1 to identify whether ammonia was a toxicant in the effluent (U.S.EPA 1993a). Concentrations of total ammonia in the whole effluent and post-zeolite effluent were measured with titration method (China NEPA 1989) at 19°C, and then were converted into the concentrations of non-ionized ammonia (Seager et al. 1988). Toxicity of non-ionized ammonia was tested at the same temperature.

Fractionation and toxicity testing procedures were employed to identify nonpolar organic toxicants (U.S.EPA 1993a). Filtered effluent sample 1 was passed through

preconditioned C₁₈ SPE column, and the post-column sample was collected two times and tested for toxicity. The column was then sequentially eluted with 25, 50, 75, 80, 85, 90, 95 and 100% methanol/water solutions. Each of these eight SPE fractions was diluted to a concentration of two times the whole effluent concentration and tested separately for toxicity. The toxic fraction was then concentrated into 100% methanol using another C₁₈ SPE column, and the concentrate was tested for toxicity by diluting it to four times the whole effluent concentration and analyzed with gas chromatography-mass spectrometer (GC-MS). The suspect nonpolar organic toxicants were identified by comparing the concentrations of compounds in the whole effluent with their corresponding LC₅₀ cited from relevant literature or gained from the laboratory test.

Concentration of total volatile phenols was measured with 4-aminoantipyrine calorimetric method (China NEPA 1989) to ensure the identification of nonpolar organic toxicants.

After the toxicity tests on individual SPE fractions were completed in Phase II, mass balance tests in Phase III toxicity confirmation procedures were initiated to determine whether all the toxicity removed by C₁₈ SPE column from effluent was accounted for in the SPE fractions (U.S.EPA 1993b). There were three separate tests that were conducted, i.e. the toxic-fraction test, the nontoxic-fraction test and the all-fraction test. In order to detect the possible toxicity increased by methanol present in SPE fractions, methanol blank toxicity test was conducted at the same time. Through comparing the toxicity removed by C₁₈ SPE column from effluent with the toxicity in all-fractions and in toxic-fractions, whether the nonpolar organic toxicants identified from toxic SPE fraction were the true toxicants was confirmed.

Concentrations of ammonia and total volatile phenols in effluent sample 2 and 3 were measured with the same methods as for sample 1. Comparison between the toxicity and concentrations of ammonia and total volatile phenols in three samples was used to further confirm whether ammonia and volatile phenols were responsible for the toxicity of the coal gasification effluent.

RESULTS AND DISCUSSION

Results of Phase I tests on effluent sample 1 are summarized in Table 1. Subdivisions represent measurements on different days that are successive.

Baseline tests on successive days revealed that the effluent toxicity decreased day by day to a certain extent, and therefore some easily degradable toxicants existed in the effluent.

EDTA chelation and sodium thiosulfate oxidant reduction manipulations had no effect on the toxicity of effluent, and so the cationic metals and oxidants were not the major toxicants in the effluent.

PH adjustment and filtration manipulations at pH 3, pH 11 and/or pH i didn't obviously reduce the toxicity of effluent. Aeration at pH 11 partly reduced the effluent toxicity, which indicated the possible presence of volatile and/or oxidizable chemicals in the effluent.

In the graduated pH tests, effluent toxicity at pH 8.5 was higher than that at pH 7.5, and the toxicity at pH 7.5 was higher than that at pH 6.5. The test results suggested that ammonia or some metals might have contributed to the effluent toxicity. Combined with the results of baseline, EDTA and aeration tests, ammonia was a suspect toxicant of the effluent.

In the pH adjustment/C₁₈SPE tests, post-column effluent were significantly less toxic than the pre-column filtrated effluent at pH 3 and pH i. This result suggested that nonpolar organic toxicants were present in the effluent.

Table 1. Phase I test results for effluent sample 1

Test	pH	24h LC ₅₀ (95%C.I.) (%) ^a
Initial	pH i ^b	13.39(7.29-24.59)
Baseline	pH i	14.32(11.02-18.61)
EDTA chelation	pH i	No effect
Oxidant reduction	pH i	No effect
pH adjustment	pH 3	17.86(13.20-23.68)
	pH 11	18.86(14.04-25.34)
Baseline	pH i	15.39(12.91-18.34)
pH adjustment/filtration	pH 3	20.31(15.85-26.02)
	pH i	17.68(-) ^c
	pH 11	20.74(15.98-26.94)
pH adjustment/aeration	pH 3	18.86(14.04-25.34)
	pH i	18.61(13.87-24.96)
	pH 11	31.22(22.47-43.37)
Baseline	pH i	17.68(-)
Graduated pH	pH 6.5	26.79(19.78-36.30)
	pH 7.5	21.02(16.07-27.05)
	pH 8.5	17.68(-)
Baseline	pH i	17.68(-)
pH adjustment/filtration	pH 3	14.87(8.24-26.82)
	pH i	17.68(6.04-51.73)
pH adjustment/ C ₁₈ SPE ^d 1	pH 3	70.71(-)
	2	59.46(-)
pH adjustment/ C ₁₈ SPE 1	pH i	70.71(-)
	2	29.73(-)

a LC₅₀ values are given in percentage of sample causing a 50% lethal response.
b pH i = 8.63.
c 95% C.I. could not be calculated due to lack of partial mortality in the test chambers.

- d Post-C₁₈ column samples: No. 1 taken after passage of 25 mL, no. 2 taken after passage of 100 mL, total passage was 150 mL.

The above results indicated that ammonia and nonpolar organic compounds were possibly key toxicants causing the effluent toxicity.

The results of toxicity tests performed in Phase II are shown in table 2.

Table 2. Phase II toxicity test results for effluent sample 1

Toxicity test	24h LC ₅₀ (95% C.I.)	24h TU
Whole effluent	22.81(16.92-30.76) % ^a	4.38
Post-zeolite effluent	26.49(13.10-53.53) %	3.78
Non-ionized ammonia	6.59 mg NH ₃ -N/L ^b	
C ₁₈ S P E ^c 1	70.71(-) %	1.41
2	29.73(-) %	3.36
50% fraction	35.36(-) %	2.83
50% fraction concentrate	158.74(128.55-196.01) %	0.63

a LC₅₀ values are given in percentage of sample causing a 50% lethal response.

b LC₅₀ value is given in concentration of sample causing a 50% lethal response.

c Post-C₁₈ column samples: No. 1 taken after passage of 25 mL, no. 2 taken after passage of 150 mL, total passage was 200 mL.

From Table 2, zeolite manipulation removed 0.60TU from the whole effluent sample, and the 24h LC₅₀ of non-ionized ammonia was 6.59 mg NH₃-N/L. The no. 1 post-C₁₈ column effluent was 2.97TU less than the whole effluent. The toxicity of no. 2 post-C₁₈ column sample revealed a slight decrease, which demonstrated that the C₁₈ column was overloaded and the toxicants might break through (U.S.EPA 1993b). Only the 50% SPE fraction among eight fractions was toxic and it was 2.83TU. The concentrate of the 50% fraction had 0.63TU, which was lower than the fraction's toxicity. This is characteristic of this concentration step and the absolute recovery was not as important as the fact that toxicity was retained in the concentrate that was analyzed via GC-MS (Norberg-King et al. 1991).

The analysis results of ammonia in the whole effluent and post-zeolite effluent sample 1 are listed in table 3.

Table 3. Concentrations of ammonia in the whole effluent and post-zeolite effluent sample 1

Sample	Total ammonia (mg NH ₃ -N/L)	Non-ionized ammonia (mg NH ₃ -N/L)
Whole effluent	81.7	11.1
Post-zeolite effluent	27.0	3.7

Table 3 indicates that zeolite removed 7.4 mg NH₃-N/L non-ionized ammonia from the effluent, which was responsible for 1.12TU based on its LC₅₀. The removal of ammonia decreased the whole effluent toxicity, and therefore ammonia was identified as one of toxicants causing the whole effluent toxicity.

Table 4. Constituents analyzed by GC-MS in the 50% fraction of effluent sample 1 and their toxicity values

Constituent	Concentration (mg/L)	LC ₅₀ (mg/L)	TU
2,4-dimethylphenol	11.023	7.01 ¹	1.57
3-ethylphenol	7.450	10.00 ²	0.75
3-methylphenol	11.583	19.23 ³	0.60
2-methylphenol	7.085	22.58 ¹	0.31
2,6-dimethylphenol	2.081	14.04 ³	0.15
2-ethylphenol	1.217	10.00 ²	0.12
3-ethyl-5-methylphenol	0.772	10.00 ²	0.08
3,4-dimethylphenol	0.899	19.70 ¹	0.05
2-ethyl-6-methylphenol	0.228	10.00 ²	0.02
N-phenylisopropylamine	0.098	8.00 ^{4a}	0.01
Benzeneacetonitrile	0.071	5.00 ^{4b}	0.01
4-methylpyridine	0.091	30.00 ⁵	<0.01
2,5-dimethylbenzaldehyde	0.105	50.00 ^{4c}	<0.01
3,4-dimethylbenzaldehyde	0.177	50.00 ^{4c}	<0.01
Phenol	0.074	64.42 ¹	<0.01
Total phenols	42.4 ⁶		3.65
Total volatile phenols	34.4 ⁷		

1 *D. magna* 24h LC₅₀ from laboratory test.

2 *D. magna* 48h EC₅₀IM for 3-ethylphenol from Sheedy et al. (1991).

3 *D. magna* 24h LC₅₀ from Devillers (1988).

4 from Glushik (1982).

4a *D. magna* 24h LC₅₀ for 2-methylaniline.

4b MATC in waters for isophthalonitrile.

4c *D. magna* 24h LC₅₀ for benzaldehyde.

5 Macroinvertebrate 48h LC₅₀ for pyridine from Phipps et al. (1984).

6 Concentration determined by GC-MS.

7 Concentration measured with 4-aminoantipyrine calorimetric method.

The concentrate of 50% SPE fraction was analyzed by GC-MS, and the results of chemical analysis, the toxicity of each constituent in the concentrate and the concentration of total volatile phenols measured with 4-aminoantipyrine calorimetric method are depicted in Table 4. Ten volatile phenols, n-phenylisopropylamine, benzeneacetonitrile, 4-methylpyridine and two dimethylbenzaldehydes were present in the effluent. Assuming the toxicity of all these 15 compounds are additive, the estimated total toxicity of all the detected volatile phenols was 3.65TU, which might account for the observed 2.83TU of the 50% fraction. Therefore, volatile phenols were identified as suspect nonpolar

organic toxicants in the effluent. Six phenols, i.e. 2,4-dimethylphenol, 3-ethylphenol, 3-methylphenol, 2-methylphenol, 2,6-dimethylphenol and 2-ethylphenol were the major toxicants with more than 0.1TU among the ten phenols. In addition, the concentration of total phenols in the effluent determined by GC-MS was 42.4 mg/L, which was close to the 34.4 mg/L measured with 4-aminoantipyrine calorimetric method. This result supported that volatile phenols were responsible for the effluent toxicity.

Results of mass balance tests in Phase III are showed in Table 5. The 24h TUs caused by toxicants in the toxic-fractions, nontoxic-fractions, and all-fractions were 2.83, 0 and 3.75, respectively. The toxicity removed by C₁₈SPE column from effluent (2.97TU, see Table 2) was recovered in the toxic-fractions and all-fractions. This result confirmed that the suspect toxicants, volatile phenols, identified from 50% toxic fraction were the real toxicants causing the whole effluent toxicity.

Table 5. Results of mass balance tests for effluent sample 1

Toxicity test	24h TU ^a	24h TU ^b	24h TU ^c
Toxic-fraction	2.83	0	2.83
Nontoxic-fraction	1.41	1.41	0
All-fraction	5.66	1.91	3.75

a Caused by toxicants and methanol.

b Caused by methanol.

c Caused by toxicants.

Table 6 shows the toxicity and concentrations of volatile phenols and ammonia in effluent sample 1 and two further samples collected at different dates. All the three samples were toxic to *D. magna* and both ammonia and total volatile phenols in these samples were present at toxic concentrations. These results further confirmed that ammonia and volatile phenols were the major chemicals responsible for the toxicity of whole effluent discharged from the Coal Gasification Plant.

Table 6. Toxicity and concentrations of volatile phenols and ammonia in three samples of the effluent

Sample	pH	24h TU	Volatile phenols (mg/L)	Total ammonia (mg NH ₃ -N/L)	Non-ionized ammonia (mg NH ₃ -N/L)
1	8.63	4.38	34.4	81.7	11.1
2	9.12	5.66	22.1	74.6	23.7
3	9.30	19.2	56.7	254	107.7

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REFERENCES

- Ankley GT, Burkhard LP (1992) Identification of surfactants as toxicants in primary effluent. *Environ Toxicol Chem* 11: 1235-1248.
- Burkhard LP, Ankley GT (1989) Identifying toxicants: NETA's toxicity-based approach. *Environ Sci Technol* 23 : 1438-1443.
- China NEPA (1989) Analytical methods for the monitoring of water and wastewater. 3rd ed, China Environmental Science Press.
- Devillers J (1988) Acute toxicity of cresols, xylenols, and trimethylphenols to *Daphnia magna* straus 1820. *The Science of the Total Environment* 76:79-83.
- Glushik Y (1982) Manuals on toxic organic chemicals in industrial wastewaters. Translated by Geng YL, Hydrocarbon Processing Press, China.
- Jin HJ, Lou X, Zhang ZH, Wang GX (1994) Ecotoxicological monitoring of major industrial effluents in Nanjing, China. In: Proceedings of 3rd Biennial International Symposium, Nanjing, P.R.C., November 3-5, 1992, U.S.EPA/600/R-94/138, U.S. Environmental Protection Agency, Athens, GE.
- Norberg-King TJ, Durhan EJ, Ankley GT (1991) Application of toxicity identification evaluation procedures to the ambient waters of the Colusa basin drain, California. *Environ Toxicol Chem* 10:891-900.
- Phipps GL, Harden MJ, Leonard EN, Roush TH, Spehar DL, Stephen CE, Pickering QH, Buikema AL (1984) Effects of pollution on freshwater organisms. *Journal WPCF* 56:725-758.
- Seager J, Wolff EW, Cooper VA (1988) Proposed environmental quality standards for list II substances in water, ammonia. TR260, Water Research Centre, Marlow, United Kingdom.
- Sheedy BR, Lazorchak JM, Grunwald DJ, Pickering QH, Pilli A, Hall D, Webb R (1991) Effects of pollution on freshwater organisms. *Journal WPCF* 63:619-696.
- U.S.Environmental Protection Agency (1990) Trimmed Spearman-Kärber(TSK) Program Version 1.5. Ecological Monitoring Research Division, Environmental Monitoring Systems Laboratory, U.S.Environmental Protection Agency, Cincinnati, OH.
- U.S.Environmental Protection Agency (1991) Methods for aquatic toxicity identification evaluations: Phase I toxicity characterization procedures. U.S. EPA-600/6-91/003, Environmental Research Laboratory-Duluth, MN.
- U.S.Environmental Protection Agency (1993a) Methods for aquatic toxicity identification evaluations: Phase II toxicity identification procedures for samples exhibiting acute and chronic toxicity. U.S. EPA-600/R-92/080, Environmental Research Laboratory-Duluth, MN.
- U.S.Environmental Protection Agency (1993b) Methods for aquatic toxicity identification evaluations: Phase III toxicity confirmation procedures for samples exhibiting acute and chronic toxicity. U.S. EPA-600/R-92/081, Environmental Research Laboratory-Duluth, MN.