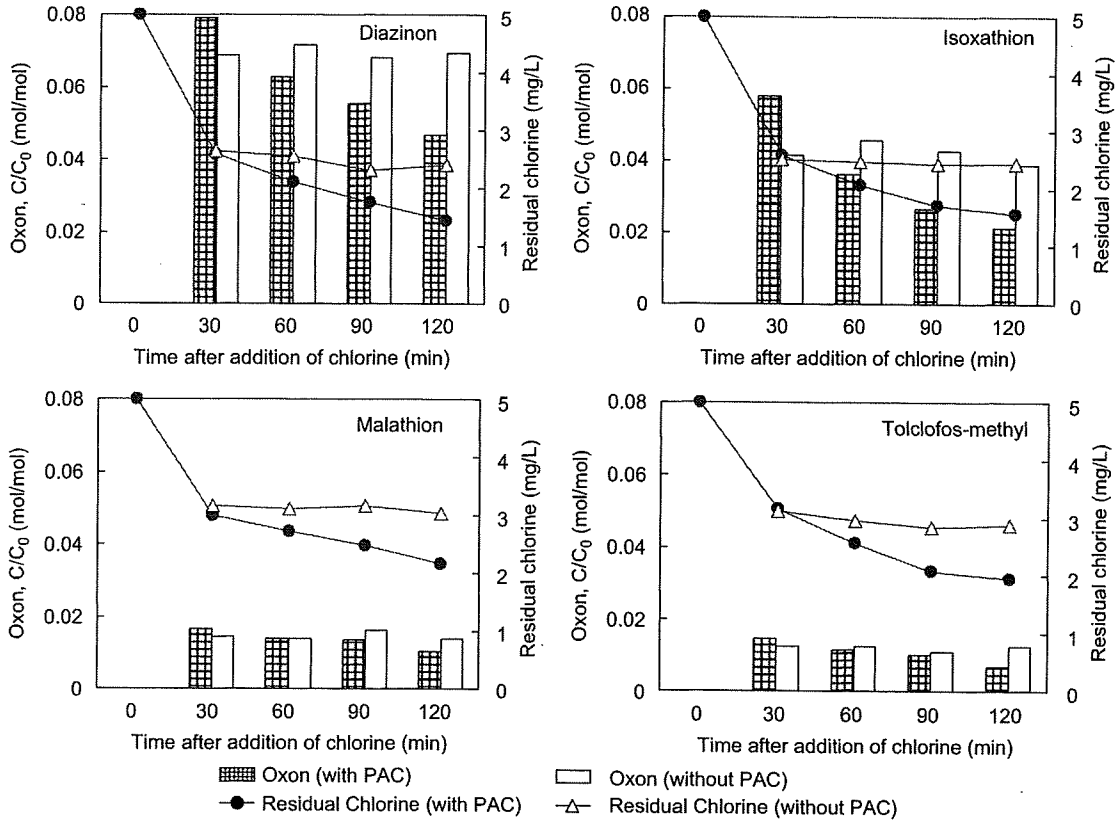

Fig. 2 – Effects of PAC on the decrease in the concentration of released oxons with chlorination time. To produce solutions without PAC, PAC was removed by filtration following 30 min of chlorination. The initial chlorine dose was 5 mg/L, and the initial concentrations of the parent pesticide solution were the same as in Fig. 1.

As shown in Fig. 2, the residual chlorine decreased with time in the solutions with PAC but not in the solutions without PAC. This is due to progressive oxidation of the PAC by chlorine. This oxidation causes a decrease in the number of adsorption sites, which may reduce the rate of oxon readsorption. To examine this effect, we performed the chlorination experiment using isoxathion, and after 30 min, we divided the solution into two parts, one of which was treated with sodium thiosulfate to quench the residual chlorine (Fig. 3). Owing to the action of PAC as a catalyst (Sontheimer et al., 1988), the oxon could be further degraded when both PAC and chlorine are present, but we found that the oxon concentration in water decreased faster when chlorine was absent. These results indicate that the dominant factor in the decrease in the released oxon concentration is not the further degradation of the oxon, but rather its readsorption to the PAC. The results also show that the residual chlorine continues to oxidize the surface of the PAC, decreasing the adsorptive capacity.

We considered that the desorption of oxons was due to the decrease of the adsorptive capacity of PAC by chlorination. The observed data also suggested that readsorption rate of oxons was slower than adsorption rate of the parent pesticides (Figs. 1–3), for almost all the parent pesticides were adsorbed on PAC for 1 h as mentioned in the result of the first

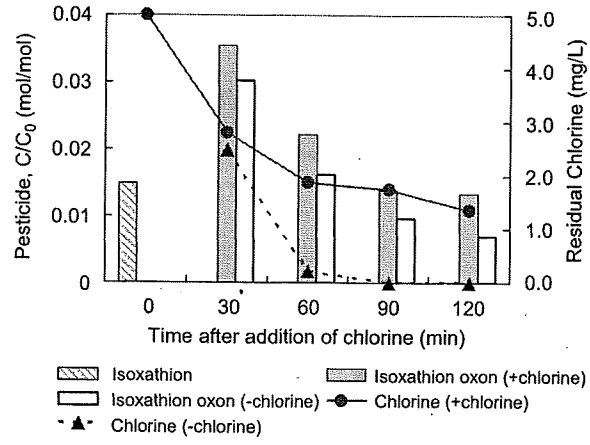

Fig. 3 – Effect of chlorine on isoxathion oxon concentrations released during 30 min of chlorination. For the condition without chlorine, chlorine was quenched after 30 min of chlorination. The initial chlorine dose was 5 mg/L, and the initial concentration of isoxathion in the solution was 2.8 μM (880 μg/L).

experiment. This slower adsorption rate of oxons can be also explained by the decrease of the adsorptive capacity. There are, however, other possible explanations for the slower

adsorption rate and for the dominant desorption of the oxons: the oxons may have slower adsorption rates. The lower adsorption capacity is consistent with the physicochemical data (Table 1): the oxons have lower octanol–water partition coefficient (K_{ow}) than the parent pesticides. Therefore, the slower readsorption rate of the oxons was considered to be due to the combination of the decrease of adsorption capacity and the lower adsorption capacity of the oxons.

3.3. *Mechanism of the desorption of oxon forms from PAC*

Our results showed that chlorination of the PAC caused the desorption of previously adsorbed organophosphorus pesticides. The desorbed substances, however, were not the parent pesticides but the oxon forms (Fig. 1). One possible explanation for these results is that chlorine oxidizes the sites where the parent pesticide was adsorbed, causing the pesticide to be released back into the water, where it is oxidized to the corresponding oxon. A second possibility is that the adsorbed parent pesticide is first oxidized to the corresponding oxon on the PAC and then released from its adsorption site. To examine these two possibilities, we performed the chlorination experiment using isoxathion solution, and measured the levels of isoxathion and its oxon after 30 min (Fig. 4). After the chlorination, ~80% of the isoxathion was degraded to the isoxathion oxon. Following the chlorination of isoxathion-adsorbed PAC, the parent isoxathion was not detected (Fig. 1). If the first explanation were correct, the parent isoxathion should have been detected. Therefore, it appears that the second explanation is correct.

To investigate the reaction of isoxathion on the PAC surface during chlorination, we extracted the compounds adsorbed on the PAC after 30 min of contact with chlorine. We found that 53% of the compound adsorbed on the PAC was isoxathion oxon and 47% was isoxathion (Fig. 5). Thus, approximately half of the isoxathion adsorbed on the PAC remained untransformed, and the remaining half was transformed to the oxon form. These findings support the idea that organophosphorus pesticides are oxidized to their oxon forms on the PAC and then released. Furthermore, if the isoxathion was released from the PAC and then transformed to the oxon form in the water phase, both the isoxathion and the oxon should have been detected in the water phase, because not all of the isoxathion in the water phase was transformed to the oxon form after 30 min of chlorination (Fig. 4). In the experiment in which isoxathion was adsorbed by PAC and chlorinated, however, isoxathion was not detected in the water phase. Thus, it is unlikely that isoxathion adsorbed on the PAC surface was released and then transformed to the oxon form in the water phase.

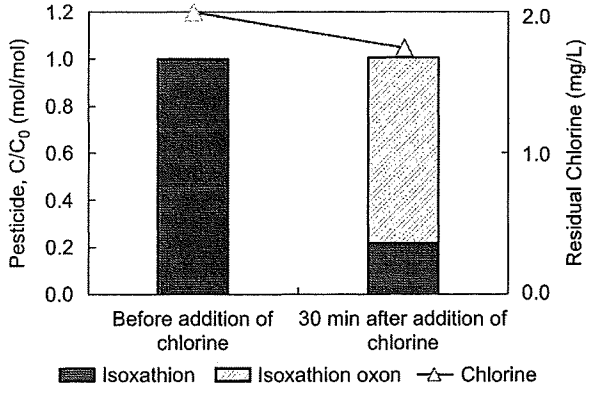

Fig. 4 – Concentrations of isoxathion and its oxon after direct chlorination in aqueous solution. The initial concentration of isoxathion in the solution was 0.20 μM (62 μg/L), and the chlorine dose was 2.0 mg/L.

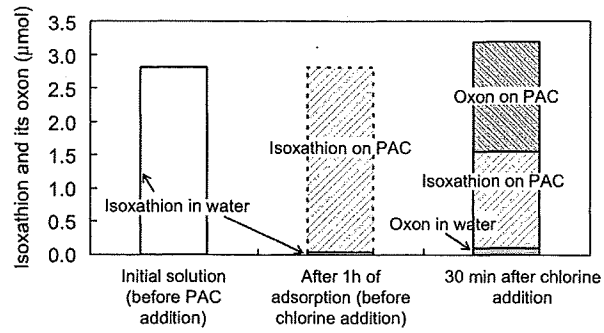

Fig. 5 – Change in the state of isoxathion and its oxon during the adsorption and chlorination experiment. The initial chlorine dose was 5 mg/L. The amount of isoxathion adsorbed on PAC after 1 h of adsorption was an estimate, whereas the amounts of isoxathion for the other samples were experimentally measured.

4. Conclusions

We investigated the effects of chlorination of organophosphorus compounds adsorbed to PAC. The oxons rather than the parent pesticides were detected in the water phase after chlorination. In addition, the concentrations of the desorbed oxons decreased with chlorination time. This result can be explained by the readsorption of oxons in the water phase by PAC. Results from additional experiments suggest that the parent pesticides adsorbed to PAC are first oxidized to the corresponding oxon, then released from the adsorption sites. In the case of isoxathion, after 30 min of chlorination, approximately half of the parent compound adsorbed to the PAC surface was converted into the corresponding oxon.

In this study, we focused on the clarification of mechanism of desorption and oxon formation of organophosphorus pesticides that were pre-adsorbed on PAC by contact with chlorine. To elucidate the mechanism more clearly, we conducted the chlorination experiments with the pesticide solutions at much higher concentrations than environmentally relevant concentrations. The reactivity of chlorine and the pesticides that are adsorbed on PAC at lower concentrations remained unrevealed. As a result of this study, however, we may provide the following recommendations for water purification facilities that have a possibility for contact

between PAC and chorine: oxon concentrations in addition to those of the parent pesticides should be monitored in finished water, and longer contact time between PAC and the desorbed oxons for readsorption should be taken.

Acknowledgment

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The behaviour and cholinesterase inhibitory activity of fenthion and its products by light and chlorination

Maiko Tahara, Reiji Kubota, Hiroyuki Nakazawa, Hiroshi Tokunaga and Tetsuji Nishimura

ABSTRACT

We established a method for quantitative analysis of fenthion (MPP) and its related compounds in water samples, using solid-phase extraction and liquid chromatography/mass spectrometry. With this method, the values of the limit of quantification ranged from 0.2 to 100 ng l^{-1}. Using this method, we examined the fate of MPP in water and the products produced by light irradiation and chlorination. MPP decreased gradually and reached 50% of the initial concentration after 48 hours in water. In particular, MPP-sulfoxide was formed. With light irradiation, MPP decomposed immediately into MPP-sulfoxide, *O,O*-Dimethyl *S*-[3-methyl-4-(methylthio)phenyl]phosphorothioate and other compounds. With chlorination, MPP decomposed into MPP-sulfoxide, MPP-sulfone, and their oxons. The concentration of oxons increased in a time-dependent manner. In their effects on organisms, MPP, MPP-sulfoxide and MPP-sulfone showed weak inhibitory activity to cholinesterase, whereas their oxons showed strong activity. It is feared that MPP and its products exist in environmental water and are produced by the disinfection treatment process. Comprehensive evaluation of the toxicity of MPP and its related compounds is important in order to understand the effects of MPP on ecosystems and human health.

Key words | ChE activity, chlorination, light irradiation, MPP, oxidized products, water

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INTRODUCTION

Fenthion (MPP) is an organophosphorus pesticide used in modern agriculture primarily as an insecticide for paddy fields. Monitoring of pesticides in natural water used as a source of drinking water has been performed at various places in Japan. Results showed that MPP was detected in natural water closely situated to paddy fields. In addition, the MPP-related compounds, such as MPP-sulfoxide and MPP-sulfone, were also detected in natural water without use as pesticide (Wang *et al.* 1987; Nagafuchi *et al.* 1994; Tsuda *et al.* 1998). Compounds in natural water are affected by environmental conditions such as irradiation by sunlight, the concentration of hydrogen ions and oxygen, microorganisms, and so on. They are also modified and oxidized through the disinfection processes of water treatment plants. Similarly, pesticides in the environment may also be affected by environmental and artificial factors. There is concern about the fate of pesticides in water sources, and their effect on ecosystems and human health (Tsuda *et al.* 1997).

In this work, we describe the methods developed for simultaneous quantitative analysis of MPP and related compounds in water samples using solid-phase extraction and liquid chromatography/mass spectrometric detection. We then examined the fate of MPP in water treated by light irradiation and chlorination. Because organophosphorus pesticides commonly inhibit nervous system cholinesterase (ChE) activity, resulting in adverse effects on organisms (Jokanović 2001), we investigated the effect of MPP and its products on ChE activity using an *in vitro* bioassay (Tahara *et al.* 2005), in order to assess how human health might be affected.

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EXPERIMENTAL

Chemicals and reagents

MPP and MPP-sulfoxide were purchased from Wako Pure Chemical Industries, Ltd (Osaka, Japan). MPP-sulfone, MPP-oxon, MPP-oxon-sulfoxide, and MPP-oxon-sulfone were purchased from Dr Ehrenstorfer GmbH (Augsburg, Germany).

Standard solutions were prepared individually in acetone at concentrations of 1,000 mg l^{-1} for MPP and MPP-sulfoxide, and 100 mg l^{-1} for MPP-sulfone. The three oxon solutions were purchased at 10 μg ml^{-1} in acetonitrile solution. All standard solutions were stored at -20°C. The working solutions were freshly prepared for every use by dilution of the standard solution with acetonitrile and/or 0.15% acetic acid, as necessary.

High quality acetone, acetonitrile, acetic acid, sodium hypochlorite solution, and L(+)-ascorbic acid sodium salt were purchased from Wako Pure Chemical Industries, Ltd. Laboratory water was purified by a Milli-Q gradient A10 and Elix with EDS polisher system water-purification (Millipore, Bedford, Massachusetts). Methanol was not used in this study, because transesterification of organophosphorus pesticides may occur in methanol (Hong & Pehkonen 1998).

Solid-phase extraction

Compounds in water samples were extracted and concentrated with solid-phase extraction (SPE) cartridges. The cartridges were equilibrated with 5 ml acetonitrile and 5 ml water, respectively. Extraction of water samples was carried out with a 10 ml min^{-1} flow rate using an automatic concentrator, Sep-Pak Concentrator Plus (Waters, Milford, Massachusetts). Air was then passed though the cartridges for 5 min. The compounds were eluted from the cartridges with 5 ml acetonitrile. The eluted solutions were concentrated to less than 0.3 ml under a gentle nitrogen stream, and for liquid chromatography/mass spectrometry (LC/MS) samples 0.15% acetic acid was added to a final volume of 1.0 ml. The final solution for LC/MS analysis was composed of 0.15% acetic acid/acetonitrile (v/v = 7:3).

Standard solutions in acetonitrile were spiked into 500 ml purified water, for final concentrations of 50 ng ml^{-1} for MPP, 0.1 ng ml^{-1} for MPP-sulfoxide, 1 ng ml^{-1} for MPP-sulfone, 0.25 ng ml^{-1} for MPP-oxon, 1 ng ml^{-1} for MPP-oxon-sulfoxide, and 0.5 ng ml^{-1} for MPP-oxon-sulfone. The recovery of compounds from water samples was performed using three cartridges: Oasis HLB Plus Extraction Cartridge, Sep-Pak Plus PS-2 Cartridge, and Sep-Pak Plus C18 Cartridge (Waters). The blank consisted of 500 ml of purified water.

Analysis with LS/MS

The target compounds were analysed by LC/MS for qualitative and quantitative analysis.

LC was carried out using an Agilent 1100 series (Agilent, Waldbornn, Germany) instrument equipped with a Rheodyne Model 7750 injector. The analytical column was Zorbax Eclipse XDB-C18 (Agilent), 4.6 mm i.d. × 250 mm, 5 μm particle size. The column oven temperature was 40°C. Mobile phases were 0.15% acetic acid (A) and acetonitrile (B) with the following gradient programme: maintaining 70% A for 5 minutes; by a linear gradient from 70% A at $t = 5$ minutes to 30% A at $t = 20$ minutes; maintaining 30% A for 5 minutes. The flow rate was set to 1.0 ml min^{-1} and the injection volume was 10 μl. The MS system was an Agilent 1100 series (Agilent) quadrupole equipped with an electrospray ionization (ESI) source. The instrument was operated in scan mode and the positive and negative ionization mode of selected ion monitoring (SIM) mode. The operating conditions for ESI were nebulizer gas (nitrogen) 60 psi; drying gas (nitrogen) flow 10 l min^{-1}; gas temperature 350°C. Capillary voltages were 4,000 V for positive and 2,000 V for negative. The fragmentor voltage was kept at 200 V. The scan mode was 50–500 m/z.

Extraction of MPP and its products from water

MPP standard solution was added to purified water to a final concentration of 0.001 mgl^{-1}. Strict pH adjustment was not performed but the extraction was conducted in neutral conditions. After stirring at room temperature for 5 minutes, a 500 ml sample was taken for the original water sample, reaction time at 0 hour. With stirring at 20°C, 500 ml samples were taken at 1, 2, 4, 6, 24 and 48 hours. MPP and its products were extracted by SPE. The operations were done at room temperature, around 25°C.

Light irradiation

Photolysis experiments were performed in purified water using an original laboratory photoreactor. An ultraviolet (UV) GL6 lamp (National, Osaka, Japan) with electrical power at 6 W and maximum wavelength of 254 nm was located at the centre of the reactor. The characteristics of this lamp were suitable to evaluate the effect in a narrow wavelength range because about 90% of the energy is concentrated in 254 nm spectrum. MPP solution of concentration 1 $\mu g\,ml^{-1}$ was put in a standard rectangular quartz cell (1 cm pathlength) and placed at a distance of 17 cm from the light source. MPP solutions were irradiated by UV light (254 nm) for 10, 20, 30, 45, 60, 90 and 120 seconds in the short irradiation experiment, and for 1, 2, 5, 10, 20 and 30 minutes in the long irradiation experiment. Sample solutions were analysed directly by LC/MS.

A 250 mg l^{-1} MPP solution was irradiated by UV light for 0.5, 1, 1.5, 2, 2.5, 3 and 4 hours for the detection of ChE inhibitory activity. The products were also analysed by LC/MS direct injection.

Chlorination

We examined the behaviour of MPP and its products in chlorine water to investigate the effect of chlorination on MPP in water treatment plants, using sodium hypochlorite solution, which was generally used as a disinfectant providing an effective barrier to many pathogens, especially bacteria at treatment plants.

The chlorination experiment for the examination of MPP behaviour was carried out at low MPP concentration having regard to the real-world situation. The preparation of samples for the evaluation of chlorination products was performed at high MPP concentration on the basis of the sensitivity of bioassay and the yield of products.

MPP standard solution was added to purified water to a final concentration of 0.001 mgl^{-1}. After stirring at room temperature for 5 minutes, a 500 ml sample was taken for the original water sample, reaction time at 0 hour. A sodium hypochlorite solution was then added to a final concentration of free chlorine of 1 mgl^{-1}. With stirring at 20°C, 500 ml samples of solution were taken at the reaction times of 5, 15, 30, 60 and 120 minutes for the short exposure experiment, and 1, 2, 4, 6, 24, 48 and 72 hours for the long exposure experiment. A 1 ml solution of sodium ascorbic acid (10 $g\,l^{-1}$) was added to the sample solutions to eliminate chlorine. MPP and its products were extracted by SPE. The operations were done at room temperature, around 25°C.

Sodium hypochlorite solution was added to an aqueous solution of 0.01 mgl^{-1} MPP, to a final concentration of free chlorine of 5 mg l^{-1}. The solution was maintained at 20°C for 0.5, 1, 2, 4 and 24 hours. Chlorine was eliminated in the sample solutions by sodium ascorbic acid. MPP and its products were concentrated 250-fold by SPE.

Evaluation of ChE activity

Stock solutions of ChE (Wako Pure Chemical Industries, Ltd) dissolved in water (1,250 IUl^{-1}) and 5-methyl -2-thenoyl-thiocholine-iodide (MTTC) (2.0 mM) were prepared. A 0.25 mM chromogen solution of 5, 5'-dithiobisnitrobenzoic acid (DTNB) was prepared in 0.1 $moll^{-1}$ phosphate buffer (pH 7.4). All chemicals were purchased from Wako Pure Chemical Industries, Ltd. They were stored at 4°C. The sample solutions were prepared in water. A solution of ChE and each appropriate sample were uniformly mixed in a ratio of 4:1, so that each sample contained 7 mIU ChE. MTTC substrate solution (63 μl) was added to 7 μl of each sample containing ChE in a 96 microwell plate, and 280 μl of the DTNB chromogen solution was added. The plate was incubated at 37°C for 7 minutes, and the absorbance was measured at 405 nm using an Ultrospec Visible Plate Reader II 96 (Amersham Biosciences, Tokyo, Japan). All experiments were performed in triplicate wells.

The mechanism of colour development is as follows: active ChE enzymatically cleaves the substrate MTTC to release thiocholine. The released thiocholine reacts with the chromogen DTNB to generate a yellow product, quantifiable at 405 nm by UV absorption, and which is impeded when ChE activity is inhibited (Karahasanoglu & özand 1967; Tahara *et al.* 2005).

RESULTS AND DISCUSSION

Calibration curves and limit of detection by LC/MS

The following six compounds were targeted for examination: MPP, MPP-sulfoxide and MPP-sulfone (containing an

oxidized thio-methyl group); MPP-oxon, MPP-oxon-sulfoxide and MPP-oxon-sulfone (three oxon forms containing P = O moiety oxidized P = S moiety of the characteristic structure for organophosphorus pesticides).

The experiments were performed using two methods: liquid chromatography with mass spectrometric detection (LC/MS) or gas chromatography with mass spectrometric detection (GC/MS). As a result of the comparison of sensitivity for detecting MPP and five related compounds, we selected the LC/MS method. The analytical conditions established for LC/MS were as shown above in the Experimental section. The target compounds were analysed in the positive and negative ionization SIM mode for qualitative and quantitative analysis by detection of the signal from the more abundant daughter ions. The daughter ion was identified in the scan mode during the acquisition of the mass spectrum. The selected ion and ionization modes are summarized in Table 1. Calibration curves were determined from the results of measurements of seven concentrations of standard solutions in the SIM mode. Standard curves show excellent linearity with correlation coefficients higher than 0.999 for all six compounds. This indicates that the established analytical conditions performed well in quantitative analysis of these compounds.

The value of limit of detection (LOD) was calculated as three times the standard deviation of the slope of the calibration curve. LOD values obtained using LC/MS for MPP, MPP-sulfoxide, MPP-sulfone, MPP-oxon, MPP-oxon-sulfoxide and MPP-oxon-sulfone were 10, 0.02, 0.2, 0.05, 0.2 and 0.1 ngml^{-1}, respectively. Concentration ranges and LOD values for the six compounds are summarized in Table 1. With LC/MS, low concentrations of the six compounds were measured at high accuracy.

Limit of quantification and recovery test by LC/MS

The value of the limit of quantification (LOQ) was determined at 10 times the value of the standard deviation and the lowest concentration that provided relative standard deviations (RSDs) of 10% or less in the recovery test. LOQ values obtained were 50 ng ml^{-1} for MPP, 0.1 ng ml^{-1} for MPP-sulfoxide, 1 ng ml^{-1} for MPP-sulfone, 0.25 ng ml^{-1} for MPP-oxon, 1 ng ml^{-1} for MPP-oxon-sulfoxide, and 0.5 ng ml^{-1} for MPP-oxon-sulfone.

The results of a comparison of recovery tests on extracting six compounds from tap water using three different types of solid-phase extraction cartridge showed that average recovery by the Oasis HLB Plus was 60.0–90.4% (RSD 1.2–9.8%), Sep-pack PS-2 58.3–83.9% (1.0–10.1%) and Sep-pack C18 39.8–86.1% (0.5–10.0%). There were discrepancies in recovery rates among the three cartridges. Oasis HLB Plus was selected to extract all the target compounds in these experiments, because it obtained satisfactory recovery rates for simultaneous analysis of all tested compounds.

The behaviour of MPP in water

MPP was added to purified water at a final concentration of 0.001 mgl^{-1}, and the behaviour of MPP and its products in water was examined at reaction times of 1, 2, 4, 6, 24 and 48

Table 1 | Analytical conditions in SIM mode of MPP and related compounds by LC/MS (P: positive mode)

Compound	MW	Monitor Ion	Retention time (min)	Range (ng ml^{-1})	Correlation coefficient	LOD (ng ml^{-1})	LOQ (ng ml^{-1})
MPP	278	279 P	18.3	10–1,000	0.999	10	50
MPP-sulfoxide	294	295 P	13.3	0.02–5	0.999	0.02	0.1
MPP-sulfone	310	311 P	16.9	0.2–20	0.999	0.2	1
MPP-oxon	262	263 P	16.2	0.05–10	0.999	0.05	0.25
MPP-oxon-sulfoxide	278	279 P	4.0	0.2–20	0.999	0.2	1
MPP-oxon-sulfone	294	295 P	6.6	0.1–20	0.999	0.1	0.5

hours. The solution pH was about 6.0 after addition of MPP and was not changed during the reaction time. Though MPP itself decreased gradually, MPP-sulfoxide was formed immediately in water and its concentration increased in a time-dependent manner. At 48 hours, MPP-oxon-sulfoxide and MPP-oxon-sulfone were detected at low levels (Figure 1). The concentration of MPP and its products was calculated using the standard curve determined by the value of the peak area obtained by SPE (Figure 2). After 24 hours, 70% of MPP remained, and 5% of MPP changed to MPP-sulfoxide. After 48 hours, 50% of MPP remained and 30% was changed. We could not detect residual MPP. It was speculated that the residual might have decomposed to other products. Chemical hydrolysis played an important role in the behaviour of MPP in an aqueous environment.

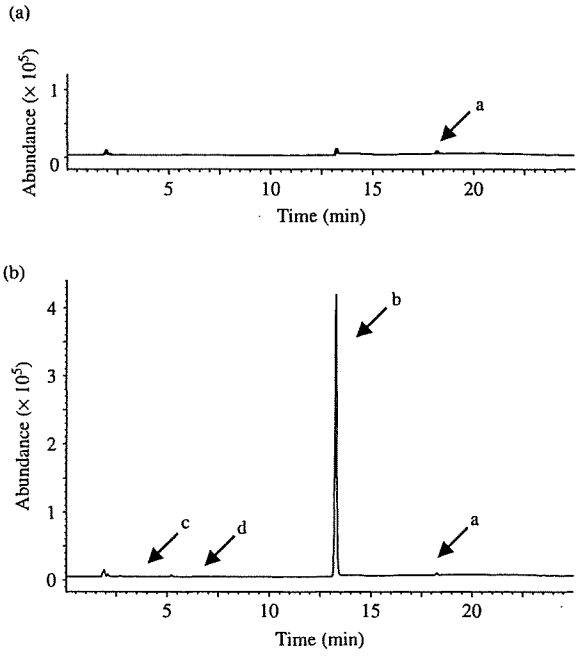

Figure 1 | Chromatograms of MPP and its products in water (a) 0 hr, (b) after 48 h. a: MPP, b: MPP-sulfoxide, c: MPP-oxon-sulfoxide, d: MPP-oxon-sulfone.

The behaviour of MPP exposed to UV

The photochemical transformation of MPP in water was studied after irradiation with UV light, because compounds in natural water are irradiated by sunlight. No change was observed under dark conditions within the timescale of these

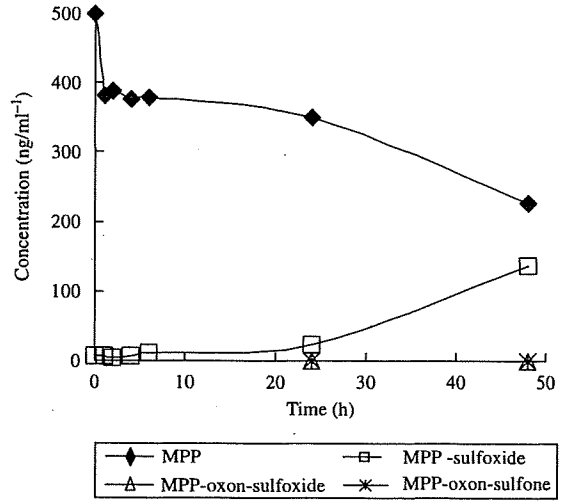

Figure 2 | Behaviour of MPP in water.

experiments. Following irradiation, MPP itself disappeared rapidly, and four main photoproducts were confirmed on the chromatogram (Figure 3). Some photoproducts of MPP have already been reported (Chukwudebe *et al.* 1989; Minelli *et al.* 1996; Huang & Mabury 2000; Hirahara *et al.* 2003; Torrisi & Sortino 2004). Two products among them were found in purified water. One identified product was MPP-sulfoxide, according to mass spectral information. It was directly produced by the oxidative reaction of MPP. The other product, detected at a 17.6 minute retention time, showed the formation $M + H^+ = 279$. It was presumed to be *O, O*-Dimethyl *S*-[3-methyl-4-(methylthio)phenyl]phosphorothioate by the fragment ions of the mass spectrum (Figure 4). It was formed by the isomerization of thiono-thiolo (e. g. RO-P = S → RS-P = O) (Lacorte & Barceló 1994; Torrisi & Sortino 2004; Zamy *et al.* 2004). It involves the lowest excited singlet state of the pesticide and a σ cation as the key intermediate in the photodecomposition of MPP (Torrisi & Sortino 2004). Both products were detected at their highest concentration at 1 minute (Figure 5). These products were also confirmed by light irradiation using a chemical lamp (6 W, maximum wavelength 352 nm). Some minor peaks were present. However, we were not able to elucidate their structure from mass fragment information. The area values of these peaks were small compared with that of the main peak.

Although the strength of the UV wavelength range of sunlight is usually weak, the solar spectral intensity is typically

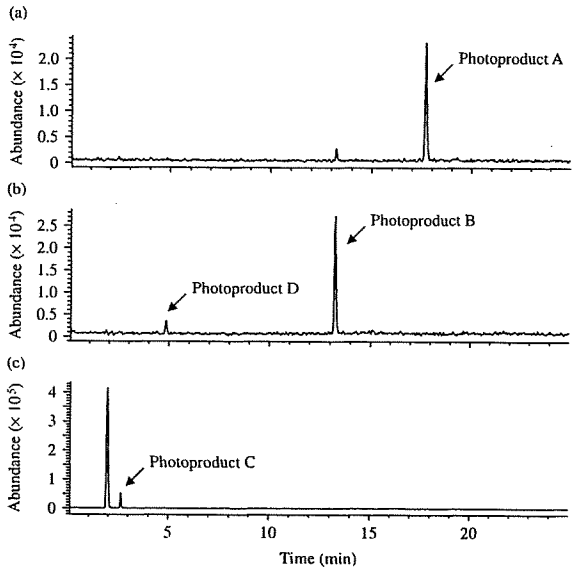

Figure 3 | Chromatograms of MPP and its products irradiated for 120 s. (a) *m/z*: 279, positive mode, (b) *m/z*: 295, positive mode, (c) *m/z*: 141, negative mode.

sufficient to break down chemical bonds of the molecule. There is a report that MPP degrades much faster under sunlight conditions than in darkness (Lartiges & Garrigues 1995). Therefore, there is concern that these compounds are formed in the environment.

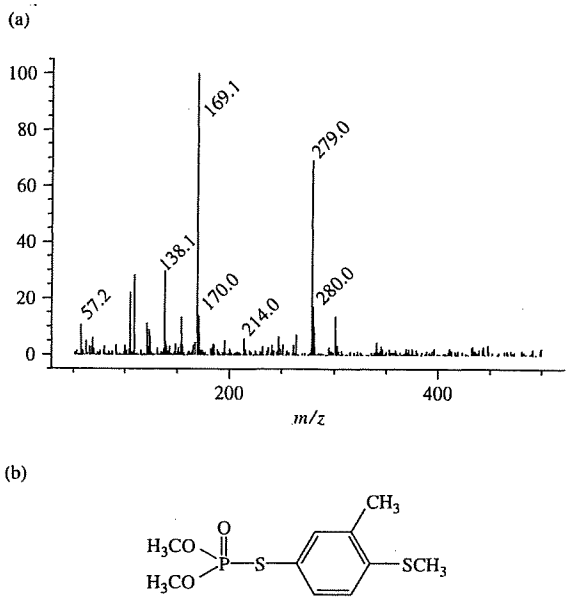

Figure 4 | Photoproduct A. (a) MS spectrum, (b) chemical structure.

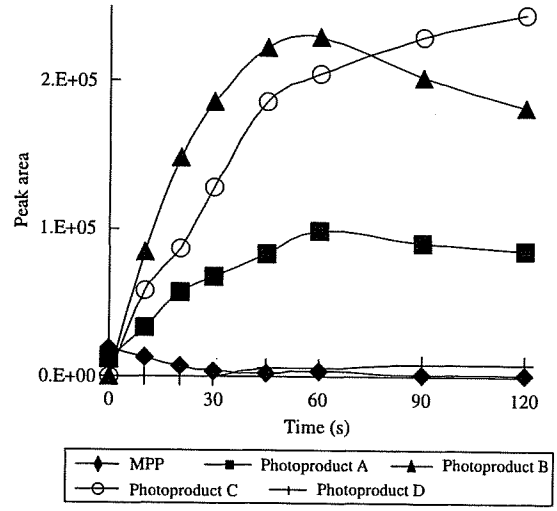

Figure 5 | Time-dependent behaviour of MPP and its products treated by light irradiation.

MPP behaviour under conditions of chlorination

MPP standard solution was added to purified water to a final concentration of 0.001 mg l^{-1}. A 500 ml sample of the solution was taken as the original water sample, reaction time at 0 h, after stirring at room temperature for 5 minutes. A sodium hypochlorite solution was added so that the concentration of free chlorine was 1 mg l^{-1}. The solution pH was about 6.0 after addition of MPP to purified water and changed to about 8.0 when the chlorine was added. However, it was resulted to get to 6.0 with the reaction time. In water containing chlorine, MPP was undetectable within 5 minutes after contact with chlorine. MPP-sulfoxide and MPP-sulfone were detectable immediately, and increased in parallel with the decrease of MPP. The concentration of these products peaked at 5 and 15 minutes, respectively. Each compound was then gradually converted to its oxon form (Figure 6). The rates of conversion from MPP-sulfoxide and MPP-sulfone to their oxon forms were slow in comparison with the rate of conversion from MPP to MPP-sulfoxide and MPP-sulfone. In this experiment, MPP-oxon was undetectable. As a result of chlorination in the long exposure experiment of 1, 2, 4, 6, 24, 48, 72 and 96 hours, MPP-oxon-sulfoxide almost disappeared by 48 hours. MPP-oxon-sulfone concentration peaked at 24 hours and maintained the same concentration level until 48 hours. The concentration of free chlorine was 0.79 mg l^{-1} after 48 hours.

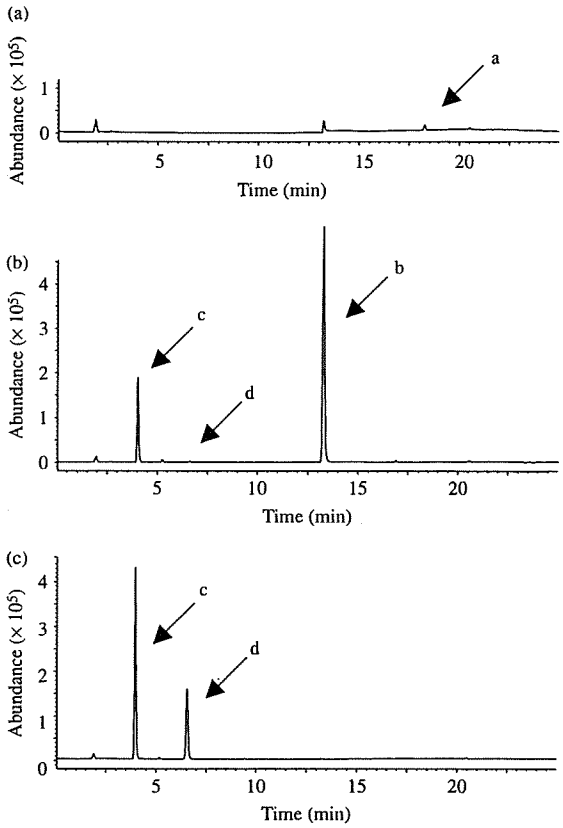

Figure 6 | Chromatograms of MPP and its products after chlorination. (a) 0 min, (b) 5 min, (c) 120 min. a: MPP, b: MPP-sulfoxide, c: MPP-oxon-sulfoxide, d: MPP-oxon-sulfone.

The concentration of MPP and detected products in the short exposure experiment was calculated using the standard curve determined by the value of peak areas obtained by SPE (Figure 7). The results indicate that under chlorination conditions, MPP changed to related compounds and converted primarily into MPP-oxon-sulfone after 48 hours.

If MPP exists in sources of drinking water, and is not eliminated sufficiently at water purification plants, it will come into contact with chlorine. MPP is rapidly oxidized to MPP-sulfoxide and MPP-sulfone, and their oxons may persist in drinking water.

ChE inhibition activity

It is known that ChE, a key neuroregulatory enzyme, is targeted and inhibited by organophosphorus pesticides and

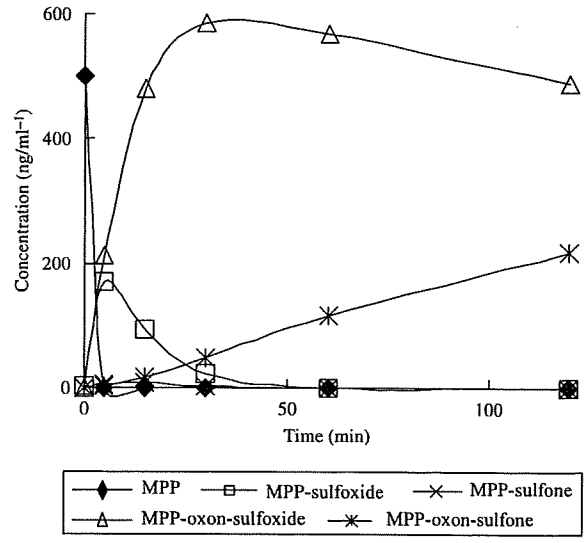

Figure 7 | Time-dependent behaviour of MPP and its products after chlorination.

their active metabolites, causing acute toxicity (Rodnitzky 1975; Soliman *et al.* 1982; Nagymajtényi *et al.* 1988). In this study, ChE inhibition activity was examined by a previously established *in vitro* method that uses MTTC as an indicator of ChE activity, in order to evaluate the effect of MPP and related compounds on organisms.

MPP, MPP-sulfoxide and MPP-sulfone showed weak inhibitory activity. However, the oxon forms showed a high inhibitory effect at ng levels. The inhibition by oxons

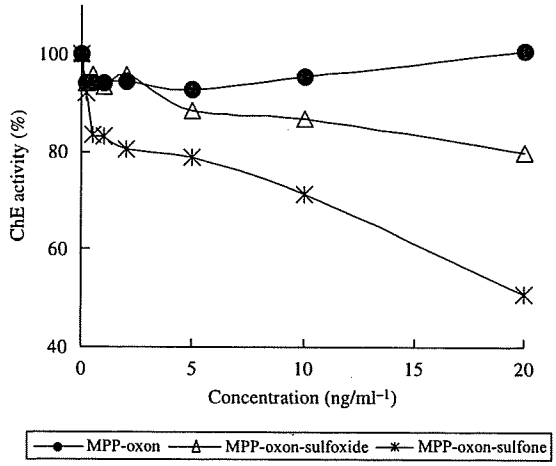

Figure 8 | Comparison of ChE inhibitory activities. The concentration at the beginning of the reaction was defined as 100%.

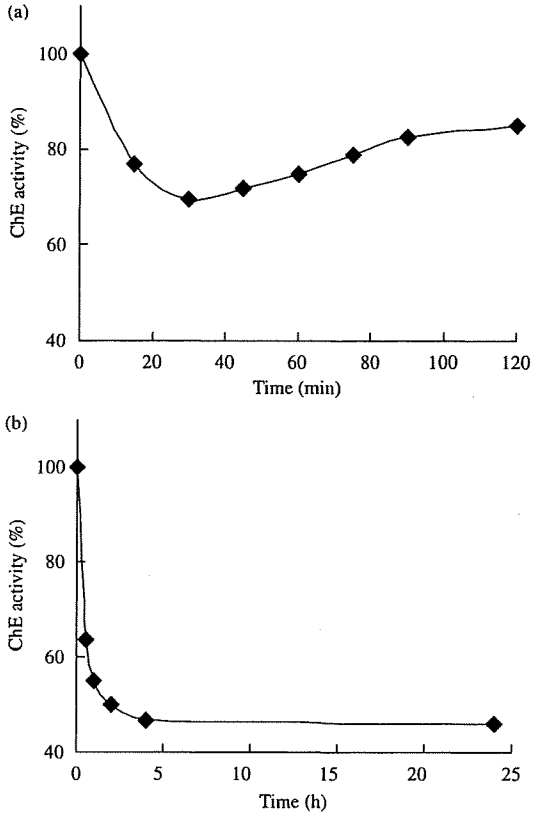

Figure 9 | Effect of reaction products mixture on ChE activity. (a) light irradiation, (b) chlorination. The activity at the non-treated MPP solution was defined as 100%.

strengthened with the degree of oxidation, in the following order: MPP-oxon, MPP-oxon-sulfoxide and MPP-oxon-sulfone (Figure 8). Concentrations of chlorpyrifos oxon, diazinon oxon, EPN oxon and fenitrothion oxon causing 20% inhibition were, respectively, 1.1, 8.9, 140 and 330 ng ml^{-1} (Tahara *et al.* 2005); and concentrations for MPP-oxon-sulfoxide and MPP-oxon-sulfone were 5.4 and 0.32 ng ml^{-1}, respectively. The inhibitory activities of MPP-oxon-sulfoxide and MPP-oxon-sulfone are high compared with other oxons of organophosphorus pesticides. Therefore, the potential for adverse effects of MPP is high, because MPP is changed to MPP-oxon-sulfoxide and MPP-oxon-sulfone.

We also studied the ChE inhibition activity of MPP solutions irradiated by UV or treated with chlorine. The treated solutions may contain mixtures of MPP and its reaction products. The results in solutions from both treatments showed strong ChE inhibitory activity in comparison with the non-treated MPP solutions (Figure 9). Photodegradation reactions may result in the formation of products with a high acute toxicity. The next step would be to isolate photodegradation products and elucidate their toxicity.

CONCLUSIONS

The present work has shown that MPP converts easily to the oxidized compounds, MPP-sulfoxide, MPP-sulfone and their oxons by photo-irradiation and by treatment with chlorine in water. In an aquatic environment, MPP may be changed by passing through different physical, chemical and biological processes. It is important to control MPP and its reaction products, in order to protect human health and the ecosystem, because these compounds have adverse effects on organisms.

ACKNOWLEDGEMENTS

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A nationwide survey of NDMA in raw and drinking water in Japan

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ABSTRACT

A nationwide survey of *N*-nitrosodimethylamine (NDMA) in both raw and finished water samples from drinking water treatment plants (DWTPs) in Japan was conducted. NDMA was analyzed by solid-phase extraction (SPE) followed by ultra performance liquid chromatography (UPLC) coupled with tandem mass spectrometry (MS/MS). NDMA was detected in 15 of 31 raw water samples collected in the summer at concentrations up to 2.6 ng/L, and in 9 of 28 raw water samples collected in winter at concentrations up to 4.3 ng/L. The NDMA concentrations were higher in raw water samples collected from treatment plants with catchment areas that have high population densities. The NDMA concentrations were higher in river water samples collected from the east and west of Japan than in those collected from other areas. NDMA was detected in 10 of 31 finished samples collected in summer at reduced concentrations of up to 2.2 ng/L, while 5 of 28 finished samples collected in winter showed NDMA concentrations up to 10 ng/L. The highest NDMA levels were detected in finished water samples collected from the Yodo River basin DWTP, which uses ozonation. Furthermore, evaluation of the process water produced at six advanced water treatment plants was conducted. Influent from the Yodo River indicated that the NDMA concentration increased during ozonation to as high as 20 ng/L, and then decreased with subsequent biological activated carbon treatment. To our knowledge, this is the first nationwide evaluation of NDMA concentrations in water conducted in Japan to date.

1. Introduction

N-Nitrosodimethylamine (NDMA) is a highly water-soluble nitrosamine that is a member of a family of extremely potent carcinogens known as *N*-nitrosoamines (Mitch et al., 2003). Some nitrosamines, including NDMA, have been classified as probable human carcinogens (B2) by the Integrated Risk Information System (IRIS) of the United States Environmental Protection Agency (US EPA, 2009a) and as 2A, probably carcinogenic, by the World Health Organization's International Agency for Research on Cancer (IARC, 2009). In the past, NDMA was used as an intermediate in the production of rocket fuel, an inhibitor of nitrification in soil, a plasticizer in the manufacture of rubber and polymers, a solvent in the fiber and plastic industry, an antioxidant, a softener of copolymers, and as an additive to lubricants (Najm and Trussell, 2001). Recently, NDMA was found to be a disinfection byproduct following chloramination or chlorination in the presence of ammonia (Mitch et al., 2003). NDMA precursors during chlorination and chloramination include nitrogenous organic compounds such as dimethylamine (DMA) and trimethylamine (TMA) (Lee et al., 2007). The US EPA has estimated that an NDMA concentration of 7 ng/L in drinking water is associated with an excess lifetime cancer risk of 10^{-5} (US EPA, 2009a), and NDMA is included among the 104 contaminants on Contaminant Candidate List 3 (CCL3) (US EPA, 2009b). Although the maximum contaminant level (MCL) for NDMA in drinking water has not been established in the USA, other regulatory agencies have established NDMA guidelines. For example, the office of Environmental Health Hazard Assessment (OEHHA) in California has set a public health goal (PHG) of maintaining NDMA at concentrations ≤ 3 ng/L based on a cancer risk of 10^{-6} (California Department of Public Health, 2009). In addition, although no MCL for NDMA in drinking water has been established to date in Canada, the Ministry of the Environment (MOE) of Ontario has set the provisional maximum allowable concentration of NDMA at 9 ng/L (Ministry of the Environment of Ontario, 2009). Although estimates of the various sources of NDMA exposure indicated that water contributes less than 10% of the overall exposure in Canada and it is less than 1% of the overall human exposure to NDMA estimated in the USA, the relative source contribution (RSC) is usually not utilized in cancer risk calculations, and no official evaluation has been conducted to determine the contributions of each source in these risk assessments (California Department of Public Health, 2009).

NDMA was first detected in drinking water in Ontario, Canada in 1989 (Charrois et al., 2007). In the USA, NDMA was first discovered as a groundwater contaminant at a Northern California aerospace facility in 1998 (Najm and Trussell, 2001). Since then, the occurrence of NDMA in drinking water treatment plants (DWTPs) has been investigated throughout Canada and the USA. A survey of quarterly samples of raw, finished, and distribution system water collected from 21 North American DWTPs indicated the presence of NDMA in concentrations greater than the method detection limit (MDL) of 0.6–1.0 ng/L in only 1 of 81 raw water samples, and that the NDMA concentrations were

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<MDL–30 ng/L in 81 finished water samples and <MDL–24 ng/L in 95 distribution system water samples (Barrett et al., 2003). However, a survey of 20 municipal drinking water distribution systems in Alberta, Canada, revealed NDMA concentrations in some systems as high as 100 ng/L. In addition, an extensive survey of 179 DWTPs in Ontario, Canada, indicated that NDMA was present in concentrations as high as 66 ng/L in one distribution system water sample (Charrois et al., 2007).

Some of recent studies have evaluated the formation of NDMA during ozonation. For example, Andrzejewski et al. (2008) reported the formation of NDMA by DMA ozonation (initial DMA concentrations, 30–700 mg/L). In addition, Schmidt and Brauch (2008) reported that the plant growth regulator, daminozide, the fungicide, tolylfluanid, and their decomposition products were NDMA precursors during ozonation. However, there have been few evaluations of NDMA in Japan.

Here, a nationwide study of the occurrence of NDMA in DWTPs, including small facilities, was conducted using ultra performance liquid chromatography (UPLC) coupled with tandem mass spectrometry (MS/MS). NDMA concentrations were also investigated at each treatment process in all six DWTPs included in the study. In addition, the concentrations of nitrogen species (total nitrogen, organic nitrogen, and inorganic nitrogen) and organic substances were also determined. This information was then used to identify the relationships between the concentrations of NDMA and water quality parameters that could be potential indicators and/or precursors of the formation of NDMA.

2. Materials and methods

2.1. Sampling

This nationwide survey of raw water and finished water of DWTPs was conducted in September to October 2007 (summer) and December 2007 to January 2008 (winter). DWTPs were selected such that the major water sources in each of the six areas (Fig. 1) could be evaluated, and they also included three water facilities that employ hypochlorite treatment at a high injection ratio. Raw water and finished water samples were collected from DWTPs and transported immediately to our laboratory in glass containers under cool and dark conditions before analysis within 10 days. The process water samples were collected in September–October 2007 at each unit process in six large advanced water treatment plants selected based on their area. Sodium thiosulfate solution, a quenching agent, was added to process water and finished water samples containing hypochlorite.

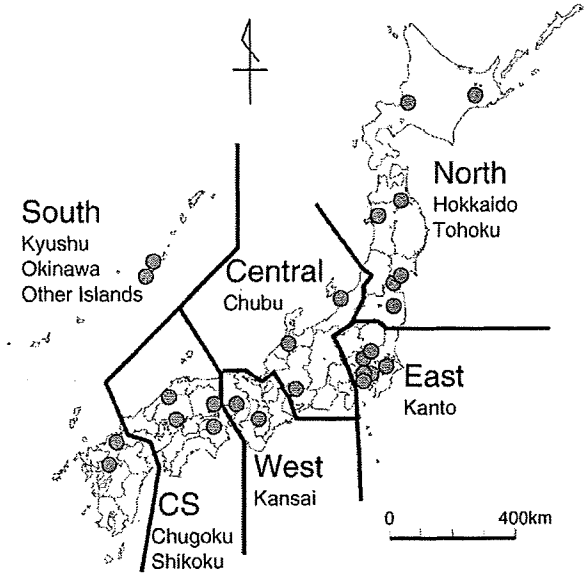

Fig. 1. Sampling areas evaluated in this study.

Table 1
NDMA analysis using UPLC/MS/MS.

UPLC/MS/MS: ACQUITY UPLC/TQD (Waters)
Column: BEH C18 (2.1 × 150 mm, Waters)
Eluent: A: 10 mM Ammonium bicarbonate, B: Acetonitrile
B: 5% (0–3.50 min) → 95% (3.85–6.35 min) → 5% (6.70–8.35 min)
Flow rate: 0.2 mL/min, injection volume: 30 μL, ionization: ESI positive
Capillary voltage: 2.2 kV, source temperature: 140 °C
Desolvation gas flow: 900 L/h, desolvation temperature: 400 °C, cone gas flow: 50 L/h,
MRM:NDMA: 74.9>43.1 (quantitative), collision: 14 eV,
74.9>57.9 (confirmative), collision: 12 eV,
NDMA-d_6: 81.0>46.0, collision: 14 eV

2.2. Reagents

All reagents used in this study were of analytical grade. Ultrapure water prepared with a Gradient A10 water purification system was used (Millipore, Bedford, MA). Furthermore, a stock solution of NDMA (40 mg/L) was prepared by diluting 2000 mg/L certified nitrosamine mix standard solutions that included NDMA in methanol (Supelco, Bellefonte, PA). Working solutions (2.0–1000 μg/L) were prepared by diluting 40 mg/L NDMA methanol solution with dichloromethane (Wako Pure Chemical, Osaka, Japan). Each working solution contained 50 μg/L NDMA-d_6 (C/D/N Isotopes, Pointe-Claire, Canada) as an isotope-labeled surrogate standard.

2.3. Sample preparation

All of the water samples described below were stored in the dark at 4 °C prior to analysis, referring to previous researches (US EPA, 2009c, for example). Sodium bicarbonate (Wako Pure Chemical) was added at a final concentration of 2 g/L to adjust the samples to approximately pH 8. In addition, the samples were spiked with known concentrations of NDMA-d_6. The 500 mL of samples were then filtered through 0.7 μm GF/F filters (Whatman, Florham Park, NJ), after which they were passed through coupled Sep-Pak® Plus AC-2 cartridges (400 mg × 2; Waters, Milford, MA) at flow rates of 3–5 mL/min under vacuum. The Sep-Pak® Plus AC-2 cartridges had been preconditioned with 20 mL of a solution of dichloromethane (Wako Pure Chemical) and diethylether (Kanto Chemical, Tokyo, Japan) (50:50 v/v), followed in sequence by 20 mL of methanol (Wako Pure Chemical) and 20 mL of ultrapure water. After passing through the cartridges, the samples were dried under nitrogen gas and then eluted with 10 mL of a solution of dichloromethane and diethylether (50:50 v/v) at a flow rate ranging from 2 to 3 mL/min. The eluent was then purified by passing through a Sep-Pak® Vac Florisil® cartridge (1 g; Waters) preconditioned with 10 mL of hexane (Wako Pure Chemical) followed by 10 mL of a solution of dichloromethane and diethylether (50:50 v/v). The combination of these two solutions was used because it enabled better separation or recovery than either methanol or dichloromethane alone. The eluate was then concentrated to around 50 μL to minimize the amount of diethylether, after which it was diluted to 200 μL with dichloromethane. Multiple reaction monitoring (MRM) chromatograms of the samples indicated that a cleanup procedure was necessary to obtain the NDMA peak in raw and finished water samples, especially following chlorination.

Table 2
Recovery of NDMA from water samples.

Category	Concentration (ng/L)	Absolute recovery (%) (RSD (%))[a]	Relative recovery (%)[b] (RSD (%))[a]
Ultrapure water	2	55 (16)	95 (17)
River water	10	59 (1.1)	102 (4.6)
Finished water	10	64 (8.4)	103 (3.4)

[a] Relative standard deviation.
[b] Recovery adjusted relative to NDMA-d_6.

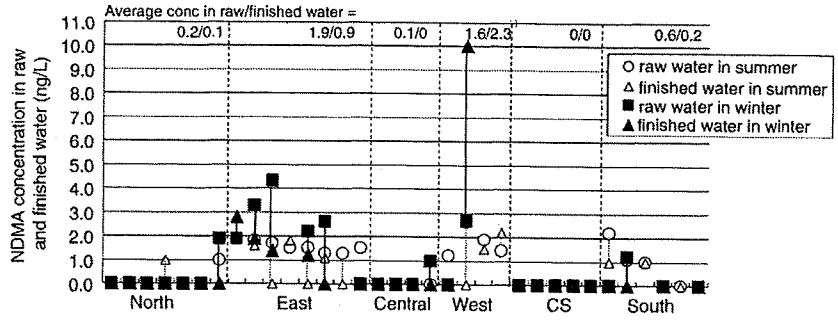

Fig. 2. NDMA in raw and finished water samples evaluated in this study. Average concentration was calculated by arithmetic mean applying zero for ND data.

2.4. Sample analysis

Separation was performed using an ACQUITY UPLC system (Waters) with a BEH C18 column (2.1 mm × 150 mm; Waters). The mobile phase was composed of 10 mM ammonium bicarbonate (Fluka, St. Louis, MO) aqueous solution (eluent A) and 100% acetonitrile (eluent B; Wako Pure Chemical). The ratio of eluent B was changed as follows: 5% for 3.5 min, which was then increased to 95% from 3.5 to 3.85 min, and then maintained at 95% for 2.5 min. The flow rate was 0.2 mL/min for all stages and the sample injection volume was 30 μL. Detection was performed using an ACQUITY TQD tandem mass spectrometer (Waters) operated in the electrospray ionization (ESI) positive-ion mode. The MRM transitions were m/z 74.9–43.1 (quantification) and m/z 74.9–57.9 (confirmation) for NDMA and m/z 81.0–46.0 for NDMA-d_6 (Table 1).

2.5. Method detection limit (MDL)

The average absolute recovery rates of NDMA in ultrapure water, river water, and drinking water samples were 55, 59, and 64%, respectively (number of replicates, $n = 5, 3, 3$, respectively) (Table 2). The relative recovery obtained using NDMA-d_6 ranged from 95 to 103%. The MDL for NDMA, which was calculated based on 3× the standard deviation of five concentrated ultrapure water samples containing 2 ng/L NDMA, was 1.0 ng/L.

2.6. Basic parameters

The total organic carbon (TOC) and dissolved organic carbon (DOC) concentrations were determined using a TOC analyzer (TOC-V CPH; Shimadzu, Kyoto, Japan). Nitrate and nitrite concentrations were determined using an ion chromatograph (DX-500; Dionex, Sunnyvale, CA). Ammonia concentrations were determined spectrophotometrically as a derivative of phenol. Total organic nitrogen (TON) concentrations were determined by subtracting the nitrate, nitrite, and ammonia concentrations from the total nitrogen (TN) concentration, which were determined spectrophotometrically after oxidation by peroxodisulfate (Japan Water Works Association, 2001).

3. Results

3.1. National survey of NDMA in raw water

Fig. 2 shows the regional distribution of NDMA concentrations in raw water samples collected for this study. NDMA was detected in 15 of 31 raw water samples collected in summer, with concentrations ranging from not detected (ND) to 2.6 ng/L. In addition, NDMA was detected in 9 of 28 samples collected in winter, with concentrations ranging from ND to 4.3 ng/L. These concentrations of NDMA are rather low in comparison to previous studies conducted in Canada and the USA (Charrois et al., 2007). Specifically, the maximum concentrations of NDMA in the studies in Canadian and the USA were 8.0 ng/L and 9.4 ng/L, respectively. However, the detection ratio of NDMA was higher in the present study than in the Canadian study, in which NDMA was detected in only 3 of 11 raw water samples.

Samples from the east and west of Japan were found to have higher concentrations of NDMA than those from other areas (Fig. 2). NDMA is often discharged from sewage treatment systems (Krauss and Hollender, 2008)), and can be present in discharge associated with industries, such as rubber manufacturing, leather tanning, pesticide manufacturing, food processing, foundries, and dye manufacturing. In the recent survey of discharge water from sewage treatment plants,

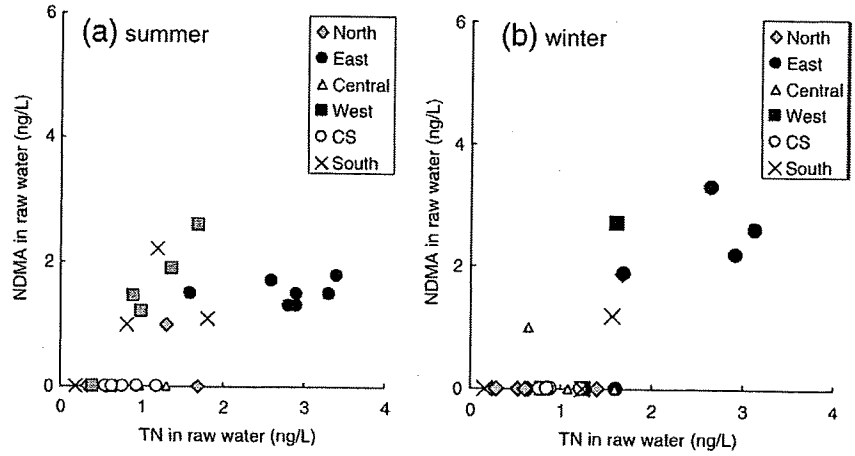

Fig. 3. TN and NDMA in raw water.

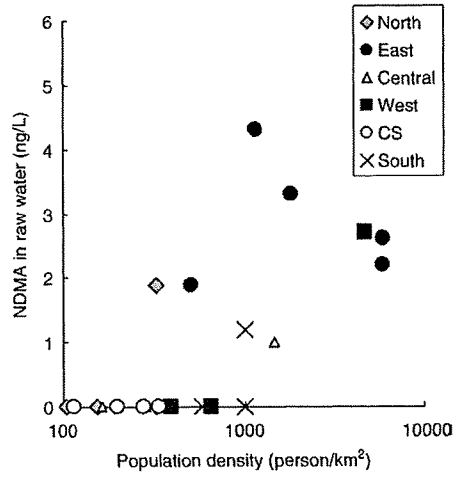

Fig. 4. Population density and NDMA concentration in raw water samples collected in winter.

NDMA was found during ozonation of water samples taken from sewage treatment plants located in the Yodo River Basin, the west area of Japan. The concentrations of NDMA before and after ozonation were 16–290 ng/L and 24–280 ng/L, respectively, and specific compounds related to the textile industry were identified in these discharges (Kosaka et al., submitted for publication). These contaminants may partially account for the NDMA in the west area.

The causes of contamination in the east and other areas are still unclear. However, NDMA levels were higher in waters containing nitrogen species. Although ammonia, nitrite, and nitrate all showed the same tendencies as NDMA concentrations, as shown in Fig. S1, TN seemed to be related to NDMA in raw water samples (Fig. 3). Although only limited data are available at present, this is probably because TN is an indicator of total nitrogen-related contamination, such as discharge from sewage treatment plants or other activities.

Fig. 4 shows the relationship between the population density of the each river basin and NDMA concentration in raw water samples. There is not a clear correlation between them because this study considered several large areas, where each area or processing plant may have source waters and treatment configurations with very different characteristics. However, NDMA was found in the water from areas in which the catchment population density was over 300 persons/km^2. Seasonal differences between summer and winter samples may exist due to flow rate, environmental fate, and/or NDMA burden. The ratio of NDMA to TN was slightly higher in the east in winter, but was higher in the west in summer. In this study, the relationship between total organic carbon and NDMA was also examined but no obvious correlation was found (data not shown).

3.2. National survey of NDMA in finished water

Fig. 2 shows the regional distribution of NDMA in finished water samples. The concentration of NDMA in the finished water samples ranged from ND to 2.2 ng/L (10/31) in summer and from ND to 10 ng/L (5/28) in winter. The concentrations of NDMA in finished water samples were generally lower than those in raw water samples. In addition, the concentration of NDMA in the finished water samples was higher in the winter than in the summer.

Additional samples containing high concentrations of hypochlorite had NDMA concentrations that were equivalent to or less than the MDL. One DWTP included in this study utilized chloramination during the treatment process; however, no NDMA was detected in samples of finished drinking water collected from this plant.

In comparison to studies performed in Canada and the USA (Charrois et al., 2007), the concentrations of NDMA observed in the finished water samples in the present study were low (max. 65 ng/L in finished water in Canada and 30 ng/L in finished water in the USA). This may have been because chloramination is not generally used in Japanese facilities; to our knowledge, there is only one water treatment plant intentionally employs chloramination to reduce the formation of trihalomethane in Japan. In addition, the level of NDMA is decreased by biological activated carbon treatment (BAC), as described in the following section, even once it has been generated.

On comparison of NDMA concentrations between raw and finished water samples, the sample with the highest concentration of NDMA (10 ng/L) was collected from a DWTP at which the treatment process included ozonation. Fig. 5 shows the relationship between TN in raw water samples and NDMA in finished water samples. The results can be divided into two categories, *i.e.*, east and other areas.

Fig. S2 shows the relationships between ammonia and nitrate in raw water and NDMA in finished water. NDMA tended to be detected more frequently in samples that contained high concentrations of each of ammonia, nitrite, and nitrate. It should be noted that the data from the finished water sample with the highest NDMA concentration from a DWTP in the Yodo River Basin, which employs ozonation, were obviously separated from the other data. Based on these results, the formation and degradation of NDMA in DWTPs appear to be dependent on the watershed in which these facilities are located, as the Yodo River is the largest water source in the western part of Japan with a population of tens of millions utilizing the river water as a

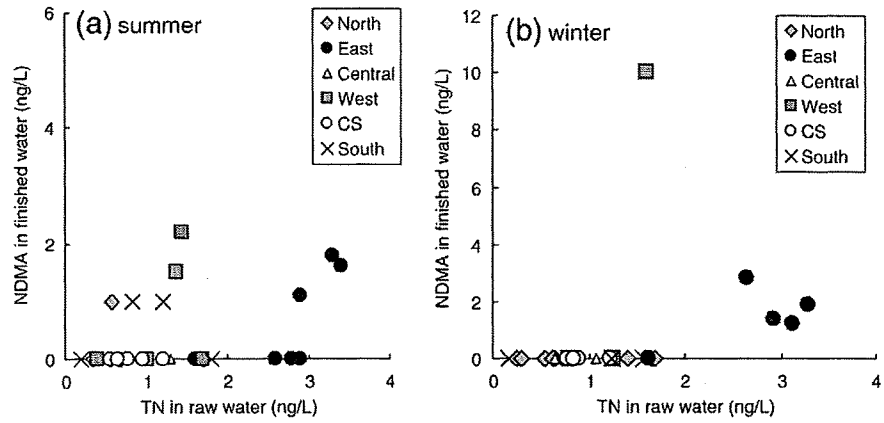

Fig. 5. TN in raw water and NDMA in finished water.

source and many water treatment facilities in this area employ ozonation.

3.3. Survey of NDMA in DWTPs

To clarify the behavior of NDMA during the purification process, NDMA concentrations were examined in each purification process in six major DWTPs (DWTP1-6) (Fig. 6). Each DWTP utilized various treatment processes, including coagulation, flocculation, sedimentation, and sand filtration. However, for convenience, these processes are simply classified here as before ozonation, after ozonation, after biological activated carbon treatment, and finished water. The NDMA concentrations of water samples following each process are shown in Fig. S3, and the concentrations before and after ozonation are summarized in Fig. 6.

The NDMA concentration increased to 7.0 ng/L following chlorination in DWTP2, which was higher than the level observed in raw water (1.5 ng/L). This increase was probably due to the use of a high dose of chlorine (4.5 mg/L) during the first stage of treatment at this facility. Although the ammonia:chlorine molar ratio of 6.8 exceeded the chlorine dose necessary for breakpoint chlorination, TOC was as high as 3.5 mg/L. Therefore, it was considered to consume chlorine so as to be combined chlorination condition at that time.

At DWTP3 and DWTP6, the NDMA concentration increased to as high as 15 ng/L following ozonation. Both of these treatment plants are located in the Yodo River Basin, and formation of NDMA likely occurred during the ozonation process.

The NDMA concentration decreased markedly (from 20 ng/L to 1.5 ng/L in DWTP3 and from 17 ng/L to 1.3 ng/L in DWTP6) following BAC treatment, regardless of the level formed by ozonation. Although it has been reported that NDMA shows poor absorption onto activated carbon due to its hydrophilic nature (Mitch et al., 2003; World Health Organization, 2008), removal of more than 90% of the NDMA following BAC treatment was observed in the present study. This finding indicates that the NDMA concentration decreased due to biological degradation during BAC treatment, similar to the findings of a previous study (Tateishi et al., 2008). However, the mechanism responsible for this decrease in NDMA concentration was not elucidated in this study. Following BAC treatment, the NDMA concentrations in the finished water samples ranged from <MDL (1.0) to 2.2 ng/L, which was below the concentration of 7 ng/L considered by the US EPA as the level in drinking water associated with an excess cancer risk of 10^{-5} (US EPA, 2009a), but was close to the public health goal of 3 ng/L proposed by California (California Department of Public Health, 2009).

As the behavior of NDMA varied among DWTPs, the relationships between NDMA concentration and other organic or inorganic water quality parameters were analyzed. The results revealed no clear relationships between the increase in NDMA concentration that occurred due to ozonation and the concentrations of organic compounds in the raw water samples (TOC, E260, and TON). In addition, no relationships were observed between the increases in NDMA concentration and pH, nitrate, nitrite, or ammonia concentrations, or the ozone dose (Fig. S4 in Appendix A).

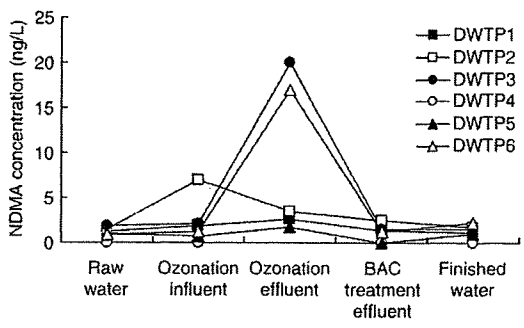

Fig. 6. NDMA concentrations at six treatment plants.

The water source for both DWTPs that showed marked increases in NDMA concentration following ozonation (DWTP3 and DWTP6) was the Yodo River, which is located in western Japan. These findings were in agreement with those of a previous study conducted to evaluate the concentrations of NDMA at DWTPs in the Yodo River Basin (Tateishi et al., 2008) and our previous study suggesting that these increases in NDMA concentration during ozonation at the two DWTPs with the Yodo River as the water source may be due to specific contaminants (Kosaka et al., submitted for publication).

4. Discussion

The present study was performed to determine the NDMA concentrations in water samples from various areas in Japan. Higher NDMA concentrations were found in water containing nitrogen species, which was consistent with the results reported previously (Mitch et al., 2003). The results of this study also indicated that NDMA formation occurs during ozonation in selected DWTPs, which was similar to the findings of other studies indicating that this phenomenon occurs in the presence of *N,N*-dimethylsulfamide (DMS), which is one of the decomposition products of tolylfluanid (Schmidt and Brauch, 2008). Coagulation polymers have also been indicated as precursors of NDMA (Mitch et al., 2003; Charrois and Hrudey, 2007). Although tolylfluanid is not approved for agricultural use and coagulation polymers are prohibited in Japan, the formation of NDMA from specific compounds was observed during ozonation in DWTPs, and further studies are required to determine the concentrations of chemicals that can lead to its formation.

Taken together, the findings of this study indicate that it is necessary to investigate the mechanism by which NDMA is formed during ozonation so that the specific compound(s) responsible for its formation in water in the western part of Japan can be identified. Accordingly, we are currently investigating the relationship between the load of NDMA precursors and its formation during ozonation in upstream sewage treatment plants (STPs) because the effluents from these STPs flow into river water that is subsequently used as the raw water supply for drinking water.

It is important to note that higher NDMA concentrations were detected in prefectures that had higher levels of human activity. Although this study provided only a rough estimate, the level of NDMA in raw water tended to increase in proportion to human activity in the area. Therefore, these activities should be regarded as area-specific and/or non-point sources of NDMA and NDMA precursors.

5. Summary

1. A national survey of *N*-nitrosodimethylamine (NDMA) levels in raw and finished water samples collected from drinking water treatment plants (DWTPs) was conducted using solid-phase extraction (SPE) followed by ultra performance liquid chromatography (UPLC) coupled with tandem mass spectrometry (MS/MS).
2. NDMA was detected in 15 of 31 raw water samples collected in winter at concentrations up to 2.6 ng/L. NDMA was also detected in 9 of 28 samples collected in summer, with a maximum concentration of 4.3 ng/L. NDMA was detected in 8 of 31 finished water samples collected in summer with a maximum concentration of 2.2 ng/L, while in winter, NDMA was detected in 5 of 28 samples with a maximum concentration of 2.8 ng/L, except in one sample with a high concentration of 10 ng/L.
3. The NDMA concentration was greater in raw water samples containing higher levels of nitrogen species. In addition, river water samples collected from the east and west of Japan showed

higher concentrations of NDMA than those collected from other areas.

4. Process water was examined during each of the processes in six advanced water treatment plants. NDMA concentrations were shown to increase during ozonation when water from the Yodo River Basin was utilized as the source water.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.scitotenv.2009.02.014.

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(8-21)モデルシミュレーションによる除草剤プレチラクロールの河川中濃度に関する感度解析

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1．はじめに

河川水中での農薬濃度や流域内での農薬の挙動を把握する一つの手段として、解析モデルは重要なツールとなる。本研究室では過去に解析モデルによって河川中の農薬濃度の推定と評価を行ってきた[1)2)]。解析モデルを用いて農薬の挙動を解析するには農薬の物性値や水文学データ、土地に関するデータ等のさまざまなデータが必要となる。それらのデータが結果に与える影響を評価する事により農薬の挙動を把握する上で役に立つ情報を得られると考えられる。よって本研究では、解析モデルを用いて農薬の挙動を推定し、入力データの変化による計算結果への影響を評価するために感度解析を行った。

2．対象流域と解析モデル

対象流域は、岩手県雫石町を含む葛根田川流域とした。この流域では農家一軒一軒の農作業データ等、詳細な情報の提供を受けることができた。観測地点より上流の対象流域面積は約191km^{2}である。流出解析モデルは農薬原体の移動モデルとその輸送媒体である水分の移動モデルによって構成されている。水と農薬原体の空間的分布を表現するため流域を1km×1kmメッシュに分割し、各メッシュに水田や河川などの特性の異なる13種類のコンパートメントを配置したコンパートメント型流出モデルを適用した。

3．農薬原体

対象とする農薬原体は、対象河川において比較的高濃度で頻度よく観測されていた除草剤のプレチラクロールとした。流域内においてプレチラクロールは5月中旬から下旬にかけて水田に散布される。モデルシミュレーションに用いたプレチラクロールの物性値は文献[3)4)]に記載された数値を用いた（表1）。

表1．プレチラクロールの物性値

	プレチラクロール
土壌中分解係数	2.3×10^{-2} [/day]
水中分解係数	3.5×10^{-3} [/day]
土壌有機炭素吸着定数	628 [L/kg]
水溶解度	50 [mg/L]

4．結果と考察

(1)農薬動態予測

解析モデルによって算出された観測地点におけるプレチラクロール濃度の時間変化を図1に示す。計算により得られた初期の流出におけるピークの時期が観測値と若干ずれた形となっているが、ピークの濃度は近い値となった。低減期に関しては計算結果が観測値に近い値をとっている結果となった。

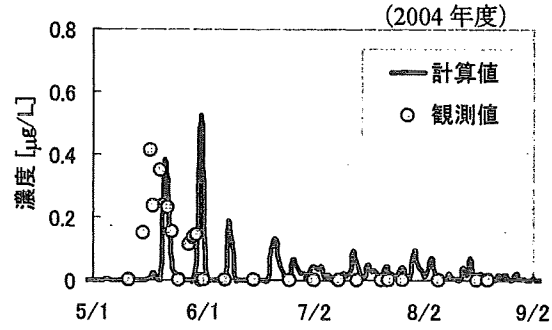


図1．プレチラクロールの濃度観測値と計算値

流域内で散布されたプレチラクロールの計算による物質収支を図2に示す。流域内に散布されたプレチラクロールの流域外への流出は9.9%に留まり、散布されたプレチラクロールの88.8%が土壌中で分解される結果となった。また水中で分解される量、流域内に残留する量は共に1%に満たなかった。

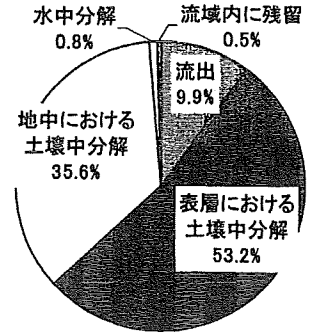
(2)感度解析による入力パラメータの評価

変化させるパラメータは散布量、土壌中分解係数、水中分解係数とした。予測値の平均濃度（以下予測値とする）は、解析モデルにより一日毎に算出されたプレチラクロールの濃度の5月から8月における平均値とした。

散布量の変化による予測値への影響を図３に示す。散布量に比例して平均濃度が高くなる結果となった。

次に、土壌中分解係数の変化による予測値への影響を図４に示す。土壌中分解係数の倍率が、0.05倍から3倍の間で、分解係数の増大と共に平均濃度が大きく下がる結果となった。土壌中分解係数を0.1倍にすると2003年では元の予測値（土壌中分解係数の倍率が1倍の予測値）の約3倍、2004年では約2.5倍となり、土壌中分解係数を10倍にすると2003年では元の予測値の約1/5、2004年では約1/2となった。プレチラクロールは土壌中分解係数の変化に比較的大きく影響される事を表す結果となった。

最後に、水中分解係数の変化による予測値への影響を図５に示す。水中分解係数を3倍以上にすると、分解係数の増大に伴い平均濃度が下がる結果となった。



2004 年度：総散布量 67.1kg
図２．プレチラクロールの物質収支

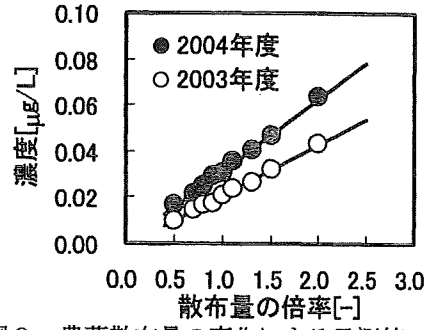


図３．農薬散布量の変化による予測値への影響

５．おわりに

解析モデルを用いて時系列の農薬濃度を算出し観測濃度と比較した結果、プレチラクロールの河川中濃度は比較的高精度で推定できる事が示された。流域内に散布されたプレチラクロールの9割は土壌中において分解され流出は1割であった。水中におけるプレチラクロールの分解量は全体の1%に満たないと推定された。

プレチラクロールの感度解析の結果、散布量に比例して平均濃度が上がる事が示された。また、土壌中分解係数の変化による影響を大きく受け、水中分解係数による影響はあまり受けないことが示された。

流域内における農薬の挙動は農薬毎に異なり、特に農薬の物性値や散布時期、水文学的要素によって変化する事が考えられる。そのため、今後他の農薬についても評価していく必要がある。

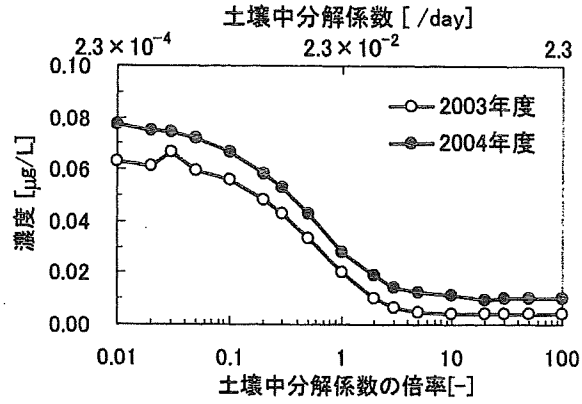


図４．土壌中分解係数の変化による予測値への影響

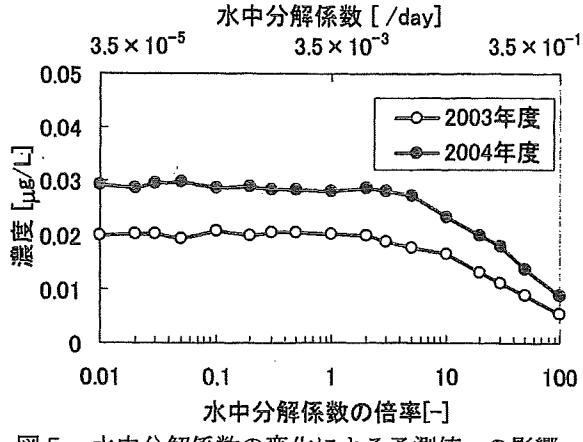


図５．水中分解係数の変化による予測値への影響

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