Major Degradation Pathway of Thiuram in Tap Water Processed by Oxidation with Sodium Hypochlorite

Shuji Kodama,* Atsushi Yamamoto, Mikiya Ohto, and Akinobu Matsunaga

Toyama Institute of Health, 17-1 Nakataikoyama, Kosugi-machi, Toyama 939-0363, Japan

Thiuram (3 μM), a fungicide, was incubated in deionized water by adding 0−100 mg/L free chlorine at 30 °C for 30 min, and the solution was analyzed by HPLC and IC. The byproducts were identified by LC/MS, EI-MS, infrared, and ^{13}C NMR spectra and a reduction technique using 2-mercaptoethanol. On the basis of these results, it was found that the oxidation of thiuram with sodium hypochlorite initially produced an intermediate dimethylthiocarbamoyl dimethylcarbamoyl disulfide, which was finally degraded to bis(dimethylcarbamoyl) disulfide, its trisulfide, and dimethylamine. Subsequently, it was suggested that monitoring of bis(dimethylcarbamoyl) disulfide, its trisulfide, and dimethylamine should be included for the management and control of thiuram in tap water processed by oxidation with sodium hypochlorite.

Keywords: *Thiuram; dimethylthiocarbamoyl dimethylcarbamoyl disulfide; bis(dimethylcarbamoyl) disulfide; bis(dimethylcarbamoyl) trisulfide; tap water; fungicide*

INTRODUCTION

Thiuram, bis(dimethylthiocarbamoyl) disulfide, is a thiocarbamate fungicide that has been widely used for the protection of seeds and vegetable crops and as an accelerator and vulcanization agent in the rubber industry. It has been reported that plate incorporation assays with *Salmonella typhimurium* demonstrated direct mutagenecity of thiuram in TA100 (Crebelli et al., 1992; Franekic et al., 1994). The Integrated Risk Information System has set up the toxicological constant of thiuram at 0.005 mg/kg/day, and Smith (1996) has established the risk-based concentration of thiuram in tap water at 180 μg/L. In Japan, a regulation of the Ministry of Health and Welfare requires the level of thiuram remaining in tap water to be <6 μg/L. There are many studies on the analysis of thiuram in river, pond, and tap water (Ohto et al., 1993; Suzuki et al., 1993; Kodama et al., 1995; Moreno-Tovar and Santos-Delgado, 1995; Kiso et al., 1996).

Miles and Moye (1988) reported that methylamine derived from thiuram by UV photolysis could be detected by fluorescene assay after reacting with *o*-phthalaldehyde and 2-mercaptoethanol. A variety of products were identified including carbon disulfide, tetramethylthiourea, *N*,*N*-dimethylthioformamide, tetramethylhydrazine, and dimethylamine by UV photolysis, photo-oxidation, and visible photosensitized oxidation of thiuram (Crank and Mursyidi, 1992), and it was concluded that C−S and S−S bond fissions were primary photochemical events in the photochemistry of dithiocarbamates. Thus, it seems that thiuram is able to be degraded under environmental conditions. Niitsuma et al. (1993) reported that thiuram was easily degraded by ozone treatment with or without UV radiation. Suzuki et al. (1993) showed that thiuram spiked in tap water containing 0.3−0.4 mg/L free available chlorine disappeared without any treatment and the loss was overcome by the addition of sodium sulfide before spiking of the pesticide. Thiuram has been known to be degraded immediately during water purification using sodium hypochlorite, and ∼30 mol of sodium hypochlorite reacted with a thiuram molecule (Ohto et al., 1993). Chew and Harpp (1993) observed that the oxidation of thiuram using thionyl chloride or chlorine gas in carbon tetrachloride gave *N*,*N*-dimethylthiocarbamyl chloride and diatomic sulfur, but, so far, little has been reported on the byproducts of thiuram in tap water processed by oxidation with sodium hypochlorite. The objective of this study was to determine the major degradation pathway of thiuram by the oxidation. We have also examined the mutagenesis of the final byproducts.

EXPERIMENTAL PROCEDURES

Chemicals. S9mix was purchased from Oriental Yeast Co., Ltd. Thiuram (pesticide grade), acetonitrile (HPLC grade), and other chemicals (reagent grade) were obtained from Wako Pure Chemical Industries Ltd. Oxidation byproducts of thiuram with sodium hypochlorite were prepared as described below.

Stock solutions (1000 μM) of thiuram and its oxidation byproducts with sodium hypochlorite were separately prepared in acetonitrile. Standard solutions were obtained by mixing together all of their stock solutions in deionized water to final concentrations of 0.03−3 μM each.

HPLC Apparatus. The HPLC system consisted of a Hitachi autosampler model AS-4000, two Hitachi pumps model L-6300, a Rheodyne manual injector, a Shimadzu photodiode array detector model SPD-M10AV, a Shimadzu column oven model CTO-10AC, and a Senshu Scientific switching valve model E1E010. Separations were attained using an RSpak DE-613 column (6 mm i.d. × 150 mm, Showa Denko), in conjunction with a 4 mm i.d. × 10 mm enrichment column packed with a polymethacrylate-based gel. The system was controlled by a Hitachi HPLC manager model D-6100. Data acquisition and processing were conducted with a Shimadzu LC workstation model CLASS-M10A.

HPLC Analysis of Oxidation Byproducts of Thiuram with Sodium Hypochlorite. HPLC was performed by a fully automated system previously described (Kodama et al., 1997). In brief, 3 mL of the standard solution or the reaction mixture was applied to an enrichment column with an autosampler. The column was then washed. The switching valve was moved

10.1021/jf9813440 CCC: $18.00
Published on Web 06/25/1999

to the injection position, and the analytes trapped on the enrichment column were desorbed with a mobile phase and transferred to an analytical column. The separated components were measured with a photodiode array detector. The mobile phase used was 50% (v/v) acetonitrile. The flow rate was 1.5 mL/min, and the column temperature was kept at 40 °C. The analytes were detected at 220 nm.

Analysis of Oxidation Byproducts of Thiuram in Tap Water. Thiuram was added to 1 L of tap water containing 0.6 mg/L residual chlorine, to a final concentration of 6 μg/L, and left to stand at 30 °C for 0.5 or 20 h. The reaction mixture was applied onto a Sep-Pak PLUS PS-2 cartridge (Waters), and the absorbed material was eluted with acetonitrile. The eluate was then analyzed by HPLC.

Preparation of Oxidation Byproducts (B, E, and D) of Thiuram with Sodium Hypochlorite. Thiuram was added to 9 L of deionized water containing 100 mg/L free chlorine to a final concentration of 20 mg/L. The solution was left to stand at 30 °C for 30 min and was applied onto a Sep-Pak PS-2 cartridge (Waters), and the absorbed materials were eluted with acetonitrile. The eluate were applied to HPLC using a manual injector, and the oxidation byproducts were separately fractionated.

IC Apparatus. The IC system consisted of a Tosoh pump model CCPD, a Rheodyne manual injector model 7125, a Tosoh conductivity detector model CM-8010, a Shimadzu UV−VIS spectrophotometer, and a Shimadzu column oven model CTO-6A. Separations of dimethylamine or HS^- were attained using a Shim-pack IC-C3 column (4.6 mm i.d. × 100 mm, Shimadzu) or an IC-anion-PW column (4.6 mm i.d. × 50 mm, Tosoh), respectively, maintained at 40 °C. Data acquisition and processing were conducted with a Shimadzu Chromatopac model C-R4A.

IC Analysis of Dimethylamine. Dimethylamine was monitored by conductivity detection. The mobile phase was 1.2 mM nitric acid. The flow rate of the mobile phase was 1 mL/min.

IC Analysis of Hydrogen Sulfide Ion (HS^-). Stock solutions (1000 μM) of thiuram and its oxidation byproducts with sodium hypochlorite were separately diluted four times with deionized water. Each diluted solution (100 μL), deionized water (700 μL), 1 N NH_4OH (100 μL), and 150 mM 2-mercaptoethanol (100 μL) was mixed and left for 45min at room temperature and then applied on IC. The HS^- liberated was monitored by UV detection at 220 nm. The mobile phase was 2 mM Na_2HPO_4 and was run with a flow rate of 1 mL/min.

Liquid Chromatography (LC)/Mass Spectrometry (MS). LC/MS was performed with a Shimadzu model QP1100EX thermospray mass spectrometer. The mobile phase was 30% (v/v) acetonitrile/70 mM ammonium acetate, and the flow rate was 1 mL/min. The vaporizer tip temperature was 229 °C. Positive ions were sampled from a scanning range of *m*/*z* 110−500. Mass spectra were obtained using the discharge mode. The oxidation byproducts of thiuram with sodium hypochlorite were dissolved in acetonitrile to obtain a concentration of 50 mg/L, and a 20 μL aliquot of the solution was applied.

Electron Impact Mass Spectrometry (EI-MS). EI-MS spectra were performed on a Shimadzu model QP1100WA mass spectrometer (70 eV) by direct introduction. In the ionization chamber, the temperature was raised by steps of 40 °C/min from 100 to 250 °C.

Infrared (IR) Spectrometry. IR spectra were recorded on a 1600 series FTIR (Perkin-Elmer) with KBr disks.

^{13}C Nuclear Magnetic Resonance (NMR) Spectroscopy. The ^{13}C NMR spectra were obtained on a JEOL EX-400 in $CDCl_3$ with 0.05% tetramethylsilane.

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

Mutagenesis Assay of Thiuram and Its Oxidation Byproducts with Sodium Hypochlorite. *S. typhimurium* strain TA100 was used in the Ames test (Ames et al., 1975) using the preincubation technique (Yahagi et al., 1977) either in the presence or in the absence of a rat liver microsomal fraction containing cofactors (known as S9mix). Thiuram and its byproducts were purified by HPLC and then dissolved in dimethyl sulfoxide, and two plates were used per dose.

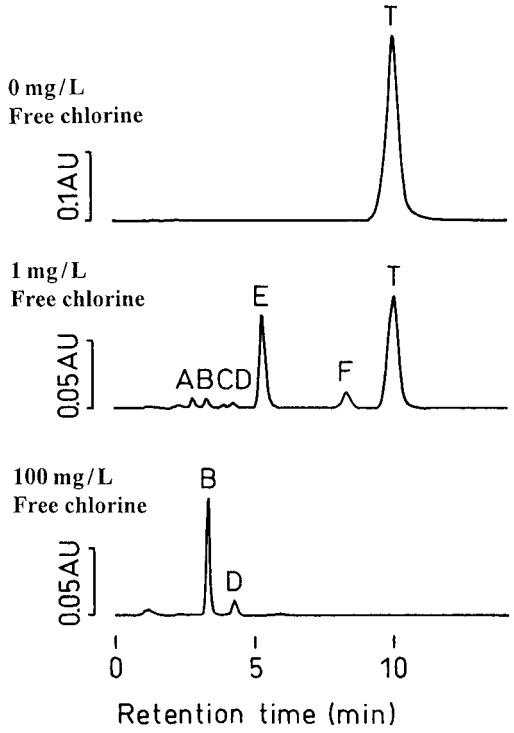

Figure 1. Typical chromatograms of the reaction mixture incubated with thiuram and 0, 1, and 100 mg/L free chlorine at 30 °C for 30 min. Peaks A−F correspond to the byproducts A−F described in the text. T indicates peak of thiuram.

RESULTS AND DISCUSSION

Degradation of Thiuram with Sodium Hypochlorite. Sodium hypochlorite was added to distilled water to obtain free chlorine concentrations of 0−100 mg/L. Thiuram was added to the chlorine solution to a final concentration of 3 μM, and the solution was incubated at 30 °C for 30 min. Three milliliters of the solution was then applied to HPLC (Figure 1). In the concentration of 1 mg/L residual chlorine, thiuram decreased and six unknown byproducts were detected. Among these byproducts, three byproducts (B, D, and E) could be isolated, but the others were unstable. Although thiuram and the other byproducts were decomposed by oxidation (100 mg/L free available chlorine) of thiuram, only byproducts B and D could be identified.

Identification of the Oxidation Byproducts of Thiuram with Sodium Hypochlorite. Thiuram and its oxidation byproducts were applied to LC/MS, and the mass spectrum of the top of each peak was obtained. The spectra of thiuram and its byproducts (B, D, and E) were characterized by $[M + H]^+$ ions at *m*/*z* 241, 209, 225, and 241, respectively. Therefore, it was suggested that the molecular weights of byproducts B, D, and E were 208, 224, and 240, respectively.

Thiuram and its oxidation byproducts were applied to EI-MS. As shown in Figure 2, the mass spectrum of thiuram showed a molecular ion peak at *m*/*z* 240 along with fragment ion peaks at 120 and 88 corresponding to dimethylthiocarbamic acid and dimethylthiocarbamoyl ion peaks, respectively. The mass spectrum of byproduct E gave a molecular ion peak at *m*/*z* 224 along with fragment ion peaks at *m*/*z* 88 and 72. The mass spectra of both byproducts B and D gave only a fragment

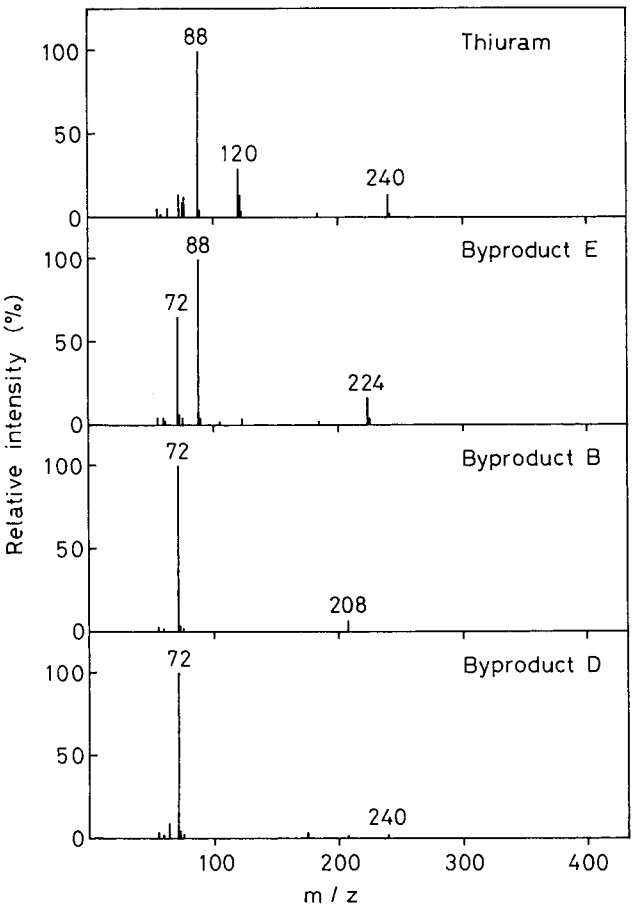

Figure 2. EI-MS spectra of thiuram and its oxidation byproducts with sodium hypochlorite.

ion peak at *m/z* 72 other than the molecular ion peaks at *m/z* 208 and 240, respectively. Molecular weights of byproducts E and B had mass deficiencies of 16 and 32, respectively, as compared with that of thiuram. A thiuram molecule has two dimethylthiocarbamoyl [$(CH_3)_2$-NC(=S)−] groups that result in the fragment ion peak at *m/z* 88. If the sulfur atom of dimethylthiocarbamoyl group can be converted by oxidation of thiuram into oxygen, the dimethylcarbamoyl [$(CH_3)_2NC(=O)-$] group resulted in the fragment ion peak at *m/z* 72. It may be assumed that the byproduct E has a dimethylthiocarbamoyl group and a dimethylcarbamoyl group and that byproduct B has two dimethylcarbamoyl groups. It also seems that byproduct D has two dimethylcarbamoyl groups, although the molecular weight of byproduct D was the same as that of thiuram.

Thiuram and its oxidation byproducts were applied to ^{13}C NMR spectroscopy (Figure 3). The ^{13}C NMR spectrum of thiuram showed three signals at 193.6 ppm (thiocarbamoyl C) and 47.5 and 42.1 ppm (dimethylamino C). On the other hand, the ^{13}C NMR data of byproduct B showed three signals at 163.8, 37.9, and 37.0 ppm, which were equal to those of dimethylcarbamoyl chloride (Pouchert and Behnke, 1993). Moreover, byproduct E showed six signals at 194.8, 163.0, 47.7, 41.8, 38.0, and 37.2 ppm that were mixtures of both signals of thiuram and byproduct B. Therefore, it was found that these data supported the above assumption.

Figure 4 shows the IR spectra of thiuram and its oxidation byproducts. In the case of both byproduct B and byproduct D, the signal at 1500 cm $^{-1}$ present in

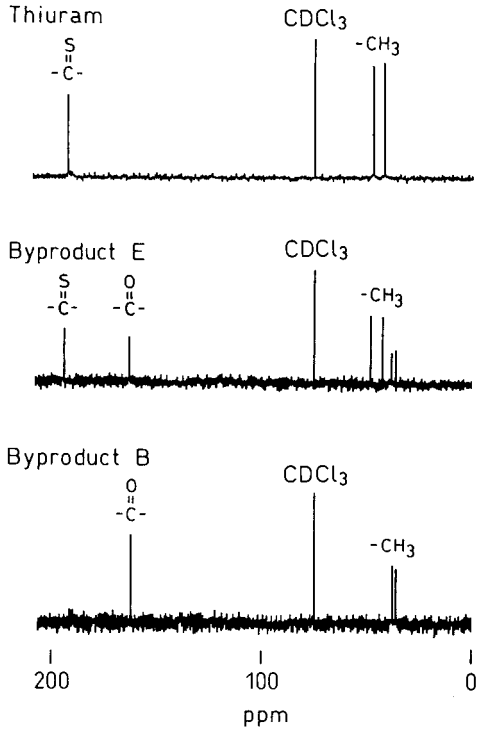

Figure 3. ^{13}C NMR spectra of thiuram and its oxidation byproducts with sodium hypochlorite.

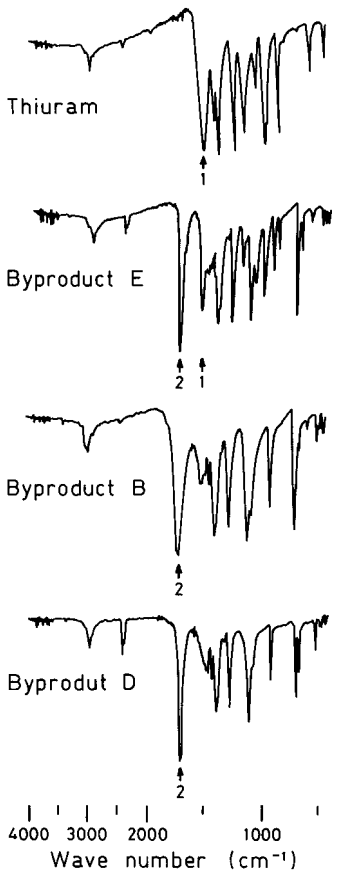

Figure 4. IR spectra of thiuram and its oxidation byproducts with sodium hypochlorite. Arrows 1 and 2 indicate wavenumbers at 1690 and 1500 cm^{-1}, respectively.

the spectrum of thiuram disappeared and a strong absorption band at 1690 cm $^{-1}$ appeared. The spectrum of byproduct E showed absorption bands at both 1500 and 1690 cm^{-1}. It was reported that the IR spectrum of

Table 1. Analysis of Oxidation Byproducts of Thiuram at Various Concentrations of Free Chlorine

free chlorine added		thiuram	byproduct B	byproduct D	byproduct E	dimethylamine	total
mg/L	μM	(μM)	(μM)	(μM)	(μM)	($\times 1/2$ μM)	(μM)
0	0	3.00	ND[a]	ND	ND	ND	3.00
0.1	2.8	2.44	ND	ND	0.12	ND	2.56
0.3	8.5	1.76	ND	ND	0.38	0.20	2.34
1	28	0.94	0.06	0.04	0.60	0.63	2.27
3	85	ND	0.22	0.14	0.12	1.34	1.85
10	280	ND	0.36	0.11	ND	2.47	2.94
30	850	ND	0.67	0.12	ND	2.30	3.09
100	2800	ND	0.83	0.11	ND	1.98	2.92

[a] Not detected (<0.03 μM for thiuram and byproducts B, D, and E; <0.1 μM for dimethylamine).

Table 2. Analysis of Oxidation Byproducts of Byproduct E at Various Concentrations of Free Chlorine

free chlorine added		byproduct E	byproduct B	byproduct D	dimethylamine	total
mg/L	μM	(μM)	(μM)	(μM)	($\times$ 1/2 μM)	(μM)
0	0	3.00	ND[a]	ND	ND	3.00
1	28	ND	0.29	0.19	0.88	1.38
10	280	ND	0.38	0.08	2.41	2.87
100	2800	ND	0.64	0.09	2.12	2.85

[a] Not detected (<0.03 μM for thiuram and byproducts B, D, and E; <0.1 μM for dimethylamine).

dimethylthiocarbamoyl dimethylcarbamoyl disulfide showed absorption bands at 1500 and 1665 cm^{-1} that could be assigned to carbonyl and thiocarbamoyl groups, respectively (El-Wassimy et al., 1983). These data also supported the above results obtained by LC/MS, EI-MS, and ^{13}C NMR spectra. Therefore, it was concluded that byproduct E was dimethylthiocarbamoyl dimethylcarbamoyl disulfide [$(CH_3)_2NC(=S)SSC(=O)N(CH_3)_2$] and that byproduct B was bis(dimethylcarbamoyl) disulfide [$(CH_3)_2NC(=O)SSC(=O)N(CH_3)_2$].

Because both the IR and EI-MS spectra of byproduct D were the same as those of byproduct B, it was suggested that byproduct D, like byproduct B, had two dimethylcarbamoyl groups. The molecular mass of byproduct D was 32 Da larger than that of byproduct B as described in Figure 1. These results may indicate that byproduct D is a trisulfide compound having two dimethylcarbamoyl groups. Fletcher and Robson (1963) reported that the reduction of a mole of bis(2-amino-2-carboxyethyl) trisulfide gave 1 mol of hydrogen sulfide and that of its disulfide did not liberate any hydrogen sulfide. The same results were obtained by using other compounds with a trisulfide or a disulfide linkage (Jespersen et al., 1994; Lundin et al., 1994). We tried to reduce thiuram and its oxidation byproducts with 2-mercaptoethnol in 0.1 N NH_4OH and to determine hydrogen sulfide ion released by IC. As a result, 0.92 mol of hydrogen sulfide ion/mol of byproduct D was detected, whereas reductions of thiuram and byproducts B and E, which have a disulfide bridge, did not liberate hydrogen sulfide ion (<0.05 mol/mol of compound). Therefore, it is suggested that byproduct D was bis(dimethylcarbamoyl) trisulfide [$(CH_3)_2NC(=O)SSSC(=O)N(CH_3)_2$].

Production of Dimethylamine from Thiuram by Oxidation with Sodium Hypochlorite. As mentioned above, oxidation of thiuram with sodium hypochlorite could result in the transformation of the thiocarbonyl group to a carbonyl group. If the substitution of a sulfur atom with an oxygen atom further proceeds, it can be thought that thiuram is further degraded to dimethylamine via unstable carbamic acid by oxidation with sodium hypochlorite. To confirm the above possibility, we applied a reaction mixture of thiuram and residual chlorine to IC. As a result, dimethylamine was apparently detected. Therefore, it was suggested that dimethylamine was also an oxidation byproduct from thiuram.

Degradation of Thiuram and Its Oxidation Byproducts at Various Concentrations of Free Chlorine. Thiuram was incubated in deionized water by adding 0−100 mg/L residual chlorine to a final concentration of 3 μM at 30 °C for 30 min. A 3 mL aliquot of the mixture was applied to HPLC, and a 100 μL aliquot of the mixture was applied to IC; the byproducts were then determined (Table 1). The concentration of dimethylamine was expressed by multiplying the value obtained by half, because a thiuram molecule has two dimethylamino groups. As free chlorine increased, thiuram decreased and it could not be detected at 3 mg/L (85 μM) free chlorine. This was the same as the result that the amount of chlorine consumed per mole of thiuram was ~30 mol (Ohto et al., 1993). Although byproduct E was increased with a decrease of thiuram, this byproduct also could not be detected at 10 mg/L free chlorine. Byproducts B and D and dimethylamine were finally produced with decreases of thiuram and byproduct E. In a low free chlorine medium (0.1−3 mg/L), the sum of the concentrations of all compounds identified was <3 μM. It appears that this difference resulted in the unstable byproducts that were unidentified at this time. In a high chlorine medium (10−100 mg/L), the sum of the concentrations of byproducts B and E and dimethylamine was almost the same as the starting concentration of thiuram.

To confirm that dimethylamine and byproducts B and D resulted from the degradation of byproduct E, we treated byproduct E with various concentrations of free chlorine. As shown in Table 2, it was confirmed that dimethylamine and byproducts B and D were produced by the chlorination of byproduct E, as well as the production of these compounds in Table 1. Furthermore, it was confirmed that neither byproduct B nor byproduct D was affected by chlorination with 100 mg/L free chlorine.

Therefore, these results suggested that oxidation with sodium hypochlorite could produce bis(dimethylcarbonyl) disulfide, its trisulfide, and dimethylamine from

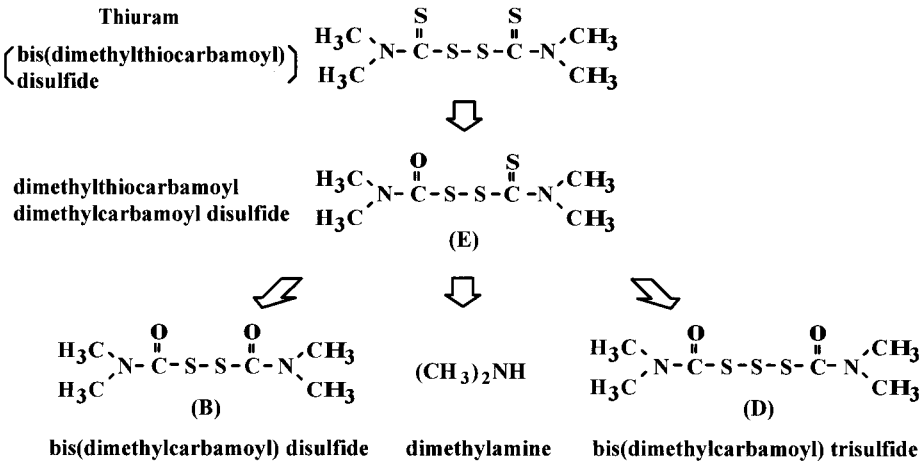

Figure 5. Major degradation pathway of thiuram by oxidation with sodium hypochlorite. B, D, and E designate each structure with corresponding letter as given in the text.

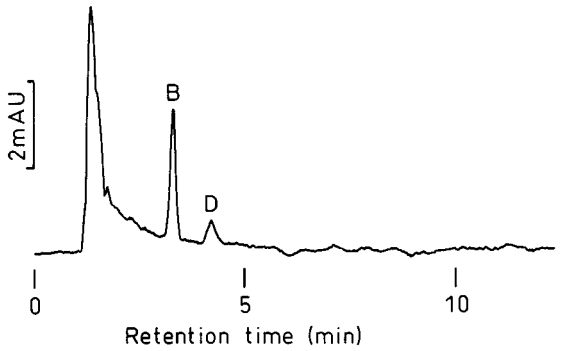

Figure 6. Typical chromatogram of thiuram incubated in tap water at 30 °C for 20 h.

thiuram via dimethylthiocarbamoyl dimethylcarbamoyl disulfide (Figure 5).

Analysis of Oxidation Byproducts of Thiuram in Tap Water. The level of thiuram remaining in tap water is required to be <6 μg/L by the Ministry of Health and Welfare in Japan. As described above, we have studied the oxidation of thiuram using very high concentrations of thiuram and free chlorine that cannot exist in environmental conditions. To confirm the above results, thiuram was added to tap water containing 0.6 mg/L residual chlorine to a final concentration of 6 μg/L and left to stand at 30 °C for 0.5 or 20 h. The reaction mixture was then analyzed by HPLC. As shown in Figure 6, both byproducts B and D were detected. This result was unaffected by the incubation time.

Mutagenic Activities. The mutagenic activities of thiuram and its byproducts B and D were assayed by the Ames test using the preincubation technique. To determine the effect of metabolic activation of these compounds, the preincubation was carried out either in the presence or in the absence of S9mix. When the thiuram concentrations were 10−30 μg/plate, the number of strain TA100 revertant colonies was induced by a factor of 1.2−1.6 as compared with the control run regardless of whether S9mix was present or absent. However, when the thiuram concentration was >30 μg/plate, either decrease or loss of the colonies that might be responsible for toxicity was observed. The same results were obtained when byproducts B and D were used. Therefore, we were unable to determine whether thiuram and its oxidation byproducts were mutagenic.

Conclusion. Thiuram was degraded to many byproducts by oxidation with sodium hypochlorite. Although there are unstable compounds among these byproducts, the major byproducts could be isolated and identified. It was found that the replacement of the sulfur atom of dimethylthiocarbamoyl group with oxygen was the first step of oxidation of thiuram with sodium hypochlorite. It was also found that the oxidation of thiuram initially produced an intermediate dimethylthiocarbamoyl dimethylcarbamoyl disulfide, and this intermediate was finally degraded to bis(dimethylcarbamoyl) disulfide, its trisulfide, and dimethylamine. Therefore, we conclude that the management and control of the fungicide thiuram in tap water processed by oxidation with sodium hypochlorite as in Japan should include monitoring of bis(dimethylcarbamoyl) disulfide, its trisulfide, and dimethylamine.

ACKNOWLEDGMENT

We are grateful to Drs. Y. Asano and Y. Kato, Biotechnology Research Center, Faculty of Engineering, Toyama Prefectural University, for performing the ^{13}C NMR spectra and for useful discussions. We thank Dr. K. Hayakawa and Mr. K. Suyama, Faculty of Pharmaceutical Science, Kanazawa University, for the Ames test and for valuable discussions. We also thank Dr. N. Takayama and Mr. S. Tanaka, Chemistry Section, Forensic Science Laboratory, Ishikawa Prefecture Police Headquarters, for performing the LC/MS spectra.

LITERATURE CITED

Ames, B. N.; McCann, J.; Yamasaki, E. Methods for detecting carcinogens and mutagens with the *Salmonella*/mammalian microsome mutagenecity test. *Mutat. Res.* **1975**, *31*, 347−364.

Chew, W.; Harpp, D. N. Chlorination of tetramethyl thiuram disulfide: A precursor to diatomic sulfur? *Sulfur Lett.* **1993**, *16*, 19−24.

Crank G.; Mursyidi A. Photochemistry of some organosulfur pesticides. *J. Photochem. Photobiol. A: Chem.* **1992**, *68*, 289−297.

Crebelli, R.; Zijno, A.; Conti, L.; Crochi, B.; Leopardi, P.; Marcon, F.; Renzi, L.; Carere, A. Further in vitro and in vivo mutagenicity assays with thiuram and ziram fungicides: Bacterial reversion assays and mouse micronucleus test. *Teratog. Carcinog. Mutagen.* **1992**, *12*, 97−112.

El-Wassimy, M. T. M.; Jorgensen, K. A.; Lawesson, S. O. The reaction of *tert*-butyl hypochlorite with thiocarbonyl com-

pound—A convenient method for the >C═S → >C═O transformation. *Tetrahedron* **1983**, *39*, 1729−1734.

Fletcher, J. C.; Robson, A. The occurrence of bis(2-amino-2-carboxyethyl) trisulfide in hydrolysates of wool and other proteins. *Biochem. J.* **1963**, *87*, 553−559.

Franekic, J.; Bratulic, N.; Pavlica, M.; Papes, D. Genotoxity of dithiocarbamates and their metabolites. *Mutat. Res.* **1994**, *325*, 65−74.

Jespersen, A. M.; Christensen, T.; Klausen, N. K.; Nielsen, P. F.; Sorensen, H. H. Characterisation of a trisulfide derivative of biosynthetic human growth hormone produced in *Escherichia coli*. *Eur. J. Biochem.* **1994**, *219*, 365−373.

Kiso, Y.; Li, H.; Shigetoh, K.; Kitao, T.; Jinno, K. Pesticide analysis by high-performance liquid chromatography using the direct injection method. *J. Chromatogr. A* **1996**, *733*, 259−265.

Kodama, S.; Matsunaga, A.; Ohto, M.; Yamamoto, A.; Mizukami E. Establishment of a fully-automated HPLC analytic system for simazine, thiram and thiobencarb in tap water. *Kankyou Kagaku* **1995**, *5*, 81−86.

Kodama, S.; Yamamoto, A.; Matsunaga, A. S-oxygenation of thiobencarb in tap water processed by chlorination. *J. Agric. Food Chem.* **1997**, *45*, 990−994.

Lundin, R. H.; Noren, B. E.; Edlund, P. O.; A convenient method for the synthesis of peptide trisulfides. *Tetrahedron Lett.* **1994**, *35*, 6339−6342.

Miles, C. J.; Moye, H. A. Postcolumn photolysis of pesticides for fluorometric determination by high-performance liquid chromatography. *Anal. Chem.* **1988**, *60*, 220−226.

Moreno-Tovar, J.; Santos-Delgado, M. J. Solid-phase extraction and determination of carbamate pesticides in water samples by reverse-phase HPLC. *An. Quim.* **1995**, *91*, 365−373.

Niitsuma, T.; Suzuki, K.; Hachiya, E.; Takemoto, Y. Degradation of six pesticides by ozone with ultraviolet radiation. *Kankyou Kagaku* **1993**, *3*, 350−351.

Ohto, M., Yamamoto, A.; Matsunaga, A.; Takayanagi, N.; Kemmei T.; Saito, Y.; Mizukami, E. Chemical changes of pesticides used for golf links in chlorinated water. *Kankyou Kagaku* **1993**, *3*, 59−64.

Pouchert, C. J.; Behnke, J. *The Aldrich Library of ^{13}C and ^{1}H ^{FT}NMR Spectra*, 1st ed.; Aldrich Chemical Co.: Milwaukee, WI, 1993; Vol. 1, p 1217.

Smith, R. L. Risk-based concentrations: prioritizing environmental problems using limited data. *Toxicology* **1996**, *106*, 243−266.

Suzuki, T.; Yaguchi, K.; Kano, I. Screening methods for asulam, oxine-copper and thiram in water by high-performance liquid chromatography after enrichment with a minicolumn. *J. Chromatogr.* **1993**, *643*, 173−179.

Yahagi, T.; Nagao, M.; Seino, Y.; Matsushima, T.; Sugimura, T.; Okada, M. Mutagenicities of N-nitrosamines on *Salmonella*. *Mutat. Res.* **1977**, *48*, 121−131.

Received for review December 14, 1998. Revised manuscript received April 14, 1999. Accepted April 30, 1999.

JF9813440