J. Pesticide Sci. **5**, 29–36 (1980)

Absorption, Translocation and Metabolism of Fluoroimide in Inu-apple Trees, *Malus prunifolia* BORKHOUSEN*

Kunihiko OGAWA, Hiroyasu AIZAWA and Fumio YAMAUCHI

Mitsubishi-Kasei Institute of Toxicological and Environmental Sciences, Midori-ku, Yokohama 227, Japan

(Received May 31, 1979)

Fate of Fluoroimide, *N*–(4–fluorophenyl)–2,3–dichloromaleimide, on/in Inu-apple trees (*Malus prunifolia* BORKHOUSEN) was investigated using two ^{14}C–labeled compounds at either benzene ring or carbonyl group. Half lives of Fluoroimide applied on the leaves and fruits were about 20 days and 30 days, respectively. It remained mainly unchanged on the applied surface and was slowly absorbed by the plant. Only 2% of applied Fluoroimide was translocated into the leaves 9 days after treatment, and 9% and 18% of applied radioactivity were absorbed and translocated into the leaves and fruits, respectively, after 93 days. A main metabolite of Fluoroimide was sodium 4–fluorodichloromaleanilate, a hydrolyzed compound of imide ring, that was identified by MS, IR, NMR and FX. Other four metabolites, such as 4–fluoroaniline, (*E*)–2,3–dichloro–*N*–(4–fluorophenyl)acrylamide, *N*–(4–fluorophenyl) maleimide and *N*–(4–fluorophenyl) succinimide, were identified by cochromatography on *tlc* and MS, but residual amounts of these metabolites were quite low both in the leaves and fruits. It is considered that succinimide moiety of a reduced metabolite was derived from dichloromaleic acid portion of Fluoroimide, but not from the plant component.

INTRODUCTION

Fluoroimide, [Spartcide®, *N*–(4–fluorophenyl) –2,3–dichloromaleimide], is used as a promising fungicide against diseases of apple (alternaria leaf spot: *Alternaria mali*, etc.), citrus (scab: *Elsinoe fawcetti*, etc.), grape (downy mildew: *Plasmopara viticola*), coffee tree (berry disease: *Colletorichum coffeanum*) and rubber plant (pink disease: *Corticium salmonicolor*).[1,2]

It has been reported that the inhibitory activity of Fluoroimide against spore germination or appressorial formation on *Pyricularia oryzae* is extremely high and this compound reacts with sulfhydryl group of cellular component.[3] As to the fate of Fluoroimide, absorption, distribution and excretion by rats have been studied.[4,5]

This paper deals with absorption, translocation and metabolism of Fluoroimide on/in Inu-apple tree which is a similar species of apples to be protected.

MATERIALS AND METHODS

1. *Plant*

The leaves and fruits of ten year-old Inu-apple trees (*Malus prunifolia* BORKHOUSEN) at falling time of blossoms and fructifing time were used for the experiments. Crossing was carried out at the flowering time in a growth chamber (Koito Ind. Ltd., Japan; about 20,000 lx). The illumination time was set for the day time (11 hr) from July to September.

2. *Chemicals*

Two kinds of ^{14}C–Fluoroimide were obtained from Daiichi Pure Chemicals Co. Ltd., Japan; one was labeled at benzene ring (BR–Fluoroimide; specific activity 5.2 mCi/mmol, radiochemical purity>99%) and the other was labeled at carbonyl group (CO–Fluoro-

* Fate of Fluoroimide, *N*–(4–Fluorophenyl)–2,3–dichloromaleimide (Part 1)
Presented at the Fourth International Congress of Pesticide Chemistry (IUPAC), July 24–28, 1978, Zürich, Switzerland.

imide; specific activity 3.9 mCi/mmol, radiochemical purity>99%). The name and structure of Fluoroimide and related compounds synthesized and their properties are shown in Table 1 and 2. β-Glucosidase (Almonds; 4.97 u/mg) was obtained from Worthington Biochem., U.S.A. Dain, a sticker, was obtained from Takeda Chemical Ind. Ltd., Japan.

3. Preparation of ^{14}C-Fluoroimide Solution

The 1,000 ppm Fluoroimide aqueous solution was prepared by homogenizing 64 mg of a wettable powder containing 78% of ^{14}C-Fluoroimide, 5% of surfactants and 17% of carrier, with one ml of water by a glass homogenizer and by diluting to 50 ml with water. For the absorption and translocation experiments, 750 ppm Dain aqueous solution was used in the place of water.

4. Radioanalysis and Autoradiography

Radioactivity was measured by liquid scintillation spectrometer (*lsc*; TRI-CARB 3385, Packard Instrument Co. Inc., U.S.A.) using 15 ml of the scintillator A (4 g PPO and 0.1 g POPOP in 1 liter of toluene) or the scintillator B (6 g PPO, 0.27 g POPOP, 112 g naphthalene, 20 ml ethylene glycol, 60 ml methanol and 920 ml of dioxane). Unextractable residues in the plant pulps were treated by an automatic sample oxidizer (TRI-CARB 306, Packard) and determined by *lsc*. Autoradiograms (*arg*) of the plant and the developed thin-layer chromatographic (*tlc*) plates were taken by exposing to X-ray film for 10 days at 4°C.

5. Thin-layer Chromatography

Precoated *tlc* plates with 0.25 mm thick silica-gel 60 F_{254} (E. Merck, W. Germany) were used. The developing solvent systems used were; A: benzene, B: ethanol/chloroform (1/19), C: ethanol/chloroform (1/3), D: acetic acid/ethyl acetate (1/99), E: ether/*n*-hexane

Table 1 Fluoroimide and its related compounds.

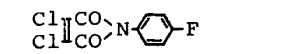
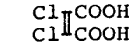
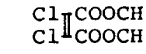
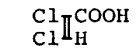
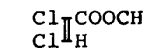
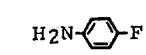
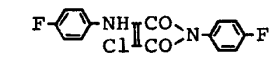
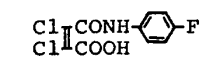
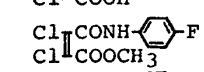
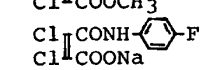
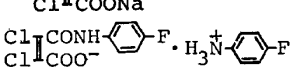
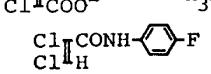
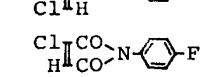
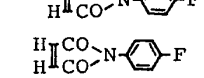
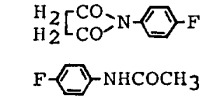
Compound No.	Chemical structure	Chemical name
Fluoroimide		*N*-(4-Fluorophenyl)-2,3-dichloromaleimide
I		Dichloromaleic acid
II		Dimethyldichloromaleate
III		(*E*)-2,3-Dichloroacrylic acid
IV		Methyl (*E*)-2,3-dichloroacrylate
V		4-Fluoroaniline
VI		2-Chloro-3-(4-fluorophenylamino)-*N*-(4-fluorophenyl) maleimide
VII		4-Fluorodichloromaleanilic acid
VIII		Methyl 4-fluorodichloromaleanilate
IX		Sodium 4-fluorodichloromaleanilate
X		4-Fluoroaniline salt of 4-fluorodichloromaleanilic acid
XI		(*E*)-2,3-Dichloro-*N*-(4-fluorophenyl)acrylamide
XII		*N*-(4-Fluorophenyl) monochloromaleimide
XIII		*N*-(4-Fluorophenyl) maleimide
XIV		*N*-(4-Fluorophenyl) succinimide
XV		4-Fluoroacetonanilide

Table 2 Physical properties of Fluoroimide and its related compounds.

Compound No.*	mp or bp (°C)	IR (cm^{-1})	MS (*m/e*)	NMR (ppm)
Fluoroimide	m: 240.5–241.8	1705, 1620, 1595 (KBr)	259	7.16 (2H, t), 7.32 (2H, m) $CDCl_3$+DMSO–d_6
I		3200, 1755, 1715, 1605 (KBr)		
II		2960, 1745, 1600 (liq. film)	212, 181	3.83 (s) CCl_4
III	m: 83–84	3400–2100, 1720, 1580 (KBr)	140,105	3.84 (s) CCl_4
IV		2960, 1750, 1600 (KBr)	154, 119	
V	b: 188	3325, 1210, 825 (liq. film)	111	
VI	m: 203–204	1780, 1710, 1665, 1610 (KBr)	334	7.12 (2H, t), 7.26 (2H, m) $CDCl_3$+DMSO–d_6
VII	m: 217	3600–2200, 1650, 1620 (KBr)	277, 111	7.00 (2H, m), 7.95 (2H, t) $CDCl_3$+MeOH–d_4
VIII	m: 148–149	1795, 1715, 1640, 1595 (KBr)	291, 259	3.86 (2H, s), 7.00 (2H, t), 7.50 (2H, q), 8.01 (1H, m) $CDCl_3$
IX		3680–2800, 1640, 1540 (KBr)	334, 123	6.97 (2H, t), 7.55 (2H, m) MeOH–d_4
X	m: 171–174	3300–2200, 1655, 1575 (KBr)	259, 111	
XI	m: 82.5	3260, 1650, 1610, 1595 (KBr)	233, 123	7.03 (2H, m), 7.52 (2H, m), 7.75 (1H, s) $CDCl_3$
XII		3110, 1790, 1730, 1605 (KBr)	225	6.78 (1H, s), 7.05 (2H, t), 7.32 (2H, m) $CDCl_3$
XIII		3110, 1715, 1705, 1585 (KBr)	191, 157	6.81 (2H, s), 7.14 (2H, t), 7.32 (2H, m) $CDCl_3$
XIV		3080, 1765, 1710, 1515 (KBr)	193, 111	1.70 (4H, s), 6.80 (2H, t), 7.12 (2H, t) Benzene–d_6
XV		3230, 1555, 1500, 1210 (KBr)	153	2.06 (3H, s), 6.92 (2H, t), 7.35 (2H, m), 7.40 (1H, s) $CDCl_3$

* cf. Table 1.

Table 3 *Rf* values of Fluoroimide and its related compounds on silica-gel *tlc*.

Compound No.*	*Rf* values in solvent system**				
	(A)	(B)	(C)	(D)	(E)
Fluoroimide	0.47	0.71	0.83	0.66	0.48
I	0.00	0.00	0.07	—	—
II	0.33	—	—	—	0.59
III	0.01	0.13	—	0.41	—
IV	—	—	—	—	0.48
V	0.11	0.40	0.71	0.50	—
VI	0.23	0.68	—	0.67	—
VII	0.00	0.00	0.19	0.07	—
VIII	0.47	0.71	0.83	0.66	—
IX	0.00	0.00	0.19	0.07	—
X	0.00	0.00	0.18	—	—
XI	0.33	0.68	0.79	0.65	0.39
XII	0.31	0.67	—	0.63	—
XIII	0.16	0.63	—	0.58	—
XIV	0.03	0.48	0.79	0.41	0.14
XV	0.01	0.18	0.67	0.56	—

* cf. Table 1.

** (A): benzene, (B): ethanol/chloroform (1/19), (C): ethanol/chloroform (1/3), (D): acetic acid/ethyl acetate (1/99), (E): ether/*n*–hexane (1/1).

(1/1). The *Rf* values for Fluoroimide and related compounds in these solvent systems are shown in Table 3.

6. Absorption and Translocation on Leaves

^{14}C–Fluoroimide solution was applied at the center of both sides of a leaf at flowering time using a pair of tweezers with urethane foam tip (1 cm×4 cm). After one, 2, 3, 9, 21 and 45 days, the leaf was excised to monitor the translocated radioactivity by *arg* method.

To determine the translocated amount of radioactivity, ^{14}C–Fluoroimide solution was applied at the rate of 50 μg (1 μCi) per leaf to basal part of either side of a leaf using microsyringe. The leaf was excised after 9 days and divided into the applied part and the translocated part which was cut one cm apart from the applied part. These two parts were extracted with 30 ml of dichloromethane and then with 30 ml of methanol.

Radioactivities in these extracts and unextractable residues were determined by *lsc*.

7. Metabolism on Leaves and Fruits

At fructifing time, ^{14}C–Fluoroimide solution was applied on the leaves and fruits by spotting 50 μg (1 μCi) per leaf or fruit by microsyringe.

7.1 Extraction

After one, 3, 9, 21, 45 and 93 days, three leaves and two fruits were excised and washed respectively by dipping in 50 ml ether for one min, and then extracted successively with 50 ml dichloromethane, 45 ml methanol and 40 ml water for 5 min at 3,000 rpm in homogenizer (Nihon Seiki Co. Ltd., Japan). Then, radioactivities of surface washes, dichloromethane extracts, methanol–water extracts and unextractable residues were measured.

7.2 Hydrolysis

A half of methanol–water extracts was evaporated to dryness, and hydrolyzed with 4.4 mg of β–glucosidase in 4 ml of 0.1 M phosphate buffer (pH 4.7) at 38°C for 3 hr and extracted three times with 3 ml ether. The residual solution was subjected to hydrolysis again under 2 N HCl acidic condition at 80°C for 3 hr and extracted in the same manner. In the case of BR–Fluoroimide, the residual aqueous solution was re-extracted with ether at pH 10.

7.3 Metabolites

The radioactive metabolites in those extracts were detected and identified by cochromatography with authentic samples on *tlc*, and analyzed by *lsc*.

Main metabolite (IX) was obtained from methanol extracts of Inu-apple leaves on which a large amount of unlabeled Fluoroimide was applied and which were excised 9 days after treatment. Since reduced metabolite (XIV) is formed more on bean plant leaves (about 3%) than on Inu-apple leaves, it was obtained from ether extracts of the bean leaves on which unlabeled Fluoroimide was applied and which were excised 3 days after treatment. These metabolites were purified by preparative *tlc* using the following solvent systems; ethanol/chloroform (1/2) [(IX) shows the *Rf* value 0.22] and benzene followed by ethanol/chloroform (1/9) [(XIV) shows the *Rf* value 0.30]. These metabolites were identified by GC–MS (Hitachi M–52, Hitachi Ltd., Japan), IR (Hitachi 295), NMR (Hitachi R–24, 60 MHz) and fluorescence X-ray (FX; Philips PW–1450–AHP, Netherlands).

RESULTS

1. Absorption and Translocation on Leaves

Fluoroimide remained at the applied portion of the leaves one day after treatment, but translocated upward slowly after 9 days and uniformly distributed in the upper part of the leaves after 21 days, as shown in Fig. 1.

The results of translocation of ^{14}C–Fluoroimide in the leaves were shown in Table 4. More radioactivity was observed in the translocated part when treated on the back of the leaves than on the face. However, it was at most 2.3% even when treated on the back, and only 1.5% was translocated as Fluoroimide. Fluoroimide decomposed into unextractable residues more easily on the back than on the face.

2. Metabolism on Leaves and Fruits

Figure 2 shows the distribution of ^{14}C in the case of leaves and fruits treatments. Eighty-five percent of the residual radioactivity was in the both surface washes after

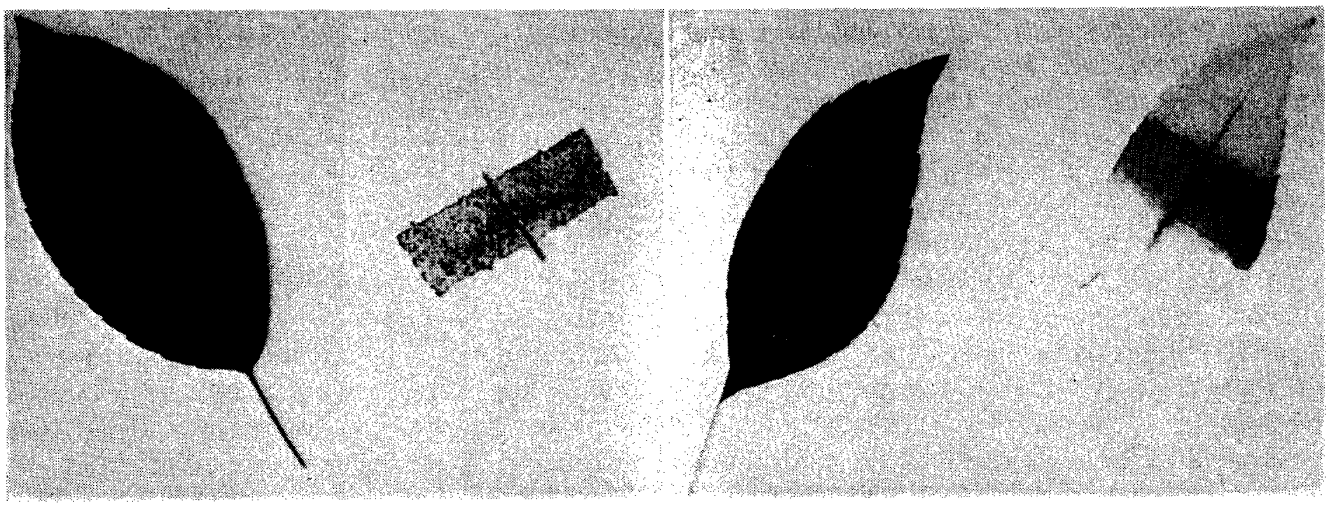


Photo. arg
After one day

Photo. arg
After 21 days

Fig. 1 Autoradiograms of Inu-apple leaf treated with ^{14}C–Fluoroimide

Table 4 Translocation of ^{14}C–Fluoroimide on leaves after 9 days.

Labeled compound*		Percent of ^{14}C			
		Face application		Back application	
		Applied part	Translocated part	Applied part	Translocated part
BR	Extracts**	95.6	1.6	92.7	2.1
	(Fluoroimide	60.9	1.0	53.9	1.5)
	Unextractable residues	2.6	0.2	5.0	0.2
	Total	98.2	1.8	97.7	2.3
CO	Extracts**	97.7	0.4	90.0	1.2
	(Fluoroimide	75.3	0.2	55.2	0.9)
	Unextractable residues	1.8	0.1	8.3	0.5
	Total	99.5	0.5	98.3	1.7

* BR: benzene ring labeled Fluoroimide, CO: carbonyl labeled Fluoroimide.
** dichloromethane and methanol extracts.

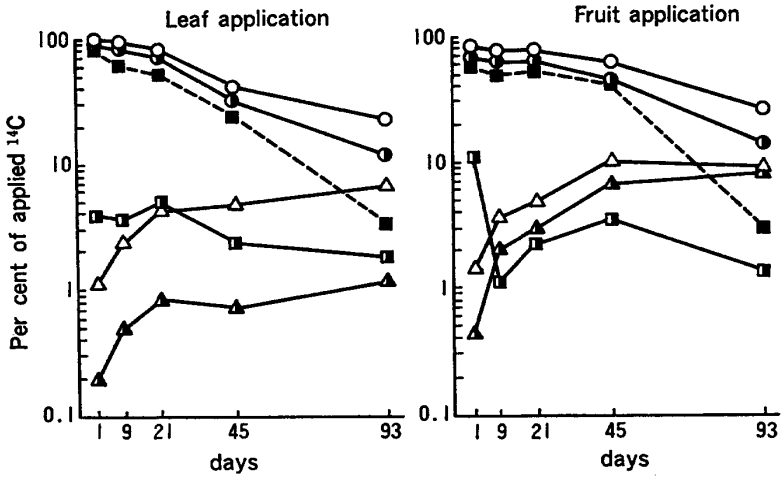


Fig. 2 Distribution of radiocarbons after treatment of ^{14}C–Fluoroimide to Inu-apple trees.
○: total ^{14}C, ◑: surface washes, ◨: dichloromethane extracts, △: methanol-water extracts,
▲: unextractable residues, ■: Fluoroimide

45 days. Methanol–water extracts and unextractable residues increased gradually and were more on the fruits than on the leaves. This shows that the fruits can absorb and metabolize Fluoroimide more easily than the leaves. When methanol–water extracts from leaves and fruits were hydrolyzed with *β*–glucosidase and then with acid, the respective radioactivities of hydrolyzed extracts from ^{14}C–(BR)–Fluoroimide were 13% and 66% and those from ^{14}C–(CO)–Fluoroimide were 8% and 33%.

Figure 2 also shows the half life of Fluoroimide, which is about 20 days on the leaves and 30 days on the fruits. About 97% of radioactivity in surface washes at the half life time was Fluoroimide.

Sixteen kinds of radioactive spots in all the extracts were detected by *tlc–arg* method, 7 of which were identified as Fluoroimide, (V), (VI), (IX), (XI), (XIII) and (XIV) by co-chromatography, MS and GC–MS.

Although (VI) was found small amount in surface washes, it can not be supported as a metabolite because it is formed about one percent from Fluoroimide after one hour on *tlc* plate[6] and the surface washes contain large amount of Fluoroimide.

The metabolite (IX) was found to have 4–fluorophenyl and dichloroethylene moieties by MS spectrum (*m/e* 111: H_2N-C$_6$H$_4$-F , *m/e* 233: ClCH=CCl-CONH-C$_6$H$_4$-F) and NMR spectrum [$\delta_{TMS}^{MeOH\text{-}d_4}$; 6.97 (2H, triplet), 7.55 ppm (2H, multiplet): -NH-C$_6$H$_4$-F], and carbonyl ion by IR spectrum (ν_{max}^{KBr}; 1620 cm^{-1}) and sodium by FX (Na; calculated 8.2%, found 6.8%). IR and MS spectra of the metabolite agreed with those of authentic sample (IX) as shown in Fig. 3. From these results, it has become apparent that the metabolite was sodium salt of (VII).

The metabolite (XIV) was found to have imide ring by IR spectrum (ν_{max}^{KBr}: 1765, 1710, 1515, 1395, 1180 cm^{-1}) and did not contain chlorine atom and was presumed to have succinimide ring and 4–fluorophenyl moieties from its MS fragment peaks (*m/e* 193, 165, 137, 111). The spectra of the metabolite agreed with those of authentic sample (XIV).

Table 5 shows the amount of extractable Fluoroimide and its metabolites in the leaves and fruits after 21 days. A main metabolite was (IX) in surface washes. (V) was obtained by acid hydrolysis of methanol–water extracts and (XIV) was detected mainly in dichloromethane extracts. U–8 (an unknown meta-

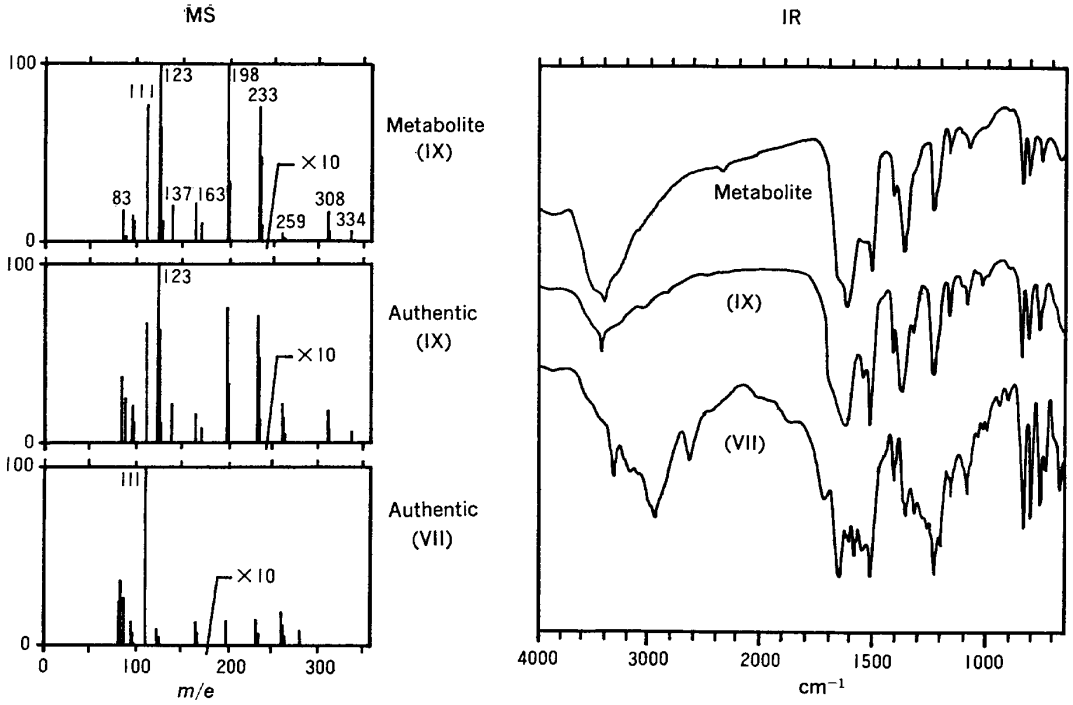


Fig. 3 MS and IR spectra of main metabolite (IX).

Table 5 Extractable* ^{14}C–Fluoroimide and its metabolites on/in Inu-apple trees after 21 days.

Fluoroimide and its metabolites	Detection**			Percent of applied ^{14}C			
				Leaf application		Fruit application	
	SW*	DM*	MW*	BR	CO	BR	CO
Fluoroimide	++	+	+	66.2	38.2	43.5	73.3
V	−	−	+	0.6	—	0.3	—
VI	+	−	−	0.1	0.1	0.0	0.3
IX	++	+	+	16.1	23.6	14.2	2.8
XI	−	−	−	0.0	0.1	0.1	0.0
XIII	−	−	−	0.1	0.1	0.0	0.3
XIV	−	+	+	0.4	0.4	1.0	0.4
U-8	−	+	+	0.2	0.1	1.0	0.6
Others	+	+	+	0.7	1.7	2.0	0.9
Total				84.4	64.3	62.1	78.6

* Surface washes (SW), dichloromethane extracts (DM) and methanol-water extracts (MW).

** −: not detected and less than 0.1%, +: 0.1–2%, ++: more than 2%, as average of the results obtained after 9, 21 and 45 days.

bolite), *Rf* values of which by solvent A and C were 0.00 and 0.11, respectively, was detected mainly in hydrolyzed extracts of methanol-water extracts.

DISCUSSION

Fluoroimide decreased to 50% in 20 and 30 days on the leaves and fruits of Inu-apple trees respectively under our experimental condition.

Eighty-five percent of residual radioactivity was derived from Fluoroimide and a main metabolite (IX). The amount of (IX) is considered to vary by extent of adherence on the plants. When in the field conditions, dissipation of these compounds from the plants should be more accelerated by such physico-chemical factors as wind, sunlight and rainfall, because (1) Fluoroimide adheres loosely, and is absorbed and translocated very little, (2) (IX) is very soluble in water. Consequently, fate of Fluoroimide and (IX) in the soils must be studied.

As reported, some *N*–phenylimides were easily hydrolyzed to form anilamic acids as a main metabolite in Ohric[7] and Sumilex,[8,9] the latter of which was unstable on silica-gel *tlc* plate where reversible reaction of ring-cleavage and -closure of the imide was observed. Half life of Fluoroimide on silica-gel *tlc* plate was 4 hr[6] and the resulting maleanilic

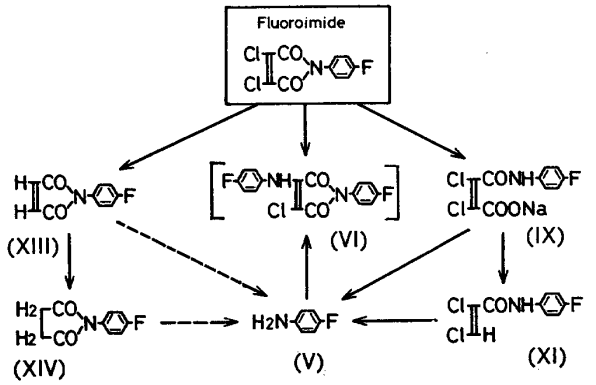


Fig. 4 Tentative metabolic pathways on/in Inu-apple trees.

acid (VII) showed the same *Rf* values with those of (IX) on *tlc*. Therefore, (IX) must be separated quickly and analyzed from Fluoroimide which decomposes to (VII) on silica-gel *tlc* plate. (IX) was formed from sodium and dissociated (VII) on the plants by water and sunlight. Sodium of (IX) was presumably derived from the plant surface, because the plants generally contain several hundred ppm of sodium ion as a usual component.

Since (XIII) and (XIV) were not detected in the surface washes but were found in the dichloromethane extracts, it is apparent that they were the resulting metabolites within the plants. Succinimide moiety of (XIV) is supposed to be derived not from the component in the plants but from the dichloroethylene moiety of Fluoroimide which might undergo

enzymatic reduction as was observed in DDT metabolites.[10] This was suggested by the fact that the amount of (XIV) produced from BR–Fluoroimide was relatively equivalent to that from CO–Fluoroimide.

Although Fluoroimide reacts with thiol compounds[3] and some of the metabolites of organochlorine pesticides are glutathione conjugates in plants,[11] such conjugates of Fluoroimide were not detected.

From these results, tentative metabolic pathways of Fluoroimide on/in Inu-apple trees are proposed as shown in Fig. 4.

ACKNOWLEDGEMENT

The authors wish to thank Mr. H. Kasugai and Mr. M. Tomita for the aids and advice about planting.

REFERENCES

1) Ministry of Agriculture and Forestry: "Nōyaku Yōran, 1977," p. 344, Japan Plant Protection Association, Tokyo, 1977 (in Japanese)
2) Kumiai Chem. Ind. Co. Ltd. and Mitsubishi Chem. Ind. Ltd.; "Japan Pesticide Information," Vol. 34, p. 26, Japan Plant Protection Association, Tokyo 1978
3) M. Tsuda, T. Nakajima, H. Kasugai, I. Yamaguchi & T. Misato: *J. Pesticide Sci.* **1**, 101 (1976)
4) H. Inoue, T. Kitagaki, R. Shinohara, T. Murata, K. Kojima, K. Matsui, H. Aizawa & K. Kusama: The Abstracts of the First Meeting of the Pesticide Science Society of Japan, No. 205 (1976)
5) R. Shinohara, H. Inoue, I. Sakurai, T. Kitagaki, K. Kojima, K. Matsui, H. Aizawa & K. Kusama: *ibid*, No. 206 (1976)
6) H. Aizawa & M. Nakazawa: Unpublished (1977)
7) H. Ohkawa, Y. Hisada, N. Fujiwara & J. Miyamoto: *Agric. Biol. Chem.* **38**, 1359 (1974)
8) N. Mikami, H. Yamamoto, Y. Tomida & J. Miyamoto: The Abstracts of the Second Meeting of The Pesticide Science Society of Japan No. 148 (1977)
9) N. Mikami, H. Satogami & J. Miyamoto: *J. Pesticide Sci.* **4**, 165 (1979)
10) C. M. Menzie: "Metabolism of Pesticides," p. 128, Bureau of Sport Fisheries and Wildlife, Washington, 1969
11) D. H. Hutson: "Bound and Conjugated Pesticide Residues," ed. by D. D. Kaufman, G. G. Still, G. D. Paulson and S. K. Bandal, p. 103, ACS, Washington, 1976

要　　約

イヌリンゴにおけるフルオルイミドの吸収，移行および代謝

小川邦彦，相沢宏保，山内文雄

殺菌剤，フルオルイミド［*N*-(4-Fluorophenyl)-2,3-dichloromaleimide，スパットサイド®］のイヌリンゴにおける挙動を芳香環またはカルボニル基を ^{14}C で標識した化合物を用いて検討した．

フルオルイミドは，葉や果実に塗布したとき，それぞれ約 20 日と約 30 日の半減期を示し，処理葉内への吸収，移行性は非常に小さく，9 日後でもわずか 2% 以下であった．吸収・移行した放射能は，93 日後の葉において約 9%，果実において約 18% に達し，取込みや代謝性が後者でよい傾向が見られた．

フルオルイミドの主代謝物はイミド環の加水分解物で，MS，IR，NMR および螢光 X 線法により sodium 4-fluorophenyldichloromaleanilate で存在することを同定，確認した．他に少量の 4 種の代謝物，4-fluoroaniline，(*E*)-2,3-dichloro-*N*-(4-fluorophenyl) acrylamide，*N*-(4-fluorophenyl) maleimide および *N*-(4-fluorophenyl) succinimide［A］，が確認でき，［A］のコハク酸部分は植物由来ではなく，ジフロルマレイミドの還元的変換生成物であることが明らかとなった．