

metabolic conditions examined. Two features probably contribute to this refractory property. First, a portion of the dose may bind to tissue components so it is isolated from the microsomal monooxygenase site. Second, it appears that only a small amount of the compound is absorbed following ingestion. These are important factors in toxicological evaluations of cyhexatin and other triorganotin derivatives.

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Plant Experiments on the Bioavailability of Unextracted [*carbonyl*-¹⁴C]Methabenzthiazuron Residues from Soil

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The extractability of methabenzthiazuron residues from soil decreases with time after application. In this study the bioavailability of this soil bound or adsorbed fraction was investigated using maize plants. A sandy soil and [*carbonyl*-¹⁴C]methabenzthiazuron (10 ppm) were incubated at 22 °C and 65% of the water holding capacity for 111 days. After extractions with H₂O/acetone/ethyl acetate/chloroform, unextracted, bound residues remaining in this soil amounted to 41% applied. Plant uptake studies were conducted in special experimental pots. After 29 days the maize shoots contained 0.7% of the radioactivity originally applied to the soil before incubation in comparison to 2.2% from the incubated soil not extracted. When the [*carbonyl*-¹⁴C]methabenzthiazuron was added to the soil immediately before the plant experiment, about 4% of the radioactivity was found in the shoots. The plant availability of the unextracted radiocarbon, as compared with the extractable portion, has decreased to about one-third and in comparison with the availability after direct methabenzthiazuron application to about one-sixth. Desorption studies with water showed similar ratios.

In experiments with certain ¹⁴C-labeled compounds, unextractable radioactivity in the soil which represents the bound residue fraction, increases with time (Führ and Mittelstaedt, 1976; Lichtenstein et al., 1977) and especially with increasing soil temperatures (Führ and Mittelstaedt, 1979; Katan et al., 1976). Thus, in the soil, pesticides appear to behave like other organic residues (i.e., straw, green manure, or roots) due to turnover processes and buildup of organic matter components (Stevenson, 1976). The organic fraction of a soil has the potential for forming strong chemical linkages with pesticides or residues arising from their partial degradation by microorganisms. Besides chemical reactions, adsorption by organic matter has been shown to be a key factor in inactivation of many pesticides in the soil (Hayes, 1970).

So far no analytical methods in soil organic matter chemistry have been developed to provide a precise separation and identification of the different compounds which contribute to this bound or conjugated pesticide residue fraction in the soil (Kaufman, 1976). Therefore

it is of interest to know if and to what extent soil-bound pesticide residues or metabolites will become bioavailable to plants and especially to what extent they are taken up by the roots of untreated rotational crops following the treated crop.

Since outdoor lysimeter experiments with ¹⁴C-labeled pesticides are not only time-consuming but also highly expensive, a plant experiment was designed to study the root uptake of unextracted pesticide residues and their turnover in the soil under standard climatic conditions. These studies were proposed at the Symposium on Bound and Conjugated Pesticide Residues at Vail, Colorado (Führ, 1976). The results from the plant experiment are discussed in relation to data from lysimeter experiments under outdoor conditions. Methabenzthiazuron [1,3-dimethyl-3-(benzthiazol-2-yl)urea, active ingredient in Tribunil (trade name, Bayer AG, Leverkusen, Germany)], a broad spectrum herbicide for weed control in cereal crops, was chosen since it was shown to be a stable compound in a lysimeter experiment with spring wheat (Führ and Mittelstaedt, 1976).

MATERIALS AND METHODS

Chemicals. [*carbonyl*-¹⁴C]Methabenzthiazuron was provided by the Bayer AG, Leverkusen (Ecker, 1975).

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Table I. Plant Experiment to Determine the Bioavailability of the Unextracted Radioactivity^a

	treatment 1 soil + MBT	treatment 2 soil + MBT incubated for 111 days	treatment 3 soil + MBT incubated for 111 days
	Radiocarbon Recovered in Percent of Soil-Applied ¹⁴ C		
extracted with H ₂ O/acetone/ethyl acetate/chloroform uptake by maize	not extracted	not extracted	56.4
shoots	3.98 ± 0.27	2.16 ± 0.07	0.69 ± 0.18
roots	0.82 ± 0.21	0.60 ± 0.06	0.30 ± 0.06
	After the Plant Experiment		
desorbed from the soil with H ₂ O	30.0 ± 2.1	24.7 ± 2.9	4.8 ± 0.5
extracted with H ₂ O/acetone/ethyl acetate/chloroform	57.7 ± 4.1	58.4 ± 10.3	12.5 ± 1.6

^a Test compound, [carbonyl-¹⁴C]methabenzthiazuron (MBT). Maize grown for 29 days in a sandy soil.

Soil. A sandy soil from Walbeck, Germany, was used. Selected chemical and physical characteristics were as follows: 1.15% C, 3.7% clay, 8.2% silt, 51.5% fine sand, 36.6% coarse sand, pH_{KCl} 6.4.

Experimental Setup. The plant experiment was conducted in special experimental pots (polypropylene, 0.8 kg of soil/pot) which allow an airtight separation of the root atmosphere (Mittelstaedt and Führ, 1975a). This experimental setup has been used successfully for 15 years to study the uptake of ¹⁴C-labeled organic compounds from soil or nutrient solution and to register the turnover processes during the experiment (Führ and Sauerbeck, 1965). The soil received fertilizer equivalent to 200 mg of N, 70 mg of P, and 100 mg of K, 5 mL of Hoagland trace element solution and was re inoculated with 20 mL of a freshly prepared garden soil extract. Maize (variety INRA 240) was chosen as experimental plant, germinated, planted into the pretreated soil, and sealed into the container lid (four plants/pot) with a durable plastic compound (Prestik). During the first week the soil was watered daily up to 80% of the maximum water holding capacity. Then the water supply was reduced to 65%. The soil was aerated with CO₂-free air and the CO₂ evolved from root and soil respiration as well as ¹⁴CO₂ derived from decomposition of the added pesticide was trapped in 1 N NaOH.

Three soil treatments, each with three replicates, were compared.

Treatment No. 1. Methabenzthiazuron (5.9 mg/kg of soil, 14 μCi/pot) dissolved in acetone was added to the soil and uniformly mixed immediately before maize was planted in the soil.

Treatment No. 2. The soil treated with methabenzthiazuron (10 mg/kg of soil, 13.6 μCi/pot) was incubated according to guidelines published by Weinmann and Schinkel (1976) at a standard temperature of 22 °C and 65% of the maximum water-holding capacity of the soil for a period of 111 days. The soil was then air-dried and used for the plant experiment.

Treatment No. 3. The soil containing added methabenzthiazuron (10 mg/kg of soil, 79 μCi/pot) was incubated as before and passed through a fourfold extraction procedure involving the use of H₂O/acetone/ethyl acetate and acetone/chloroform combinations (Cheng and Führ, 1976).

In addition, reference pots without methabenzthiazuron which had been treated the same as treatment no. 2 and 3 were included in the plant experiment. The experiment was conducted in the isotope greenhouse with a day/night

temperature fluctuation of 23 and 18 °C, respectively. After 29 days the plants were harvested, freeze-dried, and analyzed for radioactivity. Aliquots of the soil were subsequently extracted four times with water (Kerpen, 1975) or H₂O/acetone/chloroform (Cheng and Führ, 1976), followed by an extraction with 0.1 N NaOH as outlined in Figure 1. Plants were extracted with H₂O/acetone (Jarczyk, 1972). Methabenzthiazuron was determined by cochromatography on TLC (Merck silica gel, 254 nm) and high-performance liquid chromatography (Waters, 10 μ-Porasil column) (Mittelstaedt et al., 1977). Soil and plant samples were freeze-dried and combusted (Packard 306), and the radioactivity was determined in the liquid scintillation spectrometer (Packard 3385).

RESULTS AND DISCUSSION

During the preincubation of methabenzthiazuron for 111 days only 0.23% of the applied carbonyl-¹⁴C was mineralized to ¹⁴CO₂. The carbonyl carbon shows similar stability as the benzene ring carbon or the benzthiazolyl 2-carbon of the molecule, whereas both of the methyl groups on the urea moiety demethylated readily (Cheng et al., 1978).

By using H₂O/acetone/chloroform and succeeding extraction steps, 56.4% of the applied radioactivity was extracted from the soil (Table I; Figure 1). Over 98% of the extracted radioactivity was identified as the unchanged methabenzthiazuron. During the 29 days of the plant experiment, the decomposition to ¹⁴CO₂ was less than 0.1% of the applied radioactivity in all three treatments. The results of the plant experiment are shown in Table I. When methabenzthiazuron was applied immediately before planting (treatment no. 1), the maize shoots contained about 4% of the applied radioactivity compared to 2.16% from the soil after 111 days of incubation (treatment no. 2). Although only negligible degradation was observed during the preincubation, the bioavailability of the radioactivity in the soil decreased considerably to about one-half in comparison to freshly added methabenzthiazuron, while the extractability with organic solvents was a constant of approximately 58% in both treatments.

In the extracted soil, treatment no. 3, the plants showed normal growth without differences in dry matter production when compared with the reference pots. The rate of pesticide translocation to the maize shoots, however, decreased to 0.69% of the applied radioactivity. After incubation in the soil, the plant availability of the unextracted radiocarbon, as compared with the extractable

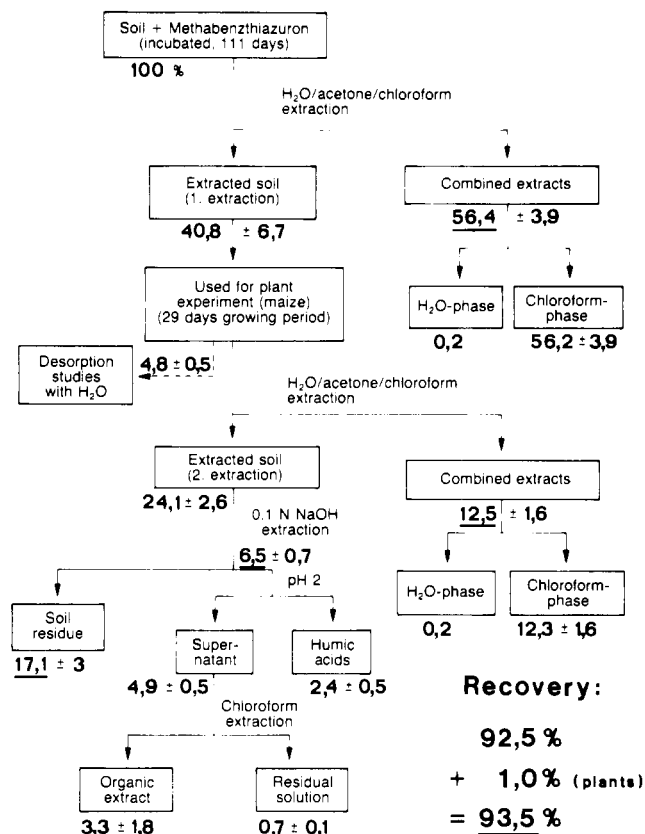


Figure 1. Flow diagram of the fractionation of methabenzthiazuron treated soil. Results of treatment 3: 800 g of soil, 10 mg of [*carbonyl-¹⁴C]methabenzthiazuron/kg of soil. Average values of three replicates and standard deviation.*

portion (treatment no. 2), decreased to about one-third, and in comparison with the availability after direct application (treatment no. 1) to about one-sixth. With organic solvents 12.5% of radioactivity could be extracted from the soil which is about one-fifth as compared to treatments no. 1 and 2.

These differences in uptake are also observed in the roots. But they are not as drastic as in the shoots, since so far no method is known to separate between root-adsorbed and adsorbed organic compounds in such uptake experiments, whereas the radioactivity found in the shoots had to pass through root exo- and endodermis to enter the conducting tissue. The analysis of the shoots revealed partly radioactive water-soluble compounds (Table II) but between 12 and 39% of the total radioactivity in the shoots represented the unchanged methabenzthiazuron. The portion of unchanged methabenzthiazuron identified by thin-layer or high-performance liquid chromatography in the plant extracts, however, decreased from 1.5% of the amount originally applied immediately before planting (treatment no. 1) to 0.15% found in the plants grown in the extracted soil (treatment no. 3). This uptake of methabenzthiazuron confirms results from short-term experiments in nutrient solution (Collet and Pont, 1974). In field experiments only traces of methabenzthiazuron could be detected in the wheat straw 111 days after spray application (Führ and Mittelstaedt, 1976).

After extraction with organic solvents, apparently an equilibrium status is built up again in the soil between bound or adsorbed pesticide residues or metabolites and such compounds in the soil solution. Data from field and laboratory studies with methabenzthiazuron labeled in various positions (Führ et al., 1977) indicate that the unextracted portion of methabenzthiazuron seems to be

Table II. Methabenzthiazuron Residues in Maize Shoots Extracted with H₂O/Acetone and Determined by Cochromatography (TLC and LC)^a

treatment	extracted with H ₂ O/acetone		methabenzthiazuron in the chloroform phase	
	H ₂ O phase, %	chloroform phase, %	LC, %	TLC, % ^c
1	37.4	47.3	39.3	38.3 (1.52)
2	40.2	18.9	12.9	12.5 (0.27)
3	24.8	37.2	22.3	21.7 (0.15)

^a Total radioactivity in maize shoots = 100%. ^b Soil + methabenzthiazuron; (2) soil + methabenzthiazuron incubated for 111 days; (3) soil + methabenzthiazuron incubated for 111 days and extracted with H₂O/acetone/ethyl acetate/chloroform. ^c Soil-applied methabenzthiazuron = 100%.

strongly adsorbed on soil organic matter binding sites rather than incorporated into or assimilated by soil organic matter. In the soil of treatment no. 3 (Figure 1), the radioactivity in the H₂O/acetone/chloroform extracts was found to be almost exclusively unchanged methabenzthiazuron. In the succeeding NaOH extract, two-thirds of the radiocarbon remained upon precipitation of the humic acids in the supernatant, which proved to be predominantly (over 90%) methabenzthiazuron. This explains that methabenzthiazuron could still be detected in the maize shoots (Table II). However, in the case of the soil-bound methabenzthiazuron (treatment no. 3) the total amount of methabenzthiazuron expressed in per cent of the soil applied is reduced to one-tenth as compared with the freshly added methabenzthiazuron (treatment no. 1). It is therefore suggested that the bioavailability of soil-bound residues may be governed by slow desorption processes thus releasing small amounts of methabenzthiazuron. The adsorption on clay or organic matter binding sites is apparently a temporary process. From studies with other pesticidal compounds it might be concluded that chemical reactions with soil organic matter components render them stable (Helling and Krivonak, 1978a; Hsu and Bartha, 1976; Mathur and Morley, 1978; Stevenson, 1976), resulting in a drastic decrease of bioavailability.

The roots can only take up organic compounds which reach the roots in the soil solution or are released by direct contact exchange from the soil sorbents. Therefore, a desorption study with water should give approximate information on the portion of radioactivity which can be expected in the soil solution of the root environment during such an uptake study. At the end of the plant experiment in treatment no. 1 (Table I), 30% of the applied radioactivity could be desorbed with water. In treatment no. 2 this portion decreased to 24.7%, while only a total of 4.8% was found in the four water extracts of treatment no. 3. This is only one-sixth of that in the extracts of treatment no. 1 and similar to that found for the uptake and translocation of radiocarbon to the maize shoots. Data of a desorption study could, therefore, be used as an estimate of the relative bioavailability of unextracted pesticide residues.

The most reliable answer on the bioavailability of soil bound pesticide residues, however, can be achieved only in field experiments. In a lysimeter study (Führ and Mittelstaedt, 1976), [*benzene-ring-U-¹⁴C]methabenzthiazuron was applied to spring wheat grown on a loess soil low in carbon (0.25% C, 19% clay, 23% silt, 57% fine sand, pH 7.3). At harvest time, 111 days after spray application, 83% of the applied radioactivity was still in the soil,*

preferentially in the upper 5-cm layer, and almost 90% of the extracted portion was identified as the unchanged methabenzthiazuron. During the following vegetation period from October 1973 till September 1974, the radioactivity in the soil decreased from 83% to about 40% of the radioactivity applied, and the portion of unextracted radioactivity increased from about 40% to 65% of the radioactivity still remaining in the soil. The rotational crop rye, grown after the treated spring wheat, contained only one-tenth of residual methabenzthiazuron equivalents and even less was found in carrots. Methabenzthiazuron could not be extracted from rye or carrots. In the laboratory experiment (Table I), the bioavailability of soil bound methabenzthiazuron residues decreased to one-sixth as compared with the uptake of methabenzthiazuron added to the soil immediately before planting. We should bear in mind that due to more drastic temperature and moisture fluctuations together with an increased energy release by decaying root and soil carbon, the biochemical reactions would be more intense under field conditions. Therefore, in laboratory studies the uptake of soil-bound pesticide residues would probably be enhanced due to the special experimental conditions prevailing in the soil such as uniform pesticide distribution, steady water supply, narrow root/soil ratio, and optimal growth conditions.

Fuhremann and Lichtenstein (1978) found in a similar study with [methyl-¹⁴C]parathion that three sets of oat plants, germinated and grown in a mixture of a clay loam soil containing bound residues with silica sand (1:20) for 14 days each, removed a total of 5.1% of the previously soil-bound radioactive residues, which was equivalent to about 1.7% of the originally applied radioactivity. Such results will differ depending on the compound applied, the soil or soil mixture used and the experimental conditions (Helling and Krivonak, 1978b). In both studies (Fuhremann and Lichtenstein, 1978; Helling and Krivonak, 1978b), the uptake rates from freshly treated soils compared to the uptake from the unextracted residue fraction was in the order of about 5:1, which confirms the results found for methabenzthiazuron. So far we have obtained results from outdoor lysimeter experiments with ten different active ingredients of herbicides (Führ and Mittelstaedt, 1976; Mittelstaedt and Führ, 1975b, and unpublished data), fungicides (Steffens and Wieneke, unpublished data), and insecticides (Steffens, unpublished data). The results lead to the conclusion that the different compounds and their residues become part of the soil organic matter pool, and their turnover rate and bioavailability during the following vegetation period are similar to those of the labile organic matter fraction of decaying plant residues (Führ and Sauerbeck, 1968; Sauerbeck and Führ, 1971). At least we have to realize that we are discussing the analytical disappearance of some 100 g of organic carbon ha⁻¹ year⁻¹ in a total carbon pool which is about 20 000 kg/ha in an average agricultural soil layer.

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