한국농화학회지 Vol. 18, No. 1, March 1975
J. Korean Agricultural Chemical Society

Decomposition of a Substituted Diphenyl Ether Herbicide(MC-4379) by Some Environmental Factors

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(Received, February 10, 1975)

環境要因에 依한 置換 Diphenyl Ether系 除草劑 MC-4379의 分解에 關한 硏究

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Introduction

The past few years have witnessed a rapidly increasing interest in the decomposition of pesticides by microorganisms, animals, higher plants, and sunlight or artificial light in connection with the environmental toxicity.

Herbicides reaching the soil, accidentally or by design, normally become dissipated or removed with time, in one or more of several ways-volatilization, photodecomposition, soil adsorption-inactivation, leaching, chemical breakdown, microbiological degradation, plant uptake, followed by metabolic degradation and/or physical removal at harvest.

MC-4379 (2,4-dichloro-3′-carboxymethyl-4′-nitro-diphenyl ether) used in this study is a new herbicide belonging to the substituted diphenyl ether, being introduced by the Mobil Chemical Company recently[39].

Nitrofen (TOK, NIP, 2,4-dichloro-4′-nitro-diphenyl ether) and CNP (MO-338, 2,4,6-trichloro-4′-nitro-diphenyl ether) are leading chemicals of this type now in wide use in this country[39].

C-6989 (Preforan, Fluorodifen,2,4′-dinitro-4-trifluoromethyl-diphenyl ether) is also a herbicide of this group which has not yet introduced to this country up to now[39].

These compounds are most favored in that they are wide spectrum herbicides, are useful for preemergence weed control in transplanted rice and other crops, and exhibit low toxiciy to fish.

Gutenmann et al.[22] reported 2,4-dichloro-4′-amino-diphenyl ether (Amino-Nitrofen) as a metabolite of Nitrofen in bovine rumen fluid.

Nitrofen was demonstrated to decompose to 2,4-dichlorophenol and p-nitrophenol in aqueous photolysis and the nitro group in the structure was shown to undergo both photoreduction and nucleophilic displaement by hydroxide ions[6].

Ichihashi et al.[30] found that X-52 (2,4-dichloro-3′-methoxy-4′-nitro-diphenyl ether), CNP (MO-338), and Nitrofen formed the reduced amino derivatives in enriched soil solution.

CNP(MO-338), Nitrofen, X-52, and MO-500 (2,4-dichloro-6-fluorophenyl-4′-nitrophenyl ether) were compared by Kuwatsuka[36] as to their degradation in soils under flooded and upland conditions.

The fate of Nitrofen was investigated by Hawton et al.[25] in some weeds. They suggested lipid-Nitrofen conjugates or Nitrofen polymers and products resulting from the ether linkage as metabolites.

The metabolic and photolytic studies on C-6989 in higher plants and under artificial light respectively were undertaken by some authors[12,13,14,15,16,39,56].

Lee[39] conducted an investigation on the products from the solution-phase photolysis of eight substituted diphenyl ether herbicides under the simulated environmental conditions. Of the chemicals used, MC-4379 and its related compounds were firstly examined in his experiment.

In this study, the degradation of MC-4379 by soil microorganisms, the crude extract of germinating rice seeds, and natural sunlight as solid-phase photolysis was investigated in relation to the environmental safety and for the comparison of its decomposition pattern.

Materials and Methods

1. Substituted diphenyl ether herbicides

MC-4379 (2,4-dichloro-3′-carboxymethyl-4′-nitro-diphenyl ether) was obtained from the Mobil Chemical Company, Edison, N.J., U.S.A. The purity of the compound was confirmed by tlc and glc.

Nitrofen (TOK, NIP, 2,4-dichloro-4′-nitro-diphenyl ether) was supplied by the Rohm and Haas Chemical Company, Independence Mall West, Philadelphia, Pa., 19105.

C-6989 (2,4′-dichloro-4-trifluoromethyl-diphenyl ether) was provided by the CIBA Agrochemical Company, Division of CIBA Corp., Vero Beach, Fla.

2. Authentic compounds

Authentic compounds were supplied also by Mobil Chemical Company and used with no purification. They include the following:

1) 2,4-dichloro-3′-carboxymethyl-4′-nitro-diphenyl ether
2) 2,4-dichloro-3′-carboxyl-4′-nitro-diphenyl ether
3) 2,4-dichloro-4′-nitro-diphenyl ether
4) 2,4-dichloro-3′-carboxyl-4′-amino-diphenyl ether
5) 2,4-dichloro-3′-carboxymethyl-4′-amino-diphenyl ether
6) 2,4-dichloro-4′-amino-diphenyl ether
7) 3-carboxymethyl-4-nitrophenol
8) 3-carboxyl-4-nitrophenol
9) p-nitrophenol
10) 3-carboxymethyl-4-aminophenol
11) 3-carboxyl-4-aminophenol
12) p-aminophenol

3. Analytical methods

Gas liquid chromatographic analyses were performed with a Tracor-550 GLC instrument equipped with a flame ionization detector.

The column packing was a mixture of 11% OV-17 and 11% QF-1 (1:1 w/w) liquid phase on 80-100 mesh Gas Chrom Q.

Operating parameters were generally as follows: Pyrex glass tube of 2 mm. i.d., 6 ft. in length: Column flow of 70 ml nitrogen gas per min. This column was conditioned at 250°C with a nitrogen flow for 48 hours. Injection temp. of 210°C; Detector temp. of 150°C; inlet temp. of 220°C; outlet temp. of 240°C; input attenuator, 10^2; output attenuator, 4; All analyses were made by temperature-programming from 100° to 250°C at a rate of a linear temperature increase of 7.5°C per min.

The ir spectra were obtained as liquid film (0.3 % chloroform solution; 0.25 mm cell) with the Shimadzu Infra Red Spectrophotometer IR-27G.

Ultraviolet (uv) *spectrum* was obtained in spectrophotometric grade solvents (Mallinckrodt Chemical Works, St. Louis, Mo.), and was recorded with a Beckman DB-G grating spectrophotometer (Beckman Instruments, Inc., Fullerton, Calif.).

Thin layer chromatography was accomplished

on analytical plates of Silica gel GF 254 (Merck, Germany) using solvent systems consisting of (A) Benzene-chloroform (1 : 1 v/v), (B) Chloroform-ethanol-acetic acid (90 : 5 : 5 v/v), and (C) Chloroform-ethylacetate-acetic acid(6 : 3 : 1 v/v). The separated substances were detected by means of iodine vapour in the glass chamber.

4. Photolysis of MC-4379

1) Photolysis in solid phase

Two grams of MC-4379 were applied in two petri-dishes with the aid of a small amount of acetone. After application, acetone was volatilized. The test materials were irradiated under natural sunlight in July-August (intensity: ca 12 photos) for 8 and 40 hrs. respectively. The photolyzed mixture was redissolved in acetone. The acetone solution was applied as a band on 20×20 cm precoated tlc plates 2.5 cm from one edge and developed in a solvent mixture of chloroform-benzene (1 : 1 v/v). Each band scraped off the plates separately was extracted with acetone. The acetone extract was concentrated to a small volume or to dryness for GLC and TLC.

2) Photolyses in suspensions of different pH's

0.2g of MC-4379 was suspended in about 200 ml of distilled water adjusted to pH 2.0 and 8.0 with IN-HCl and IN-NaOH, respectively, with the aid of a magnetic stirrer. These suspensions were exposed directly to sunlight for 40 hrs. After irradiation, the decomposed products were extracted with benzene, confirmed and compared by TLC and GLC.

3) Benzophenone-sensitized photolysis of MC-4379

A solution of 0.5 g of MC-4379 and a small amount of benzophenone in ca 20 ml of acetone was exposed to sunlight for 8 hrs. As a blank test, a benzophenone-acetone solution and an MC-4379-acetone solution were also exposed to sunlight for the same period, respectively. The decomposition products were examined by TLC and GLC.

4) Riboflavin-sensitized photolysis of MC-4379

The same method as the above was employed in this experiment.

5) Change in pH of th eMC-4379 suspension exposed to sun-light

0.341 g of MC-4379 was suspended in 100 ml of distilled water with the aid of a small amount of acetone and exposed directly to sunlight for 40 hrs. During irradiation, the pH of the sample was measured at an interval of 8 hrs to see the occurrence of dechlorination.

5. Decomposition MC-4379 by the crude enzyme of germinating rice seeds

1) Preparation of the crude enzyme solution

Rice seeds steeped in distilled water for five days were placed on wet filter paper in 10 cm Petri-dish and incubated at 30°C. After incubation for 5 days, the germinating seeds, the shoots of which were about 2~3 cm long, were used as the sample. 10 grams of the material were homogenized with 100 ml of 0.2 M-phosphate buffer solution (pH 7.00) in a blendor for 3 min and centrifuged for 30 min at 12,000 rpm. The supernatant was collected and used as the crude enzyme solution.

2) Decomposition of MC-4379

0.341g of MC-4379 dissolved in a small amount of acetone was added to 100 ml of the crude enzyme solution with Triton X-100 as surfactant. The mixture was incubated at 37°C for 8 hrs.

The reacted mixture was extracted with benzene.

The surfactant added was removed by centrifuging at 12,000 rpm for 30 min.

6. Degradation of MC-4379 by microorganisms

1) Degradation by fungi

Nine strains of fungi were cultured for 6 days in each malt extract medium containing 1,000 μg of the herbicide per milli-liter, respectively. The culture was shaken during the growth period at 30°C. The in cubated medium was extracted with benzene and the benzene solution was evaporated to a small volume on a rotary evaporator. The degradation products were confirmed by TLC, GLC, and IR.

2) Degradation by bacteria

Ten strains of bacteria were cultured for 8

days in each nutrient medium (Bacto-beef, 10g; peptone, 10g; NaCl 5g; and distilled water added to 1,000 ml; pH 7.4) containing 1,000 μg of the herbicide per milli-liter, respectively. The methods of culture and extraction were the same as the above.

Results and Discussion

1. Photoproducts of MC-4379

In order to check the light absorption of MC-4379, the absorbance of a 10^{-5} M *n*-hexane solution was measured against the wavelengths of 190 to 340 nm (Figure 1). The molar absorptivity at 300 nm is 6,500[39], which means this chemical absorbs sufficient light energy under natural sunlight to undergo photolysis. The larger wavelength band appears to be related to $n \rightarrow \pi^*$ transition of non-bonding oxygen electrons to the antibonding π^* orbital of the nitro group[4].

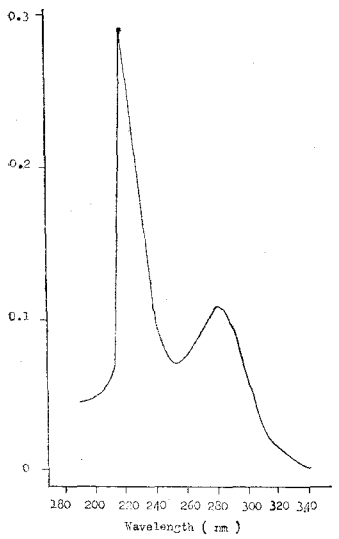

Figure 1. Ultraviolet absorption spectrum of MC-4379 (10^{-5}M in *n*-hexane)

1) Products from photolyses in solid phase

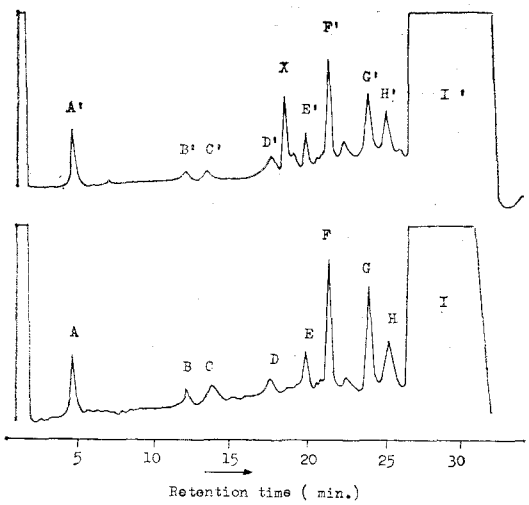

Figure 2. Gas-liquid chromatograms of products from MC-4379 photolyses in solid phase (the upper: for 8 hrs, the lower: for 40 hrs)

A: *p*-aminophenol. B: *p*-nitrophenol, C: 3-carboxymethyl-4-nitrophenol, D: 2,4-dichloro-4′-amino-diphenyl ether, E: 2,4-dichloro-3′-carboxyl-4′-amino-diphenyl ether, F: Nitrofen (2,4-dichloro-4′-nitro-diphenyl ether), G: 2,4-dichloro-3′-carboxyl-4′-nitro-diphenyl ether, H: amino-MC-4379 (2,4-dichloro-3′-carboxymethyl-4′-amino-diphenyl ether, I: MC-4379 (2,4-dichloro-3′-carboxymethyl-4′-nitro-diphenyl ether

Table I. Products from MC-4379 photolysis for 40 hrs in solid phase (GLC)

Photoproducts	Retention time(min.)
A. *p*-aminophenol	4.6
B. *p*-nitrophenol	12.2
C. 3-carboxymethyl-4-nitrophenol	13.9
D. 2,4-dichloro-4′-amino-diphenyl ether	17.8
E. 2,4-dichloro-3′-carboxyl-4′-aminodiphenyl ether)	19.9
F. Nitrofen(2,4-dichloro-4′-nitrodiphenyl ether)	21.3
G. 2,4-dichloro-3′-carboxyl-4′-nitrodiphenyl ether	23.9
H. amino-MC-4379 (2,4-dichloro-3′-carboxymethyl-4′-amino-diphenyl ether)	25.1
I. MC-4379 (2,4-dichloro-3′-carboxymethyl-4′-nitro-diphenyl ether)	26.8

Figure 2 shows the gas liquid chromatogram of the products obtained. These products identified by comparing with the authentic compounds in terms of retention time are shown in table I.

As seen in Figure 2, 2,4-dichloro-3′-carboxyl -4′-nitrodiphenyl ether (G), 2,4-dichloro-3′-carboxymethyl-4′-aminodiphenyl ether (H, Amino -MC-4379), 2,4-dichloro-4′-nitrodiphenyl ether (F, Nitrofen), and *p*-aminophenol (A) were predominant.

In general, most of the products were consistent with those obtained from the aqueous photolysis.

However, compound G was not detected in the case of aqueous photolysis in which hydroxylated products were predominant.[39]

As shown in Figure 3, the proposed radical intermediatee (I) and (I′)[45] may abstract H from the solvent (acetone and/or water in this study) to form products (G) and (F).[45]

The composition of the products of photolysis changes with the solvent. Based on the fact that proton abstraction from cyclohexane is some 20 Kcal mol^{-1} easier than from water.[34] it is more likely that proton will be more readily abstracted from acetone in which the test compound was dissolved, than from water presumably present in the air.

In addition, proton abstraction will occur more readily than hydroxyl abstraction from water[49].

The mechanism of photoreduction of nitro to amino group was already shown[5,39].

In the photolyses for 8 and 40 hrs, most products were identical, except that an unidentifiable product was detected in the 8 hr photolysis.

On the basis of the fact that this product was not detected in the 40 hr photolysis, it must be an unstable intermediate, thas is, a nitroso[42] or hydroxyl amine compound which is formed in the course of photoreduction of nitro to amino group.

Barltrop et al.[3] investigated the photoreaction mechanism of the aromatic nitro group and found that shortwave-length ultraviolet light led predominantly to complete reaction, whereas ultraviolet light of longer wavelength (>290nm) favored the production of azoxybenzene and other bimolecular products. Phenylhydroxylamine and nitroso-benzene were shown to be intermediates, but the reduction of nitroso-benzene to phenylhydroxylamine was considered to take place in a dark reaction.

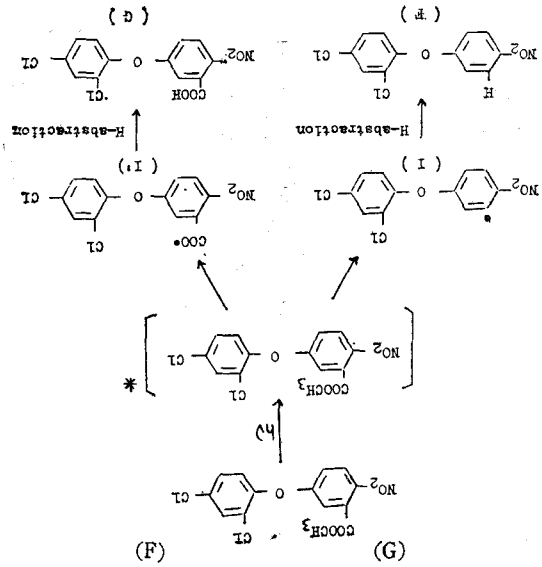

Figure 3. Postulated pathway of formation of compounds G and F from MC-4379 photolysis in solid phase

In support of the decarboxymethylation mechanism in the photoysis of the parent compound, evidence for the facile cleavage of the C-C bond in the photolysis of benzoin ethers(*α*-alkoxy-*α*-phenylacetophenone derivatives) was provided recently.[26,27,38,48]

Other types of dealkylation were investigated in the following studies.

Plimmer et al. photodecomosed the herbicide N-sec-butyl-4-tert-butyl- 2,6-dinitro aniline in water or methanol to give the dealkylated nitroso compound (4-tert-butyl-2-nitro-6-nitrosoaniline) as the product together with mixture of minor photoproducts.[50]

Several studies of the photochemistry of N-alkylated nitroanilines have been concerned with the mechanism of N-dealkylation[10,44,51]. Studies of trifluralin revealed that N-alkyl-dinitro-anilines are reduced and dealkylated by light. Of ten photoproducts obtained by irradiation of

trifluralin in hexane or methanol solution at 253.7nm, only two compounds α, α, α-trifluoro-2, 6-dinitro-N-propyl-p-toluidine and α, α, α-trifluoro-2, 6-dinito-p-toluidine, were identified[11]. Mc Mahon[42] further investigated the photochemical dealkylation. He thought that N-dealkylation might involve the transfer of an oxygen atom from the nitro group to the carbon atom directly attached to the anilino nitrogen atom analogous to the photochemical rearrangement of o-nitro-benzaldehyde to o-nitrosobenzoic acid.[4]

Trifluralin and dinitramine give complex mixtures of photoproducts, and it is noteworthy that photochemical processes such as dealkylation, reduction, and benzimidazole formation have their parallels in metabolic reactions that occur in soil or incubation with microorganisms.[11,20,37,46,52]

Meanwhile, dechlorinated products from this photolysis could not be detected. While sufficient energy to break C-Cl bonds can be derived from sunlight, it is not surprising that the longer wavelengths reaching the earth's surface do not appear to affect this test herbicide[5].

2) Photolyses in suspensions of different pH's

This experiment was done to check the effect of pH on the photolysis pattern of the MC-4379 compound. The gas-liquid chromatogram of the products was shown in Figure 4. Photoproducts in both cases were essentially identical, indicating that the possible pH difference in the environment would not affect the photolysis.

3) Benzophenone-sensitized photolysis of MC-4379

Benzophenone is a good photosensitizer(E_T= 68.5 Kcal/mole in hydrocarbon solvent)[28] frequently used in the study of photochemistry. Interestingly enough, rotenone has been reported to sensitize the photodegradation of several insecticide chemicals.[2,31]

As shown in Figure 5, p-aminophenol was noticeably detected in the chromatogram of MC-4379 with benzophenone. From this observation, there is some likelihood that benzophenone will sensitize the ether linkage cleavage followed by decarboxymethylation and photoreduction.

4) Riboflavin-sensitized photolysis of MC-4379

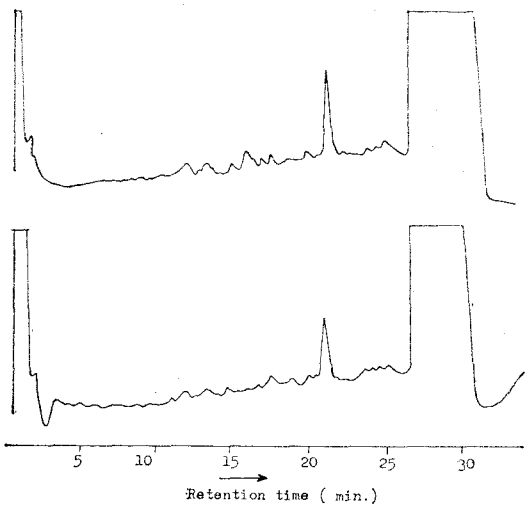

Figure 4. Gas-liquid chromatograms of photoproducts from MC-4379 suspension of different pH's(the upper: pH2. the lower:pH8)

Despite the possibility that riboflavin may play a role in photosensitizing pesticide chemicals, no appreciable difference was made on tlc.

5) Change in pH of the MC-4379 suspension

Although the solar irradiation is sufficient to break the C-Cl bond (ca. 80 Kcal/mole), it is apparently not enough for homolytic dissociation of water(116 Kcal/mole). Furthermore, in the aqueous photolysis the replaced chlorine appeared as Cl^- rather than Cl_2.[7]

So, the dechlorination is better explained as a photonucleophilic substitution[23] in which OH^- displaced chlorine from the photoexcited ring, a process already known to occur under high-energy irradiation[47].

It was believed that no dechlorination occurred under this experimental condition, based on the fact that no change in pH of the suspension was measured during the 40 hr irradiation.

2. Degradation products by the crude enzyme

Since the MC-4379 herbicide is to be used in rice plant field, it is significant to examine its possible degradation by the rice enzyme. The

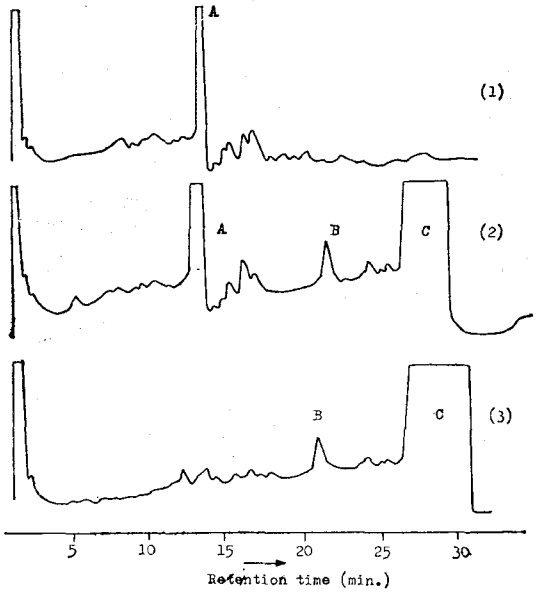

Figure 5. Gas-liquid chromatograms of photoproducts from (1) Benzophenone, (2) MC-4379 with benzophenone, and (3) MC-4379 without benzophenone
A: Benzophenone undecomposed
B: Nitrofen (photoproduct)
C: MC-4379 unchanged
D: *p*-aminophenol(photoproduct)

predominant degradation products identified by TLC and GLC include Nitrofen (2,4-dichloro-4'-nitro-diphenyl ether)and an unknown compound (presumably a polymer), in addition to the parent compound. Figure 6 shows the thin-layer chromatogram of the major products, The Rf values of unchanged MC-4379 and Nitrofen were 0.758 and 0.515, respectively.

On GLC, the retention times of unchanged MC-4379 and Nitrofen were 26.5 and 21.3 min, respectively. For some other products. attempts were not made to determine the chemical nature of these compounds because of their small amounts.

Frear et al.[19] have partially purified and characterized an aryl acylamidase from rice plants which is responsible for hydrolytic degradation of propanil.

Nineteen different genera of weeds encompass-

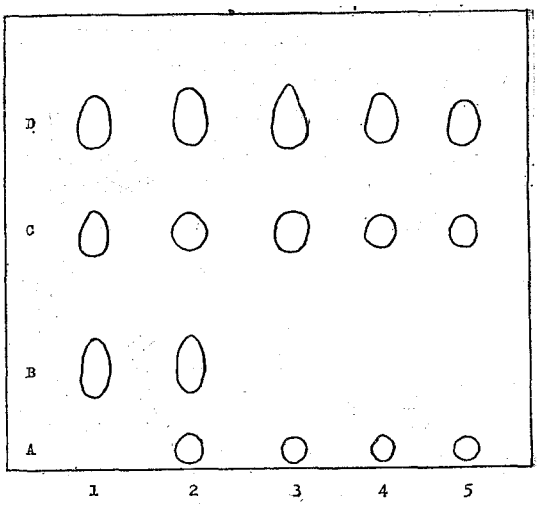

Figure 6. Thin-layer chromatogram of decomposition products of MC-4379 by environmental factors
A: Unknown tarry material
B: p-nitrophenol
C: Nitrofen
D: MC-4379
1: Authentic compounds
2: Products from decomposition by sunlight
3: Products from degradation by the crude enzyme of germinating rice seeds
4: Products from degradation by *Aspergillus ustus*
5: Products from degradation by *Pseudomonas cruciviae*
* Developing solvent: Benzene-chloroform (1:1 v/v)

ing twelve plant families were surveyed by Hoagland et al.[29] for hydrolytic enzymes which were able to metabolize the amide bonds in several groups of herbicides.

Furthermore, the present understanding of the degradation of pesticides by plant enzymes has been summarized in a review by Menzie[43] and in a symposium edited by Jaworski.[33] In the case of the MC-4379 degradation by the enzyme of germinating rice seeds, decarboxymethylation readily occurs to give Nitrofen as the predominant product. Even if the mechanism and nature of the enzyme are not known, it is noteworthy that it produces the same product as that of the solid phase photolysis.

3. Products from degradation by microorganisms

1) Degradation by fungi

Table II shows the soil-borne fungi tested for degradation of the MC-4379 herbicide. Among the four strains which showed good growth in the medium containing the herbicide, *Aspergillus ustus* was selected for the degradation study. Products prepurified by tlc and identified by tlc, glc, and ir spectrometry include unchanged MC-4379, amino-MC-4379 (2,4-dichloro-3′-carboxymethyl-4′-amino-diphenyl ether), Nitrofen, and an unknown compound (assumed as a polymer).

On tlc (Figure 6), the Rf values of unchanged MC-4379 and Nitrofen in chloroform-benzene(1:1 v/v) were 0.758 and 0.515, respectively. The Rf value of amino-MC-4379 in chloroform-ethanol-acetic acid (90:5:5 v/v) was 0.55.

Table II. The fungi tested and their response to the herbicide(1,000 ppm MC-4379)

Strains of fungi	Response to the herbicide
Aspergillus ustus	+
Aspergillus flavus	+
Fusarium moniliforme	+
Fusarium oxysporum	+
Microbispora rosea IFO 3559	−
Rhizopus hanchao Yamazaki	−
Penicillium notatum IFO 4640	−
Mucor pusillus Lindt MU-4	−
Neurospora crassa shearet	−

+: good (growth)
−: fair or inhibited
Medium: Malt extract

On glc, the retention times of unchanged MC-4379, amino-MC-4379 and Nitrofen were 26.5, 25.1 and 21.3, respectively.

Figure 7 shows the ir spectra of the authentic Nitrofen and the degradation product by *Aspergillus ustus*. Both spectra are essentially identical, suggesting that they are the same compounds. The ir spectra of the other products could not be measured because of the paucity in amounts.

2) Degradation by bacteria

The soil-borne bacteria used for the screening are shown in Table Ⅲ. Of the four strains considered to show good growth, *Pseudomonas cruciviae* was selected for the further study. This microorganism yielded the same products as those in *Aspergillus ustus*.

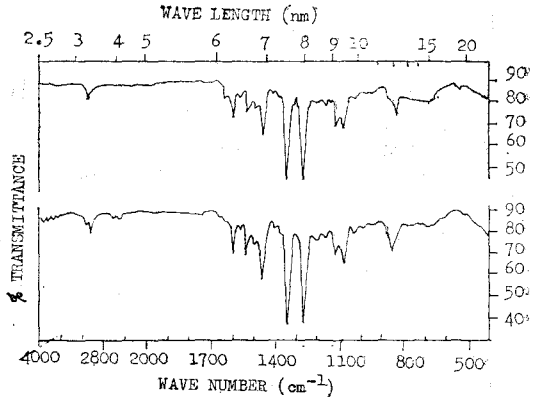

Figure 7. Infrared spectra of authentic Nitrofen (upper) and the degradation product of MC-4379 by *Aspergillus ustus* (lower); (0.3% v/v in chloroform, 0.25 mm path)

Many strains of bacteria and fungi can utilize aromatic compounds or cyclohexane derivatives as carbon sources.[1,8,9,18] The number of phenol- and phenol-carboxylic acid-utilizing organisms is essentially greater than that of the benzene-utilizing organisms. Up to now, *Pseudomonas* and

Table III. The bacteria tested and their response to the herbicide(1,000 ppm MC-4379)

Strains of bacteria	Response to the herbicide
Pseudomonas aeruginosa	+
Pseudomonas cruciviae	+
Pseudomonas fragi	+
Pseudomonas riboflavinus	+
Proteus vulgaris	−
Serratia marcescens	−
Xanthomonas campestris	−
Bacillus cereus KP 931	−
Micrococcus flavus Trevisan	−
Propionibacterium shermanii	−

+: good(growth)
−: fair or inhibited
Medium: Bacto-beef 10g, Peptone 10g, NaCl 5g, H_2O 1,000ml (pH 7.4)

Nocardia species are reported to grow on benzene as their unique C-source.[17,21,57]

Breakdown generally proceeds with bacteria by loss of the alkanoic side chain, followed by hydroxylation and aromatic ring cleavage. With an Arthrobacter sp. the first product after the removal of the side chain is the corresponding phenol.[40,41] Hydroxyl groups are introduced by microorganisms on aromatic rings[53], methyl groups attached to the furan ring system[54] and on aliphatic side chains.[55]

Nevertheless, in this experiment with *Aspergillus ustus* and *Pseudomonas cruciviae*, the decarboxymethylated product (Nitrofen) predominates over the others including the reduced product, Amino-MC-4379.

It is not known whether the incubation period of 6 and 8 days was not enough to cause the hydroxylation after the decarboxymethy lation. Anyway, it is clear that the carboxymethyl substituent in the parent compound is very labile, biologically and photochemically.

In spite of the possibility of dechlorination, preferentially of the ortho chlorine[23], no dechlorinated product was noticed in an appreciable amount on tlc in this experimental condition.

4. The effect of some substituted diphenyl ether herbicides on the growth of some plants.

1) The effect on the shoot-growth of rice plant

Each test solution was emulsified with the aid of a small amount of Triton X-100, at the rate of 1,000 μg of each compound in one milliliter of distilled water. Usually 30 slightly germinating rice seeds were placed on wet filter paper in Petri-dish with 30 ml of test solution, and grown in an incubator at 30°C. The lengths of the shoots and roots were measured at an interval of 3 days, and the results are shown at Table IV. The effect on rice plant growth was in the inhibitory order of *p*-nitrophenol>C-6989>Nitrofen>amino-Nitrofen>MC-4379.

Table IV. The effect of some substituted diphenyl ether herbicides on the shoot-growth of rice plant (average length of 30 shoots)

(shoot/root, mm)

Chemicals treated (1,000 ppm) / Growth period (days)	Control	Triton X-100	MC-4379	Nitrofen	Amino-Nitrofen	*p*-nitrophenol	C-6989
3	21/34	20/35	10/ 8	7 / 3	2 / 0	5 / 1	6/ 4
6	63/83	62/84	55/61	38/ 6	44/26	5 / 1	32/ 7
9	76/95	73/94	68/84	50/11	50/35	5 / 1	40/ 8
12	91/106	89/103	77/93	66/16	66/44	5 / 1	51/11
15	104/118	99/114	89/105	78/19	78/53	5 / 1	59/14

2) The effect on the root-growth of soybean

30 germinating roots of soybean were grown under the same condition as rice plant. All the lengths of roots were measured at the interval

Table V. The effect of some substituted diphenyl ether herbicides on the root-growth of soybean (average length of 30 root, mm)

Chemicals treated (1,000 ppm) / Growth period (days)	control	Triton X-100	MC-4379	Nitrofen	amino-Nitrofen
2	34	31	11	12	14
4	70	60	56	18	20
6	105	97	94	20	32
8	112	109	dead	dead	dead

of two days, and the results are shown at Table V. The effect on soybean was in the order of Nitrofen>amino-Nitrofen>MC-4379.

3) The effect on the shoot growth of *Amantus blitum*

Each test solution was emulsified with a small amount of Triton X-100, at the rate of 500μg of each compound in one milliliter of distilled water.

30 sprouted seeds of *Amantus blitum* were placed on wet filter paper containing petri-dish with 30ml of the upper solution, and grown in the incubator. After incubation for 3 days, all the shoots were dead.

4) The effect on the shoot growth of *Setaria viridis*

The same method as the above was employed in this experiment. After incubation for 3 days, it was observed that all the shoots of *Setaria viridis* had been dead.

SUMMARY

A substituted diphenyl ether herbicide(MC-4379) was studied on the decomposition by some environmental factors; sunlight, microorganisms, and the crude enzyme in rice plant extract.

All the decomposition products were confirmed by means of TLC, GLC, and IR. The parent compound and the decomposition products were put to the test for the effect on the growth of some plants. The results obtained are summarized as follows:

1. Photolysis

Amino-MC-4379, 2,4-dichloro-3′-carboxyl-4′-nitrodiphenyl ether, Nitrofen, 2,4-dichloro-3′-carboxyl-4′-amino-diphenyl ether, amino-Nitrofen, 3-carboxymethyl-4-nitrophenol, p-nitrophenol, p-aminophenol, etc. were confirmed as photoproducts, in addition to a relatively small amount of an unknown compound. It was confirmed that the solution-phase photolysis of MC-4379 was accelerated much more by the aid of a photosensitizer benzophenone.

2. Degradation by the crude extract of germinating rice seeds

Nitrofen was confirmed as a major degradaiton product, in addition to a relatively small amount of and unknown compound. Most of the parent compound remained unchanged.

3. Degradation by microorganisms

Nitrofen and amino-MC-4379 were confirmed as the major products, in addition to a small amount of an unknown compound.

4. The germinating rice seeds and soybean were grown in the 1,000 ppm emulsions of some chemicals, respectively. The effect on rice plant growth was in the inhibitory order of p-nitrophenol > C-6989> Nitrofen> amino-Nitrofen > MC-4379. The effect on soybean was in the order of Nitrofen > amino-Nitrofen > MC-4379.

Two weeds, *Amantus blitum* and *Setaria viridis* were grown in the 500 ppm emulsions containing the compounds, respectively. After incubation for 3 days, it was observed that all the shoots had been dead.

Acknowledgment

Dr. Kyo Chang Kim is gratefully acknowledged for providing micro-organisms used in this experiment.

要 約

環境要因으로서 日光, 微生物, 그리고 벼의 抽出液에 存在하는 粗酵素에 依한 置換 Diphenyl Ether系 除草劑의 一種인 MC-4379를 分解하여 그 分解産物을 TLC, GLC, IR등으로 分析確認하고 母化合物과 分解産物의 植物生育에 對한 阻害力을 試驗 하였다.

그 結果를 要約하면 다음과 같다.

1. MC-4379는 光分解 結果 amino-MC-4379, 2,4-dichloro-3′-carboxyl-4′-nitro-diphenyl ether, Nitrofen, 2,4-dichloro-3′-carboxyl-4′-amino-diphenyl ether, amino-Nitrofen, 3-carboxymethyl-4-nitrophenol, p-nitrophenol, p-aminophenol과 少量의 未知化合物이 生成 되었고 母化合物은 大部分 作用을 받지 않은 채로 남아 있었다. 또한 photosensitizer로 알려진 benzophenone을 加하면 光分解가 더욱 促進됨을 確認하였다.

2. 粗酵素液에 依한 MC-4379의 分解時에는 Nitrofen과 少量의 未知化合物이 確認되었으며 大

部分의 母化合物은 作用을 받지 않은 채로 存在하고 있음을 알 수 있었다.

3. *Pseudomonas cruciviae*와 *Aspergillus ustus*에 依한 分解時에는 Nitrofen과 amino-MC-4379 및 少量의 未知化合物이 生成되었으며 大部分은 作用을 받지않은 母化合物의 形態로 存在하였다.

4. MC-4379와 그 分解産物 및 他種 Diphenyl Ether除草劑가 植物의 生育에 미치는 阻害力을 試驗한 結果 벼에 있어서는 이들 各化合物(1,000 ppm 乳化液)의 生育阻害力은 p-nitrophenol> C-6989> Nitrofen> amino-nitrofen> MC-4379의 順位이었고 大豆에 있어서는 Nitrofen> amino-Nitrofen> MC-4379 의順位였다. 雜草中 비름(*Amantus blitum*)과 강아지풀(*Setaria viridis*)의 發芽된 種子를 使用하여 이들 化合物(500ppm)의 懸濁液中에서 生育시켜본 結果 3日後에 모두 枯死하는 現象을 觀察할 수 있었다.

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