Photodegradation kinetics of fenitrothion in various aqueous media and its effect on steroid hormones biosynthesis

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The photodegradation kinetics of fenitrothion in various water media were examined under both direct and indirect photolysis with respect to degradation rate, half-life, and phototransformation kinetics of fenitrothion. The effect of fenitrothion and its photoproducts on steroid hormone biosynthesis was also investigated. The results indicate that the degradation rate of fenitrothion under indirect photolysis to which nitrate was added was faster than that of direct photolysis, in both pure and natural water. The fenitrothion half-lives under indirect photolysis were 2.67 and 4.58 h, while under direct photolysis the half-lives were 3.58 and 5.66 h for natural and pure water, respectively. The phototransformation kinetics of fenitrothion in pure water showed that the identified photoproducts, such as fenitrooxon and 3-methyl-4-nitrophenol, under both direct and indirect photolysis were almost the same. This is evidence that there is no specific degradation pathway with hydroxyl radicals under indirect photolysis in fenitrothion transformation. The 1 µM~50 µM levels of fenitrothion and two of its photoproducts (fenitrooxon and 3-methyl-4-nitrophenol) altered the steroid hormones biosynthesis in bovine adrenal cultured cells, which suggests that both fenitrothion and its two photoproducts may be endocrine-disrupting compounds.

Keywords: fenitrothion, steroid hormone, photolysis, phototransformation, biosynthesis

INTRODUCTION

Recent decades have witnessed increases in the levels of contamination of water with toxic organic compounds. Among those highly toxic compounds dissolved in water are pesticides, which, through their extensive use, have become increasingly present in water. Consequently, the pesticide pollution of environmental waters is a pervasive problem with widespread ecological consequences (Koplin et al., 1996). Thus pesticides removal from the aquatic environment has become a high priority (Penuelas and Barcelo, 1998). Photolysis is one of the major transformation processes affecting the fate of pesticides in aquatic environments (Durand et al., 1992). Pesticides undergo transformation by indirect photolysis via dissolved matters in various bodies of water. This process is responsible for the disappearance and the enhanced photooxidation rates of organic compounds through the production of photoreactants such as hydroxyl radicals (Mansour et al., 1999). Therefore, photodegradation studies of pesticides allow for the modeling of pesticide behavior after application, which in return, helps in the understanding of the kinetics of pesticides degradation, increasing our knowledge about the degradation products that can form under natural conditions, and finally enabling us to assess their risk (Penuelas and Barcelo, 1996).

Nitrate was regarded for many decades as an ion, which had only a biological role in aquatic systems (Brezonik and Fulkerson-Brekken, 1998). Recent evidence indicates that the nitrate ion also promotes the photochemical oxidation of trace organic compounds in water. As reported by Zepp et al. (1987) and Torrents et al. (1997), the photolysis of nitrate, which is present in natural water as a natural component, produces hydroxyl radicals. Although hydroxyl radicals can be produced by several mechanisms, including the photolysis of hydrogen peroxide, ozone, nitrite, and dissolved organic matters, several studies have pointed to nitrate as the key source of the hydroxyl radicals in natural waters (Brezonik, 1994).

In Japan, fenitrothion is a commonly-used insecticide for various purposes, mainly for the elimination of rice stem bores in rice plants, the main crop in Japan. Thus, fenitrothion is considered to be a common river water pollutant, detected with concentrations in river water all over Japan (Numabe et al., 1992; Okumura and
Nishikawa, 1995; Itagaki et al., 2000; Kondoh et al., 2001; Tanabe et al., 2001; Sudo et al., 2002). Furthermore, fenitrothion is known for its acute toxicity toward nontarget organisms and, after application, different types of degradations affect this pesticide, such as photolysis, hydrolysis and biological degradation (Lacorte and Barcelo, 1994). In addition, for fenitrothion, many of the phototransformation products, such as fenitoxxon and 3-methyl-4-nitrophenol, may be even more toxic than the parent compound (Eto, 1974; Amoros et al., 2000). Therefore, it becomes necessary to monitor the behavior as well as the presence of fenitrothion and its photoproducts in the environment (Lacorte and Barcelo, 1994). In spite of the many degradation studies carried out using various types of water examining the photolysis and photocatalytic degradation of fenitrothion (Greenhalgh and Marshall, 1976; Mikami et al., 1985; Durand et al., 1992, 1994; Kerzhentsev et al., 1996), the concentrations used in many cases were so high (mg/l level) that the behavior of fenitrothion may not adequately reflect what happens in real environmental situations. Furthermore, the differences in direct versus indirect photolysis using nitrate, known to be a natural component of environmental water, have not been studied for fenitrothion. In this respect, there is lack of comparative degradation studies in water for fenitrothion.

Steroid hormones are involved in primary sex determination and neonatal development (Vom-Saal et al., 1992) as well as in the acquisition and maintenance of secondary sex characteristics in adults (Grumbach and Conte, 1981). On the other hand, the presence of chemicals such as pesticides that have sex hormone activities, and thus the ability to disrupt the endocrine system, is a source of concern (Kelce et al., 1998). Antiandrogenic pesticides, such as organophosphates, can disrupt male sexual differentiation by several mechanisms, including the antagonism of receptor binding, or by an inhibition of the production, transport, and/or metabolism of androgens (Leblanc et al., 1997).

Fenitrothion was found to act as an antiandrogen under both in vitro and in vivo assays and competitively antagonized human androgen receptors (Tamura et al., 2001; Turner et al., 2002). On the other hand, some organophosphate pesticides have been shown to alter normal endocrine function by inhibiting steroid hormones biosynthesis such as dichlorvos, dursban, dazinon and chlorpyrifos (Civen and Brown, 1974; Civen et al., 1977). However, the effects of fenitrothion as an organophosphate pesticide and its photoproducts on steroid hormones biosynthesis have not been previously reported.

On the basis of the previous information, we attempted in this study to compare the photodegradation kinetics of fenitrothion in various aqueous media under both direct and indirect photolysis, with respect to degradation rate, half-life time, and phototransformation kinetics of fenitrothion, and finally, to study the effects of fenitrothion and two of its most toxic metabolites on steroid hormones biosynthesis.

**Materials and Methods**

**Chemicals**

Fenitrothion and fenitoxxon with purities of 98.5 and 98.3% respectively were obtained from Kanto Chemicals Company, Japan, while a 3-methyl-4-nitrophenol standard was obtained from Tokyo Kasei Company, Japan. The standards of 3-methyl-4-nitroanisole and phosphoric acid O, O, O tri-methylene were obtained from Aldrich Chemical Company, U.S.A. Pesticide quality solvents of methanol, ethanol, acetone, chloroform, 2-propanol, acetonitrile, and dichloromethane were obtained from Nacalai Tesque, Inc, Japan. The stock solution of fenitrothion (100 ppm) was prepared by making the appropriate dilution in methanol (photochemically inert organic solvent) and stored in a refrigerator at 4°C. Working standard solutions of fenitrothion were prepared by making appropriate dilutions in Milli-Q water and storing in a refrigerator at 4°C. A nitrate stock solution (10 mM) was prepared by making appropriate dilutions in MilliQ water.

**Photodegradation experiments**

In the photodegradation experiments, a solar simulator (Oriel, Model 81160-1000) unit equipped with a 300 W Xenon lamp (ozone free, Oriel Model 81160) and special glass filters restricting the transmission of wavelengths below 300 nm was used. This Xenon lamp has been demonstrated to be equivalent to natural sunlight for conducting aqueous photolysis studies for several pesticide compounds. The wavelength range varies from 300 to 800 nm, which represents radiation very close to natural sunlight (Durand et al., 1991). As fenitrothion has

- **Table 1. Chemical composition of the Kurose River water collected for photodegradation experiments**

<table>
<thead>
<tr>
<th>Analytical item</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO₃⁻</td>
<td>201 μM</td>
</tr>
<tr>
<td>NO₂⁻</td>
<td>12 μM</td>
</tr>
<tr>
<td>SO₄²⁻</td>
<td>260 μM</td>
</tr>
<tr>
<td>DOC</td>
<td>5.47 mg Cl⁻</td>
</tr>
<tr>
<td>pH</td>
<td>7.28</td>
</tr>
<tr>
<td>Conductivity</td>
<td>136 μS</td>
</tr>
</tbody>
</table>

DOC = Dissolved organic carbon.
a limited solubility in water (14 mg/l), a water-methanol solution was employed (Durand et al., 1992). River water was collected from the Kurose River at the Hinotsume site (Derbalah et al., 2003), for which the chemical composition is given in Table 1. The water samples were filtered through a glass fiber filter (GC-50, diameter: 47 mm; pore size: 0.5 μm, Advantec) before they were used. Both the filtered river and the MilliQ water samples were spiked with fenitrothion at a concentration of 0.5 mg/l. In the case of indirect photolysis, nitrate was added to both the river and the MilliQ water samples at a level of 200 μM. This nitrate concentration was selected to be similar to that found in the collected river water samples. The irradiation of the samples was carried out in a quartz glass cell (60 ml) containing the desired amount of fenitrothion. During the irradiation, the solution in the quartz cell was well mixed with a stirring bar and the temperature was kept at 20°C. The solution from the irradiated samples was removed at regular intervals for HPLC analysis. In order to identify the photoproducts of fenitrothion, pure water samples were spiked at 0.5 and 0.01 mg/l levels of fenitrothion and irradiated for 7 hours in the same quartz glass cell under both direct and indirect photolysis, then subjected to solid phase extraction followed by GC-MS analysis. The irradiation time for photoproducts identification was selected to be 7 h because within this time about 70% of fenitrothion was degraded under direct photolysis when 0.5 mg/l of fenitrothion was irradiated (Fig. 1).

For the purpose of monitoring and estimating the concentration level of fenitrothion and its photoproducts in river water under natural conditions, river water samples were collected weekly for one month (February, 2003) from the Kurose River at the Hinotsume site, the most contaminated area with pesticides (Derbalah et al., 2003). Samples were filtered through a glass fiber filter (GC-50, diameter: 47 mm; pore size: 0.5 μm, Advantec) and then extracted using a solid phase extractor followed by GC-MS analysis. The extraction and analytical method for these samples were the same as that used in the case of the irradiated samples.

**HPLC system**

The irradiated samples were analyzed directly by the HPLC system, which consists of a pump (LC-10Ai, Shimadzu), a sample injector (Rheodyne Model 1296, sample size 50 μl) and a UV-VIS detector (SPD-10A, Shimadzu). The column was an Ultron VX-ODS (Supelcosil LC-18, particle size 5 μm; Supelco) 250 mm × 4.6 mm I.D. A guard column (Supelcosil LC-18, 5 μm, 10 mm × 4.6 mm I.D.) was fitted in the front of the analytical column. A mixture of acetonitrile (HPLC grade) and MilliQ water (60:40) was used as the mobile phase under the isocratic elution mode. The flow rate was maintained at 1.0 ml/min. The UV detector wavelength was 220 nm for the fenitrothion (Kiso et al., 1996) and 260 nm for 2-nitrobenzaldehyde.

**Determination of kinetics parameters**

In order to determine the degradation kinetics, plots of \( \ln(\text{concentration}) \) against irradiation time were made. The degradation rate constant (slope), \( k \), was calculated from the first order equation: \( C_t = C_0 e^{-kt} \) where \( C_t \) represents the concentration of the pesticide at time \( t \), \( C_0 \) represents the initial concentration, and \( k \) is the degradation rate constant. When the concentration falls to 50% of its initial amount, the half-life (\( t_{1/2} \)) can be determined by \( t_{1/2} = \frac{0.693}{k} \), according to the method described by Daley and O’Malley (1974).

As the power of the solar simulator slightly changed by time of day, the results of the present study were normalized to a clear sky, noon conditions of Higashi-Hiroshima on the 1st of May. We note that the relationship between fenitrothion degradation rate and irradiation time was linear (first order reaction). The normalization was carried out by the determination of the degradation rate constant of 2-nitrobenzaldehyde under laboratory conditions. Then, the ratio between the degradation rates of the 2-nitrobenzaldehyde under field conditions (under a clear sky, noon conditions of Higashi-Hiroshima on 1st of May) calculated by Arakaki et al. (1998) and under laboratory conditions, was used to normalize the results of the present study.

**Extraction method of fenitrothion photoproducts**

To identify the fenitrothion photoproducts, an automated SPE apparatus was used to extract the water samples after 7 hours of irradiation. The cartridges (OASIS Waters purchased from waters, Milford, MA, USA) were washed sequentially with 5 ml of dichloromethane, 5 ml of methanol and 5 ml of MilliQ water. The irradiated water samples were passed through the cartridges at

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**Fig. 1.** Percentage of remaining fenitrothion under direct photolysis as function of irradiation time.
a flow rate of about 10 ml/min. Air vacuum was employed to eliminate the water residues from the cartridges. Elution of the target compounds was carried out using 5 ml of dichloromethane. Evaporation of the solvent was performed under a stream of nitrogen until almost dryness, then redissolved in 1 ml acetone and reconcentrated to 100 µl. Then, 5 µl of the solution was analyzed by GC-MS. The concentrations of fenitrothion, fenitrooxon, phosphoric acid O, O, O tri-methylester, 3-methyl-4-nitrophenol, and 3-methyl-4-nitroanisol were measured by GC-MS under the SIM mode using their authentic standards.

The recovery test of fenitrothion and its standard photoproducts were conducted in MilliQ water at a spike level of 1 µg/l for fenitrothion, fenitrooxon 3-methyl-4-nitrophenol, 3-methyl-4-nitroanisol, and phosphoric acid O, O, O tri-methylester. Then, the spiked water samples were extracted and GC-MS analysis was carried out, as detailed above. The samples were replicated and the analysis was carried out five times.

**GC-MS analysis**

The samples were analyzed with a Hewlett-Packard HP 6890 series gas chromatograph equipped with an HP 6890 mass selective detector. An Agilent 6890 series injector was used for the injection of samples. The column was a refused silica capillary HP-5MS chromatographic column (30 m length × 0.25 mm I.D., film thickness 0.25 µm). The oven temperature was programmed to hold for 2 min at 60°C, then increase to 280°C at the rate of 8°C min⁻¹, and hold at 280°C for 8 min. The injector and transfer line temperatures were 250 and 280°C, respectively. The carrier gas (helium) was used at a constant flow rate of 1 ml/min. The standard and extracted solutions were injected in the splitless mode, according to the method described by Durand et al. (1994) with some modifications.

**Toxicological experiments**

**Cell culture** Zonae fasciculate-reticularis cells were isolated from fresh adrenals of Holstein-Friesian cows and cultured to be confluent in 12 well culture plates, as described previously by Yamazaki et al. (1992). The steroid hormones in the medium were extracted with chloroform. Before extraction, 0.2-nmol spironolactone was added to the medium as an internal standard to compensate for the extraction efficiency. The extracted steroids were separated with a silica gel column (Cosmosil 5SL, Nacalai Tesque, Tokyo, 4 mm diameter × 150 mm) with a mobile phase of hexane, which contained 0.1% of acetic acid and 7% of 2-propanol at 0–10 min, then with a linear gradient of 7 to 13% of 2-propanol at 10–17 min, followed by 13% at 17–35 min at a flow rate of 0.6 ml/min. Under these conditions, androstenedione, 17α-hydroxyprogesterone, deoxycorticosterone, deoxy cortisol, cortisol were eluted from the column at 7.5, 8.5, 12, 15, and 30 min, respectively. The amount of each steroid was estimated using the ratio of its peak area and that of the internal standard (spironolactone), which was eluted at 18 min (Kominami et al., 1989). In this experiment, the amounts of pregnenolone, 17α-hydroxyprogrenolone, and dehydroepiandrosterone were not analyzed, as these steroids quickly become converted to another UV-absorbed steroids by the intense activity of 3-beta-hydroxy-5-ene steroid dehydrogenase (3β-HSD) (Yamazaki et al., 1992).

**RESULTS AND DISCUSSION**

**Degradation kinetics in MilliQ water**

The photodegradation kinetics of fenitrothion at a concentration level of 0.5 mg/l in pure water-methanol solution under both direct and indirect photolysis was investigated by monitoring the loss in fenitrothion concentration with irradiation time using HPLC analysis. Samples were withdrawn at 0, 2, 4, 6, 8, and 10 hours after starting the irradiation. The results in Table 2 show that the photodegradation rate of fenitrothion in pure water under direct photolysis (0.12 h⁻¹) (n = 5) was slower than that of indirect photolysis (0.15 h⁻¹), which subsequently resulted in a shorter half-life of fenitrothion under indirect photolysis (4.58 h) than direct photolysis (5.66 h). In light of these results, fenitrothion was more rapidly photodegraded in pure water under indirect photolysis
Degradation kinetics of fenitrothion 205

compared to direct photolysis. This result consistently with Torrents et al. (1997) reported that in the presence of the nitrate, where the hydroxyl radical was readily formed, a faster degradation of atrazine was observed compared to direct photolysis in distilled water. Therefore, the differences in the degradation rate constant and half-lives of fenitrothion between the two aqueous media are attributed mainly to the presence of nitrate under indirect photolysis, which accelerates the degradation rate of fenitrothion compared with direct photolysis.

Degradation kinetics in natural water

The photodegradation kinetics of fenitrothion was investigated under river water condition, either with or without adding nitrate. The degradation rate constant of fenitrothion in the river water spiked with nitrate (0.26 h\(^{-1}\)) was faster than that of river water only (0.19 h\(^{-1}\)) (n = 5). Subsequently, the half-life of fenitrothion in river water spiked with nitrate (2.67 h) was shorter than that of river water alone (3.58 h) (Table 2). The differences in the degradation rate and half-lives of fenitrothion between the two aqueous media were mainly due to the presence of higher concentrations of nitrate in the spiked river water than in the river water only. Hence, the hydroxyl radicals increased proportional to the nitrate concentration (Zepp et al., 1987), resulting in an acceleration of the photodegradation rate of the fenitrothion.

Generally, according to the results of fenitrothion photodegradation in both pure and natural waters, we can find that fenitrothion was more rapidly photodegraded in natural water than pure water under direct photolysis. This finding is consistent with that previously reported for the two types of water containing fenitrothion (Lartiges and Garrigues, 1995), diazinon (Mansour et al., 1999), and thiobencarb (Vialaton and Richard, 2002). The differences in the degradation rate constant and half-lives between the two types of water are attributed firstly to the presence of nitrate as a natural component in river water that accelerates the photodegradation rate of fenitrothion by generating hydroxyl radicals (Zafiriou and True, 1979). Secondly, due to the presence of dissolved organic matter in the aqueous media of river water, which is responsible for generating photoreactants such as hydrogen peroxide through photochemical oxidation (Zepp et al., 1987; Cooper et al., 1988; Lean et al., 1994), hydrogen peroxide is known as a strong oxidant for a variety of chemical species (Cooper et al., 1994). Thirdly, due to the presence of nitrite in the river water that was considered also as a source of hydroxyl radicals via photolysis, the photodegradation rate of fenitrothion was subsequently

| Number of experiments for each sample, n = 5. SD = Standard deviation. |
|-----------------|---------------------|-----------------|

Table 2. Degradation rate constant and half-lives of fenitrothion in natural and pure waters with and without adding nitrate

<table>
<thead>
<tr>
<th>Type of sample</th>
<th>Degradation rate constant (h(^{-1}))</th>
<th>Half-life (t_{1/2}) (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MilliQ water</td>
<td>0.12 (SD = 0.007)</td>
<td>5.66 (SD = 0.37)</td>
</tr>
<tr>
<td>MilliQ water + 200 µM nitrate</td>
<td>0.15 (SD = 0.01)</td>
<td>4.58 (SD = 0.23)</td>
</tr>
<tr>
<td>River water</td>
<td>0.19 (SD = 0.01)</td>
<td>3.58 (SD = 0.28)</td>
</tr>
<tr>
<td>River water + 200 µM nitrate</td>
<td>0.26 (SD = 0.02)</td>
<td>2.67 (SD = 0.16)</td>
</tr>
</tbody>
</table>

Table 3. Name, retention time and GC-MS characteristic ions for the identified photoproducts of fenitrothion under direct and indirect photolysis

<table>
<thead>
<tr>
<th>Compound name</th>
<th>Retention time (min)</th>
<th>m/z (relative intensity %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1- Fenitrothion</td>
<td>22.4</td>
<td>277 (100), 125 (100), 109 (96), 260 (86)</td>
</tr>
<tr>
<td>2- Fenitrooxon</td>
<td>21.8</td>
<td>244 (100), 109 (100), 261 (20), 79 (14)</td>
</tr>
<tr>
<td>3- 3-Methyl-4-nitrophenol</td>
<td>17.4</td>
<td>136 (100), 77 (86), 153 (24), 53 (19)</td>
</tr>
<tr>
<td>4- 3-Methyl-4-nitroanisol</td>
<td>15.5</td>
<td>150 (100), 167 (55), 77 (39), 91 (34)</td>
</tr>
<tr>
<td>5- O,O-S-trimethyl phosphorothioate</td>
<td>8.8</td>
<td>110 (100), 79 (82), 109 (77), 156 (65)</td>
</tr>
<tr>
<td>6- Methylyparathion</td>
<td>21</td>
<td>109 (100), 125 (94), 263 (81)</td>
</tr>
<tr>
<td>7- Carbomethoxyfenitrothion</td>
<td>25.4</td>
<td>125 (100), 321 (79), 109 (74), 79 (30)</td>
</tr>
<tr>
<td>8- 5-methylfenitrothion</td>
<td>24.3</td>
<td>125 (100), 260 (100), 277 (86), 79 (55)</td>
</tr>
<tr>
<td>9- Denitrofenitrothion</td>
<td>17.2</td>
<td>232 (100), 109 (71), 93 (27), 125 (16)</td>
</tr>
<tr>
<td>10- Formylfenitrothion</td>
<td>24</td>
<td>125 (100), 109 (20), 217 (15)</td>
</tr>
<tr>
<td>11- Phosphoric acid O,O,O tri-methylester</td>
<td>6.7</td>
<td>110 (100), 79 (35), 100 (32), 140 (19)</td>
</tr>
<tr>
<td>12- Phenol 3-methyl</td>
<td>8.2</td>
<td>107 (100), 108 (95), 79 (30)</td>
</tr>
</tbody>
</table>
accelerated (Brezonik and Fulkerson-Brekken, 1998). Moreover, due to the presence of ferric ion as natural component in river water, which is responsible to generate hydroxyl radicals through the excitation Fe(OH)$^{2+}$ complex, the predominant species of ferric ion for generating hydroxyl radicals (Larson et al., 1991) beside the photolysis of Fe(III) itself generates directly hydroxyl radicals (Sun and Pignatello, 1993).

Therefore, it is clear that the photodegradation of fenitrothion as an organophosphate pesticide is greatly influenced by indirect photolysis, and this is consistent with outcomes reported by Lartiges and Garrigues (1995). Also, the degradation rates of other organophosphate pesticides are influenced by indirect photolysis (Mansour et al., 1999).

Phototransformation kinetics of fenitrothion

Qualitative information The identified photoproducts of fenitrothion that were obtained after 7 h of irradiation followed by a solid phase extraction and GC-MS analysis at a concentration level of 0.5 mg/l are listed in Table 3. The identification of the fenitrothion photoproducts was made by comparison with the available authentic standards (compounds 2–4), with the mass spectra reported in the literature and a GC-MS library search (compounds 6–11) and if no matching spectrum was found, by using a GC-MS library only (compounds 12). The spectra of the most significant ions and the chemical structures of all identified compounds under both direct and indirect photolysis are shown in Figs. 2 and 3, respectively. The identified photoproducts under both direct and indirect pho-
Degradation kinetics of fenitrothion 207 tolysis were the same except for phosphoric acid O, O, O tri-methylester, which was identified only under direct photolysis. According to the results shown in Table 3, Figs. 2, and 3, the identified compounds were as follows:

Compound (1), with a molecular weight of 277, was identified as fenitrothion, with an OH loss at m/z value of 260 and the characteristic ions of the phosphorothioate moiety at m/z values of 109 and 125 were identified (Greenhalgh and Marshall, 1976; Durand et al., 1992, 1994).

Compound (2), with a molecular weight of 261, was identified as fenitrooxon using an authentic standard library search and by it having a shorter retention time than fenitrothion (21.8 and 22.4 min, respectively). It gives ions at m/z values of 109, 244, and 261; the characteristic ion of fenitrooxon is 109, with no formation of 125, and no S is present in the molecule. Also, a significant amount of ion with an m/z value of 244 formed, corresponding to the loss of the OH, with a similar explanation as for fenitrothion, and it was chosen as the specific ion of fenitrooxon (Durand et al., 1992; Lacorte and Barcelo, 1994).

Compound (3), with a molecular weight of 153, was identified as 3-methyl-4-nitrophenol using a library search and an authentic standard. This compound had been previously identified as one of the fenitrothion photoproducts (Greenhalgh and Marshall, 1976; Durand et al., 1992, 1994); it gives an EI spectrum with characteristic ions at m/z values of 136, 153, and 77.

Compound (4), with a molecular weight of 167, was identified as 3-methyl-4-nitroanisol using a library search and an authentic standard. It gives an EI spectrum with characteristic ions at m/z values of 150 and 167 (Kerzhentsev et al., 1996).

Compound (5), with a molecular weight of 156, was identified as O, O, S-tri-methyl phosphorothioate using a library search and its mass spectrum reported in the literature. It gives an EI spectrum with m/z values of 110, 109, 79, and 156 characteristic ions. It is important to note that the methyl group does not arise from the solvent or analytical protocol as it arises from the use of the two molecules of fenitrothion (Durand et al., 1992).

Compound (6), with a molecular weight of 263, was identified as phosphorothioic acid O, O-dimethyl-O-(4-nitrophenyl)ester, better known as methyl-parathion by a library search and the mass spectrum reported in the literature (Durand et al., 1992). It gives an EI spectrum with m/z values at 125 and 109, which correspond to the phosphorothioate moiety as well as the characteristic ion at m/z value of 263 (Durand et al., 1992).

Compound (7), with a molecular weight of 321, was identified as carbomethoxyfenitrothion by its EI spectrum (Greenhalgh and Marshall, 1976; Durand et al., 1994). Besides the typical ions corresponding to the phosphorothioate moiety at m/z values of 125, 79, and 109, the EI spectrum of this compound showed a parent ion at an m/z value of 321 with two characteristic ions at m/z values of 151 and 181 being obtained. The absence of the M-17 (260) ion indicated that a change had occurred in the aryl methyl position (Greenhalgh and Marshall, 1976).

Compound (8), was identified as s-methylfenitrothion, which had the same molecular weight as its parent compound. Both structures exhibited the (CH₃S)-(CH₃O)P(O) moiety, which correspond to the same molecular weight as fenitrothion structures. Its identification was achieved based on the following evidence: (I) the lack of (109) ion in the isomer since the structure (CH₃O)₂PO (dimethoxyphosphate) does not exit in the isomer. (II) S-methylfenitrothion exhibited a longer retention time than

Fig. 2. (continued).
fenitrothion (24.3 and 22.4 min, respectively).

Compound (9), with a molecular weight of 232, was identified as denitrofenitrothion, the EI spectra of this compound showed a parent ion at m/z value of 232 and a base peak at m/z value of 109. Its EI spectrum exhibited an abundance of m/z 125, a value much lower than other ions at m/z values of 232, 109, and 93 (Greenhalgh and Marshall, 1976; Durand et al., 1992, 1994).

Compound (10), was identified as formylfenitrothion with typical ions at m/z values of 125, 79, and 109 corresponding to the phosphorothioate moiety. Other important ions were at m/z values of 217 and 261, which correspond to (M-NO₂-CO)+ and (M-NO). This EI spectrum partially agreed with that of Greenhalgh and Marshall (1976) and Durand et al. (1994).

Compound (11), with a molecular weight of 140, was identified as phosphoric acid O, O, O tri-methylester using an authentic standard and a library search. It gives an EI spectrum with m/z values of 110, 109, and 140. The ion at m/z value of 140 is considered to be the characteristic ion of this compound (Durand et al., 1992).

Compound (12), with a molecular weight of 108, was identified as 3-methylphenol using a library search of GC-MS; it gives an EI spectrum with m/z values of 108 and 107 for its characteristic ions. This compound has not been previously reported and considered to be a new photoproduct of fenitrothion that was possibly formed by the denitration of 3-methyl-4-nitrophenol.

An additional photodegradation experiment was carried out to investigate the phototransformation kinetics of fenitrothion at low concentration level (0.01 mg/l), more close to the environmental level. Under indirect photolysis, the identified photoproducts of fenitrothion at this low concentration level were the same as those identified at the high concentration level of fenitrothion (0.5 mg/l) under the same system. However, direct photolysis at this low concentration level of fenitrothion (0.01 mg/l) showed a disappearance of the following photoproducts; methyl parathion, carbomethoxyfenitrothion, denitrofenitrothion, 3-methyl-4-nitroanisol, formylfenitrothion and O, O, S tri-methyl phosphorothioate compared with direct photolysis that was carried out at a high concentration level of 0.5 mg/l. The disappearance of these photoproducts may be due to

Fig. 3. Tentative photodegradation scheme of fenitrothion in a water-methanol solution. Lables indicated for each compound correspond to compounds listed in Table 3.
the low concentration used and the slow degradation of fenitrothion under direct photolysis (Durand et al., 1991). Moreover, the disappearance of some photoproducts at this low concentration level of fenitrothion (0.01 mg/l) under direct photolysis compared with indirect photolysis at the same concentration level reflects the role of nitrate in enhancing the degradation rate of fenitrothion, resulting in the ability to identify these photoproducts.

Tentative photodegradation pathway of fenitrothion was assumed according to the identified photoproducts under both direct and indirect photolysis as shown in Fig. 3. Fenitrooxon and s-methylfenitrothion seem to be the primary products of fenitrothion, fenitrooxon was formed by substitution of sulfur by oxygen in the P=S bond, through the oxidation, while the s-methylfenitrothion is formed by isomerization, which is induced by heat (Durand et al., 1994; Lacorte and Barcelo, 1994; Castillo et al., 1997). Formylfenitrothion was formed through the oxidation of hydroxymethyl-fenitrothion, which results from the oxidation of the aryl methyl group of fenitrothion. However, hydroxymethyl-fenitrothion was not detected in this study because it is photolytically unstable and rapidly oxidized to formylfenitrothion (Greenhalgh and Marshall, 1976). Carbomethoxyfenitrothion was detected in the fenitrothion irradiation resulting from the solvolysis of the intermediate carboxyfenitrothion that formed on oxidation of the aryl methyl group of fenitrothion (Greenhalgh and Marshall, 1976). Through denitration, fenitrothion was converted to denitrofenitrothion (Greenhalgh and Marshall, 1976; Durand et al., 1994) and 3-methyl-4-nitrophenol to 3-methylphenol. Although the formation of 3-methylphenol as a fenitrothion photoproduct was not previously reported, it has been pointed out that the denitration of the NO₂ group occurs for fenitrothion photolysis, the same as in the formation of denitrofenitrothion (Greenhalgh and Marshall, 1976; Durand et al., 1992, 1994). The formation of 3-methyl-4-nitrophenol, 3-methyl-4-nitroanisole, phosphoric acid O, O, O tri-methylester, and O, O, S-tri-methyl phosphorothioate resulted from the splitting of the phosphorus-phenoxy bond through chemical hydrolysis (with or without eventual re-methylation due to the presence of methanol as a co-solvent) either with or without the removal of the nitro group (Durand et al., 1994; Kerzhentsev et al., 1996). Moreover, the formation of 3-methyl-4-nitroanisole, and O, O, S-tri-methyl phosphorothioate implies the transfer of the methyl or methoxy groups (Kerzhentsev et al., 1996).

The previous reactions illustrate that oxidation, isomerization, chemical hydrolysis, dealkylation, and denitration are considered to be the degradation routes of fenitrothion in water (Durand et al., 1992, 1994; Lacorte and Barcelo, 1994; Kerzhentsev et al., 1996; Castillo et al., 1997). These chemical processes were identically induced under both direct and indirect photolysis, which reveal no specific attachment of the hydroxyl radical generated through the photolysis of nitrate under indirect photolysis in fenitrothion phototransformation. This finding is consistent with Vialaton and Richard (2002), who reported that the hydroxyl radicals showed no selectivity in its reactivity in the transformation of the 4-chloro-2-methylphenol and nitrobenzene. Furthermore, Prevot et al. (1999) also reported that the main photoproducts of chloramben identified under direct photolysis were the same to those identified under indirect photolysis upon the addition of TiO₂.

The present work reflects more on what can happen in samples similar to real environmental samples, since the photolysis was conducted in water spiked with nitrate instead of only organic solvent or pure water, as reported previously by Greenhalgh and Marshall (1976), Mikami et al. (1985) and Durand et al. (1992, 1994). Moreover, the photodegradation was conducted using a very low concentration of fenitrothion (0.01 mg/l), so in this way, the behavior of fenitrothion reflects more closely the degradation kinetics in the real environmental situation compared to previous studies (Greenhalgh and Marshall, 1976; Mikami et al., 1985; Durand et al., 1992, 1994; Kerzhentsev et al., 1996; Castillo et al., 1997). Beside that, 3-methylphenol was identified as a new photoproduct of fenitrothion in the present study.

River water samples that collected weekly for one month from the Kurose River were analyzed without irradiation for the purpose of identifying fenitrothion and its photoproducts in natural water. Fenitrothion, fenitrooxon, 3-methyl-4-nitrophenol, and 3-methylphenol

<table>
<thead>
<tr>
<th>Compound name</th>
<th>% Relative to fenitrothion (Direct photolysis)</th>
<th>% Relative to fenitrothion (Indirect photolysis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fenitrooxon</td>
<td>14</td>
<td>6</td>
</tr>
<tr>
<td>3-Methyl-4-nitrophenol</td>
<td>7</td>
<td>12</td>
</tr>
<tr>
<td>3-Methyl-4-nitroanisole</td>
<td>2</td>
<td>4</td>
</tr>
</tbody>
</table>
were the identified compounds by GC-MS analysis. The presence of these photoproducts in the natural water is consistent with Lacorte and Barcelo (1994) and Oubina et al. (1996), who detected these photoproducts, (except 3-methylphenol), in river and rice field water, which had been treated with fenitrothion before exposure to sunlight. The detection of 3-methylphenol in the river water strongly supports our experimental results, which suggest that 3-methylphenol is one of the fenitrothion photoproducts.

**Quantitative information**

For a comparison of the photoproducts that were identified under both direct and indirect photolysis of fenitrothion, three common photoproducts (fenitrooxon, 3-methyl-4-nitrophenol, and 3-methyl-4-nitroanisol) were determined using their available standards as a percentage relative to fenitrothion concentration, under the SIM mode of the GC-MS. The results in Table 4 show that, the percentages of fenitrooxon, 3-methyl-4-nitrophenol, and 3-methyl-4-nitroanisol were determined to be 14, 7 and 2%, respectively, relative to fenitrothion under direct photolysis, while their percentages under indirect photolysis were 6, 12 and 4%, respectively. These results revealed that the percentage of fenitrooxon was decreased under indirect photolysis, while the percentages of the other photoproducts were increased compared to direct photolysis. This is due to, fenitrooxon being known as primary photoproduct, while 3-methyl-4-nitrophenol and 3-methyl-4-nitroanisol are considered to be intermediate photoproducts of fenitrothion. Therefore, under fast degradation (indirect photolysis), where hydroxyl radicals were readily formed by nitrate, the amount of the primary product decreased and the amount of intermediate products increased.

Quantitative determination of fenitrothion and its photoproducts that identified in Kurose river water without irradiation was carried out using GC-MS analysis under SIM mode. The results in Table 5 show that fenitrothion, fenitrooxon, and 3-methyl-4-nitrophenol were detected with average concentrations of 190, 72, and 110 ng/l, respectively. From these results we can realize that fenitrooxon and 3-methyl-4-nitrophenol were detected with concentration levels near the parent compound level and this reflects their risk and the importance of monitoring these transformation products beside the parent compound (fenitrothion).

A recovery test for the concerned photoproducts (fenitrooxon, phosphoric acid O, O, O tri-methylphosphate, 3-methyl-4-nitrophenol, and 3-methyl-4-nitroanisol) in this study as well as fenitrothion was carried out using their available authentic standard by solid phase extraction and GC-MS analysis. As shown in Table 6, high recoveries of fenitrothion and fenitrooxon (101 and 85%) were obtained with relative standard deviations of 14 and 23, respectively for \( n = 3 \). On the other hand, low recoveries were achieved for phosphoric acid O, O, O tri-methylphosphate, 3-methyl-4-nitrophenol, and 3-methyl-4-nitroanisol (72, 62, and 42%) with relative standard deviations of 4, 1 and 18, respectively.

**Effects of fenitrothion and two of its photoproducts on steroid hormones biosynthesis**

Beside fenitrothion as a parent compound, the photoproducts fenitrooxon and 3-methyl-4-nitrophenol were selected to investigate their toxicological effects on steroid hormone biosynthesis. The selection of these photoproducts was, firstly, based on the fact that they possess a significant toxicity to the aquatic environment, sometimes more than the fenitrothion (Eto, 1974; Amoros et al., 2000). Secondly, they are considered to be the most common photoproducts of fenitrothion, formed under both photolysis and photocatalytic degradation (Durand et al., 1992, 1994; Kerzhentsev et al., 1996; Castillo et al., 1997). Thirdly, these photoproducts were previously detected associated with the fenitrothion in both river and rice field water samples after the aerial application of fenitrothion (Lacorte and Barcelo, 1994; Oubina et al., 1996). Furthermore, in this study, these photoproducts were also detected in Kurose River water with concentration levels near to that of fenitrothion (Table 5).

To clarify the suppression mechanism of fenitrothion, fenitrooxon, and 3-methyl-4-nitrophenol on adrenal ster-

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**Table 5. Mean, minimum and maximum concentrations of fenitrothion, fenitrooxon and 3-methyl-4-nitrophenol in the Kurose River water collected on, February 2003**

<table>
<thead>
<tr>
<th>Compound name</th>
<th>Concentration (ng/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fenitrothion</td>
<td>190 (130–280)</td>
</tr>
<tr>
<td>Fenitrooxon</td>
<td>72 (50–100)</td>
</tr>
<tr>
<td>3-Methyl-4-nitrophenol</td>
<td>110 (60–230)</td>
</tr>
</tbody>
</table>

*Number of sampling times \( n = 4 \).*

**Table 6. Recovery and standard deviation of fenitrothion and its photoproducts from solid phase extraction**

<table>
<thead>
<tr>
<th>Compound name</th>
<th>Recovery%</th>
<th>RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fenitrothion</td>
<td>101</td>
<td>14</td>
</tr>
<tr>
<td>Fenitrooxon</td>
<td>85</td>
<td>23</td>
</tr>
<tr>
<td>Phosphoric acid O,O,O tri-methyl ester</td>
<td>72</td>
<td>4</td>
</tr>
<tr>
<td>3-Methyl-4-nitrophenol</td>
<td>62</td>
<td>1</td>
</tr>
<tr>
<td>3-Methyl-4-nitroanisol</td>
<td>42</td>
<td>18</td>
</tr>
</tbody>
</table>

*Number of replicates for each compound: 5.*

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\( \mu \text{g/L.} \)
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oidogenesis, the amounts of the secreted steroids (17α-hydroxyprogesterone, androstenedione, deoxycortisol, deoxycorticosterone, and cortisol) were analyzed by HPLC. As shown in Fig. 4, cortisol secretion was decreased directly after the treatments with tested compounds, starting from 1 µM until completely suppressed at 50 µM of fenitrothion and 3-methyl-4-nitrophenol treatments. However, for fenitrooxon, the cortisol secretion was suppressed almost a half by 100 µM of the compound.

Consequently, we find that all steroidogenesis were completely suppressed at 50 µM with both fenitrothion and 3-methyl-4-nitrophenol treatments. However, for fenitrooxon treatment, all steroidogenesis (except cortisol) were almost stable. Hence, fenitrothion and 3-methyl-4-nitrophenol seem to be more toxic than fenitrooxon in the adrenal cells.

We note that cell morphology and P450 scc activity were not changed by the treatment with fenitrothion and its two photoproducts at the ranges of the concentrations shown in Fig. 4. Therefore, this indicates that these compounds below their lethal toxic concentrations on the cell suppressed the steroidogenesis. In the presence of too high concentration levels, such as 100 µM of fenitrothion and 3-methyl-4-nitrophenol and 1000 µM of fenitrooxon, P450 scc activity was suppressed, cells shrunk on the culture plate and not completely dead but it was not healthy after 48 h of treatments.

In the light of Fig. 4(A), we can find that the amount of 17α-hydroxyprogesterone, which is a precursor of androgen, was increased concomitantly with the suppression of the cortisol secretion, and this disruption started at a low concentration (10 µM) of fenitrothion. On the other hand, Figs. 4(B) and (C) shows that the amount of androstenedione, which is known as a direct precursor of testosterone, the principal male sex hormone, was increased concomitantly with the suppression of cortisol secretion and the disruption started to induce at low concentrations (10 and 20 µM) of fenitrooxon and 3-methyl-4-nitrophenol, respectively. This suggests that fenitrothion and its photodegraded compounds may induce suppression of cortisol secretion and increase of male sex hormone synthesis from adrenal glands. Therefore, the endocrine system of the animals may be seriously disrupted by the increase in androgen and the decrease in glucocorticoid. This mechanism is consistent with several studies, which reported that organophosphate pesticides such as dichlorvos, diazinon, chlorpyrifos and dursban have all been shown to inhibit steroidogenesis in adrenal cells (Civen and Brown, 1974; Civen et al., 1977).

Fenitrothion pesticide is an environmental water pollutant, especially in rice field water biota, since it is extensively used for the control of rice insects in the East Asia such as Japan and China. Genlin et al. (1987) reported that the residual concentration of fenitrothion in the viscera of the common carp fish was 65.11 ppm, resulting from 0.346 ppm of fenitrothion in rice field water in the Shichuan province in China, which can be roughly converted to 235 µM. This concentration is considered to be high compared to the concentration that induces the complete suppression of the steroid hormones biosynthesis in this study (50 µM), except for fenitrooxon. Moreover, the concentration that disrupt the steroid hormone secretion in this study (50 µM) was considered to be very low compared with the lethal dose of fenitrothion (LD50 = 365 mg/kg), which can be converted to 1314 µM (Gupta et al., 1989).

Thus, although the bioaccumulation factors and metabolic pathways are not well known, these compounds may affect the hormonal system of the rice field water biota as strong endocrine disruptors at environmental concentrations. Finally, we can conclude the disruption mecha-

Fig. 4. Effect of (A) fenitrothion, (B) fenitrooxon and (C) 3-methyl-4-nitrophenol on adrenal steroid hormones secretion in bovine adrenal cultured cells.
nism of fenitrothion is not only through the action of the androgen receptor but also the suppression of adrenal glucocorticoid synthesis and the stimulation of androgen secretion. This perturbation for relative amounts of steroid hormones biosynthesis possibly occurs in gonadal and brain steroidogenesis as well as in adrenals, since the pathways of steroid hormone biosynthesis in these organs overlap each other.

CONCLUSIONS

The degradation rate of fenitrothion under indirect photolysis was faster than that of direct photolysis, which clearly indicated that the degradation rate of fenitrothion is significantly influenced by indirect photolysis in natural waters. The phototransformation kinetics of fenitrothion in pure water under direct and indirect photolysis revealed a non-specific degradation pathway with hydroxyl radicals under indirect photolysis. Fenitrothion and its two photoproduts (fenitrooxon and 3-methyl-4-nitrophenol) perturbed the activity of steroid hormones biosynthesis, thus suspected of being endocrine disruptors.

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