Uptake and Bioconcentration of Disulfoton and Its Oxidation Compounds in Carp, *Cyprinus carpio* L.

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(Received July 27, 1984)

The uptake and bioconcentration of disulfoton (0,0-diethyl S-2-ethylthioethyl phosphorodithioate) and its oxidation compounds, sulfoxide and sulfone in carp were investigated by a continuous flow water system. Disulfoton concentration in fish increases rapidly after exposure to chemical and remains constant. No increase of bioconcentration factor (BCF) was observed with longer exposure and concentration in water. The BCF of disulfoton in carp was approximately 450. On transference of fish to fresh water free of disulfoton, the chemical disappeared quite rapidly from the fish body. The BCF of disulfoton sulfoxide and sulfone which are the ultimate residue compounds in the environment showed very low values. A significant correlation was found between the BCF in fish and the partition coefficient between *n*-octanol and water.

**INTRODUCTION**

Disulfoton [0,0-diethyl S-2-ethylthioethyl phosphorodithioate, Disyston®] is a systemic insecticide and it has been shown to be effective against a wide range of insects by soil application. The metabolism and degradation studies of disulfoton have been carried out extensively in animals,\(^1,2\) plants,\(^3-6\) soil,\(^7-9\) water,\(^7,10\) and light\(^11\) using labeled or non-labeled chemical. From these metabolism studies, it is known that disulfoton is rapidly metabolized to oxidation products which have different toxicities and insecticidal activities from the parent compound. Residue compounds found in crops and soils are mainly oxidative metabolites of disulfoton: disulfoton sulfoxide, disulfoton sulfone, disulfoton oxygen analog sulfoxide and its sulfone. In the case of soil metabolism studies, disulfoton was rapidly oxidized to the corresponding sulfoxide and sulfone derivatives, while oxidative desulfurization of disulfoton occurred slightly under both upland and flooded conditions.\(^7,8\) Since it is frequently applied to the upland and paddoo field, the aquatic environment might be temporarily contaminated with the residues of disulfoton and its oxidation products. Accordingly, studies have been undertaken to clarify the fate and accumulation of disulfoton and its oxidation compounds in the aqueous environment.

This report presents the experimental results of uptake and bioconcentration of disulfoton and its oxidation compounds in carp (*Cyprinus carpio*) kept in continuous flow water containing a subacute concentration of chemicals. The concentration and ratio of a mixture tested of disulfoton and its oxidation compounds would correspond to that found in crop and soil samples of the field.

**MATERIALS AND METHODS**

1. **Chemicals**

Disulfoton [0,0-diethyl S-2-ethylthioethyl phosphorodithioate] and disulfoton oxygen analog (demeton-thiol) were used as technical grade. Disulfoton sulfoxide and its oxygen analog were prepared by oxidation with hydrogen peroxide from disulfoton and deme-
ton-thiol. Disulfoton sulfone and its oxygen analog were prepared by oxidation with potassium permanganate. Technical grade of all compounds were purified by silica gel column chromatography (Mallinkrodt Chemical Works) using a n-hexane/chloroform (7:3) mixture as the eluting solvent. These authentic compounds were identified by IR (Hitachi 260-10 type) and gas chromatograph-mass spectrometry (Shimadzu LKB-9000) as well as elemental analysis. The purity of these compounds were more than 98% by TLC analysis.

Disulfoton and a mixture of disulfoton, sulfoxide and sulfone (1:6:3), in emulsifiable concentrate were prepared as follows. Five grams of disulfoton, 2.5 g of xylene and 2.5 g of Newkalgen CP-50 (emulsifier, Takemoto Oil & Fat Co.) were mixed. One gram of disulfoton, 6 g of sulfoxide and 3 g of sulfone, 5 g of xylene and 5 g of emulsifier were mixed. One hundred mg of each 50% emulsifiable concentrate were diluted with 5 liter of distilled water (10 ppm stock solution). All solvents used for extraction and analysis were analytical reagent grade.

2. Fish
Carp, *Cyprinus carpio* L. (average fish weighing ca. 25 g and average length of ca. 11 cm), were provided by Yoshida Fish Co., Tokyo and maintained in dechlorinated running water at about 23°C for 1 week before use after disinfection by sulfametoxydiazin treatment. Fish were fed a standard pelleted gold fish food every day. Tap water dechlorinated by charcoal, about pH 6.0 were used throughout the present experiments, with constant aeration so that the dissolved oxygen was kept at ca. 6 ppm.

3. Bioconcentration Study
Bioconcentration of disulfoton and its oxidation compounds in carp was examined in a dynamic way, as in the following. Twelve fishes were acclimatized in a 50 liter glass aquarium for 5 days at 25±2°C. One ppm or 10 ppm of a mixture of disulfoton, sulfoxide and sulfone (1:6:3) was taken up from a reservoir with a micro pump (Tokyo Rikakikai MP-1001) at the rate of 3 ml/min, diluted with 300 ml/min of tap water and introduced into a 50 liter aquarium. The final concentration of the sum of disulfoton, sulfoxide and sulfone in the aquarium maintained at 0.01 ppm and 0.1 ppm throughout the experiment. In the case of pure disulfoton, 0.01 ppm solution flowed similarly into a 50 liter aquarium containing 12 carp. Thus, approximately 430 liter of fresh chemical solution per day continuously flowed into a 50 liter aquarium. The exposure period of carp was 56 days. Two carp were collected at 3, 7, 14, 28 and 56 days and residual concentration of compounds were measured. To test excretion of the compound from fish body, after the 56 days exposure period, remaining carp which had been kept in the chemical were transferred to running fresh water free of disulfoton for 2 to 4 days and analyzed for the residues of the compound. After weighing, the surface of fish body was wiped lightly with gauze and stored frozen in deep freezer until analysis. An aliquot of tank water was sampled every day during the exposure period and analyzed.

On the other hand, aqueous solution containing 1 ppm of disulfoton was prepared from the stock solution by diluting with dechlorinated water and poured into a 50 liter glass aquarium. Eight fishes were placed into a 40 liter of static water containing 1 ppm disulfoton at 25±2°C for 4 days without aeration. Two fishes were collected at 1 and 3 days, and an aliquot of aged top tank water was sampled at 0, 4, 8, 24, 48, 72 and 96 hr after preparation of disulfoton solution.

4. Analytical Method
Fish: The whole body of each tested fish was chopped with scissors and after adding 5 g of Celite 545, homogenized with 80% acetonitrile using a Polytron homogenizer. After filtration, the residue was extracted again with acetonitrile and all the filtrates were evaporated to remove most acetonitrile after adding 3 to 4 drops of 1% diethylene glycol acetone solution on a rotary evaporator *in vacuo*. The remaining aqueous phase was transferred to a separatory funnel with 150 ml of 20% sodium chloride solution and partitioned with two 100 ml portions of ethyl acetate. The combined ethyl acetate was
evaporated just to dryness and partition between n-hexane and acetonitrile was carried out to remove lipid substances in extracts of chemical. The concentrated residue of acetonitrile phase was dissolved in suitable definite volume of acetone. An aliquot of the solution was injected into gas chromatographic column for determination of disulfoton and its sulfone. For the measurement of disulfoton sulfoxide and its oxygen analog, acetone solution containing chemicals was oxidized with 20% magnesium sulfate and 1.6% potassium permanganate according to the method of Thornton and Anderson\[14\] and subjected to GLC.

Water: Five hundred ml of water was sampled and extracted with two 100 ml portions of ethyl acetate. The extracts were combined and evaporated in vacuo. The residue was dissolved with acetone and the chemicals were determined by the same procedure as described above.

GLC: Gas chromatographic determination of disulfoton and its oxidation compounds were carried out with Shimadzu GC-6A type equipped with a flame photometric detector (526 nm filter) under the following conditions.\[13\] GLC column was pyrex glass column (i.d. 3 mm, length 100 cm) packed with 10% Silicone DC 200 on Chromosorb W HP (80–100 mesh). Temperatures of column oven, injection port and detector were 240°, 270° and 300°C, respectively. The flow rate of nitrogen (carrier gas) was 60 ml/min. The retention times of disulfoton, disulfoton sulfone and its oxygen analog were 1.5, 3.3 and 2.4 min, respectively.

5. Recovery and the Detection Limit

The average recoveries and the detection limits of disulfoton and its oxidation compounds from fish and water samples are shown in Table 1. There was no necessity for fish and water to perform the chromatographic cleanup procedure, and satisfactory recovery percent of disulfoton and its oxidation compounds were obtained; more than 95% from water and 90% from fish. The detection limits of disulfoton, sulfoxide, sulfone and its oxygen analog in the fish were 0.01, 0.02, 0.02 and 0.02 ppm, respectively.

6. Measurement of Partition Coefficient

Measurement of partition coefficient of compounds between n-octanol and water was carried out at 20°C according to the method of Chiou et al.\[16\] The volumetric ratio of n-octanol and water was 1 to 10 and the partition coefficient was determined by dividing the concentration of the chemical in octanol with that in water.

**RESULTS AND DISCUSSION**

Carp were maintained for 56 days in continuously flowing water containing disulfoton at a nominal concentration of 0.01 ppm. The actual concentration of disulfoton in water and fish body was determined periodically. The data are shown in Table 2. The concentration of disulfoton in water was kept fairly constant, although it was a little lower than a nominal concentration of 0.01 ppm. The concentration of disulfoton in carp increased rapidly after exposure to disulfoton and it reached more than 3 ppm after 7 days exposure. Thereafter, disulfoton concentration constantly remained in carp. The bioconcentration factor (BCF: concentration (ppm) in fish on test day n/concentration (ppm) in water during test day 0–n) in fish was calculated. The BCF of
disulfoton in carp did not increase with longer exposure to disulfoton and the maximal factor was 525 after 28 days exposure. When once fish were transferred from disulfoton containing water to fresh clean water, disulfoton concentration in fish decrease rapidly. Disulfoton residue level diminished around 0.2ppm of about one twentieth after 4 days. Disulfoton oxidation compounds, sulfoxide, sulfone and its oxygen analogs were not detected from the fish, although in mammals disulfoton administered was rapidly metabolized to its oxidative metabolites.

For the understanding of the above results, carp were kept for 4 days in the static water conditions containing 1 ppm of disulfoton, and residue concentration of disulfoton and its oxidation compounds in water and fish were measured. As shown in Fig. 1, disulfoton in water rapidly decreased and half-life in water was calculated to be 16 hr. Disulfoton was oxidized to its sulfoxide and sulfone under the static water conditions and the amount of sulfoxide in water increased with the decrease in the amount of disulfoton. The disulfoton sulfoxide in water reached a level of more than 35% of the applied chemical after one day and disulfoton sulfone was found in the water with the time, but the amount was less than 5% of the applied one. Disulfoton was readily taken up into fish body from the surrounding water and disulfoton concentration in fish were 80.9 ppm and 97.3 ppm after 1 and 3 days exposure. Disulfoton sulfoxide and its sulfone were not detected (<0.02 ppm).

Next, carp were maintained for 56 days period in continuous flow water containing a mixture of disulfoton, sulfoxide and sulfone (1:6:3) at a nominal concentration of 0.1 ppm and 0.01 ppm as the sum of three compounds. A ratio of 1:6:3 of these compounds listed was based upon the result of soil metabolism studies of disulfoton.7,8) To cover a wide range of residues in the environment, two treatment levels of 0.1 ppm and 0.01 ppm were examined. These results are shown in Fig. 2 and Table 3. The concentration of disulfoton and its two oxidation compounds in water was kept fairly constant during 56 days exposure period, as shown in Fig. 2. The concentration of disulfoton in carp increased rapidly after exposure and reached a maximal concentration after 3 days exposure. The BCF of disulfoton in fish was 540 after 3 days exposure, which was not so different between continuous flow water and static water conditions, although the concentrations of disulfoton in water were different. Thio-oxidation products of disulfoton, sulfoxide and sulfone in fish were not detected above the detection limit of 0.02 ppm, although disulfoton in water was rapidly oxidized and thio-oxidation products were found in the static water.

Table 2 Uptake and excretion of disulfoton by fish exposed to continuously flowing water containing 0.01 ppm of disulfoton.

<table>
<thead>
<tr>
<th>Days</th>
<th>Concentration of disulfoton (ppm)</th>
<th>BCF*</th>
<th>Water</th>
<th>Fish</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>0.009</td>
<td>369</td>
<td>3.32</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>0.009</td>
<td>353</td>
<td>3.18</td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>0.010</td>
<td>525</td>
<td>5.25</td>
<td></td>
</tr>
<tr>
<td>56</td>
<td>0.009</td>
<td>407</td>
<td>3.66</td>
<td></td>
</tr>
<tr>
<td>2b)</td>
<td>—</td>
<td>—</td>
<td>0.62</td>
<td></td>
</tr>
<tr>
<td>4b)</td>
<td>—</td>
<td>—</td>
<td>0.19</td>
<td></td>
</tr>
</tbody>
</table>

*) Bioconcentration factor [concentration in fish (ppm)/average concentration (day 0-7) in water (ppm)].

b) After 56 days exposure, fresh water flowed for excretion test.

Fig. 1 Fate of disulfoton in water under the static condition containing 1 ppm of disulfoton. The concentration of disulfoton in fish were 80.9 and 97.3 ppm after 1 and 3-day exposure. Disulfoton sulfoxide and its sulfone were not detected (<0.02 ppm).
days exposure. Thereafter, disulfoton concentration in carp remained constant during the experimental period. However, on transference to running fresh water of disulfoton free, the disulfoton concentration rapidly decreased and the half-life was less than one day. On the other hand, disulfoton sulfone was found in the case of high concentration experiment and its concentration in carp ranged only from 0.1 to 0.2 ppm. The fish transferred to fresh water was not detected above the detection limit of 0.02 ppm within 2 days. Disulfoton sulf oxide, the major oxidation product, was not detected in any fish samples analyzed (detection limit: 0.02 ppm), although fish were continuously in contact with the chemical. From the present experiment, disulfoton oxygen analog sulf oxide and its sulfone which are formed by oxidative desulfurization of the P=S moiety were not found in the fish body during 56 days exposure period. In mammalian metabolism studies, disulfoton administered was readily absorbed and rapidly oxidized to the corresponding sulf oxide, sulfone and its oxygen analogs. Significant difference in the chemicals detected was observed between mammal and fish experiments. It was presumed that no disulfoton was metabolized to its oxidation compounds in the fish body. In fish exposed to continuously flowing water containing 0.01 ppm of a mixture of disulfoton, sulfoxide and sulfone (1:6:3), disulfoton content was found about 0.3 ppm (Table 3). However, disulfoton sulfoxide and its sulfone were not detected (<0.02 ppm). On these tests, fish showed no symptoms of intoxication during the experimental period.

The results of determination of the BCF in

![Graph showing concentration of disulfoton and its oxidation compounds in fish exposed to continuously flowing water containing 0.1 ppm of a mixture of disulfoton, sulfoxide and sulfone (1:6:3). Dotted line is disulfoton concentration in carp after transference to pesticides-free fresh water. Disulfoton sulfoxide in fish was not detected (<0.02 ppm).]

Table 3 Concentration of disulfoton and its oxidation compounds in fish exposed to continuously flowing water containing 0.01 ppm of a mixture of disulfoton, sulfoxide and sulfone (1:6:3).

<table>
<thead>
<tr>
<th>Days</th>
<th>Concentration in water (ppm)*</th>
<th>Concentration in fish (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Disulfoton</td>
</tr>
<tr>
<td>3</td>
<td>0.010</td>
<td>0.30</td>
</tr>
<tr>
<td>7</td>
<td>0.010</td>
<td>0.37</td>
</tr>
<tr>
<td>14</td>
<td>0.009</td>
<td>0.20</td>
</tr>
<tr>
<td>28</td>
<td>0.009</td>
<td>0.36</td>
</tr>
<tr>
<td>56</td>
<td>0.009</td>
<td>0.24</td>
</tr>
<tr>
<td>2b)</td>
<td></td>
<td>0.06</td>
</tr>
</tbody>
</table>

* The sum of disulfoton, sulfoxide and sulfone. Average concentration during test days 0–n.

b) After 56 days exposure, fresh water flowed for excretion test.
Table 4 Bioconcentration factors, water solubilities and partition coefficients of disulfoton and its oxidation compounds.

<table>
<thead>
<tr>
<th>Days</th>
<th>Compounds</th>
<th>Disulfoton Conc. in water (ppm)</th>
<th>Sulfoxide</th>
<th>Sulfone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.001</td>
<td>0.06</td>
<td>0.003</td>
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<tr>
<td>3</td>
<td>333</td>
<td>646</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>7</td>
<td>435</td>
<td>448</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>14</td>
<td>286</td>
<td>405</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>28</td>
<td>450</td>
<td>561</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>56</td>
<td>480</td>
<td>418</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Water solubility (ppm at 20°C)</td>
<td>16.3</td>
<td>&gt;4000</td>
<td>883</td>
<td></td>
</tr>
<tr>
<td>Partition coefficient log P value</td>
<td>3.78</td>
<td>1.78</td>
<td>1.93</td>
<td></td>
</tr>
</tbody>
</table>

carp, water solubilities and partition coefficients of disulfoton, sulfoxide and sulfone are shown in Table 4. The BCF of disulfoton was higher and that of sulfone showed very low values. Disulfoton sulfoxide content was always below detection limit in every fish sample analyzed. The residual concentration of disulfoton depended on the concentration in water, but the BCF was not correlated with disulfoton concentration in water, being approximately 450. Takimoto and Miyamoto reported that fenitrothion content in fish reached maximum in 1 to 3 days exposure and the BCF was 200 to 250. The BCF was not so different between fenitrothion and disulfoton, although fish species and given test conditions were different. The partition coefficient between n-octanol and water of sulfoxide and sulfone obtained only less than logarithm p value 2. The BCF of these oxidation compounds showed very low values (sulfoxide: 1, sulfone: 5), although disulfoton showed higher value. The high correlation between the BCF of organic compounds in some fishes and the water solubility or partition coefficient has already been reported by a number of researchers. Kanazawa reported that a significant correlation was found between the BCFs in fish and the water solubility or partition coefficient between n-octanol and water, when the BCF of 15 pesticides in fish were determined. In the case of disulfoton and its oxidation compounds, a significant correlation was also found between the BCF in the carp and the partition coefficient (n-octanol/water) or water solubility from these test results.

Under actual field conditions, disulfoton was rapidly oxidized to the corresponding sulfoxide, sulfone and its oxygen analogs of oxidative desulfurization of the P=S moiety. Thio-oxidation products of disulfoton were comparatively stable in soil and water. However, no bioconcentration in fish of the oxidation compounds was observed in the present experiment. On application to upland and paddy fields, disulfoton is rapidly oxidized in the soil, water and plants before it may be taken up into the fish. When disulfoton directly enters the aquatic environment, it is rapidly adsorbed to soil particles and organic matter which may be present in the water, whereas the BCF of disulfoton oxidation products, sulfoxide and sulfone which are the ultimate residue compounds in the environment showed very low values. Therefore, it seldom occurs that fish contains an appreciable amount of disulfoton and its oxidation product residues.

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要約

コイによるジスルホトンとその酸化体の取込みと濃縮

高瀬 婕, 小山寛史

ジスルホトン (O,O-diethyl S-2-ethylthioethyl phosphorodithioate) およびその酸化体スルホキシドとスルホンのコイによる取込みと濃縮性を比較した。ジスルホトン 0.01 ppm を含む流水中で飼育したコイは、ジスルホトンを急速に吸収し平衡状態に達して 56 日間暴露しても濃縮率は増加せず、生物濃縮係数は最大 525 であった。そして魚体から各酸化代謝物はまったく検出されなかった。その後、コイを清水に移すと魚体中のジスルホトンは速やかに排泄 (4 日間で 1/20) された。コイをジスルホトン・スルホキシド・スルホン (1:6:3) の合計設定濃度 0.1 ppm または 0.01 ppm に暴露すると、濃縮率は水中濃度にあまり影響されず、ジスルホトンの約 450、スルホキシドは約 10,スルホンは約 5 であり、環境における残留化合物の酸化体が魚体内で高く濃縮される傾向は見られなかった。各供試化合物の魚体濃縮性とオクタノール－水の分配係数または水溶解度との間には明らかな相関性が認められた。