Original Article

Chemical fate and mutagenic formation potentials of phenothiazine and related compounds during water chlorination

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ABSTRACT — The reactions of hetero-tricyclic aromatic hydrocarbons (H-TCAHs) with hypochlorite in an aqueous solution were investigated under conditions that simulate wastewater disinfection. H-TCAH-hypochlorite reaction products were determined by gas chromatographic-mass spectrometric (GC-MS) analyses. For 20 μ M, 10H-phenothiazine, 10H-phenoxazine, and phenoxathiin reacted rapidly with active chlorine in neutral pH (7.0), but no phenazine-hypochlorite reaction was observed over pH values of 5-9 for 1 hr. The 10H-phenothiazine-hypochlorite reaction began by oxidation with active chlorine to form its dioxides, followed by chloro-substitution in water. The extent of the reactions depended on the chlorine dose, solution pH and compound structures. Ames assays for the chlorination byproducts of 10H-phenothiazine and 10H-phenoxazine also showed to be weak mutagenicity in TA98 and TA100 strains without S9 mix, but no chlorination byproducts of phenoxathiin exhibited any mutagenicity in both tester strains with and without S9 mix.

Key words: Phenothiazine, Water chlorination, Disinfection byproduct, Mutagenicity, Ames assay

INTRODUCTION

Recent studies indicate the potential widespread occurrence of the low-level contamination (ng- μ g/l) of pharmaceuticals, hormones and other organic sewage contaminants and their metabolites in aquatic environments (Hernado *et al.*, 2006; Kasprzyk-Hordern *et al.*, 2008; Yu and Chu, 2009; Okuda *et al.*, 2009). This implies that these pharmaceuticals are incompletely eliminated in wastewater treatment plants (Nakada *et al.*, 2006, 2007; Kasprzyk-Hordern *et al.*, 2009; Klavarioti *et al.*, 2009). In Japan, municipal wastewater effluents are often disinfected with chlorine prior to their discharge. Chlorine is also frequently used as a primary or residual disinfectant during drinking water treatment. To better understand the potential for the transformation of pharmaceuticals during chlorine disinfection, it is necessary to quantify the kinetics of these reactions encountered in water and wastewater treatment systems.

When chlorine is used to disinfect filtrated or denitrified wastewater effluents or drinking water, the active forms of the disinfectant are hypochlorous acid (HOCl) and hypochlorite (OCl⁻), also known as free chlorine.

In addition to their biocidal properties, HOCl/OCl⁻ species act as oxidants or as electrophiles, selectively reacting with certain functional groups in organic compounds. One of the best characterized reactions of HOCl/OCl⁻ is the reaction of HOCl with phenols (Onodera *et al.*, 1989a, 1995 and 1998; Gallard and Gutten, 2002). In this reaction, HOCl reacts with the phenolate anion to yield mostly ortho- and para-substituted chlorophenols and their dimers. Because of presences of the dissociable protons on both the reactive species, the observed rate of reaction usually exhibits a maximum between the pKa of HOCl (i.e. 7.5) and that of the phenol.

Another well-characterized reaction of chlorine involves chlorine addition to primary and secondary amines (Antero *et al.*, 1996; Armesto *et al.*, 1996; Abia *et al.*, 1998). The reactive species in this reaction are the un-protonated amine and HOCl. As a result, the observed reaction rate typically exhibits a maximum between the pKa of HOCl and that of the amine. The N-chloro compounds formed by this reaction can be reconverted to the parent compound by reactions with strong nucleophiles, such as thiosulphate or bisulphite, which are used to dechlorinate wastewater prior to discharge. In the absence of

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a strong nucleophile, the chlorinated amines can decompose into stable products (Antero *et al.*, 1996; Armestro *et al.*, 1996).

When chlorine is used as a disinfectant in wastewater effluents that contains ammonia and organonitrogen, most of the chlorine is converted into chloramines; this is also referred to as combined chlorine. Under typical conditions, most of the chloramines generally consist of monochloramine (i.e. NH_2Cl) along with lower concentrations of dichloramine and chlorine-substituted amines (Jolley *et al.*, 1981). In addition, many drinking water plants have begun using combined chlorine as their disinfection methods in order to reduce the formation of disinfection byproducts, such as trihalomethanes. Although chloramines are much weaker oxidants than HOCl/OCl^- , they can react with some organic and nitrogenous compounds (Buttschell *et al.*, 1959).

In order to provide further insight into the possible role of organic compounds in the formation of chlorine-substituted compounds and chlorine-induced mutagens, this laboratory has continued the study of the aqueous chlorination chemistry of organic compounds (Nakamura *et al.*, 2008; Nakajima *et al.*, 2009; Kawakami *et al.*, 2009). The present report describes a detailed study of the products distribution for four hetero-tricyclic aromatic hydrocarbons (H-TCAHs), which were selected because of their widespread use in pharmaceutical and textile manufacturing industries, and as a suppressor in the polymerization of vinyl acetate, acrolein and acrylic acid.

MATERIALS AND METHODS

Chemicals

H-TCAHs, including 10H-phenothiazine, 10H-phenoxazine, phenoxathiin and phenazine, were obtained

from Wako Pure Chemical Industry (Osaka, Japan), Tokyo Chemical Industry (Tokyo, Japan), and Nacalai Tesque (Kyoto, Japan). Their molecular structures are shown in Table 1. Some of the oxygenated (phenothiazine-5,5'-dioxides and phenoxathiin-10,10'-dioxide) and chloro-substituted compounds of these H-TCAHs, which are expected to be formed during the chlorination of H-TCAHs with aqueous chlorine, were commercially available. The hypochlorite solution was prepared by diluting the sodium hypochlorite solution (ca. 10% of the available Cl, Nacalai Tesque) in a 0.1 M potassium dihydrogenphosphate-0.05 M disodium tetraborate decahydrate buffer solution of pH 7. The hypochlorite concentrations were determined by means of iodometric titration.

Aqueous chlorination of H-TCAH compounds

A mixture of 100 ml of hypochlorite solution and each H-TCAH compound dissolved in 1 ml of methanol was shaken in a separatory funnel at room temperature for 1 hr. After the reaction time, the residual chlorine was removed by the addition of an equivalent volume of sodium sulfite solution. The reaction mixture was then acidified to pH 2 with 0.1 M hydrochloric acid before extracting three 20-ml volumes of diethyl ether. The solvents were dried over anhydrous sodium sulfate and 1 ml of methanol was added to prevent the evaporation of the reaction products during concentration under vacuum at 40°C to suitable volumes for gas chromatographic (GC) and GC-mass spectrometric (GC-MS) analysis.

Analytical methods

The extracts were analyzed using a Varian CP3800 gas chromatograph coupled to a Saturn 2200 ion trap GC-MS (Varian, Walnut Creek, CA, USA) equipped with a TC-1 capillary column (30 m x 0.25 mm i.d. and a film thick-

Table 1. H-TCAHs examined in aqueous chlorination studies

Name	CAS number	Formulae	M.W.	Structure
10H-phenothiazine	92-84-2	$\text{C}_{12}\text{H}_9\text{NS}$	199.28	
10H-phenoxazine	135-67-1	$\text{C}_{12}\text{H}_9\text{NO}$	183.21	
Phenoxathiin	262-20-4	$\text{C}_{12}\text{H}_8\text{OS}$	200.26	
Phenazine	92-82-0	$\text{C}_{12}\text{H}_8\text{N}_2$	180.21	

ness of 0.12 μm film, GL Science Inc., Tokyo, Japan) under temperature ranging from 80 to 270°C at the rate of 10°C min. The helium gas flow-rate was 1 ml/min. The electron ionization conditions were as follows: ion energy, 70 eV; ion source temperature of 230°C; $m/z = 50$ –600 full scan for the qualitative analysis. A sample volume of 1 μl was splitlessly injected into the GC-MS at 270°C. The products were identified by comparison of their retention times and mass spectra with those of the authentic compounds.

Quantitative analyses were performed using a Shimadzu GC-14B gas chromatograph (Shimadzu, Kyoto, Japan) equipped with a flame ionization detector (FID). The analytical column and chromatographic conditions were the same as those described above. Both injector and detector temperatures were set at 270°C. A Shimadzu C-R6A data system was used to determine the retention times and peak areas on the chromatograms. Octadecane was used as an internal standard throughout the GC analyses of the reaction mixtures.

Mutagenicity assay

The mutagenicity of the samples was tested according to the methods of Maron and Ames (1983) with minor modifications. Because most of the mutagens formed during the reactions of organic compounds with the chlorine in water have been shown to be positive in *Salmonella typhimurium* strain TA100 without S9 mix (Onodera *et al.*, 1998), this strain was employed throughout the experiments, in addition of TA98 strain with and without S9 mix. The samples were dissolved in dimethyl sulfoxide (DMSO) and pre-incubated with a test strain at 37°C for 30 min with a phosphate buffer or S9 mix. After addition of the test samples, the plates were incubated at 37°C for 2 days. The assay was performed in triplicate for each sample and the mutagenic activity expressed as the mean of the revertants. The mutagenicity of the H-TCAHs and their chlorination byproducts were estimated by least-squares regression analysis of the initial slopes of the dose-response curves.

RESULTS AND DISCUSSION

Kinetics of the hypochlorite reactions

In a preliminary examination of the reactions of individual H-TCAHs with hypochlorite in a dilute aqueous solution, the decrease in the concentration of active chlorine (HOCl) during contact with each of these compounds was measured by using a Model RC-1 Resichlocorder (Bionics Instrument, Tokyo, Japan) at 20°C. To compare the results obtained by using the Resichlocorder measure-

ments, the concentrations of hypochlorite in the individual reactions were also followed by iodometric titrations. Furthermore, the decrease in the concentrations of these H-TCAHs was determined under the similar conditions by GC/FID analyses of the reaction mixtures.

Fig. 1A shows the time courses of the consumption of chlorine with these H-TCAH compounds (20 μM) in buffered solution at pH 7.0. Except for phenazine (which did not reveal any chlorine consumption after a reaction time of 5 hr), it can be seen that a large consumption of active chlorine occurs during the first 10 min, followed by much slower secondary reactions. Approximately 50% of the active chlorine consumed after the reaction for 1 hr for these compounds was observed within the first 10 min. In the 10H-phenothiazine-hypochlorite reaction, it was noted that the contact of the compound with active chlorine suddenly changed the solution from colorless to red-purple and then colorless again.

Fig. 1B also shows the residual concentrations of individual H-TCAHs (0.3 mM) in buffered water after treatment with hypochlorite at various molar ratios of hypochlorite per mol of the compound for 1 hr. The residual concentrations of 10H-phenothiazine and 10H-phenoxazine decreased rapidly with an increase in the hypochlorite molar ratios. Both the compounds disappeared completely when they were treated with hypochlorite at over 15 times their molar ratios. Phenoxathiin was still detected after the treatment with active chlorine at over 20 times the molar ratios. A much faster decrease in the concentrations of both active chlorine and the parent compounds were observed in the reactions of phenolic compounds (Onodera *et al.*, 1989a, 1995 and 1998) and some of the tricyclic aromatic hydrocarbons (TCAHs), such as anthracene and phenanthrene, (Onodera *et al.*, 1989b) with hypochlorite in aqueous solutions.

Formation of oxygenated and chlorine-substituted compounds of H-TCAHs

The diethyl ether extracts of aqueous H-TCAH solutions (0.3 mM) after treatment with hypochlorite (20 equivalents of HOCl per mole of the compound) at 20°C for 1 hr were analyzed by GC-MS. At least nine reaction products were observed on the chromatogram that obtained from the 10H-phenothiazine-hypochlorite reaction. Two peaks corresponding to the original material and its reaction product, and one peak corresponding to the original material were also detected on their chromatograms that were obtained from the phenoxathiin- and phenazine-hypochlorite reactions, respectively. However, no peaks were observed on the chromatogram that corresponded to any reaction products obtained from the 10H-

Fig. 1. Time courses of consumption of active chlorine with H-TCAHs (A, 20 μ M) in a buffered solution of pH 7.0 at 20°C, and residual amounts of H-TCAHs (B, 0.3 mM) in chlorinated water at various molar ratios of HOCl per mol of the compound for 1 hr.

phenoxazine-hypochlorite reaction. Some of the peaks were identified on the basis of their retention times and mass spectra when compared with those of the authentic compounds. Compounds corresponding to other peaks were determined from the mass spectral interpretations of each peak.

The major reaction product when 10H-phenothiazine was treated with hypochlorite at pH 5 for 1 hr was identified to be 10H-phenothiazine-5,5'-dioxide having the molecular ion (M^+) at $m/z = 231$ ($C_{12}H_9NSO_2^+$) and several fragment ions at m/z values of 183 ($C_{12}H_9NO^+$) and 167 ($C_{12}H_9N^+$). The product in the 10H-phenothiazine-hypochlorite reaction was also identified to be 10Cl-phenothiazine-5,5'-dioxide having the molecular ion (M^+) at $m/z = 265$ ($C_{12}H_8ClNSO_2^+$) and several fragment ion at m/z values of 230 ($C_{12}H_8NSO_2^+$), 217 ($C_{12}H_8ClNO^+$), 201 ($C_{12}H_8ClO^+$), and 166 ($C_{12}H_8N^+$). The same molecular ion at $m/z = 265$ was observed for another small peak with a somewhat different fragmentation pattern. The compound corresponding to another small peak exhibited a larger retention time and broad tailing in the chromatogram. The molecular ion, its fragmentation, and chromatographic behaviors indicate the occurrence of monochloro-substituted 10H-phenothiazine-5,5'-dioxide in the diethyl ether extract.

Other reaction products corresponding to the other

peaks were determined from the mass spectrum of each compound in the same manner as described above. These GC-MS studies demonstrated the occurrence of dichloro-, trichloro-, tetrachloro-substituted 10Cl-phenothiazine-5,5'-dioxide and 10H-phenothiazine-5,5'-dioxide, in the diethyl ether extract of the 10H-phenothiazine-hypochlorite solution. In addition, the GC-MS studies indicate the occurrence of phenoxathiin-10,10'-dioxide in the diethyl ether extract of the phenoxathiin-hypochlorite solution. A summary of the chlorination products identified or determined from their relative retention times and mass spectrometric characteristics is listed in Table 2. These findings lead to the conclusion of the aqueous chlorination of 10H-phenothiazine solutions with hypochlorite yields not only 10H-phenothiazine-5,5'-dioxide but also those of the chlorine substituents.

The occurrence of chlorine-substituted and oxygenated compounds in chlorine-treated TCAH solutions has been confirmed in previous papers (Oyler *et al.*, 1978; Onodera *et al.*, 1989b). Similar compounds have also been discovered to be present in chlorinated leachate from coal tar (Alben, 1980) and drinking water (Shiraishi *et al.*, 1995; Onodera, 1991). However, no oxygenated and chlorine-substituted compounds have been reported in H-TCAHs-hypochlorite reaction in the literatures (Oyler *et al.*, 1978; Alben, 1980; Shiraishi *et al.*, 1985; Onodera *et al.*,

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Table 2. Mass spectrometric characteristics of the reaction products of phenothiazine with hypochlorite in neutral solution at 20°C for 1 hr

Products identified or determined No.	Formulae	Chemical name	Mass spectrometric characteristics
			Molecular (M^+) and fragment ions (m/z , relative intensity)
1	$C_{12}H_8ClNSO_2$	10Cl-phenothiazine-5,5'-dioxide	265 (M^+ , 100), 230 (M^+-Cl , 8), 217 (M^+-SO , 22), 201 (M^+-SO_2 , 19), 166 (M^+-SO_2-Cl , 16)
2	$C_{12}H_7Cl_2NSO_2$	10Cl-Cl-phenothiazine-5,5'-dioxide	299 (M^+ , 100), 264 (M^+-Cl , 19), 251 (M^+-SO , 27), 235 (M^+-SO_2 , 17), 200 (M^+-SO_2-Cl , 13)
3	$C_{12}H_9NSO_2$	10H-phenothiazine-5,5'-dioxide	231 (M^+ , 100), 213 (M^+-H_2O , 10), 183 (M^+-SO , 20), 167 (M^+-SO_2 , 28)
4	$C_{12}H_7Cl_2NSO_2$	Isomer of product 2	299 (M^+ , 100), 264 (M^+-Cl , 7), 251 (M^+-SO , 21), 235 (M^+-SO_2 , 11), 200 (M^+-SO_2-Cl , 18)
5	$C_{12}H_6Cl_3NSO_2$	10Cl-Cl ₂ -phenothiazine-5,5'-dioxide	333 (M^+ , 100), 298 (M^+-Cl , 15), 285 (M^+-SO , 20), 269 (M^+-SO_2 , 9), 234 (M^+-SO_2-Cl , 12)
6	$C_{12}H_7Cl_2NSO_2$	Isomer of product 2	299 (M^+ , 100), 264 (M^+-Cl , 13), 251 (M^+-SO , 18), 235 (M^+-SO_2 , 13), 200 (M^+-SO_2-Cl , 16)
7	$C_{12}H_5Cl_4NSO_2$	10Cl-Cl ₃ -phenothiazine-5,5'-dioxide	367 (M^+ , 100), 332 (M^+-Cl , 8), 319 (M^+-SO , 13), 303 (M^+-SO_2 , 8), 268 (M^+-SO_2-Cl , 8), 207 ($M^+-SO_2-2Cl-CN+H$, 48)
8	$C_{12}H_8ClNSO_2$	10H-Cl-phenothiazine-5,5'-dioxide	265 (M^+ , 100), 230 (M^+-Cl , 5), 217 (M^+-SO , 12), 201 (M^+-SO_2 , 17), 166 (M^+-SO_2-Cl , 22)
9	$C_{12}H_6Cl_3NSO_2$	Isomer of product 5	333 (M^+ , 100), 298 (M^+-Cl , 8), 285 (M^+-SO , 13), 269 (M^+-SO_2 , 8), 234 (M^+-SO_2-Cl , 16)

1989b, 1991), although it has been shown that chlorine, ozone and chlorine dioxide treatments lead to a reduction in some pharmaceuticals in water treatment system (Ternes *et al.*, 2002; Pinkston and Sedlak, 2004; Huber *et al.*, 2005; Nakada *et al.*, 2006; Bedner and Maccrehan, 2006; Suzuki, 2006).

Effects of experimental conditions on the H-TCAHs-hypochlorite reactions

In addition to the identification of the products of H-TCAH-hypochlorite reactions, it is also important to discern how the distribution of products varies with reaction time. As already mentioned, a large consumption of active chlorine with 10H-phenothiazine and 10H-phenoxazine occurred during the initial 10 min of the reactions (Fig. 1A). Further, a marked reduction in the original amounts of both the compounds occurred with an increase in the molar ratio of hypochlorite to the compound in water (Fig. 1B). Therefore, the 10H-phneothiazine-hypochlorite reaction was investigated in more detail at the various molar ratios of hypochlorite per mol of the compound and for a constant time period.

Fig. 2 shows the results of the GC determination of diethyl ether extracts from aqueous 10H-phenothiazine solutions after treatment with hypochlorite at the various molar ratios of chlorine per mol of the compound for 1

Fig. 2. Residual amounts of the reaction products in aqueous phenothiazine solution (0.3 mM, pH 5) after treatment with hypochlorite at various molar ratios of chlorine per mol of compound for 1 hr. The yields were derived from GC peak areas, relative to the area of the original compound.

hr. Only the oxygenated compound (10H-phenothiazine-5,5'-dioxide) was found to be present in high concentrations in water at low molar ratios of chlorine. At moderate molar ratios, monochloro- and dichloro-substituted compounds of the oxygenated product were detected as intermediates for the reaction of 10H-phenothiazine solution

Fig. 3. Effect of the solution pH values on the residual amounts of products in the reaction of H-TCAHs (0.3 mM) with hypochlorite in an aqueous solution at 20°C for 1 hr. Other details are the same as that in Fig. 2.

with hypochlorite in water. At molar ratios of hypochlorite to the compound of over 20, trichloro-substituents of the oxygenated product were found to be present at high concentrations in water.

GC analysis of the diethyl ether extracts also indicated that the reactions of H-TCAHs with hypochlorite in aqueous solutions are strongly dependent on structural features (Fig. 3). The oxygenated compound and its chlorine-substituted products were detected at high concentrations when 10H-phenothiazine was treated with excess

hypochlorite under acidic condition (pH 5). Low concentrations of these oxygenated and chlorine-substituted compounds were observed under alkaline condition (pH 9). Unknown compounds were also found to be present at low concentrations during the aqueous chlorination of phenoxazine solutions at values of pH 5, 7 and 9, respectively.

Only phenoxathiin-10,10'-dioxide was found to be present at high concentrations when phenoxathiin was treated with excess hypochlorite under acidic and neutral

conditions (Fig. 3). The occurrence of oxygenated compound and its chlorine-substituted products were also observed in the phenoxathiin-hypochlorite reaction of pH 9. An exception was observed for the reaction of phenazine with hypochlorite in water (Fig. 3).

For typical ranges of pH found during the course of most water treatment processes (pH 5-9), the activated chlorine species can range from entirely hypochlorite [OCl^-] to entirely hypochlorous acid [HOCl , $\text{pK}_a = 7.5$ at 20°C]. It has been observed that chlorine is more readily incorporated into aromatic systems at low pH values (Onodera *et al.*, 1989b), a result that is in good agreement with the observation of increasing disinfection ability with decreasing pH (White, 1972). Therefore, it appears that higher concentrations of chlorine-incorporated compounds would be formed when acidic industrial water effluents were treated with an excess of hypochlorite.

On the basis of the results present above, it may be concluded that the chlorination with hypochlorite of 10H-phenothiazine with hypochlorite in an aqueous solution takes place by the following competing reactions, as shown in Fig. 4.

Mutagenicity tests

H-TCAHs and related compounds have been reported to be non-mutagens in the literatures (Jose, 1979; Speit and Vogel, 1979; Sono *et al.*, 1981). In this study, Ames assays were therefore re-performed for 10H-phneothiazine, 10H-phenoxazine, and phenoxathiin by using the *S. typhimurium* TA98 and TA100 to compare the mutagenic responses between the parent compounds and their chlorination byproducts. Aqueous H-TCAHs solutions were treated with hypochlorite at 20 equivalents $\text{HOCl}/\text{compound}$ at pH 5, 7 and 9 for 1 hr. Under these conditions, a complete decomposition of the parent compounds 10H-phenothiazine and 10H-phenoxazine, with the except for

phenoxathiin, was observed in the chlorinated solutions (Fig. 1B) and some oxygenated and chloro-substituted compounds, as their chlorination byproducts, occurred in the diethyl ether extracts (Figs. 2 and 3). Results of the Ames assay conducted for these original compounds and their chlorination products by using the TA98 and TA100 strains without rat liver homogenate (S9 mix) were summarized in Table 3.

None of the H-TCAHs (10H-phenothiazine, phenoxazine and phenoxathiin) before aqueous chlorination were any mutagenic in the tester strains TA98 and TA100 without the S9 mix (Table 3), although some TCAHs having substituent at 9 position, i.e., 9,10-dimethylantracene and 9-chloroanthracene, have been shown to be mutagenic on Ames assays (LaVoie *et al.*, 1983, 1985; Löfroth *et al.*, 1985; Onodera *et al.*, 1994). Aqueous chlorination at pH 7, however, generated not only flameshift (TA98) but also base-pair substitution mutagens (TA100) from the non-mutagenic parent compounds, with the except for the phenoxathiin-hypochlorite reaction, against the tester strains without the S9 mix (Table 3). In addition, mutagenic effects of 10H-phenothiazine and 10H-phenoxazine solutions were observed when these compounds were treated with an excess hypochlorite even at pH 5 and 9 for 1 hr.

Fig. 5 also shows the mutagenic responses obtained by exposing the tester strains TA98 and TA100 with and without S9 mix to diethyl ether extracts from the chlorinated aqueous 10H-phenothiazine, 10H-phenoxazine and phenoxathiin solutions (0.3 mM) at pH 7 for 1 hr. As expected for the most chlorination byproducts (Onodera *et al.*, 1995, 1998), addition of the S9 mix reduced the mutagenic effects of these chlorination byproducts on the tester strains. No mutagenic effects on the tester strains was observed for the diethyl ether extract from the phenoxathiin-hypochlorite reaction (Fig. 5), although phe-

Fig. 4. Proposed pathways for reaction of 10H-phneothiazine with hypochlorite in aqueous solution.

Table 3. Mutagenic effects of phenothiazine, phenoxazine, and phenoxathiin and their chlorination products on *S. typhimurium* strains of TA98 and TA100 without S9 mix

Compound/products tested*	Revertants per plate							
	Dose (μg), as starting material				Dose (μg), as starting material			
	50	100	200	300	50	100	200	300
	TA98 -S9 mix				TA100 -S9 mix			
Phenothiazine standard	21 ± 2	20 ± 1	22 ± 2	18 ± 4	105 ± 10	101 ± 12	102 ± 9	105 ± 10
Chlorination products at pH 5	49 ± 5	81 ± 11	45 ± 5	28 ± 3	280 ± 11	405 ± 18	287 ± 11	230 ± 13
Chlorination products at pH 7	56 ± 5	86 ± 8	40 ± 5	18 ± 3	270 ± 15	400 ± 25	265 ± 13	230 ± 10
Chlorination products at pH 9	49 ± 10	78 ± 9	37 ± 9	19 ± 5	273 ± 18	390 ± 22	279 ± 21	225 ± 22
Phenoxazine standard	21 ± 1	19 ± 3	19 ± 4	18 ± 3	101 ± 12	105 ± 12	98 ± 15	99 ± 10
Chlorination products at pH 5	49 ± 8	85 ± 5	101 ± 3	75 ± 2	301 ± 12	553 ± 21	772 ± 25	370 ± 21
Chlorination products at pH 7	52 ± 5	83 ± 10	96 ± 12	72 ± 5	295 ± 18	513 ± 18	711 ± 32	303 ± 17
Chlorination products at pH 9	45 ± 3	78 ± 6	102 ± 3	67 ± 3	293 ± 18	495 ± 11	655 ± 18	326 ± 13
Phenoxathiin standard	22 ± 2	19 ± 3	18 ± 4	21 ± 2	100 ± 4	110 ± 3	107 ± 8	100 ± 4
Chlorination products at pH 5	21 ± 1	22 ± 1	20 ± 2	21 ± 1	110 ± 20	115 ± 15	113 ± 8	101 ± 15
Chlorination products at pH 7	21 ± 2	20 ± 2	18 ± 3	17 ± 5	98 ± 18	97 ± 13	102 ± 3	100 ± 3
Chlorination products at pH 9	18 ± 5	21 ± 2	19 ± 1	18 ± 5	110 ± 20	100 ± 10	106 ± 16	108 ± 12
Phenazine standard	20 ± 2	19 ± 2	17 ± 5	19 ± 0	98 ± 12	100 ± 4	105 ± 2	111 ± 1
Spontaneous (DMSO)	20 ± 5				112 ± 20			
Positive control, AF-2, 0.05 μg NaN ₃ , 0.5 μg	271 ± 15				616 ± 25			

* Each phenothiazine compound (0.3 mM) was treated with hypochlorite (20 equivalents HOCl per mol of compound) at 20°C for 1 hr and at various pH values.

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Fig. 5. Mutagenic responses of H-TCAHs: phenothiazine, phenoxazine and phenoxathiin, and their chlorination byproducts on *S. typhimurium* TA98 and TA100 strains with and without S9 mix. The chlorination byproducts were obtained by the reaction of 20 eq. HOCl/H-TCAHs solutions (0.3 mM) at room temperature for 1 hr at pH 7.0.

phenoxathiin-10,10'-dioxide was detected at high concentration in the ether extract (Fig. 3). The mutagen formation potentials (TA100) of 10H-phenothiazine and phenoxazine during aqueous chlorination calculated from the dose-response curves given in Fig. 5 were to be c.a. 0.59 and 0.73 net revertants/nmol, respectively; their activity were the same as those reported for chlorophenols in the previous paper (Onodera *et al.*, 1998).

In the present study, the compounds responsible for the mutagenic response could not be confirmed from the diethyl ether-extractable products of the reactions of 10H-phenothiazine or 10H-phenoxazine with hypochlorite in water. However, the results of Ames assays presented in Table 3 and Fig. 5 suggest that treatment with hypochlorite of these compounds in water produces mutagenic compound(s) in Ames *Salmonella/microsome* assays. Therefore, further investigation will be conducted to determine the exact structure and mutagenic response of unknown compound(s) after separating for the reaction mixture of 10H-phenothiazine or 10H-phenoxazine and hypochlorite in water.

In conclusion, aqueous chlorine is used to disinfect sewage water and raw water that are destined for human consumption. H-TCAHs have been shown to produce not only many oxygenated and chlorinated aromatic hydrocarbons but also weak mutagen(s) after reaction with hypochlorite in water. The production of these compounds is greatly dependent on the molar ratios of hypochlorite to the compound, the compound structures and the reaction pH, with higher concentrations at moderate chlorine doses and acidic conditions. Acidic chlorinated waters are generally adjusted to neutral conditions or diluted in the receiving water at the water treatment station. There-

fore, most of the chlorinated aromatic compounds are destroyed by this process, except for phenoxathiin and phenazine, and public health hazard may be substantially decreased. However, the oxygenated and chlorine-substituted compounds are partially interesting since they are present in chlorine-treated H-TCAH solutions. In fact, chlorpromazine and related phenothiazine drugs have been used in human and veterinary medication for more than 50 years, predominantly as psychotropic agents.

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