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Invited Lecture

Use of Radioactive Isotope Technique in Weed Science

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Radioactive isotope(RI) technique has been widely used in many fields of sciences and gives a big advantage for advancing sciences and technologies. Unfortunately, however, it has not fully been applied to weed research yet. In the present paper it is discussed how RI tenchnique can possibly be utilized in weed science and what kinds of information can be obtained from it.

Sources of radioisotopes

In most cases β-ray radioisotopes such as ^{3}H, ^{14}C, ^{32}P, ^{33}P, or ^{35}S are used because of thier adequaet energy of β-ray, long half lives and chemical stability. Detection and determination systems for β-ray radioativity have been remakably developed with increase of their utilization. Especially ^{14}C-laveled herbicides are really powerfyul weapons of weed researches, and at present synthesized by industrial companies simultaneously with development of their products.

Sorts of researches required RI technique

RI technique is useful and efficient especially in(1) studies on modes of action of herbicides and (2) residue studies in plant-soil environments.

Generally speaking, radioisotopes in either plants or soils can be detected even in an extremely small quantity. Least significant levels of determination (LSD) for analysis of pesticides by gaschromatography, for example, are estimated as approximately 0.005 ppm, while determination by RI technique can be made in much less concentration than 1/10 or 1/100 of them.

Secondly, RI labeled compounds are distinguished from plant constituents or from herbicides which have been incorporated beforehand into plant tissues. We can trace an newly applied herbicide and clarify its behaviour, especially its chemical transformation by metabolism of plants and soil micro-organisms.

Thirdly, RI is essential for identification of metabolic products of herbicides in plants and soils as well as proposal of metabolic pathways.

Finally, radioautograph technique provides us visible and semi-quantitative patterns of herbicide distribution and accumulation.

Use of RI in studies on modes of action of herbicides

First of all, I figure out a mechanism on herbicidal effects on plant growth(Fig. 1), which includes processes of absorption, translocation, and chemical transformation as well as of reaction on metabolism of plants. Several examples are to be given in each process.

1. Absorption process

(a) absorption of ^{14}C-simetryn by shoots of vaious gramineous species.

Rates of absorption by shoots of various gramineous species are shown different from each other (Fig. 2). The difference is obtained even among closedly classified species and often contributes to produce

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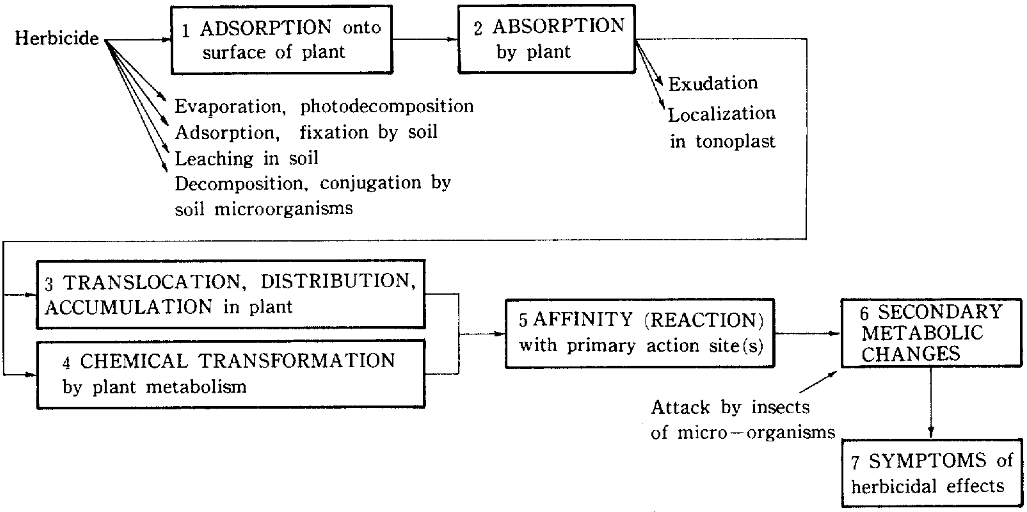


Fig. 1. Herbicide behaviours and activities in plants[8].

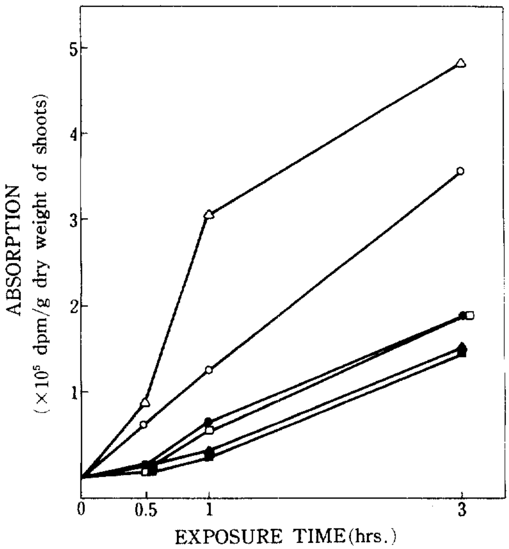


Fig. 2. Absorption of ^{14}C–simetryn by shoots of gramineous plants.

—△— Large crabgrass, —▲— Wheat,
—○— Barnyardgrass, —●— Rice,
—□— Finger millet, —■— Corn.

selectivity among them.

(b) absorption of ^{14}C–barban by leaves of wheat and oat.

A rate of absorption by whole intact leaves of wheat is shown much less than that of oat(Fig. 3). while rates of absorption by sectioned leaves of both plant species are similar(Fig. 4). A kind of absorption

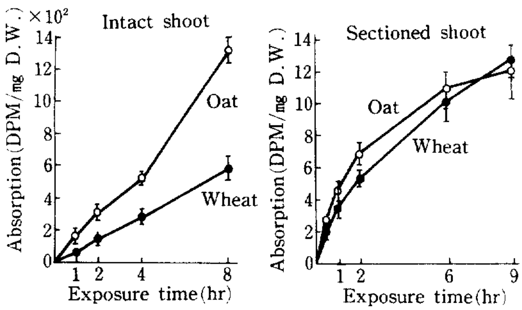


Fig. 3 and 4. Absorption of ^{14}C–labeled barban by oat and wheat seedlings[13].

barrier is assumed in surface areas of leaves.

(c) effect of formulating agents on absorption.

Surfactants or other formulating agents enhance sometimes rates of absorption(Fig.5). Herbicides, which is absorbed very limitedly by weeds such as glyfosinate, may possibly be improved much efficience by increasing the absorption rates.

(d) absorption of ^{14}C–propanil by rice leaves.

By tracing its absorption, hydrolytic decarboxylation of propanil is assumed in leaves of rice seedlings, but not in barnyardgrass(Fig.6). The rusult also indicates that simultaneous spray of propanil with organic phosphorous insecticides shold be avoided(Fig.7).

(e) high temperature effect on rates of absorption of ^{14}C–simetryn.

Under high temperature circumstances, growth of

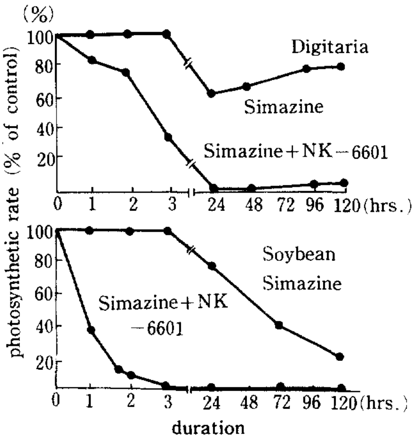


Fig. 5. Simazine effects on the photosynthetic rates as affected by shrfactants. [NK-6601 : nonionic surfactant, Konnai]

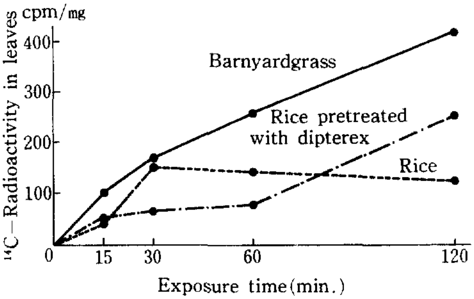


Fig. 6. Absorption of carbonyl−^{14}C−propanil into leaves of rice and barnyardgrass plants(dipped)[3].

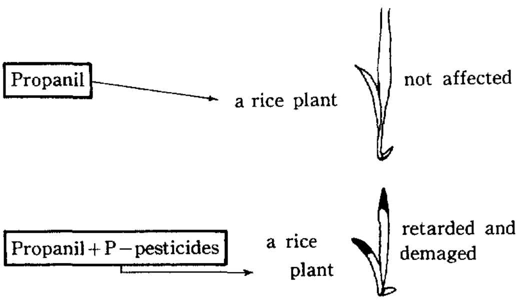


Fig. 7. Effect of organic phosphate pesticides on a herbicidal activity of propanil[3].

rice seedlings is occasionally affected by simetryn applied to sandy paddy soil. Under high temperature condition, absorption by rice roots is enhanced compared with lower temperature condition, while its translocation and metabolism in plants are not much effected(Fig.8).

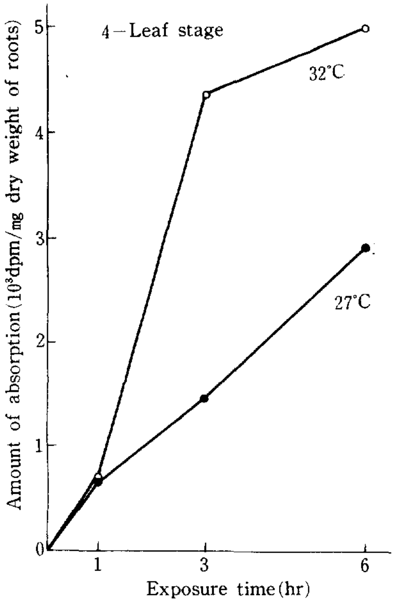


Fig. 8. Amount of absorption of ^{14}C−simetryn by roots of rice plants under different temperature conditions[25].

2. **Translocation process**

(a) Translocation of diphenylether herbicides from roots to shoots. Diphenylethers such as chlornitrofen and chlormethoxynil hardly move from to shoots of almost all species(Fig.9). Foliar spray or soil surface treatment is only applicable for this type of herbicides. Translocation barrier is assumed to locate in tissue between root surface and xylem. In contrast, simetryn moves very quickly from roots to shoots.

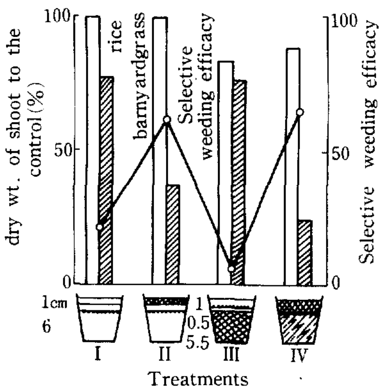


Fig. 9. Relationships between application site and weeding efficacy. [Note. Netted : treated, soil, Blank : untreated soil, ● : Seeding level, ○ : water level]

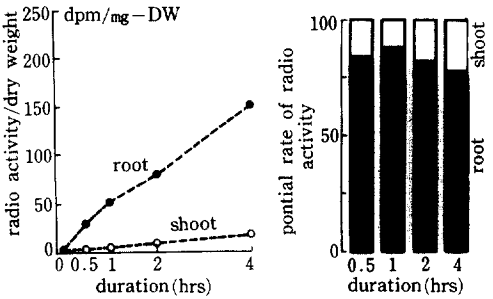


Fig. 10. absorption and partial distribntion of ALA applicated by root of rice .

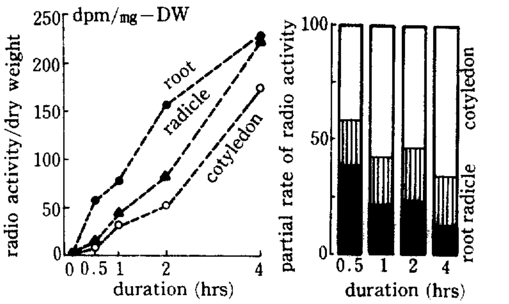


Fig. 11. absorption and partial distribntion of ALA applicated by root of cucumber

(b) Downward translocation of herbicides.

For control of perenial weeds or tall grass-weeds, systemic herbicides are required. Mechanism of downward translocation, especially into tubers should be clarified.

(c) ^{14}C−aminolevulenic acid(ALA) tanslocation.

ALA is known as a "herbicide and effective in the light. ^{14}C−ALA applied to roots of cucumber (susceptible) moves to shoots to remarkable extent, while ^{14}C−ALA applied to roots of rice(tolerant) moves to extremely slight extent(Fig.10 and 11).

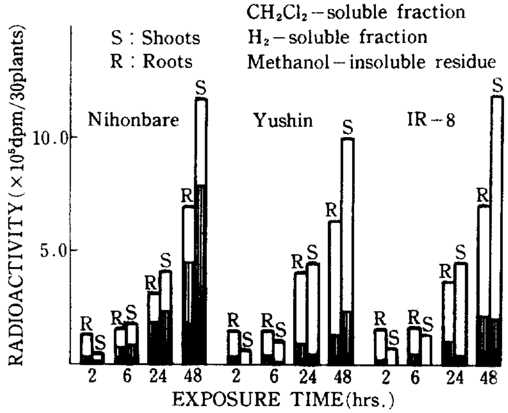


Fig. 12. Metabolic rate of root−applied ^{14}C−simetryn in shoots and roots of rice cultivars.

3. **Degradation and integration process**

(a) Degradation of simetryn in rice cultivars.

Simetryn is degradated in japonica−type "Nihonbare"cultivar much faster than in indica−type "IR−8"cultivar and i×j hybrid "Yushin"cultivar (Fig.12). DPX−F5384, sulfonylurea, is degradated in different rates among rice cultivars, but slower in japonica cultivars.

(b) Conjugation of MY−15 with glucose in rice seedlings.

Conjugation of herbicides with plant constituents such as glucose or glutathion has been reported many and studied in closed relation to selective activity of them. Once MY−15 is incorporated into

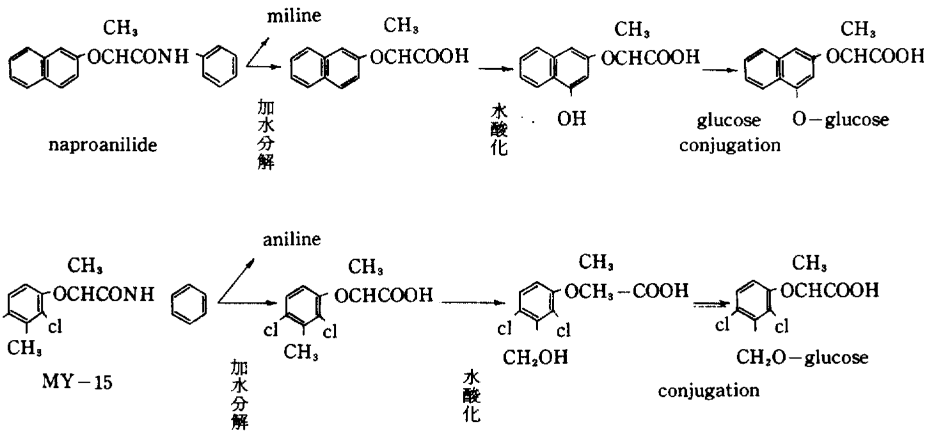


Fig. 13. Metabolism of MY-15 and Naproanilide in glants.

plants it is hydrolyzed immediately, followed by oxidation of phenyl–side chain methyl grounp. In contrast to tomato(susceptible), rice(tolerant) metabolizes the oxidized product, 2, 4–dichlorophenyl–3–hydroxy–methyl–phenoxypropionate, to its glucoside(Fig.13 and Table.1).
Atrazine conjugation with chloroplast peptide of resistant biotype of common groundsel, or solan binding with chlorophyll and so forth are interesting examples for future studies.

4. **Responces of plant metabolism to herbicides.** (a) effects of atrazine on plant metabolism of a common groundsel biotype. Ashton, California Univ., proposed use of ^{14}C labeled procursors for analysis of respiration, photosynthesis, biosynthesis of protein, nucleic acid and lipid(Table.2). By tracing effects of herbicides on metabolic turn–over of these precursors, modes of herbicide action are clarified well.

Use of RI in residue studies of herbicides in plant–soil environments

Nowadays problems of bioaccumulation and food–chain of pesticides are of public concerns. Concentrations of herbicides in edible parts of crops and vegitables are determined by RI technique,

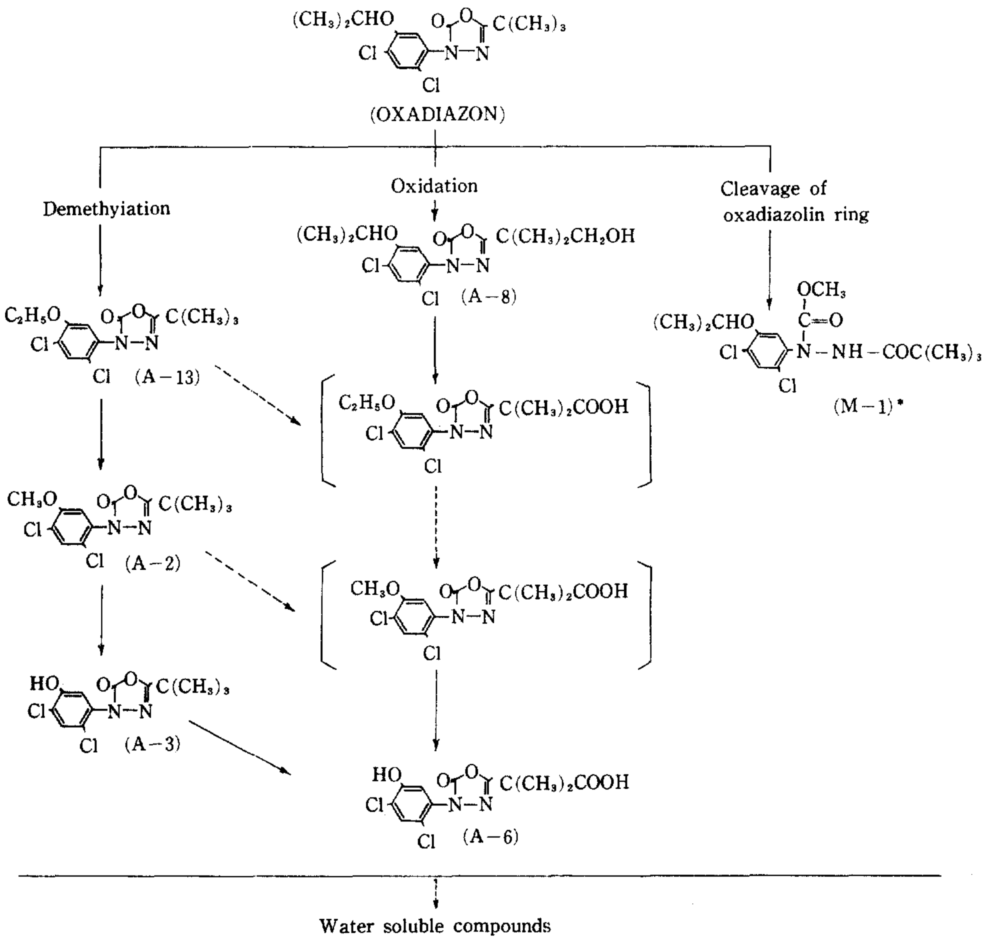


Fig. 14. Proposed Metabolic Pathways of Oxadiazon in Rice Plants.
* M–1 was identified later. See the following paper.
→, identified ; →→, assumed ; [compounds], assumed.

Table 1. Distribution of ^{14}C-radioactivity in the hydrolysed water soluble extracts from roots of rice and tomato applied with either ^{14}C-MY-15 or ^{14}C-MY-COOH

Applied compounds	MY-15		MY-COOH	
Compunds detected	Rice	Tomato	Rice	Tomato
MY-COOH	19.9	45.9	15.8	15.6
Mytyl ester		10.4		
DCC		5.8		
6OH-MY-COOH	1.1			
$3CH_2OH$-MY-COOH	70.2	24.2	33.5	8.1
Unknown I			2.8	3.4
Unknow III	0.9		12.3	9.8
Others	7.9	13.7	31.3	57.9
Origin	2.5		5.3	5.2
Total	100.0	100.0	100.0	100.0

simultaneously with determination of metabolic products.

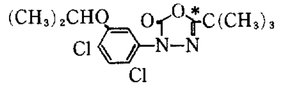
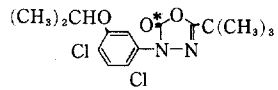
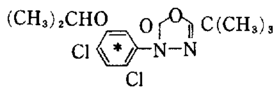
An example of residue study of oxadiazon in rice plants is given here. Three sorts of ^{14}C- labeled oxadiazon which are labeled in different positions of the chemical structure from each others are available(Table 3). Herbicides are generally accumulated much less in grains compared with insecticides and fungicides. RI technique clarifies concentrations of not only the parent compound but also metabolic products in grains(Table 4 and 5). Those concentrations were less than 0.005 ppm, yet acute toxicity and carry-over of the major metabolite were also checked.

Table 2. The effect of atrazine on net CO_2 fixation, CO_2 evolution, RNA synthesis, protein synthesis and lipid synthesis in isolated leaf cells of resistant and susceptible common groundsel biotypes expressed as percentage of the controls.[a]

	Expo-sure times	Net CO_2 fixation (CPM fixed/mg chlorophyll) μM Atrazine				CO_2 evolved/mg (CPM evolved/mg chlorophyll) μM Atrazine				Uracil incorporation (CPM incorporated/mg chlorophyll) μM Atrazine				L-leucine incorporation (CPM imcorporated/mg chlorophyll) μM Atrazine				Acetate incorporation (CPM incorporated/mg chlorophyll) μM Atrazine			
Biotype	(mm)	0.01 (%)	0.1 (%)	1.0 (%)	10.0 (%)	0.01 (%)	0.1 (%)	1.0 (%)	10.0 (%)	0.01 (%)	0.1 (%)	1.0 (%)	10.0 (%)	0.01 (%)	0.1 (%)	1.0 (%)	10.0 (%)	0.01 (%)	0.1 (%)	1.0 (%)	10.0 (%)
Resistant	15	113	91	96	108	101	85	107	103					104	100	95	95	105	82	92	97
Resistant	30	102	92	95	93	100	91	104	104	96	96	106	90	99	106	100	100	97	90	94	100
Resistant	60	102	91	100	100	98	92	97	97	83	95	101	90	96	100	93	93	102	94	104	101
Suscept	15	112	83	10	11	106	102	108	108					104	94	87	78	109	135	133	58
Suscept	30	105	81	10	6	107	91	102	105	98	105	84	78	108	116	98	96	105	152	141	53
Suscept	60	94	85	7	3	100	94	102	120	97	97	54	52	97	98	93	95	111	158	145	53

[a] LSD (0.01) for CO_2 fixation at 15, 30, and 60 min = 36, 25 and 16% respectively. LSD (0.05) for CO_2 evolution at 15, 30, and 60 min = 40, 30 and 27% respectively. LSD (0.05) for Uracil incorporation at 30 and 60 min = 25 and 21% respectively. LSD (0.05) for L-leucine incorporation at 15.30 and 60 min = 21, 20 and 14% respectively, LSD (0.05) for acetate incorporation at 15, 30 and 60 min = 41, 60 and 50% respectively.

Table 3. RADIOCHEMICAL PROPERTIES OF ^{14}C-LABELED OXADIAZON

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

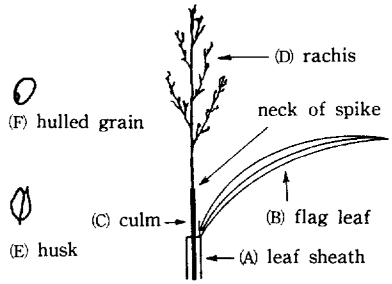
* ; indicates the position labeled with carbon-14.

Table 4. ACCUMULATION OF ^{14}LABELED OXADIAZON (Oxa−2) AND ITS METABOLITES IN RICE STRAWS, HUSKS AND HULLED GRAINS

Percentage(%) of radioactivities of oxadiazon and its metabolites			
Plant parts / Compounds[a]	Straw	Husk	Hulled grain
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.

Table 5. DISTRIBUTION OF ^{14}C−LABELED OXADIAZON AND ITS METABOLITE M−1 IN TISSUES OF RICE PLANTS



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

Concluding remarks

Although special training, facilities and equipments are necessary for RI utilization, importance of using them in weed science is getting more and more remarkable. It is hoped that whenever industrial companies develope a new herbicide, they are ready to provide ^{14}C−labeled compound to international cooperative research on the herbicide.