Absorption, Translocation and Metabolism of 2-*tert*-Butyl-4-(2,4-dichloro-5-isopropoxyphenyl)-4²-1,3,4-oxadiazolin-5-one (Oxadiazon) in Rice Plants

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Received January 30, 1975

Absorption, translocation and metabolism of 2-*tert*-butyl-4-(2,4-dichloro-5-isopropoxyphenyl)-4²-1,3,4-oxadiazolin-5-one (oxadiazon) in rice plants were investigated. Three types of ¹⁴C-labeled oxadiazon were used in this study. ¹⁴C-Labeled oxadiazon was applied to soil under submerged conditions and plants were taken for the preparations at various stages of growth and development. Oxadiazon was translocated remarkably to shoots and accumulated in the lower leaves and stems. A small portion of oxadiazon intaken in plants was translocated to head parts. Oxadiazon was chemically transformed in plants to produce dealkylated compounds, oxidized alcohol and carboxylic acid as metabolites. In addition, two unidentified metabolites were detected, one of which was translocated to head parts from leaves and stems. Translocation and accumulation of oxadiazon and its metabolites were discussed in relation to physiological conditions of rice plants.

The problem of pesticide residues in harvested crops is becoming a matter of great concern, especially with regard to public health. Investigation of metabolism of pesticides and determination of their terminal residues in plants are considered to be important for the problem of the pesticide residues.

Rice is one of the most popular cereals in the world, and producers have kept yields of rice high by using fertilizers and pesticides in large quantities, combined with their improved agricultural techniques. For proper use of pesticides in rice production, intensive studies are necessary not only to determine their efficiency as pesticides, but also to clarify their behaviour in rice plants as well as in the environment where they might accumulate.

The three steps needed in this type of investigation should be emphasized. The first step is to make a quantitative determination of the concerned pesticide in rice plants, especially in the edible portion, the second is to trace the

metabolic products of the pesticide in the plants, and the third is to clarify plant-physiological significance of the results obtained on the fate of the pesticide.

2-*tert*-Butyl-4-(2,4-dichloro-5-isopropoxyphenyl)-4²-1,3,4-oxadiazolin-5-one (oxadiazon), a product of Société des Usines Chimiques Rhône-Poulenc, has been developed as a herbicide in rice fields by Nissan Chemical Industries, Ltd. Oxadiazon, which contains a N-heterocyclic ring structure, is a new type of a herbicide in regard to chemical structure and few works have been published on its behaviour in plants.^{1,3,7)}

In the present work the fate of oxadiazon in rice plants is studied with respect to its absorption, translocation and metabolic transformation concerned with the development and growth of the plants.

MATERIALS AND METHODS

1) *Plants.* Seeds of rice (*Oryza sativa* L. v. *kimmaze* or v. *nihombare*) were surface-sterilized with 0.1% aqueous solution of methoxyethylmercuric chloride fungicide (Uspurun) for 6 hr. After washing

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several times with water, they were seeded in a seedling-tray and grown in a controlled environmental room (phytotron). About a month later, rice seedlings (*O. sativa* L. v. *kimmaze*) at the 4 to 5 leaf-stage were transplanted to 1/2000 are-sized Wagner's round pots (25 cm in diameter $\times$ 30 cm deep) which were filled with submerged soil. The plants were grown to the ripening stage in the phytotron. Temperature in the phytotron was regulated between 18°C and 35°C adjusted properly for the development and growth of the rice plants under natural light. In each pot 7 kg of Tanashi volcanic-ash soil, passed through 5 mm sieve, were filled in addition to 3 kg of sand and 3.5 kg of gravel in the bottom, and 6 g of a compound fertilizer (N 15-P 15-K 15) and 5 g of 20% super phosphate were applied. Three bundles of rice consisting of 3 seedlings were separately planted. No leaching of water from the bottom of the pot was tried during the rice culture.

For water culture rice seedlings (*O. sativa* v. *nihom-bare*) at the 4 to 5 leaf-stage were transplanted to culture solution from a seedling-bed and kept grown further in the phytotron. Water culture was carried out by the Kasugai's cultural system²¹ in 1/5000 are-sized Wagner's round pots.

2) ¹⁴C-Labeled oxadiazon. Three types of oxadiazon labeled with carbon-14 at the different positions in the molecule were used. They were oxadiazolin-2-¹⁴C, oxadiazolin-5-¹⁴C and phenyl-universal-¹⁴C labeled oxadiazon. Their radiochemical properties are listed in Table I. The labeled compounds abbreviated as Oxa-1 and Oxa-3 in Table I were synthesized and purified by Nissan Chemical Industries, Ltd. and the one as Oxa-2 by Société des Usines Chimiques Rhône-Poulenc. Oxa-2 was not purified further and contained about 2.5% of impurities.

3) *Radioassay.* ¹⁴C-Labeled compounds were assayed by a Beckman LS-150 liquid scintillation spectrophotometer. For measurement, an aliquot of 0.1 to 1.0 ml solution containing radioactive carbons was mixed with 15 ml of scintillator solution (4.0 g of 2,5-diphenyloxazole (POP) and 100 mg of 1,4-bis-2-(5-phenyloxazolyl) benzene (POPOP) dissolved in 1 liter of toluene). Quenching occurred by water, ethanol or plant-originated coloured substances existing in sample preparations was corrected by the external standard method. Macro-radioautography of rice plants was carried out with Fuji no screen X-ray films. X-ray films were exposed ordinarily for 30 days, and in particular cases for 80 days.

4) *Isotope dilution method.* Quantitative determination of oxadiazon in hulled rice grains was precisely conducted by means of the isotope dilution method, of which procedures are described in Fig. 1.

5) *Thin-layer chromatography (TLC).* Thin-layer chromatography was performed on plates coated with silica-gel GF 254 (Merk) in 0.5 mm thickness. A mixture of (i) cyclohexane, chloroform and acetic acid (11:9:1 v/v) was mainly used as a developing solvent and supplementally were used other solvents such as (ii) *n*-hexane and acetone (4:1 v/v), (iii) benzene and ethylacetate (4:1 v/v), (iv) cyclohexane and chloroform (1:1 v/v) and (v) cyclohexane and ethylacetate (9:1 v/v). Detection of compounds was conducted by UV absorption and identification was made by means of co-chromatography. Chemical structures and *R_f* values of oxadiazon and its related compounds are shown in Table II and Table III. Out of compounds listed in Table II from A-4 to A-15 were supplied by Société des Usines Chimiques Rhône-Poulenc and others were supplied by Nissan Chemical Industries, Ltd.

TABLE I. RADIOCHEMICAL PROPERTIES OF ¹⁴C-LABELED OXADIAZON

Abbreviation	¹⁴ C-Labeled oxadiazon	Specific activity	Radiochemical purity
Oxa-1	oxadiazolin-2- ¹⁴ C	1.8 mCi/mmole	99%
Oxa-2	oxadiazolin-5- ¹⁴ C	42 mCi/mmole	97.5%
Oxa-3	phenyl-U- ¹⁴ C	0.57 mCi/mmole	99%

* Indicates the position labeled with carbon-14.

FIG. 1. Quantitative Determination of Oxadiazon in Hulled Grain by the Isotope Dilution Method.

6) *Column chromatography.* For identification of compounds a technique of column chromatography was supplementarily adopted. Substituted compounds of 4-phenyl- β^2 -1,3,4-oxadiazolin-5-one by hydroxyl or carboxyl group were in most cases adsorbed by activated florisil. Florisil (100~200 mesh) was activated at 140°C for 4 hr and applied on a glass-column (10 mm i.d. $\times$ 30 cm long). A small quantity of anhydrous sodium sulfate was laid on the top of florisil column. Elution was carried out with either (i) benzene, (ii) *n*-hexane and dichloromethane (1:1 v/v), (iii) benzene and ether (9:1 v/v), or (iv) benzene and ethylacetate (4:1 v/v). Derivatives of 4-phenyl- β^2 -1,3,4-oxadiazolin-5-one, which are substituted by either hydroxyl or carboxyl group, are hardly eluted by these solvents.

7) *Extraction and analytic methods for plant preparations.* ^{14}C -Labeled oxadiazon was applied to soil under paddy field condition a day before transplanting. One and 2 months after transplanting, plants were sampled from the pots and washed with water to remove soil particles from roots. Then plants were separated into roots and shoots. Each plant tissue preparation was cut into small pieces and dipped in methanol for

a week. The methanol extract was filtered through a glass filter (25G-4) by suction and residual tissues were washed and extracted with methanol again. The methanol extract combined with methanol-washing was concentrated *in vacuo* at 40°C or below to a definite volume. An aliquot of 0.1 to 1.0 ml of the concentrated extract was taken into a vial for the measurement of radioactivity by a liquid scintillation spectrophotometer.

Oxadiazon and its metabolic products in the methanol extracts were separated by thin-layer chromatography. TLC was carried out under the conditions mentioned before. For comparison, authentic oxadiazon and its related compounds were spotted on both ends of the silica-gel coated plate. The plate having developed was exposed to an X-ray film to make a radioautography. Detection by UV absorption was also conducted. Silica-gel in the areas of oxadiazon and its metabolites on the TLC plates was scraped off separately and extracted with acetone, and their radioactivities were measured.

8) *Extraction and analytic methods for rice grains and straws.* The rice plants applied with ^{14}C -labeled oxadiazon (Oxa-1) on 2nd August, 1971 were harvested on 23rd November, 1971 and those applied with Oxa-2 and Oxa-3 on 16th September, 1971 were harvested on 23rd March, 1972. In both cases, rice plants grew well and obtained good yields equivalent to 60 kg/a as hulled grains. Hulled rice grains, husks, rachises, culms, leaf-sheaths and flag-leaves were separated for further preparations and crushed respectively by a mill. These materials were dipped for a week in a sufficient amount of acetone and ethanol, combined with a little amount of hydrochloric acid (1:1:0.01 v/v). The extract was collected by filtration through a glass filter, and the plant tissues were washed again with a mixture of acetone and ethanol. Identification and quantitative determination of oxadiazon and its related compounds were made as same as mentioned in 7).

As radioactivity in hulled rice grains was very low and difficult to be determined, application of the isotope dilution method was helpful to confirm the precise quantities of oxadiazon in the hulled rice grains.

9) *Absorption, translocation and metabolism studies with ^{14}C -labeled oxadiazon in water culture*

a) *Absorption and translocation by roots.* An emulsifiable concentrate of 10% oxadiazon was prepared by mixing oxadiazon (Oxa-1), Sorpol 2680 R (emulsifier) and xylene in a weight ratio of 10:5:85. This stock emulsion was diluted with water to contain 0.1 ppm of the active ingredient of oxadiazon. Roots of 3 intact seedlings at the 4 to 5 leaf-stage grown by water culture were dipped in the emulsified oxadiazon solution (2.2 liters) for definite periods. Five hours, 1, 4, 7 and 10 days after the application ^{14}C -radioactivities in various parts of plant tissues were determined by

TABLE II. A LIST OF OXADIAZON AND ITS RELATED COMPOUNDS
SYNTHESIZED FOR THIS STUDY

Abbreviation	Chemical structure	R 1	R 2	R 3
A-1 (oxadiazon)		-OCH(CH ₃) ₂	-C(CH ₃) ₃	
- 2		-OCH ₃	-C(CH ₃) ₃	
- 3		-OH	-C(CH ₃) ₃	
- 4		-OCH(CH ₃) ₂	-C(CH ₃) ₂ COOH	
- 5		-OCH ₃	-C(CH ₃) ₂ COOH	
- 6		-OH	-C(CH ₃) ₂ COOH	
- 7		-OCHCH ₃ CH ₂ OH	-C(CH ₃) ₃	
- 8		-OCH(CH ₃) ₂	-C(CH ₃) ₂ CH ₂ OH	
- 9		-OCH(CH ₃)COOH	-C(CH ₃) ₃	
-10		-OH	-C(CH ₃) ₂ CH ₂ OH	
-11		-OCH(CH ₃) ₂	-CH(CH ₃) ₂	
-12		-OCH(CH ₃) ₂	-CH ₂ CH ₃	
-13		-OCH ₂ CH ₃	-C(CH ₃) ₃	
-14		-OCH ₂ CH ₃	-CH(CH ₃) ₂	
-15		-OCH ₂ CH ₃	-CH ₂ CH ₃	
B- 1		-OCH(CH ₃) ₂		
- 2		-OCH ₃		
- 3		-OH		
C- 1		-OCH(CH ₃) ₂		
- 2		-OCH ₃		
D- 1		-OCH(CH ₃) ₂		-NO
- 2		-OCH(CH ₃) ₂		-NO ₂
- 3		-OCH(CH ₃) ₂		-NH ₂

TABLE III. *R_f* VALUES OF OXADIAZON AND ITS RELATED COMPOUNDS
IN THIN-LAYER CHROMATOGRAPHY

Solvents ^{a)}	(i)	(ii)	(iii)	(iv)	(v)
Compounds ^{b)}					
A-1 (oxadiazon)	0.42	0.60	0.68	0.35	0.41
A- 2	0.32	0.47	0.65	0.26	0.27
A- 3	0.22	0.21	0.43	0.02	0.07
A- 4	0.21	0	0.02	0	0
A- 5	0.18	0	0.01	0	0
A- 6	0.03	0	0	0	0
A- 7	0.21	0.24	0.30	0	0.03
A- 8	0.21	0.24	0.27	0	0.03
A- 9	0.20	0	0.02	0	0
A-10	0.04	0.05	0.06	0	0
A-11	0.34	0.55	0.64	0.31	0.32
A-12	0.27	0.47	0.62	0.23	0.24
A-13	0.39	0.55	0.67	0.32	0.34
A-14	0.35	0.49	0.64	0.26	0.26
A-15	0.28	0.43	0.61	0.21	0.18
B- 1	0.24	0.34	0.43	0.04	0.10
B- 2	0.21	0.28	0.35	0.04	0.07
B- 3	0.11	0.12	0.20	0	0.02
C- 1	0.30	—	0.50	—	0
C- 2	0.27	—	0.43	—	0
D- 1	0.13	0.19	0.42	0	0.08
D- 2	0.56	0.66	0.69	0.57	0.53
D- 3	0.29	0.42	0.55	0.28	0.22

^{a)} (i) cyclohexane, chloroform and acetic acid (55:45:5). (ii) *n*-hexane and acetone (4:1). (iii) benzene and ethylacetate (4:1). (iv) cyclohexane and chloroform (1:1). (v) cyclohexane and ethylacetate (9:1).

^{b)} See Table II.

liquid scintillation spectrophotometer and radioautography. Plants for metabolism studies were prepared by exposure to ^{14}C -labeled oxadiazon solution for definite periods.

b) *Metabolism in shoots.* An emulsifiable concentrate of 2% oxadiazon was prepared by mixing oxadiazon (Oxa-2), Sorpol 2680 R, and xylene in a weight ratio of 2:5:93. Oxa-2 was purified and confirmed radiochemically to be a spot in TLC. 0.3 ml of this stock solution added with 0.2 ml of Sorpol W 150 was diluted with 1000 ml of water to contain 5 ppm of the active ingredient of oxadiazon. Actually the concentration of oxadiazon in the diluted solution was exactly determined as 4.87 ppm.

Shoots of 30 intact seedlings at the 4 to 5 leaf-stage were dipped in the diluted solution for 2 hr. Two replications were carried out. Roots of the plants were protected from drying by covering with wetted paper during the application. After the shoots were removed from the solution and washed with water, the treated plants were transferred to a cultural solution and kept grown furthermore. Two, 5, 10, 24, 48, 72, 120 and 168 hr after the transferring, the shoot parts of the plants were cut into small pieces and extracted with 80% methanol. After evaporation of methanol from the extract *in vacuo*, it was diluted approximately 10 times with water and partitioned with dichloromethane.

The dichloromethane fraction was used for further preparations of TLC and quantitative determination was made almost similarly as mentioned in 7). Water fraction was concentrated by lyophilization and ^{14}C -radioactivities in them were determined.

c) *Absorption and translocation by shoots.* A paste containing 100 ppm of oxadiazon was prepared by mixing ^{14}C -labeled oxadiazon (Oxa-1) and vaseline. The paste was painted on either leaf-blades or leaf-sheaths of rice plants. The plants were kept growing further for 1, 3, 5 or 7 days and then used for preparation of radioautography.

10) *Absorption, translocation and metabolism studies with ^{14}C -labeled oxadiazon under paddy field conditions.* An emulsifiable concentrate of 10% oxadiazon was prepared as mentioned before by mixing ^{14}C -labeled oxadiazon (Oxa-1, Oxa-2 or Oxa-3), Sorpol 2680 R and xylene. Three g of the oxadiazon stock solution were diluted with 1000 ml of water and 10 ml of the diluted emulsion were applied to surface of soil filled in a 1/2000 are-sized Wagner's pot and tried to be distributed evenly in soil of 5 cm below the surface by stirring the soil layer. The amount of oxadiazon applied corresponded to 6 g/a. Soil used in this study was collected from a paddy field and air-dried soil was passed through 5 mm sieve. Three ~ four weeks before the application of oxadiazon sieved soil had been kept under submerged

condition in a pot.

A day after the application of oxadiazon, rice seedlings were transplanted and submerged water was kept 3 cm deep. Plants were kept growing in the phytotron until the harvest time. Temperature, humidity and day length were regulated to suitable conditions for the growth development of rice plants. Plants were sampled for preparations of radioautography and metabolism studies 1, 2, 3, and 4 weeks and 2, 4 and 5 ~ 6 months after transplanting.

RESULTS AND DISCUSSION

1) *Absorption and translocation of oxadiazon by roots of rice seedlings*

When diphenyl ether herbicides such as nitrofen* or CNP,* which were considered to have the same phyto-toxicological activity as oxadiazon,³⁾ were applied to germinating shoots directly, they gave injurious brown spot symptoms to stems of rice seedlings as well as growth retardation of whole plants.⁴⁾ On the contrary, when nitrofen or CNP of the same concentration were applied to germinating roots, neither growth retardation nor brown spot symptoms were occurred. Furuya and Arai assumed that nitrofen or CNP was either hardly absorbed by roots or translocated very slightly from roots to shoots. In addition, it was investigated that oxadiazon applied to upland fields was hardly absorbed by roots of crop plants.⁵⁾

In an attempt to clarify whether oxadiazon was intaken by roots of rice seedlings and whether it was distributed to shoot parts in an amount enough for bringing about growth retardation or brown spot symptoms, absorption and translocation of oxadiazon were studied by using seedlings grown in water culture. Root parts of the seedlings were dipped in oxadiazon emulsified solution. As shown in Fig. 2, oxadiazon in a culture solution was absorbed linearly by roots of rice seedlings, although in this particular case only about 5% of oxadiazon applied were absorbed by roots within 10 days. On the other hand, it was observed that oxadiazon once intaken into root

* nitrofen: 2,4-dichlorophenyl-4'-nitrophenyl ether.

* CNP: 2,4,6-trichlorophenyl-4'-nitrophenyl ether.

FIG. 2. Absorption of ^{14}C -Labeled Oxadiazon by Roots of Rice Plants Grown in Water Culture.

tissues was considerably translocated to shoots and distributed in both young and aged leaves. The brown spot symptoms were shown on stems of the treated seedlings.

One, 4 and 10 days after the oxadiazon application, radioautographs of treated plants were taken (Fig. 3-A, -B and -C). Oxadiazon which was translocated from roots to shoots was distributed in aged leaves more heavily than in younger leaves and also distributed more densely in a basal part than in an apical or subapical part of a leaf. In the present study there was little possibility that oxadiazon moved from roots to shoots superficially on the surface of stems by means of surface tension, because a treatment with vaseline on surface of the basal parts of the stems to interrupt the superficial movement of oxadiazon was conducted. During 7 days' exposure to the oxadiazon solution, more than half of

oxadiazon intaken into roots was translocated to shoots (Table IV). It was found that ^{14}C -radioactivity was heavily accumulated in the areas of the brown spots and more than 90% of the radioactivity found there were identified as oxadiazon. Figure 3-B and 3-C show heavy accumulation of radioactivity in the areas of brown spot symptoms.

Translocation of oxadiazon from leaf-blades and leaf-sheaths was surveyed. Oxadiazon mixed with vaseline paste was painted on leaf-blades or leaf-sheaths (Fig. 4-A and -B).

Translocation from the site of application to the other parts was detected more in leaf-sheaths than in leaf-blades. Acropetal translocation was observed, while a little basipetal translocation was detected in both blades and sheaths. It was observed that oxadiazon applied to the sheaths was translocated neither to the roots nor to the tillered shoots.

2) Distribution of oxadiazon in rice plants at various growth stages

Behaviour of oxadiazon in rice plants was investigated at various stages of their growth and development. As mentioned before,⁶⁾ the direction of translocation of substances in plants is influenced by physiological conditions of the plant tissues. For example, at the milk-ripening stage of the development of rice plants the accumulation of carbohydrates into heads is conducted for the most parts by translocation of the carbohydrates photosynthesized in flag-leaves, not in the lower leaves.

TABLE IV. TRANSLOCATION OF ^{14}C -LABELED OXIDIAZON FROM ROOTS TO SHOOTS OF RICE PLANTS

Periods (days)	Amounts of ^{14}C -radioactivity									
	0.2		1		4		7		10	
Parts	dpm ^{a)}	ppm ^{b)}	dpm	ppm	dpm	ppm	dpm	ppm	dpm	ppm
Shoots	1,900	0.2	2,600	0.3	13,200	1.5	42,300	3.1	59,400	3.6
Roots	6,300	2.4	10,400	4.2	25,900	9.4	35,500	10.5	70,800	14.4
Rate ^{c)}	23.1		20.1		33.8		54.4		45.6	

^{a)} Total radioactivities detected.

^{b)} Concentrations (ppm) were calculated from radioactivities in either shoots or roots, based on the specific radioactivities of ^{14}C -labeled oxadiazon.

^{c)} A rate of translocation = (dpm in shoots / dpm absorbed by plants) $\times$ 100 (%).

FIG. 3.

FIG. 4.

FIG. 3. A Radioautograph (right) and a Photograph (left) of a Rice Plant Treated with ^{14}C -Labeled Oxadiazon (Oxa-1).

Oxadiazon was applied to the roots of a plant grown in water culture.

3-A. The plant was left further for 1 day after the application.

3-B. The plant was left further for 4 days after the application.

3-C. The plant was left further for 10 days after the application.

Areas of the brown spot symptoms are marked with arrows in the Figures.

FIG. 4. A Radioautograph (right) and a Photograph (left) of a Rice Plant Treated with ^{14}C -Labeled Oxadiazon (Oxa-1).

The plants were grown in water culture.

4-A. Oxadiazon was applied to the stem marked with arrows in the figure. The plant was left further for 3 days after the application.

4-B. Oxadiazon was applied to the leaf marked with an arrow in the figure. The plant was left further for 5 days after the application.

FIG. 5.

FIG. 5.

In the present work rice plants were grown under paddy field conditions in the phytotron, where temperature, humidity and length of day-time were regulated in a way as similarly as possible to the ones in the practice of agriculture around Tokyo in Japan. A day after the application of emulsion containing ^{14}C -labeled oxadiazon to submerged soil in a Wagner's pot, rice seedlings at the 4 to 5 leaf-stage were transplanted in the pot. One, 2, 4 and 5~6 months after transplanting, which corresponded to the seedling, the maximum tillering, the milk-ripening and the ripening stages respectively, the rice plants were taken for the preparations of radioautography and metabolism studies.

A week after the transplanting a radioautograph of rice plants was taken. The plants had formed already fresh roots. Figure 5-A shows that oxadiazon was absorbed by both roots and stems. Oxadiazon once intaken into plants was distributed all over the entire shoots, and much more accumulation was observed in the more aged leaves than the younger. Since the submerged water was about 3 cm deep and the stems of plants contacted in parts with the surface water and soil which contained ^{14}C -labeled oxadiazon, the more aged leaves had more opportunity to contact with ^{14}C -labeled oxadiazon compared to the younger leaves. However it was shown that oxadiazon, which was applied definitely to roots only through culture solution, was translocated to shoots and distributed more heavily in the aged leaves than the younger just as same as in the soil culture.

Two weeks after the transplanting, a radioautograph of rice plants cultured in soil was

taken (Fig. 5-B). The rice plants were at the vegetative growing stage, and oxadiazon was incorporated in plants and distributed more in the aged leaves. Brown spot symptoms occurred and heavy accumulation of oxadiazon was observed in these areas on stems. The symptom was marked with an arrow in the Figure.

A month or two after the transplanting, rice plants were at the tillering or maximum tillering stage. Partially heavy accumulation of oxadiazon in the more aged leaves became much clear (Fig. 5-C and -D).

At the milk-ripening stage, a very slight degree of radioactivity was found in parts of heads (Fig. 5-E). It is necessary to point out that a little accumulation of oxadiazon was observed in flag-leaf. A flag-leaf is the youngest among leaves of a plant.

At the ripening stage, a slight degree of radioactivity was detected in flag-leaves and a much less degree in heads (Fig. 5-F).

A quantitative analysis of oxadiazon in plant tissues was conducted. From the radioactivities existing in plant tissues amounts of oxadiazon were calculated, of which results are shown in Table V. The amounts many include ^{14}C -labeled metabolic products in them. It was clarified that concentrations of oxadiazon in both shoots of plants at the seedling stage and straws at the harvesting were about 1 ppm, but the concentration in husks was about 1/25 to 1/50 of those in shoots and straws and that in hulled grains was about 1/100 to 1/250 of them. Concentrations of oxadiazon decreased in the plant parts in the order; leaves and stems $\gg$ husks $\gg$ hulled grains. Translocation barriers are postulated

FIG. 5. A Radioautograph (right) and a Photograph (left) of a Rice Plant Treated with ^{14}C -Labeled Oxadiazon (Oxa-2).

The plants were grown in soil culture. The X-ray films were exposed for 30 days, but 60 and 80 days in the case of Fig. 5-E and 5-F.

5-A. The plant was at a week after the transplanting.

5-B. The plant was at two weeks after the transplanting.

5-C. The plant was at a month after the transplanting.

5-D. The plant was at two months after the transplanting.

5-E. The plant was at the milk-ripening stage.

5-F. The plant was at the harvest-time.

Areas of the brown spot symptoms are marked with arrows in the Figures.

TABLE V. CONCENTRATION (ppm)^{a)} OF ¹⁴C-LABELED OXADIAZON AND ITS METABOLITES IN PLANT TISSUES AT THE VARIOUS GROWTH STAGES

Growth stages Parts	Months after transplanting		At harvest time		
	1	2			
Type ^{b)} of oxadiazon	Shoot	Shoot	Straw	Husk	Hulled grain
Oxa-1	0.56	1.31	0.74	0.040	0.0099
Oxa-2	1.27	1.29	1.14	0.022	0.0039
Oxa-3	1.79	1.21	0.81	0.045	0.0080

^{a)} Concentrations (ppm) were calculated from radioactivities, based on the specific activity of ¹⁴C-labeled oxadiazon.

^{b)} See Table I.

as existing between stems and heads and between husks and grains.

To the milk-ripening stage, plants had incorporated oxadiazon linearly at constant rates in proportion to increase of their dry weight. The result that the concentration of oxadiazon in straws at harvesting was lower than in shoots of seedlings was considered to be caused by detachment of withered aged leaves.

The concentration in hulled grains was given lower in the experiment with Oxa-2 than with Oxa-1 or Oxa-3, but it was ascertained that chemical transformation of oxadiazon in plants gave no difference among experiments with Oxa-1, Oxa-2 and Oxa-3.

Concentrations in rice grains obtained in the present study resulted higher than a concentration obtained in a field experiment.⁷⁾ In experiments conducted in a greenhouse or phytotron, in general, amounts of accumulation of pesticides in rice grains tend to be higher than those in field experiments, because UV irradiation to plants is restricted and leaching of the pesticides by rainfall is eliminated in a greenhouse or phytotron.

3) Chemical transformation of oxadiazon in plants during their whole life

Metabolic transformation of oxadiazon once incorporated into rice plants was surveyed (Table VI and Table VII). ¹⁴C-Labeled oxadiazon was applied to soil as mentioned before, and plants were taken for preparations of meta-

bolism studies 1 and 2 months after transplanting, and at harvest time.

TABLE VI. METABOLISM OF ¹⁴C-LABELED OXADIAZON (Oxa-2) IN RICE SHOOTS AND ROOTS

Percentage of radioactivities of oxadiazon and its metabolites (%)				
Plant parts Com- pounds ^{a)}	Months after transplanting			
	Shoot	Root	Shoot	Root
A-1	75.5	93.7	83.0	93.1
A-2	1.4	0.8	3.0	1.3
A-3	0.7	0.9	1.4	1.9
A-6 ^{b)}	3.2	1.2	3.3	1.2
A-8	1.1	0.9	1.5	0.9
M-1	17.4	2.4	7.3	1.5
M-2	0.7	0.1	0.5	0.1

^{a)} See Table II.

^{b)} A-6 is a fraction found in the origin-location in TLC.

In shoots of seedlings and straws of plant at harvesting, the 4-phenyl-*A*²-1, 3, 4-oxadiazolin-5-one structure of oxadiazon was found to be stable in rice plants. Metabolic reactions in plants occurred mainly in the side chain structures of oxadiazon to produce dealkylated compounds (designated as A-2, A-3 and A-6 in Table II), oxidized alcohol (A-8) and carboxylic acid (A-6). In addition, two unidentified metabolites (M-1 and M-2) were detected by thin-layer chromatography.

Seventy-five to 80% of radioactivity found in shoots or straws were identified as oxadiazon,

while more than 90% of radioactivity in roots were detected as the parent compound. The rate of metabolic transformation was considered to be relatively of small value in roots.

TABLE VII. ACCUMULATION OF ^{14}C -LABELED OXADIAZON (Oxa-2) AND ITS METABOLITES IN RICE STRAWS, HUSKS AND HULLED GRAINS

Percentage (%) of radioactivities of oxadiazon and its metabolites			
Plant parts	Straw	Husk	Hulled grain
Compounds ^{a)}			
A-1	78.0	32.6	22.0 (19.6) ^{b)}
A-2	2.0	5.6	
A-3	3.6	5.0	
A-6 ^{c)}	7.0	3.3	22.9
A-8	2.5	4.8	
M-1	5.6	46.2	46.5
M-2	1.3	2.5	

^{a)} See Table II.

^{b)} Percentage of radioactivities of oxadiazon in hulled grains determined by isotope dilution method.

^{c)} A-6 is a fraction found in the origin-location in TLC.

Amount of M-1 in shoots of plants at a month after the transplanting resulted to be bigger in the experiment with Oxa-2 than Oxa-1 or Oxa-3. In the experiments with Oxa-1 and Oxa-3, amounts of M-1 in shoots were found to be approximately 4 to 7%. It might be because Oxa-2 had impurities in it, one of which corresponded to M-1 in *Rf* value of thin-layer chromatography.

Amount of oxadiazon in rice grains was so little that a precise measurement was conducted by means of the isotope dilution method. The results obtained by the method corresponded well to the results determined directly by a liquid scintillation spectrophotometry of a thin-layer chromatographed fraction.

It is noteworthy that in both grains and husks an unidentified metabolic product (M-1) was found as the most in quantity out of the parent compound and its metabolites. The percentage of M-1 in the radioactivity extracted from husks or grains, in most cases, exceeded

TABLE VIII. METABOLISM OF ^{14}C -LABELED OXADIAZON (Oxa-1) IN RICE PLANTS GROWN FOR 10 DAYS IN WATER SOLUTION (0.1 ppm)

Percentage (%) of radioactivities of oxadiazon and its metabolites					
Compounds ^{a)}	A-1	A-3	A-6 ^{b)}	A-8	M-1
Plant parts					
Root	94.2	2.0	1.8	0.8	1.2
Shoot	91.1	—	3.1	3.3	2.5

^{a)} See Table II.

^{b)} A-6 is a fraction found in the origin-location in TLC.

the percentage of the parent compound. Since we ascertained that no M-1 was detected in soil which had been applied with oxadiazon, and that M-1 was found in extracts from plants grown in water culture containing Oxa-1 (Table VIII), M-1 was considered to be a product by plant metabolism.

Although the amount of M-1 existing in rice grains was less than 0.001 ppm (the detectable limit by gas-liquid chromatography) in field experiments, a toxicological investigation for M-1 is thought to be necessary. For this purpose, it is essential to identify the chemical structure of M-1.

4) Translocation and distribution of the metabolite M-1 in plants

Since M-1 was found as one of the metabolic products in plant tissues and accumulated in head parts in the experiment with Oxa-2, an investigation was tried with Oxa-1 and Oxa-3 for confirmation (Table IX). It was clarified that ^{14}C -labeled M-1 was detected in husks as one of the major metabolites in an experiment with Oxa-1 and Oxa-3 as well as Oxa-2. Amounts of oxadiazon and M-1 in grains were determined by radioassay only with Oxa-2 of which specific radioactivity was considerably high. In all the experiments with Oxa-1, Oxa-2 and Oxa-3, percentages of M-1 in radioactivities in straws were less than 10%, but those in husks and grains were shown 30 to 50%.

Distribution of ^{14}C -labeled oxadiazon and M-1 in a flag-leaf, leaf-sheath, culm, rachis, husk and grain was investigated in detail

TABLE IX. ACCUMULATION OF OXADIAZON AND ITS METABOLITES IN RICE PLANTS

Percentage of radioactivities of oxadiazon and its metabolites (%)							
Oxadiazon	Oxa-1		Oxa-2		Oxa-3		
Parts	Straw	Husk	Straw	Husk	Grain	Straw	Husk
Compounds ^{a)}							
A-1	84.3	52.7	78.0	32.6	22.0 (19.6) ^{b)}	76.0	48.2
A-2	—	—	2.0	5.6	—	—	—
A-3	4.6	—	3.6	5.0	—	4.6	—
A-6 ^{c)}	3.4	5.0	7.0	3.3	22.9	3.5	3.4
A-8	—	—	2.5	4.8	—	—	—
M-1	4.9	31.1	5.6	46.2	46.5	9.8	36.1
M-2	—	—	1.3	2.5	—	—	—
Others	2.8	11.2	—	—	—	6.1	12.3

^{a)} See Table II.^{b)} Determined by isotope dilution method (oxadiazon in grains).^{c)} A-6 is a fraction found in the origin-location in TLC.TABLE X. DISTRIBUTION OF ¹⁴C-LABELED OXADIAZON AND ITS METABOLITE M-1 IN TISSUES OF RICE PLANTS

Concentration (ppm) of oxadiazon (A-1) and its metabolite M-1					
Parts	(A)+(B)	(C)	(D)	(E)	(F)
Compounds					
Oxadiazon	0.479	0.216	0.129	0.008	0.001
M-1	0.018	0.008	0.012	0.010	0.002

(Table X). Concentrations of oxadiazon decreased in the order; leaf and leaf-sheath > culm > rachis >> husk >> grain. On the contrary, concentrations of M-1 in these tissues did not decrease much in the order. It was concluded that oxadiazon and M-1 were translocated at different rates from each other, both from leaf to head and from rachis to husk, while there

was little difference in the rates of translocation from husk to grain between oxadiazon and M-1. It was suggested that M-1 had a distinctive characteristic of moving easily in-between plant tissues compared to oxadiazon, and postulated that there was a kind of translocation barrier against oxadiazon between rachis and husk.

5) Metabolic pathways of oxadiazon in rice seedlings

In an attempt to trace metabolic transformation of oxadiazon in rice plants and to propose its metabolic pathways, a quantitative determination of oxadiazon and its metabolic products in rice seedlings was conducted within short periods of oxadiazon treatment. Shoot parts of intact seedlings were dipped in emulsified oxadiazon solution for 2 hr and the plants were transferred to culture solutions to be kept grown further. Then the treated shoots were harvested after various periods for the determination. Oxadiazon once incorporated into shoots from the applied solution was transformed chemically to produce several metabolic products.

Changes in time course of amounts of the

FIG. 6. Changes in Time Course of Metabolic Transformation of Oxadiazon in the Shoots of Rice Seedlings.

* The values are calculated as=(an amount of the concerned metabolite/an amount of total metabolites) $\times 100(\%)$.

●—●, A-2; ○—○, A-3; ▲—▲, A-6; △—△, A-8; ○—○, A-13; ■—■, M-1; □—□, M-2; □—□, M-3; ●—●, water soluble compounds.

metabolic products are shown in Fig. 6. A demethylated product (A-13) of the isopropoxy group of oxadiazon was found in a comparatively large amount in early metabolism of oxadiazon and unidentified metabolites M-1* and M-3 were also early detected considerably. Later on, oxidized products (A-8 and A-6) of the *tert*-butyl group became gradually detectable. Sequent demethylation reactions of the isopropoxy group and oxidation reactions to alcohol and carboxylic acid of the *tert*-butyl group of oxadiazon are considered to be main pathways of the oxadiazon metabolism. In addition, unidentified routes to produce M-1 and M-3 are estimated as contributory. Metabolic pathways of oxadiazon in rice plants are proposed as shown in Fig. 7.

6) Concluding remarks

In conclusion, we would emphasize the need for both plant physiological and biochemical approaches in further studies on the residue problems of the pesticides in rice plants. Oxadiazon, which is applied to the surface water or surface layer of soil in paddy fields at the seedling stage, is in contact with rice plants for a long period of time. Of the herbicide incorporated into rice plants, a little is translocated to the head parts and the rest remains in shoots and roots, especially in the lower leaves and stems. During the rice plants are growing and developing, oxadiazon is transformed chemically by metabolic reactions of rice plants. Since these metabolites may behave differently from the mother compound, they should be carefully traced both from the toxicological and plant physiological points of view.

Acknowledgement. The authors wish to express their gratitude for the kind help of Mr. S. Takano, Nissan Chemical Industries, Ltd., and also to thank Mr. F. Suzuki and Mr. I. Kawakami, Nissan Chemical Industries, Ltd., for their kind synthesis of ^{14}C -labeled oxadiazon and oxadiazon-related compounds, and Dr. T. Saito, Sagami Chemical Research Center, for his kind guidance in isotope dilution techniques.

* M-1 was identified as 1-(2,4-dichloro-5-isopropoxyphenyl)-1-methoxycarbonyl-2-trimethylacetylhydrazine. Details will be mentioned in later publication.

FIG. 7. Proposed Metabolic Pathways of Oxadiazon in Rice Plants.

* M-1 was identified later. See the following paper.

—→, identified; ---→, assumed; [compounds], assumed.

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