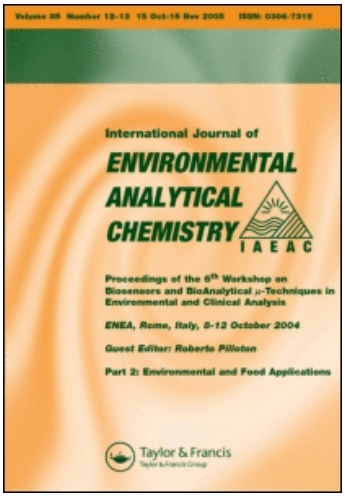


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# Comparative Studies on the Formation of Bound Residues in Soil in Outdoor and Laboratory Experiments

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# COMPARATIVE STUDIES ON THE FORMATION OF BOUND RESIDUES IN SOIL IN OUTDOOR AND LABORATORY EXPERIMENTS

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To evaluate the formation of bound residues in soil, standardized degradation experiments were designed with soil-plant systems such as pot experiments in the greenhouse and lysimeter experiments under outdoor conditions using $^{14}$C-labelled metamitron,* and methabenzthiazuron.† During the experiments different losses of $^{14}$C occurred due to mineralization of the labelling positions. More than 80% of the remaining radioactivity applied in pot and lysimeter experiments was detected in the 0–10 cm soil layer. Thirteen weeks after application of $^{14}$C-metamitron, 20% of the applied $^{14}$C remained in the lysimeter soils after extraction with organic solvents or 0.01 M $CaCl_2$ solution while 50% remained unextractable in the soils kept at 22°C and 50% of the maximum water holding capacity of the soil.

In the case of methabenzthiazuron, 25% of the applied radioactivity could not be extracted from the lysimeter soils with organic solvents, whereas, 30–40% remained in the soils of the laboratory studies. The results from the soil in the pot experiments were intermediate between the outdoor and laboratory experimental values.

The study indicates that small-scale laboratory experiments do not provide realistic data about the formation of bound residues under practical field conditions.

## INTRODUCTION

Most of the pesticides applied reach the soil by preemergence spraying with herbicides, by seed dressing and spraying with fungicides and insecticides, by soil fumigation with nematicides, by wash-off, and by working in treated plants. The pesticides are subjected to degradation processes in soil which, depending on the physico-chemical behaviour of the active ingredient and the prevailing soil and climatic conditions, lead to different mineralization and fixation rates in the soil.[1] Chemical and biochemical degradation processes compete with sorption processes in which the active ingredients or the metabolites are sorptively attached, complexed or chemically incorporated at binding sites of the soil organic matter or clay minerals.[2] These bound pesticide residues partly defy chemical analysis because they can no longer be extracted from soil by conventional extraction

*Goltix (70% w.p.), trademark, Bayer AG Leverkusen, FRG
†Tribunil (70% w.p.), trademark, Bayer AG Leverkusen, FRG

methods. The commission of pesticides chemistry of the International Union of Pure and Applied Chemistry (IUPAC) defined non-extractable pesticide residues as follows:[3,4]

"Non-extractable residues (sometimes referred to as 'bound' or 'non-extracted' residues) in plants and soils are defined as chemical species originating from pesticides, used according to good agricultural practice, that are unextracted by methods which do not significantly change the chemical nature of these residues. These non-extractable residues are considered to exclude fragments recycled through metabolic pathways leading to natural products."

The quantification of these bound residues in soil requires detailed studies with $^{14}C$-labelled active ingredients. The experiments are usually carried out in the laboratory under standardized conditions using Erlenmeyer flasks or micro-ecosystems.[5,6] Although the results thus obtained are able to describe the influence of individual parameters such as temperature, moisture and soil type on the formation of bound residues, data from such experiments cannot be transferred to the real field situation without reservation.[7,8] Since it has been demonstrated that lysimeter experiments can simulate pesticide behaviour in soil and plant under field conditions,[9,10] the application of $^{14}C$-labelled active ingredients also allows detailed studies on the formation of bound residues under conditions of normal agricultural practices. To evaluate the magnitude of the fixation processes which lead to soil-bound residues, laboratory, greenhouse and lysimeter experiments were carried out as a comparative study employing two $^{14}C$-labelled herbicides.



## MATERIALS AND METHODS

For all experiments, an alfisol-type clayey loess soil was used representing a valuable arable soil widely distributed in the Federal Republic of Germany. The composition of the soil was as follows:

| Total C | Sand | % | Silt | Clay | $pH_{(KCl)}$ |
|---|---|---|---|---|---|
| 1.1 | 5.5 | | 84.6 | 9.9 | 7.1 |

The following experimental units were used:

- Lysimeters with 0.5 $m^2$ surface area containing an undisturbed soil profile 80 or 110 cm in depth either set up overground or embedded into the ground within a control plot of 25 $m^2$ under outdoor conditions.[11]
- Kick-Brauckmann vessels with a surface area of 360 $cm^2$ containing 8 kg of dry soil sieved $<5$ mm and watered daily up to 50–60% of the maximum water holding capacity (WHC = 42%) of the soil in the greenhouse.[12]
- Microecosystems[13] containing 1.5 kg of dry soil and watered daily to 50–60% water holding capacity in the laboratory (22 °C). The soil-root area is aerated with $CO_2$-free air and mineralized $^{14}CO_2$ as well as $CO_2$ is trapped in 1 N NaOH.

**Table 1** Experimental units and applied amounts of active ingredients (a.i.) and radioactivity

| *Experiment* | *Active ingredient* | *g a.i./ha* | *kBq/mg a.i.* | *Replications* |
|---|---|---|---|---|
| Lysimeter | Metamitron | 3500 | 92 | 2 |
| | | 7000 | 46 | 2 |
| | Methabenzthiazuron | 1400 | 266 | 2 |
| | | 2800 | 133 | 2 |
| Kick-Brauckmann vessel | Metamitron | 3500 | 68 | 4 |
| | | 7000 | 34 | 4 |
| | Methabenzthiazuron | 1400 | 296 | 4 |
| | | 2800 | 149 | 4 |
| Microecosystems | Metamitron | 3500 | 20 | 4 |
| | | 7000 | 10 | 4 |
| Erlenmeyer flasks Laboratory | Metamitron | 3500 | 460 | 4 |
| | | 7000 | 230 | 4 |
| | Methabenzthiazuron | 1400 | 1770 | 4 |
| | | 2800 | 870 | 4 |
| Erlenmeyer flasks Outdoor | Metamitron | 3500 | 460 | 4 |
| | | 7000 | 230 | 4 |

- Erlenmeyer flasks without plants containing 100 g of dry soil and held at 50% WHC in the laboratory (22 °C) or in an additional experiment under outdoor conditions embedded into the surface soil.

A preemergence spraying of (3-$^{14}$C)-metamitron (4-amino-3-methyl-phenyl-1,2,4-triazine 5-(4H)-one) to sugar beets was carried out in lysimeters and Kick-Brauckmann vessels in May 1983. The (3-$^{14}$C)-metamitron was mixed in the soil in the Erlenmeyer flasks and the microecosystems.

In April 1984 (carbonyl-$^{14}$C)-methabenzthiazuron (1,3-dimethyl-3-(2-benzthiazolyl)-urea) was sprayed post emergence onto winter wheat in lysimeters and Kick-Brauckmann vessels. In addition a laboratory experiment using Erlenmeyer flasks was started. The radioactivity was mixed into the soil or applied on the soil surface using a pipette.

The amounts of active ingredients applied corresponded to agricultural practice and are given in Table 1 together with the specific radioactivity used. The amounts for the laboratory experiments were calculated on the basis of a specific soil weight of 1.3 g/cm$^3$.

Soil samples were taken from the lysimeter soils and the soils in the Kick-Brauckmann vessels using a soil borer (2.1 cm i.d.). The samples were sectioned every 10 cm of depth, homogenized by 30 min shaking, and sieved (< 2 mm). The whole soil of the experiments with microecosystems and Erlenmeyer flasks was used for the investigations.

Portions (100 g) of metamitron-treated soil were extracted by shaking first 1 h with 250 ml acetone/water (4:1, v/v), then with 250 ml of ethylacetate, and at last with 250 ml of chloroform, respectively. To detect the active ingredient and the main metabolite desamino metamitron, the solutions were combined, and after the water had been removed, the organic fraction was evaporated to dryness, redissolved in 5 ml of diethylether, methylated and examined by gas chromatogra-

phy (Hewlett Packard 5840 A) using a N-FID and a glass column (2.2 mm i.d.) filled with 8% OV 61 on Chromosorb W 17 W/DMCS. The injection port temperature was 320 °C and the column temperature was 250 °C

In addition, extraction studies were carried out by shaking 10 g of dry soil 5 times with 50 ml of 0.01 M $CaCl_2$ solution for 12 h each.

Portions (100 g) of methabenzthiazuron-treated soil were extracted by first 5 h shaking with 200 ml of acetone, 15 h with 250 ml of ethylacetate and finally 1 h with 250 ml of chloroform, respectively. To determine the residues of the active ingredient together with the main metabolite (1-methyl-1-(2-benzthiazolyl)-urea), the solutions were combined, and after the water had been removed, the organic fraction was evaporated to dryness, redissolved in 6 ml of chloroform, and cleaned up by elution with 200 ml of chloroform in glass columns filled with inactivated Florisil (8% $H_2O$, 60–100 mesh). The eluate was evaporated, redissolved in 2 to 4 ml of acetone, and examined by gas chromatography using an N-FID and a glass column (2.2 mm i.d.) filled with 1.5% OV 101/0.5% Versamid 900 on Gaschrom Q 80/100. The injection port temperature was 265 °C and the column temperature was 190 °C.

For both methods described the smallest detectable concentration was 0.01 mg/kg.

Radioactivity in the solutions was detected using an LSC (Packard Tec. 460C) with automatic quench correction. To evaluate the radioactivity in soil, samples were oxidized in an incinerator (Packard Tec. 306) after extraction, and the $^{14}CO_2$ contained in a mixture of Permafluor and Carbosorb (12:9) was determined as described above.



## RESULTS

$^{14}C$-studies of the soils showed no statistical differences concerning the applied amounts of active ingredients. Therefore, the following results are mean values of the replications and amounts of herbicides.

*Metamitron.* Different losses of radioactivity occurred in the experiments. The remaining $^{14}C$-activities in the soil of the different experimental units and dates of sampling are given in Figure 1. Most of the remaining radioactivity in the lysimeter and Kick-Brauckmann experiments was still detected in the 0–10 cm soil layer (Figure 1). The detectable $^{14}C$ in the 20–30 cm soil layer of the lysimeter experiments was 1% of the applied amount 20 weeks after application.

Results after the extraction of the 0–10 cm soil layer from the lysimeter and Kick-Brauckmann vessels and from soil of the laboratory experiments are given in Figure 2 together with the results from the extraction studies using 0.01 M $CaCl_2$ carried out in parallel. There were no differences between both types of extraction of the soil specimen. After 8 weeks of application, only the amount of non-extractable $^{14}C$ in the soil of the microecosystems differed statistically from the other experimental units.

Significant differences occurred between the lysimeter experiment and the experiments with Erlenmeyer flasks 13 weeks after application. No significant differences occurred comparing the results of the 2 extraction methods used for the

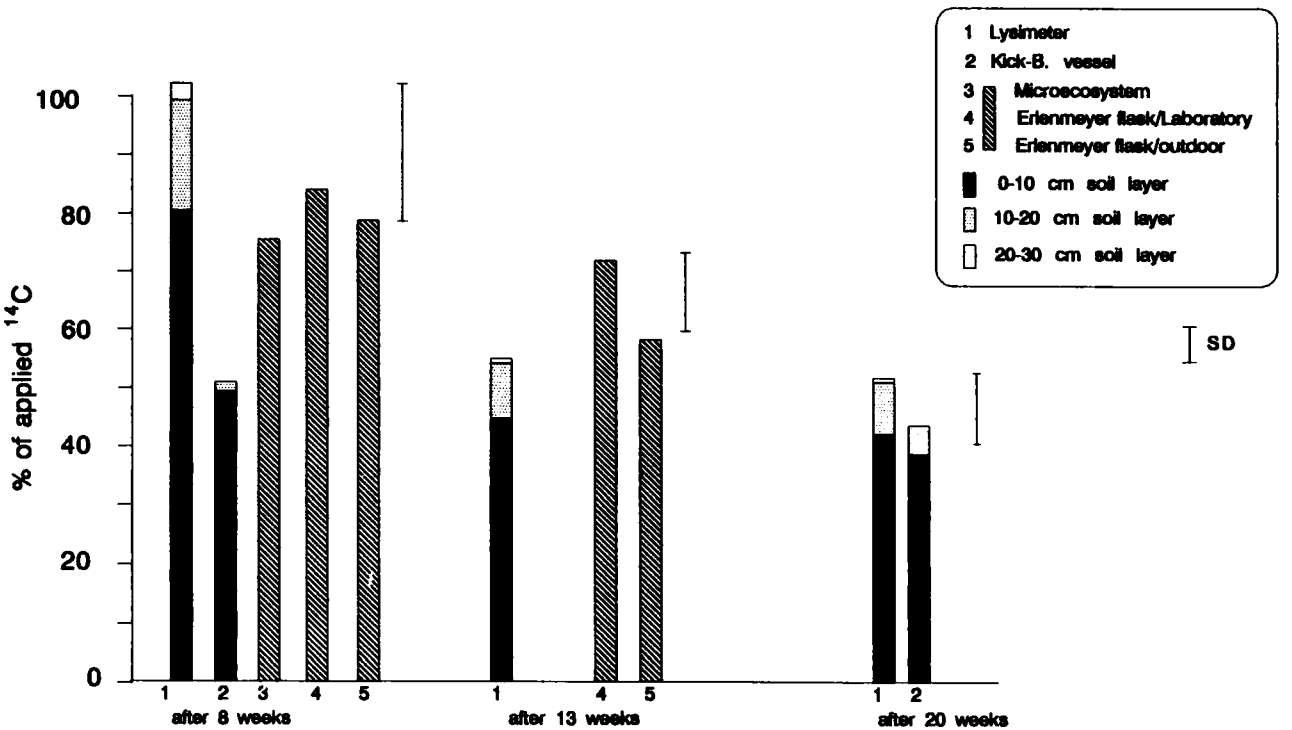


**Figure 1** Experiments with (3-$^{14}$C) Metamitron. Remaining radioactivity in the soil of all experimental units.

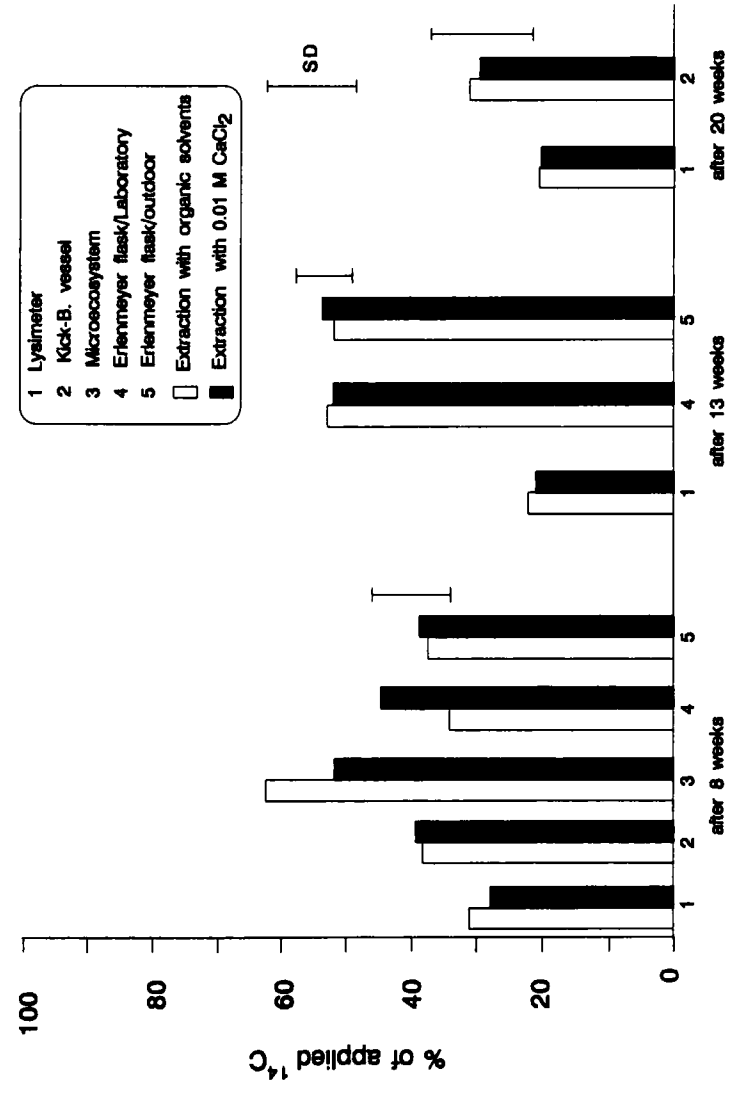


**Figure 2** Experiments with (3-$^{14}$C) Metamitron. Remaining radioactivity in the soil of all experimental units after extraction or desorption.

lysimeter soils after 13 and 20 weeks. At these times, 18 to 25% of the applied $^{14}C$ were extractable while 8 weeks after application this amount was significantly higher (Figure 2).

*Methabenzthiazuron.* In the case of winter wheat harvest, after 16 weeks of application, about half of the applied $^{14}C$ was still detectable in the soil of the lysimeter and Kick-Brauckmann vessels, most of it still located in the 0–10 cm soil layer. Only a small amount of about 1% of the applied $^{14}C$ was translocated into the 20–30 cm soil layer in the 16-week period.

However, 80% remained in the soil of the Erlenmeyer flasks when the radioactivity was mixed into the soil while no loss of the applied radioactivity was observed after application on the surface of the soil using a pipette (Figure 3).

The amounts of non-extracted radioactivity differed statistically between the outdoor lysimeter soil and the soil from the Erlenmeyer experiments (Figure 4). In the lysimeter soils 25% of the applied $^{14}C$ remained non-extractable while up to 40% remained in the soils of experiments with Erlenmeyer flasks under laboratory conditions.

## DISCUSSION

Apart from the physico-chemical behaviour of the active ingredient and the type of soil, the changing climatic conditions, the influence of plant roots and the type of application have a great effect on degradation processes and the formation of bound residues.[8] These parameters led to different amounts of non-extracted radioactivity in the soil of the outdoor and laboratory experiments (Figures 1, 3). The losses of $^{14}C$ in all experiments were due to mineralization processes leading to the formation of $^{14}CO_2$.[9,14] The two different extraction studies with metamitron-treated soils led to similar amounts of non-extractable radioactivity. Metamitron is degraded rapidly to desamino-metamitron and converted to further unstable metabolites in soil resulting in the mineralization of the molecule.[9] Only 8 weeks after application in all experiments metamitron and the major metabolite desamino-metamitron were identified by gas chromatography. The detectable residues were 6 to 9% of the applied metamitron in the lysimeter experiments compared to 21% in the standardized degradation experiments using Erlenmeyer flasks.[9]

In the case of methabenzthiazuron, half of the remaining radioactivity represented extractable active ingredient and the main metabolite (1-methyl-1-(2-benzthiazolyl)-urea).[9]

The most important result is the difference in non-extracted radioactive compounds remaining in the soil of the lysimeter study compared to the situation in the soil of the Erlenmeyer flasks. Significantly less $^{14}C$ always remained unextractable in the lysimeter soil (Figures 2, 4) although the content of humic substances, the main binding sites for both herbicides,[9] was the same in all experiments (1.9%). The results reflect the disadvantages of the experiments under standardized conditions:

- A closer ratio of roots to soil (microecosystems) or the lack of plant roots

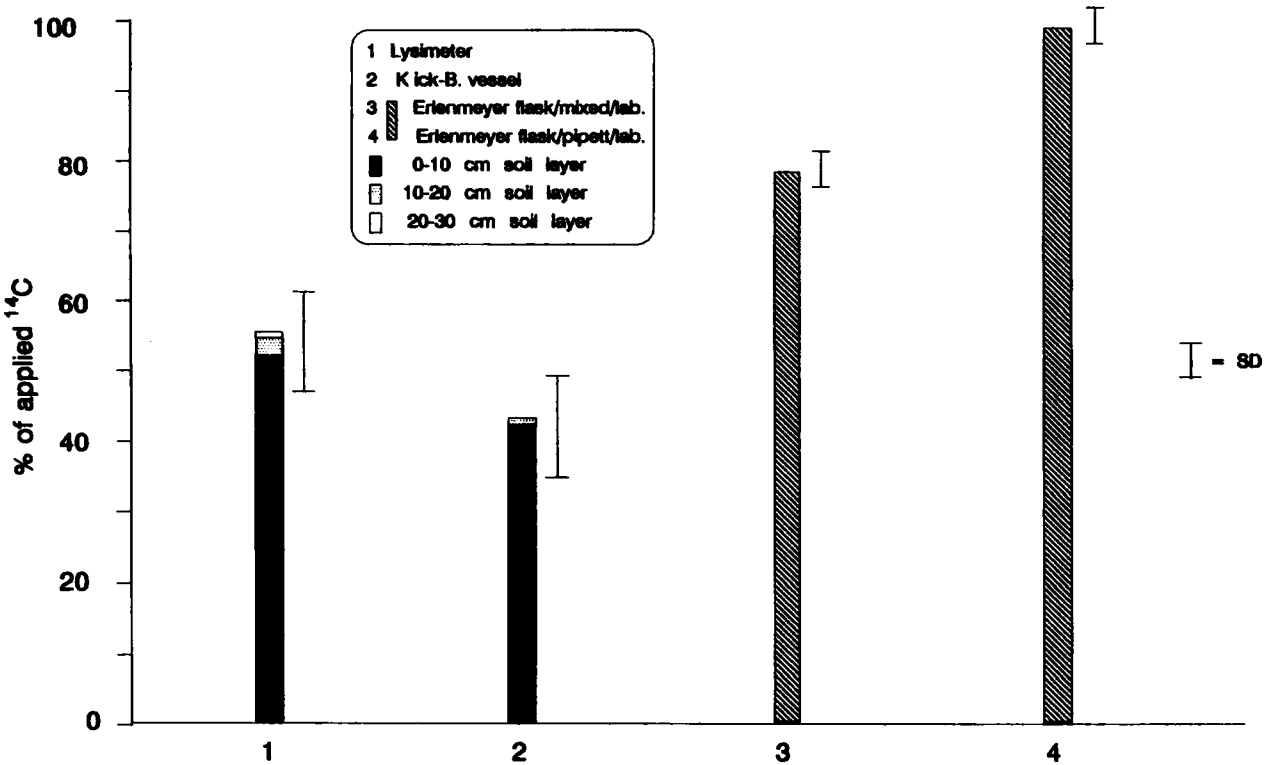


**Figure 3** Experiments with (carbonyl-$^{14}$C) Methabenzthiazuron. Remaining radioactivity in the soil of all experimental units, 16 weeks after application.

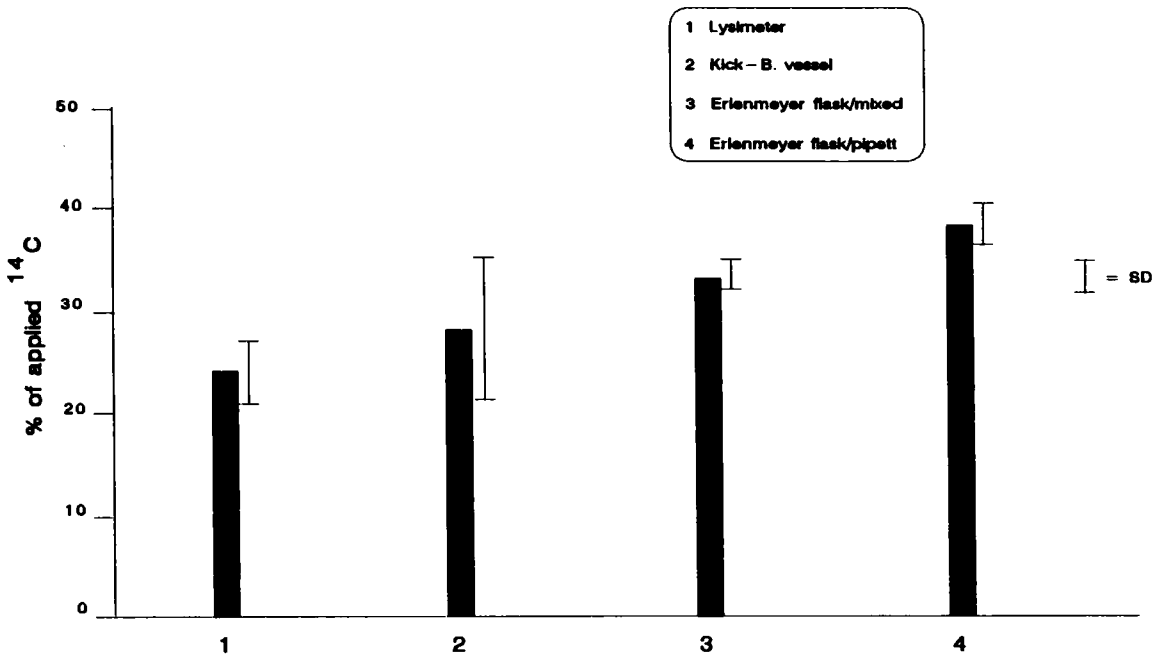


**Figure 4** Experiments with (carbonyl-$^{14}$C) Methabenzthiazuron. Remaining radioactivity in the soild of all experimental units after extraction.

(Erlenmeyer flasks) combined with standardized experimental conditions led to energetic situations for micro-organisms which were completely different from outdoor experiments. Soil biomass and its biological activity are the most important factors for degradation and fixation processes in soil.[15]

- Precipitation in the lysimeter experiments led to a distribution of the radioactive compounds in the 0–10 cm soil layer. Changing temperatures and cultivated plants influence degradation and sorption processes. For example, the changing temperatures in the outdoor experiment with metamitron-treated soil in Erlenmeyer flasks led to similar amounts of residual $^{14}C$ compared with the lysimeter results 13 weeks after application (Figure 1) and the closer soil root relationship in the Kick-Brauckmann vessels resulted in faster degradation processes 8 weeks after the application of metamitron.[9] (Figure 1).
- The experimental duration and the opportunities for sampling were limited, and long-term studies—especially important for bound residue studies—were not possible.

The data indicate that small-scale laboratory experiments do not reflect the real field situation, especially if the formation of bound residues is a major object of research. Realistic data however can be expected from lysimeter experiments employing $^{14}C$-labelled pesticides. The degradation and translocation in soil as well as the uptake by plants can be investigated over several years.[16] Apart from the quantification of unextracted $^{14}C$-compounds further attempts were made to identify the remaining radioactivity in soil.[17,18] It was found that for both herbicides, up to 60% of the non-extracted $^{14}C$-activity was associated with the humin fraction and not extractable with 0.1 M $Na_4P_2O_7$.



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