A Study on the Evolution of 3,4-DCA and TCAB in Some Selected Soils (Part II)

Degradation of ^{14}C-3,4-DCA and ^{14}C-TCAB

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數種土壤中에서 3,4-DCA 및 TCAB의
變化에 關한 研究(第二報)

^{14}C-3,4-DCA 및 ^{14}C-TCAB의 分解

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SUMMARY

In an attempt to elucidate the fate of 3,4-DCA and TCAB in various French soils, uniformly ^{14}C-ring-labeled 3,4-DCA and TCAB were utilized and the following results obtained.

1) The rate of breakdown of ^{14}C-3,4-DCA into ^{14}CO$_2$ was relatively higher in the early stage than that in the later stage. In 6 months of incubation in alkaline soil (pH 7.9), the rate was as high as 6.5% at dose 1 (1.5 ppm) and as low as 1.92% at dose 2(94 ppm), whereas in organic acid soil (pH 5.5) the rate was 4.91% at dose 1 and 4.24% at dose 2, respectively, without making any great difference between the two levels.

2) At dose 1, 47.70% of the initial radioactivity of ^{14}C-3,4-DCA was bound to soil in organic acid soil and 29.49% bound in alkaline soil, whereas at dose 2, 38.40% in organic acid soil and 20.30% in alkaline soil, respectively.

3) The amount of formation of ^{14}C-TCAB from ^{14}C-3,4-DCA seems to depend largely on the concentration of 3,4-DCA applied rather than on soil types. At dose 2, the amount was 50% of the total radioactivity extracted in organic acid soil and 30% in alkaline soil, corresponding to 1.8% and 1.4% of the initial radioactivity applied to soil, respectively. Cis-TCAB also seemed to be formed at dose 2 in both soils. Meanwhile, at dose 1, even though ^{14}C-TCAB was detected in trace on tlc and glc in both soils, the amount does not exceed 2 to 3% of the radioactivity extracted,

corresponding to 0.05 to 0.1% of the initial radioactivity.

4) The rate of breakdown of ^{14}C-TCAB into $^{14}CO_2$ ranged from 0.05 to 0.20% in all the four soils. Most of the applied ^{14}C-TCAB remained intact after 3 months, not producing any detectable metabolites.

5) The fact that much more ^{14}C-TCAB was adsorbed to alkaline soil than to the other soils strongly indicates that in alkaline condition trans-isomer was converted tocis-isomer which has the higher adsorption affinity than the former.

INTRODUCTION

3,4-Dichloroaniline-based herbicides, the phenylureas, the phenylcarbamates, and the acylanilides produce 3,4-dichloroaniline (3,4-DCA) as an intermediate in their microbial degradation in soil.[4,14,15,23,24] Moreover, not only are some chloroanilines quite persistent in soil[1] and toxic,[8,23,33] but it appears that a substantial amount of the chloroaniline may condense in soil to form chloroazobenzenes.[2,3,4,5,7]

It has been suggested that microbiological action[2] and more specially peroxidase activity[4,8,16] are involved in the condensation of DCA to TCAB in soil. Peroxidases have a wide distribution in nature and occur in soil.[8,18,19,27] The formation of chloroazobenzene is now of concern, because some of them are known to be carcinogens.[34] Nevertheless, there is a contradictory report that rats fed TCAB at a level of 4 mg per week for an initial 3 week period and at 10 mg per week for an additional 37 weeks produced no tumors when the animals were sacrificed at the 60th week.[9] In laboratory studies, ^{14}C-TCAB supplied to rice roots in nutrient solution was absorbed and translocated to the shoots, though the amount absorbed was fairly low.[31] In addition, in field surveys conducted on rice-producing soils in Stuttgart, Ark., U.S.A., TCAB was detected only at low concentrations(<0.1 ppm).[26] Sprott et al.[30] and Hughes et al.[22] have reported on the formation of TCAB in some Canadian soils treated with 3,4-DCA. Chisaka et al.[12] reported on the metabolism of Propanil in soils in relation to ^{14}C-3,4-DCA degradation, indicating that ring $^{14}CO_2$ evolution was slower and amounted to less than 3% of

the original ^{14}C.

The present investigation was undertaken to determine the rate of degradation of 3,4-DCA and TCAB over a longer period of incubation, at different concentration levels in different French soils which exhibited wide differences in texture, organic carbon, organic matter, nitrogen, C/N ratio, pH, and C.E.C. To this end, uniformly ^{14}C-ring-labeled 3,4-dichloroaniline (^{14}C-3,4-DCA) and 3,3',4,4'-tetrachloroazobenzene (^{14}C-TCAB) were utilized for tracing the ultimate fate and persistence of the herbicide-derived chemicals in soil in connection with environmental contamination and public health.

MATERIALS AND METHODS

Incubation of soils with uniformly ^{14}C-ring-labeled 3,4-DCA and TCAB.

^{14}C-3,4-DCA and ^{14}C-TCAB were applied in 0.5 ml acetone to an amount of each refrigerator-stored soil which was equivalent to 50 gr by dry weight. After thorough mixing, the soil was moistened to 80% of its water holding capacity, and was incubated with peristaltic pumps at 18-25°C in the special apparatus described earlier.[17] The radioactivity of $^{14}CO_2$ trapped in 0.2N-NaOH solution was measured as a function of incubation period. The possible volatilized ^{14}C-3,4-DCA was trapped in 0.5M-H_2SO_4 solution. At the end of the incubation, the soils were extracted with acetone for subsequent metabolic studies, according to the following procedure.

Soils used.

Four different types of soils were collected from different areas in France. As a matter of convenience, they were designated as organic acid soil, organic neutral soil, argillaceous soil,

<div align="center">Table 1. Physical characteristics of soils</div>

Soils	Texture	Clay (%)	Silt (%)	Fine sand(%)	Coarse sand(%)
Organic acid soil		19.4	19.5	15.8	45.3
Organic neutral soil		17.5	47.3	33.8	1.4
Agrillaceous soil		12.8	15.4	36.5	35.3
Alkaline soil		12.5	15.6	34.3	37.6

<div align="center">Table 2. Chemical characteristics of soils</div>

Soils	Chemical characteristics	Organic carbon (%)	Organic matter (%)	N (Kjeldahl) (%)	C/N Ratio	pH (H_2O)	C.E.C. (m.e./100gr)
Organic acid soil		4.48	7.71	0.3	14.93	5.5	19.8
Organic neutral soil		56.8	97.6	5.35	10.61	6.9	35.9
Argillaceous soil		8.8	15.1	0.84	10.47	7.6	6.4
Alkaline soil		1.21	2.08	0.133	9.10	7.9	6.3

and alkaline soil respectively. The soils vary in texture, organic carbon, organic matter, nitrogen, C/N ratio, pH, and C.E.C. Their physico-chemical characteristics are presented in Table 1 and 2.

Treatment of ^{14}C-3,4-DCA.

^{14}C-3,4-DCA was treated to soils as seen in Table 3.

<div align="center">Table 3. Treatment levels of ^{14}C-3,4-DCA to soils</div>

Doses \ Soils	Organic acid soil (pH 5.5)	Alkaline soil (pH 7.9)
Dose 1	72.9 γ (1.5 ppm)	72.9 γ (1.5 ppm)
Dose 2	4,700 γ (94 ppm)	4,700 γ (94 ppm)

Each experiment was carried out in duplicate.

Treatment of ^{14}C-TCAB.

Soils used include organic acid soil, organic neutral soil, argillaceous soil, and alkaline soil. Each treatment was done in duplicate using 176 μg of ^{14}C-TCAB(3.52 ppm) dissolved in 0.1 ml of acetone.

Extraction of soils treated with ^{14}C-3,4-DCA and ^{14}C-TCAB.

For metabolic studies, at the completion of each incubation period, 90 ml of acetone and ca. 5 gr of anhydrous $MgSO_4$ were added to each soil phial and stirred thoroughly with a magnetic stirrer for 2 hrs. After standing in a refrigerator, the supernatant was pipetted out to collect. The same procedure was repeated 5 times to exhaustively extract the remaining residues. The pooled extracts were then flash-evaporated to dryness, and the resulting residue was taken up in 10 ml of n-hexane for the subsequent tlc and glc analyses.

Measurement of radioactivity.

The radioactivity was measured with a Packard Model 3375 Tri-Carb Scintillation Spectrometer. The specific activity was determined by placing 10 μl of each sample in scintillation vials containing 10 ml of toluene scintillation fluid.[28] The initial specific activities of ^{14}C-3,4-DCA and ^{14}C-TCAB were 3.5μ Ci/μ mole and 1.01μCi/μ mole, respectively.

For measuring the activity of $^{14}CO_2$-trapping solutions, duplicate 1 ml samples of them were added to 10 ml of scintillation solution. The spots on tlc plates which were completely separated from all other uv-absorbing martials were quantitatively transferred to counting vials.

GLC analysis.

Gas-liquid chromatographic analyses were performed with an F & M Scientific 5750 Research Chromatograph (Hewlett-Packard) equipped with a flame ionization detector. The column-packing was 5% SE-30 (methyl) on 60-80 mesh Chromosorb W. Operating parameters were as follows: stainless steel column of 9 ft×1/8 in. outer diam.; injector temp. of 250°C; detector temp. of 230°C; nitrogen carrier flow of 23 ml/min. Temp-programming was done from 80°C (post-injection interval : 1 min.) to 220°C (upper limit interval : 10 min.) at a rate of a 6°C increase per min. Chart speed was 5 mm per min.

TLC and Autoradiography.

Thin-layer chromatographic separations were performed with 5196494 X, Art. 5554, DC-Aluf-olien, Kieselgel 60 F_{254} (25 Folien 20×20 cm), Schichtdicke 0.2 mm, E. Merck, Darmstadt. The eluent was n-hexane. Autoradiograms were obtained with the acetone extracts of soils incubated with ^{14}C-3,4-DCA for 106 days by being exposed for 3 weeks.

Measurement of mineralized radioactivity.

The radioactivity of ^{14}C-3,4-DCA and ^{14}C-TCAB adsorbed to soils was measured by treating each soil sample with an oxidation mixture consisting of sulfuric acid, potassium dichromate, and silver sulfate.[32]

^{14}C-3,4-DCA and ^{14}C-TCAB.

^{14}C-ring-labeled 3,4-DCA (specific activity: 3.5 μ Ci/μ mole; melting point, 72-73°C) was supplied by the Radiochemical Centre, Amersham, England. ^{14}C-TCAB was synthesized from ^{14}C-3,4-DCA.[28] They had a radiochemical purity, as determined by thin-layer chromatography and scanning, of more than 99%.

RESULTS AND DISCUSSION

Breakdown of ^{14}C-3,4-DCA into ^{14}CO$_2$ in soils.

In organic acid and alkaline soils, the percentage of the initial radioactivity liberated in the form of ^{14}CO$_2$ at two levels of treatment is shown as a function of incubation periods in Table 4. As seen in Table 4, the breakdown of ^{14}C-3,4-DCA into ^{14}CO$_2$ is rather faster in the early stage than in the later stage. In 187 days of incubation, in alkaline soil (pH 7.9) the rate

Table 4. ^{14}CO$_2$ evolution from ^{14}C-3,4-DCA in soils (% of the initial radioactivity liberated in the form of ^{14}CO$_2$)

Incubation days	Organic acid soil dose 1 (1.5 ppm)		Alkaline soil dose 1		Organic acid soil dose 2 (94 ppm)		Alkaline soil dose 2	
Replicate	1	2	1	2	1	2	1	2
7	0.59	0.46	1.01	0.82	0.54	0.46	0.07	0.06
14	1.28	0.76	1.68	1.43	1.00	0.88	0.14	0.14
21	1.77	1.14	2.27	1.96	1.45	1.31	0.23	0.24
28	2.01	1.42	2.61	2.29	1.69	1.54	0.33	0.31
35	2.38	1.73	2.94	2.59	1.95	1.77	0.43	0.40
42	2.60	1.92	3.21	2.87	2.04	1.86	0.50	0.47
49	2.78	2.07	3.44	3.16	2.32	2.11	0.61	0.58
63	3.15	2.37	3.93	3.75	2.64	2.40	0.76	0.72
74	3.50	2.64	4.42	4.34	2.95	2.70	0.93	0.88
88	3.91	2.93	5.09	5.08	3.39	3.06	1.17	1.12
106	4.00	3.19	5.70	5.80	3.71	3.36	1.46	1.33
130	4.32		5.99		3.90		1.69	
150	4.59		6.16		4.07		1.81	
187	4.91		6.47		4.24		1.92	

of $^{14}CO_2$ evolution was as high as 6.5% at dose 1 (1.5 ppm) and as low as 1.92% at dose 2 (94 ppm).

Meanwhile, in organic acid soil(pH 5.5), the rates of $^{14}CO_2$ evolution were 4.91% and 4.24% at dose 1(1.5 ppm) and dose 2 (94 ppm), respectively. In consequence, there is not much difference between dose 1 and dose 2 in organic acid soil which contains larger amounts of organic matter(7.71%) and C.E.C. (19.8 m.e./100 gr) than alkaline soil.

On the other hand, in the case of alkaline soil (pH 7.9), the low rate of $^{14}CO_2$ evolution at dose 2 (94 ppm) suggests the possibility of the inhibition of microbial activity by the toxic ^{14}C-3,4 -DCA.[3,23,29,33] However, at dose 1 (1.5 ppm), the higher rate of degradation of ^{14}C-3,4-DCA to $^{14}CO_2$ is suggestive of the feasibility that 3,4-DCA would not be enoughly bound to soil humus, because of its scanty amount of organic matter. Meanwhile, the result in organic acid soil seems to prove the fact that humus-bound DCA residues, even at excessive concentrations, do not inhibit microbial respiration [20] and can be regarded as temporarily detoxified as well as immobilized.[21]

In this connection, it will be reasonable to believe that the relatively slower rate of $^{14}CO_2$ release in the later stage will be due to the formation of the 3,4-DCA-humus complexes.[20,21] Hsu et al.[20] showed that in experiments with soil-bound radiolabeled 3,4-DCA, $^{14}CO_2$ was released from soil at 1% per week and the rate of $^{14}CO_2$ release was not influenced significantly by variations of pH from 5.0 to 8.0 or by moisture contents between 50% and 70% of the holding capacity. In the present experiment the rate of $^{14}CO_2$ evolution was much less than that of Hsu et al., when it was done over a longer period of incubation. The percentage of $^{14}CO_2$ evolution from ^{14}C-3,4-DCA in soils was plotted against incubation periods as shown in Fig. 1.

The percentage of the radioactivity recovered after 6 months of incubation of ^{14}C-3,4-DCA in two types of soils in presented in Table 5. As
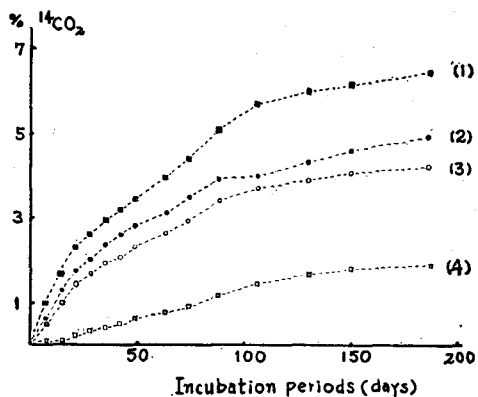

Fig. 1. $^{14}CO_2$ evolution from ^{14}C-3,4-DCA in soils(% of $^{14}CO_2$ liberated as a function of incubation periods)
(1): Alkaline soil (dose 1)
(2): Organic acid soil(dose 1)
(3): Organic acid soil(dose 2)
(4): Alkaline soil(dose 2)

seen in Table 5, at dose 1 (1.5 ppm), 47.70% of ^{14}C-3,4-DCA became bound to soil in organic acid soil(pH 5.5) and 29.49% of it bound in alkaline soil (pH 7.9) in 6 months of incubation. Whereas, at dose 2(94 ppm), 38.40% in organic acid soil and 20.30% in alkaline soil were bound, respectively. These results confirm the fact that solvent-extractable residue formation depends on the 3,4-DCA concentration, as shown by Chisaka et al.[12] and Bartha.[11]

At high concentrations, polymerization reactions predominate, but at low concentrations binding sites on the soil organic matter compete successfully for the chloroaniline moieties. Accordingly, the proportion of solvent-extractable metabolites decreases, and most chloroaniline becomes soil-bound.[11] In Table 5, a considerable amount of radioactivity was not extracted, depending on the soil types and doses.[12] It would probably be caused during the application to soils, extarction therefrom with acetone, and concentration procedures.[6] Based on the fact that the radioactivity trapped in 0.2 N-H_2SO_4 solution throughout the entire incubation period was negligible, it is unlikely that the loss will

Table 5. The radioactivity after 6 months of incubation of ^{14}C-3,4-DCA in two types of soils (% of the radioactivity recovered)

| Origin of the radioactivity	Organic acid soil (pH 5.5)		Alkaline soil (pH 7.9)	
	Dose 1 (1.5 ppm)	Dose 2 (94 ppm)	Dose 1 (1.5 ppm)	Dose 2 (94 ppm)
I. $^{14}CO_2$ liberated	4.91	4.24	6.47	1.92
II. Mineralization(before extraction with acetone)	77.80	67.02	56.66	47.17
III. Acetone extract	6.58	6.70	3.43	7.68
IV. Mineralization(after extraction)	47.70	38.40	29.49	20.30
Total I+II	82.71	72.26	63.13	49.09
Total I+III+IV	59.19	50.34	39.39	29.90

be due to volatilization. May be other mechanisms of disappearance might be associated with the formation of high molecular weight compounds as described by Kearney et al.[25] Chisaka et al.[12] have proposed that low recoveries of anilines from soils could be caused by binding of the aniline, or possibly its alteration products, to soil particles. They also demonstrated that the recovery of aniline from treated soils was a function of the soil type and the initial concentration of aniline added.

Identification of ^{14}C-TCAB from soils treated with ^{14}C-3,4-DCA.

Fig. 2 shows the autoradiogram of the acetone extract of soils.

As seen in the autoradiogram, trans-TCAB(Rf 0.35) was strikingly formed from ^{14}C-3,4-DCA in both soils at dose 2 (94 ppm). Meanwhile, in the case of dose 1 (1.5 ppm), it was formed only in trace. Quantitatively, the spots on the tlc plate corresponding to trans-TCAB were scraped off and their radioactivities were measured. At dose 2 (94 ppm), the amounts of trans-TCAB formed are 50% in organic acid soil and 30% in alkaline soil, respectively, of the total radioactivities of the soil extracts. These figures correspond to 1.8% and 1.4%, respectively, of the original radioactivities applied to soils. In both soils treated with ^{14}C-3,4-DCA at dose 1 (1.5 ppm), ^{14}C-TCAB was also formed in trace. The percentage of radioactivities does not exceed 2 to 3% of the total radioactivity of the extracts,
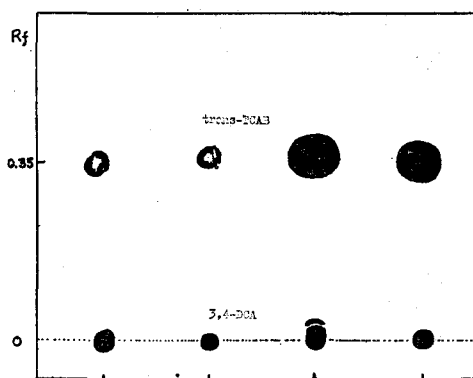
Fig. 2. Autoradiogram of the acetone extract of soils treated with ^{14}C-3,4-DCA for 106 days
1: Dose 1(Organic acid soil)
2: Dose 1(Alkaline soil)
3: Dose 2(Organic acid soil)
4: Dose 2(Alkaline soil)
Time exposed: 3 weeks
Eluent: n-Hexane

being equivalent to 0.05 to 0.1% of the original radioactivities applied to soils. Hughes et al.[22] reported that air-drying soils, as well as extended periods of storage of freshly collected soils at 4°C, reduced the formation of TCAB. In their study, the greatest concentration of TCAB formed from applied 3,4-DCA was 22.5% conversion(soil pH, 4.5; concentration applied, 100 ppm). They also concluded that soils which fall within a rather narrow range of pH 4.5 to 5.5 resulted in much higher production of azoben-

enes. In this respect, the low yield of ^{14}C-TCAB formation (trans isomer) in our experiment will be attributed to the use of long-stored soil samples. It seems that the amount of TCAB formed is related to the clay content and accordingly the C.E.C., not much to the soil pH, based on the fact that organic acid soil which has more TCAB formed contains 19.8 m.e./100 gr of C.E.C. (clay content: 19.4%), whereas alkaline soil has 6.3 m.e./100 gr(clay content: 12.5%)

Chisaka[12] et al. also reported that a clay soil formed the largest amount of TCAB by glc analysis. The value using freshly collected soil samples for these types of microbial transformations of aniline residues has been discussed by Bartha.[10]. In addition, Bartha[5] has also shown that peroxidase activity was decreased through air-drying of soils. Low yields of TCAB from several soils, the pH values of which fell within the optimum pH range for TCAB formation were also discussed to result from the use of air-dried soil samples in the experiment of Hughes et al.[22]

Additionally, undecomposed ^{14}C-3,4-DCA and an unknown product were observed in the four extracts. It also seems that trace amounts of cis-TCAB were detected in the two soils treated with dose 2 (94 ppm).

Identification by glc of ^{14}C-TCAB formed from ^{14}C-3,4-DCA.

The gas-liquid chromatograms of acetone extracts of soils treated with ^{14}C-3,4-DCA are shown in Fig. 3 and 4.

As seen in Fig. 3, trans-TCAB(Retention time: 27 min.) was remarkably formed in organic acid soil, whereas in alkaline soil in Fig. 4, only a small amount of trans-TCAB was recognized.

Degradation of ^{14}C-TCAB.

In an attempt to follow the fate and persistence of TCAB to be formed from 3,4-DCA in soil, ^{14}C-TCAB was incubated with four types of soils and analyzed. The percentage of the initial radioactivity liberated in the form of [14]CO_2 is presented in Table 6.

As seen in Table 6, the degradation rate of

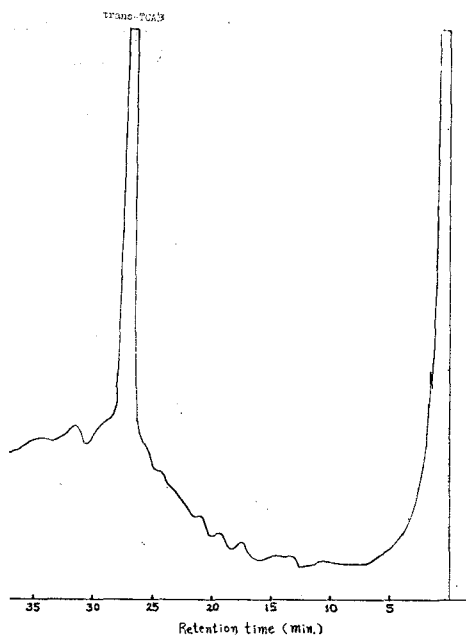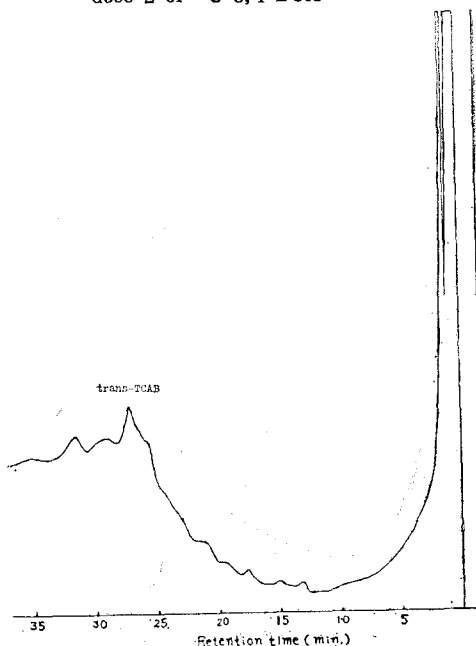Fig. 3. Gas-liquid chromatogram of the acetone extract of organic acid soil treated with dose 2 of ^{14}C-3,4-DCA

Fig. 4. Gas-liquid chromatogram of the extract of alkaline soil treated with does 2 of ^{14}C-3,4-DCA

ring-labeled ^{14}C-TCAB was very low, based on the amount of [14]CO_2 evolution and in 6 months of incubation, organic acid soil decomposed it by 0.1%, organic neutral soil, 0.05%, argillaceous soil, 0.20%, and alkaline soil, 0.19%.
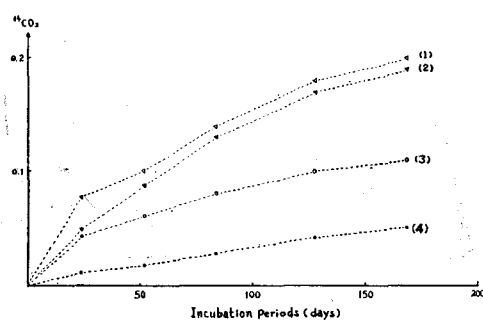
Table 6. $^{14}CO_2$ evolution from ^{14}C-TCAB in soils(Percentage of the initial radioactivity liberated in the form of $^{14}CO_2$)

Incubation days \ Soils Replicate	Organic acid soil (pH 5.5) 1	2	Alkaline soil (pH 7.9) 1	2	Organic neutral soil(pH 6.9) 1	2	Argillaceous soil (pH 7.3) 1	2
24	0.042	0.035	0.048	0.042	0.011	0.028	0.076	0.055
52	0.06	0.054	0.087	0.075	0.018	0.041	0.10	0.070
84	0.08	0.070	0.13	0.12	0.028	0.053	0.14	0.11
128	0.10		0.17		0.042		0.18	
169	0.11		0.19		0.051		0.20	

Table 7. The radioactivity after 6 months of incubation of ^{14}C-TCAB in four types of soils (Percentage of the initial radioactivity)

Origin of the radioactivity \ Soils	Organic acid soil (pH 5.5)	Organic neutral soil(pH 6.9)	Argillaceous soil(pH 7.3)	Alkaline soil (pH 7.9)
$^{14}CO_2$ liberated	0.11	0.05	0.20	0.19
Acetone-extractable radioactivity	86.55	86.83	86.53	78.57
Mineralization	6.59	8.61	7.11	15.39
Total	93.25	95.49	93.84	94.15

respectively. It was also reported that there was no apparent decomposition of TCAB after 105 days of incubation.[12] As a result, TCAB turned out to persist in most soils, irrespective of their physico-chemical properties. The degradation of ^{14}C-TCAB into $^{14}CO_2$ is plotted against the incubation periods as seen in Fig. 5.

Fig. 5. $^{14}CO_2$ evolution from ^{14}C-TCAB in four types of soils as a function of incubation periods
(1): Argillaceous soil
(2): Alkaline soil
(3): Organic acid soil
(4): Organic neutral soil

Degradation products of ^{14}C-TCAB were not detectable in 3 months of incubation and most of it remained intact. Table 7 shows the balance sheet of the radioactivity of ^{14}C-TCAB utilized to soils.

As seen in Table 7, much more ^{14}C-TCAB (15.39%) which could not be extractable with acetone is thought to be adsorbed to alkaline soil, compared with those adsorbed to the rest three soils. Of all the physico-chemical properties available, soil pH is believed to be largely related to this result. That is, the amount of the cis-isomer of ^{14}C-TCAB in alkaline soil is expected to be more than those in the other soils which have the lower pH values.[13] Accordingly, the cis-^{14}C-TCAB which has the higher adsorption affinity[35] than the trans-isomer would be adsorbed so strongly that it could not be extracted with acetone.

The high yields of radioactivity recovery from the four types of soils (>93%) and the failure to detect any noticeable degradation products of ^{14}C-TCAB confirm that ^{14}C-TCAB remained

intact for at least a period of 6 months.

要　　約

佛蘭西의 數種 土壤中에서 3,4-DCA 및 TCAB 의 변화과정을 구명하기 위하여 環標識된 ¹⁴C-3,4 -DCA 및 ¹⁴C-TCAB를 사용하여 실험을 行한 바 다음의 結果를 얻었다.

(1) ¹⁴C-3,4-DCA가 ¹⁴CO₂로 分解되는 速度는 培養初期에는 比較的 빠르고 後期에는 완만하다. 배양 6개월 後에 alkaline soil(pH=7.9)에서 dose 1(1.5 ppm)에서 最高 6.5%, dose 2(94 ppm)에서 最下 1.92%의 分解率을 보였다. organic acid soil (pH=5.5)의 경우 dose 1에서 4.91%, dose 2에 서 4.24%가 分解되었으며 兩者間에는 大差가 없 었다.

(2) Dose 1로 3,4-DCA를 6개월동안 배양할 때 organic acid soil에서는 47.70%, Alkaline soil에 서는 29.49%가 토양에 결합되었다. 한편 dose 2 의 경우 organic acid soil에서는 38.40%, alkaline soil에서는 20.30%가 結合되었다.

(3) 土壤中에서 3,4-DCA로부터 生成되는 TC- AB의 量은 土壤의 種類보다는 3,4-DCA의 使用濃 度에 依存하는것 같다. dose 2에서 生成된 TCAB 의 量은 organic acid soil에서는 抽出液의 總放射 能의 50%, alkaline soil에서는 30%에 해당하며 이것은 土壤試料에 첨가한 最初의 放射能의 1.8% 와 1.4%에 各各 해당된다. 반면 dose 1에서는 抽 出液의 總放射能에 比하여 두 土壤 共히 2~3% 를 넘지 못하며 最初의 總放射能의 0.05~0.1% 를 超過하지 못한다.

(4) ¹⁴C-TCAB가 ¹⁴CO₂로 分解되는 速度는 매 우 느리며 배양 6개월후에 四種의 土壤에서 모두 0.05%~0.20%의 分解率을 보였고 배양 3개월후 에 뚜렷한 分解產物을 檢出할 수 없었으며 大部 分 未分解된 상태로 存在하였다.

(5) Alkaline soil에서 다른 토양에서 보다 훨씬 많은 量의 ¹⁴C-TCAB가 土壤中에 吸着된 것으로 보아 Alkali土양 조건하에서 trans-TCAB→cis- TCAB의 轉換이 일어나 이 吸着性이 더 강한 cis 異性體가 土壤中에 많이 吸着된 것으로 生覺된다.

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