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# Formation of Bentazon Residues in a German and Korean Agricultural Soil

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# 독일과 한국토양 중에서 Bentazon 잔류물의 형성

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## Abstract

Benzene-ring-labelled $^{14}$C-Bentazon(3-isopropyl-2, 1, 3-benzothiadiazin-4-one-2, 2-dioxide) incubated aerobically in a German and a Korean soil at application rates of 5.51 and 25.05mg/kg was mineralized to $^{14}CO_2$ at average rates of 0.6% and 0.2%/week, respectively, in both soils, in the absence of plants. Distilled water was the most suitable solvent for the extraction of Bentazon-treated soils. Extraction results disclosed that higher percentages of non-extractable residues were formed in the lower concentration of 5.51mg/kg than in the higher concentration of 25.05mg/kg, relative to the initial concentrations.

## Introduction

Bentazon, 3-isopropyl-2, 1, 3-benzothiadiazin-4-one-2, 2-dioxide, developed by BASF(Limburgerhof, West Germany) in 1970 is a selective herbicide used in the cultivation of soybean, cereals and rice. It is generally used as a post-emergence herbicide by foliar application and its primary mode of action is reported to be photosynthetic inhibition[1]. Mine and Matsunaka[2] investigated the absorption, translocation, and metabolism of $^{14}$C-Bentazon by several plant species to determine the mechanism of Bentazon selectivity. They concluded that the main mechanism governing the selectivity appeared to be a difference between the plant species in their ability to metabolize and detoxify Bentazon. Mahoney and Penner[3] reported that Bentazon metabolism was more rapid in Bentazon-tolerant plant species than in susceptible ones. It was also concluded that translocation of $^{14}$C-Bentazon in these species was not related to susceptibility. Hayes and Wax[4], in their studies of the differential responses of soybean cultivars to Bentazon, revealed a slightly greater translocation and about a two-fold greater foliar absorption in the sensitive cultivar than in the tolerant one. They also concluded that differential Bentazon metabolism appeared to be the

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main cause of the differential responses of these soybean cultivars to Bentazon. Müller and Sanad[5] studied the distribution and behaviour of various combinations of herbicides containing Bentazon in winter wheat under field conditions. Otto et al.[6] investigated the degradation of Bentazon in plants and soils. They reported that the degradation of Bentazon by micro-organisms and plants proceeds mainly via hydroxylation of the aromatic ring in the 6-and 8-positions. Still relatively little information is published and available on degradation of Bentazon in soil. Therefore, in this study, soil degradation was evaluated using two soils representing a major agricultural soil type in Germany and Korea, respectively. Mineralization rates and the formation of non-extractable bound Bentazon residues were examined.

## Materials and Methods

### Chemicals

Ring-labelled $^{14}$C-Bentazon (specific activity: 10.81mCi/mMol) and cold Bentazon (>99.5% purity) were provided by the BASF Corporation, Limburgerhof, West Germany. The purity was again verified by thin-layer chromatography (TLC) prior to use. Some metabolites which were also supplied by BASF include anthranilic acid isopropylamide (>99.5% purity), 2-[[[(1-methylethyl)amino]sulfonyl]amino]-benzoic acid (>98% purity), 6-hydroxybentazon (>98% purity), and 8-hydroxybentazon. The chemical structure of Bentazon and the $^{14}$C-labeling position are shown in Fig. 1.

### Soils used

A German parabraun soil and a Korean soil were used after being air-dried and put through a 2-mm Tyler sieve. The physico-chemical characteristics of the two soils were as follows: sand, 7.0 and 64.4%; silt, 85.3 and 31.2%; clay, 7.7 and 4.4%; textural class, sandy loam both; pH (KCl), 7.1 and 5.9; carbon, 1.10 and 4.11%; total nitrogen, 0.05 and 0.06%; maximum water-holding capacity, 44 and 46 g/100g soil, respectively. The parabraun soil (alfisol) represents one of the most fertile soils in agricultural use in Germany[7].

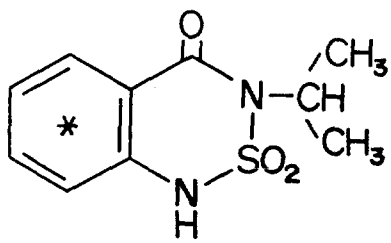

**Fig. 1. Structural formula and labeled position(*) of Bentazon (3-isopropyl-2, 1, 3-benzothiadiazin-4-one-2, 2-dioxide)**

### Incubation of soils treated with $^{14}$C-Bentazon

In order to investigate the degradation of Bentazon in soil and to characterize its residue formation, two concentration levels of $^{14}$C-Bentazon were applied to each soil for an aerobic incubation. For treatment 1 (T-1), the initial radioactivities and active ingredient concentrations of the parabraun and Korean soil were 25$\mu$Ci (5.51 mg/kg) and 25.13 $\mu$Ci (5.51mg/kg), respectively, for 5 kg of soil on a dry weight basis. Meanwhile, for treatment 2 (T-2), the initial radioactivities and active ingredient concentrations added to the parabraun and Korean soil were 100 $\mu$Ci (25.05 mg/kg) and 100.5 $\mu$Ci (25.05 mg/kg) for 5kg of soil on a dry weight basis, respectively (Table 1). After $^{14}$C-Bentazon, dissolved in a small amount of MeOH, was added to each soil, the solvent was allowed to evaporate by flushing the container with nitrogen gas. The mixed soil was put in desiccators and incubated aerobically by passing through it a stream of $CO_2$-free air. Throughout the incubation, the moisture contents were maintained at 50% of the maximum water-holding capacity of each soil. The incubation temperature was kept at 23±1°C for 105 days. The $^{14}CO_2$ evolved during incubation was absorbed in

**Table 1. Treatment levels of $^{14}$C-Bentazon in the soil pre-incubation experiment. 5kg soil at 23±1°C at 50% of the maximum water-holding capacity of the soil.**

| Treatment | Parabraun soil | | Korean soil | |
|---|---|---|---|---|
| | mg/kg | $\mu$Ci/kg | mg/kg | $\mu$Ci/kg |
| T-1 | 5.51 | 5.0 | 5.51 | 5.0 |
| T-2 | 25.05 | 20.0 | 25.05 | 20.1 |

1 N NaOH and measured at intervals of one week.

**Extraction of the soils**

At the completion of the incubation, the soils of T-1 were air-dried and those of T-2 were exhaustively extracted with distilled water and/or a simulated soil solution of 0.01M $CaCl_2$, since the latter solution gave good sedimentation. Nevertheless, distilled water gave the best extractability among the solvents tested. The exitracted soils were also air-dried for a subsequent plant experiment. The water extracts of T-2 for both soils were concentrated to dryness on a rotary evaporator and the residues were redissolved with MeOH, passed through a florisil column, and concentrated again under a stream of nitrogen for the elucidation of possible metabolites formed during the incubation.

**Formation of non-extractable bound residues of $^{14}$C-Bentazon**

10g each of T-1 and T-2 which had been treated with $^{14}$C-Bentazon of different concentrations and incubated for 105 days were exhaustively extracted with five 50-ml aliquots of distilled water. The extracts were then adjusted to pH 1 with conc. hydrochloric acid. After 40ml of the extract had been extracted with the same volume (40ml) of ethyl acetate, the radioactivities of aqueous and organic phases were measured. The soil-bound $^{14}$C residues were again extracted with five 50-ml aliquots of 0.1M sodium pyrophosphate. Centrifugation at 13,000 rpm for 10 min. removed the insoluble material, humin, and acidification of the supernatant with conc. hydrochloric acid caused flocculation of the humic acid fraction. The fulvic acid fraction remained in solution. The humic acid precipitate was redissolved with 1N-NaOH and the radioactivity was measured. Radioactivity in the humin fraction was measured by combustion with a Packard Tri-Carb Sample Oxidizer (Packard 306).

**High-performance liquid chromatography**

The concentrated soil extracts were separated and partially purified using a 7 RP-18 column(Hibar 250—4, E. Merck, Darmstadt). A Waters Associates high-performance liquid chromatograph with a Model 440 detector (UV detector with fixed wavelength of 254 nm), two pumps Model 6,000-A, and a solvent programmer Model 660 were used for separation. The radioactivity was monitored on-line with a Berthold radioactivity monitor Model LB 503. The HPLC conditions were: room temperature (23~24°C); a gradient elution of 35% acetonitrile/65% water (2.5% acetic acid) to 100% acetonitrile in 15 min. using program #8; the flow rate was 1ml/min.

**Thin-layer chromatography**

The extracts from the soils were developed on TLC plates (0.2 mm thickness, Kieselgel, E. Merck) with an F 254 fluorescent indicator for autoradiography and the subsequent identification. Developing solvents were chloroform-MeOH(7 : 3, V/V) and Benzene-EtOH-$NH_4OH$ (40 : 59 : 1, V/V).

## Results and Discussion

**Mineralization of $^{14}$C-Bentazon in soil**

The amounts of $^{14}CO_2$ evolved during the incubation period of 105 days are shown in Fig. 2. As can be seen in this figure, the percentage of mineralization to $^{14}CO_2$ is greater in T-1 (5.5 mg/kg) than in T-2 (25 mg/kg) by a factor of almost 3. In T-1, almost 9% (based on $^{14}$C added) of the ring carbon of the Bentazon molecule was mineralized to $^{14}CO_2$ within 105 days, compared with 3.2 ~3.4% in T-2. This result seemed to be either due to the inhibition of the responsible microorganisms by the higher concentration of Bentazon(T-2) or due to the limit of their degrading power. No differences existed between the two soils. The absolute quantities of $^{14}$C mineralization to $^{14}CO_2$ were greater in T-2 than in T-1 in both soils.

**Solvent extractability**

In order to select a suitable solvent for the extraction of $^{14}$C-Bentazon-treated soils, various solvents were tested (Table 2) and also the soil conditions (wet soil versus dry soil) were changed for the extractability (Table 3). Neither MeOH nor a successive extraction with MeOH, acetone, and MeOH showed a higher extraction efficiency com-

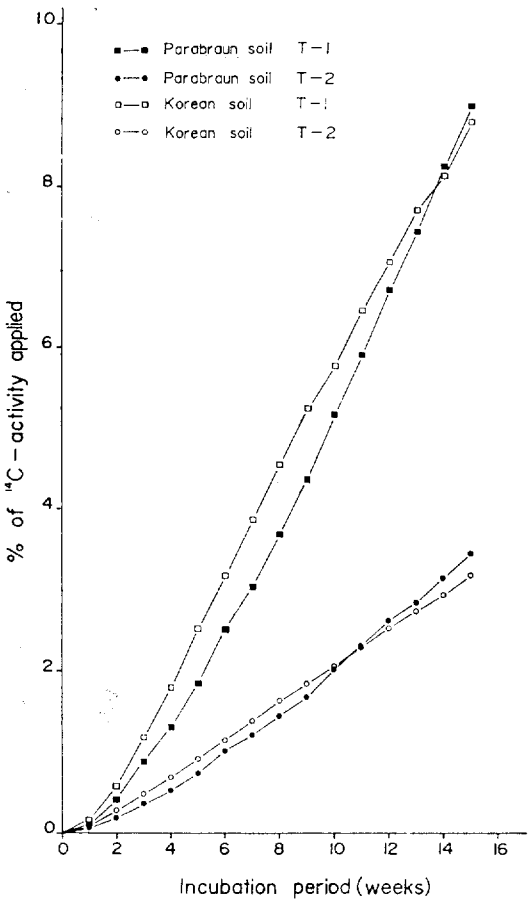


**Fig. 2. Evolution of $^{14}CO_2$ during incubation of soils with $^{14}$C-Bentazon(Applied $^{14}$C-activity=100)**

**Table 2. The efficiency of some solvents in extracting Bentazon residues from a parabraun soil which was incubated with $^{14}$C-Bentazon (10mg/kg) for 70 days at 22°C and 50% maximum water-holding capacity. Wet soil was used.**
**$^{14}$C-activity applied=100%**

| Solvent tested | Extraction | | | |
|---|---|---|---|---|
| | 1st+2nd% | 3rd% | 4th% | total% |
| 50% MeOH (50ml×4) | 74.6 | 1.2 | 0.8 | 76.6 |
| Distilled water (50ml×4) | 75.9 | 1.1 | 0.4 | 77.4 |
| MeOH (50ml×2) | 73.4 | | | |
| Acetone (50ml) | | 1.0 | | 76.2 |
| MeOH (50ml) | | | 1.8 | |

pared with distilled water(Table 2). TLC of extracts verified that all solvents extracted the same materials, indicating that only a slight degradation occurred. As can be seen in Table 3, the extractability of each solvent varies with the soil conditions. In the case of MeOH, wet soil was extracted better than the air-dried soil. In the cases of 0.01M $CaCl_2$ and distilled water, more radioactivity was extracted from the air-dried soil than from the wet soil. Overall, distilled water was the most efficient for both wet and air-dried conditions, suggesting that it would be most suitable for the preparation of non-extractable bound residues without significantly altering soil conditions[8]. Never-

**Table 3. Extractability of some solvents tested for the extraction of the parabraun soil which was treated with $^{14}$C-Bentazon, incubated aerobically for 105 days, and planted with maize for 21 days.**
**Soil : 10g**
**Solvent : 50ml×4**
**Radioactivity in the soil (0.035$\mu$Ci/10g soil)=100%**

| Solvent | Soil condition | Extracted(%) | Non-extractable*(%) | Recovery(%) |
|---|---|---|---|---|
| MeOH | Wet | 12.95 | 82.38 | 95.33 |
| | Air-dried | 10.02 | 85.13 | 95.15 |
| 0.01M | Wet | 13.81 | 79.21 | 93.02 |
| $CaCl_2$ | Air-dried | 15.89 | 78.91 | 94.80 |
| Distilled | Wet | 17.13 | 75.70 | 92.83 |
| water | Air-dried | 19.18 | 74.19 | 93.37 |

* Measured by soil combustion

**Table 4. Comparison between distilled water and 0.01M $CaCl_2$ solution in terms of extractability of residual Bentazon ring carbon from soil.**
**Air-dried soil : 10g**
**Solvent : 50 ml×5=250 ml**
**Extraction time : 15 hrs, each**
**$^{14}C$-activity in the soil before extraction=100%**

| Soil | State* | Extracted(%) | |
|---|---|---|---|
| | | Distilled water | 0.01M $CaCl_2$ |
| Parabraun | A | 37.76 | 35.42 |
| | B | 18.49 | 16.47 |
| | C | 12.23 | 10.45 |
| Korean | A | 48.31 | 41.15 |
| | B | 20.24 | 16.49 |
| | C | 16.78 | 13.36 |

* implies $^{14}C$-Bentazon residues of different backgrounds in soil.

theless, 0.01M $CaCl_2$ was also used for the extraction of soil, because it provided good sedimentation and it would not affect the soil microorganisms as much during extraction. Otto et al.[6] used MeOH in the extraction of the Bentazon-degrading soils to solubilize degradation products. Since distilled water and 0.01M $CaCl_2$ solution were the most promising solvents, they were compared more closely in Table 4 using the soils which were used in the subsequent plant experiment. In Table 4, it is readily recognized that distilled water was far better than 0.01M $CaCl_2$ in the two soils and their treatments, especially in the Korean soil.

**Formation of non-extractable bound residues of $^{14}C$-Bentazon in soil**

After an aerobic incubation of $^{14}C$-Bentazon at two concentration levels for 105 days, 10 g of each soil on a dry weight basis were exhaustively extracted with 50 ml of distilled water for 15 hrs. The same extraction procedure was repeated 5 times. The non-extractable residues which remained were measured by soil combustion and are presented in Table 5. Based on this result, the soils which were treated with a lower concentration of 5.51 mg/kg on a dry weight basis produced higher percentages of non-extractable residues, 46.55 and 56.69% in the German and Korean soils, respectively, relative to the initial radioactivity applied. The soils which were treated with a higher concentration of 25.05 mg/kg produced 25.94 and 43.99% bound $^{14}C$ in the German and Korean soils, respectively. As indicated by the $^{14}CO_2$ evolution (Fig. 2) in the concentration T-1 (5.51mg/kg soil), the higher turnover rate of Bentazon led not only to a higher mineralization but also to a relatively higher production of bound residues. Although 55~72% of $^{14}C$-Bentazon or its metabolites were extracted from soils of higher concentration, the absolute values of radioactivities of the soils of higher concentration were still greater than those of lower concentration in both soils.

Fig. 3 shows the radioactivity distribution of the non-extractable residues in fulvic acid, humic acid, and humin. Abernathy and Wax[9] used 12 soils from Illinois/USA containing organic matter contents ranging from 0.7 to 8.5% to evaluate Bentazon mobility and adsorption. They reported that Bentazon movement in soil columns was more retarded by high organic matter than high clay content. It is quite reasonable then that in the experiment described here, more non-extractable residues were formed in the Korean soil which has a higher carbon content and a lower pH than does the German parabraun soil. In addition, Abernathy and Wax[9] concluded that Bentazon was not adsorbed by any of the 12 selected Illinois soils, due to its high water solubility(500 ppm) and strong anionic characteristics (pKa=3.2), based on the

**Table 5. Comparison of the non-extractable bound residues of $^{14}$C-Bentazon formed in soils after an aerobic incubation for 105 days at 23±1°C and 50% of the maximum water-holding capacity of the soil in the dark.**

**$^{14}$C-activity applied=100%**

| Soil | Initial conc. (mg/kg soil) | Mineralization to $^{14}CO_2$(%) | $^{14}$C-activity extracted with water (%) | $^{14}$C-activity remaining in soil (%) | Recovery (%) |
|---|---|---|---|---|---|
| Parabraun | 5.51 | 8.98 | 41.71 | 46.55 | 97.24 |
| | 25.05 | 3.44 | 71.76 | 25.94 | 101.14 |
| Korean | 5.51 | 8.79 | 30.95 | 56.69 | 96.43 |
| | 25.05 | 3.18 | 54.80 | 43.99 | 101.97 |

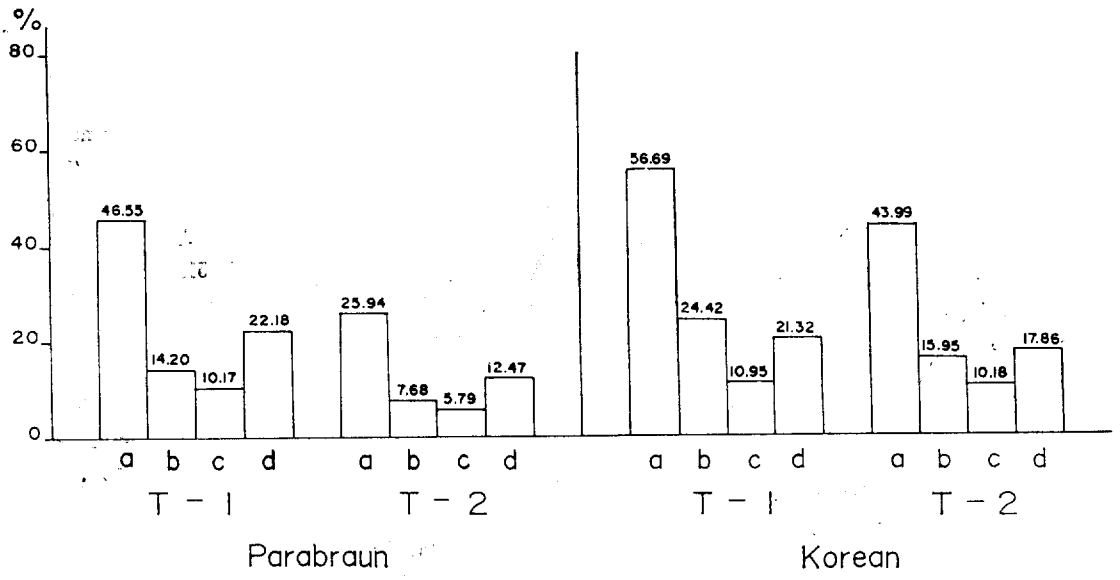


**Fig. 3. Distribution of the radioactivity of non-extractable bound residues of $^{14}$C-Bentazon in humic substances**

**a : Non-extractable bound residue**
**b : Fulvic acid**
**c : Humic acid**
**d : Humin**

result obtained from 1g of air-dried soil and 10 ml of Bentazon solutions shaken and allowed to equilibrate for 24 hrs at 30°C. However, the formation of non-extractable $^{14}$C residues of Bentazon, as presented, after 105 days of incubation in soil, could not be interpreted by the same mechanism as demonstrated above.

**Elucidation of the extracts from the $^{14}$C-Bentazon-treated soils**

Fig. 4 shows the distribution of the radioactivity extracted with distilled water after 105 days' aerobic incubation of $^{14}$C-Bentazon in soil. In both soils, it is clear that greater quantities of polar products were formed at the lower concentration (T-1) of $^{14}$C-Bentazon compared with the higher concentration (T-2), as evidenced by the radioactivity distributed in the aqueous phase relative to that of the ethyl acetate phase. For further elucidation, the extracts from T-2 of the soils were subjected to high performance liquid chromatography. About 95% of the initial $^{14}$C-Bentazon remained intact in

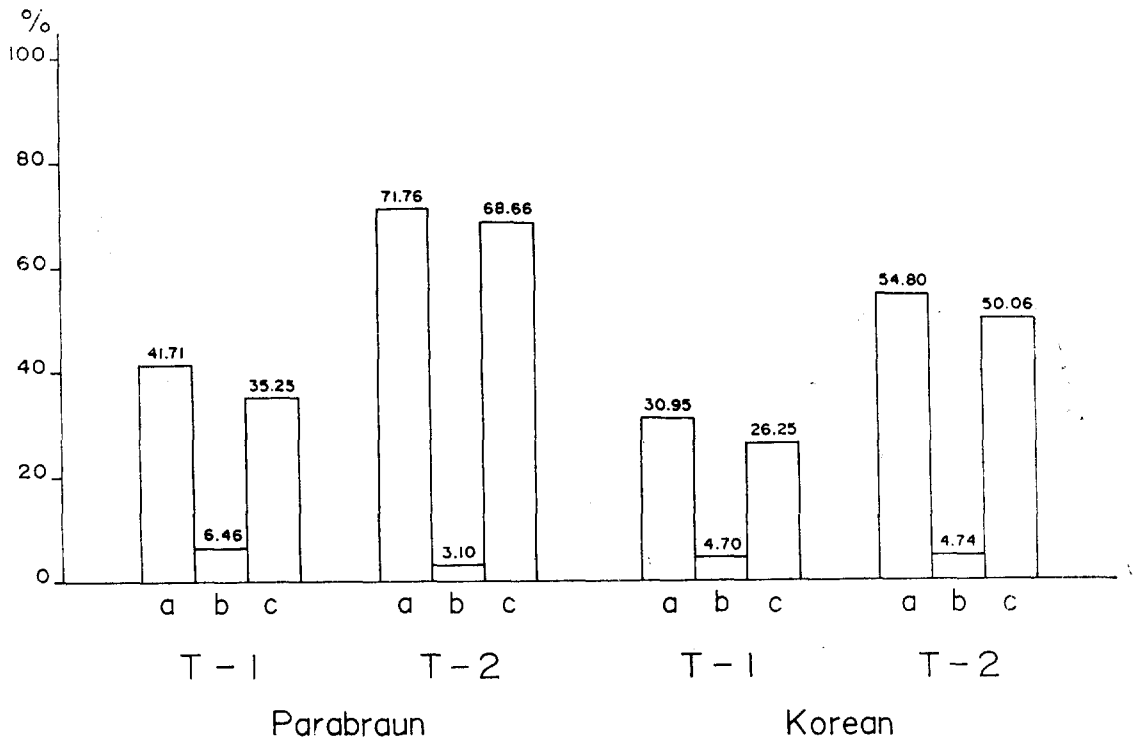


**Fig. 4. Distribution of $^{14}C$-Bentazon and its metabolites extracted with distilled water, between aqueous and organic phase.**

**a : Extracted(%)**
**b : Aqueous phase(%)**
**c : Ethyl acetate phase(%)**

the German parabraun soil, and about 93% was unchanged in the Korean soil. The rest which degraded during incubation was composed of both more and less polar products than Bentazon. Approximately half of these materials were more polar and half were less polar than the parent compound. These degradation products have not been identified. Even if the difference in the degradation of Bentazon in the two soils is slight, it might be due to the different contents of organic matter which affects the soil microorganisms involved.

## 要 約

Benzene環이 균일하게 표지된 제초제 $^{14}C$-Bentazon (3-isopropyl-2, 1, 3-benzothiadiazin-4-one-2, 2-dioxide)을 서독과 한국의 토양에 5.51 mg/kg과 25.05 mg/kg의 두 수준으로 처리하여 23±1°C에서 105일간 호기적으로 배양했을때 두 토양에서 모두 $^{14}CO_2$로 분해되는 속도는 주당 0.6%와 0.2%이었다. Bentazon을 처리한 토양을 용매로 추출한 결과 증류수가 가장 우수한 용매임이 밝혀졌고 용매로 추출이 불가능한 Bentazon 토양 잔류물은 25.05 mg/kg을 처리한 토양에서 보다는 5.51 mg/kg을 처리한 토양에서 모두 최초 시용량에 대하여 보다 높은 비율로 형성되었다.

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