# Bioavailability of the fungicides carbendazim and iprodione in soil, alone and in mixtures

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ABSTRACT

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Measures to protect soil ecosystems require research data on the effects of toxic chemicals on soil organisms. In the co-operative MIXTOX project, the effect of mixtures of toxic chemicals on some animal species in soil is studied. Mutual interactions in the behaviour of toxic chemicals in soil may be expected to affect the exposure of the organisms to the mixtures. The fungicides carbendazim and iprodione compete in the adsorption on soils, which increases their bioavailability in mixtures. The transformation of the fungicides is slowed-down in mixtures, which also increases their bioavailability. The exposure of soil animals to the fungicides in soil solution is much higher in the mixtures than when a fungicide alone is present in soil. The inclusion of copper in the mixture has little additional effect on the bioavailability of the fungicides in pore water. Chemical analyses in the framework of the dose-response experiments of the research partners serve as a check on the dose and on unexpected losses of the fungicides.

Keywords: adsorption, chemical analysis, combination, copper, degradation, exposure, interaction, pesticides, pore water, soil quality, toxic chemicals, transformation.

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# Contents

# Preface

This report presents procedures and results of Alterra Research Project No 87708 titled: 'Bioavailability of toxicant mixtures to soil organisms (MIXTOX)'. The studies were carried out in the framework of the co-operative MIXTOX project: 'Conceptualizing the effect assessment of toxicant mixtures to soil organisms (MIXTOX)'. The co-operative project was funded by the EC in the framework of the EC R&D Environment and Climate Programme-II, Contract ENV4-CT97-0507. The project leader of the whole MIXTOX project was Dr Ir J.E. Kammenga of Wageningen University.

Supplementary funding of the Alterra contribution to MIXTOX was provided by the Dutch Ministry of Agriculture, Nature Management and Fisheries, in the framework of Research Program No 359 'Pesticides and the Environment'.

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The research was carried out in the period October 1997 to October 2000. Work plans and progress of the research were discussed at the MIXTOX project meetings in Wageningen (August 1997), Lund (August 1998), Amsterdam (August 1999) and Wageningen (February 2000). The Alterra project consisted of three main parts: a) method development for experiments and analyses, b) own laboratory experiments and c) chemical analyses in the framework of the dose-response experiments of the research partners. Two fungicides were selected for the studies: carbendazim and iprodione; besides copper was included in the mixtures. The three soils used in the studies were: Speyer 2.2, Lincolnshire and Droevendaal. Annual progress reports were prepared at the end of each of the three years of the project.

## Summary

Present-day human life entails the large-scale use of various chemicals. Sooner or later large fractions of many of these chemicals reach the soil, where they may have adverse effects on essential soil functions. These functions include the production of crops, the support of natural vegetation and food chains, the recycling of nutrients, and the purification of percolating water (e.g. used for drinking water supply). Healthy soils contain dense populations of many species of soil organisms, which should be protected from undue damage by the chemicals. Tests and procedures have been developed to measure and evaluate the effects of single chemicals in soil systems. However, often various chemicals are present in the soil at the same time and it is not known how these interact in behaviour and effects.

The MIXTOX project was set up to study the effects of mixtures of toxic chemicals on soil organisms, as compared to the effects of the chemicals alone. Mixtures of chemicals in soil may be expected to interact in chemical behaviour and thus in the exposure of the soil organisms. When evaluating the dose-response relationship of mixtures, it is essential to know how the exposure of the soil organisms to each chemical is influenced by the presence of the other compounds in the mixture. The present part of the MIXTOX project concentrates on the interactions in the behaviour of chemicals in soil and on the resultant interactions in the exposure of the soil organisms to the chemicals via pore water.

Two widely-used fungicides were selected for the present study: carbendazim and iprodione. Further, copper was involved in the mixtures. Physico-chemical data needed for understanding the behaviour of the fungicides in soils were collected from the literature. Both sets of data on fungicide-soil interactions show a wide range of variation, so specific measurements for the fungicide-soil combinations are needed for quantitative studies.

The adsorption of carbendazim and iprodione on three soils, alone and in combinations, was measured by batch equilibration. The chemicals were analysed by liquid chromatography (HPLC). The adsorption at three concentration levels could be described by the Freundlich equation, with rather low Freundlich exponents. The adsorption of the fungicides on the Speyer 2.2 and Droevendaal soils is significantly higher than that on the Lincolnshire soil, which is related to the higher organic matter contents of the former two soils. The adsorption of carbendazim was reduced significantly by the presence of iprodione. The reduction of iprodione sorption by the presence of carbendazim was even stronger. Copper did not affect the adsorption of carbendazim, but it increased the adsorption of iprodione somewhat.

Carbendazim and iprodione were incubated in the three soils at 20 $^{o}$C to measure their rate of transformation, alone and in combinations. In most cases, the course of the transformation in time could be described reasonably well by first-order kinetics, but in some other cases the rate coefficient decreased with time in a later stage. The

rate of transformation of carbendazim in the Speyer 2.2, Lincolnshire and Droevendaal soils was of the same order of magnitude (half-lives 107 to 141 days). The presence of iprodione and copper in the Speyer soil decreased the rate of transformation of carbendazim only to a low extent.

The rate of transformation of iprodione in the Speyer 2.2 soil at 20 °C (half-life of 167 days) was distinctly lower than that in the Lincolnshire and Droevendaal soils (half-lives of 37 and 38 days). The presence of carbendazim and copper substantially decreased the rate of transformation of iprodione in the Speyer 2.2 soil.

The bioavailability of the fungicides in soil solution was measured by centrifugation of pore water after different times of incubation in the soil. When carbendazim alone was incubated in the soil, its concentration decreased continuously to low values at the end the two-month period. In the presence of iprodione, the decrease of carbendazim in solution proceeded more slowly, especially after the first two weeks. The effect of iprodione can be explained partly from its retardation of the transformation of carbendazim. After the first two weeks there was no clear shift in the soil/water distribution ratio for carbendazim. Copper had little additional effect on the decrease of carbendazim in soil solution.

The concentration of iprodione in pore water, when applied alone, decreased gradually to low values after two months. In the presence of carbendazim, the concentration of iprodione decreased in the first two weeks, but after that there was hardly any further decrease. The presence of carbendazim distinctly retarded the transformation of iprodione in soil. There was no clear shift in the soil/water distribution ratio for iprodione after the first two weeks. The presence of copper had no distinct additional effect on the concentration of iprodione.

Concentrations of the fungicides in soil solution were measured while their contents in soil ranged up to the high levels needed in some dose-response studies. At the high contents, the concentrations in solution approached the solubility of the fungicides in water, as expected. Application of the fungicides in powder form slowed down their release to the soil solution, as compared to that after application in acetone solution.

Various series of analyses for the fungicides in the dose-response experiments of the research partners were performed to check the initial content at various levels and to check the extent of transformation in the course of the studies. On the basis of the details on the procedures in each of the dose-response experiments, the results of these analyses can be evaluated in more detail. The fungicide measurements play an important part in the interpretation of the dose-response experiments with the various soil animals. The results on the interactions between the fungicides with respect to their adsorption, transformation and bioavailability in soil have to be translated to the divergent conditions in the various dose-response studies.

# 1 Introduction

Soil ecosystems have to be protected against the adverse effects of toxic chemicals produced by mankind and then distributed in the environment. Such chemicals may disturb the functions of the soil with respect to the production of food and raw materials, the support of natural vegetation and food chains, the recycling of the nutrients, the purification of the percolating water, etc. Healthy soils have dense populations of a diversity of soil organisms, which should be protected for maintaining the soil functions. The protection of soil ecosystems against pollution is a main target in the policy of the European Union as a whole, of its Member States and of various other countries.

In the framework of soil protection regulations, the effects of toxic chemicals on soil organisms and soil processes are being studied. The dose-response tests and evaluation procedures deal with the effect of individual compounds on the soil organisms. For example, progress has been made in developing tests and procedures to be used in the regulation of agricultural pesticides.

In practice, various toxic chemicals may occur in soil at the same position and time. For example, various pesticides are applied during the growth of a crop. In agricultural soils, metals like copper and zink occur on a large scale. They may have been distributed as a constituent of pesticides or they may have other sources (e.g. manure or compost). The toxic effects of combinations of chemicals on soil organisms have hardly been studied. It is not known how the risk of combinations of chemicals should be evaluated, as compared to the risk of the individual compounds. As a consequence, there is no policy yet to protect the soil ecosystems against contamination by combinations of chemicals.

The MIXTOX project has been set up to study the effects of mixtures of toxic chemicals on soil organisms. The principal aim is to develop general concepts for the evaluation of the effects of combinations of chemicals on the organisms, as compared to the effects of the chemicals alone. The following toxic substances were selected for the whole project: a) the fungicides carbendazim and iprodione, and b) the metals copper, zinc, cadmium and lead. The test organisms in the project were species of springtails, earthworms, potworms and nematodes.

In ecotoxicological research it is essential to study the actual exposure of the organisms to the chemicals in soil. Due to the abundant mass of soil, processes like adsorption, transformation and spatial distribution in soil play a dominant part. Various soil organisms are mainly exposed to the toxic chemicals in soil solution (pore water). Besides, other pathways of exposure may be relevant, e.g. those via food and solid phase. Progress has been made in the measurement and evaluation of the exposure of soil organisms to single chemicals.

Each of the toxic chemicals in a combination may affect the behaviour of one or more of the other chemicals. Possible examples deal with interactions in the adsorption and transformation of the chemicals in soil. The chemicals may compete for the adsorption sites and mechanisms, thus reducing the adsorption of each of them. One substance may inhibit the micro-organisms responsible for the transformation of another substance in the combination, thus increasing its persistence in soil. In combinations of organic chemicals and metals, processes like complexation may occur. Such interactions may have a large effect on the exposure of soil organisms to the chemicals, as compared to the exposure to the chemicals alone.

The aim of the present contribution to the co-operative MIXTOX project is to study the interactions in the behaviour of some chemicals (carbendazim, iprodione, copper) which occur simultaneously in soil. Firstly the mutual effects on adsorption and transformation of the chemicals in soils are studied. Secondly, mutual effects on the bioavailability of the chemicals in soil solution are measured. Finally, attention is paid to other factors and processes which may affect the exposure of soil animals, first of all in the dose-response experiments of the research partners.

In Chapter 2, the physico-chemical properties of the fungicides carbendazim and iprodione, relevant for their behaviour in soil systems, are presented. Results of measurements on the adsorption of the fungicides to soils, both when present alone and in combinations, are given in Chapter 3. The rate of transformation of the fungicides was measured (Chapter 4) when incubated a) alone, b) in combination with the other fungicide and c) in combination with the other fungicide plus copper. The course in time of the bioavailability of the fungicides in soil solution, when incubated alone and in combinations, is described in Chapter 5. The effect of the limited solubility of the fungicides on their concentration in solution at comparatively high contents in soil is described (Chapter 6). Several chemical analyses were carried out in the framework of the dose-response experiments for soil animals by the research partners (Chapter 7).

# 2 Physico-chemical properties of the fungicides

## 2.1 Carbendazim

The benzimidazole compound carbendazim is a systemic fungicide used for the protection of many agricultural crops from fungus diseases. Carbendazim controls the pathogenic fungi by inhibiting the development of germ tubes, the formation of appressoria and the growth of mycelia. The chemical name (IUPAC) of carbendazim is methyl benzimidazol-2-ylcarbamate.

The vapour pressure of carbendazim is reported to be 0.09 mPa at 20 $^{o}$C (Tomlin, 1997). However, other sources give lower values. (CTB, 2000) states the vapour pressure to be < 0.15 mPa (at 25 $^{o}$C). Presumably, carbendazim can be classified to be moderately volatile.

At comparatively low pH-values, the carbendazim molecule becomes protonated: the $pK_a$-value is 4.2 (Tomlin, 1997). As a consequence, various properties of carbendazim (weak base) are pH-dependent.

The solubility of carbendazim in water at 24 $^{o}$C (Tomlin, 1997) is 29 $\mu g/cm^3$ (at pH 4) and 8 $\mu g/cm^3$ (at pH 7). The compound can be classified as slightly soluble in water at neutral pH-values.

As the Henry coefficient for the partitioning of carbendazim between air and water is calculated to be 1.0 $10^{-6}$ or lower, its volatilization from wet surfaces is expected to be slow.

The values reported (Tomlin, 1997) for the octanol/water partitioning coefficient $K_{ow}$ are 24 (pH 5) and 32 (pH 7).

The adsorption of carbendazim on two silty loam soils was measured by Süss and Pritzl (1977). The Freundlich coefficients were 7 and 23 $cm^3/g$, respectively. The values of the coefficient $K_{om}$ for the adsorption on soil organic matter were calculated to be 170 and 340 $cm^3/g$, respectively. The comparatively high adsorption by the second soil may be related to its low pH value of 4.9, which caused partial protonation of carbendazim.

Dios Cancela et al. (1992) measured the adsorption of carbendazim on a series of eight soils. The Freundlich adsorption coefficient ranged from 2.0 to 21.6 $cm^3/g$. The extent of adsorption was closely correlated with soil organic matter content: this factor explained 85% of the variation. The correlation of adsorption with clay content of the soil was weaker (explained 72% of the variation). The average value of the coefficient $K_{om}$ for the adsorption of carbendazim on soil organic matter was calculated to be 230 $cm^3/g$ (n = 8; s.d. = 77 $cm^3/g$).

The adsorption of carbendazim on four soils was measured and the $K_{om}$-values were calculated (CTB, 2000; no details). The average $K_{om}$ was 145 $cm^3/g$ and its range was 127 to 156 $cm^3/g$.

Carbendazim is only very slowly hydrolysed in water buffered at pH-values of 5 and 7; its half-life (22 °C) is more than a year (Tomlin, 1997; CTB, 2000). Photolysis of carbendazim in aqueous solution exposed to sunlight is only slow (CTB, 2000).

The rate of transformation of carbendazim in four moist soils at 27 °C was measured by Yarden et al. (1985, 1987). Translated to 20 °C, their results correspond to an average half-life of 28.4 days (n = 4; s.d. = 6.6 days). Carbendazim in soil is mainly transformed by microbial activity (Yarden et al., 1985). Soils that had been treated previously with carbendazim showed highly accelerated transformation of the fungicide (Yarden et al., 1987).

The DT50 (50%-transformation time) of carbendazim in 16 soils at 20 °C (different studies) showed the wide range of 11 tot 302 days; the average DT50-value was 94 days (CTB, 2000; no details).

2-Aminobenzimidazole has been found as a minor metabolite in soil (Tomlin, 1997; CTB, 2000).

## 2.2 Iprodione

The systemic fungicide iprodione belongs to the group of dicarboximide compounds. It is used for the protection of many food and ornamental crops against fungus diseases. Iprodione inhibits germination of spores and growth of fungal mycelia. The chemical name (IUPAC) of iprodione is 3-(3,5-dichlorophenyl)-N-isopropyl-2,4-dioxoimidazolidine-1-carboxamide.

The vapour pressure of iprodione is reported to be 5 $10^{-4}$ mPa at 25 °C (Tomlin, 1997). Iprodione can be classified as slightly volatile.

The solubility of iprodione in water (20 °C) is 13 $\mu g/cm^3$ (van de Plassche and Linders, 1989; Tomlin, 1997). Thus the compound can be classified as moderately soluble.

The octanol/water partitioning coefficient $K_{ow}$ of iprodione (at 22 °C) is 1260 (van de Plassche and Linders, 1989). Another reported value (Tomlin, 1997) for $K_{ow}$ is 1000 (at pH 3 and pH 5).

The adsorption of iprodione on soil could be estimated from leaching studies with soil columns. The adsorption coefficient $K_{om}$ was estimated to be on average 281 $cm^3/g$, with a range from 164 to 361 $cm^3/g$ (van de Plassche and Linders, 1989).

The coefficient for the adsorption of iprodione on soil organic carbon $K_{oc}$ is reported to be 373 to 1551 $cm^3/g$ (Tomlin, 1997; no details); this corresponds to a range of $K_{om}$-values of 213 to 884 $cm^3/g$.

The half-life of iprodione hydrolysis in buffered water at 25 °C was >35 days (pH 3), 20 days (pH 6) and < 1 day (pH 9) (van de Plassche and Linders, 1989).

The rate of transformation of iprodione in some soils showed a wide range; the average half-life (at 20 °C) was estimated to be 41 days (van de Plassche and Linders, 1989). Tomlin (1997) gives a range of 50%-transformation times (DT50-values) in soils in the laboratory of 20 to 80 days (no details). Various transformation products are formed from iprodione.

Iprodione was found to be susceptible to accelerated transformated after repeated application to the soil. Walker et al. (1986) found a progressive increase in the rate of transformation of iprodione with successive treatments of the soil with the fungicide.

The transformation of iprodione in soils with comparatively low pH (< 5.5) tends to be slower than that in soils with higher pH (Walker et al., 1986). Further, the induction of accelerated microbial transformation in a soil with pH 5 was slow.

# 3 Adsorption of the fungicides on soils, alone and in combinations

## 3.1 Introduction

The adsorption of the fungicides carbendazim and iprodione on three soils has been measured. The soils were indicated by Speyer 2.2, Lincolnshire and Droevendaal. Adsorption of the fungicides on the Speyer soil has been measured for:
a) the fungicides individually,
b) the combination carbendazim + iprodione,
c) the combination carbendazim + iprodione + copper.

The adsorption of the fungicides individually on the Lincolnshire and Droevendaal soils was measured for comparison with their individual adsorption on the Speyer soil.

## 3.2 Procedures

### 3.2.1 Sorption experiment

A mass of 40 g moist soil was weighed into glass centrifuge tubes (90 $cm^3$). The initial moisture content of the Speyer 2.2 soil was 14.0%, that of the Lincolnshire soil was 13.2% and that of the Droevendaal soil was 21.5%. A volume of 40 $cm^3$ of fungicide solution in water (with 0.01 mol $CaCl_2$ per $dm^3$ and max. 3.8% acetone) was added to each of the tubes. The tubes were sealed and clipped on a disk at an angle of 1.4 rad, which was slowly rotated at 20 rotations per minute (r.p.m) for 24 hours. After this equilibration (at 20 $^oC$), the tubes were centrifuged at 2000 r.p.m. for 20 min (also at 20 $^oC$). A subsample of the water layer was collected for chemical analysis.

The adsorption of each of the fungicides alone on the Speyer 2.2 soil was measured in triplicate at each of three concentration levels. The adsorption of the fungicides alone on the Lincolnshire and Droevendaal soils was measured in triplicate at the highest concentration level. The adsorption of the fungicides in the combination carbendazim + iprodione and in the combination carbendazim + iprodione + $CuCl_2$ on the Speyer soil was also measured in triplicate at the highest concentration level. The masses of chemical applied to the tubes correspond to 12.1 $\mu g/g$ (carbendazim), 10.7 $\mu g/g$ (iprodione) and 4.4 $\mu g/g$ ($Cu^{2+}$) on dry soil basis.

The pH may have an effect on the adsorption, especially on that of the weak base carbendazim. After equilibration, the pH in the water layer was measured to be pH = 6.6 (n = 24; s = 0.2) for the Speyer 2.2 soil and pH = 6.3 (n = 6; s = 0.3 ) for the Droevendaal soil.

### 3.2.2 Properties of the soils

Information on the composition of the soils and their pH-value is given in Table 3.1. The composition of the Speyer and Droevendaal soils was measured by the Laboratory for Soil and Crop Testing in Oosterbeek, The Netherlands. The new values for the Speyer soil are close to the values given earlier by LUFA Speyer. The original data on the Lincolnshire soil, as measured in the UK, are included. The batch of Lincolnshire soil, as received, was quite heterogeneous.

*Table 3.1 Properties of the three soils used in the adsorption and transformation experiments*

| Soil property | Soil name | | |
|---|---|---|---|
| | Speyer 2.2 | Lincolnshire | Droevendaal |
| Clay (< 2 μm; %) | 6.4 | 6.3 | 3.1 |
| Silt (2 to 50 μm; %) | 18.0 | 47.3 | 8.0 |
| Textural class | Loamy sand | Sandy loam | Sand |
| Organic matter (%) | 4.2 | 1.4 | 2.7 |
| $CaCO_3$ (%) | 0.2 | 8.2 | 0.1 |
| Value of pH(KCl) | 5.7 | 6.2 | 5.6 |

### 3.2.3 Chemical analysis

The fungicides in the water layer after sorption-equilibration were analyzed by HPLC. The conditions were:

| Compound: | carbendazim | iprodione |
|---|---|---|
| Separation column: | Waters Novapak C18 | Waters Novapak C18 |
| Oven temperature (°C): | 40 | 40 |
| Mobile phase (v/v): | acetonitrile/water (30/70) | acetonitrile/water (60/40) |
| Flow rate ($cm^3$/min): | 0.7 | 1.0 |
| UV detection at wavelength (nm): | 285 | 210 |

### 3.2.4 Adsorption isotherm and equations

The adsorption isotherm may be linear or the adsorption is considered at one concentration level only. Then the pesticide distribution coefficient $K_d$ is used:

$$X = K_d \, c_L \qquad \text{(Eq. 3.1)}$$

with $X$ = content of pesticide adsorbed on soil, μg/g;
$K_d$ = distribution coefficient, $cm^3$/g;
$c_L$ = concentration in the liquid phase, μg/$cm^3$.

Usually, the adsorption isotherm is curved: the distribution coefficient decreases as the concentration in the liquid phase increases. Then the Freundlich adsorption equation can often be used. The following type of Freundlich equation is used in this study:

$$X = K_f \, c_{L,r} \, (c_L / c_{L,r})^n \qquad \text{(Eq. 3.2)}$$

with: $K_f$ = Freundlich adsorption coefficient, $cm^3/g$;
$c_{L,r}$ = reference concentration in the liquid phase, $\mu g/cm^3$;
n = Freundlich exponent, -

The value for the reference concentration is taken to be 1 $\mu g/cm^3$ in this study. The value of the exponent n depends on the pesticide-soil combination; its value is usually somewhat below 1.0. If n = 1.0, the adsorption isotherm is linear and $K_f$ equals $K_d$.

The Freundlich coefficients were calculated by linear regression for the data points ln(X) versus $\ln(c_L)$, using the Genstat 5 (Release 3.2) software package.

The measured organic matter contents allow calculation of the adsorption coefficient $K_{om}$ ($cm^3/g$) based on organic matter:

$$K_{om} = K_d / omc \qquad \text{(Eq. 3.3)}$$

with: omc = organic matter content, g/g.

## 3.3 Results

### 3.3.1 Fungicides individually

The adsorption of carbendazim and iprodione alone on the Speyer 2.2 soil was measured at three concentration levels. The isotherms were curved, so the Freundlich equation was used to describe the results. The Freundlich plot for carbendazim is shown in Figure 3.1. The corresponding plot for iprodione is given in Figure 3.2.

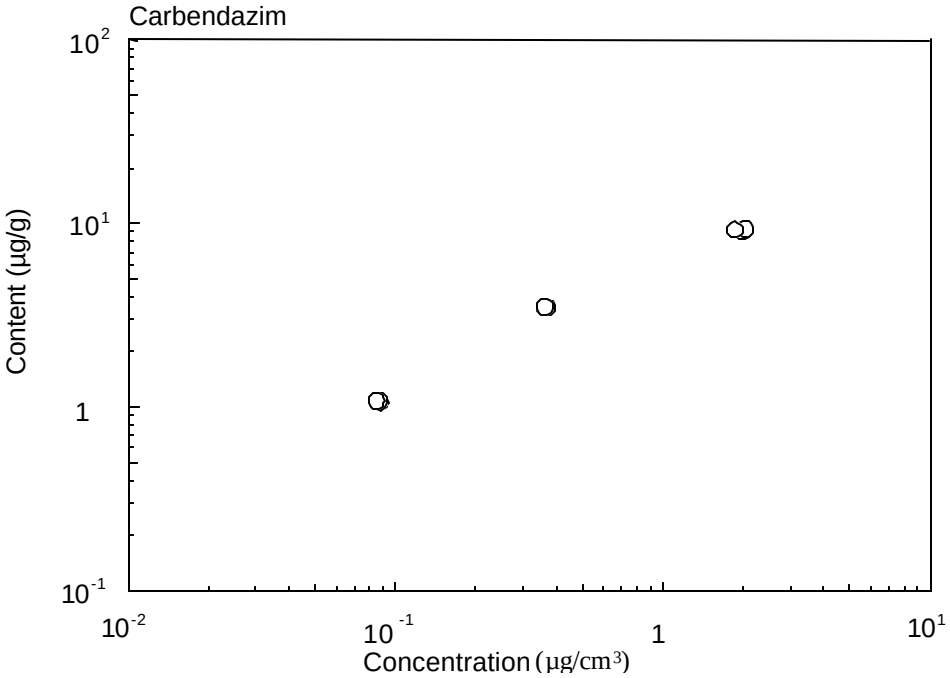


*Figure 3.1. Freundlich plot of the adsorption of carbendazim to Speyer soil at three levels.*

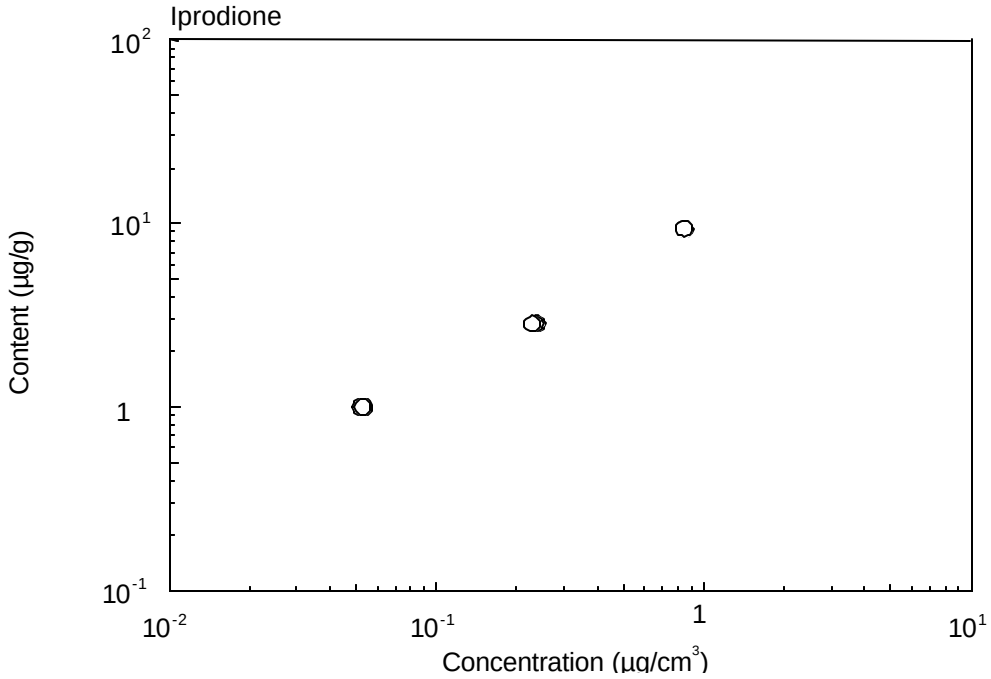


*Figure 3.2. Freundlich plot of the adsorption of iprodione to Speyer soil at three levels.*

The Freundlich coefficients obtained by regression are given in Table 3.2. The percentages of variance accounted for by the regression were 98.8% and 99.3%, respectively.

*Table 3.2 Freundlich coefficients for the adsorption of carbendazim and iprodione, as single compounds, to the Speyer 2.2 soil*

| Compound | Coefficient $K_f$ ($cm^3/g$) | Exponent n |
|---|---|---|
| Carbendazim | 6.14 | 0.69 |
| Iprodione | 10.07 | 0.80 |

The higher value of $K_f$ for iprodione shows that its adsorption to the soil is stronger than that of carbendazim. The adsorption isotherm for carbendazim is most strongly curved, as is shown by the comparatively low value of Freundlich exponent n.

The adsorption of carbendazim and iprodione alone on the Lincolnshire and Droevendaal soils was measured only at the highest concentration level (in triplicate). The resulting average values of $K_d$ are shown in Table 3.3. For comparison, the average values of $K_d$ for the adsorption of the fungicides on the Speyer 2.2 soil at the highest concentration level are included.

*Table 3.3 Coefficients $K_d$ for the adsorption of carbendazim and iprodione alone, at the highest concentration level, to the Speyer 2.2, Lincolnshire and Droevendaal soils. Figures with different letters are significantly different according to Student's t-test at $\alpha = 0.05$*

| Compound | Soil | Equilibrium concentration ($\mu g/cm^3$) | Coefficient $K_d$ ($cm^3/g$) |
|---|---|---|---|
| Carbendazim | Speyer 2.2 | 1.97 | 4.69a |
| | Lincolnshire | 3.23 | 2.30b |
| | Droevendaal | 2.03 | 4.64a |
| Iprodione | Speyer 2.2 | 0.85 | 11.0c |
| | Lincolnshire | 1.43 | 6.14d |
| | Droevendaal | 0.98 | 10.0c |

The adsorption of carbendazim to the Speyer 2.2 and Droevendaal soils is significantly stronger than its adsorption on the Lincolnshire soil. The same holds for the differences in adsorption of iprodione on the soils.

### 3.3.2 Carbendazim plus iprodione

The adsorption of carbendazim and iprodione, when applied in combination to the Speyer 2.2 soil, was measured at the highest concentration level (in triplicate). The results are shown in Table 3.4. The adsorption of carbendazim was reduced significantly by the presence of iprodione: to about 70% of its adsorption as single compound. The reduction of the adsorption of iprodione by the presence of carbendazim was even stronger: to about 30% of the adsorption of iprodione as single compound.

*Table 3.4 Coefficient $K_d$ for the adsorption of carbendazim and iprodione to Speyer 2.2 soil, when equilibrated alone and in combinations. Figures with different letters are significantly different according to Student's t-test at $\alpha = 0.05$*

| Compound | Combination | Equilibrium concentration ($\mu g/cm^3$) | Coefficient $K_d$ ($cm^3/g$) |
|---|---|---|---|
| Carbendazim | Alone | 1.97 | 4.69 a |
| | Carb+ipro | 2.68 | 3.23 b |
| | Carb+ipro+Cu | 2.70 | 3.20 b |
| Iprodione | Alone | 0.85 | 11.0 c |
| | Ipro+carb | 2.34 | 3.31 d |
| | Ipro+carb+Cu | 1.54 | 5.72 e |

### 3.3.3 Carbendazim plus iprodione plus copper

The results for the adsorption of the fungicides in the presence of copper are also given in Table 3.4. Copper did not have a significant effect on the adsorption of carbendazim in the presence of iprodione. However, the adsorption of iprodione in the presence of carbendazim was increased significantly by copper. Possibly, iprodione is subject to some kind of complexation with copper.

### 3.3.4 Adsorption on the basis of soil organic matter

The coefficients $K_d$ for the adsorption of the individual fungicides to the three soils (Table 3.3) were converted to the sorption coefficients $K_{om}$ on the basis of soil organic matter. If a pesticide is mainly sorbed to soil organic matter, the $K_{om}$-value should show less variation between soils than the $K_d$-value. The $K_{om}$-values calculated for carbendazim and iprodione in the present study are given in Table 3.5.

*Table 3.5 Coefficients $K_{om}$ for the adsorption of carbendazim and iprodione on the basis of soil organic matter*

| Compound | Soil | Coefficient $K_{om}$ ($cm^3/g$) |
|---|---|---|
| Carbendazim | Speyer 2.2 | 112 |
| | Lincolnshire | 164 |
| | Droevendaal | 172 |
| Iprodione | Speyer 2.2 | 262 |
| | Lincolnshire | 439 |
| | Droevendaal | 370 |

Using the measurements on the adsorption of carbendazim to two silty loam soils by Süss and Pritzl (1977), the values of $K_{om}$ can be calculated to be 170 and 340 $cm^3/g$, respectively. The comparatively high sorption by their second soil may be related to its low pH value of 4.9, which may be expected to cause partial protonation of carbendazim. Two of the $K_{om}$ values obtained in the present study correspond to the lowest value of 170 $cm^3/g$ calculated for the first soil (pH 7.3) in the Süss and Pritzl study, whereas one of our values was lower.

The measurements of Dios Cancela et al. (1992) on the adsorption of carbendazim to eight loamy soils resulted in a range of $K_{om}$-values of 132 to 344 $cm^3/g$ (average 230 $cm^3/g$). The $K_{om}$-values obtained in the present study (Table 3.5) are in the lower part of this range or even somewhat lower.

As compared to the range of $K_{om}$-values of 121 to 156 $cm^3/g$ (average 145 $cm^3/g$) for the adsorption of carbendazim to the organic matter in four soils (CTB, 2000), one of our values (Table 3.5) was lower and two were higher.

Tomlin (1997) indicated the range of $K_{om}$-values for the adsorption of iprodione to soil organic matter to be 213 to 884 $cm^3/g$. The results for the three soils in the present study (Table 3.5) fall in the lower half of this range.

Evidently, the use of a $K_{om}$-value for a pesticide from the literature only allows to make a very rough estimate of the adsorption coefficient for a particular soil. For quantitative assessments, it is advised to measure the adsorption for the particular pesticide-soil combinations.

## 3.4 Conclusions

* The coefficient for the soil/water distribution of both fungicides is strongly dependent on the concentration level, which is described well by the Freundlich sorption equation.

* Iprodione is adsorbed more strongly to the three soils than carbendazim.

* Adsorption of the fungicides on the Lincolnshire soil (lowest organic matter content) is lower than that on the other two soils.

* The adsorption of carbendazim is somewhat reduced by the presence of iprodione.

* The adsorption of iprodione is strongly reduced by the presence of carbendazim.

* Copper does not have an effect on the adsorption of carbendazim in the presence of iprodione. However, it increases the adsorption of iprodione in the presence of carbendazim.

* The adsorption coefficient $K_{om}$ based on soil organic matter, as derived from literature data, only gives a rough indication on the adsorption of the pesticide on other soils. Consequently, quantitative assessments require adsorption measurements for the particular pesticide-soil combinations.

# 4 Rate of transformation of the fungicides in soil, alone and in combinations

## 4.1 Introduction

Carbendazim and iprodione were incubated in the Speyer 2.2 soil to measure their rate of transformation. The following incubations were carried out:
– carbendazim alone;
– iprodione alone;
– carbendazim in the presence of iprodione;
– iprodione in the presence of carbendazim;
– carbendazim in the presence of iprodione and copper;
– iprodione in the presence of carbendazim and copper.

For comparison, the rates of transformation of carbendazim and iprodione when incubated individually in the Lincolnshire and Droevendaal soils were also measured.

## 4.2 Procedures

### 4.2.1 Incubation study

Three soils were used for the incubation experiments:
– Speyer 2.2 soil, provided by the Landwirtschaftliche Untersuchungs- und ForschungsAnstalt (LUFA) Speyer, Germany;
– Lincolnshire soil, provided by the Centre for Ecology and Hydrology, Monks Wood, Huntingdon, England;
– Droevendaal soil, collected in December 1997 from an arable field at the Droevendaal experimental farm near Wageningen, The Netherlands.
Properties of the soils are given in Table 3.1 (Chapter 3).

After the soil batches were received or collected, they were stored at 15 °C for less than 2 months. The incubation study started in the beginning of February 1998. Subsamples of 39 g moist soil were weighed into large centrifuge tubes (90 $cm^3$) and they were pre-incubated at 20 °C for 6 days. The moisture contents of the soils were measured by drying other subsamples to constant mass at 105 °C; they were 14% (Speyer), 13% (Lincolnshire) and 22% (Droevendaal).

Carbendazim solution in acetone (1.5 $cm^3$; 263 $\mu g/cm^3$) was trickled with a glass syringe on the enlarged soil surface in tilted tubes. The acetone was allowed to evaporate. Then the contents of the tubes were mixed intensively. The tubes were covered with aluminium foil with a small hole and placed in a box in a constant-temperature cabinet at 20 °C. The box contained a layer of water and was covered with a lid to keep the soil moist. The initial contents of carbendazim in the soils (on

dry soil basis) were 11.5 μg/g (Speyer), 11.4 μg/g (Lincolnshire) and 12.2 μg/g (Droevendaal).

Iprodione was applied to the soil batches in acetone solution (1.0 $cm^3$; 432 $\mu g/cm^3$), using the same method as for carbendazim. The initial contents of iprodione in the soils (on dry soil basis) were 12.5 μg/g (Speyer), 12.4 μg/g (Lincolnshire) and 13.3 μg/g (Droevendaal).

In the combined incubations, carbendazim and iprodione were dosed in the same mass and in the same way as in the incubations of the compounds alone. A volume of 0.5 $cm^3$ $CuCl_2$-solution in water (1037 $\mu g/cm^3$) was applied to the Speyer soil in two series of tubes as described above. The initial content of $Cu^{2+}$-ions in the soil batches (on dry soil basis) was 7.1 μg/g.

At ten times in a period of 148 days of incubation in the Speyer soil, two tubes with soil from each incubation series were taken and stored in deep-freeze until the extraction. The duration of the incubations in the Lincolnshire and Droevendaal soils was up to 77 days (seven extraction times; duplicate or singular tubes).

### 4.2.2 Extraction and analysis

The soil stored in deep-freeze was thawn and then the 39 g moist soil in each tube was extracted by shaking for 1 hour with 50 $cm^3$ ethyl acetate. A volume of 100 $mm^3$ of the extract was transferred to an HPLC-vial and the solvent was evaporated. The drying residue was redissolved in 1 $cm^3$ HPLC-water by repeated shaking and ultrasonic vibration.

Carbendazim and iprodione were analysed by liquid chromatography (HPLC). A volume of 100 or 200 $mm^3$ was injected by an autosampler (Waters 717 plus). The mobile phase was pumped with a Perkin Elmer 410 LC pump at a flow rate of 1 $cm^3$/min. The first column (length 20 cm; inner diam. 3.9 mm) and the second column (length 15 cm; inner diam. 4.6 mm), connected in series, both contained Nova-Pak C18 (4 μm; Waters Millipore) and they were mounted in an oven at 40 °C. The compounds were detected by a UV detector (Perkin Elmer LC 90 UV), whose signal was processed with the Multichrom package (VG Data Systems). Standard solutions in the range of 0.05 to 1.00 $\mu g/cm^3$ were injected for the construction of the calibration line.

In the analysis of carbendazim, the mobile phase was acetonitrile + HPLC-water (3+7 by volume). Carbendazim was detected at a wavelength of 285 nm. Its retention time in the columns was 4 min.

The mobile phase for the analysis of iprodione was acetonitrile + HPLC water (6+4 by volume). Iprodione was detected at a wavelength of 210 nm. Its retention time was 5 min.

The recovery of carbendazim in the analytical procedure was calculated from the amounts of the fungicide measured at days 0 and 1 of the incubation, as compared to the dose. The average recovery of carbendazim was found to be 88.1% (n = 19; s.d. = 8.4%). In the same way, the recovery of iprodione from the soils was found to be on average 90.4% (n = 20; s.d. = 5.3%).

### 4.2.3 Calculations

Attempts were made to describe the transformation of the pesticides by first-order kinetics:

$$dM/dt = - k_t M \qquad \text{(Eq. 4.1)}$$

with: $M$ = mass of pesticide, µg;
$t$ = time, d;
$k_t$ = first-order rate coefficient, $d^{-1}$.

The relevant solution of this equation is:

$$M = M_o \exp(-k_t t) \qquad \text{(Eq. 4.2)}$$

with: $M_o$ = initial mass, µg.

This solution is transformed into its logarithmic form:

$$\ln(M/M_o) = - k_t t \qquad \text{(Eq. 4.3)}$$

The rate coefficient $k_t$ was calculated by linear regression of $\ln(M/M_o)$ against time t using the GENSTAT 5 statistical package (Release 3.2). The percentage of variance accounted for by the regression and the standard error of the rate coefficient are given.

The half-life $t_{1/2}$ (d) of the pesticides is calculated by:

$$t_{1/2} = (\ln 2)/k_t \qquad \text{(Eq. 4.4)}$$

## 4.3 Results

### 4.3.1 Carbendazim

The percentages of carbendazim remaining in the Speyer soil at various times of incubation are given in Figure 4.1. At some times, the duplicate measurements (almost) coincided. In the first period of about 77 days, the course of the transformation could be approximated well by first-order kinetics. Thereafter, the rate coefficient for the transformation of carbendazim seemed to decrease.

Approximating all the measurements by first-order kinetics, the first-order rate coefficient $k_t$ was calculated to be 0.00647 $d^{-1}$. The regression accounted for 89.9% of the total variance of the measuring points. The corresponding half-life of carbendazim was 107 days.

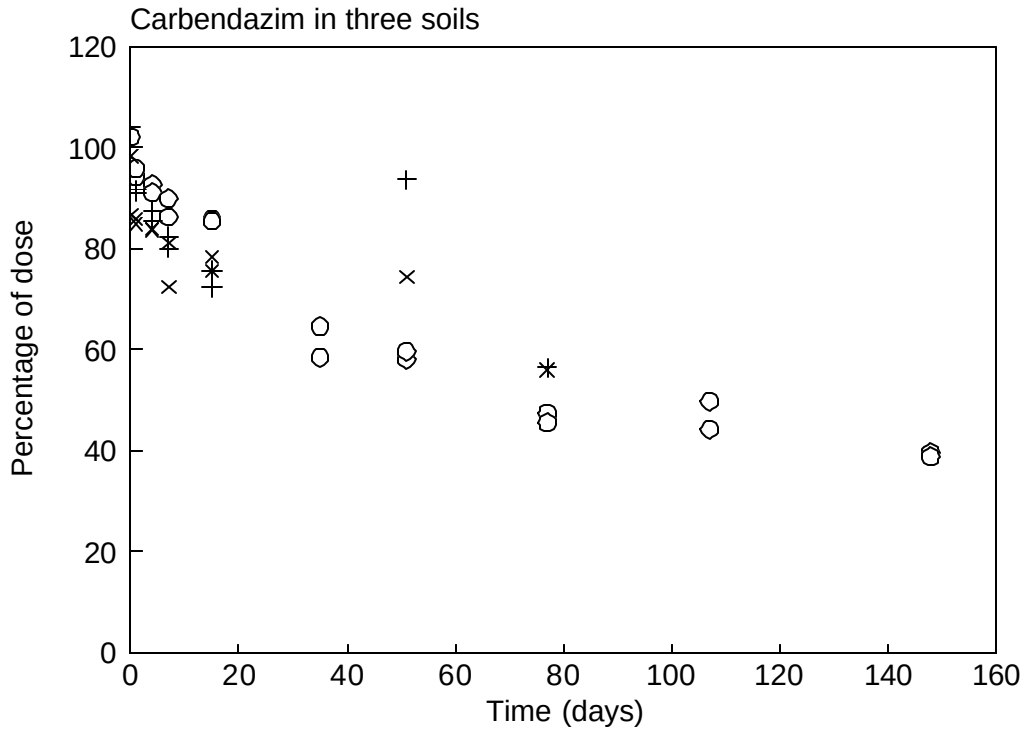


*Figure 4.1 Rate of transformation of carbendazim in the Speyer (O), Lincolnshire (+) and Droevendaal (x) soils at 20 °C*

Figure 4.1 also shows the course of the transformation of carbendazim in the Lincolnshire soil. The rate coefficient of the transformation decreased in time. At 51 days of incubation, the percentage remaining was unexpectedly high. When approximating all measurements, a first-order rate coefficient $k_t$ = 0.00645 $d^{-1}$ was calculated. The regression accounted for 77.5% of the total variance. The corresponding (extrapolated) half-life of carbendazim was 108 days.

The results of the incubation of carbendazim in the Droevendaal soil are also given in Figure 4.1. The percentages of the dose measured to remain in the initial period were mostly low, as compared to the dose. The rate coefficient of the transformation seemed to decrease in time. Description of the course of the transformation of carbendazim by first-order kinetics resulted in a rate coefficient $k_t$ of 0.00490 $d^{-1}$. The regression accounted for 71.3% of the total variance of the measuring points. The corresponding (extrapolated) half-life of carbendazim was 141 days.

The results of the incubation of carbendazim in the Speyer soil in the presence of iprodione are given in Figure 4.2. The amounts measured in the first few days were

lower than expected from the dose. After that, the course of the transformation approximately followed first-order kinetics. Using all the measuring points, the course of the transformation of carbendazim was described by the first-order rate equation, with rate coefficient $k_t$ = 0.00535 $d^{-1}$. The regression accounted for 93.4% of the total variance in the measuring points. The corresponding half-life of carbendazim was 130 days.

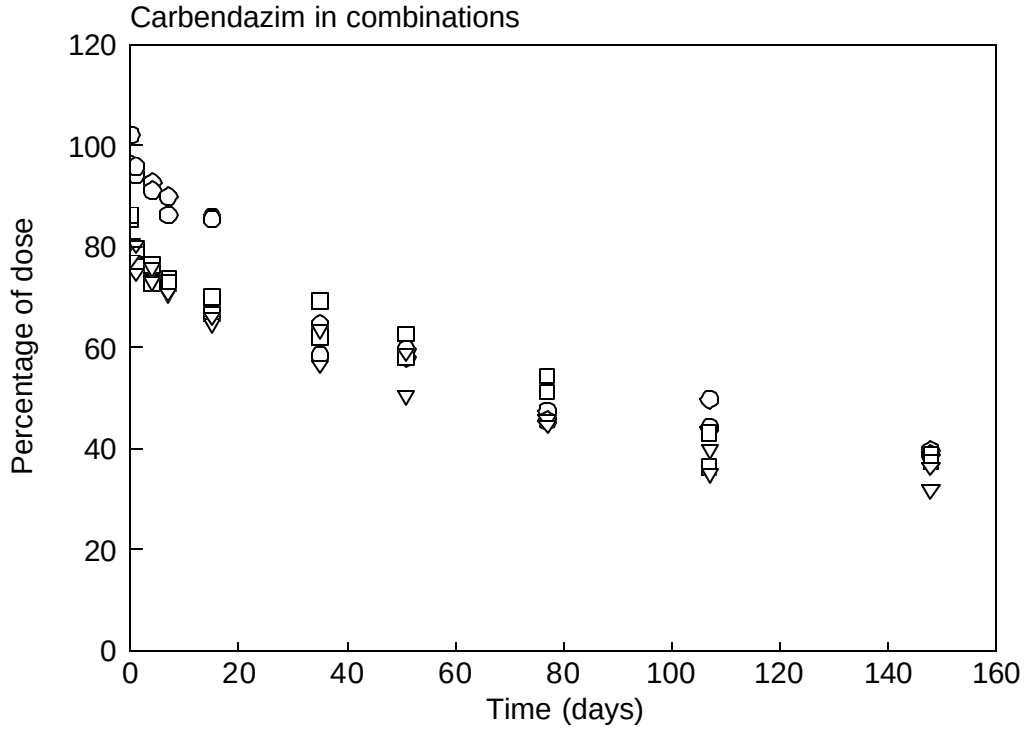


*Figure 4.2 Rate of transformation of carbendazim in the Speyer soil at 20 °C, alone (O), in combination with iprodione (□) and in combination with iprodione plus copper (Ñ)*

When carbendazim was incubated in the Speyer soil in the presence of both, iprodione and copper, results were obtained as shown also in Figure 4.2. Again, the amounts of carbendazim measured in the first few days were lower than expected from the dose. The course of the transformation in time can be approximated by first-order kinetics, with a tendency of the rate coefficient to be lower in the second half of the incubation period. Description of the transformation by first-order kinetics, using all the measuring points, resulted in a rate coefficient of $k_t$ = 0.00589 $d^{-1}$. The regression accounted for 94.9% of the total variance. The corresponding half-life of carbendazim was 118 days.

The rate coefficients and half-lives of carbendazim in the five incubation series are taken together in Table 4.1. The rate coefficients were of the same order of magnitude. There is no indication that the rate coefficient of carbendazim in the Speyer soil strongly deviates from those in the other soils. None of the three soils shows accelerated transformation of carbendazim due to microbial adaptation. There may be some slowing down of the transformation of carbendazim by the presence of

iprodione; more incubations are needed to check whether this effect is significant. Presence of the combination of iprodione and copper seemed to have little effect on the rate of transformation of carbendazim.

*Table 4.1 First-order rate coefficients and half-lives for the transformation (at 20 °C) of carbendazim in three soils, alone and in combinations*

| Soil | Additional substance | Rate coefficient ($d^{-1}$) | Standard error ( $d^{-1}$) | Half-life (d) |
|---|---|---|---|---|
| Speyer | | 0.00647 | 0.00050 | 107 |
| Lincolnshire | | 0.00645 | 0.00114 | 108 |
| Droevendaal | | 0.00490 | 0.00092 | 141 |
| Speyer | Iprodione | 0.00535 | 0.00033 | 130 |
| Speyer | Iprodione and copper | 0.00589 | 0.00031 | 118 |

### 4.3.2 Iprodione

The course of the transformation of iprodione in the Speyer soil with time, when incubated alone, is presented in Figure 4.3. At some times, the duplicate measurements (almost) coincided. The gradual transformation of iprodione could be approximated by first-order kinetics in the whole incubation period. The rate coefficient was calculated to be 0.00416 $d^{-1}$. The regression accounted for 90.4% of the total variance of the measuring points. The corresponding half-life (extrapolated) of iprodione was 167 days.

The results of the incubation of iprodione in the Lincolnshire soil are also given in Figure 4.3. The transformation rate coefficient was comparatively high in the beginning; thereafter it decreased with time. Description of iprodione transformation by first-order kinetics (for comparison) resulted in a calculated rate coefficient $k_t$ of 0.0185 $d^{-1}$. The regression accounted for 88.2% of the total variance of the measuring points. The corresponding half-life of iprodione was 37 days.

When iprodione was incubated in the Droevendaal soil, the transformation pattern was measured as shown also in Figure 4.3. In the first period, the measurements were lower than expected from the dose; further they were quite variable. The transformation of iprodione was approximated by first-order kinetics, which resulted in a rate coefficient $k_t$ of 0.0181 $d^{-1}$. The regression accounted for 84.1% of the total variance of the measuring points. The corresponding half-life of iprodione was 38 days.

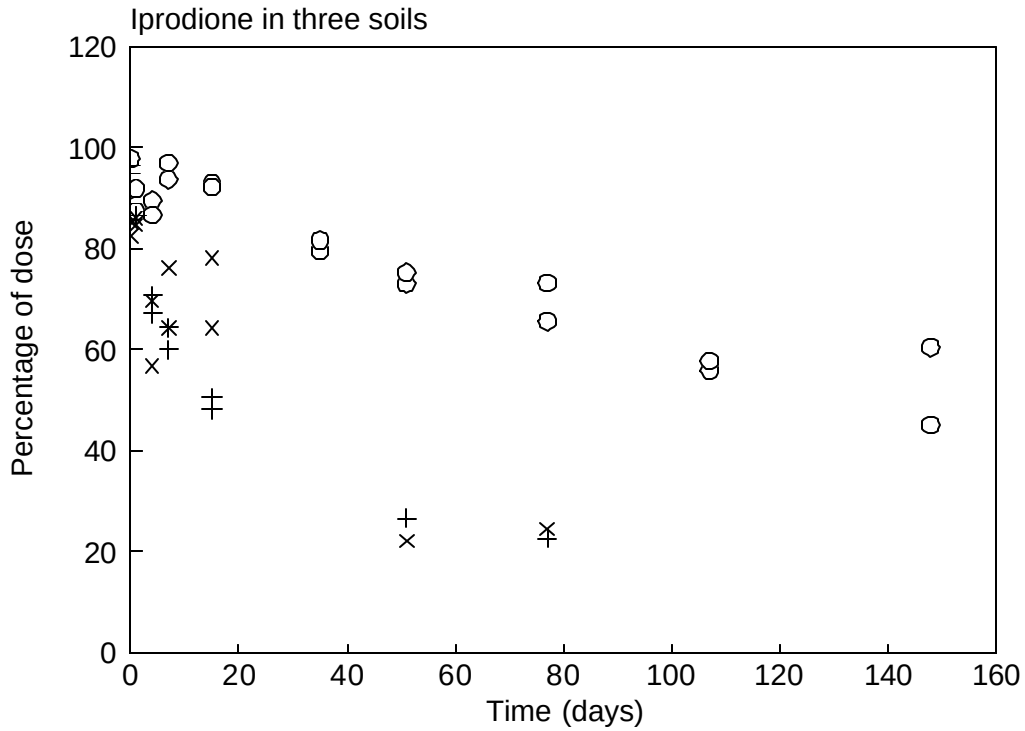


*Figure 4.3 Rate of transformation of iprodione in the Speyer (O), Lincolnshire (+) and Droevendaal (x) soils at 20 °C*

Figure 4.4 shows the course of the transformation of iprodione when incubated in the Speyer soil, in the presence of carbendazim. The transformation of iprodione proceeded only gradually. When the transformation of iprodione was approximated by first-order kinetics, the rate coefficient was calculated to be 0.00269 $d^{-1}$. The regression accounted for 81.0% of the total variance of the measuring points. The corresponding half-life (extrapolated) of iprodione was 257 days.

The results on the transformation of iprodione in the Speyer soil, when incubated together with carbendazim and copper, are also given in Figure 4.4. The transformation proceeded only gradually. The course of the transformation was approximated by first-order kinetics and the resulting rate coefficient for iprodione was calculated to be 0.00169 $d^{-1}$. The regression accounted for 53.2% of the total variance of the measuring points. The corresponding half-life (extrapolated) of iprodione was 410 days.

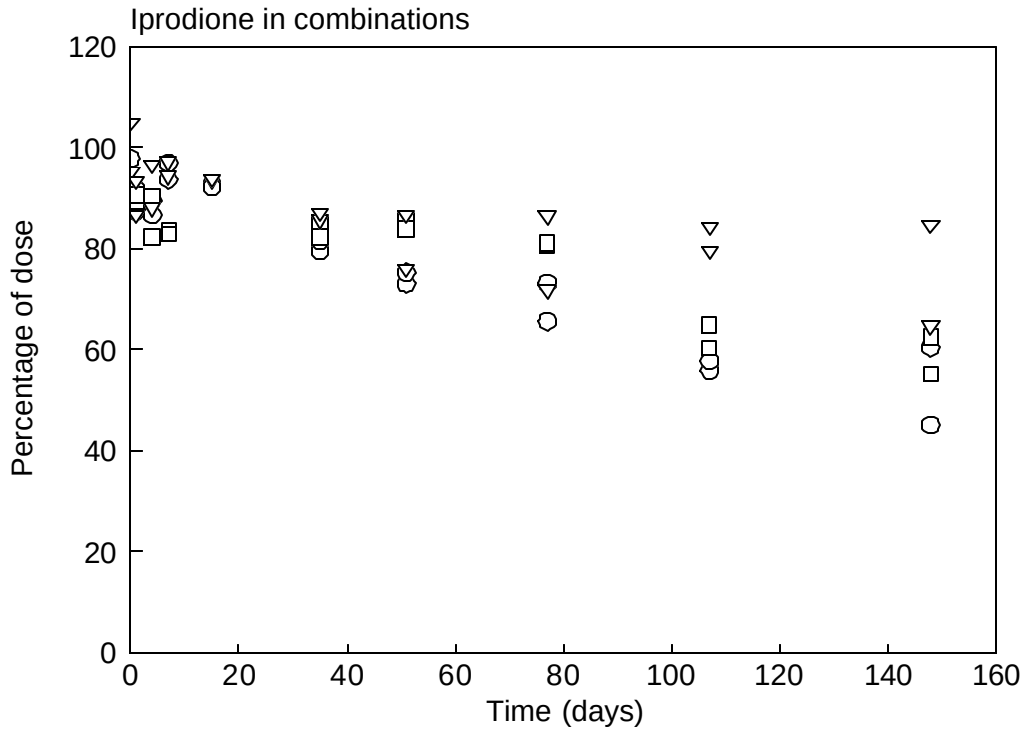


*Figure 4.4 Rate of transformation of iprodione in the Speyer soil at 20 °C, alone (O), in combination with iprodione ( □ ) and in combination with iprodione plus copper ( Ñ )*

The rate coefficients and half-lives for the transformation of iprodione in the soils are collected in Table 4.2. The rates of transformation in the Lincolnshire and Droevendaal soils were distinctly higher than that in the Speyer soil. This illustrates the natural variation that may occur in the rate of transformation of a pesticide in different soils. The presence of carbendazim in the Speyer soil seemed to reduce the transformation rate of iprodione. Further incubations are needed to check whether this effect is significant. The presence of copper, in addition to the presence of carbendazim, further reduced the rate of transformation of iprodione.

*Table 4.2 First-order rate coefficients and half-lives for the transformation (at 20 °C) of iprodione in three soils, alone and in combinations*

| Soil | Additional substance | Rate coefficient ($d^{-1}$) | Standard error ($d^{-1}$) | Half-life (d) |
|---|---|---|---|---|
| Speyer | | 0.00416 | 0.00031 | 167 |
| Lincolnshire | | 0.0185 | 0.0020 | 37 |
| Droevendaal | | 0.0181 | 0.0024 | 38 |
| Speyer | Carbendazim | 0.00269 | 0.00031 | 257 |
| Speyer | Carbendazim and copper | 0.00169 | 0.00036 | 410 |

## 4.4 Discussion

The half-lives of carbendazim in the three soils of the present study are substantially longer than the average half-life (translated to 20 $^{o}$C) of 28.4 days for four soils in the studies of Yarden et al. (1985, 1987). The range of half-lives compiled in the Dutch registration procedure (CTB, 2000) is very wide (11 to 302 days). The half-lives for carbendazim in the present study are somewhat shorter than the middle of that range.

The half-lives for iprodione in the Lincolnshire and Droevendaal soils are close to the average half-life (at 20 $^{o}$C) of 41 days, calculated for some soils by van de Plassche and Linders (1989). They are well within the range of 20 to 80 days given by Tomlin (1997). The half-life of iprodione in the Speyer soil is much longer than both the average of 41 days and the range of 20 to 80 days.

Cases of substantial retardation of the transformation of carbendazim by other fungicides, viz. thiram and fentin acetate, in the soil were described by Yarden et al. (1985). Transformation of carbendazim was retarded to a greater extent as the content of the other fungicides was higher.

## 4.5 Conclusions

### 4.5.1 Carbendazim

* The transformation of carbendazim in the Speyer 2.2 soil is quite slow (half-life of 107 days). After a dose-response experiment of a month about 82% of the dose would be left.

* The rate of transformation of carbendazim in the Speyer, Lincolnshire and Droevendaal soils is of the same order of magnitude. None of the soils shows accelerated transformation due to microbial adaptation.

* The presence of iprodione may decrease the rate of transformation of carbendazim in Speyer soil, but the significance of this effect still has to be checked.

The presence of copper seems to have little effect on the rate of transformation of carbendazim in Speyer soil in the presence of iprodione.

### 4.5.2 Iprodione

* The transformation of iprodione in Speyer 2.2 soil is rather slow (half-life of 167 days). After a dose-response experiment of a month, about 88% of the dose would be left.

* The transformation of iprodione in the Lincolnshire and Droevendaal soils (half-lives of 37 and 38 days) is much faster than that in the Speyer soil. This illustrates the natural variation in transformation rate for a pesticide in different soils.

* The presence of carbendazim seemed to decrease the rate of transformation of iprodione in the Speyer soil.

* The presence of copper decreased the rate of transformation of iprodione in the Speyer soil in the presence of carbendazim.

# 5 Concentration of the fungicides in pore water, alone and in combinations

## 5.1 Introduction

The concentration of pesticides in soil solution (pore water) may be a suitable measure of their bioavailability to various soil organisms. The course of this concentration in time is affected by the adsorption-desorption kinetics of the pesticides in soil. This kinetics may lead to a gradual shift to stronger adsorption in time. Further, pesticide transformation (often by microbial activity) affects the course of pesticide bioavailability in soil. Knowledge about the bioavailability of pesticides in soil is essential in the evaluation of their toxic effects at various content levels.

In the present project, the effects of mixtures of substances on soil organisms are studied. The presence of one substance may have an effect on the behaviour of other substances. The adsorption of one compound on soil can be influenced by the presence of another compound (Chapter 3). Similarly, the presence of one substance may affect the rate of transformation of another substance in soil (Chapter 4). Interactions between substances with respect to their bioavailability in soil solution can thus be expected to occur.

Various methods can be used to estimate the concentration of a substance in pore water in soil. Centrifugation of the moist soil batches, as they are used in the experiments, allows isolation of pore water with minimal disturbance. When pressing solution out of a soil sample in a pressure cell, there may be interactions between the pesticide and the membrane. Adding water to the soil sample (as a kind of extraction) causes shifts in the composition of the soil solution.

In the first type of experiment in the present study, the course of the concentration of carbendazim and iprodione in soil solution, when applied alone to soil, is measured. The overall rate of transformation of the fungicides (applied alone) is also measured. In this way it should be possible to establish the role of both, sorption kinetics and transformation kinetics, in the bioavailability of the fungicide.

In the second type of experiment, soil solution concentration and overall transformation are measured for the combinations a) carbendazim plus iprodione and b) carbendazim plus iprodione plus copper. This enables the study of the effect of the presence of one or two other substances on the bioavailability of a particular fungicide. In the evaluation of the effect of combinations of substances on soil organisms, the interactions with respect to bioavailability in soil solution should be known.

## 5.2 Procedures

Incubation experiments with the fungicides carbendazim and iprodione were carried out with Speyer 2.2 soil (batch of September 1998). Four portions of 1000 g soil (moisture content 6.8%) were spread out in shallow stainless-steel trays. First, 10 $cm^3$ solution of $CuCl_2$ in water (387 μg $Cu^{2+}$ per $cm^3$) was trickled with a syringe on the whole soil surface in Tray 4. This was followed by intensive mixing of the soil. The initial content of $Cu^{2+}$-ions in soil was 4.13 μg/g (dry soil basis). Then 35 $cm^3$ of solution of carbendazim in acetone (268 $\mu g/cm^3$) was evenly spread with a syringe over the soil surface of Trays 1, 3 and 4. The acetone was allowed to evaporate. The initial content of carbendazim in soil was 10.0 μg/g. Subsequently, 35 $cm^3$ solution of iprodione in acetone (259 $\mu g/cm^3$) was evenly spread on the soil surface in Trays 2, 3 and 4; again the acetone was allowed to evaporate. The initial content of iprodione in soil was 9.7 μg/g. Finally, water was added to moisten the soil batches to 30% moisture and the soil in each tray was mixed intensively for another time.

In summary, the four treatments were:

Tray 1: carbendazim;
Tray 2: iprodione;
Tray 3: carbendazim plus iprodione;
Tray 4: carbendazim plus iprodione plus $CuCl_2$.

The soil in each tray was collected in a glass jar, loosely covered with aluminium foil. The jars were put in a box with water layer (covered with a lid) to prevent the soil from drying out. The box was placed in a constant-temperature cabinet at 15 °C.

The measurements were made at 0, 1, 7, 14, 41 and 62 days after the start of the experiment. At each time, duplicate subsamples of the soil in each flask were subjected to:
a) isolation of pore water followed by chemical analysis of the water;
b) extraction of the remaining soil followed by chemical analysis of the extract

To isolate pore water, 56 g of moist soil was placed in a large stainless-steel centrifuge tube with fritted-glass filter. The tubes were centrifuged at 7000 r.p.m. for 30 min (thermostat at 15 °C). About 7 $cm^3$ of solution per subsample was collected in the vessel at the bottom end. The remaining soil was combined with 10 $cm^3$ of water and 50 $cm^3$ of ethyl acetate, and then extracted by shaking for 1 hour. The concentration in the ethyl acetate extract was measured by HPLC. The masses of dry soil and water in each subsample were determined by weighing.

The water samples were analysed directly for carbendazim and iprodione by HPLC. A subsample of 0.1 $cm^3$ of the soil extract in ethyl acetate was put in a glas vial, after which the solvent was evaporated. The drying residue was taken up in 1 $cm^3$ HPLC-water. For each analysis, 0.1 $cm^3$ of the solution in water was injected in the liquid chromatograph. The analytical column contained C18 reversed-phase sorbent (Merck).

The mobile phase for carbendazim analysis was acetonitrile/water (30/70; v/v) at a flow rate of 1.0 $cm^3$/min. Carbendazim was detected by UV absorption at 285 nm. Its retention time was 3.2 min.

The mobile phase for iprodione analysis was acetonitrile/water (60/40; v/v) at a flow rate of 1.0 $cm^3$/min. Iprodione was detected by UV absorption at 210 nm. Its retention time was 4.2 min.

Five concentrations in the range of 0.5 to 5.0 $\mu g/cm^3$ were injected for the construction of the calibration line. The response of the detector was processed with the Multichrom data system (VG Data Systems).

The average recovery of carbendazim in the analyses on days 0 and 1 was 93% (n = 12; s.d. = 5%). For iprodione, the average recovery of the analyses on the first two days was 101% (n = 12; s.d. = 3%).

## 5.3 Results and discussion

### 5.3.1 Carbendazim

The concentrations of carbendazim measured in the pore water of the Speyer soil as a function of time are presented in Figure 5.1. In the first day, the concentrations increased. Presumably, this is caused by the additional wetting of the soil in the beginning of day 0. The net-desorption of carbendazim into the freshly-added water seemed to be incomplete on the first day.

When carbendazim was applied alone to the soil, its concentration decreased continuously in the period of 1 tot 62 days, to low values at the end (Figure 5.1).

When carbendazim was applied in combination with iprodione, its concentration in pore water decreased gradually in the period day 1 to day 14 (Figure 5.1). After that, the decrease in concentration was very slow. At 62 days after the start, about half of the concentration at day 1 was still present.

The concentration of carbendazim in pore water at day 1 was comparatively low when it was applied in combination with iprodione plus copper (Figure 5.1). Again, there was a gradual decrease in the concentration of carbendazim in the period day 1 to day 14, which was followed by a period with slow decrease. From day 14 on, the concentrations of carbendazim in pore water for the two combinations were at the same level.

The general pattern (Figure 5.1) is that iprodione substantially slowed-down the decrease of the concentration carbendazim in pore water after the first 2 weeks. The addition of copper (besides iprodione) had little additional effect on the decrease of carbendazim.

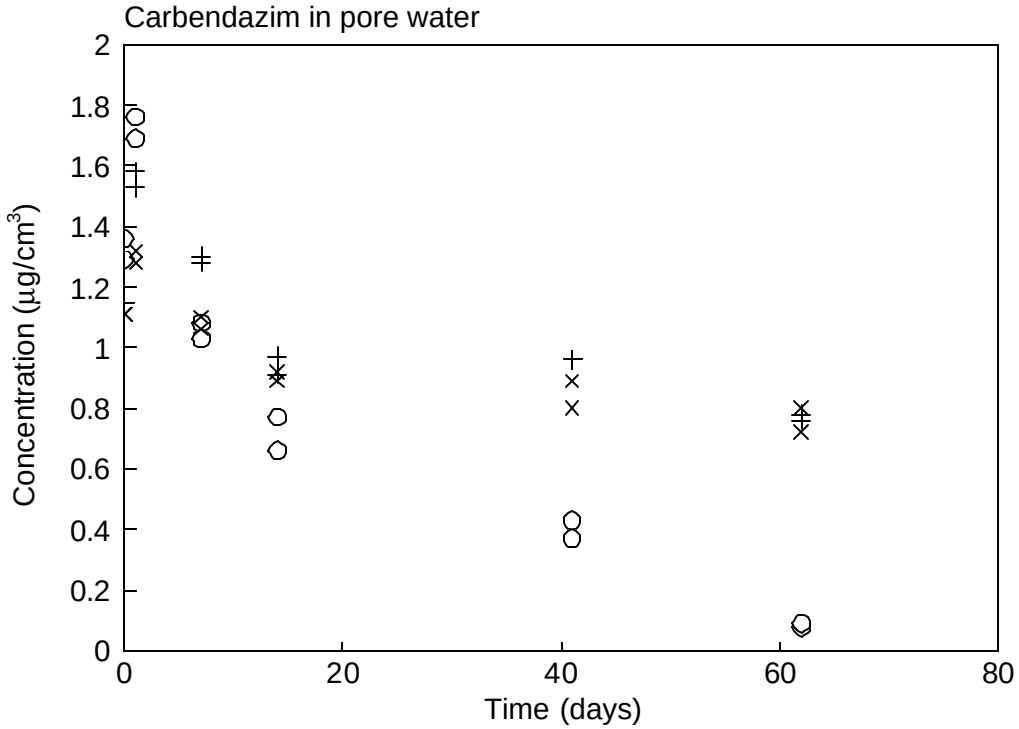


*Figure 5.1 Concentration of carbendazim in pore water of the Speyer soil, when present alone (O), in combination with iprodione (+) and in combination with iprodione plus copper (x)*

The content of carbendazim adsorbed to the Speyer soil, as a function of time, is given in Figure 5.2. This content represents by far most of the carbendazim in the system, because the pore water contained on average 5.0% of the amount in soil.

When carbendazim was applied alone to the soil, it was transformed at a substantial rate in the first two weeks, to less than half of its dose (Figure 5.2). However, in the period from day 14 to day 62, the transformation was comparatively slow.

When carbendazim was applied in combination with iprodione, its rate of transformation in the first 2 weeks was almost the same as that for carbendazim applied alone (Figure 5.2). Thereafter, carbendazim transformation rate was distinctly slower in the presence of iprodione.

The presence of copper (besides iprodione) did not have much additional effect on the course of the transformation of carbendazim in soil (Figure 5.2).

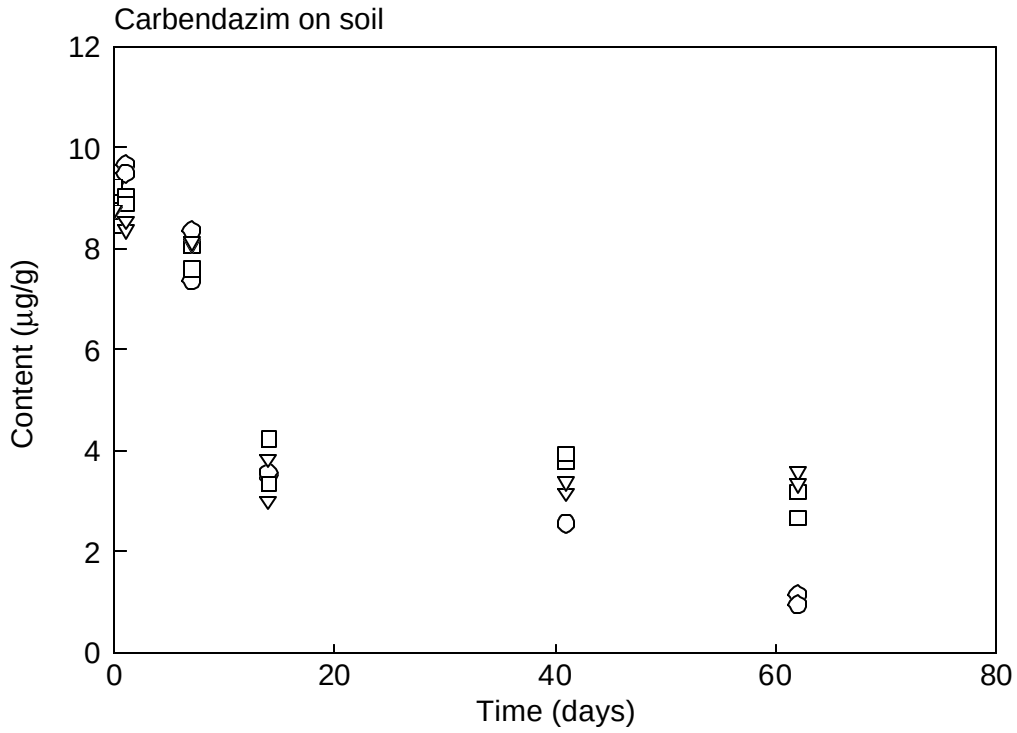


*Figure 5.2 Content of carbendazim adsorbed to Speyer soil, when present alone (O), in combination with iprodione ( □ ) and in combination with iprodione plus copper (Ñ)*

The higher concentration of carbendazim in the pore water in the presence of iprodione (Figure 5.1) can be partly explained by its slower transformation in soil when iprodione is present (Figure 5.2). When incubated alone, the concentration of carbendazim in pore water (Figure 5.1 ) decreases to roughly the same extent as the amount in soil (Figure 5.2). In the first two weeks of the combined incubations, the concentration in pore water tended to decrease less than the amount in soil. After that, the soil/water distribution ratio for carbendazim was almost constant.

The non-distinct additional effect of copper holds for both, the concentration of carbendazim in pore water and its amount in soil.

### 5.3.2 Iprodione

The concentrations of iprodione measured in the pore water of the soil are given in Figure 5.3. In two out of three cases, the concentration increased from day 0 to day 1. This indicates that the net-desorption of iprodione to the freshly-added water was incomplete on the first day.

When iprodione was applied alone to the soil, its concentration in pore water showed a continuous decrease from day 1 to day 41 (Figure 5.3). Thereafter, the decrease was comparatively slow.

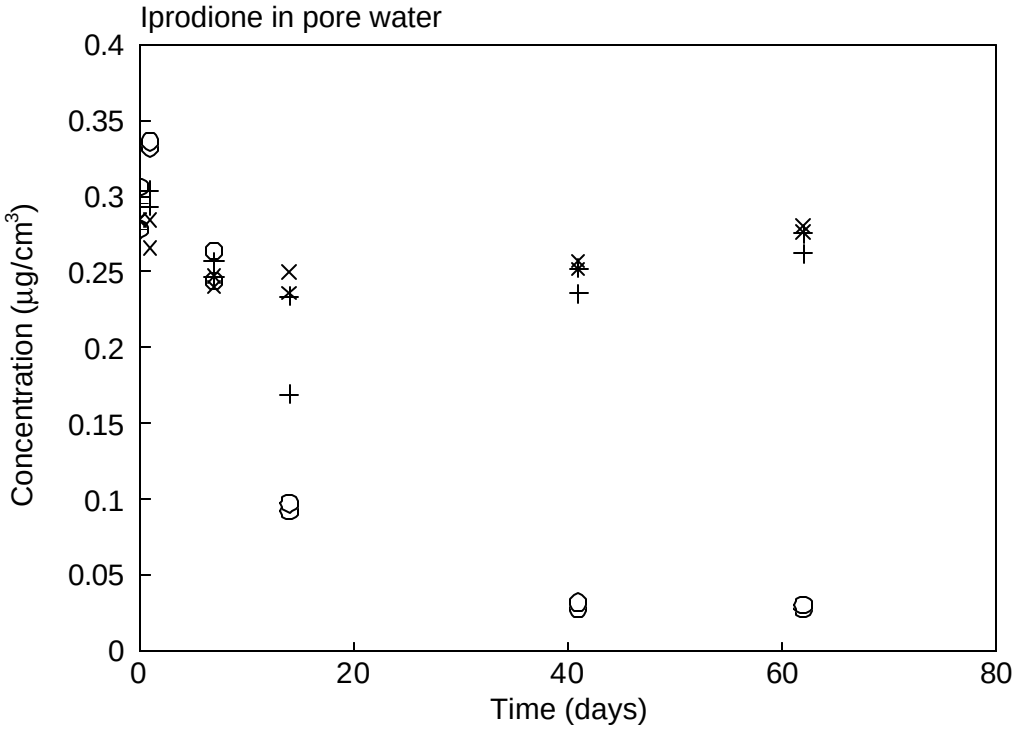


*Figure 5.3 Concentration of iprodione in pore water of the Speyer soil, when present alone (O), in combination with carbendazim (+) and in combination with carbendazim plus copper (x)*

In the presence of carbendazim, the concentration of iprodione in the pore water decreased in the first week, at a rate similar to that for iprodione alone (Figure 5.3). However, there was hardly any further decrease in this concentration from day 7 to day 62.

When iprodione was incubated in soil in the presence of carbendazim plus copper, the concentrations in the pore water were usually close to those in the presence of carbendazim (Figure 5.3). This indicates that copper had little additional effect on the concentration of iprodione in pore water.

Figure 5.4 shows the content of iprodione adsorbed to the soil as a function of the time of incubation. This content represents by far most of the iprodione in soil; on average 0.7% of the iprodione was present in the pore water.

When iprodione was applied alone to the soil, its amount gradually decreased during the incubation (Figure 5.4). About 35% of the dose was left after 62 days.

Incubation of iprodione in the presence of carbendazim resulted in very slow transformation (Figure 5.4). At 62 days after the start, about 90% of the dose of iprodione was left in the soil.

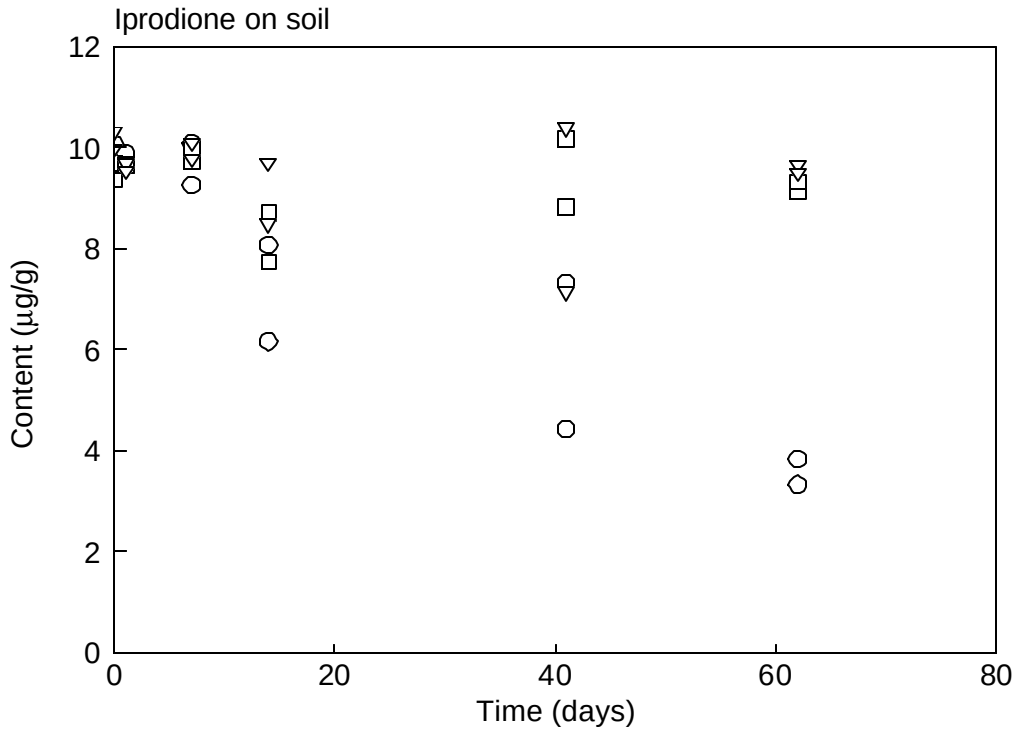


*Figure 5.4 Content of iprodione adsorbed to Speyer soil, when present alone (O), in combination with carbendazim ( □ ) and in combination with carbendazim plus copper (Ñ)*

When iprodione was incubated in soil in the presence of both, carbendazim and copper, its transformation was slow (Figure 5.4). At 62 days after the start, more than 90% of the dose was left. At two times, there was substantial variation in the measuring points. The presence of copper had little effect on the (low) rate of transformation of iprodione.

The comparatively high concentrations of iprodione in pore water in the presence of carbendazim (Figure 5.3) can be partly explained from the reduction in iprodione transformation rate by carbendazim (Figure 5.4). When incubated alone, the concentration of iprodione in pore water (Figure 5.3) decreased to a greater extent than its total amount in soil (Figure 5.4). This means that the distribution ratio soil/water for iprodione increased in time. In the presence of carbendazim (and of copper) such a shift to a higher soil/water ratio for iprodione was not clear.

The addition of copper to the soil had little additional effect on both the concentration of iprodione in pore water (Figure 5.3) and its transformation rate in soil (Figure 5.4).

# 6 Concentrations of the fungicides in soil solution at high contents in soil

## 6.1 Introduction

The fungicides carbendazim and iprodione show rather low toxicity to some of the soil animals used in the project. Then, high fungicide contents are mixed in the soil in the dose-response experiments. It is expected that, at the highest fungicide contents in soil, the concentration in soil solution will be close to the solubility in water. If so, the exposure of the soil animals via the soil solution does not increase if the content in soil is increased above a certain level. Another question is whether the fungicides can be applied in powder form to the soil (instead of in acetone solution) at such high contents.

## 6.2 Procedure

Carbendazim and iprodione were mixed separately with the Speyer soil (batch September 1998; 6.8% moisture) in a series of four increasing contents. The target contents in the duplicate experiment were 10, 40, 160 and 640 μg/g. The total soil batch for each addition was 100 g. The fungicides were applied in acetone solution (263 μg/cm$^3$ for carbendazim; 325 μg/cm$^3$ for iprodione) to 20 g of each soil batch. The solution was added in portions of at most 20 cm$^3$. After each addition, the acetone was allowed to evaporate. The treated 20 g of soil was mixed intensively with the other 80 g of soil, in a flask (Schott, 250 cm$^3$). The water content of the soil was increased to 30% (dry mass basis) and the soil was mixed again. The soil batches were incubated under moist condition at 15 °C in the dark. Mixing of the soil in the flasks was repeated at 1 and 2 days after the start. At the highest two contents in soil, the fungicides showed local precipitation on the soil surface and on the glass wall.

Pore water (about 16 cm$^3$) was collected at 3 days (two lower contents) or 6 days (two higher contents) after the addition of the fungicides. The soil batches were centrifuged at 7000 r.p.m. for 30 min (thermostat at 15 °C). The isolated pore water was diluted if necessary and the concentrations of the fungicides were measured by HPLC.

At the highest target content of 640 μg/g, the fungicides were also applied as powder of the technical product. A mass of 69400 μg of fungicide powder was added to 20 g of the soil and mixed-in. After that, the 20 g soil was added to the other 80 g of the soil batch and thorougly mixed. Further, the procedure was the same as for the addition of the fungicides in acetone solution.

## 6.3 Results

The concentrations of carbendazim in soil solution at increasing contents in the Speyer soil (on dry mass basis) are given in Figure 6.1. In a range of comparatively low contents in soil, the concentration in pore water increases linearly, with a steep slope. However, at contents above 100 μg/g, the slope decreases substantially. Possibly, the concentrations in solution at the highest contents in soil approach the solubility of carbendazim in water (reported to be 8 $\mu g/cm^3$ at pH 7; Tomlin, 1997). It should be noted that the solubility of carbendazim in pore water may deviate from that in pure water, because of the effect of other substances present in the pore water.

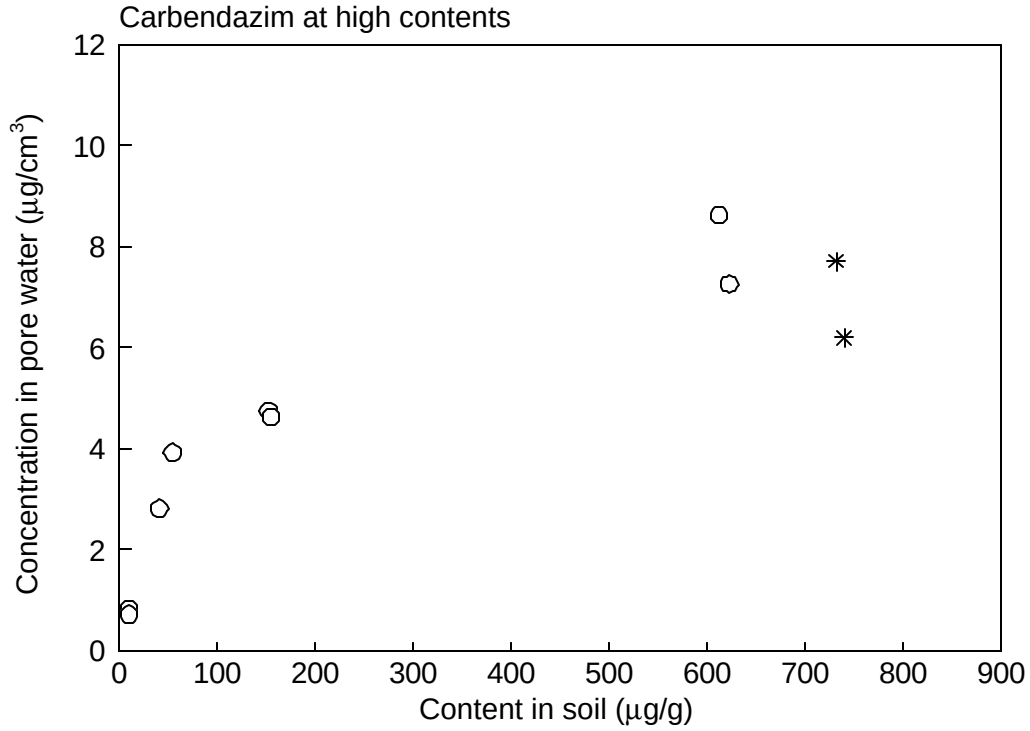


*Figure 6.1 Concentrations of carbendazim in pore water at high contents in soil. Applied as solution in acetone (O) or as powder (*)*

The concentrations of carbendazim in solution, when applied as a powder to a high content in soil, are also shown in Figure 6.1. These concentrations are somewhat lower than those measured after application as a solution in acetone.

In a range of comparatively low contents of iprodione in soil, its concentration in soil solution increases linearly with its content (Figure 6.2). The slope in the relationship is lower for contents above 100 μg/g. The concentration of iprodione at the higher contents could gradually approach its solubility in pore water. The solubility of iprodione in pure water is reported to be 13 $\mu g/cm^3$ at 20 °C (Tomlin, 1997). In the case of iprodione too, other substances in the pore water may affect its solubility.

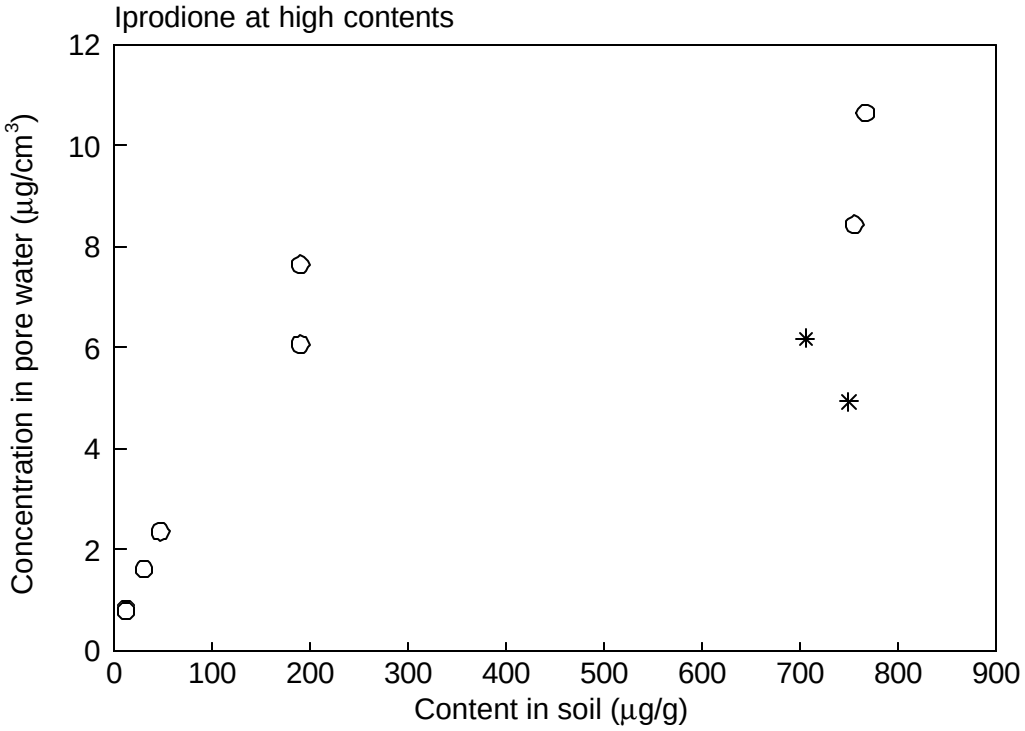


*Figure 6.2 Concentrations of iprodione in pore water at high contents in soil. Applied as solution in acetone (O) or as powder (*)*

Figure 6.2 also shows the concentrations measured for iprodione in soil solution, when applied as a powder. They are distinctly lower than the concentrations measured after application as a solution in acetone. It seems that the powder form slows-down the release of the fungicides to the soil solution, as compared to the release after application in acetone.

So when the contents of the fungicides in soil are increased to rather high values (above 100 µg/g), their concentration in soil solution increases less than proportional. At contents around 600 µg/g and higher, non-uniform distribution of the fungicides in the soil batches by local precipitation was observed. Then the concentration in soil solution approaches the solubility of the fungicides in water. So the exposure of soil animals via the pore water hardly increases when increasing fungicide content in the high range. Besides, the animals are exposed locally to solid particles of the fungicides then.

# 7 Chemical analyses for the dose-response experiments

## 7.1 Introduction

In the dose-response experiments of the research partners, soil animals were exposed to different levels of the chemicals alone and in mixtures. The levels were obtained by applying target doses (calculated) to the exposure systems. However, the actual doses may deviate from the target doses because of unexpected phenomena and inaccuracies.

When handling the present pesticides carbendazim and iprodione in the laboratory, some special problems may be encountered. It was found to be difficult to dissolve carbendazim in water, even at levels well below its maximum solubility in water. The same problem may occur with other solvents. Further, carbendazim tends to precipitate from its dissolved state, also below its maximum solubility in water.

Iprodione in aqueous solution was found to interact with some kinds of glass (tubes, vials), which leads to fast transformation (catalysis). Check measurements for such interactions are thus needed for the glassware used in the experimental and analytical procedures.

It is necessary to have checks on the contents of the fungicides at the end of the dose-response experiments. On the basis of a low rate of transformation of carbendazim and iprodione in soil one might expect that much of the dose is left then after e.g. a few weeks. However, the rate of fungicide transformation is influenced by various factors, so occasional checks are needed. Potential loss processes for organic chemicals, besides transformation, are volatilization, binding to (synthetic) materials and photochemical transformation.

A protocol was set up and distributed for handling the soil samples to be analysed for the fungicides in the framework of the dose-response experiments. The soil batches could be obtained from the experiments themselves or from a parallel experiment in which the soil was treated in the same way as in the main experiment. Preferably, the moist soil batches had to correspond to at least 5 g of dry soil and they had to be stored in glass flasks or tubes in deep-freeze. The soil samples collected in a period of e.g. 3 months had to be dispatched to the laboratory in Wageningen for chemical analysis. The dispatch at ambient temperature should take only a few days or less; some correction for the transformation in such a short period seemed possible.

The soil batches had to be extracted with ethyl acetate in all-glass systems, e.g. flasks or tubes with ground-glass stoppers. In case of rather dry soil, some distilled water had to be added to reach about 50% soil water content (on dry soil basis). The mass of wet soil and the volume of ethyl acetate (ratio about $1/1\ g/cm^3$) had to be shaken mechanically for 1 hour, thus keeping the soil plus water plus solvent in full motion.

A clear layer of ethyl acetate had to be obtained, e.g. by settling overnight (cold room) and/or by centrifugation. An exactly-known volume of at least 1 to 2 $cm^3$ of the ethyl acetate layer had to be collected, e.g. in an HPLC or GLC vial which is thoroughly sealed. The extracts could be stored for a short time in a refrigerator until chemical analysis.

In the next sections, the chemical analyses in the framework of the dose-response experiments of the research partners are presented. A brief description is given of known aspects of the experimental procedure. The results of the analyses are presented in tables.

## 7.2 Measuring series WU-6-98

The soil samples (Speyer 2.2; 40 and 50 g, respectively) were received from the Department of Nematology of Wageningen University in June 1998. The dose-response experiment with the nematodes had been carried out in spring 1998. Typical treatments of the soil in the framework of the dose-response experiments were:
a) 2 min in the microwave at 100%;
b) addition of Acinetobacter in yeast extract;
c) addition of standard pore water;
d) exposure of the nematodes at 20 °C for 3 weeks.

The present samples were obtained from a parallel experiment with larger soil masses. The samples were rather wet: soil moisture content (on dry soil basis) was 30%. The target contents of the fungicides in the dose-response experiments had been calculated on wet soil basis. The soil samples were stored in deep-freeze from collection in the dose-response experiment (nematodes) until extraction and analysis.

The complete soil samples were extracted by shaking with a known volume of about 31 mL ethyl acetate for 1 hour. A subsample of the extract (diluted if necessary) was evaporated and the drying residue was taken up in 1 $cm^3$ HPLC water by shaking and ultrasonic vibration. All concentrations in the procedure of extraction and analysis were below the solubilities of carbendazim and iprodione in ethyl acetate and water.

The results of the measurements for the fungicides in the soil samples are given in Table 7.1.

*Table 7.1 Contents (on dry soil basis) of carbendazim and iprodione measured in the soil samples of Wageningen University received in June 1998*

| Compound | Sample Code | Content (µg/g) |
|---|---|---|
| Carbendazim | C.0.t0.pr1 | 0.01 |
| | C.0.t0.pr2 | 0.00 |
| | C.10.t0 | 22.5 |
| | C.10.t1 | 15.1 |
| | C.10.t2 | 15.7 |
| | C.10.t3 | 14.6 |
| | C.100.t3 | 99.2 |
| Iprodione | I.0.t0 | 0.00 |
| | I.30.t0 | 38.9 |
| | I.30.t1 | 40.4 |
| | I.30.t2 | 36.8 |
| | I.30.t3 | 33.5 |
| | I.100.t0 | 61.2 |
| | I.100.t1 | 64.7 |
| | I.100.t2 | 53.7 |
| | I.100.t3 | 55.2 |

## 7.3 Measuring series LU-3-99

The soil samples were sent by the Department of Ecology, Lund University, on 23 February 1999 and they were received in Wageningen on 26 February 1999. The average mass of wet soil in Series A was 21.1 g (n = 25; s.d. = 3.8 g). The samples contained on average 15.4 g dry soil (s.d. = 2.8 g). The average moisture content of the soil samples in Series A was 36.8% (s.d. = 0.7%). The average mass of wet soil in Series B was 21.5 g (n = 24; s.d. = 2.3 g). The samples contained on average 15.9 g dry soil (s.d. = 1.7 g). The average moisture content of the soil samples in Series B was 35.6% (s.d. = 3.8%).

The samples in Series A originate from an experiment in Lund started on 2 February 1999. First, $CuCl_2$ solution was added and the soil was incubated at 15 °C (dark) for 15 days. On 17 February 1999 the fungicides dissolved in acetone were added, after which the acetone was allowed to evaporate overnight. The soil was moistened to about 35%, put in glass flasks and placed in deep-freeze until dispatch. Thus, these are samples from the beginning of a dose-response experiment for the Enchytraeids.

The samples in Series B were taken from an experiment started in Lund on 12 October 1998. $CuCl_2$ solution was added and the soil was incubated at 15 °C (dark) for 14 days. On 26 October 1998, the fungicides, dissolved in acetone, were added and the solvent was allowed to evaporate overnight. Then the soil was moistened to about 35% and rolled oats (as food) were mixed with the soil. Adult Enchytraeids were exposed in the soil at 15 °C in the dark for 1 week. On 3 November 1998, the adults were removed from the soil and the evaporated water replenished. Layed

cocoons were then incubated in the soil under the same conditions. One week later, evaporated water was replenished and rolled oats were spread over the soil surface. On 17 November only the evaporated water was supplemented. At the end of the experiment on 24 November 1998, the treated soil was was put in 25 $cm^3$ vials and placed in deep-freeze until dispatch.

The complete soil samples (about 21 g) were extracted by shaking with ethyl acetate (24 $cm^3$) for 1 hour. A subsample of the ethyl acetate layer was put in a vial, the solvent was evaporated and the drying residue taken up in HPLC water (1 $cm^3$). This solution was diluted, if necessary, after which the concentrations of carbendazim and iprodione were measured by HPLC.

A problem was encountered in the analyses of the highest contents of carbendazim and iprodione in soil. In one step of the analytical procedure, it was attempted unintentionally to exceed the solubility of the fungicides in water. In these cases, the actual content of the fungicide in soil may have been higher than measured. In the tables, these results are preceded by the symbol for 'greater than or equal to'. On the basis of this experience, the protocol for the extraction and analysis of the fungicides was approved and all previous measurements of comparatively high contents were checked for this problem.

The results of the analyses for carbendazim in the soil samples from Lund University are given in Table 7.2.

*Table 7.2 Contents (on dry soil basis) of carbendazim measured in the soil samples received from Lund University in February 1999*

| Sample code | Content (µg/g) | Sample code | Content (µg/g) |
|---|---|---|---|
| A8 | 3.54 | B8 | 2.28 |
| A10 | 0.75 | B10 | 0.58 |
| A11 | 4.74 | B11 | Broken |
| A13 | 9.14 | B13 | 11.3 |
| A14 | 0.68 | B14 | Broken |
| A15 | 8.17 | B15 | 5.25 |
| A17 | 2.22 | B17 | 1.89 |
| A18 | 7.36 | B18 | 5.83 |
| A20 | ≥16.0 | B20 | 10.8 |
| A21 | 1.95 | B21 | 1.77 |
| A22 | 11.2 | B22 | 5.25 |
| A24 | 3.05 | B24 | 3.64 |
| A25 | ≥11.0 | B25 | ≥12.6 |
| A27 | ≥25.8 | B27 | 9.76 |
| A28 | 3.20 | B28 | 2.11 |
| A43 | Lost | B43 | 5.40 |
| A44 | 10.2 | B44 | 7.28 |
| A46 | ≥20.7 | B46 | 9.02 |
| A48 | ≥29.7 | B48 | ≥21.0 |
| A49 | ≥12.6 | B49 | ≥22.3 |

Table 7.3 shows the results of the analyses for iprodione in the soil samples received from Lund University.

*Table 7.3 Contents of iprodione measured in the soil samples received from Lund University in February 1999*

| Sample code | Content (µg/g) | Sample code | Content (µg/g) |
|---|---|---|---|
| A8 | 70.4 | B8 | 53.5 |
| A10 | 86.2 | B10 | 55.8 |
| A11 | 17.8 | B11 | Broken |
| A13 | 15.6 | B13 | 21.3 |
| A14 | 135 | B14 | Broken |
| A15 | 130 | B15 | 68.2 |
| A17 | 163 | B17 | 67.6 |
| A18 | 41.2 | B18 | 29.8 |
| A20 | 44.5 | B20 | 21.7 |
| A21 | 156 | B21 | 167 |
| A22 | ≥450 | B22 | 67.4 |
| A24 | ≥238 | B24 | ≥253 |
| A25 | 67.5 | B25 | 38.9 |
| A27 | 59.5 | B27 | 52.4 |
| A28 | 17.3 | B28 | ≥256 |
| A50 | 131 | B50 | 37.1 |
| A51 | 130 | B51 | 185 |
| A53 | ≥234 | B53 | 174 |
| A55 | ≥350 | B55 | ≥203 |
| A56 | ≥427 | B56 | ≥205 |
| A57 | 0.1 | B57 | 23.9 |

## 7.4 Measuring series VU-6-99

The soil samples were received from the Vrije Universiteit in Amsterdam on 1 June 1999. The mass of the moist soil samples was 10 or 20 g, respectively. Moisture content of the samples was on average 24.0% (n =12; s.d. = 0.8%).

The soil samples were extracted in centrifuge tubes (90 $cm^3$) by adding 10 $cm^3$ of distilled water and 25 $cm^3$ of ethyl acetate, which was followed by shaking for 1 hour. A volume of 10 $mm^3$ of the clear ethyl acetate layer was transferred to a vial and the solvent was evaporated. The drying residue was taken up in 1 $cm^3$ HPLC water by repeated shaking and ultrasonic vibration. The concentrations of carbendazim and iprodione were measured by HPLC.

One extraction of carbendazim provided only about one-half of the expected amount. Therefore, the soil samples were extracted another two times with 25 $cm^3$ ethyl acetate. The second extraction provided another large fraction of carbendazim, while the third extraction only provided a small additional fraction. The results of the three extractions together are presented in Table 7.4.

*Table 7.4 Contents (on dry soil basis) of carbendazim measured in the soil samples received from the Vrije Universiteit in Amsterdam on 1 June 1999*

| Sample code | Sample date | Content (µg/g) |
|---|---|---|
| 100carb | 27/1 | 77.9 |
| 1TUipro+100carb | 27/1 | 96.0 |
| 1TUipro+100carb | 26/2 | 111 |
| 1TUCu+1TUipro+100carb | 27/1 | 110 |
| 1TUCU+1TUipro+100carb | 26/2 | 113 |

It can be estimated from the measurements that the maximum concentation of carbendazim in the ethyl acetate layer after the first extraction was about 30 µg/cm$^3$. This is much lower than the solubility of carbendazim in ethyl acetate, which is reported to be 135 µg/cm$^3$ (Tomlin, 1997). Possibly, the tendency of carbendazim to dissolve in the ethyl acetate layer is decreased, because this layer contains some water (3.3 g/g) and other substances from the soil sample. These constituents seem to make the ethyl acetate layer more polar.

The results of the measurements for iprodione are presented in Table 7.5.

*Table 7.5 Contents (on dry soil basis) of iprodione measured in the soil samples received from the Vrije Universiteit in Amsterdam on 1 June 1999*

| Sample code | Sample date | Content (µg/g) |
|---|---|---|
| 1TUipro | 12/1 | 196 |
| 1TUipro | 11/2 | 132 |
| 1TUCu+1TUipro | - | 203 |
| 0.125TUipro | 27/1 | 27.7 |
| 1TUipro | 27/1 | 219 |
| 1TUipro | 26/1 | 210 |
| 4TUipro | 27/1 | 723 |
| 1TUipro+100carb | 27/1 | 187 |
| 1TUipro+100carb | 26/2 | 91.1 |
| 1TUCu+1TUipro+100carb | 27/1 | 203 |
| 1TUCu+1TUipro+100carb | 26/2 | 186 |

No problem with the dissolution of iprodione in the ethyl acetate layer is expected because the solubility of this fungicide in ethyl acetate is very high (Tomlin, 1997).

## 7.5 Measuring series CEH-4-00

These samples were obtained from the dose-response experiments by the Centre for Ecology and Hydrology (CEH) at Monks Wood, Huntingdon, UK. The fungicides were added as a solution in acetone and the solvent was allowed to evaporate for 24 hours. The jars with soil were left at room temperature (with lid on) for 2 weeks after dosing, before the earthworms were added. Soil moisture content was adjusted to about 30%. At 1 day after the start of the exposure, each jar was supplied with 3 g horse manure, placed on top of the soil. After 2 weeks of exposure of the worms, the same amount of manure was added again. The jars were emptied after 4 weeks of exposure to collect the worms. The soil and cocoons were placed back into the jars

for another 3 weeks. The soil batches for the analyses were dried to a certain extent and then stored in deep-freeze.

The soil samples were sent by CEH on 7 April 2000; they were received in Wageningen on 11 April 2000. The soil batches of about 30 g had been packed in small plastic bags. One series of soil samples was taken before the exposure experiment ('Before') and the other series was taken after the exposure experiment ('After'). In Wageningen, the samples were stored in deep-freeze until extraction and analysis.

The soil batches contained both fine material and small clods; they were refined with a stamper in a mortar (carefully cleaned each time) and then mixed. A mass of 15 g well-mixed soil was weighed into glass tubes (90 $cm^3$). Volumes of 10 $cm^3$ water and 25 mL ethyl acetate were added. The tubes were sealed and shaken for 1 hour on a mechanical shaker. After settling of the soil particles, most of the ethyl acetate layer was collected. The total volume of the ethyl acetate layer was calculated accounting for the dissolution of some water in the ethyl acetate and some ethyl acetate in the water. Other subsamples were used to determine the soil moisture contents.

A well-known volume of the ethyl acetate layer was transferred into an HPLC vial and the solvent was evaporated. The drying residue was taken up in a known volume of water. The volumes of the liquids were taken such that the expected concentration never exceeded one-quarter of the solubility of the fungicide.

Carbendazim and iprodione were analysed by HPLC. Some details of the analysis:

***Carbendazim :***
The separation column (length 150 mm; inner diam. 4.6 mm) was filled with Waters™ X-terra MSC18 (particles 3.5 μm). The pre-column of the same type was 22 mm long. The mobile phase was acetonitrile/water (30/70; v/v); its flow rate was 1 $cm^3$/min. Carbendazim was detected by measuring UV absorption at a wavelength of 285 nm. The retention time was 3.73 min.

***Iprodione:***
The separation column (length 150 mm; inner diam. 4.6 mm) was filled with Waters™ X-terra MSC18 (particles 3.5 μm). The pre-column of the same type was 22 mm long. The mobile phase was acetonitrile/water (60/40; v/v) and its flow rate was 1 $cm^3$/min. Iprodione was detected by measuring UV absorption at a wavelength of 210 nm. The retention time was 5.57 min.

The contents of carbendazim and iprodione measured in the soil samples are given in Table 7.6.

*Table 7.6 Contents (on dry soil basis) of carbendazim and iprodione measured in soil samples from dose-response experiments by the Centre for Ecology and Hydrology at Monks Wood, Huntingdon, UK*

| Date | Jar number | Content in soil (µg/g) of | | | |
|---|---|---|---|---|---|
| | | Carbendazim | | Iprodione | |
| | | Before | After | Before | After |
| 8 Feb. 1999 | 21 | < 0.01 | < 0.01 | < 0.01 | < 0.01 |
| | 25 | 0.22 | 0.16 | 3.81 | 1.98 |
| | 29 | 1.05 | 0.66 | 12.3 | 5.55 |
| | 33 | 1.45 | 1.38 | 30.6 | 7.59 |
| | 39 | 1.84 | 1.86 | 42.1 | 38.8 |
| 9 Feb. 1999 | 22 | < 0.01 | < 0.01 | < 0.01 | < 0.01 |
| | 26 | 0.24 | 0.14 | 1.80 | 2.17 |
| | 30 | 1.06 | 0.68 | 12.9 | 3.69 |
| | 34 | 1.50 | 1.37 | 18.2 | 8.82 |
| | 38 | 1.86 | 0.79 | 49.6 | 24.6 |
| 10 Feb. 1999 | 23 | < 0.01 | < 0.01 | < 0.01 | < 0.01 |
| | 27 | 0.24 | 0.18 | 2.91 | 1.29 |
| | 31 | 1.15 | 0.70 | 4.30 | 4.78 |
| | 35 | 1.09 | 1.34 | 21.1 | 7.03 |
| | 37 | 0.94 | 2.07 | 19.1 | 30.2 |
| 11 Feb. 1999 | 24 | < 0.01 | < 0.01 | < 0.01 | < 0.01 |
| | 28 | 0.31 | 0.17 | 3.62 | 1.63 |
| | 32 | 1.23 | 0.66 | 13.4 | 2.99 |
| | 36 | 1.17 | 1.30 | 24.9 | 5.93 |
| | 40 | 2.29 | 0.92 | 58.7 | 19.9 |
| 17 Feb. 1999 | 60 | 4.12 | 1.53 | < 0.01 | < 0.01 |
| | 62 | 4.29 | 1.13 | < 0.01 | < 0.01 |
| | 57 | 6.19 | 2.22 | < 0.01 | < 0.01 |
| 18 Feb. 1999 | 41 | < 0.01 | < 0.01 | < 0.01 | < 0.01 |
| | 43 | < 0.01 | < 0.01 | < 0.01 | < 0.01 |
| | 59 | < 0.01 | < 0.01 | 59.4 | 9.46 |
| | 58 | < 0.01 | < 0.01 | 50.3 | 38.0 |
| | 61 | < 0.01 | < 0.01 | 37.0 | 109 |

## 7.6 Measuring series WU-9-00

These soil samples (received in September 2000) originated from the dose-response experiments for nematodes by Wageningen University. Before the experiment, the soil was heated in a microwave (3 min; 100%). The fungicides were applied in acetone solution to 10% of the total soil batch, after which the solvent was allowed to evaporate. The treated soil was mixed intensively with the other 90% of the soil batch, which was then divided into smaller batches. Small volumes of standard pore water and bacteria in yeast extract were mixed into the soil. The small soil batches per treatment consisted of about 5 g moist soil. After adding the chemicals, the soil

was pre-incubated at 20 °C (dark) for 2 weeks, before starting the exposure of the nematodes. The samples from the dose-response experiment were stored in deep-freeze.

The soil samples were extracted by adding 10 $cm^3$ water and 25 $cm^3$ ethyl acetate, followed by shaking for 1 hour. A subsample of the clear ethyl acetate layer was put in a vial and the solvent was evaporated. The drying residue was taken up in HPLC-water and diluted, if necessary. The fungicides were analysed by HPLC, as described before.

The results of the analyses for carbendazim in the framework of this dose-response experiment with nematodes are given in Table 7.7.

*Table 7.7 Measurements of carbendazim for the dose-response experiment with nematodes by Wageningen University, received in September 2000. Contents expressed on the basis of dry soil*

| Tube No | Content (µg/g) | Tube No | Content (µg/g) |
|---|---|---|---|
| 1 | 0.00 | 44 | 1.80 |
| 2 | 0.00 | 45 | 1.81 |
| 3 | 1.90 | 46 | 1.86 |
| 4 | 2.03 | 47 | 2.27 |
| 5 | 3.76 | 48 | 2.38 |
| 6 | 3.77 | 49 | 4.92 |
| 7 | 7.56 | 50 | 5.08 |
| 8 | 7.91 | 51 | 8.17 |
| 9 | 19.9 | 52 | 8.23 |
| 10 | 20.9 | 53 | 8.35 |
| 11 | 27.2 | 54 | 8.52 |
| 12 | 27.0 | 55 | 14.8 |
| 25 | 0.00 | 56 | 16.3 |
| 26 | 0.00 | 57 | 21.6 |
| 27 | 0.83 | 58 | 22.8 |
| 28 | 0.99 | 59 | 0.80 |
| 29 | 2.78 | 60 | 0.75 |
| 30 | 2.50 | 61 | 2.06 |
| 31 | 3.20 | 62 | 2.18 |
| 32 | 3.04 | 63 | 2.58 |
| 33 | 7.46 | 64 | 2.56 |
| 34 | 7.87 | 65 | 5.26 |
| 35 | 8.90 | 66 | 4.94 |
| 36 | 10.3 | 67 | 8.37 |
| 37 | 14.7 | 68 | 9.55 |
| 38 | 14.1 | 69 | 9.73 |
| 39 | 20.2 | 70 | 10.4 |
| 40 | 22.3 | 71 | 19.5 |
| 41 | 26.0 | 72 | 19.9 |
| 42 | 25.3 | 73 | 31.9 |
| 43 | 0.69 | 74 | 33.5 |

Table 7.8 presents the results of the analyses for iprodione in the framework of the dose-response experiment with nematodes by Wageningen University.

*Table 7.8. Measurements of iprodione for the dose-response experiment with nematodes by Wageningen University, received in September 2000. Contents expressed on the basis of dry soil*

| TubeNo | Content(µg/g) | TubeNo | Content(µg/g) |
|---|---|---|---|
| 13 | 0.00 | 61 | 116 |
| 14 | 0.00 | 62 | 87.6 |
| 15 | 60.2 | 63 | 55.5 |
| 16 | 61.7 | 64 | 73.0 |
| 17 | 186 | 65 | 82.4 |
| 18 | 177 | 66 | 75.4 |
| 19 | 363 | 67 | 72.1 |
| 20 | 316 | 68 | 23.9 |
| 21 | 418 | 69 | 70.7 |
| 22 | 435 | 70 | 66.4 |
| 23 | 397 | 71 | 82.9 |
| 24 | 503 | 72 | 92.4 |
| 25 | 0.00 | 73 | 82.7 |
| 26 | 0.00 | 74 | 83.2 |
| 59 | 77.8 | 75 | 59.5 |
| 60 | 72.2 | 76 | 77.4 |

## 7.7 Discussion

The addition of comparatively high contents of the fungicides to the soils presented problems. Application in aqueous solution would require the addition of large volumes, followed by repeated and time-consuming drying of the soil. When the powder itself is mixed with the soil, there is a great chance that larger particles of the fungicide remain present (Chapter 6). Then the exposure of the animals to the fungicide may be lower than expected.

A comparatively high concentration of the fungicides can be applied with acetone. Fungicide solution in acetone was first applied to a sub-batch of 10% of the mass of soil in each object (repeatedly if needed). After the acetone had evaporated, the fungicide in the sub-batch of soil was mixed intensively with the other 90% of the soil batch. This method assures that the fungicides are finely distributed throughout the soil, without maltreating the whole soil batch with acetone.

The concentrations of the pesticides in the solutions should be kept substantially below the reported solubility in the solvent. Otherwise, the pesticides dissolve difficultly and they tend to precipitate from the solvent. At somewhat higher concentrations, repeated shaking and ultrasonic vibration is needed to keep the pesticide in solution.

Fast transformation of iprodione in aqueous solution was found to occur in contact with certain types of HPLC vials and glass tubes. The peak of a transformation product appeared in the chromatogram. Cleaning of the vials did not resolve this problem. Certain glass types could thus not be used in the experiments and analyses.

Some transformation may have occurred while the samples were not in the freezer (e.g. during transport). If this was limited to a few days, the extent of transformation (which can be estimated) was likely to be small.

Most pesticides are stable in the freezer at minus 20 °C. However, there are a few reports on possible transformation (of one or two other pesticides) under such conditions. There was no indication that there was a problem with carbendazim and iprodione stability when freezing the soil samples from the incubation experiments for up to a few months. Especially when freezing soil samples for a long time it is advisable to check for the stability of the pesticide.

Some soil samples were received in rather dry condition; they were dried after the experiment. This may result in volatilization of moderately to highly volatile compounds. However, the vapour pressures of carbendazim (0.09 mPa at 20 °C) and iprodione (0.0005 mPa at 25 °C) are low (Tomlin, 1997). Drying the soil before extraction and analysis is not needed; it may reduce the extractibility of the fungicides at a later stage.

In new experiments, it is advisable to include measurements on the extent to which the fungicides are distributed homogeneously within the soil batches. A possible heterogeneous distribution of the fungicide may be expected to increase the variability in the dose-response results.

The procedures used in the dose–response experiments by the various researchers were different. The conditions were adapted to obtain optimum conditions for the test organisms (e.g. the optimal moisture condition). Further the habits in the different laboratories in dealing with the soil batches seem to be different. In some exposure experiments, it is necessary to subject the soil to specific treatments beforehand. An example is the microwave treatment of the soil before the experiments with nematodes. Such treatments may affect the adsorption, transformation and bioavailability of the toxicants in the period of exposure of the test animals.

On the basis of the details on the procedures in each of the dose-response experiments, the results of the present chemical analyses can be evaluated in more detail. The present results are expected to play an important part in the interpretation of the dose-response experiments with the various soil animals.

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