A screening study on the mutagen formation potential of 44 pesticides

Hirokazu Takanashi, Misako Kishida, Kazuya Abiru, Takashi Kondo, Takashi Kameya, Taku Matsushita, Tsunenori Nakajima and Akira Ohki

ABSTRACT

The mutagenicity and the mutagen formation potential (MFP) of seven fungicides, 15 herbicides and 22 insecticides upon chlorination were measured with the Ames *Salmonella* assay. All the pesticides except for thiram and dichlorvos were non-mutagenic. However, 75% of the tested pesticides showed significant MFP. This indicated that the chlorination by-products of some pesticides are mutagenic, although the pesticides themselves are not. No significant mutagenicity or MFP was observed for 20% of the pesticides tested in the present study. Thiram and dichlorvos, representing 5% of the tested pesticides, had significant mutagenicity and MFP. The average MFP of asulam, which had the highest MFP in this study, was 860 times greater than the average MFP of propyzamide, which had the lowest MFP (below the detection limit). In addition, the chemical structures of pesticides which had significant MFPs were compared to each other in order to see if there are common characteristics among them, but we could not predict whether pesticides are capable of forming mutagens from their chemical structures, use types or mode of actions. MFP measurements are indispensable in order to know whether pesticides are capable of forming mutagens.

Key words | Ames assay, chlorination by-product, mutagen formation potential (MFP), mutagenicity

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INTRODUCTION

Pesticides are sprayed onto plants, weeds or soil for insect or weed control, and some of the pesticides will transfer to the water environment. These pesticides may undergo various reactions such as hydrolysis, photolysis and biodegradation in the water environment. Pesticides and their degradation products have been found in natural water (Lambropoulou *et al.* 2002; Cerejeira *et al.* 2003; Palma *et al.* 2004; Rebich *et al.* 2004; Donald *et al.* 2007; Leong *et al.* 2007; Maloschik *et al.* 2007; Hladik *et al.* 2008; Chiron *et al.* 2009; Gao *et al.* 2009; Vryzas *et al.* 2009; Iwafune *et al.* 2010; Abiru *et al.* 2011). Natural water containing these pesticides and their degradation products will often be chlorinated at water purification plants, and distributed as tap water.

The authors reported the survey results of the mutagenicity of Japanese tap water (Takanashi *et al.* 2009, 2011), which was measured using *Salmonella typhimurium* TA100 strain without metabolic activation (S9). In these surveys, a remarkable seasonal difference of mutagenicity was observed at a sampling point. Some possible factors for this difference are the seasonal variations of precipitation and water temperature. Meanwhile, substances mingled into raw water supplies from non-natural sources may also affect the mutagenicity of tap water. The substances we are focusing on are pesticides.

Pesticides must be registered in accordance with legislation in each country before they can be used. For example, in Japan and in the USA, the production, sale, and usage of pesticides are regulated by Japan's Agricultural Chemicals Regulation Law and America's Federal Insecticide, Fungicide, and Rodenticide Act. Before pesticides are

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permitted to be registered, they must undergo various toxicity tests. In Japan, the Ames assay is mandatory before any pesticide is registered. According to the legislation, the major derivatives of pesticides, such as hydrolysates or metabolic products produced by vegetation, are also required to be tested. However, substances produced as a result of chlorination are exempt from the legislation. These facts intrigued our interests on whether pesticides produce mutagens when they react with chlorine.

There are many reports on the chlorination by-products and chlorination degradability of pesticides (Miles & Oshiro 1990; Takahashi & Morita 1993; Magara *et al.* 1994; Arai *et al.* 2005; Kamoshita *et al.* 2007; Kamel *et al.* 2009). However, there are only a few studies on changes in the mutagenicity of pesticides through chlorination; the mutagenicity of thiram was decreased by chlorination (Setsuda *et al.* 1992), while the mutagenicity of organophosphorus pesticides was increased (Onodera *et al.* 1995a; Kishida *et al.* 2010). Although a few studies have identified the chlorination by-products of certain pesticides and revealed their mutagenicity (Inoue *et al.* 1995; Onodera *et al.* 1995b; Kodama *et al.* 1997, 1999; Kamoshita *et al.* 2010; Kishida *et al.* 2010), none of them have confirmed strong mutagens. As a result, the number of studied pesticides is limited, and it is unknown whether many other pesticides could produce mutagens during chlorination.

In the present study, the mutagenicity caused by mutagens, which are produced from pesticides aqueous solution through chlorination conducted in the same manner as water purification processes and can be concentrated by using CSP800 resin (Takanashi *et al.* 2001), is defined as the mutagen formation potential (MFP) of a pesticide upon chlorination. We measured the MFPs of seven fungicides, 15 herbicides and 22 insecticides to determine if mutagens can be produced through the chlorination of raw water containing commonly found pesticides.

MATERIALS AND METHODS

Chemicals

For this study, 44 pesticides were selected from 491 pesticides registered in Japan (Japan Plant Protection Association 2009). Out of 44 pesticides, 41 were selected from the 'Complementary Items for Setting the Targets for Water Quality Management in Japan' document by the Ministry of Health, Labour and Welfare Japan (2010), which is used as a category to set water standards for tap water quality management. The pesticides in this document are frequently found in raw water supplies.

Various types of pesticides, such as organophosphorus, organochlorine, and amide, were tested in the present study. All the chemical reagents were purchased from Wako Pure Chemical Industries, Ltd (Osaka, Japan). All the pesticides tested were purchased in the purity grade of 'Standards for Pesticide Residue Analysis'.

Sample preparation

To prepare the initial individual concentrations of aqueous solution for the 44 tested pesticides, each pesticide was dissolved into ethanol and this ethanol solution was added to two bottles, each of which contained 2 L of distilled water. The initial concentrations of the chemicals are shown in Table 1. The prepared sample solutions were stirred for 24 hours under light-shading at room temperature. One bottle of each sample solution was adjusted to pH 2.0 ± 0.2 with 2.5 M H_2SO_4, and mutagens in the sample solution were concentrated 1,000 times using a Sep-Pak Plus CSP-800 cartridge (Nihon Waters K. K., Tokyo, Japan).

The other bottle of each sample solution was adjusted to pH 7.0 ± 0.2 with 0.1 M NaOH or 0.1 M H_2SO_4, then 3 mg-Cl_2 mg-C^{-1} of sodium hypochlorite was added to the sample solution. This chlorine dosage was decided by examining chlorine demands for the MFP measurement. The sample solution was stirred for 24 hours under light-shading at room temperature. At least 0.1 mg-Cl_2 L^{-1} of chlorine remained after chlorination, as confirmed by the DPD (*N*, *N*-diethyl-*p*-phenylendiamine) colorimetric method. The mutagens produced by chlorination were concentrated 1,000 times by a Sep-Pak Plus CSP-800 cartridge and used for the Ames assay (Takanashi *et al.* 2001).

The Ames *Salmonella* mutagenicity assay

The preincubation method of the Ames assay was adopted according to the guidebook published by

Table 1 | Summary of mutagenicity test results

Use type	Pesticide	Determined value (net rev μmol^{-1})[a]	Average (net rev μmol^{-1})[a]	MR value (–)[a]	Result[b]	Detection limit (net rev μmol^{-1})	Dose (mg plate^{-1})	CCRIS TA100 –S9[b]	CCRIS TA1535 –S9[b]	Initial conc. (mg L^{-1})
Fungicide	Ferimzone	(8.1)	(8.1)	(1.2)	N (1)	16	0.25–1	–	–	10
	Thiram	880–910	890	2.2–4.0	P (2)	110–290	0.0125–0.1	P (4)	P (3)	10
	Mepronil	(9.2)	(9.2)	(0.94)	N (1)	21	0.25–1	–	–	10
	Pyroquilon	(5.0)	(5.0)	(1.1)	N (1)	9.9	0.25–1	–	–	10
	Iprobenfos (IBP)	(9.8)	(9.8)	(0.97)	N (1)	20	0.25–1	–	–	10
	Edifenphos (EDDP)	(20–25)	(23)	(0.92–1.1)	N (2)	40–49	0.25–0.5	–	–	10
	Tricyclazole	(6.2–7.4)	(6.8)	(0.89–0.91)	N (2)	12–15	0.25–1	–	–	10
Herbicide	Asulam	(6.2)	(6.2)	(0.90)	N (1)	13	0.25–1	–		10
	Diuron (DCMU)	(16)	(16)	(0.85)	N (1)	16	0.25–0.5	–		10
	Bentazone	(8.4)	(8.4)	(1.1)	N (1)	17	0.25–1	N (1)	N (1)	10
	Mecoprop (MCPP)	(4.1)	(4.1)	(1.3)	N (1)	8.2	0.25–1	N (1)	–	10
	Flazasulfuron	(18)	(18)	(1.1)	N (1)	36	0.25–0.5	–	–	10
	Molinate	(4.1)	(4.1)	(0.85)	N (1)	8.2	0.125–0.25	N (1)	N (1)	10
	Thiobencarb	(4.9)	(4.9)	(0.84)	N (1)	9.8	0.25–1	–	–	10
	Atrazine	(4.7)	(4.7)	(1.1)	N (1)	9.5	0.25–1	N (8)	N (3)	10
	Pretilachlor	(5.4)	(5.4)	(1.3)	N (1)	13	0.25–1	N (1)	–	10
	Dimethametryn	(5.6)	(5.6)	(0.94)	N (1)	11	0.25–1	–	–	10
	Simetryn	(7.5)	(7.5)	(1.1)	N (1)	15	0.25–1	–	–	10
	Dichlobenil (DBN)	(3.3)	(3.3)	(1.0)	N (1)	6.5	0.25–1	–	–	10
	Triclopyr	(4.9)	(4.9)	(1.2)	N (1)	9.7	0.25–1	–	–	10
	Napropamide	(8.1)	(8.1)	(1.0)	N (1)	16	0.25–1	–	–	10
	Propyzamide	(5.6)	(5.6)	(1.1)	N (1)	11	0.25–1	–	–	10
Insecticide	Isoxathion	(67)	(67)	(1.3)	N (1)	130	0.0375–0.15	–	–	1.5
	Pyridaphenthion	(11)	(11)	(0.62)	N (1)	22	0.25–1	–	–	10
	Malathion	(7.6)	(7.6)	(1.1)	N (1)	15	0.25–1	N (1)	N (1)	10
	Fenitrothion (MEP)	(7.8–9.7)	(8.9)	(0.97–1.2)	N (4)	15–19	0.25–1	N (2)	N (1)	10
	Isoprocarb (MIPC)	(5.2)	(5.2)	(0.97)	N (1)	11	0.25–1	–	–	10
	Fenthion (MPP)	(23)	(23)	(0.90)	N (1)	45	0.1–0.4	–	–	4.2
	Isofenphos	(12)	(12)	(1.1)	N (1)	25	0.25–1	–	–	10
	Carbaryl (NAC)	(3.8)	(3.8)	(1.2)	N (1)	7.6	0.25–1	N (1)	N (2)	10
	Mevinphos	(4.9)	(4.9)	(1.2)	N (1)	9.9	0.25–1	–	–	10
	Methidathion (DMTP)	(10)	(10)	(0.73)	N (1)	21	0.25–1	–	–	10
	Diazinon	(9.4)	(9.4)	(0.88)	N (1)	19	0.25–1	N (1)	N (2)	10
	Fenobcarb (BPMC)	(8.9)	(8.9)	(1.1)	N (1)	18	0.125–0.5	–	–	10
	Disulfoton	(8.8)	(8.8)	(1.0)	N (1)	18	0.25–1	–	P (1)	10
	Monocrotophos	(4.9)	(4.9)	(1.2)	N (1)	9.8	0.25–1	N (2)	P (1), N (1)	10

(*continued*)

Table 1 | continued

Use type	Pesticide	Determined value (net rev μmol^{-1})[a]	Average (net rev μmol^{-1})[a]	MR value (–)[a]	Result[b]	Detection limit (net rev μmol^{-1})	Dose (mg plate^{-1})	CCRIS TA100 –S9[b]	CCRIS TA1535 –S9[b]	Initial conc. (mg L^{-1})
	Trichlorfon (DEP)	(5.7)	(5.7)	(1.3)	N (1)	11	0.25–1	P (1), N (6)	N (4)	10
	Phenthoate (PAP)	(11)	(11)	(1.1)	N (1)	23	0.25–1	–	N (1)	10
	Methomyl	(3.7)	(3.7)	(0.92)	N (1)	7.5	0.25–1	–	–	10
	Dichlorvos (DDVP)	(7.1)–42	28	(1.2)–2.5	P (2), N (1)	9.9–15	0.25–1	P (4), N (1)	–	10
	β-Endosulfan	(530)	(530)	(1.2)	N (1)	1,100	0.00625–0.025	–	–	0.25
	Buprofezin	(190–200)	(200)	(0.97–1.1)	N (2)	390	0.0125–0.05	–	–	0.5
	Acephate	(5.8)	(5.8)	(1.3)	N (1)	12	0.25–1	N (1)	N (1)	10
	Dimethoate	(4.7–6.9)	(5.8)	(1.2–1.3)	N (2)	9.2–14	0.25–1	P (1), N (1)	N (3)	10

P: A result is determined to be positive in the present study or CCRIS (Chemical Carcinogenesis Research Information System). N: A result is determined to be negative in the present study or CCRIS. –: No information is available.
[a]Negative results are in parentheses.
[b]Number of samples or studies are in parentheses.

Japan's Ministry of Health, Labour and Welfare (Ministry of Labour Japan 1991). The assay was performed using *Salmonella typhimurium* TA100 strains without metabolic activation (–S9), and was performed with three or four dose steps using duplicate plates for each step. Quadruplet plates were used for the negative control tests. The mutagenicity and the MFP of the samples was evaluated as net revertant per μmol (net rev μmol^{-1}), which was calculated from the slope of their dose-response lines. In order to confirm the strains' specific activity, 4-nitroquinoline-1-oxide (4NQO) was used as the positive control substance.

At 9,000–11,000 net rev μg-4NQO^{-1}, the strains' specific activity was consistent through all the runs. The negative control test results were also consistent, showing 88–209 rev plate^{-1}. From these results, all the mutation ratio (MR) values attained in the different runs of the Ames assay could be compared with each other. The MR value was calculated according to Equation (1):

$$\mathrm{MR} = \frac{R_d}{R_0} \tag{1}$$

where R_d is the maximum average number of revertant colonies within the linearly responsive range (rev plate^{-1}), and R_0 is the average number of revertant colonies in the spontaneous tests (rev plate^{-1}). In this study, test results with an MR value over 1.4 were deemed positive, because quadruplet plates were used for the negative control steps (Takanashi & Urano 1998). The mutagenicity and the MFP with an MR value over 1.4 were defined as significant in this study. The detection limit of the assay (DL) was calculated according to Equation (2):

$$\mathrm{DL} = \frac{0.4R_0}{D_\mathrm{m}} \tag{2}$$

where D_m (mg plate^{-1}) is the maximum dosage in the sample test. Mutagenicity under the DL was described as N. D. (not detected) in the present study. The minimum mutagenicity was assumed to be half the DL value in order to calculate the average mutagenicity.

RESULTS AND DISCUSSION

Mutagenicity of pesticides

The mutagenicity of the selected pesticides was measured and the results are summarized in Table 1. All the pesticides except for thiram and dichlorvos (DDVP) were not mutagenic. These results were compared with the information in the Chemical Carcinogenesis Research Information System (CCRIS) database, which has peer-reviewed mutagenicity test results (United States National Library of Medicine 2010). Almost all of the test results for thiram and DDVP using the TA100 strain were also positive in CCRIS, which is consistent with the results obtained in the present study. As for disulfoton, a positive result was found in CCRIS; however, a negative result was obtained in this study. Partial positive results for monocrotophos, dimethoate and trichlorfon were found in CCRIS, but no significantly mutagenic results were observed in the present study. These differences can be attributed to the difference of the maximum dosage of test compounds in the assay system: the tests in CCRIS employed 5 mg plate^{-1} as the maximum dosage, while the maximum dosage in the present study was 1 mg plate^{-1}. The purpose of this study is not to examine whether pesticides have mutagenicity, but to determine if mutagens can be produced through the chlorination of raw water containing pesticides.

The negative results for the other pesticides obtained in the present study were consistent with CCRIS. As for pesticides having no information in CCRIS, the negative results also obtained in some reports (Nihon Ciba-Geigy 1986a, b, 1992; Nihon Nohyaku Co. Ltd 1986; Eli Lilly Japan K. K. 1989; Shionogi & Co. Ltd 1990; Takeda Pharmaceutical Company Limited 1994) are consistent with the results obtained in the present study.

Formation of mutagens by chlorination of pesticides

Figure 1 shows the mutagenicity and the MFP of malathion as an example. As shown in Figure 1, the mutagenicity of malathion was not significant, while the MFP was. This indicates that the chlorination by-products of malathion are mutagenic, even though malathion is not. The results of the mutagenicity measurements are summarized in Table 1 and the MFP measurements are in Table 2 (their dose-response lines are not shown due to limitations of paper space). All duplicated data were almost consistent for each pesticide.

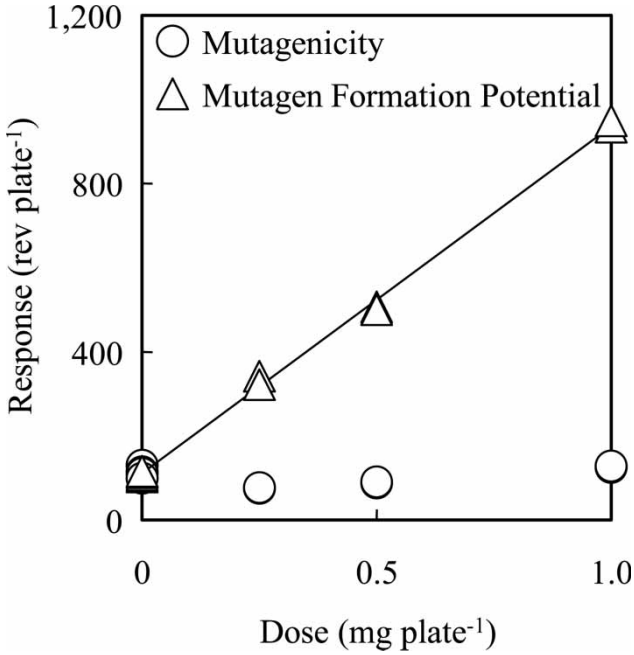

Figure 1 | An example of dose-response line (malathion).

We separated the results into three groups: Group A where 20% of the pesticides had no significant mutagenicity or MFP, Group B where 75% had no significant mutagenicity but had significant MFP, and Group C where 5% had significant mutagenicity and significant MFP. The MFPs of thiram and DDVP (Group C) decreased compared to their mutagenicity, but the differences were statistically insignificant ($p > 0.086$ and $p > 0.57$, respectively). Therefore, mutagens were formed by the chlorination of many pesticides, and there were no cases where the mutagenicity significantly decreased because of chlorination in the present study.

In the experiments for Group B, the initial concentrations of aqueous solutions of pesticides and the concentration factors were identical when measuring the mutagenicity and the MFP. Due to different tolerances of the TA100 strain to the samples, some samples were diluted with dimethyl sulfoxide (DMSO). As a result of different dilution, there were some cases where the maximum dosages of pesticide per plate in the mutagenicity tests differed from those in the MFP tests. Nevertheless, for all the pesticides, the maximum dosages in the mutagenicity tests were equal to or greater than those of the MFP tests (the sensitivity of the experimental

Table 2 | Summary of mutagen formation potential test results

Use type	Pesticide	Determined value (net rev μmol^{-1})[a]	Average (net rev μmol^{-1})[a]	MR value (–)[a]	Result[b]	Detection limit (net rev μmol^{-1})	Dose (mg plate^{-1})	Initial conc. (mg L^{-1})	Water solubility[c] (mg L^{-1})
Fungicide	Ferimzone	530–1,600	940	8.1–10.9	P (3)	29–40	0.1–0.4	10	–
	Thiram	550–650	600	1.7–3.6	P (2)	100–290	0.025–0.1	10	18
	Mepronil	57–71	66	2.1–2.9	P (3)	14–26	0.25–1	10	12.7
	Pyroquilon	43–68	58	1.8–3.5	P (3)	11–20	0.25–1	10	4,000
	Iprobenfos (IBP)	35–45	40	1.8–1.9	P (2)	18–20	0.25–1	10	430
	Edifenphos (EDDP)	(10–12)	(11)	(1.3)	N (2)	20–25	0.25–1	10	56
	Tricyclazole	(7.4–12)	(9.7)	(0.98–1.1)	N (2)	15–25	0.25–1	10	1,600
Herbicide	Asulam	3,600–6,400	4,800	2.4–2.8	P (4)	1,000–1,400	0.0025–0.01	10	5,000
	Diuron (DCMU)	(820)–5,300	2,300	(1.3)–2.9	P (3), N (1)	100–1,600	0.0025–0.0125	10	36.4
	Bentazone	710–800	760	8.0–8.1	P (2)	40–44	0.0625–0.25	10	570
	Mecoprop (MCPP)	350–530	450	2.3–3.9	P (4)	65–120	0.01–0.2	10	734
	Flazasulfuron	150	150	1.5	P (2)	130–130	0.05–0.2	10	2,100
	Molinate	(5.1)–150	84	(1.3)–7.9	P (3), N (1)	8.2–12	0.25–1	10	990
	Thiobencarb	80–82	81	3.2–4.4	P (2)	9.8–15	0.25–1	10	30
	Atrazine	33–37	35	2.5	P (2)	8.8–9.5	0.25–1	10	33
	Pretilachlor	23–24	24	1.5–1.7	P (2)	13–19	0.25–1	10	50
	Dimethametryn	(8.2)–31	22	(1.3)–2.0	P (2), N (1)	11–17	0.25–1	10	50
	Simetryn	15–17	16	1.4–1.9	P (2)	8.1–15	0.25–1	10	400
	Dichlobenil (DBN)	(5.2)–16	11	(1.1)–2.0	P (1), N (1)	6.5–10	0.25–1	10	14.6
	Triclopyr	(7.7)–9.2	(8.5)	(1.2)–1.4	P (1), N (1)	9.7–15	0.25–1	10	408
	Napropamide	(8.1)	(8.1)	(1.0)	N (1)	16	0.25–1	10	73
	Propyzamide	(5.6)	(5.6)	(1.1)	N (1)	11	0.25–1	10	15
Insecticide	Isoxathion	220–4,100	1,500	1.8–8.7	P (3)	110–210	0.0375–0.15	1.5	1.9
	Pyridaphenthion	(92)–980	630	(1.3)–3.6	P (2), N (1)	150–220	0.025–0.1	10	100
	Malathion	250–270	260	4.8–8.6	P (3)	15–26	0.25–1	10	145
	Fenitrothion (MEP)	100–310	170	3.4–4.5	P (4)	15–38	0.25–1	10	14
	Isoprocarb (MIPC)	(6.2)–410	120	(1.3)–8.9	P (4), N (1)	11–20	0.05–1	10	265
	Fenthion (MPP)	120	120	2.4	P (1)	35	0.1-0.4	4.2	4.2
	Isofenphos	60–120	81	1.7–2.1	P (3)	25–46	0.1–1	10	11
	Carbaryl (NAC)	68–88	78	1.5–1.7	P (2)	48–56	0.0625–0.25	10	120
	Mevinphos	22–120	72	1.9–4.1	P (4)	8.5–15	0.25–1	10	–
	Methidathion (DMTP)	(7.9)–170	51	(1.3)–4.1	P (2), N (2)	16–22	0.25–1	10	200
	Diazinon	38–60	49	1.8–2.8	P (2)	13–19	0.25–1	10	60
	Fenobcarb (BPMC)	48–50	49	1.7–2.1	P (2)	18–27	0.125–0.5	10	610

(*Continued*)

Table 2 | continued

Use type	Pesticide	Determined value (net rev μmol $^{-1}$)[a]	Average (net rev μmol $^{-1}$)[a]	MR value (–)[a]	Result[b]	Detection limit (net rev μmol $^{-1}$)	Dose (mg plate $^{-1}$)	Initial conc. (mg L $^{-1}$)	Water solubility[c] (mg L $^{-1}$)
	Disulfoton	24–91	46	1.5–3.1	P (4)	15–19	0.25–1	10	25
	Monocrotophos	32–35	34	1.8–2.4	P (2)	9.8–15	0.25–1	10	–
	Trichlorfon (DEP)	30–36	33	1.7–2.2	P (3)	12–18	0.25–1	10	120,000
	Phenthoate (PAP)	(10)–30	23	(1.3)–1.5	P (2), N (1)	21–23	0.25–1	10	10
	Methomyl	19–25	22	2.1–2.4	P (3)	7.1–7.5	0.25–1	10	57,900
	Dichlorvos (DDVP)	12–25	20	1.5–2.0	P (3)	9.7–12	0.25–1	10	18,000
	β-Endosulfan	(450)	(450)	(1.1)	N (1)	890	0.00625–0.025	0.25	0.33
	Buprofezin	(160–220)	(190)	(1.1–1.3)	N (3)	330–440	0.0125–0.05	0.5	0.9
	Acephate	(6.0)–21	(9.9)	(1.1)–1.5	P (1), N (3)	12	0.25–1	10	790,000
	Dimethoate	(4.7–7.1)	(5.9)	(1.3)	N (1)	9.2–14	0.25–1	10	23,800

P: A result is determined to be positive. N: A result is determined to be negative.
[a]Negative results are in parentheses.
[b]Number of samples or studies are in parentheses.
[c]Data taken from Summary and Individual Agricultural Chemicals Data Base for Revision of Water Quality Standard.

system determined by the initial concentrations of pesticides, the concentration factors and the dilution factors in the mutagenicity tests was equal to or greater than those of the MFP measuring tests). Therefore, for the pesticides in Group B, the detection of MFPs was not due to a difference in sensitivity between the tests. In conclusion, mutagens were formed by the chlorination of many pesticides.

The MFP of asulam, which had the highest MFP in this study, was 860 times greater than that of propyzamide, which had the lowest MFP (below the DL). The wide variation of the MFPs allows us to examine whether we can lower overall MFP in raw water by using low-MFP pesticides instead of high MFP-pesticides.

Since the tested pesticides have various chemical structures, they have a broad range of modes of action. For example, ferimzone, which had a high MFP, shows an effect on rice blast fungus, while edifenphos (EDDP) and tricyclazole, which had MFPs below the DL, also show the same effect. For another example, asulam, which had the highest MFP in the present study, can be used against annual weed species in Japanese lawn grass, while napropamide and propyzamide, which had MFPs below the DL, also have an effect on the same applicable crop and target weed. The environmental load (load in raw water) of mutagens from using a pesticide will depend on not only the MFP (specific activity) of the pesticide but also the quantity used, which is related to the effectiveness of the pesticide on insects or in weed control. Detailed discussions about this are difficult for the authors due to a lack of information on pesticide efficacy, but these examples suggest that the environmental load of mutagens from pesticide usage may be lowered by using low MFP-pesticides instead of high MFP-pesticides.

The chemical structures of pesticides which had significant MFPs were compared in order to see if there are common characteristics among them. The result was that many of the pesticides have a benzene ring in their chemical structures. In the case of chlorination with sodium hypochlorite solution, a chloronium ion (Cl^+) may be added to a nucleophilic site in a benzene ring, which is available for electrophilic addition, so chlorine substitutions may occur via the formation of a π-complex and a σ-complex. Therefore, pesticides which have a benzene ring are likely to cause chlorine substitutions, resulting in the probable formation of organochlorines. However, some of the

pesticides, whose benzene rings were substituted by chlorine before chlorination, were non-mutagenic in both the present study and the experiments described in CCRIS.

Heteroaromatic compounds and pesticides which have an aromatic ring that cannot be substituted by chlorine in their chemical structures are unlikely to cause an electrophilic addition. Unfortunately, some of them, such as diazinon and atrazine, still significantly formed mutagens. Non-aromatic compounds like trichlorfon and mevinphos also significantly formed mutagens.

Moreover, although the relationship between the use type of pesticides and their respective MFPs were studied, as well as the relationship between MFP and mode of action, no clear relationships were found. We could not predict the MFP of pesticides from their chemical structure, use types or mode of action. In conclusion, MFP measurements are indispensable in order to know whether pesticides are capable of forming mutagens through chlorination.

CONCLUSIONS

By measuring the mutagenicity and the MFP of seven fungicides, 15 herbicides and 22 insecticides, it was clear that many pesticides produced mutagens through chlorination conducted in the same manner as water purification processes, even though they were not mutagenic before chlorination. Some of the pesticides tested in the present study, however, did not produce mutagens through chlorination. These results indicate that we can lower the amount of mutagens in raw water by using low-MFP pesticides instead of high MFP-pesticides.

We were unable to use the chemical structures, use types or modes of action of pesticides to predict whether they were capable of forming mutagens. MFP measurements from testing are therefore indispensable in order to identify which pesticides are capable of forming mutagens through chlorination.

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