J. Pesticide Sci. **9**, 471–480 (1984)

Original Article

Degradation of the Herbicide Alloxydim-sodium in Soil

Shigeo ONO, Hironori SHIOTANI, Kanji ISHIHARA,*
Masanori TOKIEDA and Yoshinori SOEDA*

Fine Chemicals Research Laboratory, Nippon Soda Co., Ltd., Takada, Odawara 250–02, Japan
**Nisso Institute for Life Science, Nippon Soda Co., Ltd., Oiso, Naka-gun, Kanagawa 255, Japan*

(Received January 23, 1984)

Alloxydim-sodium (ADS), sodium salt of 2-(1-allyloxyaminobutylidene)-5,5-dimethyl-4-(methoxycarbonyl)cyclohexane-1,3-dione, ^{14}C-labeled at butylidene-1 and ring-1,5, respectively, was degraded with a half-life of about 5 days in Oiso soil at 25°C in the dark. The following were identified as degradation compounds by thin-layer cochromatography and mass spectrometry: deallyloxylated compound (**I**), two isomers of oxo-tetrahydrobenzoxazoles (**II** and **III**), deallyloxyaminated compound (**VI**), demethoxycarbonylated compound (**VII**) and ring-opened dicarboxylic acid (**XX**). These compounds further decomposed to $^{14}CO_2$ for the extent of 20% of applied radioactivity at 28 days. Moreover, the evolution of $^{14}CO_2$ was much faster in the case of **XX** incubation. In the sterilized soil, ADS degraded slowly and the oxazoles accumulated up to 43% at 28 days. The half-life of ADS under outdoor winter conditions was about 2 days and major degradation compound was **I**. The amount of soil-bound materials of ADS reached the maximum around 2 months after treatment, but thereafter decreased gradually.

INTRODUCTION

Alloxydim-sodium (ADS), sodium salt of 2-(1-allyloxyaminobutylidene)-5,5-dimethyl-4-(methoxycarbonyl)cyclohexane-1,3-dione, is an active ingredient of herbicides Kusagard® (Japan) and Fervin® (Europe), which are being used for the control of gramineous weeds in tolerant broad leaf crops such as sugar beet and soybeans.[1] It has been reported that ADS degrades in sugar beets and soybean plants to three major compounds: deallyloxylated compound and two isomers of oxo-tetrahydrobenzoxazoles.[2–4] These transformations also occur physicochemically by light and heat. The formation of the two oxazoles is explained by a Beckmann rearrangement of the oxime structure followed by cyclization to form the five member ring.[2] The residue analytical methods for ADS in crops and soils were also reported.[5]

A major part of the pesticide sprayed in fields falls down to the soil surface. From the view point of environmental safety evaluation, it is important to know the fate of pesticide in soil. In this paper, the fate of ADS in soil is described through laboratory studies on the degradation in soils incubated in the dark and on the effect of soil sterilization upon this fate. Comparisons are made with its fate under outdoor conditions.

MATERIALS AND METHODS

1. Chemicals

Two kinds of labeled compounds, [7-^{14}C] *i.e.* [butylidene-1-^{14}C]ADS and [ring-1,5-^{14}C]ADS, were synthesized at Fine Chemicals Research Laboratory, Nippon Soda Co., Ltd. and at New England Nuclear Corporation, USA, respectively. The specific activities were 1.9 and

10.5 mCi/mmol for [7-^{14}C] and [1,5-^{14}C], respectively. The radiochemical purities were 99% for [7-^{14}C]ADS and 98% for [1,5-^{14}C]ADS as determined by thin-layer chromatography (TLC) using silica gel plates (Eastman Chromagram sheet 6060®) and a developing solvent of *n*-hexane–acetone (7 : 3). The following labeled compounds were also synthesized from [1,5-^{14}C]ADS and had the same specific radioactivity 10.5 mCi/mmol: deallyloxylated ADS (**I**), a mixture of oxo-tetrahydrobenzoxazoles (**II** and **III**), and 3,3-dimethyl-2-methoxycarbonylglutaric acid (**XX**).

For the identification of metabolites, authentic standards (**I** to **VIII**) were prepared as reported previously[2] and their abbreviated names are listed in Table 1 with the *Rf* values of the TLC. A ring-opened compound (**XX**) was newly prepared by refluxing ADS in 5% H_2O_2 followed by purification by column chromatography. The chemical structure of this standard was confirmed by CI-MS and NMR after conversion into its methyl ester by refluxing it with conc. H_2SO_4 in methanol. MS (isobutane), *m/z* 247 (M+1, base peak), 216 (M+1−OCH_3). NMR ($CDCl_3$, TMS), δ 1.20 (s, 6 H, $C(CH_3)_2$), 2.58 (s, 2 H, CH_2), 3.59 (s, 3 H, C-$COOCH_3$), 3.62 (s, 6 H, $C(COOCH_3)_2$), 5.21 ppm (s, 1 H, CH).

Table 1 *Rf* values of alloxydim-sodium and its potential metabolites.

Compound[a]	TLC solvent system[b]			
	A	B	C	D
Alloxydim	0.43	0.50	—	—
I	0.11	0.36	—	—
II	0.24	0.49	—	—
III	0.16	0.48	—	—
IV	0.55	0.66	—	—
V	0.55	0.66	—	—
VI	0.44	0.28	—	—
VII	0.54	0.67	—	—
VIII	0.13	0.19	—	—
XX	0.03	0.03	0.57	—
XX methyl ester	—	—	—	0.65

[a] See Fig. 1 for the structures of alloxydim, **I**, **II**, **III**, **VI**, **VII**, and **XX**. Chemical names of other compounds are as follows. **IV**: 6,6-dimethyl-5-methoxycarbonyl-3-propyl-4-oxo-4,5,6,7-tetrahydrobenzoisoxazole, **V**: 6,6-dimethyl-7-methoxycarbonyl-3-propyl-4-oxo-4,5,6,7-tetrahydrobenzoisoxazole, and **VIII**: 4-methoxycarbonyl-5,5-dimethyl-2-propionamidocyclohexane-1,3-dione.

[b] A: benzene–ethyl acetate (4 : 1), Merck Art. 5554.
B: *n*-hexane–acetone (7 : 3), Eastman Chromagram sheet 6060.
C: *n*-butanol–acetic acid–water (4 : 1 : 1), Merck Art. 5554.
D: chloroform–acetone (1 : 1), Merck Art. 5554.

2. *Soils*

Soils were collected at fields in Oiso, Kanagawa prefecture, and Shiojiri, Nagono prefecture. The content of organic matter was determind by the Robinson method. Characteristics of the soils were as follows. Oiso sandy clay loam, volcanic ash soil: sand 63.6%, silt 11.5%, clay 24.9%, organic matter 10.5%, cation exchange capacity (CEC) 31.6 meq/100 g and pH (H_2O) 5.9. Shiojiri sandy clay loam, volcanic ash soil: sand 61.1%, silt 16.4%, clay 22.5%, organic matter 10.1%, CEC 31.3 meq/100 g and pH (H_2O) 5.7. The soil was passed through a 2 mm sieve before the application of the compounds.

3. *Radioactivity Measurement*

The radioactivity in the liquid samples was measured by a scintillation spectrometer (Packard Tri-Carb 3320®) equipped with an automatic external standard. The radioactivity of the soil residue was determined by the same spectrometer after converting it to $^{14}CO_2$ by a sample oxidizer (Packard Tri-Carb 306®).

4. *Spectroscopy*

Mass spectra were recorded on a spectrometer (Hitachi M-80®) fitting with a data processing system (Hitachi M-003®). Direct injection (DI) method was adopted at 20 eV (EI) or 100 eV (CI with isobutane). NMR spectra were determined in deuterated chloroform at 270 MHz on a spectrometer (JMM FX-270®).

5. *Thin-Layer Chromatography (TLC)*

Two kinds of precoated silica gel plate (Merck Art. 5554® and Eastman Chromagram sheet 6060®) were developed with each solvent

system: A, benzene–ethyl acetate (4 : 1); B, *n*-hexane–acetone (7 : 3); C, *n*-butanol–acetic acid–water (4 : 1 : 1) and D, chloroform–acetone (1 : 1). Spots of authentic standards were detected by UV fluorescence quenching (254 nm). The *Rf* values of the authentic standards are shown in Table 1. Autoradiograms of TLC plate were prepared on medical X-ray films (Fuji Photo Film Co., Ltd.). The radioactivity of each spot on the TLC plates was determined by the spectrometer after scraping the corresponding gel regions into scintillation vials.

6. Degradation of ADS in Unsterilized and Sterilized Soils Incubated at 25°C (Experiment A)

Oiso soil was adjusted to 60% of the maximum water holding capacity (MWHC) and preincubated at 25±1°C in the dark for 6 days. The incubated soil (6 g as dry weight basis) was placed in cotton-plugged and sterilized test tubes (ϕ 11 mm×150 mm) after the soil moisture was checked. The test tubes containing soil were divided into two groups, unsterilized and sterilized. The soil in one group was mixed with the aqueous solution (0.2 ml) of [7-^{14}C]ADS or [1,5-^{14}C]ADS formulated as a 75% soluble powder. By this treatment, the soil moisture was adjusted to 60% of MWHC. The other group of soil was autoclaved at 120 °C for 30 min and then mixed with the ^{14}C-ADS solutions as mentioned above. The 75% soluble powder consisted of ADS (75%) and a succinate type surfactant (25%). The concentration of ADS in the soil was 3 ppm for dry weight basis and the applied radioactivity was around 1.0×10^5 dpm for both ^{14}C-ADS in a test tube. The treated soils were incubated at 25°C in the dark for 28 days. Sterilized water (0.15 ml) was added weekly to maintain the initial moisture content.

At incubation times of 0 (30 min), 1, 3, 7, 14, 21 and 28 days, the soils in two test tubes for each condition were separately extracted with a mixed solvent of ethyl acetate–methanol–water (1 : 2 : 2, 80 ml×3 times). Both samples of soil were separated off by centrifugation (1000 rpm×20 min). The combined supernatants were added to water (50 ml) and acidified with HCl to pH 1. The aqueous solution was extracted with ethyl acetate (50 ml× 2 times). The radioactivity in the extracts and soil residues was counted. The extract with ethyl acetate was concentrated and subjected to TLC analysis.

7. $^{14}CO_2$ Trapping Experiment Using Unsterilized and Sterilized Oiso Soils (Experiment B)

The unsterilized and sterilized soils were prepared, mixed with each of two kinds of ^{14}C-ADS and incubated as mentioned above. In this case, a side arm of Thumberg tube containing 3 ml of 20% KOH was attached to the test tube. The alkaline solutions were replaced every week and counted for radioactivity, before and after acidifying (*ca.* pH 2) with conc. HCl. At the same time, sterilized water was supplied to maintain the initial moisture content, 60% of MWHC.

8. Another $^{14}CO_2$ Trapping Experiment Using Unsterilized Shiojiri Soil (Experiment C)

Shiojiri soil with 60% of MWHC was incubated in the dark at 25°C for a week. The preincubated soil (5 g as dry weight basis) was placed in a 50 ml Erlenmeyer flask with a ground glass stopper. A small glass beaker containing 3 ml of 10 N NaOH was placed in this flask to trap $^{14}CO_2$ evolved from the soil. [1,5-^{14}C]ADS (69 μg), ^{14}C-**I** (61 μg), a mixture of ^{14}C-**II** and ^{14}C-**III** (61 μg), and ^{14}C-**XX** (48 μg) were added into the soil separately. After capping, the flasks were kept at 25°C in the dark for 30 days. At each incubation period of 4, 7, 14 and 30 days, the alkali trap was replaced with a new one and 0.5 ml of the trap solution in each flask was counted for the radioactivity before and after acidifying with conc. HCl.

9. Isolation and Structural Determination of Degradation Compounds (Experiment D)

This experiment was carried out to isolate a sufficient amount of the degradation compounds to elucidate the chemical structures by spectrometry. Shiojiri soil (500 g) placed in a plastic box was moistened so as to be 60% of MWHC and preincubated for 7 days at 25°C in the dark. Then, a water solution (2.5 ml) containing [1,5-^{14}C]ADS (0.1 mg, 7.0×10^6 dpm)

and an unlabeled one (50 mg) was added to the soil, which was incubated for a further 30 days under the same condition. The incubated soil was extracted with a mixture (1 $l \times 2$ times) of methanol and 0.1 N NH_4Cl (8 : 2), and filtered off by suction. The filtrate was acidified and extracted with dichloromethane. After drying the extract with Na_2SO_4 and evaporating, the concentrate was chromatographed on the Merck plate with solvent system A. The autoradiographic spots corresponding to authentic alloxydim, **I**, **II**, **III**, **VI** and **VII** were scraped off and their EI-MS were determined.

Furthermore, the soil residue remaining after methanol-0.1 N NH_4Cl extraction was shaken with 0.05 N NaOH (1 $l \times 2$ times). The alkaline solution was adjusted to pH 3.5 to exclude precipitates (humic acid fraction). The aqueous layer (fulvic acid fraction) was again washed with dichloromethane to extract remaining low-polar compounds. Then, the aqueous layer was neutralized and passed through an Amberlite XAD-4 (300 ml) column. The resin-bound materials were eluted with methanol (350 ml). The methanol eluate was concentrated and the residue was esterified by refluxing in methanol containing a small amount of conc. H_2SO_4. Thus obtained methyl ester of **XX** was purified by the Merck plate and solvent system D and subjected to CI-MS determination.

10. Degradation of ADS in Soil Placed Outdoors and Indoors (Experiment E)

[7-^{14}C]ADS (7.5 mg) and a succinate type surfactant (2.5 mg) were dissolved in water (10 ml). The solution (0.2 ml, 8.39×10^5 dpm) was mixed with Oiso soil (5 g) and the concentration of ADS was about 30 ppm. The treated soil was placed upon the same soil (45 g) in a 100 ml beaker (ϕ 5 cm $\times$ 7 cm). The depth of the combined sample was about 2.5 cm. The combined soil was kept under two different conditions from Jan. 27th to Mar. 27th, 1976. One was kept outdoors in the daytime (sunny 33 days and cloudy 21 days) and indoors at night and on rainy days (7 days). The other was kept in a dark place indoors. Water was sprayed periodically to avoid dryness. At post-treatment times of 0 (30 min), 1, 3, 7, 14, 30 and 60 days, the whole sample in each of two beakers for each condition was separately extracted and analyzed for each metabolite by the method in Experiment A.

11. Long Term Experiment for Degradation of ADS in Soil (Experiment F)

Oiso and Shiojiri soils containing 60% moisture of MWHC were preincubated for 10 days. These soils, each of 600 g, were placed in each of 10 plastic containers (ϕ 11 cm $\times$ 10 cm). In addition, another 20 g of the soil was mixed with 1 ml of aqueous solution of [1,5-^{14}C]ADS 75% formulation so as to make a 30 ppm concentration of ADS. The treated soil was placed on the same soil in the container. The soil samples were kept outdoors from May 30, 1977, except on rainy days and nights, when they were kept indoors. The weight of the soil including the tare was measured and 10 to 50 ml of water a day was added to the soil to keep the 60% moisture of MWHC. The temperature varied between -5°C and 35°C over one year. The entire sample in each of two containers was separately analyzed 1, 2, 3, 6, 9 and 12 months after treatment. A part (100 g) of the soil was extracted in duplicate with the mixed solvent which was used in Experiment A and the radioactivity in the extract and the soil residue was counted as mentioned before.

RESULTS AND DISCUSSION

Fate study of pesticides in soil poses difficulties because of the many factors involved in dealing with the various conditions. In this report, two different types of soils were used for degradation study. Furthermore, one soil was sterilized before the addition of the pesticide to ascertain the contribution of microbial activity for the degradation. The soils treated with ^{14}C-ADS were routinely incubated at 25°C in the dark. Beside the above constant condition, they were placed indoors and outdoors for a maximum of one year so as to simulate behavior in the actual field as much as possible. In Japan, 1000-fold aqueous solution of the ADS formulation of 75% soluble powder is usually sprayed at the rate of one m^3/ha in fields. When the compound spreads uniformly

Table 2 Balance of ^{14}C-compounds in unsterilized Oiso soil treated with [7-^{14}C] or [1,5-^{14}C] alloxydim-sodium (Expt. A).

Compound	Day	Solvent extract								Bound residue	Total
		Alloxydim	I	II	III	VII	Unknowns	Origin	Subtotal		
[7-^{14}C] ADS	0	75.5[a)]	1.0	1.7	1.3	0.6	5.9	0.7	86.7	8.4	95.1
	1	72.5	1.3	3.3	3.0	0.6	3.4	1.5	85.6	5.1	91.7
	3	63.5	2.0	6.5	5.0	0.6	2.9	1.8	82.3	7.4	89.7
	7	46.6	2.9	7.3	5.1	0.7	3.4	3.0	69.0	16.6	85.6
	14	17.8	2.9	9.5	7.1	0.7	3.7	5.0	46.7	21.6	68.3
	21	6.1	4.0	7.6	5.6	0.3	3.2	6.0	32.8	25.5	58.3
	28	2.2	3.1	5.6	4.7	0.0	2.4	5.4	23.4	27.6	51.0
[1,5-^{14}C] ADS	0	75.9	1.9	1.5	1.6	1.1	5.2	1.8	89.0	6.9	95.9
	1	78.1	2.6	4.2	3.6	1.1	5.0	2.1	96.7	6.2	102.9
	3	62.7	3.2	5.5	4.3	1.1	4.1	2.5	83.4	11.9	95.3
	7	42.9	3.5	8.0	5.3	0.6	4.4	3.7	68.4	21.3	89.7
	14	15.4	3.4	8.5	5.5	0.4	2.9	6.3	42.4	29.3	71.7
	21	5.3	4.4	6.4	5.7	0.2	3.4	6.2	31.6	31.2	62.8
	28	2.3	3.5	5.3	4.9	0.0	2.5	6.6	25.1	34.5	59.6

[a)] Figures are % of radioactivity applied.

in surface soil of 10 cm depth, the concentration in soil corresponds to 7.5 ppm. The dosage in this study was designed for these practical conditions.

Extractability of ^{14}C-ADS from soil was surveyed for various solvents and the following yields were obtained by the extraction procedure described in METHODS: 82% with methanol, 63% with acetone, 90% with ethyl acetate, 33% with 0.1 N HCl solution, and 91% with a mixture of ethyl acetate–methanol–water (1 : 2 : 2). Considering this extractability, the mixed solvent was employed for extraction, except for the isolation of degradation compounds (Expt. D).

1. *Degradation of ADS in Unsterilized Soil Incubated in the Dark*

ADS applied to Oiso soil behaved as shown in Table 2 (Expt. A). Both compounds, [7-^{14}C] and [1,5-^{14}C]ADS, were degraded in nearly the same pattern over 28 days of incubation. Little difference between two ^{14}C-ADSs was found in the total recovery of the radioactivity. The decrement of total radioactivity recovered may mean that radioactive volatiles were evolved and released from the soil. The radioactivity remaining in the soil after the extraction, *i.e.* bound residues, increased gradually up to 28 days of incubation. Major parts of the extracted ^{14}C-compounds were identified and quantified by the TLC method. ADS persisted as a main residue before 21 days of incubation. The half-life of ADS was 5 to 6 days under the dark incubation. Identified metabolites were **I**, **II**, **III** and **VII**. **IV**, **V**, **VI** and **VIII** were not found in this experiment. As shown in Fig. 1, **II** and **III** were in the relation of isomeric oxazoles which formed after Beckmann rearrangement.[2)] Huhtanen and Dorough suggested the Beckmann type rearrangement in the metabolic degradation of an oxime carbamate methomyl.[6)] The nature of the unknowns, consisting of 3 to 4 compounds and the materials unmoved from the starting point on the TLC plate, were not investigated in this study.

$^{14}CO_2$ released from the soil was trapped with an alkaline solution in Thumberg tube in another Experiment B (Table 3). All trapped radioactivity was accounted for by CO_2 because the radioactivity disappeared by acidifying the trapping solution. The evolution of $^{14}CO_2$ differed a little between [7-^{14}C] and [1,5-^{14}C]ADS. Cumulative amounts of $^{14}CO_2$ were 22.6% for [7-^{14}C]ADS and 16.7% for [1,5-^{14}C]-ADS over 28 days of incubation. This suggests that both the sidechain and the cyclohexane ring of ^{14}C-ADS can be utilized by soil

Table 3 Cumulative $^{14}CO_2$ evolved from ^{14}C-ADS treated into Oiso soil (Expt. B).

Condition	Compound	Days after treatment			
		7	14	21	28
Unsterilized	7-^{14}C	4.6[a)]	13.6	18.8	22.6
	1,5-^{14}C	3.6	9.5	13.6	16.7
Sterilized	7-^{14}C	0	0	0.1	0.1
	1,5-^{14}C	0	0	0.2	0.2

a) Figures are % of radioactivity applied.

microorganisms.

Instrumental identification of degradation compounds was carried out in Experiment D, using an extract from the soil sample incubated for 30 days. From aqueous methanol extract containing 27% of applied ^{14}C, **I**, **II** and **III** were cleaned up and identified with each authentic standard by EI mass spectrometry: *m/z* 267 (M^+, base), 208 (M^+—COOMe) for **I**, 265 (M^+), 206 (M^+—COOMe, base) for **II**, and 265 (M^+, base), 206 (M^+—COOMe) for **III**. Nature of the soil bound residue remaining after mixed solvent extraction was investigated by alkali extraction as commonly employed. In a preliminary experiment it was observed that **I** is easily convertible to **VI** and further to demethoxycarbonylated **VI** at 0.5 N NaOH extraction. In the case of 0.05 N NaOH treatment, conversion of **I** to **VI** mostly diminished and color substance in the extract decreased. Thus applied 0.05 N alkali fraction contained 20% of applied ^{14}C, which was further fractionated into humic acid fraction 4%, fulvic acid fraction 17% and soil residue fraction 27%. Chloroform extractable compounds in the fulvic acid fraction were again **I**, **II** and **III**, and the aqueous layer contained **XX**, which was identical on cochromatogram with the authentic compound. This compound was derivatized into a methyl ester, then identified by TLC cochromatography and CI-MS. The abundance of **XX** was about the half of the radioactivity in the fulvic acid fraction.

2. *Effect of Sterilization of Soil on the Degradation of ADS*

Oiso soil was autoclaved and incubated with [7-^{14}C] and [1,5-^{14}C]ADS for 28 days under the same conditions as those of the above dark incubation (Expt. A). The results of two experiments are shown in Tables 3 and 4. Number of microorganisms in the sterilized soil was counted weekly by the dilution plate methods using albumin agar and rosebengal agar for bacteria and fungi respectively. No colony was found through 14 days. A slight contamination by fungi was observed at 21 and 28

Table 4 Balance of ^{14}C-compounds in sterilized Oiso soil treated with [7-^{14}C] or [1,5-^{14}C] alloxydim-sodium (Expt. A).

Compound	Day	Solvent extract								Bound residue	Total
		Alloxydim	I	II	III	VII	Unknowns	Origin	Subtotal		
[7-^{14}C] ADS	1	74.2[a)]	1.9	7.6	4.8	0.6	4.0	0.8	93.9	3.5	97.4
	3	71.1	1.9	9.3	7.5	0.6	4.2	1.0	95.6	4.6	100.2
	7	62.1	2.9	14.6	11.0	0.4	3.3	1.5	95.8	6.7	102.5
	14	43.6	2.8	22.3	15.4	0.3	2.4	0.8	87.6	15.6	103.2
	21	30.2	7.1	25.4	14.3	0.2	2.1	1.9	81.2	18.0	99.2
	28	17.5	7.3	27.8	13.0	0.0	3.6	2.9	72.1	19.7	91.8
[1,5-^{14}C] ADS	1	78.8	2.5	5.8	4.0	1.2	4.3	1.4	98.0	4.3	102.8
	3	69.4	4.7	6.1	4.8	1.2	4.6	1.5	92.3	5.7	98.0
	7	65.5	2.0	14.1	10.5	0.5	2.8	0.8	96.2	7.9	104.1
	14	42.4	3.2	19.6	13.8	0.8	3.0	1.9	84.7	12.2	96.9
	21	31.1	7.4	23.9	15.0	0.6	1.9	1.6	81.5	16.5	98.0
	28	22.3	8.2	28.7	15.0	0.2	0.7	1.4	76.5	25.3	101.8

a) Figures are % of radioactivity applied.

Table 5 Cumulative $^{14}CO_2$ evolved from Shiojiri soil (Expt. C).

Treated compound	Days after treatment				
	4	7	14	21	30
^{14}C-ADS	—	2.3[a)]	5.7	—	11.4
^{14}C-**I**	—	1.9	3.7	—	10.6
Mixture of ^{14}C-**II** and **III**	—	0.9	—	5.3	8.0
^{14}C-**XX**	24.2	33.1	—	—	—

a) Figure are % of radioactivity treated.

days of incubation.

The behavior of the radioactivity was almost the same between two ^{14}C-ADSs under the sterilized conditions. Nearly all of the applied radioactivity was recovered from the sterilized soil, but that from the unsterilized soil decreased with time (Tables 2 and 4). This difference is coupled with the fact that $^{14}CO_2$ was produced in unsterilized soil but not in the sterilized one (Table 3). The half-life of ADS in the sterilized soil was about double (10 to 11 days) of that in the unsterilized soil. On the other hand, the amounts of **II** and **III** increased with time and reached about 42% of applied radioactivity at 28 days. The formation of these two metabolites was in the ratio of 3 : 2 as reported in the chemical transformation of ADS.[2)] Comparing this with the results in Table 2, it may be understood that **II** and **III** are also decomposed by soil microorganisms under unsterilized conditions. Actually, in Experiment C using Shiojiri soil, $^{14}CO_2$ derived from ^{14}C-**I** and the mixture of ^{14}C-**II** and **III** was detected at nearly the same rate as that from ^{14}C-ADS (Table 5). Moreover, the ring-opened dicarboxylic acid **XX** degraded rapidly in the soil, compared to the ring-unopened compounds. This fact shows that **XX** is an important intermediate in the metabolic pathway of ADS to CO_2 (Fig. 1).

3. *Degradation of ADS in Soil Placed Outdoors and Indoors*

Apart from the above condition-controlled incubation (25°C in the dark), the fate of [7-^{14}C]ADS under nearly natural conditions was investigated using 10 times the amount of soil (50 g) in a beaker (Expt. E). The radioactivity balance under outdoor and indoor conditions is summarized in Table 6. Under outdoor conditions, the total recovery of radioactivity decreased gradually over 60 days to about 56% of the applied radioactivity. In parallel with this, the extractable ^{14}C-compounds decreased to 56.7 and 21.1% at 14 and 60 days respectively. Meanwhile, the bound residue reached about 34% at the same periods. The behavior of ^{14}C-ADS in indoor soil was similar to that of outdoors, but the decrease of the recovered radioactivity was slower in the former than in the latter. Comparing these results with those in Table 2, the total ^{14}C-recoveries in the outdoor soil were higher than in 25°C dark-incubation soil. This difference may be caused by the lower temperature in the outdoor experiment, which was carried out during winter, and by the faster formation of bound residues. The bound residues are thought to persist longer than the solvent extractable residues.

Within 30 days, the major ^{14}C-compound was alloxydim under both conditions. At 60 days alloxydim was replaced by **I** in outdoor soil, while it was still the major ^{14}C-compound in the indoor soil. The half-lives of ADS were about 2 days outdoors and 4 days indoors. Metabolites identified by the co-chromatography were **I**, **II**, **III**, **VI** and **VII**. The amounts of these metabolites under indoor conditions were small, less than 5% of applied radioactivity, and showed a similar tendency to the case of 25°C incubated soil. But in the outdoor soil, **I** reached the maximum 14.2% at 14 days and then decreased with time. As reported in the previous paper,[2)] ADS is sensitive to UV light and transformable to **I**. Thus it is understood that **I** could also be formed by sunlight exposure. As other major products, isomeric oxazoles **II** and **III** were always persistent during the experiment.

To determine the fate of ADS over a longer period and the seasonal effect on ADS degradation, Experiment F was conducted by determining the radioactivity in the solvent extract and soil residues (Table 7). The radioactivity in the solvent extract in this experiment was less than that in the outdoor experiment as shown in Table 6 at 1 and 2 months of incubation. The rates of binding of the

Table 6 Balance of ^{14}C-compounds in Oiso soil applied with [7-^{14}C]alloxydim-sodium (Expt. E).

Condition	Day	Solvent extract									Water solubles	Bound residue	Total
		Ethyl acetate extract											
		Alloxydim	I	II	III	VI	VII	Unknowns	Origin	Subtotal			
Outdoors[b]	0	84.6[a]	0.5	1.3	1.2	0.0	0.5	3.4	0.9	92.4	0.1	7.0	99.5
	1	63.5	4.5	2.0	1.8	0.4	0.9	9.4	2.4	85.0	0.4	17.2	102.6
	3	38.6	10.7	3.5	3.2	0.4	0.9	11.6	5.2	74.1	1.5	23.9	99.5
	7	22.7	12.5	4.5	4.0	0.7	0.6	8.4	5.2	58.6	1.5	32.9	93.0
	14	18.2	14.2	4.3	3.9	0.7	0.5	7.7	5.8	55.3	1.4	33.7	90.4
	30	14.1	11.6	3.0	2.4	0.9	0.4	3.5	5.4	41.3	1.2	33.5	76.0
	60	0.6	10.3	1.6	1.1	0.7	0.0	1.9	4.1	20.3	0.8	34.8	55.9
Indoors	0	84.6	0.5	1.3	1.2	0.0	0.5	3.4	0.9	92.4	0.1	7.0	99.5
	1	69.3	1.7	1.4	1.2	0.4	0.6	9.9	3.7	88.2	0.2	12.0	100.4
	3	54.9	2.2	1.7	1.5	0.7	0.9	13.6	5.6	81.1	0.3	15.5	96.9
	7	39.2	2.8	3.1	2.8	0.9	0.8	15.1	9.1	73.8	1.2	18.8	92.8
	14	24.2	3.4	3.9	3.5	1.0	0.4	13.4	10.3	60.1	1.2	28.1	89.4
	30	19.3	4.2	4.6	4.2	0.8	0.1	11.1	10.9	55.2	1.3	30.2	86.7
	60	12.7	4.8	5.0	4.8	0.6	0.0	4.6	10.3	42.8	1.4	33.0	77.2

[a] Figures are % of radioactivity applied and the average of duplicates.

[b] Cumulative sunlight exposing time to sampling date; 1 day: 2 hr, 3: 14, 7: 35, 14: 63, 30: 119 and 60: 245.

Table 7 Behavior of [1,5-^{14}C]ADS in soils incubated outdoors for one year (Expt. F).

Month	Oiso soil			Shiojiri soil		
	Solvent extract	Bound residue	Total recovery	Solvent extract	Bound residue	Total recovery
1	16.6[a]	27.4	44.0	20.4	36.2	56.6
2	6.5	30.4	36.9	10.2	36.4	46.6
3	2.9	29.3	32.2	5.0	37.3	42.3
6	1.9	23.5	25.4	3.0	31.2	34.2
12	1.2	22.7	23.9	1.4	25.5	26.9

[a] Figures are % of radioactivity applied.

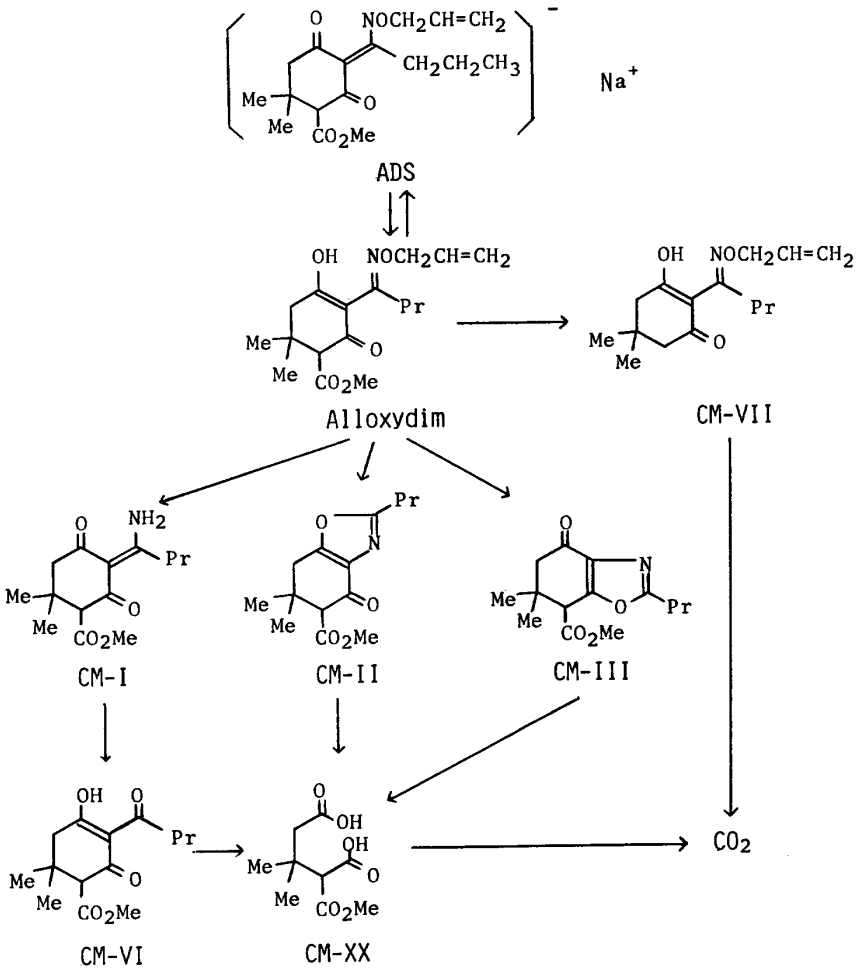

Fig. 1 Proposed degradation pathways of ADS in soil.

radioactivity into the bound residue were also lower by several percent. The bound materials reached maximum abundance after around 2 or 3 months of incubation, but after that, they decreased gradually, probably by microbial degradation to CO_2.

In conclusion, it was shown that ADS is degradable in soil with half-life of less than 2 days in the environment. ADS and its degraded compounds such as **I**, **II** and **III** are gradually decomposed to carbon dioxide *via* **XX** as shown in Fig. 1.

ACKNOWLEDGEMENTS

The authors express their thanks to Professor Izuru Yamamoto, Tokyo University of Agriculture, for his valuable suggestion during the course of this study. Thanks are also due to their colleagues for useful advice and help.

REFERENCES

1) I. Iwataki & Y. Hirono: "Advances in Pesticide Science," ed. by H. Geissbühler, Part 2, Pergamon Press, Oxford & New York, p. 235, 1979
2) Y. Soeda, K. Ishihara, I. Iwataki & H. Kamimura: *J. Pesticide Sci.* **4**, 121 (1979)
3) Y. Hashimoto, K. Ishihara & Y. Soeda: *J. Pesticide Sci.* **4**, 299 (1979)
4) Y. Hashimoto, K. Ishihara & Y. Soeda: *J. Pesticide Sci.* **4**, 375 (1979)
5) S. Ono, N. Toyama, K. Sugioka, M. Tokieda, O. Nomura & S. Tsukada: *J. Pesticide Sci.* **5**, 575 (1980) (in Japanese)
6) K. Huhtanen & H. W. Dorough: *Pestic. Biochem. Physiol.* **6**, 571 (1976)

要　　約

除草剤アロキシジムの土壌における分解

小野成男，塩谷広範，石原莞爾
時枝正則，添田吉則

アロキシジム (alloxydim-sodium, ADS) の butylidene-1 および ring-1, 5 に別々に ^{14}C で標識された化

合物を用い，土壌における分解を調べた．25°C のインキュベータ内に置いた大磯土壌で，ADS は約 5 日の半減期で消失し，分解物として脱アリルオキシ体 (**I**)，二つのオキサゾール体 (**II** および **III**)，脱アリルオキシアミノ体 (**VI**)，脱メトキシカルボニル体 (**VII**) および開環して生じたグルタール酸誘導体 (**XX**) が同定された．これらの分解物はさらに $^{14}CO_2$ まで分解され，処理 28 日後に $^{14}CO_2$ は約 20% に達した．**XX** からの $^{14}CO_2$ 発生は，ADS 処理の場合に比べ，さらに速やかであった．野外条件下の土壌における ADS の半減期は約 2 日であり，主分解物は **I** であった．土壌結合残留物の量は処理後 2 カ月で最高となるが，その後は徐々に減少した．