S-Oxygenation of Thiobencarb in Tap Water Processed by Chlorination

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Thiobencarb, an herbicide, was incubated in tap water containing 0.7 mg/L residual chlorine at 30 °C, and the solution was analyzed by HPLC. Thiobencarb could not be detected after 3 h, and only a byproduct was detected. The increase in concentration of the byproduct correlated well with the decrease in that of thiobencarb. In a high free chlorine medium (more than 10 mg/L), the byproduct degraded to *p*-chlorobenzyl alcohol, *p*-chlorobenzaldehyde, and *p*-chlorobenzyl chloride, but not in a low free chlorine medium (0.1–10 mg/L). The byproduct was identified to be thiobencarb sulfoxide by LC/MS, infrared, and NMR spectra, and a reduction technique using 2-mercaptoethanol. Chiral HPLC analysis made it clear that the sulfoxide was a racemic compound. The sulfoxide was mutagenic regardless of the presence or absence of S9mix in the Ames *Salmonella typhimurium* TA100 assay. It was suggested that the management and control of thiobencarb in tap water processed by chlorination should include monitoring of thiobencarb sulfoxide, using HPLC.

INTRODUCTION

Thiobencarb (*S*-*p*-chlorobenzyl diethylthiocarbamate) is a carbamate herbicide that has been widely used for weed control in rice crops. It was moderately toxic to aquatic invertebrates and fishes in acute toxicity tests (Sanders and Hunn, 1982). The Integrated Risk Information System has set up the toxicological constant of thiobencarb at 0.01 mg/kg/d, and Smith (1996) has set the risk-based concentration of thiobencarb in tap water at 370 μg/L. In Japan, the level of thiobencarb remaining in tap water is being regulated at less than 20 μg/L by the Ministry of Health and Welfare. There are many studies on the analysis of thiobencarb in river water, soil, and tap water (Takahashi and Morita, 1988; Redondo *et al.*, 1994; Kodama *et al.*, 1995).

It was reported that irradiation of thiobencarb at 300 nm in aqueous solution yielded 30 photoproducts containing thiobencarb sulfoxide, *N*-desethyl and *N*-acetyl derivatives of thiobencarb, and so on (Ruzo and Casida, 1985). Thus, it seems that thiobencarb is able to be degraded under environmental conditions. It was reported that thiobencarb was detected in raw water but not in tap water processed by chlorination (Takahashi and Morita, 1988). Takahashi and Morita (1993) and Magara *et al.* (1994) have reported that thiobencarb was degraded by chlorination to produce *p*-chlorobenzyl alcohol, *p*-chlorobenzaldehyde, *p*-chlorobenzyl chloride, *p*-chlorotoluene, and *p*-chlorobenzoic acid as chlorination byproducts. They concluded that the management and control of pesticides in drinking water should include testing for chlorination byproducts.

In this paper, we have found that thiobencarb disappeared by incubation with tap water containing 0.7 mg/L of residual chlorine and only a byproduct was detected by high-performance liquid chromatography (HPLC). We have also studied the identification and the mutagenesis of the byproduct.

EXPERIMENTAL PROCEDURES

Chemicals. Distilled water, acetone, hexane, and methanol were of HPLC grade from Kanto Chemical Co., Inc. S9mix was purchased from Oriental Yeast Co., Ltd. Thiobencarb (pesticide grade), acetonitrile (HPLC grade), and other chemicals (reagent grade) were obtained from Wako Pure Chemical Industries Ltd.

Stock solutions (2000 mg/L) of thiobencarb, thiobencarb sulfoxide, *p*-chlorobenzoic acid, *p*-chlorobenzyl alcohol, *p*-chlorobenzaldehyde, *p*-chlorotoluene, and *p*-chlorobenzyl chloride were separately prepared in acetonitrile or acetone. Standard solutions were obtained by mixing together all of their stock solutions in 10 mM phosphoric acid to final concentrations of 1–100 μg/L each.

HPLC Apparatus. The HPLC system consisted of a Hitachi autosampler model AS-4000, two Hitachi model L-6300 pumps, a Rheodyne manual injector, a Shimadzu photodidoe array detector model SPD-M10AV, a Shimadzu column oven model CTO-10AC and a Senshu Scientific model E1E010 switching valve. Separations were attained using RSpak DE-613 column (6 mm i.d. × 150 mm, Showa Denko) maintained at 40 °C, in conjunction with a 4 mm i.d. × 10 mm enrichment column packed with a polymethacrylate-based gel. For the chiral resolution, Chiralcel OB column (4.6 mm i.d. × 250 mm, Daicel Chemical Industries, Ltd.) and both a Shimadzu photodiode array detector and a Jasco model OR-990 chiroptical detector were used. The system was controled by a Hitachi HPLC manager model D-6100. Data acquisition and processing were conducted with a Shimadzu LC model CLASS-M10A workstation.

HPLC Analysis of Chlorination Byproducts of Thiobencarb. HPLC was performed by a fully-automated system previously described (Kodama *et al.*, 1995). That is, 3 mL of the standard solution or the reaction mixture injected by using an autosampler was applied onto an enrichment column followed by washing. The switching valve was moved to the injection position, and the analytes trapped on the enrichment column were desorbed with a mobile phase and transferred to the analytical column. The separated components were measured with a photodiode array detector. The mobile phase used was 53% (v/v) acetonitrile containing 10 mM phosphoric acid. The flow rate was 1.5 mL/min, and the column temperature was kept at 40 °C.

Preparation of a Chlorination Byproduct of Thiobencarb (Thiobencarb Sulfoxide). Thiobencarb was added to 9 L of distilled water containing 3 mg/L free chlorine to a final concentration of 4 mg/L. The solution was left to stand at 30 °C for 3 h and was dechlorinated with 2 mM sodium ascorbate. The dechlorinated solution was applied onto a SEP PAK PLUS PS-2 cartridge (Waters), and the adsorbed material was eluted with 50% (v/v) acetonitrile. The eluate was applied to HPLC
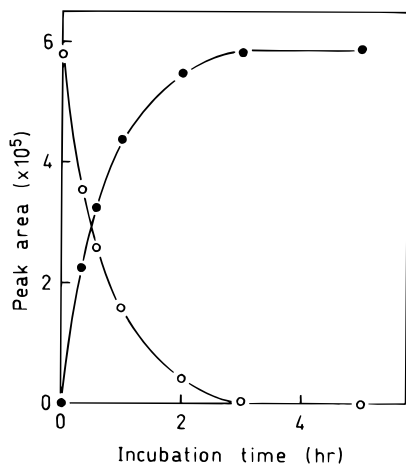
Figure 1. Time course of chlorination of thiobencarb in tap water. ○, Thiobencarb; ●, chlorination byproduct of thiobencarb.

using a manual injector, and the chlorination byproduct of thiobencarb was fractionated.

Liquid Chromatography (LC)/Mass Spectrometry (MS). LC/MS was performed with a Shimadzu model QP1100EX thermospray mass spectrometer. The mobile phase was 50% (v/v) acetonitrile−50 mM ammonium acetate and the flow rate was 1 mL/min. The vaporizer tip temperature was 212 °C. Positive ions were sampled from a scanning range of m/z 150−400. Mass spectra were obtained using the discharge mode. The chlorination byproduct of thiobencarb was dissolved in acetonitrile to obtain a concentration of 20 mg/L, and a 20 μL aliquot of the solution was applied.

Infrared (IR) Spectrometry. IR spectra were recorded in neat with a 1600 series FTIR (Perkin Elmer).

¹H-Nuclear Magnetic Resonance (NMR) Spectroscopy. The ¹H-NMR spectra were obtained on a JOEL EX-400 in CDCl₃ with tetramethylsilane as an internal standard.

Optical Resolution of a Chlorination Byproduct of Thiobencarb (Thiobencarb Sulfoxide) by Using Chiral HPLC. Thiobencarb sulfoxide was dissolved in ethanol−hexane (5:95) to obtain a concentration of 100 mg/L. 10 μL of the solution was applied onto a Chiralcel OB column at 40 °C, and absorbance at 220 nm was measured. The mobile phase used was ethanol−hexane (5:95) at a flow rate of 1 mL/min. For the chiroptical detection, 10 μL of 4000 mg/L thiobencarb sulfoxide was used.

Measurement of Residual Chlorine. Residual chlorine was measured by the diethyl *p*-phenylenediamine method (Japan Waterworks Association, 1993).

Mutagenesis Assay of Thiobencarb and Its Chlorination Byproduct (Thiobencarb Sulfoxide). *Salmonella typhimurium* strains TA98 and TA100 were used for the Ames test (Ames *et al.*, 1975) using the preincubation technique (Yahagi *et al.*, 1977) either in the presence or absence of a rat liver microsomal fraction containing cofactors (known as S9mix). Thiobencarb and its sulfoxide samples were purified by HPLC and then dissolved in dimethyl sulfoxide, and two plates were used per dose.

RESULTS AND DISCUSSION

Chlorination of Thiobencarb in Tap Water. Thiobencarb was added to tap water containing 0.7 mg/L residual chlorine to a final concentration of 100 μg/L, and left to stand at 30 °C for 0−5 h. After dechlorination with 2 mM sodium ascorbate, 3 mL of the solution was applied to HPLC. Figure 1 shows the time course of the peak areas of thiobencarb and its chlorination byproduct. Thiobencarb decreased with incubation time, and only an unknown byproduct was detected. The increase in concentration of the byproduct correlated well with the decrease in that of thiobencarb and within 3 h reached a plateau.

The byproduct was different from the compounds such as *p*-chlorobenzyl alcohol, *p*-chlorobenzaldehyde, or *p*-chlorobenzyl chloride previously reported (Takahashi and Morita, 1993). The byproduct was stable in water for at least 1 month. It seemed that the byproduct was more hydrophilic, as the retention time of the byproduct was significantly shorter than that of thiobencarb on the reversed-phase column.

Identification of the Chlorination Byproduct of Thiobencarb. The chlorination byproduct of thiobencarb was applied to LC/MS, and the mass spectrum on the top of its peak was obtained (Figure 2). The spectrum of the byproduct was characterized by ions at m/z 274/276 and 291/293. It was reported that a ratio between the protonated molecular adduct [M + H]⁺ and the ammonia-cationized molecular adduct [M + NH₄]⁺ depended on the chcaracteristics of the compound (Chiu *et al.*, 1989). It was suggested that ions at m/z 274/276 and 291/293 corresponded to [M + H]⁺ and [M + NH₄]⁺ species, respectively. Therefore, it was also suggested that the molecular weight of the byproduct was 273/275 and the byproduct contained a chlorine atom.

Figure 3 shows the infrared spectra of thiobencarb and its chlorination byproduct. Both spectra seemed to be similar except that strong 1060 cm⁻¹ absorbance in the byproduct appeared and the carbonyl band at 1641 cm⁻¹ for thiobencarb shifted to absorbance at 1695 cm⁻¹ for the byproduct. It was suggested that the strong 1060 cm⁻¹ absorbance resulted in sulfoxide (Tseng *et al.*, 1977). It seems that the production of sulfoxide affected the environment near the carbonyl residue to shift the carbonyl band at 1695 cm⁻¹.

¹H-NMR spectrum data of thiobencarb was as follows: σ 7.28 (d, J = 8.6 Hz, 2H, aromatic H), σ 7.24 (d, J = 8.6 Hz, 2H, aromatic H), σ 4.10 (s, 2H, SCH_2), σ 3.36 (br d, J = 34.2 Hz, 4H, NCH_2), σ 1.15 (t, J = 6.8 Hz, 6H, NCH₂CH_3). The data of the chlorination byproduct of thiobencarb were as follows: σ 7.34 (d, J = 8.3 Hz, 2H, aromatic H), σ 7.26 (d, J = 8.3 Hz, 2H, aromatic H), σ 4.23 (d, J = 12.7 Hz, 1H, SOCH_2), σ 4.18 (d, J = 12.7 Hz, 1H, SOCH_2), σ 3.35 (q, J = 7.1 Hz, 2H, NCH_2), σ 3.25 (m, 1H, NCH_2), σ 3.12 (m, 1H, NCH_2), σ
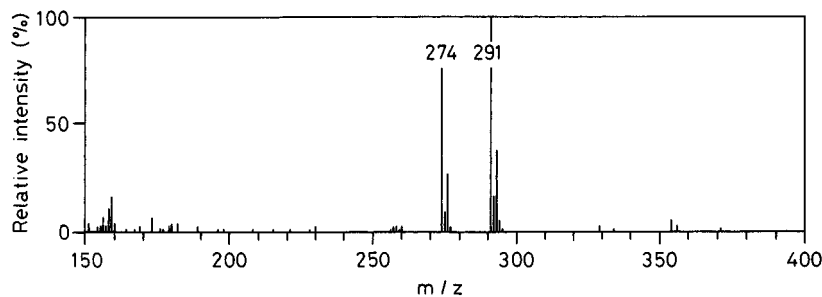

Figure 2. Thermospray liquid chromatography mass spectrum of a chlorination byproduct of thiobencarb.
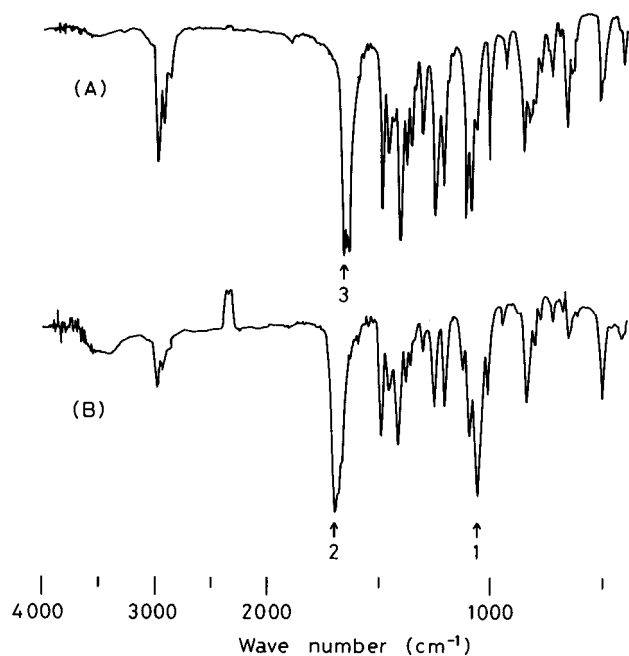
Figure 3. Infrared spectra of thiobencarb and its clorination byproduct. (A) Thiobencarb; (B) chlorination byproduct of thiobencarb. Arrows 1, 2, and 3 indicate wavenumbers at 1060, 1695, and 1641 cm^{-1}, respectively.
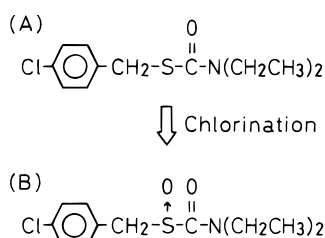

Figure 4. Structure formulas of thiobencarb and its chlorination byproduct (thiobencarb sulfoxide). (A) Thiobencarb; (B) thiobencarb sulfoxide.

1.14 (t, $J = 7.1$ Hz, 3H, NCH$_2$CH_3), σ 0.94 (t, $J = 7.1$ Hz, 3H, NCH$_2$CH_3). This was nearly the same as the data of thiobencarb sulfoxide previously reported (Cashman *et al.*, 1990) except for the methylene resonance of NCH_2. On the basis of the data obtained, it is proposed that the structure of thiocarbamoyl group in thiobencarb sulfoxide under the NMR measurement condition is $-$S($\rightarrow$O)$-$C($-$O$^-$)$=$N$^+$(C$_2$H$_5$)$_2$, but not $-$S($\rightarrow$O)$-$C-($=$O)$-$N(C$_2$H$_5$)$_2$.

Jori *et al.* (1968) reported that methionine sulfoxide was reduced back to methionine by treatment with 2-mercaptoethanol. The chlorination byproduct of thiobencarb was also reduced to thiobencarb by treatment with 5% (v/v) 2-mercaptoethanol in distilled water at 30 °C for 20 h. This result also supported the finding that chlorination of thiobencarb produced thiobencarb sulfoxide (Figure 4).

Therefore, it was shown that thiobencarb sulfoxide was produced not only by photochemical reaction (Draper *et al.*, 1981; Ruzo *et al.*, 1985) or enzyme reaction (Cashman *et al.*, 1990), but also by chlorination of thiobencarb.

Optical Resolution of Thiobencarb Sulfoxide by Chiral HPLC. Compounds containing sulfoxide may be optically active. Thiobencarb sulfoxide obtained by chlorination was applied to HPLC with a RSpak DE-613 column and a chiroptical detector, but it did not show any optical activity. Figure 5 shows the optical
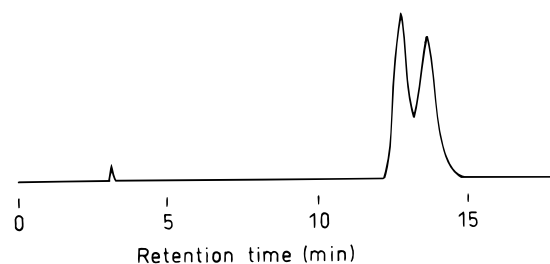

Figure 5. Optical resolution of thiobencarb sulfoxide.

resolution of the sulfoxide carried out by a Chiralcel OB column. The former peak area was almost the same as the latter one. The optical activities of the former and the latter peaks were negative and positive, respectively. Therefore, it is shown that the thiobencarb sulfoxide produced by chlorination of thiobencarb is a racemic compound.

Chlorination of Thiobencarb and Its Sulfoxide at Various Concentrations of Free Chlorine. Sodium hypochlorite was added to distilled water to obtain free chlorine concentration of 0$-$300 mg/L. Thiobencarb was added to the chlorine solution to a final concentration of 100 μg/L. After incubation of the solution at 40 °C for 3 h, 60 mM sodium ascorbate and 10 mM phosphoric acid were added. 3 mL of the mixture was applied to HPLC, and the byproducts were determined (Table 1). As free chlorine increased, thiobencarb decreased and it could not be detected at 0.7 mg/L free chlorine. On the other hand, production of thiobencarb sulfoxide proceeded in a similar manner to parallel the decrease of thiobencarb, and its sulfoxide decreased gradually at more than 10 mg/L free chlorine. *p*-Chlorobenzyl alcohol, *p*-chlorobenzaldehyde, and *p*-chlorobenzyl chloride were detected when thiobencarb was treated with more than 10 mg/L free chlorine. However, *p*-chlorobenzoic acid and *p*-chlorotoluene could not be detected. According to Takahashi and Morita (1993), *p*-chlorobenzyl alcohol, *p*-chlorobenzaldehyde, and *p*-chlorobenzyl chloride were mainly produced by chlorination (140 mg/L free chlorine) of thiobencarb, and these compounds accounted for 3$-$20% of the total thiobencarb. Our result obtained by incubation of thiobencarb and free chlorine (more than 100 mg/L) was identical with their results.

To confirm whether the compounds such as *p*-chlorobenzyl alcohol resulted in a degradation of thiobencarb sulfoxide, the sulfoxide was treated with various concentrations of free chlorine. As shown in Table 2, it was confirmed that *p*-chlorobenzyl alcohol, *p*-chlorobenzaldehyde, and *p*-chlorobenzyl chloride were produced by the chlorination of the sulfoxide, as well as the production of these compounds in Table 1.

Mutagenic Activities of Thiobencarb and Its Sulfoxide. Mutagenic activities of thiobencarb and its sulfoxide were assayed by the Ames test using the preincubation technique (Figure 6). In order to know the effect of metabolic activation of these compounds, the preincubation was carried out either in the presence or absence of S9mix. Neither of the samples showed any mutagenic activity in the presence or absence of S9mix against strain TA98 at the concentrations of 30$-$1000 μg/plate. In the case of strain TA100, however, both the samples were mutagenic regardless of being in the presence or absence of S9mix. It seemed that the decrease of strain TA100 revertant colonies in concentrations of 300$-$1000 μg/plate thiobencarb was responsible for the toxicity. Cashman and Olsen (1990)

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Table 1. Analysis of Chlorination Byproducts of Thiobencarb at Various Concentrations of Free Chlorine

free chlorine added (mg/L)	thiobencarb [$t_R{}^a$ = 22.9 min]		thiobencarb sulfoxide [t_a = 10.2 min]		p-chlorobenzyl alcohol [t_R = 10.9 min]		p-chlorobenzaldehyde [t_R = 13.52 min]		p-chlorobenzyl chloride [t_R = 21.1 min]		total (nM)
	(µg/L)	(nM)	(µg/L)	(nM)	(µg/L)	(nM)	(µg/L)	(nM)	(µg/L)	(nM)	
0	100	389	ND	ND	ND	ND	ND	ND	ND	ND	389
0.1	65	252	33	121	ND	ND	ND	ND	ND	ND	373
0.3	9	35	89	326	ND	ND	ND	ND	ND	ND	361
0.7[b]	ND[c]	ND	98	359	ND	ND	ND	ND	ND	ND	359
1	ND	ND	99	363	ND	ND	ND	ND	ND	ND	363
3	ND	ND	96	352	ND	ND	ND	ND	ND	ND	352
10	ND	ND	81	297	1	7	ND	ND	ND	ND	304
30	ND	ND	55	201	3	21	ND	ND	1	6	228
100	ND	ND	18	66	5	35	2	14	2	12	127
300	ND	ND	ND	ND	7	49	5	36	2	12	97

[a] Retention time. [b] Chlorination in tap water containing 0.7 mg/L of resudal chlorine. [c] Not detected (less than 1 µg/L).

Table 2. Analysis of Chlorination Byproducts of Thiobencarb Sulfoxide at Various Concentrations of Free Chlorine

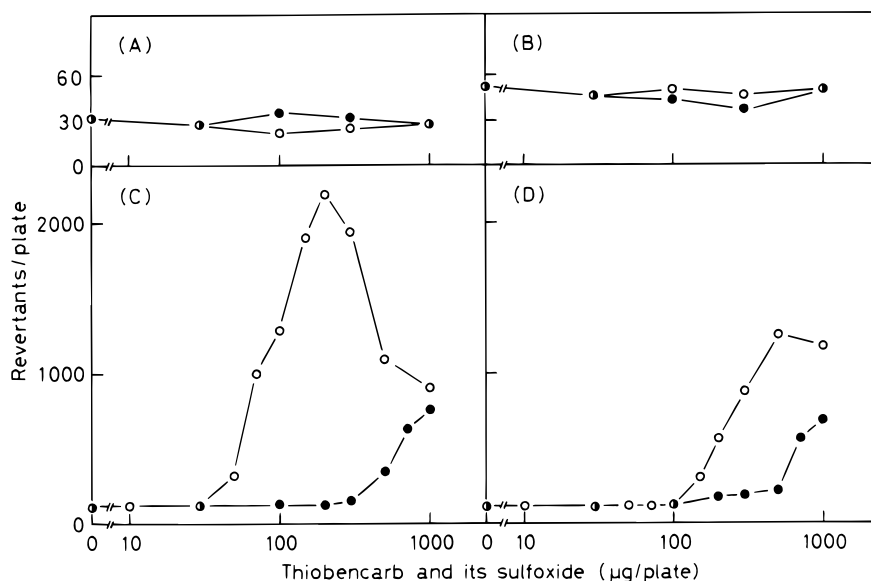| free chlorine added (mg/L)	thiobencarb sulfoxide		p-chlorobenzyl alcohol		p-chlorobenzaldehyde		p-chlorobenzylchloride		total (nM)
	(µg/L)	(nM)	(µg/L)	(nM)	(µg/L)	(nM)	(µg/L)	(nM)	
0	100	366	ND[a]	ND	ND	ND	ND	ND	366
1	100	366	ND	ND	ND	ND	ND	ND	366
10	86	315	ND	ND	ND	ND	ND	ND	315
100	31	113	4	28	1	7	1	6	154
300	3	11	6	42	5	36	2	12	100

[a] Not detected (less than 1 µg/L).

Figure 6. Dose−response curve of thiobencarb and its sulfoxide by the Ames TA98 and TA100 assay. Mutagenic activities of thiobencarb (○) and its sulfoxide (●) were measured by the Ames TA98 (A, B) and TA100 (C, D) assay in the presence (B, D) or absence (A, C) of S9mix.

reported that thiobencarb sulfoxide was the principal metabolite of thiobencarb in the presence of hepatic microsomes from striped bass, and accounted for 98% of the total thiobencarb. They also reported that this reaction was catalyzed largely by flavin-containing monooxygenase and to a lesser extent by cytochromes P-450. The mutation frequency (1.0 rev/µg) of thiobencarb sulfoxide in the presence of S9mix was almost the same as that (1.2 rev/µg) in the absence of S9mix. In contrast, the mutation frequency (3.0 rev/µg) of thiobencarb in the presence of S9mix was lower than that (14.3 rev/µg) in the absence of S9mix. This might suggest that thiobencarb was S-oxygenated in the presence of S9mix during the preincubation time, because S9mix contained microsomal fraction. Therefore, it could be considered that thiobencarb and its sulfoxide were mutagenic and both their mutagenic types were base-pair substitutions but were not frameshift.

CONCLUSION

It has been found that thiobencarb sulfoxide is produced by a reaction of thiobencarb and residual chlorine contained in tap water. In our experiments, the reaction occurred at low residual chlorine concentration in a range of 0.1−10 mg/L. In high residual chlorine concentration (more than 10 mg/L), the produced sulfoxide degraded followed by yielding p-chlorobenzyl alcohol, p-chlorobenzaldehyde, and p-chlorobenzyl chloride. On the basis of the above results, it seems that thiobencarb sulfoxide is a major byproduct from thiobencarb in tap water processed by chlorination. And it was found that thiobencarb sulfoxide was mutagenic despite its mutagenic activity being lower than that of thiobencarb. In Japan, the level of thiobencarb remaining in tap water is being regulated at less than 20 µg/L by the Ministry of Health and Welfare. In our preliminary study, thiobencarb was detected but its

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sulfoxide was not detected by GC/MS using DB-1 or DB-17 column (J&W Scientific), because the sulfoxide may be thermally unstable (Draper and Crosby, 1981). By using a fully-automated HPLC analysis system, linearity (r > 0.999) was demonstrated in a range of 1−100 μg/L by standard curves of thiobencarb and its sulfoxide. Therefore, it was suggested that the management and control of the herbicide thiobencarb in tap water processed by chlorination as in Japan should include monitoring of thiobencarb sulfoxide, using HPLC.

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