

Original Articles

Oxadiazon Residues in Fish and Shellfish

Masaaki IMANAKA, Kazuyoshi MATSUNAGA, Ayamichi SHIGETA
and Tatsuo ISHIDA

Okayama Prefectural Research Center of Environment and Public Health,
Uchio, Okayama 701-02, Japan

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Studies were made on an analytical method for determining residues of oxadiazon herbicide in fish and shellfish, consumption of which has recently increased, and the levels of residues in crucian carps were investigated. In order to eliminate interference, particularly of PCBs and organochlorine insecticides, which had contaminated freshwater and marine fishes at high levels, conditions of Florisil column chromatography and ECD gas chromatography were examined and one method was found for routine analysis of oxadiazon where a recovery of 96.0% could be obtained. When the flesh of crucian carps, collected in Lake Kojima 2 months, 4 months and 9 months after application of oxadiazon, was analyzed by this method, 0.442 ppm, 0.046 ppm and 0.017 ppm were detected, respectively. This suggests the possibility that oxadiazon may have a strong persistence in fish. Identification was carried out by means of mass spectrometry and mass chromatography.

INTRODUCTION

The use of oxadiazon (2-*tert*-butyl-4-(2,4-dichloro-5-isopropoxyphenyl)-1,3,4-oxadiazolin-5-one), which is one of the oxadiazole herbicides, has recently increased and its 48-hour *tlm* to carp (*Cyprinus carpio*) is 3.2 ppm.¹⁾ Although the water supply to the paddy fields is stopped for 2 days after submerged application of the herbicide,²⁾ oxadiazon draining into the rivers is inevitable. Therefore, its use will certainly have a strong impact on the aquatic ecosystem. However, there have been few reports³⁻⁵⁾ on its fate in the aquatic environment, and especially on the residue levels in fishes and shellfishes.

0.442 ppm of oxadiazon on a wet basis was detected in crucian carps (*Carassius cuvieri*) which were caught in Lake Kojima on August 13, 1980, approximately 2 months after the herbicide was applied. In addition, fishes caught on October 21, 1980 and April 7, 1981, still showed rather high levels of contamination (0.046 ppm and 0.017 ppm). The Lake Kojima is the second largest artificial fresh-

water lake in the world and has an area of 1,100 ha.

METHODS

1. Analytical Method⁶⁾

Fifty grams of the flesh of fishes or shellfishes was placed in a 300 ml flask, dehydrated with 70 g of anhydrous Na₂SO₄, and extracted with *n*-hexane (100 ml × 1, 50 ml × 4), by homogenizing it sufficiently with Polytron (Kinematica, Switzerland). After drying the combined *n*-hexane extracts the solvent was evaporated under reduced pressure at 30°C to render fat, which was dissolved in *n*-hexane (20 ml), and extracted with CH₃CN saturated with *n*-hexane (30 ml × 3). Further, CH₃CN phase (90 ml) was washed with 10 ml of *n*-hexane. The obtained CH₃CN phase was poured into 500 ml of 2% NaCl solution and shaken with *n*-hexane (100 ml × 2). The *n*-hexane phase was washed with 2% NaCl solution (50 ml), dried, concentrated to ca. 5 ml under reduced pressure and chromatographed on a Florisil column (2.2 × 30 cm) which was prepared by packing 10 g of Florisil,

activated for about 13 hours at 130°C, as slurry with *n*-hexane and 5 g of anhydrous Na₂SO₄ on the top. After flowing 150 ml of *n*-hexane, the fraction containing oxadiazon was eluted with 150 ml of 30% diethyl ether in *n*-hexane. The eluted solution was again concentrated to an appropriate volume and was used as a sample for ECD gas chromatography.

2. Gas Chromatography

Apparatus, Shimadzu GC-4BM equipped with ⁶³Ni ECD; operating conditions are listed in Table 1.

3. Gas Chromatograph-Mass Spectrometry (GC-MS)

Apparatus, Shimadzu LKB 9000 with MASPAC 90; column, 1.0 m × 3 mm *i.d.*, glass; stationary phase, 2% DEGS+0.5% H₃PO₄; support, Gaschrom Q, 80-100 mesh; carrier gas, He (20 ml/min); column temp., 200°C; inj. and separator temp., 250°C; ion source temp., 290°C; electron energy, 70 eV; accelerating voltage, 3.5 kV; trap current, 60 μA; selected ion at mass chromatography 302, 304, 344 and 346 (*m/e*).

RESULTS AND DISCUSSION

1. Analytical Method

Fishes and shellfishes have been contaminated with PCBs and organochlorine insecticides (BHC, DDT and *etc.*) at quite a high level, therefore these chemicals are supposed to interfere with the determination of oxadiazon. PCBs especially, contain a component which

has a peak of the same retention time as oxadiazon under all examined GC conditions (Table 1 & Fig. 1). Consequently, the first *n*-hexane fraction (150 ml, PCBs are eluted) must be discarded in Florisil column chromatography. On the other hand, some of the organochlorine insecticides are eluted in the same fraction as oxadiazon, but can be completely separated from it by GC columns with the stationary phase having strong polarity (Fig. 1).

When 2 μg of oxadiazon (0.04 ppm) was

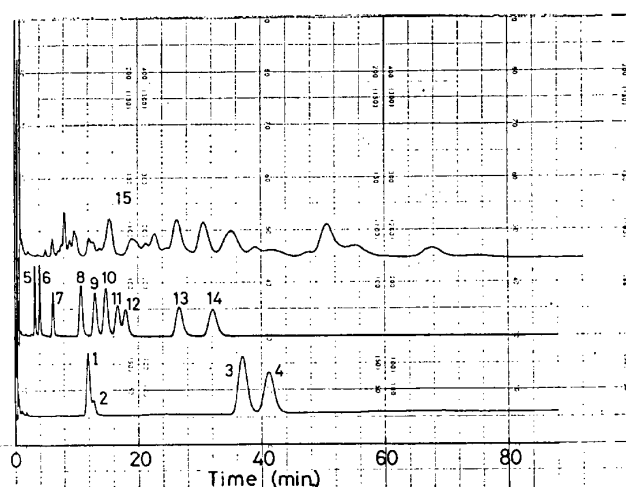


Fig. 1 ECD gaschromatograms of pesticides standard and PCBs.

1: Oxadiazon, 2: Swep (MCC), 3: Nitrofen, 4: CNP (chlornitrofen), 5: Aldrin, 6: α -BHC, 7: γ -BHC, 8: *p,p'*-DDE, 9: Dieldrin, 10: *o,p'*-DDT+Endrin, 11: δ -BHC, 12: β -BHC, 13: *p,p'*-DDT, 14: *p,p'*-DDD, 15: PCBs (KC 300: KC 400: KC 500: KC 600=1:1:1:1)
GC conditions are listed in No. 1 of Table 1.

Table 1 GC columns and conditions.

No.	Stationary phase	Support	Temp. (°C)		N ₂ flow (ml/min)
			Inj-Det.	Column	
1	2% DEGS+0.5% H ₃ PO ₄	Gaschrom Q (80/100)	245	195	60
2	5% Thermon 1000 +0.5% H ₃ PO ₄	W (80/100)	260	215	45
3	5% Thermon 3000	W (80/100)	260	215	45
4	5% FFAP	Gaschrom Q (60/80)	260	215	45
5	2% OV-1	W (60/80)	250	200	45

Column: Glass column, 3 mm × 1.5 m.

W: Chromosorb W (AW-DMCS).

added to 50 g of Japanese littleneck (*Tapes (Amygdala) philippinarum*) and analyzed by the method described above, the recovery was $96.0 \pm 2.32\%$ (3 replications).

2. Oxadiazon Residues in Fishes and Shellfishes

To secure irrigation water for reclaimed land, the Lake Kojima was made in 1959 as a closed water area by damming up the eastern part of Kojima Bay into which the second-rate rivers, the Sasagase and Kurashiki, flow through a typical farming region in Okayama Prefecture (Fig. 2). Hence the lake can be regarded as a most suitable model for our study on the influence of pesticides on the aquatic environment. The Lake Kojima has also been an important fishing ground for crucian carps, catfish and eels *etc.*, and therefore, research on pesticide residues is considered important from the point of sanitation.

Figure 3 shows an ECD gaschromatogram of the *n*-hexane extracts from the flesh of crucian carps which were caught in a central area of the Lake Kojima on August 13, 1980 and October 21, 1980. (In both cases a mixture of the flesh of 7 fishes whose length was about 15 cm and body weight was about 100 g

was used for the determination).

Oxadiazon was detected in both cases at the following levels: 0.442 ± 0.007 ppm (3 replications) in the former and 0.046 ± 0.002 ppm (3 replications) in the latter. Furthermore, the crucian carps collected on April 7, 1981 also contained $0.017 \text{ ppm} \pm 0.002 \text{ ppm}$ (3 replications) of oxadiazon. Although oxadiazon is applied to paddy fields just before the rice-planting season, crucian carps which were caught more than 9 months after its application were still contaminated at high levels. On the other hand, the level in the surface lake water taken at the same time was 0.024 ppb which was much lower than that in the carps. These results show the possibility that oxadiazon may have a long persistence in fish. Therefore, further study regarding in this field should be carried out immediately.

The identification was verified by comparison of mass spectra of a standard oxadiazon with those of the compound in *n*-hexane extract from the carps collected on August 23, 1980 in Lake Kojima (Fig. 4). Figure 5 shows total ion chromatogram and mass chromatogram of the extract, which confirms that the crucian carps are surely contaminated with oxadiazon.

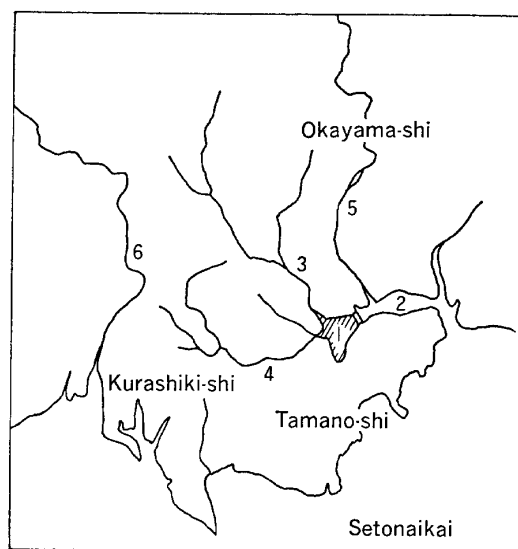


Fig. 2 Map showing Lake Kojima area of Okayama prefecture, Japan.

1: Lake Kojima, 2: Kojima Bay, 3: Sasagase River, 4: Kurashiki River, 5: Asahi River, 6: Takahashi River.

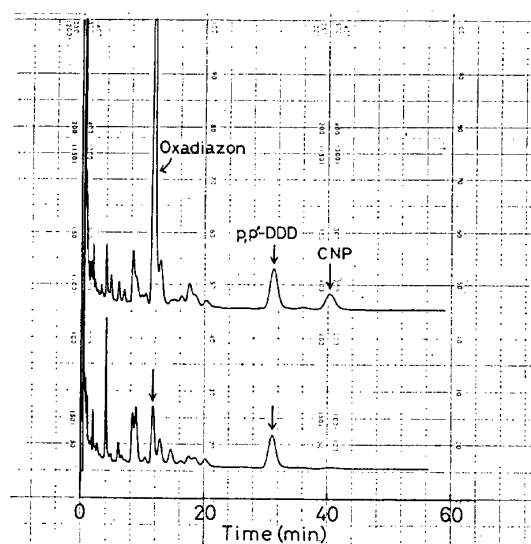


Fig. 3 ECD gaschromatograms of *n*-hexane extracts from crucian carps after fractionation on Florisil column.

Top: Crucian carps were collected on August 13, 1980.

Bottom: Collected on October 21, 1980.

GC conditions are listed in No. 1 of Table 1.

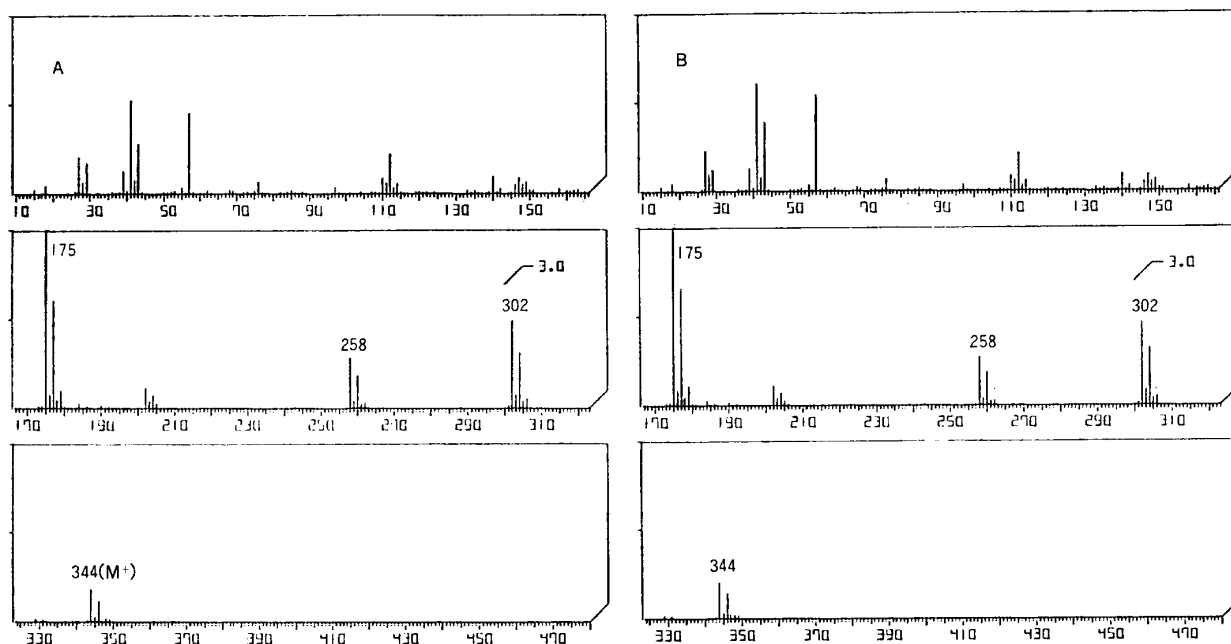


Fig. 4 EI mass spectra for identification of oxadiazon.

A: Oxadiazon standard,

B: Peaks corresponding to oxadiazon in *n*-hexane extract from crucian carps collected on August 13, 1980.

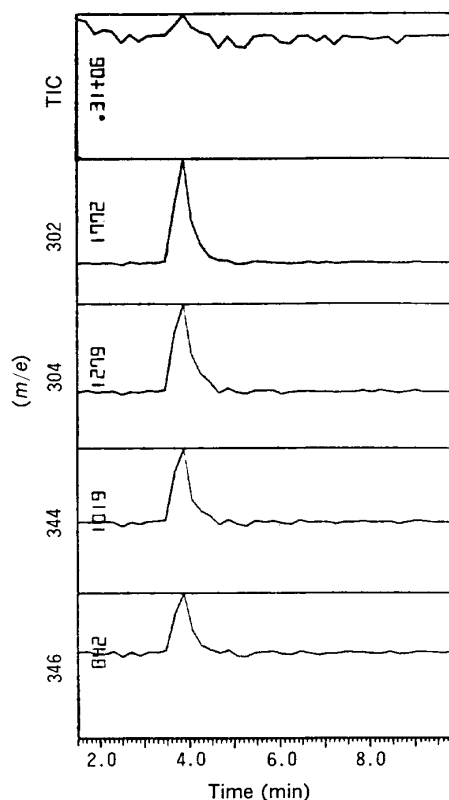


Fig. 5 Mass chromatogram of *n*-hexane extract from crucian carps collected on August 13, 1980.

TIC: Total ion chromatogram, Scan time: 8 sec, Interval: 12 sec.

Oxadiazon ranging from trace to 0.066 ppm with 100% detection rate was also detected in three Japanese littlenecks and three pale chubs (*Zacco platypus*) caught in June and July from different areas in Okayama Prefecture.

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要 約

除草剤オキサジアゾンの魚介類への残留

今中雅章, 松永和義, 繁田文通, 石田立夫
近年使用量が急増している除草剤オキサジアゾンについて, 魚介類中の分析方法を検討するとともに実態調査を実施した. とくに, 魚介類中かなりのレベルで残留している PCB と *p,p'*-DDE, ディルドリン等の有機塩素系農薬による影響を除去すべく, フロリジルカラムによるクリーン・アップ条件, GC カラム充填剤の種類な

どに検討を加えた結果, 妨害を受けることなく, 回収率 96.0% で定量することが可能になった. この方法によって, 散布後 2 カ月, 4 カ月 および 9 カ月経過した時点で児島湖より採取したフナを分析したところ, おのおの 0.442 ppm, 0.046 ppm, 0.017 ppm のオキサジアゾンが検出され, 魚体内での残留性が強い可能性が示唆された. また, EI マススペクトルとマスキングクロマトグラフィーによって, フナ中の残留物はオキサジアゾンであることを同定した.