Original

Studies on Pesticide Degradation Products in Pesticide Residue Analysis

(Received April 24, 1998)

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In order to apply the multiresidue screening method to degradation products of *N*-trihalomethylthio fungicides (captan, captafol, folpet, dichlofluanid, tolylfluanid) and oxidized organophosphorus insecticides generated during sample preparation or in plants, the recovery of sixteen of these degradation products and metabolites from 3 agricultural products was investigated. The recoveries ranged from 74 to 129%, and these data suggest that the degradation products can be determined simultaneously with the parent pesticides by this method.

The degradation ratios of 5 *N*-trihalomethylthio fungicides, demeton-S-methyl and isofenphos were examined during sample preparation from 5 agricultural products and during GC injection. Cabbage, spinach and pumpkin readily caused the degradation of *N*-trihalomethylthio fungicides, and the degradation ratios of captan were 100, 100 and 81%, respectively. Decreases of the parent pesticides corresponded closely to the formation of the degradation products.

Key words: pesticide degradation products; multiresidue analysis; GC/MS; agricultural products; *N*-trihalomethylthio fungicide

Introduction

The number of regulated pesticides in the Food Sanitation Law¹⁾ has been increasing annually since 1993. The pesticide multiresidue method is an efficient way to screen a large number of food samples. It is well known that *N*-trihalomethylthio fungicides (captan, captafol, folpet, dichlofluanid, tolylfluanid) are degraded by chemical reaction with thiol groups (-SH) of the plant matrices during sample grinding²⁾, and demeton-S-methyl and isofenphos are oxidized to sulfone or oxon analogues³⁾. Some analytical modifications, such as addition of phosphoric acid⁴⁾ or hydrochloric acid⁵⁾, have been proposed to prevent such degradation. These degradation products are produced not only during the sample preparation step, but also in the plant itself after pesticide application. Several studies have been reported on the disappearance of pesticides during extraction pro-

cedures^{*1, 2}, but degradation products have not been well studied quantitatively^{*3}.

Aiming at the simple and rapid screening of pesticide residues, we investigated the simultaneous determination of both parent pesticides and their degradation products. This paper reports the application of our previous findings^{6, 7)} to degradation products produced during sample preparation, or in plants, and also reports the effect of matrices on the degradation

*¹ Iwata, H., Arimura, Y., Matsuhira, Y., Imaizumi, N., Shibuya, T., Maekawa, Y.: Abstracts of the 70th Meeting of the Food Hygienic Society of Japan, p. 44 (1995. 10. Nagoya).

*² Iwata, H., Arimura, Y., Matsuhira, Y., Imaizumi, N., Shibuya, T., Maekawa, Y.: Abstracts of the 71st Meeting of the Food Hygienic Society of Japan, p. 41 (1996. 5. Ibaraki).

*³ Iwahori, M., Imaizumi, N., Shibuya, T., Maekawa, Y.: Abstracts of the 65th Meeting of the Food Hygienic Society of Japan, p. 51 (1993. 5. Tokyo).

of pesticides during sample preparation and GC injection.

Materials and Methods

1. Food samples

Brown rice, lemon, spinach, cabbage, pumpkin, and kiwi fruit were used in this study.

2. Analytical standards

Pesticides and their degradation products: Folpet, demeton-S-methyl, demeton-S-methyl-sulfone, isofenphos oxon, isoxathion oxon, parathion oxon, parathionmethyl oxon, malathion oxon, *N,N'*-dimethyl-*N*-phenylsulfonyldiamide (degradation product of dichlofluanid), and *N,N'*-dimethyl-*N*-*p*-tolylsulfonyldiamide (degradation product of tolylfluanid) were purchased from Hayashi Pure Chemical Industries (Osaka, Japan), and captafol, captan, dichlofluanid, tolylfluanid, isofenphos, diazinon oxon, fenitrothion oxon, prothiofos oxon, EPN oxon, 2-chlorobenzonitrile (degradation product of clofentezine), 4,4'-dichlorobenzophenone (degradation product of dicofol), 1,2,3,6-tetrahydropthalimide (degradation product of captan and captafol), and phthalimide (degradation product of folpet) were from Wako Pure Chemical Industries (Osaka, Japan).

Standard solutions: Individual stock standard solutions (250 µg/mL) were prepared in acetone. Working solutions were prepared from stock solutions at 25 µg/mL with acetone. Mixed standard solutions for GC/MS analysis were freshly diluted with *n*-hexane-acetone (4:1) for parent pesticides and degradation products.

3. Instrumentation

Gas chromatography with mass-selective detection was performed on a Hewlett Packard HP 5890 Series II GC system with an HP5972 MSD system. The following analytical conditions were used: a 5% phenyl-methyl silicone column (DB-5ms, 30 m × 0.25 mm i.d., 0.10 µm film thickness); initial oven temperature 80°C, hold 3 min, linear gradient 30°C/min to 170°C, hold 4 min, then linear gradient 10°C/min to 270°C, hold 15 min; injector temperature 250°C; interface temperature 280°C; initial carrier gas (He) pressure 30 psi (4 mL/min), hold 1 min, decrease immediately to 8 psi, keep constant flow (0.9 mL/min); injection volume 4 µL (splitless mode); purge off time 1 min; ionization energy 70 eV. The pesti-

Scheme 1. Sample preparation method for multi-pesticide residue analysis

cides were detected in the SCAN mode (50~450 amu, 1.8 scans/sec).

4. Recovery test for degradation products and metabolites

Twenty-five gram portions of chopped samples (lemon and spinach) and ground samples (brown rice) were fortified with 0.1 mL of mixed working solution (25 µg/mL each of pesticide degradation products and metabolites). The fortified samples were analyzed as described previously⁷⁾, that is, blended with acetonitrile, then cleaned up through ODS and PSA mini-columns (Scheme 1).

5. Degradation during sample preparation

Twenty-five gram portions of chopped or ground samples (cabbage, pumpkin, spinach, brown rice and kiwi fruit) were fortified with 0.2 mL of mixed working solution of parent pesticides (25 µg/mL each of captan, folpet, dichlofluanid, tolylfluanid, demeton-S-methyl and isofenphos). Captafol was separately added to cabbage and pumpkin with 0.2 mL of working solution (25 µg/mL). The control was performed by making up the volume to 25 mL with purified water, without sample matrices. The samples were analyzed according to the procedure

Fig. 1. Mass spectra and structural formulas of pesticide degradation products

shown in Scheme 1. Parent pesticides and degradation products were quantified by GC/MS.

6. Degradation at GC injection

The cleaned-up sample solutions of cabbage, pumpkin, spinach, brown rice and kiwi fruit were used as matrix modifiers. They were dried under an N_2 stream, and dissolved in the same volume of mixed standard solution (captan, folpet, dichlofluanid, tolylfluanid, demeton-S-methyl and isofenphos) or captafol solution (1 $\mu\text{g/mL}$ in *n*-hexane-acetone (4:1)). The modified standard solution was injected into the GC/MS and the quantities of parent pesticides and their degradation products were compared with those in the standard solution free from matrices.

Results and Discussion

1. Application of multiresidue method to degradation products

N-Trihalomethylthio fungicides release a tri-

halomethylthio group by chemical reaction with thiol groups (-SH) in plant matrices⁸. Dichlofluanid is unstable in acetone solution⁹ and clofentazine is easily photodegraded². Fig. 1 showed the structural formulas of these degradation products and mass spectra obtained from GC/MS of their standard solutions.

These degradation products and sulfone or oxon analogues of organophosphorus insecticides which are produced both during analysis and in the plant itself, were added to 3 kinds of agricultural products (brown rice, lemon and spinach) and recovered by the reported multiresidue screening methods⁷. The recoveries were 73.9–129.4% except for degradation products of captan, captafol and folpet in lemon, owing to interfering peaks (Table 1). These degradation products and metabolites could thus be analyzed simultaneously with their parent pesticides.

2. Pesticide degradation in the presence of plant

Table 1. Recoveries of Metabolites of Pesticides Added to Agricultural Products

Pesticide	Recovery (% , Mean $\pm$ S.D., $n=3$)		
	Brown rice	Lemon	Spinach
1,2,3,6-Tetrahydrophthalimide	115.3 $\pm$ 4.3	—	112.1 $\pm$ 4.3
Phthalimide	89.7 $\pm$ 4.7	—	96.7 $\pm$ 0.8
<i>N,N'</i> -Dimethyl- <i>N</i> -phenylsulfonyldiamide	117.8 $\pm$ 2.6	116.2 $\pm$ 6.5	117.8 $\pm$ 0.8
<i>N,N'</i> -Dimethyl- <i>N-p</i> -tolylsulfonyldiamide	97.5 $\pm$ 5.0	94.1 $\pm$ 3.1	100.8 $\pm$ 1.5
4,4'-Dichlorobenzophenone	101.4 $\pm$ 2.9	125.2 $\pm$ 3.4	105.6 $\pm$ 2.7
2-Chlorobenzonitrile	103.7 $\pm$ 5.1	113.7 $\pm$ 14.0	91.7 $\pm$ 5.8
Demeton-S-methylsulfone	90.7 $\pm$ 3.1	73.9 $\pm$ 5.6	78.3 $\pm$ 2.8
Diazinon oxon	95.4 $\pm$ 5.1	114.6 $\pm$ 6.7	94.2 $\pm$ 2.1
Parathionmethyl oxon	123.8 $\pm$ 4.5	98.8 $\pm$ 5.6	112.7 $\pm$ 6.0
Fenitrothion oxon	122.5 $\pm$ 6.9	110.9 $\pm$ 2.9	112.1 $\pm$ 1.4
Malathion oxon	104.9 $\pm$ 4.8	99.3 $\pm$ 7.4	97.1 $\pm$ 2.3
Parathion oxon	107.7 $\pm$ 3.2	104.3 $\pm$ 13.3	110.5 $\pm$ 2.1
Isofenphos oxon	103.1 $\pm$ 6.3	109.5 $\pm$ 10.3	105.1 $\pm$ 4.6
Prothiofos oxon	95.1 $\pm$ 4.1	95.6 $\pm$ 3.1	97.8 $\pm$ 1.7
Isioxathion oxon	117.5 $\pm$ 9.9	129.4 $\pm$ 9.5	114.2 $\pm$ 2.9
EPN oxon	101.4 $\pm$ 3.6	76.2 $\pm$ 4.9	99.1 $\pm$ 5.2

matrices

We investigated the degradation ratios of 5 *N*-trihalomethylthio fungicides (captan, captafol, folpet, diclofluanid, tolylfluanid) and the alteration ratios of demeton-S-methyl to its sulfone analogue and isofenphos to its oxon analogue in the presence of cabbage, pumpkin, spinach, brown rice and kiwi fruit.

Gas chromatograms of standard solutions are shown in Fig. 2. The general tendency was observed that the degradation products of *N*-trihalomethylthio fungicides generated during GC injection were the same as those formed during sample preparation. Stained insert liners and columns in the GC system greatly affect the formation of degradation products¹⁰⁾. In GC using clean apparatus, degradation of components of a standard solution was almost negligible.

The following recoveries and degradation ratios were observed during sample preparation and GC injection.

(1) Captan, captafol, folpet, diclofluanid and tolylfluanid

Recoveries and degradation ratios of the parent pesticides are shown in Table 2. When these products were made up to a volume of 25 mL with purified water, all pesticides were recovered as the parent pesticides. In the case of cabbage, the parent pesticides disappeared com-

Fig. 2. Total ion chromatograms of pesticides and their degradation products prepared at 1 μ g/mL in *n*-hexane-acetone (4 : 1) standard solution

1: Demeton-S-methyl, 2: Dichlofluanid, 3: Tolyfluanid, 4: Isofenphos, 5: Captan, 6: Folpet, 7: Captafol, 8: Phthalimide, 9: 1,2,3,6-Tetrahydrophthalimide, 10: *N,N'*-Dimethyl-*N*-phenylsulfonyldiamide, 11: *N,N'*-Dimethyl-*N-p*-tolylsulfonyldiamide, 12: Demeton-S-methylsulfone, 13: Isofenphos oxon

pletely and more than 80% were recovered as degradation products. In the case of pumpkin, 39–81% of pesticides were recovered as degra-

Table 2. Recoveries and Degradation Ratios of Pesticides Added to Agricultural Products

Pesticide	Degradation product	Recovery (%; Mean $\pm$ S.D., $n=3$)					
		Blank ¹⁾	Cabbage	Pumpkin	Spinach	Brown rice	Kiwi fruit
Dichlofluanid		79.5 $\pm$ 6.2	0.0 $\pm$ 0.0	36.6 $\pm$ 9.1	2.0 $\pm$ 0.6	50.0 $\pm$ 3.0	76.7 $\pm$ 3.2
<i>N,N'</i> -Dimethyl- <i>N</i> -phenylsulfonylethylidamide		0.0 $\pm$ 0.0	80.4 $\pm$ 2.4	56.9 $\pm$ 4.3	71.1 $\pm$ 1.9	31.0 $\pm$ 1.2	11.0 $\pm$ 0.8
Tolylfluanid		82.5 $\pm$ 4.7	0.0 $\pm$ 0.0	54.5 $\pm$ 9.7	3.5 $\pm$ 1.8	58.3 $\pm$ 2.6	72.1 $\pm$ 4.8
<i>N,N'</i> -Dimethyl- <i>N</i> - <i>p</i> -tolylsulfonylethylidamide		0.0 $\pm$ 0.0	108.3 $\pm$ 3.8	46.3 $\pm$ 5.9	93.6 $\pm$ 2.5	23.5 $\pm$ 1.7	9.0 $\pm$ 0.2
Folpet		71.5 $\pm$ 3.8	0.0 $\pm$ 0.0	70.8 $\pm$ 8.9	11.5 $\pm$ 2.7	67.5 $\pm$ 4.3	33.0 $\pm$ 5.2
Phthalimide		0.0 $\pm$ 0.0	97.7 $\pm$ 13.1	39.2 $\pm$ 4.0	65.9 $\pm$ 5.7	20.0 $\pm$ 1.0	34.2 $\pm$ 2.6
Captan		76.0 $\pm$ 7.4	0.0 $\pm$ 0.0	29.2 $\pm$ 4.7	1.3 $\pm$ 1.3	58.2 $\pm$ 4.7	60.8 $\pm$ 5.5
1,2,3,6-Tetrahydrophthalimide		0.0 $\pm$ 0.0	100.3 $\pm$ 5.6	80.7 $\pm$ 6.9	100.3 $\pm$ 2.6	35.0 $\pm$ 2.3	20.2 $\pm$ 1.1
Captafol		83.0 $\pm$ 5.1	0.0 $\pm$ 0.0	43.0 $\pm$ 8.3	—	—	—
1,2,3,6-Tetrahydrophthalimide		0.0 $\pm$ 0.0	102.7 $\pm$ 7.8	66.6 $\pm$ 6.5	—	—	—
Demeton- <i>S</i> -methyl		76.3 $\pm$ 13.1	127.4 $\pm$ 10.0	39.7 $\pm$ 30.2	75.2 $\pm$ 4.9	86.5 $\pm$ 4.8	77.2 $\pm$ 7.0
Demeton- <i>S</i> -methylsulfone		0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	20.5 $\pm$ 10.3	4.2 $\pm$ 0.8	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
Isofenphos		77.9 $\pm$ 3.1	92.6 $\pm$ 4.0	80.3 $\pm$ 2.7	83.8 $\pm$ 2.2	72.5 $\pm$ 2.2	73.1 $\pm$ 4.3
Isofenphos oxon		0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0

¹⁾ Made up to a volume of 25 mL by adding purified water without sample matrices.

Table 3. Recoveries and Degradation Ratios of Pesticides during GC Injection with Sample Extracts

Pesticide	Degradation product	Recovery (%)				
		Cabbage	Pumpkin	Spinach	Brown rice	Kiwi fruit
Dichlofluanid		73.3	105.2	113.0	85.3	79.9
<i>N,N'</i> -Dimethyl- <i>N</i> -phenylsulfonyldiamide		20.7	0.0	0.0	0.0	7.4
Tolylfluanid		73.8	101.1	105.8	83.1	73.1
<i>N,N'</i> -Dimethyl- <i>N-p</i> -tolylsulfonyldiamide		24.2	0.0	0.0	0.0	5.5
Folpet		81.9	114.4	104.6	96.9	45.2
Phthalimide		18.8	0.0	0.0	0.0	24.2
Captan		72.4	105.4	100.3	114.5	98.1
1,2,3,6-Tetrahydrophthalimide		22.9	8.3	0.0	0.0	9.3
Captafol		64.1	98.1	89.1	120.5	23.6
1,2,3,6-Tetrahydrophthalimide		36.1	16.1	28.2	0.0	52.1
Demeton-S-methyl		131.4	117.5	111.0	103.1	105.4
Demeton-S-methylsulfone		0.0	0.0	0.0	0.0	0.0
Isofenphos		106.8	101.3	96.4	83.5	81.6
Isofenphos oxon		0.0	0.0	0.0	0.0	0.0

degradation products and total recoveries of parent pesticides and degradation products were 94–110%. Recoveries as degradation products were 66–100% for spinach, 20–35% for brown rice, and 9–34% for kiwi fruit. Total recoveries were 73–102% except for folpet added to kiwi fruit (67%). Thus, disappeared pesticides could be detected as degradation products.

It was reported that captan, captafol, diclofluanid and tollyfluanid added to cabbage and pumpkin completely disappear immediately after homogenization^{*1,2}. But in this study, samples were homogenized with acetonitrile, and the parent pesticides in pumpkin were not completely lost.

Pesticides were also supposed to be degraded during GC injection owing to the coexistence of plant ingredients¹⁰. Pesticides added to the cleaned-up extracts from agricultural products were compared by GC/MS analysis with standard solutions (Table 3). Degradation ratios of *N*-trihalomethylthio fungicides were 18–36% with cabbage extracts, and 5–52% with kiwi fruit extracts. Captafol was the most degradable, and the degradation ratios were 16% with pumpkin extracts and 28% with spinach extracts, although no degradation products were detected with brown rice extracts.

From these results, the alterations to degradation products shown in Table 2 were considered to have been caused mainly during the extraction process and partly during GC injection with

cabbage and kiwi fruit extracts.

(2) Demeton-S-methyl and isofenphos

The recovery of demeton-S-methyl added to cabbage was 127% owing to interfering peaks and its sulfone analogue was not detected, but in the case of pumpkin, the sulfone analogue was detected and the recoveries varied widely for both demeton-S-methyl and demeton-S-methyl-sulfone (Table 2). Modification of pesticides to sulfoxide and sulfone analogues during extraction was reported for aldicarb and ethionfencarb^{*3}. For demeton-S-methyl, its sulfoxide analogue, which is an intermediate product in the formation of the sulfone analogue, was expected to be generated during extraction. In the case of spinach, a small amount of demeton-S-methyl was converted to its sulfone analogue. But demeton-S-methyl was not oxidized to its sulfone analogue during GC injection with any sample extract (Table 3).

Isofenphos added to 5 kinds of agricultural products was not changed to its oxon analogue during sample preparation and GC injection.

3. Pesticide residue monitoring

The degradation products of *N*-trihalomethylthio fungicides generated during residue analysis were detected quantitatively with the multiresidue screening method. In F.Y. 1997 151 (89 domestic and 62 imported) agricultural products were analyzed. They included 12 cereals, 6 beans, 66 vegetables and 67 fruits. Captan and its degradation product were detect-

Table 4. Pesticide Residues Found in Agricultural Products

Sample (producing district)	Pesticide	Residue (ppm)	MRL ¹⁾ (ppm)
Apple (Nagano pref.)	Captan	0.10	5.0 ²⁾
	1,2,3,6-Tetrahydrophthalimide	0.06	
Grape (Chile)	Captan	0.13	5.0 ³⁾
	1,2,3,6-Tetrahydrophthalimide	0.11	
Grape (Chile)	Captan	0.03	5.0 ³⁾
	1,2,3,6-Tetrahydrophthalimide	0.05	
Tomato (Miyazaki pref.)	Dichlofluanid	0.11	15 ²⁾
	<i>N',N'</i> -Dimethyl- <i>N</i> -phenylsulfonyldiamide	0.18	
Japanese pear (Hyogo pref.)	Dichlofluanid	N.D. ⁴⁾	5.0 ²⁾
	<i>N',N'</i> -Dimethyl- <i>N</i> -phenylsulfonyldiamide	0.01	

¹⁾ Maximum Residue Limit²⁾ Pesticide Residues Standards under Food Sanitation Law³⁾ Standards for Withholding Registration of Pesticides under Agricultural Chemicals Regulation Law⁴⁾ Less than 0.01 ppm

ed in 1 sample of domestic apples and 2 of imported grapes, while dichlofluanid and its degradation product were detected in 1 sample of domestic tomatoes, and the degradation product of dichlofluanid was detected in 1 sample of domestic Japanese pears. Details are shown in Table 4. Parent pesticides and their degradation products were detected simultaneously, except for one sample with a low residue level. In all cases, residue levels were less than the maximum residue limits (MRLs).

Conclusion

The degradation products of *N*-trihalomethylthio fungicides and oxidized organophosphorus insecticides were determined simultaneously with their parent pesticides by the multi-residue screening method. The reduction of parent pesticides from fortified samples was almost quantitatively matched by the increase of their degradation products. Degradation ratios varied depending on the combination of pesticides and sample matrices.

By including the degradation products in routine analysis, the residue levels of degradable pesticides have been roughly estimated by a simple and rapid screening method. An official method for *N*-trihalomethylthio fungicides, which includes acidification with phosphoric

acid or hydrochloric acid, is essential only when these pesticides and degradation products are detected in large quantities with the multi-residue method.

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