Chlorination Byproducts of Epoxy Resin Hardener and Mutagenic Assay of Their Products

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The reaction of 4,4′-methylenedianiline (MDA) with chlorine in aqueous solution was investigated by gas chromatography/mass spectrometry (GC/MS) in order to determine its chemical changes in water. MDA was shown to react readily with chlorine under conditions likely to be found during water treatment disinfection. The reaction of MDA with chlorine in water was dependent on the solution pH and the molar ratios of chlorine to MDA. The disinfection products of *p*-benzoquinone, *p*-chloroaniline, 2,4-dichloroaniline, 2,4,6-trichloroaniline, and *p*-aminophenol were identified and them of *p*-aminobenzyl chloride, *p*-aminobenzaldehyde and *p*-methoxyaniline were estimated in the chlorination MDA solution. The mutagenic activity of the chlorination byproducts extracted from the chlorination MDA solution was evaluated using *umu*-test.

Key words —— 4,4′-methylenedianiline, epoxy resin hardener, chlorination, mutagenic assay, water

INTRODUCTION

Epoxy resin paint has been used to coat the inside of water supply distribution pipes, and particularly reconditioning pipes in buildings. This paint has been used with hardeners such as aliphatic and aromatic amines, toluene diisocyanate, and polythiol compounds to promote the drying process after painting.

4,4′-methylenedianiline (MDA) had been an epoxy resin hardener used to dry the paint quickly until 1986. MDA has been judged by the International Agency for Research on Cancer (IARC)[1,2] as being carcinogenic to experimental animals, inducing tumors primarily in liver and thyroid. MDA is not permitted to use for drying the paint quickly now.

If coated water pipes are not completely dry when used, MDA will dissolve in the running water and form chlorinated compounds through its reaction with chlorine. It is known that water chlorination has the potential to produce several mutagenic substances.[3] It is therefore of interest to establish which compounds may be present in drinking water after chlorination. Recently, there has been concern that carcinogenicity in blue water may have originated from chlorination MDA solution.

This paper describes the chlorination byproducts of MDA that were identified and estimated by gas chromatography/mass spectrometry (GC/MS) analysis, and the proposed reaction pathways for the chlorination of MDA. In addition, a mutagenic assay was carried out on MDA and chlorination MDA solution using *umu*-test.

MATERIALS AND METHODS

Materials —— MDA, used in the model study of the chlorination of water, was obtained from Wako Pure Chemical Industry Co. (Osaka, Japan). Several chlorination byproducts of MDA used were commercially available reagents.

Hypochlorite solutions were prepared by diluting a sodium hypochlorite solution (*ca.* 16% available chlorine, Wako Pure Chemical Industry Co.) with distilled water, and the resulting solutions were adjusted to the required pH by the addition of 0.05 M Na_2HPO_4-KH_2PO_4 buffer solution.

Umulac AT was purchased from JIMRO Co. (Gunma, Japan).

Treatment with Hypochlorite Solution Containing MDA and Specimen Preparation —— MDA 2 mg dissolved in 2 ml methanol was added to

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0.05 M phosphate buffer solution (pH 7) and 3 equivalents (equiv.) of HOCl per mole of MDA (3 equiv. of HOCl/MDA) in the specimen solution. After undergoing reaction with chlorine for 1 hr at room temperature, the unreacted chlorine was removed by the addition of sodium thiosulphate solution. Sodium hydroxide in water was added to alkalify the solution (pH 8.5), then extracted with dichloromethane (30 ml × 2 times). The extracts were dried over anhydrous sodium sulphate and concentrated by vacuum evaporation and N_2 purge. The chlorination byproducts of MDA in the reacted extracts were confirmed by GC/MS analysis.

GC/MS Analysis —— GC/MS was used for the identification and quantification of chlorination byproducts of MDA. A Shimadzu model GC-17A/QP5050 (Shimadzu, Kyoto, Japan) equipped with a data processing system was used under the following conditions. The ion source of electron inpact ionization (EI) was operated with an electron energy of 70 eV. A SPB^{TM}-35 column, 0.25 mm × 29 m and cross-linked with 0.25 μm, was used. The column temperature was programmed to increase from 40°C to 200°C at 10°C/min, and from 200°C to 270°C at 5°C/min.

Mutagenic Assays —— The mutagenicity of MDA, the chlorination MDA solution, and the chlorination byproducts specimens was determined by *umu*-test using *Salmonella typhimurium* strains NM2009. 2-Aminoanthracene (2-AA) for the specimen with S9 mix and furylfuramide (AF-2) for the specimen without S9 mix were used as positive control mutagens. The solvent dimethyl sulfoxide (DMSO) was used as a negative control. MDA in pH 5, 7, 9 buffer solution were treated with hypochlorite (3, 5, 10 equiv. of HOCl/MDA) for 1 hr. The extract of chlorination MDA solution was dissolved in DMSO. The assay was performed in triplicate for each sample.

RESULTS AND DISCUSSION

GC/MS Analysis of Chlorination Byproducts of MDA

A typical GC/MS (total ion current) trace of dichloromethane extracts of neutral MDA solution after treatment with hypochlorite (3 equiv. of HOCl/MDA) for 1 hr is shown in Fig. 1. At least 9 chlorination byproducts can be seen on the chromatogram (upper panel). The reaction products were identified as follows on the basis of comparison with their retention time and mass spectra:

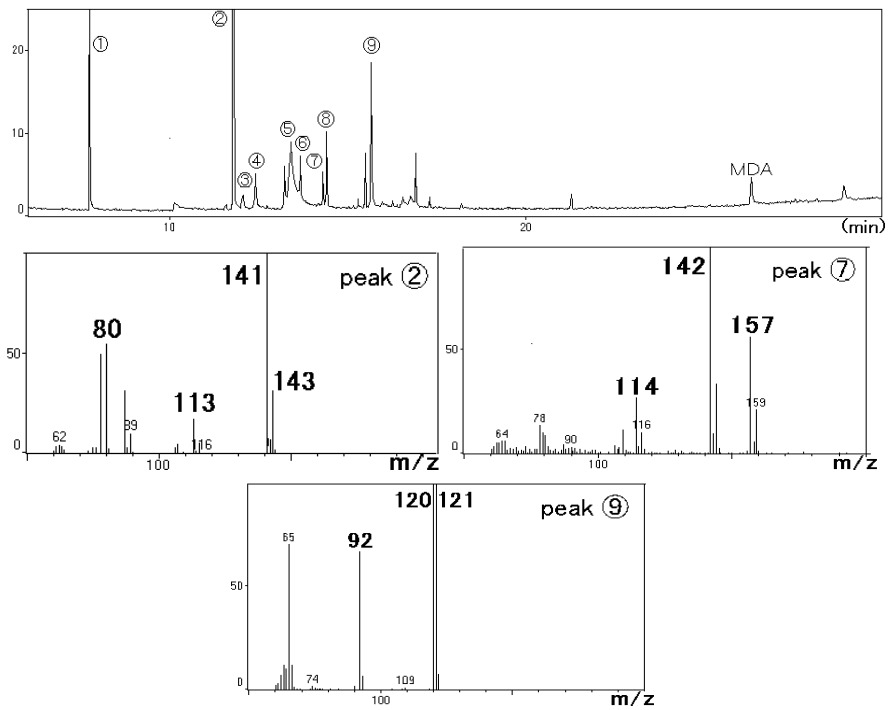

Fig. 1. Total Ion Chromatograms of Dichloromethane Extracts from Chlorine Treated MDA Solutions (Upper Panel) and Mass Spectrum of the Peaks ②, ⑦, ⑨

p-benzoquinone (peak 1); *p*-chloroaniline (peak 3); *p*-methoxyaniline (peak 4); *p*-aminophenol (peak 5); 2,4-dichloroaniline (peak 6); and 2,4,6-trichloroaniline (peak 8). The mass spectrum of peak 2 gave a molecular ion at m/z = 141 and other fragmentation ions at m/z = 143, 113 and 80, and was estimated as *p*-aminobenzyl chloride. The mass spectrum of peak 7 gave a molecular ion at m/z = 157 and other fragmentation ions at m/z = 144, 142, 116 and 114, and was estimated as 2-chloro-4-methoxyaniline. The mass spectrum of peak 9 gave a molecular ion at m/z = 121, and other fragmentation ions at m/z = 120 and 92, and was estimated as *p*-aminobenzaldehyde.

Chlorination of MDA has been reported,[4] but some problems remain in the identification and estimation of chlorination byproducts.

A summary of the chlorination byproducts identified or estimated based on their GC retention times and mass spectra in the present study is presented in Table 1.

Effect of Contact Time on MDA Reaction with Hypochlorite in Aqueous Solution

The effect of contact time on the reaction of MDA with chlorine under 5 equiv. of HOCl/MDA is shown in Fig. 2.

MDA disappeared within 15 min. With the exception of peak 1 (*p*-benzoquinone), the concentration of each chlorination byproduct was a maximum at 30 min or 60 min. When MDA was treated with hypochlorite for 120 min, each chlorination byproduct showed an equal or decreased concentration compared to its values at 30 min and 60 min. *p*-Benzoquinone, on the other hand, increased at 120 min. This could be explained by the production

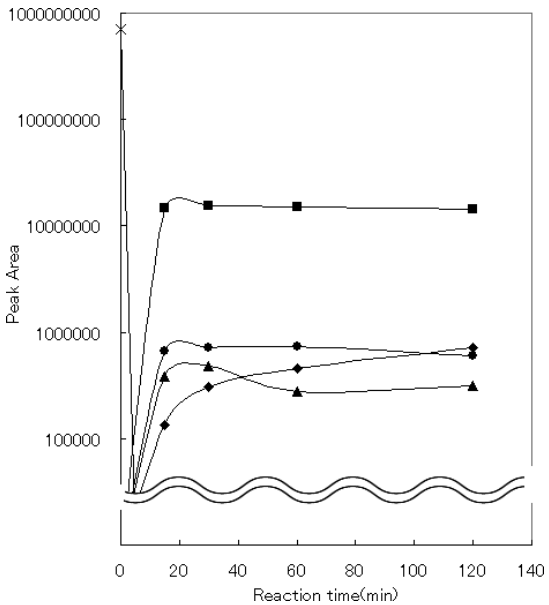

Fig. 2. Reaction of MDA with Hypochlorite in pH 7 Buffered Solution, as a Function of Time

Initial concentration of hypochlorite: 35 mg/l. Initial concentration of MDA: 40 mg/l. ◆ = peak 1, ■ = peak 2, ● = peak 8, ▲ = peak 9 and × = MDA. Peak numbers correspond to those in Table 1.

Table 1. Summary of MS Data (EI = 70 eV) for the Chlorination Byproducts of MDA

	Rt (min)	MS fragments (m/z)	Proposed product
peak ①	7.76	108 [M^+ 100%] 82 [M^+–CO 45%] 80 [M^+–CH_2O 35%]	*p*-benzoquinone
peak ②	11.81	141 [M^+ 100%] 113 [M^+–H_2NCH 19%] 80 [M^+–$CHCH_2Cl$ 51%]	*p*-aminobenzyl chloride
peak ③	12.07	127 [M^+ 100%] 100 [M^+–CH_2N 19%] 92 [M^+–Cl 28%]	*p*-chloroaniline
peak ④	12.41	123 [M^+ 60%] 108 [M^+–CH_3 100%] 80 [M^+–$CHOCH_3$ 32%]	*p*-methoxyaniline (*p*-anisidine)
peak ⑤	13.42	109 [M^+ 100%] 80 [M^+–CHOH 43%]	*p*-aminophenol
peak ⑥	13.69	161 [M^+ 100%] 126 [M^+–Cl 16%] 90 [M^+–HCl 31%]	2,4-dichloroaniline
peak ⑦	14.31	157 [M^+ 56%] 142 [M^+–CH_3 100%] 114 [M^+–$COCH_3$ 27%]	2-chloro-4-methoxyaniline
peak ⑧	14.43	195 [M^+ 100%] 159 [M^+–HCl 17%] 124 [M^+–HCl_2 39%]	2,4,6-trichloroaniline
peak ⑨	15.66	121 [M^+ 100%] 120 [M^+–H 100%] 92 [M^+–CHO 67%]	*p*-aminobenzaldehyde
MDA	26.32	198 [M^+ 100%] 180 [M^+–NH_4 27%] 106 [M^+–$C_6H_5NH_2$ 63%]	

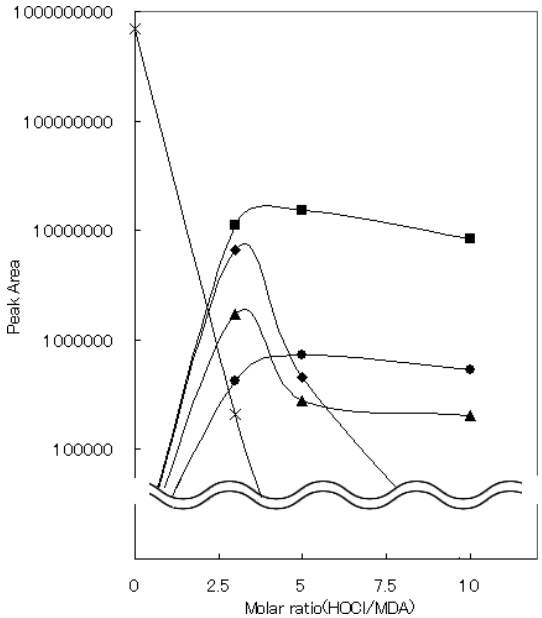

Fig. 3. Effect of Chlorine Doses on the Formation of MDA Chlorination Byproducts in pH 7 Buffer Solution Chlorine Treated for 1 hr

Initial concentration of MDA: 40 mg/l. Symbols for peaks are the same as in Fig. 2.

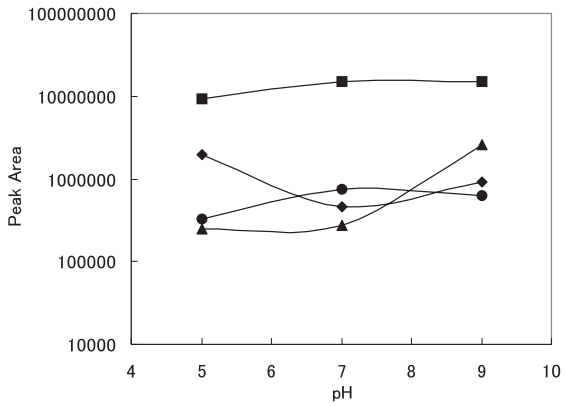

Fig. 4. Effect of pH on Chlorination Byproduct Concentration in MDA Solutions Chlorine Treated for 1 hr

Initial concentration of hypochlorite: 35 mg/l. Initial concentration of MDA: 40 mg/l. Symbols of peaks are the same as in Fig. 2.

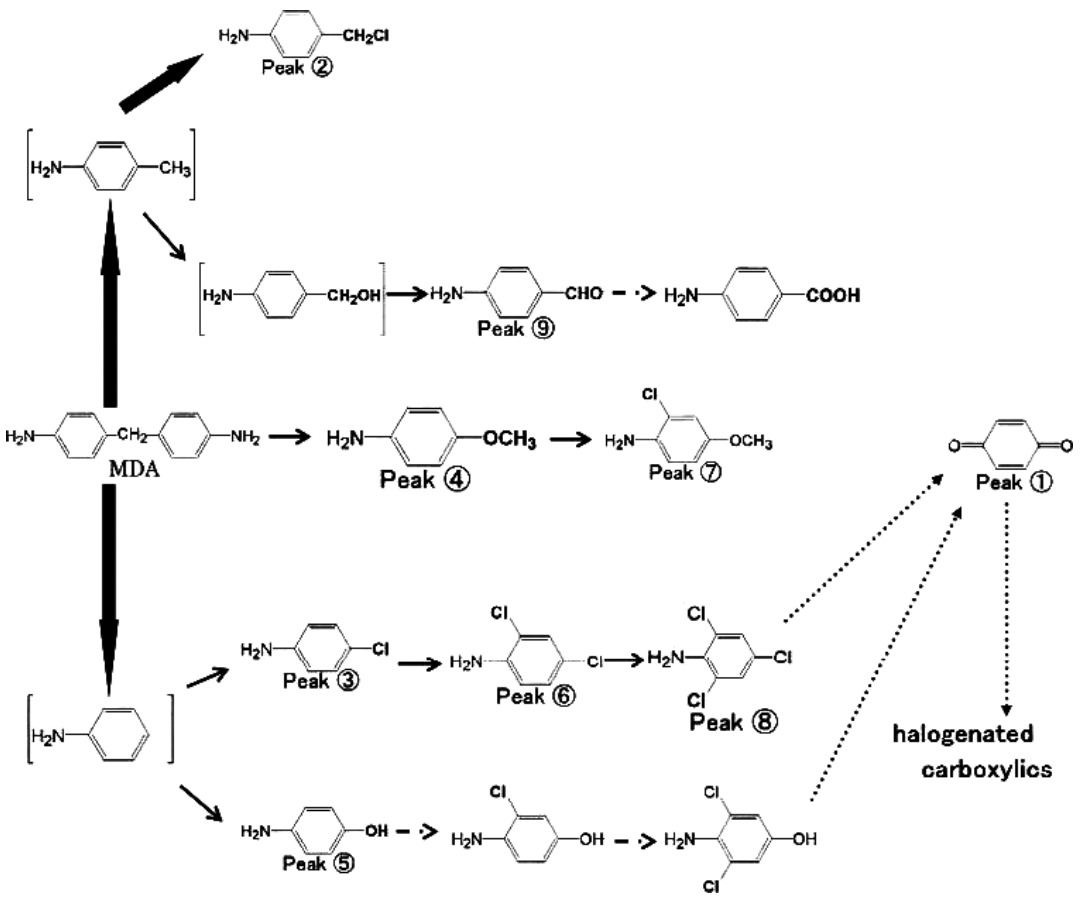

Chart 1. Proposed Reaction Pathways for Chlorination of MDA in the Buffered Solution

➡ : main path, ⟶ : identified path, - -> : estimated path, ⋯⋯➝ : estimated path based on published work[6–9]

of *p*-benzoquinone via other chlorination byproducts.[5, 6)]

From among the chlorination byproducts shown in Fig. 2, those with peaks 3–7 were detected in small amounts and were not dependent on contact time.

Effect of Chlorine Doses on MDA Reaction with Hypochlorite in Aqueous Solution

The effect of chlorine dose on the reaction of MDA with chlorine is shown in Fig. 3.

MDA remained at 3 equiv. of HOCl/MDA and disappeared at more than 5 equiv. of HOCl/MDA. MDA thus reacts with chlorine readily. Peak 2 (*p*-aminobenzyl chloride) is the main product of the chlorination of MDA for 1 hr. Under conditions of more than 5 equiv. of HOCl/MDA, *p*-benzoquinone decreased rapidly, while the other identified chlorination byproducts shown in Fig. 3 decreased slowly. This phenomenon is interpreted in terms of the competition between the production of halogenated compounds and their destruction giving more oxidized products such as halogenated carboxylics.[7)]

Effect of pH Conditions on MDA Reaction with Hypochlorite in Aqueous Solution

The effect of solution pH on the reaction of MDA with chlorine is shown in Fig. 4.

p-Aminobenzyl chloride is produced as the main product under each pH condition. *p*-Benzoquinone was present at high concentrations under acidic conditions. This finding suggests that further reactions of hypochlorite with other chlorination byproducts occur under acidic conditions. On the other hand, peak 9 (*p*-aminobenzaldehyde) was detected at high concentrations under alkaline conditions. This finding suggests that further reactions of hypochlorite with other chlorination byproducts occur under alkaline conditions.

Taking the present results and those of earlier investigations together,[5, 6)] it may be concluded that chlorination with hypochlorite of MDA in aqueous solution takes place as a result of the competing reactions shown in Chart 1. The chlorination byproducts were confirmed as *p*-benzoquinone, *p*-chloroaniline, *p*-aminophenol, 2,4-dichloroaniline, and 2,4,6-trichloroaniline for specimen aniline, 2-chloro-4-methoxyaniline for specimen *p*-methoxyaniline, *p*-aminobenzaldehyde for specimen *p*-aminobenzyl alcohol, *p*-aminobenzyl chloride and *p*-aminobenzaldehyde for specimen *p*-toluidine.

Table 2. Mutagenic Effects of Dichloromethane Extracts of the Chlorination MDA Solution on *Salmonella typhimurium* NM2009 Strains with and without S9 Mix

Experimental conditions					
Compound	HOCl/MDA	pH	Spiked (μl)	NM2009 −S9	NM2009 +S9
MDA	3	5	4	−	−
			2	−	−
			1	−	−
		7	4	−	−
			2	−	−
			1	−	−
		9	4	−	−
			2	−	−
			1	−	−
	5	5	4	−	−
			2	−	−
			1	−	−
		7	4	−	−
			2	−	−
			1	−	−
		9	4	−	−
			2	−	−
			1	−	−
	10	5	4	±	−
			2	±	−
			1	±	−
		7	4	±	−
			2	±	−
			1	±	−
		9	4	±	−
			2	±	−
			1	−	−

Compound	Dose (μg)	NM2009 −S9	NM2009 +S9
o-chloroaniline	8	−	−
m-chloroaniline	8	−	−
p-chloroaniline	8	−	−
2,4-dichloroaniline	8	−	−
2,4,6-trichloroaniline	8	−	−
p-benzoquinone	8	−	−
MDA	8	−	−
4-amino-3-chlorophenol	8	−	−
m-aminophenol	8	−	−
p-aminophenol	8	−	−
2,6-dichloro-4-aminophenol	8	−	−
3-chloro-4-methoxyaniline	8	−	+

−S9· · · −: under 0.03 μg/ml for AF-2, ±: 0.03–0.1 μg/ml for AF-2, +: over 0.1 μg/ml for AF-2.

+S9· · · −: under 0.11 μg/ml for 2-AA, ±: 0.11–0.3 μg/ml for 2-AA, +: over 0.3 μg/ml for 2-AA.

AF-2: furylfuramide, 2-AA: 2-aminoanthracene, HOCl/MDA: molar concentration ratio, pH 5, 7, 9: pH of the chlorination MDA solution.

Mutagenicity of MDA, Chlorination MDA Solution, and Chlorination Byproducts Specimens

MDA is reported to be mutagenic when assayed in the *Salmonella*/mammalian microsome test.[8, 9] However, virtually nothing is known about the toxicity of MDA chlorination byproducts.[4] The mutagenicity of MDA, the chlorination MDA solution, and chlorination byproducts specimens was therefore tested by *umu*-test (Table 2).

The chlorination MDA solution showed weak mutagenic activity for strain NM2009 without S9 mix. However, this result was not clear because many precipitates were present in the mixture.

For the chlorination byproducts specimens and their isomers, 3-chloro-4-methoxyaniline showed mutagenic activity for strain NM2009 with S9 mix. The compound is 2-chloro-4-methoxyaniline isomer, and is not contained in the chlorination MDA solution.

In conclusion, MDA was shown to react readily with chlorine under conditions likely to be found during water treatment disinfection. The reaction of MDA with chlorine in water was dependent on solution pH and the molar ratios of chlorine to MDA. GC/MS analyses suggested that the disinfection products of *p*-benzoquinone, *p*-aminobenzyl chloride, *p*-aminobenzaldehyde and six other products were present in the chlorination MDA solution. The mutagenic activity of the chlorination byproducts extracted from the chlorination MDA solution was evaluated using *umu*-test. The chlorination MDA solution showed weak mutagenic activity for strain NM2009 without S9 mix. However, this result was not clear because many precipitates were present in the mixture.

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