Detection of Fenitrothion and its Degradation Product 3-Methyl-4-nitrophenol in Water Environment

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ABSTRACT

The organophosphate pesticide fenitrothion and its degradation product 3-methyl-4-nitrophenol (3M4NP), which possesses greater mutagen formation potential (MFP) than fenitrothion, were monitored. A total of 109 samples were collected from 41 sites of 12 rivers in the Tokyo metropolitan area and 71 samples were collected from 4 sites of 2 streams close to paddy fields in Kanagawa, Japan. The concentrations of fenitrothion and 3M4NP were determined by GC/MS with selected ion monitoring after solid-phase extraction and derivatization of 3M4NP with N,O-bis-(trimethylsilyl)trifluoroacetamide (BSTFA). Both fenitrothion and 3M4NP were below 1 µg/L (0 – 0.083 µg/L of fenitrothion and 0 – 0.156 µg/L of 3M4NP) in the river water samples, whereas fenitrothion and 3M4NP reached 0.16 and 1.6 µg/L, respectively, in the stream water samples. Seasonal and areal fluctuations were also observed in the streams. Nevertheless, the maximum concentration of 3M4NP appeared in late May at all sites. Furthermore, among the samples in which fenitrothion and/or 3M4NP were detected, 3M4NP largely contributed to the potential in 56% and 67% of the river and stream samples, respectively. These results strongly support the need for simultaneous monitoring of various pesticides and their degradation products, identification of degradation products and their MFP, and characterization of their behavior in water purification processes.

Keywords: degradeate, environmental monitoring, fenitrothion, GC/MS, 3-methyl-4-nitrophenol, mutagen formation potential

INTRODUCTION

Fenitrothion (O, O-dimethyl O-(3-methyl-4-nitrophenyl) phosphorothioate), shown in Fig. 1, is a typical organophosphorus insecticide, and has been widely applied in agricultural use and household use as an alternative to persistent organochlorine pesticides and parathions with high mammalian toxicity. Fenitrothion affects many kinds of insects and crustaceans through inhibition of acetylcholinesterase, and is categorized as a “moderately hazardous compound, WHO class II” (Meaklim et al., 2003). Due to the rapid degradation of fenitrothion, the concentration of fenitrothion in water environment is generally low.

As also shown in Fig. 1, 3M4NP is a typical degradation product of fenitrothion, and shows significant toxicity such as inhibition of enzymes in DNA synthesis and cell division (Wright et al., 1982), estrogenic activity (Taneda et al., 2004b; Furuta et al., 2005; Kanaly et al., 2005), anti-androgenic activity (Taneda et al., 2004b; Li et al., 2006), and reproductive toxicity (Li et al., 2008). Therefore, 3M4NP has been brought
under the review of the OECD (Organisation for Economic Co-operation and Development) HPV (High Production Volume) Chemicals Programme due to its genotoxicity presuming carcinogenicity (OECD, 1994). Because the oxon form of fenitrothion more strongly inhibits acetylcholinesterase, 3M4NP often shows stronger toxicity than fenitrothion (Baarschers et al., 1980). In addition, 3M4NP also shows higher MFP than fenitrothion (Kishida et al., 2010b). The MFP is an index of mutagen formation in the process of chlorination in water purification plants. Therefore, it has become necessary to analyze the fate of fenitrothion and to monitor the presence of 3M4NP in water environment.

The environmental behavior of both fenitrothion and its degradation product 3M4NP has been reported as a prevalent issue for organophosphorus pesticides and their transformation products (Baarschers et al., 1983; Misra et al., 1993; Lacorte and Barceló, 1994; Oubina et al., 1996; Castillo et al., 1997; Kanaly et al., 2005; Zayed et al., 2008) in Canada, Spain, India, and Egypt. Although fenitrothion has been used in many countries such as Japan, Canada, and Australia, the environmental situation of fenitrothion and 3M4NP in Japan has rarely been reported.

This paper presents the results of the simultaneous monitoring of fenitrothion and 3M4NP in public water environment in Japan. The concentration level of both chemicals was compared among several dozens of water samples collected from public environmental monitoring sites assigned by local governments as well as those from paddy areas. In addition, the environmental impact shown as MFP, which was changed by the degradation of fenitrothion and the formation of 3M4NP, were analyzed.

**MATERIALS AND METHODS**

**Chemicals**

Fenitrothion (≥ 99%, traceable reference material) and 3M4NP (≥ 98%, for residue pesticide anal.), sodium chloride (≥ 99.5%, JIS extra-pure grade), acetone (≥ 99.5%, for residual pesticide and PCB anal.), acetone (≥ 99.5%, JIS extra-pure grade), hexane (≥ 96%, for residual pesticide and PCB anal.), and BSTFA (≥ 95%, for environmental anal.) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Pure water was obtained by purifying tap water using ion exchange and activated carbon adsorption columns.

**Sample Preparation**

River water sampling was conducted from spring to winter of 2011 in the Kanto area of
Japan. The location of the sampling sites is shown in Fig. 2. In total, 109 river water samples were collected from 41 sites of 12 rivers, although the sampling season of each site was not unified for reasons of voluntary provision of water samples and weather condition among others. All of these sites are assigned as environmental monitoring sites by local governments, and changes in detection frequency and concentration level were investigated at these sites. In addition, 71 samples were collected from 4 sites of 2 tributary streams in a paddy field area in Kanagawa Prefecture next to Tokyo, and the time changes of the concentration levels of fenitrothion and 3M4NP in the cropping season were investigated at these sites.

At the beginning of the solid-phase extraction (SPE) method, pre-conditioning of Sep-Pak® Plus PS-2 cartridge (Nihon Waters K.K., Japan) and Sep-Pak® Plus AC-2 cartridge (Nihon Waters K.K., Japan) was necessary. The cartridges were washed with 10 mL of acetone (JIS extra-pure grade, 10 mL/min × 1 min) and 100 mL of pure water (20 mL/min × 5 min).

The procedure of the SPE method used in this study is shown in Fig. 3. The collected water samples were filtrated using a 1-μm glass fiber filter, and 25 g/L of sodium...
chloride and 4 mL/L of 0.5M-H₃PO₄ buffer were added. A total of 1 L of the sample solution was supplied to the series of PS-2 and AC-2 cartridges at a flow rate of 15 mL/min × 67 min. After drying up by nitrogen gas purging, 10 mL of acetone (JIS extra-pure grade, 2 mL/min × 5 min) was supplied from the reverse direction to each cartridge. The extracted solutions were collected in a glass tube, and were concentrated by nitrogen gas purging, and then were adjusted to 1 mL with hexane. Internal standards, acenaphthene-d₁₀ for fenitrothion and phenanthrene-d₁₀ for 3M4NP, were added, and BSTFA was also added as a derivatization reagent. With this method, the water samples were concentrated up to 1,000 times. The recovery ratios using this method were investigated and good results were confirmed: 95% (CV = 2.5%; n = 3) for fenitrothion and 103% (CV = 3.4%, n = 3) for 3M4NP. All of the values satisfied the guideline of the Ministry of Environment, Japan (recovery ratio: 70 – 120% and CV: < 15%).

**GC-MS analysis for fenitrothion and 3M4NP**

Quantitative analysis was performed with a gas chromatograph electron impact ionization mass spectrometer (GC-EI-MS, QP-2000 plus, Shimadzu). The conditions for the analysis are summarized in Table 1.

**Estimation of mutagen formation potential based on fenitrothion and 3M4NP in water samples**

The MFP based on fenitrothion and 3M4NP in water samples were estimated from their measured concentrations and their MFP per weight, for which the mutagen test had been performed using *Salmonella typhimurium* TA100 strains without exogenous activation (S9) (Kishida *et al.*, 2010b). The MFP per weight (net rev./mg) of fenitrothion and 3M4NP is defined as the mutagenicity in an aqueous solution, which is chlorinated under the same conditions of chlorine dosage to carbon, pH, and reaction duration in water works, and is shown as a net number of revertant colonies compared with negative control (Takanashi *et al.*, 2001).

### Table 1 - Summary of GC-EI-MS conditions.

<table>
<thead>
<tr>
<th>Item</th>
<th>Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>J&amp;W DB-5ms (30m×0.25mm, 0.25µm)</td>
</tr>
<tr>
<td>column oven</td>
<td>50ºC (2 min) → 10ºC/min (12 min) → 170ºC (0 min) → 3ºC/min (10 min) → -200ºC (0 min) → 100ºC/min (10 min) → 300ºC (0 min)</td>
</tr>
<tr>
<td>Temperature</td>
<td>220ºC</td>
</tr>
<tr>
<td>vaporizing chamber</td>
<td>250ºC</td>
</tr>
<tr>
<td>interface</td>
<td>230ºC</td>
</tr>
<tr>
<td>ionization source</td>
<td></td>
</tr>
<tr>
<td>Injection mode</td>
<td>split less</td>
</tr>
<tr>
<td>Sampling period</td>
<td>2 min</td>
</tr>
<tr>
<td>Carrier gas</td>
<td>He</td>
</tr>
<tr>
<td>Linear velocity</td>
<td>40 cm/sec</td>
</tr>
<tr>
<td>Ionization method</td>
<td>EI</td>
</tr>
<tr>
<td>Mass scan</td>
<td>Fenitrothion m/z 125, 109, 217</td>
</tr>
<tr>
<td></td>
<td>3M4NP m/z 210, 73, 209</td>
</tr>
<tr>
<td>Injection volume</td>
<td>1 µL acetone solution</td>
</tr>
</tbody>
</table>
RESULTS AND DISCUSSION
Reliability and sensitivity of GC-EI-MS analysis
For the environmental monitoring of chemical contaminants, confirming the limit of
detection (LOD) and the limit of quantification (LOQ) is essential; therefore, the
reliability and sensitivity of GC-EI-MS analysis were first accurately investigated. The
spectra of GC-EI-MS analysis for fenitrothion and 3M4NP are shown in Fig. 4. The
instrumental LODi (mg/L-injection) and the instrumental LOQi (mg/L-injection) were
investigated, and they are shown in Table 2. The chromatographic peaks were very
sharp, and specific quantification ions could be observed for fenitrothion and 3M4NP,
respectively. The derivatization of 3M4NP with BSTFA was very beneficial for raising
the sensitivity 100-fold. Using the selected ion monitoring method, the LODi was
0.0015 mg/L for fenitrothion and 0.00003 mg/L for 3M4NP (+BSTFA). Therefore, it
was confirmed that accurate detection was available for fenitrothion in 1.5 ng/L river
water and 3M4NP in 0.03 ng/L river water, because the river water could be
concentrated up to 1,000 times with the SPE method. Calibration curves were made in
the concentration range of 0.01 mg/L to 5 mg/L for both fenitrothion and 3M4NP in this
study, and therefore, higher reliability of accurate quantification monitoring could be
achieved.

Detection at public environmental monitoring sites
The detection frequencies of fenitrothion and 3M4NP at 109 public environmental
monitoring sites assigned by local governments are summarized in Table 3. The total
percentage of sites where fenitrothion and/or 3M4NP were detected reached 57% (62

![Fig. 4 - Spectra of GC-EI-MS analysis for fenitrothion and 3M4NP.](image)

Table 2 - Instrumental LODi and LOQi for fenitrothion and 3M4NP.

<table>
<thead>
<tr>
<th>Mode</th>
<th>Limit</th>
<th>Fenitrothion</th>
<th>3M4NP</th>
<th>3M4NP + BSTFA</th>
</tr>
</thead>
<tbody>
<tr>
<td>TIC</td>
<td>LODi</td>
<td>0.01</td>
<td>0.15</td>
<td>0.0015</td>
</tr>
<tr>
<td></td>
<td>LOQi</td>
<td>0.03</td>
<td>0.5</td>
<td>0.005</td>
</tr>
<tr>
<td>SIM</td>
<td>LODi</td>
<td>0.0015</td>
<td>0.003</td>
<td>0.00003</td>
</tr>
<tr>
<td></td>
<td>LOQi</td>
<td>0.005</td>
<td>0.01</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

TIC: Total ion chromatogram
SIM: Selected ion monitoring
sites) in this study. Among these, the percentage of sites where the concentration of 3M4NP was equivalent or higher than that of fenitrothion was 45% (28 sites), and the percentage of sites where only 3M4NP was detected was 16% (10 sites). These results show that 3M4NP, a degradation product of fenitrothion, frequently exists in public water environments at higher concentrations than fenitrothion. The seasonal changes in the detection of fenitrothion and 3M4NP are summarized in Table 4 with Kanagawa Prefecture as example. Both fenitrothion and 3M4NP could be observed in all experimental seasons: June, October, and December. The percentage of sites where fenitrothion and/or 3M4NP were detected was higher in June than in October and December. Fenitrothion and 3M4NP were often detected together. Unfortunately, the reasons for this cannot be clarified in detail without reaction rate analysis and dynamic material flow analysis of the regions. Here, diesel exhaust particles (DEP) from rain and/or air have been reported as a source of 3M4NP (Taneda et al., 2004a; Ganranoo et al., 2010). In this study, a period when 3M4NP was not detected (< c. a. 3ng/L water sample) could be observed. Therefore, the effect of DEP was thought to be small in the sites selected in this study.

The concentration distribution of fenitrothion and 3M4NP at public environmental monitoring sites of local governments is shown in Fig. 5. In Kanagawa Prefecture, a concentration of more than 0.1 µg/L river water was often observed for both fenitrothion and 3M4NP, while almost all of the concentrations in Yamanashi and Chiba Prefectures were less than 0.1 µg/L river water. Meanwhile, several tens or more µg/L river water of fenitrothion and 3M4NP were detected in previous studies (Oubina et al., 1995; Kishida et al., 2010a; Abiru et al., 2011). In particular, all of the data for more than 0.1 µg/L river water were observed in June. It was considered that fenitrothion is widely used in the cropping season from spring to summer in Kanagawa Prefecture. Here, the European Union (EU) has set a maximum admissible concentration of 0.1 µg/L for individual pesticides and their related compounds in drinking water (EU, 1998), while the United States Food and Drug Agency (USFDA) has set a threshold value of

Table 3 - Detection frequency at environmental monitoring sites of local governments.

<table>
<thead>
<tr>
<th>Chemicals</th>
<th>Kanagawa</th>
<th>Yamanashi</th>
<th>Chiba</th>
<th>Saitama</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fenitrothion only</td>
<td>16</td>
<td>3</td>
<td>8</td>
<td>0</td>
<td>27</td>
</tr>
<tr>
<td>Fenitrothion &gt; 3M4NP</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Fenitrothion = 3M4NP</td>
<td>6</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td>Fenitrothion &lt; 3M4NP</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>3M4NP only</td>
<td>6</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>Sub-total</td>
<td>36</td>
<td>12</td>
<td>12</td>
<td>2</td>
<td>62</td>
</tr>
<tr>
<td>% of detected sites</td>
<td>51%</td>
<td>71%</td>
<td>60%</td>
<td>100%</td>
<td>57%</td>
</tr>
<tr>
<td>Not detected</td>
<td>34</td>
<td>5</td>
<td>8</td>
<td>0</td>
<td>47</td>
</tr>
</tbody>
</table>

Table 4 - Seasonal change in detection (Kanagawa Prefecture).

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Fenitrothion</td>
<td>61</td>
<td>(14/23)</td>
<td>25</td>
<td>(6/24)</td>
<td>39</td>
<td>(9/23)</td>
</tr>
<tr>
<td>3M4NP</td>
<td>57</td>
<td>(13/23)</td>
<td>13</td>
<td>(3/24)</td>
<td>13</td>
<td>(3/23)</td>
</tr>
<tr>
<td>Fenitrothion and/or 3M4NP</td>
<td>78</td>
<td>(18/23)</td>
<td>33</td>
<td>(8/24)</td>
<td>39</td>
<td>(9/23)</td>
</tr>
<tr>
<td>Not detected</td>
<td>22</td>
<td>(5/23)</td>
<td>67</td>
<td>(16/24)</td>
<td>61</td>
<td>(14/23)</td>
</tr>
</tbody>
</table>
0.75 µg/L for individual contaminants in drinking water. According to this case study, the environmental concentration level of 3M4NP cannot be ignored as a contaminant for a drinking water source in some cases.

Detection in paddy areas in the cropping season
The changes in concentration of fenitrothion and 3M4NP in paddy areas in the cropping season are shown in Fig. 6. The concentration often exceeded 0.1 µg/L in some cases, but the disappearance of both fenitrothion and 3M4NP was very rapid, within only a week. The rapid degradation of fenitrothion and/or 3M4NP has also been reported in many previous studies (Misra et al., 1993; Lacorte and Barceló, 1994; Oubina et al., 1996; Baroja et al., 2004), and the result of this present study conforms with them. It was, therefore, found that the concentration levels of fenitrothion and 3M4NP were strongly dependent on the site and timing of the sampling, and that the simultaneous monitoring of the pesticide and its degradation product is necessary for their exposure assessment.

Mutagen formation potential estimated from fenitrothion and 3M4NP
The MFP estimated from the concentrations of fenitrothion and 3M4NP in public water environment is shown in Fig. 7 with Yamanashi Prefecture as an example. The MFP based on 3M4NP was dominated by 3M4NP in most cases because the MFP per weight of 3M4NP was about 35 times stronger than that of fenitrothion (Kishida et al., 2010b). This result indicates that a degradation product may become a major contaminant if its toxic activity is much stronger and/or its concentration in water environments is much higher than that of its parent chemical. And it was shown that the simultaneous monitoring of pesticides and their degradation products is effective in assessing the risk of chemicals in environmental water.
Fig. 6 - Concentration of fenitrothion and 3M4NP in paddy areas in the cropping season.

Fig. 7 - Mutagen formation potential estimated from fenitrothion and 3M4NP. (Yamanashi Prefecture).
CONCLUSIONS
The environmental concentration of 3M4NP often exceeded that of fenitrothion in public water in Japan, and the level cannot be ignored as a contaminant for a drinking water source in some cases. In the cropping season in a paddy field, the concentration often becomes higher, but the disappearance of fenitrothion and 3M4NP is very rapid, within only a week. The MFP in public environmental water was dominated by 3M4NP in most cases because of the stronger specific mutagen activity of 3M4NP than that of fenitrothion. From the results of this case study, it was shown that the simultaneous monitoring of a pesticide and its degradation products is effective in assessing the risk of chemicals in environmental water.

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REFERENCES


