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*Original Research*

# Carbendazim Residues in the Soil and Their Bioavailability to Plants in Four Successive Harvests

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**Abstract**

In this work, long-term outdoor lysimeter investigations using $^{14}$C-labeled carbendazim (MBC) were carried out. At the start of the experiment a single application of MBC into the soil was performed, then barley as a test plant was sowed in four vegetative seasons. The disappearance of MBC in the soil comprised two phases. In the first phase, disappearance of extractable and the formation of bound residues occurred simultaneously. In the second phase, further degradation of both kinds of residues was observed. Approximately 33% of the applied radiocarbon was retained in the top soil layer, even four years after application (mostly 'soil-bound'). The residues taken up by plants depended mainly on the level of extractable MBC in the soil, but the residues in plants were detected as extractable and bound as well. In the barley harvested in the first growing season, the residues were the highest and were present in all parts of the plant. After the fourth season only barley roots were contaminated with bound residues.

**Keywords:** carbendazim, soil, extractable residues, bound residues, bioavailability

## Introduction

One of the unintended and unfavorable effects of pesticide use is soil contamination. Regardless of coverage of the soil surface with plants and the treatment techniques used, a certain amount of the applied pesticide reaches the soil. The concentration of the chemical remaining on the soil surface gradually decreases due to vaporization into the atmosphere, while light-sensitive compounds can also decompose under exposure to the sunlight. In the soil environment xenobiotics interact simultaneously with various biotic (enzymes, bacteria, fungi, microfauna) and abiotic factors (clay minerals, hydroxides, oxides, noncrystalline components, organic matter), which may be involved in the same transformative reactions [1]. These two kinds of interactions have a significant impact on the processes of pesticide degradation and mineralization in the soil. But numerous studies have shown that regardless of the factors causing decomposition of an active ingredient, a considerable amount of the parent compound and/or products of its metabolism remain in the soil structure in the form of either 'free' or 'bound' residues. It is estimated that between 20-70% of applied pesticides or their degradation products are retained in the soil matrix and therefore are not extracted from soil by water and organic solvents [2-4]. As a potential environmental hazard, this persistent remainder has

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become the subject of much research. Discussion regarding biological availability of pesticide residues remaining in the soil, carry-over into subsequent crops, leaching to groundwater, and potential long-term effects on soil quality is still open [5-8]. The behavior of crop protection chemicals in the soil is usually studied under laboratory conditions using radio-labeled compounds. However, the results obtained under controlled conditions do not always reflect the actual situation in arable fields. Investigating the fate of pesticides in field lysimeters with undisturbed soil monolith is more transferable to natural field conditions.

In the present study, tests were carried out with carbendazim (methyl benzimidazole-2-ylcarbamate, MBC), a systemic fungicide used to control a wide range of pathogens of cereals, fruits, and vegetables. Carbendazim is also the main degradation product of two other compounds: benomyl and thiophanate methyl. For this reason, benomyl and thiophanate methyl tolerances are generally expressed as MBC tolerance. These fungicides are either applied directly into the soil or sprayed over the crop. The pesticide that does not reach the target contributes to soil pollution. Because of possible health effects, the widespread use of MBC created the need for development of reliable test methods for assessing the side-effects of MBC residues. To our knowledge, there are only a few papers published concerning the fate of radiolabeled MBC in soil [9] and plants [10], but none describes such long-term studies.

Therefore, the objectives of the present study were:

(1) determination of the disappearance dynamics of extractable MBC residues and the formation of bound residues in the soil within four growing seasons, and
(2) determination of the bioavailability of MBC residues from the soil using barley as a test plant.

## Experimental Procedures

The behaviour of MBC fungicide in the soil/plant system was investigated in lysimeters with a surface area of 0.8 $m^2$ and a depth of 2.0 m. Physiochemical properties of the soil were as follows: organic matter 3.74%; sand 63.00%; silt 21.00%; clay 16.00%; WHC 34.00g/100g; pH 6.20. The labeled $^{14}C$ – MBC ($^{14}C$ located at carbon 2 in the benzimidazole ring, specific radioactivity 2.5 MBq/mg) and 'cool' compound in total amount of 420 mg were dissolved in a water/methanol mixture. The contribution of the $^{14}C$-labeled compound to the total amount was 13.1 mg. The treatment solution was applied on the soil surface by spraying and then mixed uniformly to a depth of 0-15 cm. Spring barley (cultivar Diva) was sown each year. Sowing density complied with the standards used in Polish agricultural production. A lysimeter with soil of the same characteristic parameters, but without MBC fungicide treatment, was used as control. The disappearance of extractable MBC and the formation of bound residues in soil layer of 0-15 cm, and the contamination of mature plants were determined. At harvest, the whole plants were collected. The aboveground parts of barley were separated from roots and divided into seeds, ears without seeds, and straw. The roots were carefully separated from the soil by washing on a sieve with tap water. After 24 h of drying at 60°C the plant material was ground, passed through a 20-mesh screen (Arthur H. Thomas Co., Scientific Apparatus, US), and analyzed for extractable and bound radioactivity. Soil samples taken after specific periods and dried plant samples were exhaustively extracted with methanol using a Soxhlet apparatus. The bound $^{14}C$ residues were analyzed by combustion of post-extracted materials in a Biological Oxidizer OX-500 (RJHarvey, US), using liquid scintillator Oxysolve C-400 (Zinsser Analytic, Germany) for absorption and counting of $^{14}CO_2$. The radioactivity of liquid samples (methanol extracts and trap-solutions for $^{14}CO_2$) were analyzed by liquid scintillation counting on a LS 5000TD (Beckman, US). The standard deviation (SD) for the radioactivity extracted with methanol ranged between 1.0-3.4%, bound to soil 0.8-3.9%. The SD for extractable $^{14}C$ in plant material ranged from 0.7-1.6%, and 0.2-0.8% for bound residues. The concentrated methanol extracts from the soil and plant samples were also analyzed by TLC. The extracts were applied on 0.25 mm silica gel precoated plates (Pierce Chemical Co., US) then developed in chloroform/acetone/methanol (7:2:1). The radioactive spots were localized by scanning on a LB 2723 radioscanner (Berthold, Germany). For further confirmation of identity of MBC several methanol extracts of silica gel zones were analyzed by HPLC (Milton Roy with computer MP3000E, US) using a $C_{18}$ reverse-phase column, and UV detection at 280 nm.

## Results and Discussion

The changes of residue levels in the soil within four vegetative seasons are presented in Fig. 1. In the first growth season the rapid disappearance of extractable residues and formation of bound MBC was observed. The total initial concentration of MBC in the treated soil, sampled to a depth of 15 cm, was about 4.2 mg/kg (3.84 mg/kg extractable and 0.36 mg/kg bound). After 6 weeks the levels of bound and extractable residues were about equal (1.81 mg/kg). During the following 3 months the extractable residues were reduced to 0.47 mg/kg, whereas the bound MBC residues increased to 2.75 mg/kg. Plant uptake of MBC residues from the soil are summarized in Table 1.

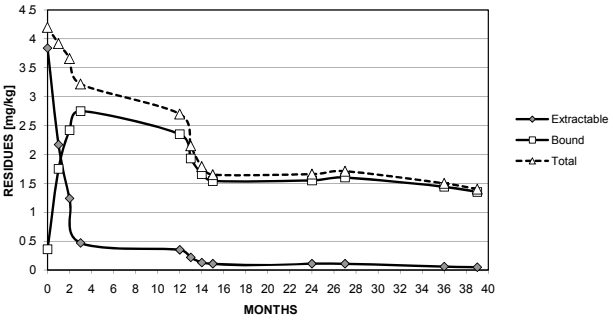


Fig. 1. Disappearance dynamic of MBC residues in the soil during four growing seasons.

Table 1. Extractable and bound MBC residues in mature barley plants.

| Part of plant | | Residues [mg/kg] | | | |
|---|---|---|---|---|---|
| | | in the 1st season | in the 2nd season | in the 3rd season | in the 4th season |
| Seeds | extractable | 0.19 | 0.28 | < 0.01 | < 0.01 |
| | bound | 0.26 | 0.07 | 0.14 | < 0.01 |
| Ears | extractable | 0.11 | 0.13 | < 0.01 | < 0.01 |
| | bound | 0.24 | 0.20 | 0.12 | < 0.01 |
| Straw | extractable | 0.25 | 0.15 | < 0.01 | < 0.01 |
| | bound | 0.98 | 0.17 | 0.09 | < 0.01 |
| Roots | extractable | 1.30 | 0.17 | 0.04 | < 0.01 |
| | bound | 7.65 | 0.71 | 0.35 | 0.37 |

The highest residue levels in harvested barley were found after the first growing season. All parts of plants contained $^{14}C$, mostly in the form of bound residues. The roots accumulated about 80% of the total uptaken residues. Total radioactivity of roots, expressed as parent compound, was 8.95 mg/kg. In parts of plants above the soil surface the residues were significantly lower. In the straw, ears after threshing, and seeds the levels of residues were 1.23 mg/kg, 0.35 mg/kg, and 0.45 mg/kg, respectively. The transport of MBC from water solution to wheat grain was studied by Michel and Buszewski using unlabeled compound [11]. The results of both studies are a good illustration of systemic action of MBC fungicide and its transport within the plant.

During the second season, the disappearance of both forms of MBC residues in the soil was observed. The bound residues were especially reduced from 2.35 to 1.25 mg/kg. Simultaneously, the level of extractable MBC decreased from 0.35 to 0.11 mg/kg. Radioactive residues taken up by plants were also lower, but both extractable and bound $^{14}C$ was still detected. The contamination of straw, ears and seeds with $^{14}C$ residues was similar, and corresponded to MBC residues ranging between 0.32-0.35 mg/kg. Total radioactivity found in the barley roots was greater and approximately equal to 0.88 mg/kg.

The concentrations of extractable and bound residues in the soil reached at the end of the second season remained almost unchanged until the end of the experiment.

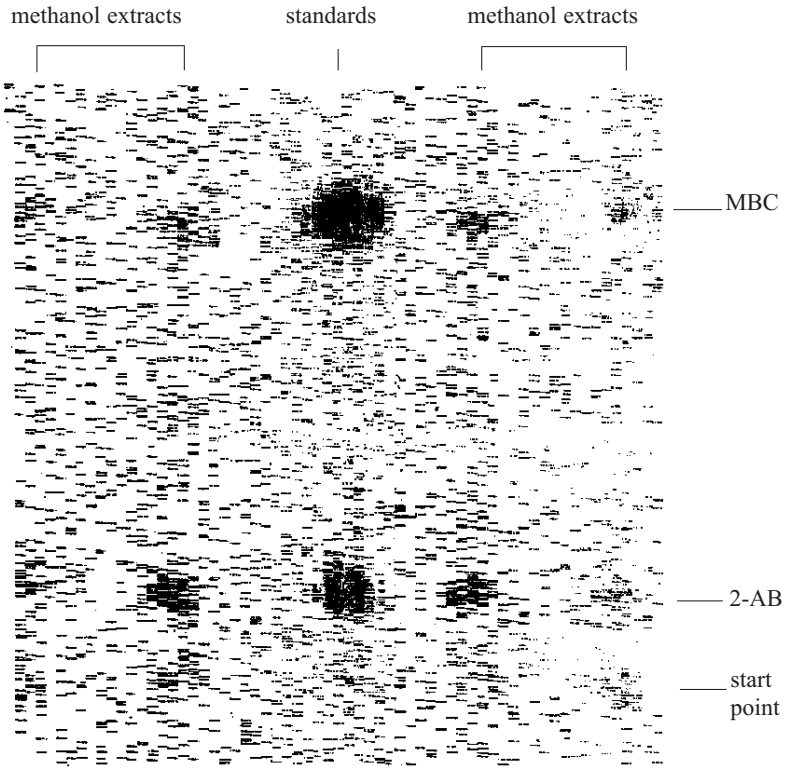


Fig. 2. Radioscanning of TLC plates after analysis of the methanol extracts of soil samples.

In the third season, in the above-ground parts of harvested barley, only small amounts of bound $^{14}C$ residues were determined, which expressed as parent MBC ranged between 0.09-0.14 mg/kg. The radioactivity of the extractable residues was under the detection limit, i.e. < 0.01 mg/kg. These were the only roots to be contaminated with both forms of $^{14}C$ residues, and the bound radioactivity was 10-fold times higher than the extractable. Total radioactivity accumulated in the roots corresponded to 0.39 mg/kg of MBC. In plants cultivated in the 4$^{th}$ season after MBC treatment only a small amount of bound residues was detected in the roots (0.37 mg/kg), whereas straw, ears, and seeds were free from residues. It should be noted that in plants, bound $^{14}C$ residues dominated over extractable. These results are in agreement with the previous findings by Barak et al. [10] and Kumar et al. [12]. In their works, the tendency of systemic fungicides to bind to plant tissues has indicated that lignin, a constituent of the xylem, is the main component binding pesticides in the plants.

Generally, it appears that the levels of MBC residues taken up by barley mainly depend on the levels of extractable residues present in the soil. Similar observations were made for other xenobiotics. Führ and Mittelstaedt [13], for instance, determined the soil-bound residues of [$^{14}C$]methabenzthiazuron to maize plants and the uptake from soil containing both bound and extractable residues, and the uptake from soil freshly treated with the radioactive compound as well. The proportion of the respective uptakes was 1:3:5. In the studies of Fuhremann and Lichtenstein [14] the ratio of the uptake from soil containing only bound residues of [$^{14}C$]parathion to the uptake from freshly treated soil was 1:5. The same relationship was determined by Helling and Krivonak [15] in a work with six dinitroaniline herbicides. Also, similar results were obtained by Dec et al. [16] in laboratory investigations with cyprodinil fungicide. These observations clearly indicated that the rate of plant uptake greatly depended on the level of extractable residues. The data obtained in MBC experiment are in agreement with the aforementioned findings, suggesting that extractable residues are more easily taken up by plants than the soil-bound.

In the TLC analysis of the methanol extracts from the soil, three radioactive spots were detected, at $R_f$ 0.05, 0.16, and 0.80 (Fig. 2). According to co-chromatography with known $^{14}C$ standards, the major spot at $R_f$ 0.80 represented unchanged MBC, and the small radioactivity at $R_f$ 0.16 appeared to be its metabolite, 2-aminobenzimidazole (2-AB). In the extracts from plant material only MBC was found. The presence of intact MBC in the extracts was confirmed by HPLC, while the metabolite 2-AB was not detected. At the end of the experiment, after four growth seasons, total radioactivity remaining in the soil amounted to 33% of the applied $^{14}C$. The obtained results suggest that the MBC residues in soil are susceptible to long-term persistence. The contribution of extractable $^{14}C$ to total radioactivity of the soil was evaluated to be only 1%.

Binding of pesticide residues and other xenobiotics is a common phenomenon in soil. As already mentioned, the competition of various soil factors affects the formation of bound residues, giving chemical and physical bonds of various strengths. Also, inclusion of residues within humus aggregates is possible due to steric conditions [2, 7, 17]. A frequently asked question is whether bound organic compounds are permanently 'locked' in the soil matrix, or if bound residues serve as a pool of toxicity that may be released in the future [3, 18-22].

From an environmental point of view the presence of MBC residues in agricultural soils may be undesirable for many reasons. Field studies have reported MBC to cause significant reduction in earthworm populations and decreased cocoon production [23-29]. Also, the soil bacterial community is affected by MBC [30]. The chemical structure of the MBC molecule favors adsorption processes in the soil matrix. It is a known fact that benzimidazoles possess an imidazole ring containing both acidic and basic nitrogen atoms. Under suitable conditions, the molecule may be protonated ($pK_a$ ~5–6) or deprotonated ($pK_a$ ~12) [31]. MBC strongly adsorbs to most soil types and is moderately to highly persistent, especially in soils with high organic content and low pH [23, 32, 33]. The acidity increases the adsorption of carbendazim to soil particles and thereby hinders its degradation and leaching. On the other hand, under field conditions pH equilibrium is disturbed by continuous modification of the soil environment by fertilization, crop rotation, and other agronomic practices. It could be expected that previously immobilized MBC residues may be released into soil solution liming of the soil, thus making the residues more bioavailable and/or mobile. Bioavailability to plants and soil-inhabiting organisms of pesticides retained in soil is probably a major source to the food chain and an important route of exposure to humans and animals. Some recalcitrant pesticides are considered non-toxic at the concentrations found in the environment, but they can reach hazardous levels when they become biomagnified in natural food chains [34-36].

## Conclusions

This study has provided a unique opportunity to observe the levels of extractable and bound residues in the soil in correlation with contamination of successive plant growths over several years. Most of the fungicide residues were retained in plant roots, whereas small amounts were carried to the above-ground parts. This study also indicates that MBC is relatively persistent in the soil environment and that accumulation of its bound residues in the soil is possible by repeated fungicide application. Therefore, the tendency of MBC to accumulate in acid soils and its possible lime-induced release should not to be overlooked.

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