Degradation of CNP, a Diphenyl Ether Herbicide, in Flooded Soil under Oxidative and Reductive Conditions*

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Degradation of CNP, 2,4,6-trichlorophenyl 4'-nitrophenyl ether, in a flooded soil under oxidative (aerobic) and reductive (anaerobic) conditions was studied in the laboratory using ^{14}C -CNP labelled at each of the benzene rings. The degradation rate of CNP differed depending upon the redox potential of the soil. The half-life period was 6 to 7 days under oxidative conditions and 1 to 2 days under reductive conditions. Degradation of CNP under oxidative conditions started after a short lag-period. The degradation products were identified as 2,4,6-trichlorophenyl 4'-aminophenyl ether, 2,4,6-trichlorophenyl 4'-acetylamino phenyl ether, 2,4,6-trichlorophenyl 4'-hydroxyphenyl ether, 2,4,6-trichlorophenol, *p*-nitrophenol, and *p*-aminophenol by *tlc*. Major products were the acylamino derivatives which under reductive conditions reached about 15.5% of applied radioactivity after 2 days and then decreased thereafter. The other products were detected only in small amounts. Most of the radioactivity was strongly adsorbed to the soil within a few days and was unextractable with organic solvents. This was attributed to complex formation between the amino derivative and soil constituents. More $^{14}\text{CO}_2$ was liberated under oxidative, than reductive, flooded conditions.

INTRODUCTION

Diphenyl ether herbicides such as CNP, nitrofen and chlomethoxynil are extensively used as pre-emergence herbicides in paddy fields in Japan and other Asian countries. The amount of CNP (2,4,6-trichlorophenyl 4'-nitrophenyl ether) consumed has been the largest among all pesticides used in Japan during the past several years.

Investigations on the degradation of diphenyl ether herbicides such as fluorodifen,²⁾ CNP,³⁾ chlomethoxynil³⁾ and bifenox⁴⁾ in soil have been reported. Degradation of CNP, nitrofen, chlomethoxynil and CFNP (2,4-dichloro-6-

fluorophenyl 4'-nitrophenyl ether) in soils were much faster under flooded, than upland, conditions.³⁾ Their half-life periods ranged from 6 to 20 days under flooded conditions but more than 50 days under upland conditions. These compounds were rapidly converted to their amino derivatives in flooded soils. The persistence of fluorodifen in upland soils²⁾ and CNP⁵⁻⁷⁾ in paddy fields have also been investigated. Considerable amounts of amino derivatives remained in paddy soils, and were extracted from soil after heating in a conc. alkali solution.⁸⁾

In this study the degradation of CNP in a soil under oxidative and reductive flooded conditions was investigated in the laboratory experiments using ^{14}C -CNP. Degradation products in the soil is also reported. The absorption and metabolism of CNP and its amino derivative in rice plant was already reported by Shimotori and Kuwatsuka.⁹⁾

* Most part of this study was presented at the Meeting of the Society of Science of Soil and Manure, Japan, 1974.¹⁾

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MATERIALS AND METHODS

1. Radioactive CNP

Two radioactive samples of ^{14}C -CNP, 2,4,6-trichloro- ^{14}C -phenyl 4'-nitrophenyl ether (ring A- ^{14}C) and 2,4,6-trichlorophenyl 4'-nitro- ^{14}C -phenyl ether (ring B- ^{14}C) were synthesized in Daiichi Pure Chemicals Co. Ltd., and kindly supplied by Mitsui Toatsu Chemicals Inc. No radioactive impurity of the sample of ring B- ^{14}C -CNP was found on thin layer chromatogram. The sample of ring A- ^{14}C -CNP containing small amounts of radioactive impurities was purified by thin layer chromatography using silica gel plate and *n*-hexane : ethyl acetate (3 : 2 v/v). The specific activity of ring A- ^{14}C -CNP and ring B- ^{14}C -CNP were 0.96 mCi/mmol and 0.70 mCi/mmol, respectively, and the radioactive purity of both samples was more than 99.9% determined by the dilution method.

2. Non-radioactive Chemicals

Non-radioactive compounds of CNP, mp 106–7°C, with 99.9% of purity, the amino derivative (CNP-NH₂, 2,4,6-trichlorophenyl 4'-amino phenyl ether), mp 108–9°C, with 99.9% of purity, the acetyl amino derivative (CNP-NHCOCH₃, 2,4,6-trichlorophenyl 4'-acetylaminophenyl ether), mp 214–5°C, the hydroxy derivative (CNP-OH, 2,4,6-trichlorophenyl 4'-hydroxyphenyl ether), mp 137–8°C, and the azo-compounds (CNP-N=N-CNP, *p,p'*-bis(2,4,6-trichlorophenoxy) azobenzene, mp 285–7°C, and *p,p'*-hydroxy azobenzene, mp 215–6°C) were supplied by Mitsui Toatsu Chemicals Inc. 2,4,6-Trichlorophenol, *p*-nitrophenol and *p*-aminophenol were obtained commercially. Synthetic methods of these compounds are reported elsewhere.¹⁰⁾

3. Soil Sample

The soil used was sampled from the plough layer of a paddy rice field in Paddy Field Experimental Farm of Aichi-ken Agricultural Research Center, at Anjo, Aichi Prefecture in October 1973, finely crushed, passed through a 2 mm sieve, and stored at 4°C.

The incubation experiments were performed by the early summer of 1974. The physico-chemical properties of the soil were as follows:

kaolin clay mineral, clay content 45.2%, light clay, maximum water holding capacity 53.5%, pH (H₂O) 6.2, total carbon 1.75%, total nitrogen 0.15%, cation exchange capacity 10.0 m.e./100 g.

4. Soil Conditioning

4.1 Oxidative flooded conditions

Fifty grams (oven-dried weight basis) of the soil sample in a 500-ml Erlenmeyer flask was flooded with water up to 1 cm deep and its soil layer was 5 mm deep. The mouth of the flask was covered with aluminum foil and the soil was pre-incubated at 30°C in the dark for 5 weeks. Redox potential of the incubated soil (pH 6) was +650 mV, and was kept constant throughout the whole incubation period.

4.2 Reductive flooded conditions

Fifty grams of the soil sample was placed in a test tube (20 cm × 3 cm i.d.). The soil was submerged with water up to 1 cm deep and the soil layer was 90 mm deep. The mouth of the test tube was capped with aluminum foil and the soil was pre-incubated in the same way as described for the oxidative condition. The redox potential of incubated soil at pH 6 was –250 mV, and was kept constant throughout the incubation period.

5. Application of ^{14}C -CNP and Incubation

Each of ring A- ^{14}C -CNP (33.8×10^5 dpm) and ring B- ^{14}C -CNP (25.0×10^5 dpm) dissolved in 0.5 ml of acetone was mixed well with the pre-incubated soil at a concentration of 10 ppm on dry soil basis.

For the absorption of released $^{14}\text{CO}_2$, a vial containing 5-ml solution of 4 N KOH was placed in the soil. The mouth of the flask or test tube was capped with aluminum foil, and the soil was incubated under the same conditions as described for the pre-incubation conditions described above for 1 hr, 2, 5, 10 and 20 days.

6. Extraction of Radioactive Compounds

The incubated soil was transferred into a 300-ml bottle, and 100 ml of acetone was added. The bottle was shaken for 1 hr. The soil was filtered with glass filter (G-4), and washed twice with 50 ml portions of acetone. The filtrate was combined and the acetone was

distilled off by concentration using a rotary evaporator. The residual water was extracted three times with 50 ml portion of ether. The extracted solutions and $^{14}\text{CO}_2$ absorbed by the 4 N KOH solution were subjected to radioassay. The soil residue was also radioassayed by the wet combustion method.¹¹⁾

7. Radioassay

Radioactivity of the extracted solution was measured in 10 ml of dioxane liquid scintillator and for $^{14}\text{CO}_2$ in Bray's scintillator (PPO 4 g, POPOP 0.2 g, naphthalene 60 g, methanol 100 ml, ethyleneglycol 20 ml, and dioxane, to make 1000 ml), using a liquid scintillation spectrometer, Packard Model 3350. The quenching was determined by the external standard method.

8. Thin Layer Chromatography

Ether extracts from the soil were dried with anhydrous sodium sulfate and concentrated. An aliquot of each concentrated solution was spotted on silica gel plate (Merck 60 F 254, pre-coated, 0.25 mm thick, 20 × 20 cm) and reference compounds dissolved in acetone were spotted over the sample spot. The plates were developed using two dimensional chromatography with *n*-hexane-ethyl acetate (2:1 v/v) for the first development and chloroform for the second one, and *n*-hexane for the third one to the same direction as the second one. Other solvent systems were also used, if necessary. *R_f* values of the reference compounds are shown

in Table 1. Spots of the reference compounds were detected by irradiation with ultraviolet rays (254 nm), and the radioactive spots were detected by radioautograms on X-ray films (Fuji Kx, Medical).

RESULTS AND DISCUSSION

Figure 1 shows change with time in the radioactivity in each fraction. The radioactivity of each fraction extremely differed between oxidative conditions and reductive conditions of the flooded soil. The difference in degradation products between ring A- ^{14}C -CNP and ring B- ^{14}C -CNP were hardly observed except 2,4,6-trichlorophenol and *p*-nitrophenol. The degradation rate of CNP was largely different between both redox conditions of soil. In the oxidative flooded soil the degradation started after a short lag-period and the half-life period of CNP was 6 to 7 days. On the other hand, no lag period was observed under reductive conditions and the half-life was 1 to 2 days. The radioactivity of the degradation products of ^{14}C -CNP in ether-soluble fractions reached about 30 to 35% of applied radioactivity after 10 days under oxidative flooded conditions, about 55 to 60% after 2 to 5 days under reductive conditions, and then decreased.

The acetone unextractable radioactivity remaining in the soil, originated from both ring A- ^{14}C -CNP and ring B- ^{14}C -CNP, and increased slowly to reach about 35 to 40% after 10 to 20 days under oxidative flooded conditions. Under reductive conditions, the retained radioactivity

Table 1 *R_f* values of CNP and the reference compounds.

Compounds	Solvent systems ^{a)}						
	A	B	C	D	E	F	G
CNP	0.84	0.78	0.85	0.86	0.70	—	—
2,4,6-Trichlorophenyl 4'-aminophenyl ether	0.59	0.48	0.64	0.69	0.11	—	—
2,4,6-Trichlorophenyl 4'-acetylaminophenyl ether	0.19	0.15	0.32	0.38	0	—	—
2,4,6-Trichlorophenyl 4'-hydroxyphenyl ether	0.67	0.28	0.65	0.60	0.06	—	—
<i>p,p'</i> -Bis(2,4,6-trichlorophenoxy)azobenzene	0.86	0.82	0.90	0.91	0.81	—	—
<i>p,p'</i> -Hydroxy azobenzene	0.31	0.01	0.21	0.34	0	—	—
2,4,6-Trichlorophenol	0.72	0.44	0.53	0.70	0.32	—	—
<i>p</i> -Nitrophenol	0.52	0.17	0.49	0.41	0.02	0.76	0.68
<i>p</i> -Aminophenol	0.06	0.02	0.09	0.11	0	0.56	0.28

^{a)} Solvent systems: A, *n*-hexane-ethyl acetate (2:1 v/v); B, chloroform; C, benzene-chloroform-acetone (7:2:1 v/v); D, benzene-methanol-acetic acid (45:5:1 v/v); E, *n*-hexane-toluene (1:1 v/v); F, benzene-acetone (3:1 v/v); G, chloroform-acetone (1:1 v/v).

Fig. 1 Change in radioactivity of various fractions during ^{14}C -CNP degradation in soil.

○ total radioactivity, ● ether-extractable, ■ water-soluble, □ unextractable from soil, ○ CNP, △ total ether-extractable metabolites

Fig. 2 Thin-layer chromatogram of ether-extracts of ^{14}C -CNP degradation products in soil.

[I] CNP, [II] CNP-NH_2 , [III] CNP-NH-COCH_3 , [IV] CNP-OH , [V] 2,4,6-trichlorophenol, [VI] *p*-nitrophenol, [VII] *p*-aminophenol

Numerals in brackets shows the same numbers for chemical structure in Fig. 5.

increased rapidly within 2 days and reached a maximum after 5 to 10 days. Radioactivity of the acetone-extracts was almost soluble in ether. The radioactivity of water-soluble fractions in acetone extracts was relatively small in all cases.

The radioautograms of the ether extracts on *tlc* (Fig. 2) revealed CNP (I), the amino derivative (CNP-NH_2 , II), the acetylamino derivative (CNP-NHCOCH_3 , III), the hydroxy derivative (CNP-OH , IV), 2,4,6-trichlorophenol (V), *p*-nitrophenol (VI), *p*-aminophenol (VII), and several unidentified products.

Among these compounds CNP-NHCOCH_3 was most abundant. However, the acetylamino derivative was not clearly separated from a minor unidentified product by several solvent systems tested. Therefore, these were scraped off together from the *tlc* plate and the total radioactivity was measured. The unidentified product was presumed to be the formylamino derivative of CNP (CNP-NHCHO) from the relative *R_f* values on *tlc* of the acetylamino and formylamino derivatives of chlomethoxynil.³⁾

p-Nitrophenol and *p*-aminophenol was separated clearly by *tlc* with the solvent systems F and G in Table 1. *p,p'*-Bis(2,4,6-trichlorophenoxy) azobenzene had a similar *R_f*-value as the other unknown product using solvent systems F and G, but this product was not identical with the azocompound on *tlc* using the other solvent systems (A-E). *p,p'*-Hydroxy azobenzene was not detected.

The change in amount of the acetylamino derivative containing the minor unidentified product presumed to be CNP-NHCHO was more rapidly produced under reductive, than oxidative, flooded conditions, by incubating with both ^{14}C -CNP labelled samples (Fig. 3). These acetylamino derivatives of CNP were produced in greater amounts than the acetylamino derivatives of chlomethoxynil. Especially, under reductive flooded conditions, the amounts of the acetylamino derivatives from CNP was 3 to 5 times of those from chlomethoxynil. These results seem to indicate that the acetylamino derivatives of chlomethoxynil are degraded additively by demethylation of the methoxy group.

Each of CNP-NH_2 , CNP-OH , 2,4,6-trichlorophenol and CO_2 was produced in larger

Fig. 3 Change in amount of the acetylamino derivative containing a small amount of an unidentified product.

○ ring A- ^{14}C , Δ ring B- ^{14}C

Fig. 4 Change in amounts of CNP-NH₂, CNP-OH, 2,4,6-trichlorophenol, and CO₂.

○ CNP-NH₂, ● CNP-OH, ▲ CO₂, Δ 2,4,6-trichlorophenol

amount under reductive flooded conditions than oxidative conditions, but the amounts did not exceed 1% in all cases (Fig. 4). The amount of CNP-NH₂ recovered was extremely smaller than that reported in a previous study, in which CNP-NH₂ was extracted with hot conc. alkali solution from the soil.⁶⁾ It is presumed that CNP-NH₂ is present as complexes between the

amino group and soil constituents. It was also determined in previous studies.^{12,13)} The amount of CNP-NH₂ shown in Fig. 4 may result only from the quantity of "free" amino derivative in the soil. The complex or the "Bound residue" of CNP-NH₂ is reported elsewhere.¹²⁾ CNP-OH was produced in larger amount under reductive, than oxidative, flooded conditions in amounts less than 0.5% of applied radioactivity. The amount of 2,4,6-trichlorophenol produced was less than 0.2%. *p*-Nitrophenol and *p*-aminophenol were detected only in trace amounts. The other unidentified products in the ether-extracts were recovered in small amounts. No products accumulated in the ether-extractable fraction. $^{14}\text{CO}_2$ was liberated more abundantly under oxidative flooded conditions than reductive conditions. However, $^{14}\text{CO}_2$ produced was only about 2 to 3% of applied radioactivity after 20 days even under oxidative flooded conditions.

The degradation pathways of CNP postulated from the identified compounds are shown in Fig. 5. The degradation products of CNP in flooded soil may be present largely as complexes of the amino derivative. The reduction of nitro group to amino group may be the dominant reaction in the flooded soils, similar to the degradation of chlomethoxynil³⁾ and bifenox.⁴⁾ The amino derivative, however, may rapidly form complexes with soil particles. It has been reported that the reduction of the nitro group of CNP is due to soil microorgan-

Fig. 5 Proposed pathways of CNP degradation in flooded soil.

Numerals in brackets correspond to the numerals in Fig. 2.

isms¹⁴⁾ and also occurs non-biologically by ferrous ions¹⁵⁾ produced under reductive soil conditions.

Diphenyl ether-, carbonilate-, acylanilide-, and phenylurea-herbicides are degraded to produce the aniline derivatives in soil. Acetylation is probably the major pathway of amino derivatives. CNP-NH₂ was converted to its acylamino derivatives by several soil microorganisms.¹⁴⁾ The amino derivatives of chlo-methoxynil and bifenox were also acetylated only in non-sterilized soil but not in sterilized soil. On the other hand, propanil,¹⁵⁾ CMMP¹⁶⁾ and other acylaniline compounds are easily hydrolyzed to the corresponding aniline derivatives in soil. Therefore, the acylation of CNP-NH₂ may also be a reversible reaction performed by soil microbes.

The nitro group was also substituted by hydroxy group. This hydroxy derivative is produced probably from CNP-NH₂ by a chemical reaction in which CNP-NH₂ reacts with nitrite in soil to form the diazo-compound that is decomposed to the hydroxy compound. ¹⁴C-2,4,6-Trichlorophenol was detected in the soil treated with ring A-¹⁴C-CNP (Fig. 4). ¹⁴C-*p*-Nitrophenol and its reduced product, ¹⁴C-*p*-aminophenol, were also produced in the soil treated with ring B-¹⁴C-CNP, but these were detected only in trace amounts. This is prob-

ably because *p*-nitrophenol produced was rapidly reduced to *p*-aminophenol, which is strongly adsorbed to soil particles to become unextractable with aqueous acetone in the same way as CNP-NH₂ described here and 3,4-dichloroaniline reported.¹⁷⁾ These phenolic compounds produced by cleavage of ether linkage of CNP were detected only in small or trace amounts compared with non-cleaved degradation products such as CNP-NH₂, CNP-NH-COR, and CNP-OH, under both reductive and oxidative conditions. These simple phenolic compounds may be decomposed rapidly to form aliphatic acids.

It has been reported that 3,4-dichloroaniline, a major degradation product of propanil, was transformed to 3,3',4,4'-tetrachloroazobenzene,¹⁷⁾ 3,3',4,4'-tetrachloroazoxybenzene,^{18,19)} 4-(3,4-dichloroanilino)-3,3',4'-trichloroazobenzene, and 1,3-bis(3,4-dichlorophenyl)triazene²⁰⁾ in the soil. In CNP degradation, however, such azo and related condensation products were not detected.

¹⁴CO₂ was liberated by cleavage of the ¹⁴C-benzene ring. Greater amount was produced under oxidative conditions, but the amounts did not exceed 0.3% of applied radioactivity after 20 days of incubation (Fig. 4). This may be because CNP-NH₂ transformed from CNP is present in relatively stable state against microbial attack by being adsorbed on soil particles. CNP degradation was slower and ¹⁴CO₂ liberation was more rapid in oxidative than reductive flooded soil. Therefore, it is presumed that CNP is retained for longer time in the surface layer than in the sub layer of field soil, and the degradation of benzene rings of CNP to CO₂ occurs more rapidly in the surface layer where most part of the treated CNP is held in the field. These may result that CNP acts more effectively to weed seedlings in surface soil and is more safe to rice roots in deeper soil.

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要 約

酸化的および還元的土壌条件下の湛水土壌中におけるジフェニルエーテル系除草剤 CNP の分解

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室内実験により、好気的および嫌氣的湛水土壌中における CNP の分解について、ベンゼン環のそれぞれを標識した ^{14}C -CNP を用いて研究した。CNP は、好氣的（酸化的）湛水土壌中では短い休止期間ののち分解し、半減期は 6~7 日であった。嫌氣的（還元的）湛水土壌中では急速に分解し、半減期は 1~2 日であった。tlc によって同定された分解生成物は、CNP のニトロ基がアミノ基、アセチルアミノ基、水酸基で置換された化合物および 2, 4, 6-トリクロルフェノール、*p*-ニトロフェノール、*p*-アミノフェノールなどであった。アセトン抽出液中の分解物としてはアセチルアミノ体が最も多量に生成し、還元的湛水土壌中では、ホルミルアミノ体と推定される微量の未同定化合物との含量で添加した放射能の 15% に達した。アセトン抽出物には集積する分解生成物はみられなかった。有機溶媒で抽出できない土壌残渣画分の放射能は添加量の 35~40% に達したが、大部分は土壌粒子に強く結合した CNP アミノ体と推定した。アミノ体が縮合して生成するアゾおよびアゾキシ化合物などは検出されなかった。最終的にはベンゼン環も開裂し、 $^{14}\text{CO}_2$ の生成がみられるが、20 日間の実験期間中の $^{14}\text{CO}_2$ の生成は少なかった。CNP の減衰は還元的条件下のほうが速いが、 $^{14}\text{CO}_2$ の生成は酸化的条件下のほうが速かった。