Metabolic Transformation and Accumulation of *O*-Ethyl *S,S*-Diphenyl Phosphorodithiolate (Hinosan®) in Rice Plants[†]

Iwao TAKASE,* Ken Ei TAN and Kozo ISHIZUKA**

***The Institute of Physical and Chemical Research, Wako-shi, Saitama, Japan*

Received October 24, 1972

³²P-labeled organophosphorus fungicide Hinosan was sprayed on rice plants at various growth stages, and metabolic fate of the pesticide in the rice plants was studied. Identification of Hinosan and its metabolites in the *n*-hexane and water extracts was conducted by TLC and GLC (FPD).

The rate of hydrolysis of Hinosan in rice plants seemed to be slower than that of other organophosphorus pesticides. Hexane-soluble components, which were detected throughout the experimental period, consisted mainly of Hinosan. Among the water-soluble metabolites identified were *O*-ethyl *S*-phenyl phosphorothioic acid and *S,S*-diphenyl phosphorodithioic acid, which were detected one to four days after the application, and ethyl phosphate and phosphoric acid which increased with the lapse of time.

Upon examination of the radioactivity of Hinosan and its metabolites in rice grains, a certain level was detected in husk, but very little in hulled rice and polished rice.

The importance of investigations on various aspects of the behavior of pesticides in plants was pointed out in the previous paper.¹⁾ Such investigations are considered to be essential for clarification of the primary mode of action of the concerned pesticide and also for understanding secondary effects on the metabolism of plants treated with the pesticide. As for the secondary effects of pesticides, for instance, it was reported that *O*-ethyl *S,S*-diphenyl phosphorodithiolate (Hinosan) showed a remarkable property to increase the chlorophyll content in leaves of rice plants,²⁾ or it was demonstrated by our group that Hinosan had a strong inhibitory effect on acyl-arylamidase of rice plants.

It is also necessary to study whether metabolic products of Hinosan have any effect on plant metabolism and on the primary pesticidal activity of Hinosan.

In addition, the problem of residual pesti-

cides in harvested crops is recently a matter of great concern in regard to public health. The necessity of intensive studies on the metabolism of pesticides and on their terminal residues in plants is ever increasing.

Translocation and absorption of Hinosan were surveyed in the previous paper.¹⁾ The present paper is concerned with metabolic transformation of Hinosan in rice plants and accumulation of Hinosan and its metabolic products in rice grains by using ³²P-labeled Hinosan.

MATERIALS AND METHODS

(1) ³²P-labeled Hinosan. Hinosan (*O*-ethyl *S,S*-diphenyl phosphorodithiolate) labeled with ³²P was synthesized by Bayer AG. and supplied through Nihon Tokushu Noyaku Seizo K.K. A Scanogram of a thin-layer chromatograph (TLC) of the supplied Hinosan is shown in Fig. 1. The supplied Hinosan of technical grade had a radiochemical purity of 88.2% (Table I). Further purification was conducted as previously mentioned¹⁾ by TLC using a mixture of benzene and methyl ethyl ketone (5:1 v/v) as a developing solvent. The purified material was shown to be radiochemically pure by TLC and infrared analysis with a specific activity of 0.86 mCi/mmol. The radiochemical purity obtained was calculated to be 99.5%.

[†] A work of this paper was presented at the Annual Meeting of Agricultural Chemical Society Japan, held on April 4 1969.

* Present Address: Institute for Agricultural Chemicals, Nihon Tokushu Noyaku Seizo K.K., Hino-shi, Tokyo.

** Mailing address.

³²P-labeled Hinosan *O*-Ethyl *S,S*-Diphenyl PhosphorodithiolateFIG. 1. Scannogram of a Thin-layer Chromatogram of the Supplied ³²P-labeled Hinosan.

Radioactivity was detected by a gas flow scanner for thin-layer chromatograms. Chromatography was carried out with petroleum ether and acetone (4:1 v/v) as a developing solvent.

TABLE I. RADIOCHEMICAL CONSTITUTION OF THE SUPPLIED ³²P-LABELED HINOSAN

Chromatograph was carried out with benzene and methyl ethyl ketone (5:1 v/v) as a developing solvent.

<i>R_f</i> -value in TLC	Compound identified	Percentage of Hinosan and its metabolites (%)
0		2.98
0.05		0
0.16		0.56
0.35		1.34
0.43	$(C_2H_5O)_2P(O)S-$	3.58
0.59	$C_2H_5OP(O)(S-$ $)_2$ (Hinosan)	88.2
0.80	$P(O)(S-$ $)_3$	2.77
0.92		0.57

(2) *Hinosan and its metabolites.* i) A non-³²P-labeled standard sample of Hinosan was obtained from Nihon Tokushu Noyaku Seizo K.K. and purified by silica gel column chromatography using *n*-hexane and chloroform (3:1) as eluent. Other possible breakdown products, such as thiophenol and diphenyl disulfide, were obtained from commercial sources.

Metabolites and hydrolysis compounds of Hinosan were prepared by the method described below. Their purity was determined by elementary analyses of phosphorus, infrared spectrum, thin-layer chromatography, and gas liquid chromatography after methylation with diazomethane.

ii) *Disodium ethyl phosphate* $C_2H_5OP(O)(ONa)_2$ (W-2).^{*} W-2 was obtained by the reaction of ethyl phosphorodichloridate and sodium hydroxide in water for about 2 hr, followed by evaporation to dryness. Resulting crystals were dissolved in ethanol and recrystallized. Phosphorus analysis: calculated, 18.2%, found, 18.7%.

iii) *Potassium ethyl S-phenyl phosphorothiolate* $(C_2H_5O)(C_6H_5S)P(O)OK$ (W-4).^{*} W-4 was prepared by mixing *O,O*-diethyl *S*-phenyl phosphorodithiolate with potassium ethyl xanthate in ethanol and refluxed at 70°C to 80°C for 3 hr. The reaction mixture was added ethyl ether for washing. The aqueous reaction mixture was evaporated to leave crystals, which were recrystallized from ethanol. Phosphorus analysis: calculated, 11.4%, found 11.7%

iv) *Sodium S,S-diphenyl phosphorodithiolate* $(C_6H_5S)_2P(O)ONa$ (W-5).^{*} W-5 was prepared by hydrolyzing *S,S,S*-triphenyl phosphorotrithiolate with an equimolar amount of sodium hydroxide with stirring for 1 hr and after washing with benzene, the aqueous solution was evaporated to dryness, and resulting crystals were recrystallized twice from ethanol. Phosphorus analysis: calculated, 10.2%, found, P=9.99%

(3) *Radioactivity assay.* Radioactivities of ³²P-labeled Hinosan and its hydrolyzed compounds were assayed on a Beckman LS-150 liquid scintillation counter. For the radioactivity assay, aliquots of 0.1 ~ 1.0 ml of *n*-hexane solutions of ³²P-labeled materials were mixed with 15 ml of a scintillator solution which consisted of 4.0 g of 2,5-diphenyl oxazole (POP) and 100 mg of 1,4-bis-2-(5-phenyloxazolyl) benzene (POPOP) in 1000 ml of toluene, or 0.1 ~ 1.0 ml of aqueous solutions of radioactive samples were mixed with 20 ml of another scintillator solution consisting of 7.0 g of POP, 100 mg of POPOP and 100 g of Naphthalene in 1000 ml of dioxane.

Quenching due to water and *n*-hexane or plant-originated colored substances in sample preparations was corrected by the external standard technique.

(4) *Thin-layer chromatography (TLC).* Thin-layer chromatography (TLC) was performed on plates coated with silica gel G prepared with 1% oxalic acid aqueous solution (20×20 cm, 0.5 mm thick). Thi

* See Fig. 3.

FIG. 2. Autoradiograph of a Thin-layer Chromatogram of a *n*-Hexane Extract from Rice Plants Applied with ^{32}P -labeled Hinosan.

The solvent system was a mixture of benzene and methyl ethyl ketone (5: 1 v/v).

*H represents a *n*-hexane-soluble fraction.

preparation of silica gel G with oxalic acid protected Hinosan from hydrolysis on a glass plate of TLC.

A mixture of benzene and methyl ethyl ketone (5: 1 v/v) was mainly used as developing solvent and other solvent systems were also used (Fig. 2 and Table II). Water-soluble fractions from plant materials containing hydrolyzed products of Hinosan were separated by TLC on plates coated with silica gel. Developing solvents used were (i) isopropanol and concentrated ammonia (9: 1 v/v), (ii) isopropanol and concentrated ammonia (3: 1 v/v), and (iii) *n*-butanol, methanol and water (4: 1: 2 v/v). (Fig. 3 and Table II).

(5) *Gas liquid chromatography (GLC).*⁴⁾ A Micro Tek 160 gas liquid chromatograph equipped with a flame photometric detector (FPD) which was especially sensitive to phosphorus and sulfur, was used. A column of Pyrex glass (120 cm $\times$ 4 mm i.d.) was packed

FIG. 3. A Thin-layer Chromatogram of a Water-soluble Fraction from Rice Plants, Using a Solvent System of Isopropanol and Concentrated Ammonia (9: 1 v/v).

*W represents a water-soluble fraction.

with 10% silicone DC 200 on Gas Chrom Q (80~100 mesh). Column temperature was either 150°C or 190°C.

The temperature of the injection port and that of the detector were 250°C and 200°C, respectively. Set flow rates of gas in ml per minute were as follows: nitrogen (carrier) 50, oxygen 40, hydrogen 160 and air 80 (Fig. 4).

(6) *Application of Hinosan on rice plants.* Rice seeds (*Oryza sativa* L. var. hatsuhinode) were planted in soil flats in a greenhouse. About a month later, they were transplanted in 1/2000 are sized Wagner's round pots and kept to grow further in the greenhouse. Five and ten weeks after transplantation, the plants were used for the studies on dissipation of Hinosan.

The 30% emulsifiable concentrate of Hinosan was prepared by mixing Hinosan, Sorpol 2020 (as emulsifier) and xylene in the weight ratio of 30: 15: 55. This Hinosan concentrate was further diluted 1000 fold with water for application. The whole shoot parts of the plants in every three ports were sprayed uniformly with 150 ml of the diluted Hinosan.

The purified ^{32}P -labeled Hinosan was used for the determination of dissipation and metabolic fate of Hinosan in rice leaves and results obtained with it were completely corresponded with results of ^{32}P -labeled Hinosan of technical grade. The metabolic

TABLE II. *R_f* VALUES OF HINOSAN AND ITS METABOLITES IN THIN-LAYER CHROMATOGRAPHY

Authentic compounds were chromatographed on a thin layer plate of silica gel coated with 1% oxalic acid and spots were detected with a spray reagent of 0.5% PdCl₂ or ammonium phosphomolybdate.

Compounds	Developing solvents (ratio v/v)		Solvent systems			
	Benzene-MEK (5: 1)	<i>n</i> -Hexane-acetone (9: 1)	<i>n</i> -Hexane-MEK-dioxane (6: 1: 1)	Iso-PrOH-NH ₄ OH (9: 1)	Iso-PrOH-NH ₄ OH (3: 1)	<i>n</i> -BuOH-MeOH-H ₂ O (4: 1: 2)
-SH,	0.92	0.79	0.80	0.95	—	—
P(O)(S-) ₃	0.80	0.70	0.72	0.95	—	—
C ₂ H ₅ OP(O)(S-) ₂	0.59	0.33	0.40	0.93	—	—
(C ₂ H ₅ O) ₂ P(O)S-	0.43	0.21	0.24	—	—	—
C ₂ H ₅ OP(O)(S-) ₂ (SH)	—	—	—	0.68	—	—
HOP(O)(S-) ₂	0.05	—	—	0.55	0.84	—
C ₂ H ₅ OP(O)(S-) ₂ (OH)	0.02	—	—	0.35	0.76	0.1~0.3
C ₂ H ₅ OP(O)(OH) ₂	0	—	—	0	0.03	0.07
H ₃ PO ₄	—	—	—	0	0	0

changes of Hinosan in rice plants at various growth stages were surveyed with ³²P-labeled Hinosan of technical grade. The results are discussed on consideration of the constitution of ³²P-labeled Hinosan of technical grade.

³²P-labeled Hinosan was applied either once, *i.e.*, on the 13th of August (booting stage), two times, *i.e.*, on the 13th of August and the 25th of August and the 2nd of September (ripening stage). Rice grains were harvested on the 25th of September.

FIG. 4. Gas Chromatogram of *n*-Hexane-soluble and Water-soluble Fractions of 10% Silicone DC 200 at 190°C (Flame photometric detector used 526 nm filter and 394 nm filter for sulfur detection).

The water-soluble fraction was applied to GLC after being methylated with Diazomethane.

In another experiment, rice plants (*Oryza sativa* L. var. jukkoku) were grown up in 1/2000 are sized pot in the greenhouse as previously mentioned. ^{32}P -labeled Hinosan was sprayed on the 18th of September. Rice grains were harvested on the 25th of September, and were processed to make fractions such as husks, hulled rice grains and polished rice grains.

(7) *Extraction and analytical methods for rice plants.* After application of ^{32}P -labeled Hinosan, rice plants were kept in the greenhouse and sampled after 1, 4, 7, 10 and 14 days, and separated into leaf blades and leaf sheaths. Each sample was homogenized in methanol for 5 min with a Waring blender. The homogenate was filtered through filter paper by suction, and the residue was washed and extracted again by homogenization in 80 per cent methanol for 5 min. After filtration, the residue was further homogenized in 10 per cent trichloroacetic acid. All the filtrates were combined, and concentrated under reduced pressure in a vacuum rotary evaporator below 45°C to remove most methanol. The remaining aqueous solution was transferred to a separatory funnel and extracted with *n*-hexane three times. The hydrolysates of Hinosan in the plants were extracted into this aqueous solution, and Hinosan itself was in the *n*-hexane fraction.

The aqueous solution was concentrated to a definite volume and an aliquot of 0.1~1.0 ml of the fraction was taken into a vial for the assay of total radioactivity in a liquid scintillation spectrometer. Then the aqueous solution was evaporated to dryness under reduced pressure below 50°C , and the residue was dissolved in 2 to 5 ml of methanol. 1 ml of the methanol solution was applied on silica gel plate (20×20 cm) for TLC.

The hydrolysates of Hinosan in this methanol solution were separated by thin-layer chromatography using a mixture of isopropanol and concentrated ammonia (75:25 v/v) as a developing solvent. For comparison, authentic samples of the Hinosan hydrolysates were spotted on both ends of the plate. After being developed, the plate was immediately sprayed with 0.5% palladium chloride ethanol solution. The chromatographed plate was exposed to an X-ray film to make a radioautograph. Radioactive metabolites of Hinosan on TLC were located by referring to the radioautogram.

The areas of the Hinosan metabolites on the silica gel plate were scraped off and extracted with methanol, and their radioactivity was measured. After counting all the extracts were combined and evaporated almost to dryness. Methylation of the compounds in the residue was conducted with diazomethane in ethyl ether, according to the method of St. Hohn, and Lisk.³⁾ The methylated products were identified with authentic compounds by GLC equipped with a flame photo-

metric detector.

The *n*-hexane soluble fraction was concentrated to 10 ml, and its radioactivity was counted.

Hinosan and its metabolites in the *n*-hexane fraction were separated by TLC on silica gel plates (20×20 cm), using a mixture of benzene and methyl ethyl ketone (5:1) as a developing solvent.

After being developed, the chromatographed plate was sprayed with 0.5% palladium chloride ethanol solution, and exposed to an X-ray film for radioautography. The areas corresponding to Hinosan and its metabolites was scraped off, and extracted with benzene for determination of radioactivity. Then, the *n*-hexane soluble fraction was concentrated and subjected to GLC for identification of Hinosan and its metabolites as described above.

(8) *Extraction and analytical methods for rice grains.* The rice plants used in the present work were harvested on the 25th of September, 1968, and husks, hulled rice and polished rice were prepared from them. Each material was crushed and homogenized in 80% methanol with a Waring blender. After the supernatant of the homogenate was separated by centrifugation, 10% trichloroacetic acid was added to the residue and homogenized again in a Waring blender.

All the extracts were combined and concentrated under reduced pressure to remove methanol. The concentrated aqueous solution was transferred to a separatory funnel and partitioned with *n*-hexane into an aqueous and a *n*-hexane fraction.

An aliquot each of the *n*-hexane and aqueous fractions was taken into a vial for the assay of radioactivity. For identification of Hinosan and its metabolites with authentic compounds, the *n*-hexane- and water-soluble fractions were separated on TLC by the method described above, and their radioactivity was measured.

RESULTS AND DISCUSSION

Time course of metabolism of Hinosan in rice plants

For the purpose of studying metabolism of Hinosan in rice plants, ^{32}P -labeled Hinosan was applied to rice leaves. After a definite time for absorption, the leaves of the rice plants were washed with water and methanol to remove ^{32}P containing compounds on the surface of the leaves. Then, ^{32}P containing compounds in the leaf tissue were extracted and partitioned between *n*-hexane and water. The former fraction consisted mostly of Hinosan. ^{32}P -labeled Hinosan once taken

FIG. 5. Metabolic Fate of ^{32}P -labeled Hinosan in Rice Leaves at the Tillering Stage.

□, *n*-Hexane-soluble fraction; ■, Water-soluble fraction
*Concentration (ppm) of radioactivity of Hinosan and its metabolites

up into rice leaves was transformed chemically in some part and the metabolites were found in a water extract from the tissues, although most of Hinosan applied to the rice leaves dissipated by evaporation or photodecomposition before absorption into the plant tissues as previously mentioned.¹⁾

Metabolic products of Hinosan in an aqueous extract increased with time (Fig. 5) in contrast with a decrease of Hinosan in the *n*-hexane extract. The rate of metabolic

reactions of Hinosan to give water-soluble compounds in rice plants was shown to be relatively small, compared with *O,O*-diethyl-*O-p*-nitrophenyl phosphorothiolate (Parathion) and *S*-benzyl *O,O*-diethyl phosphorothiolate (Kitazin®). These three organophosphorus pesticides were sprayed on the leaves of rice plants and their amounts found in the leaf tissue were measured after a definite time by the method previously mentioned. As shown in Table I of the previous paper,¹⁾ Parathion and Kitazin, once taken up into the leaf tissue, decreased rapidly with time. For instance, the amount of parathion found in the tissue 2 hr after application was 61.4 ppm, and 3 days after application 80 to 90% of the applied amount was transformed to other compounds. On the contrary, in the case of Hinosan only about 10% was transformed to other compounds in the leaf tissue. As ^{32}P radioactivity was scarcely found in the residue of the leaf tissues after repeated extraction with water and methanol, the possibility to form any conjugates or integrated compounds of Hinosan insoluble in water or methanol seems to be eliminated.

The rate of metabolic transformation of Hinosan to water-soluble compounds in the rice leaf tissue was compared between the plants of five and ten weeks old after transplantation, which corresponded to vegetative and reproductive stages of rice plants respectively. There was no difference in the ability

TABLE III. CHANGES IN QUANTITIES OF HINOSAN AND ITS METABOLITES IN *n*-HEXANE AND AQUEOUS EXTRACTS FROM RICE PLANTS AFTER AN APPLICATION OF ^{32}P -LABELED HINOSAN OF TECHNICAL GRADE TO THE LEAVES

Fractions extracted	Percentage of radioactivities of Hinosan and its metabolites										
	<i>n</i> -Hexane-soluble fraction						Water-soluble fraction				
	H-1	H-2+3+4	H-7	H-8 (Hinosan)	H-9	H-10	W-1	W-2+3	W-4	W-5	W-6+7
Days after application											
1	2.8	1.4	3.1	89.3	3.0	0.4	4.2	5.4	30.7	29.4	13.6
7	0.8	1.3	3.6	83.2	3.7	1.7	10.3	6.5	20.7	21.5	13.4
10							27.9	8.5	31.8	22.9	8.9

* The compounds listed here as H- and W-numbers were identified by TLC and GLC. See Figs. 2, 3 and 4. The rice plants used were 10 weeks old (at the tillering stage).

of the transformation between the plants at different growth stages.

Metabolism of Hinosan in leaves of rice plants

Since it was clarified that Hinosan was transformed to water-soluble compounds in rice leaves, the water-soluble compounds were surveyed in detail in addition to the *n*-hexane-soluble compounds.

The *n*-hexane extract from the leaf-tissue was chromatographed by GLC and TLC, and the results obtained are shown in Table III. One day and 7 days after application, Hinosan was estimated as 89.3% and 83.2% of the total radioactivity in the *n*-hexane fraction. Next major compounds, *S,S,S*-triphenyl phosphorotrithiolate (represented as H-9 in Fig. 2) and *O,O*-diethyl *S*-phenyl phosphorothiolate (represented as H-7 in Fig. 2) were found to be about 3% respectively. However, this particular experiment was conducted with ^{32}P -labeled Hinosan of a technical grade which included these two compounds as impurities.

An experiment with purified ^{32}P -labeled Hinosan was conducted in order to confirm the results obtained with ^{32}P -labeled Hinosan of technical grade. On 1 day after the application, only Hinosan was detected as a ^{32}P radioactive compound in the *n*-hexane extract. On 7 days after the application, the two compounds (H-7, and H-9) were also detected though their amounts were extremely small. Therefore, metabolic transformation of Hinosan to these two compounds remains to be investigated, but these reaction pathways, if any, should be minor in the Hinosan metabolism.

The water-soluble fraction was analyzed by means of TLC and GLC accompanied by methylation. Major compounds in the water-soluble fraction were identified as *O*-ethyl *S*-phenyl phosphorothioic acid and *S,S*-diphenyl phosphorothioic acid, the amounts of which were 30% each of the total ^{32}P in the fraction. With lapse of time, ethyl phosphoric acid and phosphoric acid increased, while *S,S*-diphenyl phosphorothioic acid decreased.

FIG. 6. Proposed Metabolic Pathways of Hinosan in Rice Plants.

—, Identified; ----, Assumed.

O-Ethyl-*S*-phenyl phosphorothioic acid and *S,S*-diphenyl phosphorothioic acid are products of des-*S*-phenylation and desethylation, respectively, of Hinosan, and ethyl phosphate and phosphoric acid, which increased with time, are considered to be the terminal products of Hinosan. Since no integrated compounds of Hinosan were found in the leaf tissue, and no unexpected substances for the chemical structure of Hinosan were detected, the following metabolic pathways of Hinosan are proposed (Fig. 6).

In the Fig. 6, the existence of *S*-phenyl phosphorothioic acid was postulated as a des-*S*-phenylation product of *S,S*-diphenyl phosphorothioic acid but it was not proved because the authentic compound had not been synthesized and was not available for co-chromatography.

Accumulation of Hinosan and its metabolites in rice grains

Harvested rice grains were separated into husks and hulled rice grains, and the latter were further separated into polished rice grains and bran. The amounts of Hinosan and its metabolites found in the *n*-hexane and aqueous extracts from these parts are shown in Tables IV and V. For the purpose of clari-

TABLE IV. DISTRIBUTION OF ^{32}P -LABELED HINOSAN AND IT'S METABOLITES IN RICE GRAINS INCLUDING HUSKS

Portion	Treatment ^{c)} Fraction ^{a)}	Concentration of Hinosan and it's metabolites (ppm) ^{d)}							
		A		B		C		D	
		H ^{a)}	W ^{a)}	H	W	H	W	H	W
Husk		0.11	0.04	0.17	0.12	0.28	0.14	2.125	0.342
Hulled Rice		trace	0.02	trace	<0.01	0.06	0.03	0.310	0.054
Polished Rice		<0.01 ^{b)}	<0.01	<0.01	<0.01	trace	trace	trace	—

^{a)} H represents the *n*-hexane-soluble fraction and W represents the water-soluble fraction.

^{b)} The detectable limit is 0.01 ppm.

^{c)} Application of ^{32}P -Hinosan was conducted as below:

Treatment A; Application once on August 13, Treatment B; Application twice on August 13 and August 25, Treatment C; Application three times on August 13, August 25 and September 2, Treatment D; Application once on September 18.

Rice plants were harvested on September 25, 1968

^{d)} Concentrations (ppm) were calculated from radio-activities of the fractions, based on the specific activity of ^{32}P -labeled Hinosan.

TABLE V. ACCUMULATION OF HINOSAN AND IT'S METABOLITES IN THE HUSKS OF RICE GRAINS

Compounds	Percentages of radioactivities of Hinosan metabolites	
	C	D
Water soluble fraction		
unknown (<i>Rf</i> 0.8)	9.6	13.4
HOP(O) (S- ₂)	10.9	19.4
C ₂ H ₅ OP(O)(S- ₂)(OH)	37.6	29.1
C ₂ H ₅ OP(O)(OH) ₂ .H ₃ PO ₄	41.8	38.1

* Treatments of ^{32}P -labeled Hinosan were conducted as below.

C; Application three times on Aug. 13, Aug. 25 and Sept. 2, 1968.

D; Application once on Sept. 18, 1968.

TABLE VI. ACCUMULATION OF HINOSAN IN HULLED RICE GRAINS IN A FIELD EXPERIMENT

Results in this table were obtained from field experiments, referred to *Noyaku Seisan Gijutsu*, **23**, 31 (1971) by I. Takase *et al.*⁴⁾

Days between application and harvest time	No. of samples	Concentration of Hinosan in average (ppm)
0~15	3	0.010
16~30	9	<0.005
31~45	4	<0.005
46~	6	<0.005

fyng translocation and accumulation of Hinosan and its metabolites into rice grains, an extremely heavy dose schedule such as repetition of applications three times was conducted in addition to the practical way of application.

In the cases of one application at the booting stage and two times applications at the booting and milk-ripening stages of rice plants, the *n*-hexane extract from husks included 0.11 ppm and 0.17 ppm (calculated as Hinosan) of ^{32}P -containing compounds, while the *n*-hexane extracts from both hulled and polished rice grains included ^{22}P -compounds in trace amounts or below the limit of detection (0.01 ppm). In the case of three times applications the *n*-hexane extract from husks showed a content of 0.28 ppm. These results are considerably higher than a concentration obtained in a field experiment. In experiments conducted in a greenhouse, in general, amounts of accumulation of pesticides in rice grains tend to be higher than those in field experiments or in agricultural practice, because ultraviolet irradiation to plants is cut off by glass windows and drifting of air around the plants is restricted compared with field experiments and leaching of pesticides by rain-fall is eliminated in a greenhouse. Results in field experiments were obtained by Takase

*et al.*⁴⁾ and a part of them is shown in Table VI.

In these *n*-hexane extracts Hinosan was identified as the major compound which amounted to 90% or more.

As for water-soluble compounds, 0.14 ppm of ³²P-containing substances, calculated as Hinosan, was measured in husks in the experiment of the three times application schedule, whereas 0.03 ppm was found in hulled rice. In polished rice only trace amounts of phosphorus-containing substances were detected in the water-soluble fraction.

³²P-containing compounds in these water extracts from the husks were identified as *O*-ethyl *S*-phenyl phosphorothioic acid and a mixture of ethyl phosphate and phosphoric acid which occupied about 40% each of the total ³²P in the fraction as shown in Table IV.

Hinosan and its metabolites found in rice grains are considered to be derived either from direct adsorption of the sprayed compound or from translocation from other parts of the plants. The relationship between the amount of accumulation of Hinosan in grains and time length of the period from the time of application to the harvest time was such that the shorter the time period, the more accumulation was observed.

The amount of Hinosan in husks or in hulled grains was greater in the experiment with the treatment D than with the treatment A as shown in Table III. When the period between application and harvest was short as in the case of treatment D, Hinosan found in husks and grains is considered to be derived from

the absorbed material after spraying. On the other hand, the amount of ³²P-containing substances in the water extract from hulled rice grains in the treatment D was 0.054 ppm and the differences from the corresponding values with the treatment A, B, and C were small. It is likely that most of the Hinosan metabolites were not produced in rice grains or husks but produced in and translocated from the leaves or the stems.

In conclusion, some of Hinosan applied to the shoot and its metabolic products are translocated to the husks of rice grains, but only trace amount of them penetrate into the edible parts of rice grains.

Acknowledgement. The authors wish to acknowledge the kind advice of Dr. Yasuhiko Uesugi, National Institute of Agricultural Science and to thank Mr. Yoshiharu Umeda, Nihon Tokushu Noyaku Seizo K. K., for his helpful activity of liaison among the Institutes of the authors, of Nihon Tokushu Noyaku Seizo K. K., and of Bayer AG.

REFERENCES

- 1) K. Ishizuka, I. Takase, K. E. Tan and S. Mitsui, *Agr. Biol. Chem.*, **37**, 1307 (1973).
- 2) T. Nakahara, K. E. Tan and K. Ishizuka, Abstracts of Papers, 45th Annual Meeting of Agr. Chem. Soc. Japan (1970).
- 3) L. E. St. John Jr. and D. J. Lisk *J. Agr. Food Chem.*, **16**, 48 (1968); *ibid.*, **16**, 408 (1968).
- 4) I. Takase, H. Tsuda, S. Nakamura and S. Kuyama, *Noyaku Seisan Gijutsu* **22**, 13 (1970); I. Takase, S. Nakamura, H. Tsuda and I. Ueyama, *ibid.*, **23**, 31 (1971).