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$$P = c_n - \frac{k_1 c_n}{(k_1 + k_2 c_n)e^{k_1 t} - k_2 c_n} + c_b(1 - e^{-k_3 t})$$

Inge S. Fomsgaard

Ph.D. dissertation

The mineralisation of pesticides in surface and subsurface soil – in relation to temperature, soil texture, biological activity and initial pesticide concentration

Ministry of Food, Agriculture and Fisheries

Danish Institute of Agricultural Sciences

Ph.D. dissertation

The mineralisation of pesticides in surface and subsurface soil – in relation to temperature, soil texture, biological activity and initial pesticide concentration

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Foreword

The work behind this Ph. D. thesis was performed at the Royal Danish School of Pharmacy, Institute of Analytic Chemistry with Dr. Eng., Dr. Scient. Sven Erik Jørgensen as my principal tutor. I have been an external Ph.D. student, as I have been employed at the Danish Institute of Agricultural Sciences (former the Danish Institute of Plant and Soil Science) during the whole period. Dr. Agro. Arne Helweg has been my co-tutor. The Ph.D. project was financed by the Danish Ministry of Agriculture, the Danish Agricultural and Veterinary Research Council and the Danish Environmental Protection Agency.

I wish to express my particular gratitude to Dr. Sven Erik Jørgensen, whose inspiration in relation to the use of mathematical models as a scientific tool has been inestimable, and to Dr. Arne Helweg, a pioneer in the field of degradation of pesticides in soil, who generously allowed me to draw on his experience.

I owe a debt of gratitude to laboratory technician Helle Priess, who joined me during the whole period with her clever effort in the technical field. My thanks are also due to laboratory technicians Alice Binder and Marianne Nielsen for their clever efforts in parts of the project. Our laboratory technician students were engaged in the project during their period of training. With their sympathy and kindness, all my other colleagues at the laboratory created an environment, which to a high extent promoted my project. Jette Jeppesen, Ellen Marie Bentsen, Sonja Graugaard, Maria Lange Lehmann, Phyllis Rasmussen and Mariann Naundrup assisted me with their linguistic qualifications. Henny Rasmussen drew all the figures I asked for, both in the papers and in the synopsis with patience and creativity, and Kristian Kristensen was a fabulous sparring partner in the area of statistics and modelling.

Last but not least very special thanks to my husband, Gunnar, whose love and patience was – and is – most essential to me.

The structure of the thesis

The present Ph.D. thesis consists of a synopsis and 6 scientific papers. The thesis begins with the Background (Chapter 1) for the performed research and a declaration of the Purpose (Chapter 2). After that comes chapter 3, Summary of the included scientific papers; chapter 4, Synopsis of the investigated pesticides; chapter 5, Synopsis of the results and the discussions; and finally chapter 6, Synopsis of the conclusions. A short description of the used methods can be seen in the summaries of the included publications. A thorough description of the methods can be seen in the publications. In chapter 5, Synopsis of the results and the discussions, the most important results from the included scientific papers are presented and discussed in relation to each other and to other current research in the area. Each of the scientific papers comprises results and discussion both of the pesticide mineralisation kinetics and of the pesticide mineralisation rate. The synopsis was therefore divided into two sub-chapters 5.1, The mineralisation kinetics in relation to geo-environmental factors and 5.2, The mineralisation rate in relation to geo-environmental factors. In both sub-chapters, I discuss the results of the publications in relation to each other. In chapter 6, Synopsis of the conclusions the results are connected to the purpose of the project and the needs in future research in the area is discussed.

The scientific papers, which are included in the thesis, are numbered **I-VI** and enclosed in their full length in chapter 11, Enclosures. I had the main responsibility for the work in papers **I, II, III, V** and **VI** and the responsibility for the collation of data in paper **IV**.

With the presentation of a summary (chapter 3) of the included scientific papers I hope the reader will benefit from reading the synopsis even without reading the whole papers. It is of course impossible to express in a short summary what was described in a paper of i.e. 23 pages. The reader will therefore only have the full benefit of the thesis, if the included scientific papers are read firstly.

1. Background

1.1 Pesticides in the environment

For a number of years the tendency of the development of Danish agriculture has been to raise the efficiency and the yield. Consequently, a total amount of 184,011, 622 of kg of pesticides (measured as active ingredient) of the 200 most-sold compounds was used in the years 1956-1993 (Miljøstyrelsen, 1997a). The approval of pesticides for use in Danish agriculture is undertaken by the Danish Environmental Protection Agency according to the guidelines given in "Rammer for vurdering af plantebeskyttelsesmidler" (Miljøstyrelsen, 1994). Beyond a broad toxicological assessment, the persistence in and sorption to soil of the compounds are evaluated. Compounds considered to be leachable to ground water are not approved.

It was thus against most people's expectations that pesticide residues above 0.1 $\mu g\ l^{-1}$ were detected in ground water in the extensive ground water monitoring programmes performed in the beginning of the '90s (GEUS, 1997). Yet Helweg (1984) already pointed out the risk.

The numerous finds of pesticide residues in ground water raised public concern and prompted the population to demand reduction of ground water contamination. A substantial need for investigating the fate of pesticides in soil was built up. The guidelines for approval of pesticides (Miljøstyrelsen, 1994) concerning degradation rates of pesticides, demand that degradation studies must be performed in three different plough layer soils and that in none of the three soils the half life time, DT_{50}, must exceed 90 days.

A number of indicators have been developed where the purpose is to rank the risk for pesticides leaching into ground water on the basis of few parameters for each compound. The GUS—index ranks pesticides exclusively on the basis of inherent properties, degradability (measured as half-life time, DT_{50}) and sorption (measured as K_{OC}), and thus, gives a measure of the leaching potential. Lindhardt et al. (1998) showed that ranking of 11 pesticides according to the GUS-index on the basis of the half-life times and K_{OC} values reported to the Danish Environmental Protection Agency, resulted in a high degree of uncertainty, caused by the great variation in the data material.

1.2. Modelling

Models have been applied in natural science as long as natural science has existed. Newton's laws i.e. are models which describe the influence of gravity on bodies (Jørgensen, 1994). A mathematical description of the kinetics according to which a pesticide is degraded is also a model, like the mathematical description of the process that takes place when a pesticide is sorbed to soil is a model. Such models have been used in pesticide research for decades. With the progress of advanced computer techniques, it has become possible to develop dynamic models, which can simulate transport and degradation of xenobiotic compounds in the environment. Dynamic models, used to simulate pesticide leaching to ground water, consist of

a number of submodels, all of them containing mathematical descriptions of the processes which are relevant to transport pesticides in soil into the actual compartments. Such submodels could be: a soil model describing the structure of the soil layers, a hydrological model describing the transport of water through the soil layers, an evapo-transpiration model, a run-off model, a model for pesticide sorption, a model for pesticide degradation, a model for pesticide application and a plant growth model. Of 9 frequently used dynamic pesticide leaching models, PRZM-2, PRZM, PELMO, GLEAMS, PESTLA, VARLEACH, LEACHM, MACRO and PLM, 8 of them use a submodel for pesticide degradation which assume that the degradation follows first order kinetics (Boesten et al., 1995). They all assume that the degradation rate depends on temperature and/or soil depth and/or soil water content.

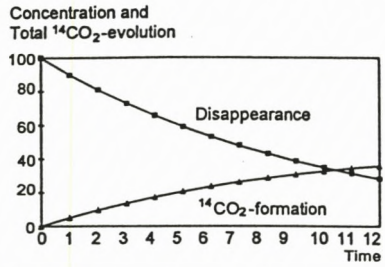
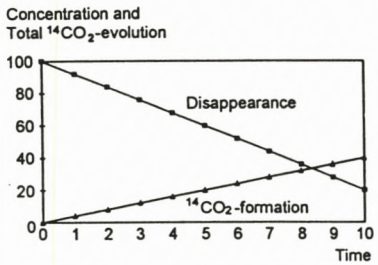


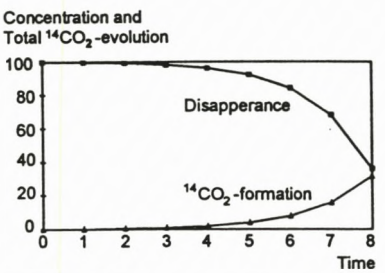
FIGURE 3 General diagramme of models for pesticide degradation.
A. first-order reaction kinetics[30]

Rate equation: $-\frac{dc}{dt}=kc$ Disappearance: $c=c_0e^{-kt}$. Formation: $P=c_0(1-e^{-kt})$.



B. Zero-order reaction kinetics[27]

Rate equation: $-\frac{dc}{dt}=k_0$ Disappearance : $c=c_0-k_0t$. Formation: $P=k_0t$.



C. Degradation with growth

Rate equation, log. growth[33]: $-\frac{dc}{dt}=k_1c(c_0+x_0-c)$

Disappearance, log. growth[33]: $c=\frac{c_0+x_0}{1+\left(\frac{x_0}{c_0}\right)e^{k_1(c_0+x_0)t}}$

Formation, log. growth[33]: $P=c_0-\frac{c_0+x_0}{1+\left(\frac{x_0}{c_0}\right)e^{k_1(c_0+x_0)t}}$

Rate equation, exp. growth[27]: $-\frac{dc}{dt}=ke^{rt}$

Disappearance, exp. growth[27]: $c=c_0-\frac{k(e^{rt}-1)}{r}$

Formation, exp. growth[27]: $P=k\frac{(e^{rt}-1)}{r}$

Figure 1.1. Diagrams describing degradation of pesticides, where disappearance of pesticide as well as formation of mineralisation product $^{14}CO_2$ is shown. A. First order kinetics, B. Zero order kinetics and C. Kinetics with growth of micro-organisms. (Figure 3 from **IV**).

1.3. Degradation kinetics

Depending on experimental conditions, degradation rates are reported differently. When the amount of mineralisation product $^{14}CO_2$ evolved from ^{14}C-labelled pesticides during time is measured, either the amount of evolved $^{14}CO_2$ after a certain number of days, or the rate constant from the kinetic process describing the mineralisation is reported. When the amount of residual pesticide during time is measured, the kinetic process is analysed, and the rate constant for the kinetic process is reported. Figure 1.1 shows three examples of a graphical presentation of a degradation process, where the amount of residual parent compound as well as the formation of mineralisation product (i.e. $^{14}CO_2$) is measured. In the following text, I will distinguish between measurement of degradation, degradation studies and measurement of mineralisation, mineralisation studies both for my own studies as for studies from references. Yet in more general discussions I will use the expression degradation.

In the literature, the kinetics for the degradation of xenobiotic compounds in the environment has been described with two different bases. One basis is the power-rate models; the other is the hyperbolic rate model, as described by Hamaker (1972), whose publication still is used by many authors as a reference. The power rate model, when residues of parent compound are measured, is expressed as

$$-\frac{dc}{dt} = kc^n \qquad (1.1)$$

where c is the concentration of pesticide, k is the rate constant, and n is the order of reaction.

The hyperbolic rate model, which is founded on Michaelis-Menten enzyme kinetics, is expressed as

$$-\frac{dc}{dt} = \frac{k_1 c}{(k_2 + c)} \qquad (1.2)$$

where c is the concentration of pesticide, k_1 is the maximum reaction rate obtained with growing concentrations, and k_2 is a pseudo-equilibrium constant, also called the half saturation constant. The hyperbolic rate model was used to describe the degradation of pesticides in aquatic solutions by for instance Simkins & Alexander (1984) and Schmidt et al. (1985). Hamaker (1972) and Parker & Doxtader (1982) used the hyperbolic rate model to describe the degradation of pesticides in soil, while Scow et al. (1986), Brunner & Focht (1984) and Jacobsen & Pedersen (1992) excluded the use of the hyperbolic rate model, either based on the results of empirical trials or on the theoretical considerations that in the very complex soil environment an equilibrium situation would never occur.

The power rate model was often used to describe the degradation of pesticides in soil. Kempson-Jones & Hance (1979) and Moorman & Harper (1989) determined both the rate constant k and the reaction order n in their degradation studies, where the reaction order was $\neq 1$.

In many published studies it was shown that the degradation followed first order kinetics where n=1 in the power rate model. In other published studies it was assumed that the degradation process should follow first order kinetics, the degradation only depending on the pesticide concentration.

With basis in a first order degradation, the degradation rate can be given as half life time (DT_{50} or $t_{½}$)

$$-\frac{dc}{dt} = k \cdot c \qquad (1.3)$$

In integrated form it is written as

$$c(t) = c_0 \cdot e^{-k \cdot t} \qquad (1.4)$$

where
$c(t)$ = concentration of pesticide at time t, c_0 = start concentration of pesticide, k = rate constant

In the pesticide approval procedure (Miljøstyrelsen, 1994) degradation rates are mainly given as half-life time, which traces back to first order kinetics. Fomsgaard (1998) went through all the degradation studies reported to the Danish Environmental Protection Agency for 12 compounds and stated that in many cases the degradation did not follow first order kinetics. Pseudo first order kinetics, empirical one and a half order kinetics, half order kinetics or power rate kinetics with $n \neq 1$ were reported. In several cases, both in the pesticide approval documents (Fomsgaard, 1998), in Hill & Schaalje (1985) and in Gustafson & Holden (1990) it was shown that the degradation reaction took place in several compartments, for which reason the mathematical description consisted in several first order terms and then no longer was a simple first order process.

Another important reason for not being able to anticipate a first order degradation, is that a lag-phase can occur in the degradation process. In the lag-phase the micro-organisms adapt to the presence of the pesticide, whereupon the micro-organisms achieve energy from the degradation process. Achieving energy, the micro-organisms grow and the degradation rate increases (Torstensson, 1988). Linders et al. (1994) examined reports for 243 pesticides and corrected the reported half-life times, leaving out the lag-phase.

Figure 1.1 shows three theoretical examples of degradation and mineralisation, a) according to first order kinetics, b) according to zero kinetics and c) according to kinetics with growth. The last example, kinetics with growth, could for instance be logistic or exponential growth, as explained in the text of the figure.

No matter how pesticide degradation is measured, quantifying residues of parent compound or formation of a mineralisation product, i.e. $^{14}CO_2$ from ^{14}C-labelled pesticides, it is essential to analyse and describe the kinetics of the process, to get to know the parameters needed for comparing degradation rates.

1.4. Degradation rate

In **I**, I examined published degradation studies in subsoil and concluded that for many pesticides, results from subsoil were very limited and that the great variation in techniques, used for the studies, made it difficult to compare the results. Almost all the published studies were performed with pesticide concentrations, which were unrealistic compared to theoretical concentrations of pesticides in subsoil after normal agricultural use of the pesticides. At the same time, the studies were not performed in concentrations high enough to simulate situations, where the pesticides could be present in subsoil because of point-source contamination.

Many studies showed that soil depth influenced the degradation rate of the pesticides due to different chemical and biological conditions at varying depth (Dictor et al., 1992; Mueller et al., 1992; Minton et al., 1990; Moorman & Harper, 1989; Pothuluri et al., 1990).
The effect of temperature on the degradation rate of pesticide was also well described (Helweg, 1993; Helweg, 1987; Matoba et al., 1995; Ismail & Lee, 1995; Walker et al., 1996). Walker et al. (1996) reviewed a high number of degradation studies and calculated mean Q_{10} values. Water content of the soil was also often described as having importance for the degradation rate (Ismail & Lee, 1995; Helweg, 1993; Helweg, 1987), as well as the initial concentration of pesticide (Helweg, 1993; Helweg, 1987; Reffstrup et al., 1998; Jacobsen & Pedersen, 1992; Parker & Doxtader, 1982; Mueller et al., 1992). Temperature, soil water content and soil depth are the factors that are considered to have an effect on the pesticide degradation rate in the 9 frequently used dynamic leaching models, PRZM-2, PRZM, PELMO, GLEAMS, PESTLA, VARLEACH, LEACHM, MACRO and PLM (Boesten et al., 1995). Microbial activity/biomass was often measured and related to the degradation rate of pesticides, either directly or through the variation in soil depth (Anderson, 1984; Torstensson & Stenström, 1986; Monrozier et al., 1993; Dictor et al., 1992). The amount of organic matter - also frequently related to soil depth – and its influence on the degradation of xenobiotic compounds was also investigated (Reddy et al., 1995; Duah-Yentumi & Kuwatzuka, 1980; Greer & Shelton, 1992; Knaebel et al., 1994). Walker (1976a, 1976b, and 1976c) studied the effect of temperature, soil water content and pesticide concentration in soil from plough layer and developed simulation models.

FOCUS (FOrum for the Co-ordination of pesticide fate models and their USe) – a working party under the EU (Boesten et al., 1995) compared 9 dynamic leaching models and concluded that a better description of subsoil degradation is necessary, to improve the predictive value of the models. As regards degradation rates, the models only include the

dependence on soil depth and/or temperature and/or soil water content, and 8 of 9 models assume that the degradation follows first order kinetics.

A better description of the degradation kinetics not only in subsoil, but also in plough layer, is required for the further development of the dynamic leaching models in order to obtain a better simulation of actual conditions. Knowing the great variation in degradation rates and the high number of factors which influence the degradation, makes it necessary to study the concurrent influence of these factors and develop models, which describe this influence.

2. Purpose

The aim of this Ph. D. project was to describe the mineralisation kinetics for pesticides in soil and to develop and validate a predictive mineralisation model, which could describe the effect of external geo-environmental factors on the mineralisation.

A complete validation of the model would entail a study of all relevant pesticides in combination with all relevant external geo-environmental factors. That is obviously not possible within a realistic time scale. Four characteristic pesticides were therefore selected on the grounds of consumption, the risk of leaching and the formation of metabolites. The degradation of these pesticides was investigated for various combinations of external geo-environmental factors, which support the model. The following studies were carried out:

- the mineralisation of 4 characteristic pesticides: mecoprop, bentazon, isoproturon and maneb, and 1 metabolite of maneb, ETU
- the effect on mineralisation of
  - the depth of soil (0-75 cm)
  - the biological activity
  - the concentration of the pesticide
  - the content of organic carbon in the soil
  - the temperature
  - the texture of the soil
  - the content of nutritive salts in the soil

3. Summary of the scientific papers I-VI

I.

I.S. Fomsgaard, 1995. Degradation of pesticides in subsoil - a review of methods and results. International Journal of Environmental Analytical Chemistry 58, 231-245.

As the starting point of the whole project, in the present paper, I examined the published degradation studies in soil from the unsaturated zone. During the search for published studies it became clear that the number of studies performed in subsoil was substantially smaller, than the number of studies from the plough layer. Only for the pesticides: mecoprop, 2,4-D, atrazin, alachlor, aldicarb, carbofuran, linuron, oxamyl, methomyl, MCPA, dichlorprop, monochlorprop, dichlorphenol, TCA, parathion, metribuzin, metolachlor and fenamiphos, subsoil studies were reported.

Going through the publications, I firstly focused on the used methods to be able to make a clear decision on which method to use myself. The main part of the published studies was performed as laboratory studies, where the soil was dried and sieved prior to the studies. In almost all the examined studies, pesticides were added to soil in concentrations of 0,5-5 $\mu g\ g^{-1}$ dry soil. In part of the studies the degradation was followed by measuring the concentrations of residual pesticide during time. In another part of the studies, ^{14}C-labelled compounds were used and the degradation (mineralisation) was followed by measuring the development of $^{14}CO_2$. In the last-mentioned studies the mineralisation rate was reported as % $^{14}CO_2$ developed after a certain number of days, which made a comparison of mineralisation rates between studies difficult.

I concluded that degradation studies in the laboratory should be performed under conditions that are as close to natural circumstances as possible. Disturbance of the micro-organisms that cause the degradation of most pesticides is avoided, by using undisturbed soil samples from subsoil. Degradation studies should be performed in concentrations close to the actual probable concentrations in soil, since the concentration of the pesticide can effect the micro-organisms and thus the degradation rate of the pesticide. Furthermore, I have concluded that studies which include both the development of $^{14}CO_2$ from ^{14}C-labelled pesticide and those where residual concentrations of the pesticide are determined, should be preferred. Measuring $^{14}CO_2$, the total mineralisation of the compound is measured. Since soil is a very heterogeneous environment, it is furthermore of high importance that degradation studies are performed with replicates.

Secondly, while going through the published studies I focused on the description of degradation kinetics and the influence of the soil environment on the degradation rate. Part of the studies showed that the degradation followed first order kinetics, while another part

simply assumed that the degradation followed first order kinetics. Those studies reported the degradation rate by means of the half-life time. However, some of the studies showed that the degradation did NOT follow first order kinetics, but could better be described by means of empirical equations or by means of a power rate model with a reaction order different from first order.The factors mentioned as being of importance for the degradation rate of the pesticides were biological activity, soil temperature, soil water content, oxygen content in soil air, pesticide concentration, soil type and adaptation of the micro-organisms after repeated use of a pesticide. The influence of all the mentioned factors on the degradation of the pesticides were all described separately.

The reading of the published studies made me give a high priority to the following a) to study the degradation in plough layer as well as in subsoil b) to perform my own studies in subsoil with undisturbed soil samples c) to perform the degradation studies at very low concentrations (which was only possible by using ^{14}C-labelled compounds) when the fate of the pesticides after normal agricultural use was to be examined and d) to find a standardised way both to perform the studies and to describe the results.

II.

I.S. Fomsgaard, 1997. Modelling the mineralisation kinetics for low concentrations of pesticides in surface and subsurface soil. Ecological Modelling, 102, 175-208.

In the present publication I described mineralisation studies for mecoprop, ETU and bentazon in concentrations as low as 0.04 $\mu g\ g^{-1}$, 0.07 $\mu g\ g^{-1}$ and 0.08 $\mu g\ g^{-1}$, respectively. Such low concentrations of pesticides could typically be present in subsoil after normal agricultural use of the compounds. The low concentrations will naturally be present in plough layer too, at a certain time after the application. The experiments were performed with the addition of ^{14}C-labelled compounds to the soil samples, incubation at 10°C, and the mineralisation was followed by measuring the evolved $^{14}CO_2$. The data was shown as mineralisation curves, depicting the accumulated amount of $^{14}CO_2$ as a function of days. The experimental set-up made it possible to follow the mineralisation at very low concentrations and to follow the mineralisation in each single soil sample, without the need for taking out aliquots (which is not possible when the soil samples are incubated with a natural water content). The experiments were performed in sandy soil, sampled two different years in three different fields in Denmark and in soil with varying clay content from Germany, Spain and Italy. Degradation studies were performed in soil from plough layer (0-15 cm) as well as in soil from varying depths, determined by the ground water level at each site. The degradation studies in soil from plough layer were performed in disturbed samples (mixed and sieved), while undisturbed soil samples were used for the studies in subsoil. In most of the published studies, in which $^{14}CO_2$ was measured, the results were presented as % $^{14}CO_2$ evolved after a certain number of days. Such results are difficult to compare. Therefore the purpose of the work in the present paper was to find a mathematical model, which could describe the

mineralisation. A number of mathematical descriptions of transformation kinetics, used by other authors either to describe degradation of pesticides or degradation of other xenobiotic compounds, were tried out with the mecoprop, ETU and bentazon mineralisation data. 18 different models were used of which some were a) models without growth of micro-organisms, expressing cometabolic degradation (first order, zero order, two-compartment first order, combined first + zero order, sequential first order, simple Monod kinetics) b) models with growth of micro-organisms (linear growth, logarithmic growth, logistic growth, exponential growth) and c) empirical models. Models, which were used in the literature to describe the disappearance of added pesticide, were converted to express the formation of mineralisation product $^{14}CO_2$ coming from ^{14}C-labelled pesticides. The models were fitted to the data using non-linear regression.

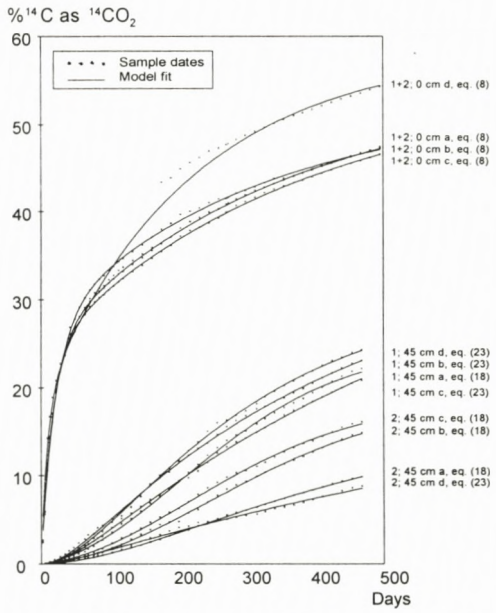


Figure 3.1. Mineralisation of 0.08 μg g^{-1} ^{14}C-bentazon in Spanish soil. Depth (0 and 45 cm), replicate number and model equation from paper **II** shown at the end of each curve. (Figure 2b from **II**).

The work showed that a number of mathematical models were useful for the description of the mineralisation of the investigated pesticides. There was a clear difference between mineralisation kinetics with and without growth. With few exceptions, the mineralisation kinetics in plough layer soil samples showed to be without growth of micro-organisms (cometabolic mineralisation). In subsoil – with only a few exceptions as well – the mineralisation kinetics showed to be with growth of micro-organisms (metabolic). The cometabolic mineralisation is seen in the depicted mineralisation curve as a gradual rise in the

accumulated amount of $^{14}CO_2$ followed by a deflection whereupon the curve turns flat (Figure 3.1. – 0 cm). The metabolic mineralisation results in mineralisation curves having a sigmoidal form, with a slow evolution in the beginning (lag-phase), followed by a heavy increase in the formation of $^{14}CO_2$ for a period whereupon the mineralisation curves turns flat (Figure 3.1. - 45 cm). Mecoprop can be degraded both through cometabolic and metabolic processes according to literature. It was therefore not a surprise that cometabolic degradation occurred in plough layer where a high amount of other organic matter is present, which can serve as a nutrient for the micro-organisms that carry out the degradation of mecoprop. Bentazon and ETU have been reported as compounds which can only go through cometabolic degradation. The metabolic mineralisation seen in the present study could be due to the formation of metabolites, which could give rise to propagation of micro-organisms. Another explanation could be that degradation of low concentrations of bentazon and ETU do follow kinetics with growth of micro-organisms because of the special living conditions for micro-organisms in subsoil (e.g. presence of dormant micro-organisms).

III.

I.S. Fomsgaard, H. Johannesen, J. Pitty & R. Rugama, 1998. Degradation of ^{14}C-maneb in sediment from a Nicaraguan estuary. The International Journal of Environmental Studies B, 55, 175-198.

In a joint project in Nicaragua the influence on an estuarine environment of the use of pesticide in the drainage basin were to be investigated. As part of the project, mineralisation studies with maneb in sediment from the estuary were performed. Maneb is used as a fungicide in Nicaragua in the cultivation of onions, beans, maize, tobacco and tomatoes. The mineralisation studies were performed with a concentration of maneb of 0.08 μg g^{-1} sediment (dry weight), covered by 2 cm water from the sampling site. The studies were performed both in July and September 1994. Sediment samples were taken at five sites, site 1 closest to the mouth of the river and site 5 in the upper part of the river. A number of mathematical models taken from **II** were fitted to the mineralisation data. The best fit to the mineralisation experiments from the month of July was obtained with mathematical models describing kinetics with growth. The experiments from September could be described with both models describing kinetics with growth as well as with no-growth kinetic models. The model

$$P = c_0 - \frac{k_1 c_0}{(k_1 + k_2 c_0)e^{k_1 t} - k_2 c_0} \qquad (3.1)$$

where

P = amount of pesticide mineralised at time t (% ^{14}C as $^{14}CO_2$)
c_0 = total amount of pesticide converted to $^{14}CO_2$ according to the modelled process
k_1 = rate constant

k_2 = rate constant
t = time in days

was fitted to all the data curves, and a two-way analysis of variance (ANOVA) was applied to compare c_0 and k_1. A significant difference in c_0 between stations was seen where the highest amount of ^{14}C-maneb was converted to $^{14}CO_2$ at the sites 4 and 5 in the upper part of the river, probably because of a higher biological activity. After 150 days of incubation all the soil samples from the experiments from September were extracted and the amount of residual ^{14}C-ETU in the extract was measured. Less than 2.72 % was found. Therefore it must be concluded that the formation of ETU as a metabolite, after the use of maneb in the drainage basin, is not a problem.

IV.

A. Helweg, I.S. Fomsgaard, T.K. Reffstrup & H. Sørensen, 1998. Degradation of different pesticide concentrations in soil. International Journal of Environmental Analytical Chemistry, 70(1-4) 133-148.

Pesticides can appear in the soil environment with a wide range of concentration depending on which source they come from. Normal agricultural use of pesticides (except the new low-dose products) can lead to concentrations in the plough layer of about 1 μg g^{-1} and to concentrations in subsoil several times lower. Direct contamination, waste disposal by burying etc. can lead to extremely high concentrations of pesticides in the soil environment. Many published studies have shown that the degradation rate of pesticides is influenced by the initial concentration of the compound. In the present study, mineralisation studies in concentrations from 0.0005 to 5000 μg g^{-1} for ^{14}C-mecoprop and from 0.001 to 5000 μg g^{-1} ^{14}C-isoproturon for isoproturon were performed. All studies were performed in soil from plough layer as well as in soil from 40-60 cm's depth. The experiments were performed by adding the ^{14}C-labelled compound to soil samples, where each soil sample was mixed thoroughly with the compound. The mineralisation was measured by collecting $^{14}CO_2$ and measuring it in a scintillation counter. The mineralisation curves, total amount of ^{14}C as $^{14}CO_2$ in function of number of days, were fitted to a number of mathematical models, models describing kinetics with growth as well as models describing kinetics without growth The mineralisation of mecoprop at 0.0005 μg g^{-1} followed first order kinetics both in plough layer and in subsoil. The rate constant for the mineralisation process was significantly higher in plough layer than in subsoil, probably due to the lower biological activity in subsoil. Kinetics with growth was seen at the concentration of 5 μg g^{-1} in both plough layer and subsoil and of 50 μg g^{-1} in plough layer. At the concentrations of 50 and 500 μg g^{-1} in subsoil and of 5000 μg g^{-1} in plough layer (concentrations which probably have been toxic to the micro-organisms) the mineralisation was very slow. For that reason the curves could not be fitted with any model. The mineralisation of isoproturon followed kinetics without growth in all concentrations. At the highest initial concentration of isoproturon the mineralisation was slow, but measurable. A clear difference between mineralisation rates in soil from different

depths was seen. The mineralisation in plough layer was faster than in soil from 40-60 cm's depth, probably because of lower biological activity in subsoil.

V.

I.S. Fomsgaard and K. Kristensen, 1998. ETU mineralisation in soil under influence of organic carbon content, temperature, concentration and depth. Toxicological and Environmental Chemistry 70, 195-220.

ETU is a toxic water-soluble metabolite of the EBDC fungicides. In the present study the mineralisation of ^{14}C-ETU was investigated in soil from two different depths (15 and 75 cm), with two different concentrations of ^{14}C-ETU (0.07 and 2.0 μg g^{-1}), at two temperatures (5 and 20°C), and with two different amounts of soluble carbon in the soil (a) natural: only water was added to obtain 50% WHC and b) added: an extract of soluble soil-carbon was added to obtain 50% WHC). Undisturbed soil samples were used, and the mineralisation was followed by collection and quantifying the mineralisation product $^{14}CO_2$. In the review in paper **I** it was said that in most published subsoil degradation studies, the influence of geo-environmental factors on the degradation was investigated, one factor at a time. Contrary to this, the present study was designed as a 2^4 factor study, where the effect on the mineralisation rate of ^{14}C-ETU was investigated for all the combinations of the two levels of all 4 factors.

As described earlier, other publications have shown that is has not been possible to find a mathematical expression, which could describe the mineralisation of xenobiotic compounds under all circumstances. My conclusions in the papers **II**, **III**, and **IV** were that one mathematical model which could describe all types of mineralisation curves did not exist. Yet with different mathematical expressions it was possible to describe all types of mineralisation curves. One of the mathematical models, used in papers **II**, **III** and **IV** to describe mineralisation with growth of micro-organisms, was further developed in the present study to

$$P = c_n - \frac{k_1 c_n}{(k_1 + k_2 c_n)e^{k_1 t} - k_2 c_n} + c_b(1 - e^{-k_3 t}) \qquad (3.2)$$

where

P = total amount of evolved mineralisation product ($^{14}CO_2$), equivalent to the total amount of mineralised ^{14}C-pesticide at time t (measured as % ^{14}C evolved as $^{14}CO_2$)

c_n = total % ^{14}C-pesticide converted to $^{14}CO_2$ according to the Liu & Zhang-model (Liu & Zhang, 1986)

c_b = total % ^{14}C-pesticid converted to $^{14}CO_2$ according to the first order model

k_1, k_2 =rate constants

$k_1 = k(m_0 + \lambda c_n)$

$k_2 = -k\lambda$

k_3 = rate constant for the first order process

λ = growth rate of the micro-organisms

m_0 = the initial amount of degradation micro-organisms

The model consists of two terms, in which the first term describes the mineralisation of the ^{14}C-ETU that was available for immediate decomposition, while the second term describes the first order mineralisation of organic material, in which ^{14}C from the pesticide had been built in. Two variants of the model were used. Model A, in which $c_n + c_b = 100\%$ and model B, in which $c_n + c_b < 100\%$. The developed model showed to fit all mineralisation curves, both when a long lag-phase followed by a vigorous rise was seen and when the inspection of the curve resulted in doubts whether a lag-phase was present or not. A very useful mineralisation model was thus developed, which probably would be useful for the description and the comparison of the mineralisation curves of other xenobiotic compounds.

Since the study was built up as a 2^k factor study, it was possible to investigate the interaction effects between the examined factors. A three-way interaction effect depth*concentration*temperature was seen for both c_n, k_1, k_2 and λ/m_0. The interaction between two of those factors (depth*concentration, depth*temperature, concentration*temperature) thus depended on the level of the third factor. The three-way interaction effect depth*concentration*suspension was only seen for c_n, while a two-way interaction effect concentration*suspension was seen for k_1 and k_2. It was thus concluded that an investigation of the interactive effects of the factors which influence the mineralisation rate, is important when the mineralisation of ^{14}C-ETU is to be described. Such investigations would probably be important for other compounds as well.

VI.

I.S. Fomsgaard and K. Kristensen, 1999. Influence of microbial activity, organic carbon content, soil texture and soil depth on mineralisation rates of low concentrations of ^{14}C-mecoprop – development of a predictive model. Ecol. Mod. 122, 45-68.

This publication continues the modelling work carried out in paper **I**. We worked with all the mecoprop mineralisation studies from Danish soil and used the model which was developed in paper **V**:

$$P = c_n - \frac{k_1 c_n}{(k_1 + k_2 c_n)e^{k_1 t} - k_2 c_n} + c_b(1 - e^{-k_3 t}) \qquad (3.2)$$

where

P = total amount of evolved mineralisation product ($^{14}CO_2$), equivalent to the total amount of mineralised ^{14}C-pesticide at time t (measured as % ^{14}C evolved as $^{14}CO_2$)
c_n = total % ^{14}C-pesticide converted to $^{14}CO_2$ according to the Liu & Zhang-model (Liu & Zhang, 1986)
c_b = total % ^{14}C-pesticide converted to $^{14}CO_2$ according to the first order model
k_1, k_2 =rate constants
$k_1 = k(m_0 + \lambda c_n)$

$k_2 = -k\lambda$

k_3 = rate constant for the first order process

λ = growth rate of the micro-organisms

m_0 = initial amount of degrading micro-organisms

The parameters c_n, c_b, k_1, k_2 and k_3 were estimated. The model gave useable fits for the mecoprop mineralisation curves from plough layer as well as from subsoil and thus fulfilled our expectations after having seen the applicability of the model in paper **V**. The relation between parameters c_n, c_b, k_1, k_2, k_3 and the following geo-environmental factors: biological activity, MPN-number, % humus, % clay, % sand, % silt, pH, soluble C (mg kg^{-1}) NO_3-N (mg kg^{-1}), NH_4-N (mg kg^{-1}), soil depth was determined. The biological activity was determined as the rate constant $k_{1\text{-}naac}$ for the mineralisation of ^{14}C-Na-acetate. It was concluded, that the mineralisation of mecoprop at the same temperature and initial concentration depends both on humus content, clay content, biological activity and soil depth. A full model describing the parameters c_n, c_b, k_1, k_2, k_3, as a function of soil depth, % humus, biological activity and % clay was constructed and subsequently validated with mecoprop mineralisation results from German soils. The used functions were:

$$\log_e k_1 = \alpha_1 + \beta_1 \cdot \log_e \frac{\%humus}{100 - \%humus} + \beta_2 \cdot ploughlayer \qquad (3.3)$$

$$k_2 = \alpha_2 + \beta_3 \cdot ploughlayer \qquad (3.4)$$

$$k_3 = \alpha_3 + \beta_4 \cdot ploughlayer \qquad (3.5)$$

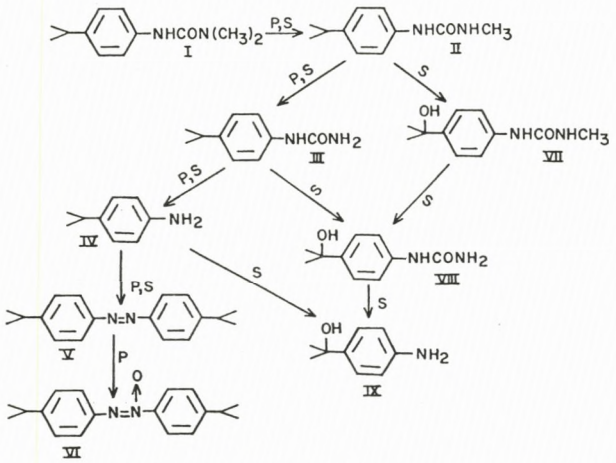
$$\log_e \frac{c_n}{100 - c_n} = \alpha_n + \beta_5 \cdot \log_e k_1\_naac \qquad (3.6)$$

$$\log_e \frac{cb}{100 - cb} = \alpha_b + \beta_6 \cdot \log_e \frac{\%clay}{100 - \%clay} + \beta_7 \cdot ploughlayer \qquad (3.7)$$

The prediction of the initial lag-phase resulting from the model was not optimal, however, the model was able to predict the time, when no mecoprop was left. It was thus shown that it is possible to develop a mineralisation model for mecoprop, with which the mineralisation rate can be predicted on the basis of easier measurable parameters.

4. Synopsis of the investigated pesticides

The choice of which pesticides to investigate was based on their use, leachability and/or existence of important metabolites. Formerly mecoprop was used in high amounts in Denmark in the autumn. Degradation at low temperatures is therefore particularly interesting.



Proposed pathway of photodecomposition (P) and degradation of isoproturon in soil(s)

I = N-(4-isopropyl phenyl) -N',N'-dimethyl urea
II = N-(4-isopropyl phenyl) -N'-methyl urea
III = N-(isopropyl phenyl) urea
IV = 4-(isopropyl) aniline
V = 4,4'-diisopropyl azobenzene
VI = 4,4'-diisopropyl azoxybenzene
VII = N-(4-(2-hydroxyisopropyl) phenyl) -N'-methylurea
VIII = 4-(2-hydroxy isopropyl) phenyl urea
IV = 4-(2-hydroxy isopropyl phnyl) aniline

Figure 4.1. Proposed metabolic pathways for isoproturon in soil (S) and by photolysis (P) (Kulshrestha & Singh, 1995. (Copyright Gordon and Breach Publishers. Reproduced with permission).

Bentazon has proved to be leachable in several countries, among them Sweden (Kreuger, 1997), for which reason the degradation rate must be known both in plough layer and in subsoil. According to the literature, the fungicide maneb is readily degraded to the metabolite ETU, which is supposed to be carcinogenic (National Research Council, 1987). The amount of isoproturon, used in Denmark, has increased during the last years, because isoproturon in many crops replaced the phenoxyacids, of which the use was restricted years ago.

Table 4.1 shows the chosen pesticides, the sales figures, toxicity and physical-chemical properties. Proposed degradation pathways for the first steps of the degradation of isoproturon and bentazon are shown in Figures 4.1 and 4.2. Figure 4.3 shows the total mineralisation of mecoprop and Figure 4.4 shows proposed pathways for the total mineralisation of the EBDC fungicides maneb and mancozeb.

Table 4.1. Summary of properties of investigated pesticides.

Name	Mecoprop	Bentazon	Maneb	Ethylene thiourea (ETU)	Isoproturon
Chemical formula					
Systematic name IUPAC	2-(4-chloro-o-tolyloxy)propionic acid	3-isopropyl-1H-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide	manganese ethylenebis(dithiocarbamate)	4,5-dihydroimidazole-2(3H)-thione	3-p-cumenyl-1,1-dimethylurea
Herbicide(H), Fungicide(F), Metabolite(M)	H	H	F	M	H
Sales figures (kg a.i.) Denmark 1994	291.402 [b]	69.352 [b]	256.072 [b]	-	346.767 [b]
Sales figures (kg a.i.) Denmark 1995	313.287 [c]	93.326 [c]	251.246 [c]	-	453.168 [c]
Sales figures (kg a.i.) Denmark 1996	210.838 [d]	80.577 [d]	0 [d]	-	523.547 [d]
Sales figures Nicaragua	-	-	?	-	-
Vapour pressure	0.00031 Pa[a]	0.00046 Pa[a]	neg[a]	-	
Solubility water (25°C)	0.62 g l^{-1} [a]	0.5 g l^{-1} [a]	0.16 g l^{-1} [a]	20 g l^{-1} [g]	0.055 g l^{-1} [a]
Use	Cereals/ grass for seed production [h]	cereals/grass [h]	onions, beans, maize, tobacco, tomatoes [i])	-	cereals [h]
LD_{50} mammals mg kg^{-1}	1166 [a]	1710 [a]	750 [a]	-	1800 [a]
LD_{50} birds mg kg^{-1}	5000 [a]	5000 [a]	5000 [a]	-	1000 [a]
LD_{50} worms mg kg^{-1}		1000 [a]	1000 [a]	-	1000 [a]
LC_{50} fish mg l^{-1}	100 [a]	100 [a]	0.22 [a]	-	9 [a]
LC_{50} daphnia mg l^{-1}	420 [a]	125 [a]	0.52 [a]	-	100000 [a]
LC_{50} algae mg l^{-1}	220 [a]	47 [a]	0.43 [a]	-	0.03[a]
Cancerogenity	-	-	-	cancerogenic and teratogenic in laboratory animals[f]	-
References	[b])Miljøstyrelsen, 1995; [c])Miljøstyrelsen, 1996; [d])Miljøstyrelsen, 1997; [a])PC-Planteværn: [f])National Research Council, 1987; [g]) IUPAC, 1977				

[h]) In Denmark, [i]) In Nicaragua

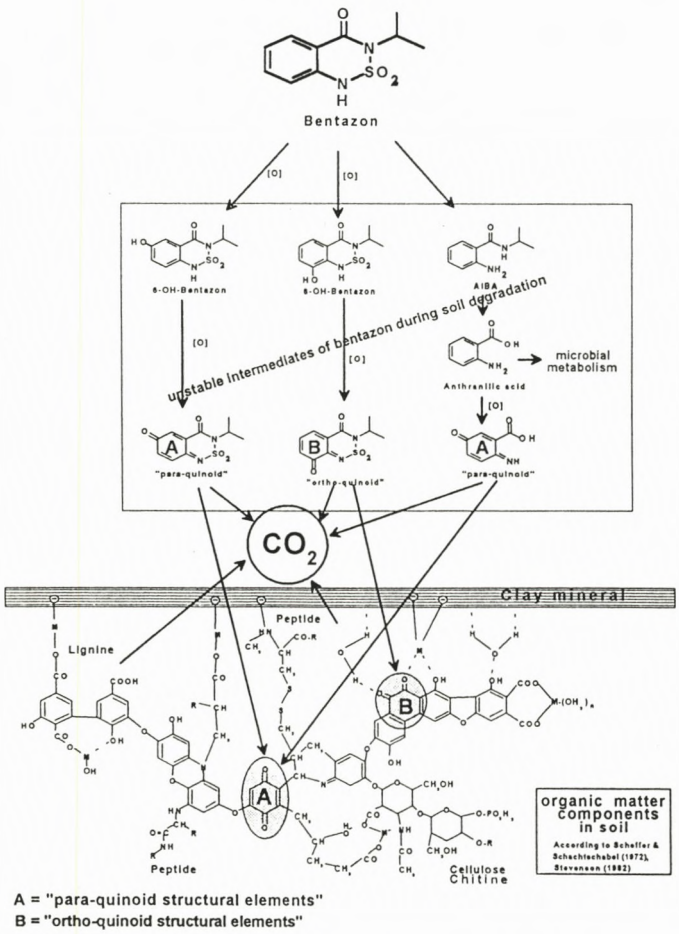


Figure 4.2. Proposed degradation pathways for bentazon in soil (Huber & Otto, 1994). (Copyright Springer-Verlag. Reproduced with permission).

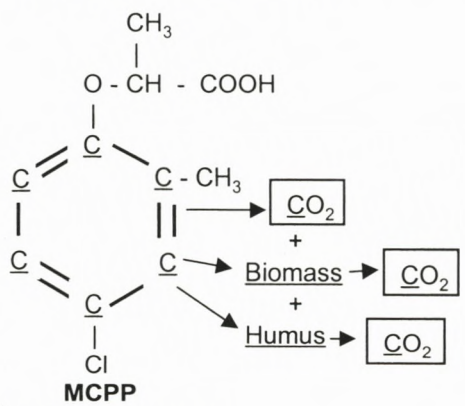


Figure 4.3. Mineralisation of mecoprop in soil.

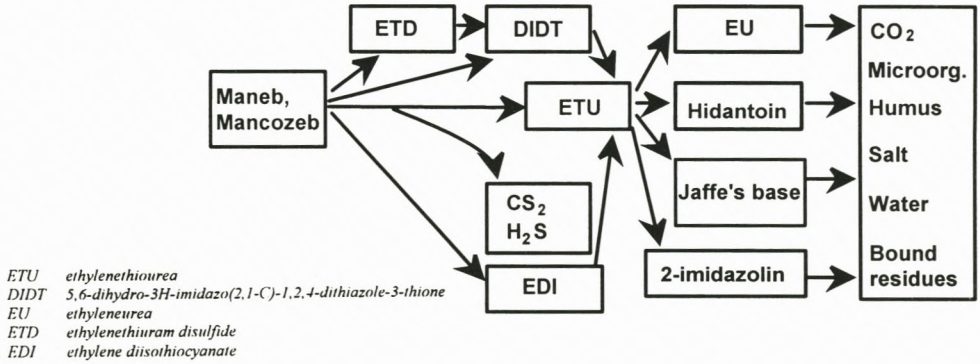


Figure 4.4. Degradation pathways for EBDC fungicides in soil. Adapted from WHO (1988) and IUPAC (1977). (Figure 12 from **III**).

5. Synopsis of the results and the discussions

5.1. The mineralisation kinetics in relation to geo-environmental factors

5.1.1. The experiments

The pesticide mineralisation experiments in soil were performed by following the evolution of $^{14}CO_2$ from ^{14}C-labelled pesticide. Soil samples from the plough layer were mixed with the added ^{14}C-labelled pesticide and incubated in Erlenmeyer flasks. Subsoil samples were taken as undisturbed samples in metal tubes and the ^{14}C-labelled pesticide was added to the soil by injection or by dripping before incubation. The use of ^{14}C-labelled pesticide assured that the compound could be quantified in very low concentrations. Investigations of the degradation of pesticides in very low concentrations are important, since other studies earlier showed that the degradation of xenobiotic compounds in the soil environment develops differently at different initial concentrations of the compound (Helweg, 1993; Stenström, 1988; Jacobsen & Pedersen, 1992). Common agricultural use of a pesticide leads normally only to low concentrations of the pesticide in soil below the plough layer. A representative aliquot of a soil sample cannot be taken during incubation. The use of ^{14}C-labelled compound can therefore furthermore assure that the mineralisation in each soil sample can be followed during time, by measuring the evolved $^{14}CO_2$. Figure 5.1, 5.2 and 5.3 show the mineralisation of mecoprop in German soil, bentazon in German soil and ETU in Danish soil.

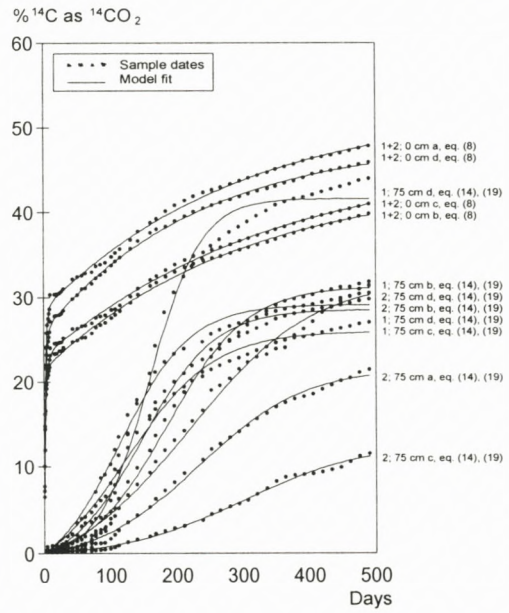


Figure 5.1. Mineralisation of 0.04 µg g^{-1} ^{14}C-mecoprop in German soil. Depth (0 and 75 cm), replicate and equation number from paper **II** is shown at the end of each curve (Figure 1h from **II**).

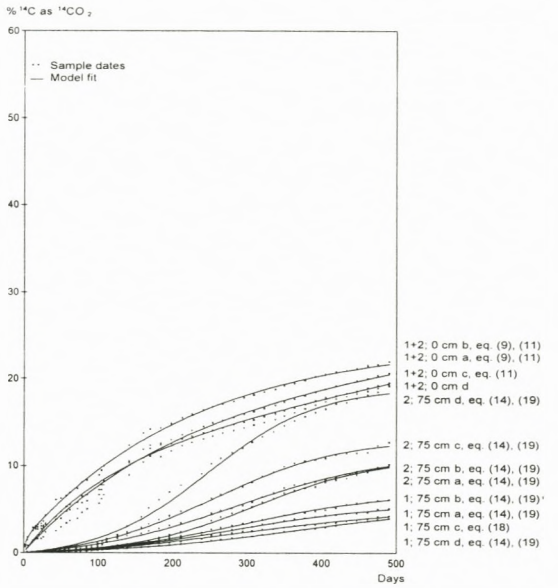


Figure 5.2. Mineralisation of 0.08 μg g^{-1} bentazon in German soil. Depth (0 and 75 cm), replicate and equation number from paper **II** is shown at the end of each curve (Figure 2c from **II**).

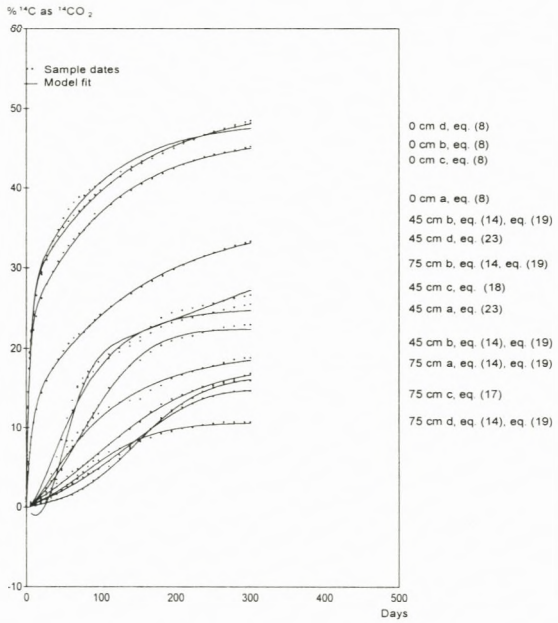


Figure 5.3. Mineralisation of 0.07 μg g^{-1} ETU in Danish soil (FB3_II). Depth (15, 45 and 75 cm), replicate and equation number from paper **II** is shown at the end of each curve (Figure 3b from **II**).

5.1.2. The choice of kinetic models

Brunner & Focht (1984), Jacobsen & Pedersen (1992), Stenström (1988), Scow et al. (1986) and Reffstrup et al. (1998) all stated that under varying circumstances (several soil depths, different concentrations) a one and only mathematical model describing all the mineralisation curves did not exist. Brunner & Focht (1984) declared that with their different models at least one of them fitted to their mineralisation curves. Jacobsen & Pedersen (1992), Scow et al. (1986) and Reffstrup et al. (1998) pointed out that even if they used the models given by Brunner & Focht (1984) or the further developed models presented by Focht & Brunner (1985) cases were seen in which no model at all could fit the mineralisation curves. The existence of a mathematical description of the mineralisation is of decisive importance for a trustworthy comparison of mineralisation rates. Therefore, I examined whether a number of theoretically as well as empirically founded mathematical expressions, used in the literature to describe the degradation kinetics of xenobiotic compounds in soil and water (Table 5.1), were useful for describing the mineralisation kinetics for mecoprop (**II**), ETU (**II**), bentazon (**II**) and maneb (**III**) in extremely low concentrations, and of mecoprop and isoproturon in a wide range of concentrations (**IV**). Table 5.1 furthermore contains the models which were subsequently developed (**V** and **VI**).

When the degradation is followed by measuring the evolved $^{14}CO_2$ from ^{14}C-labelled pesticide, the results cover the total mineralisation of the added, as already explained. However, the mineralisation is likely to proceed through several steps or to occur in various compartments. The very simple mathematical expressions will therefore seldom be useful for the description of mineralisation results.

Table 5.1. Mathematical models used to describe the mineralisation of ^{14}C-labelled pesticides in paper **II, III, IV, V,** and **VI.**

Model	Equation		Equation no. in paper	Growth/no-growth	References
First order	$P = c_0(1 - e^{-kt})$	(5.1)	**II**-6, **IV**-2	no growth	Knaebel et al., 1994; Simon et al., 1992; Mueller et al., 1992;
	P = amount of pesticide mineralised at time t (% ^{14}C as $^{14}CO_2$) c_0 = total amount of pesticide converted to $^{14}CO_2$ k = rate constant for the mineralisation t = time in days				
First order (c_0=100)	$P = 100(1 - e^{-kt})$	(5.2)	**II**-7	no growth	**II**
	P = amount of pesticide mineralised at time t (% ^{14}C as $^{14}CO_2$) k = rate constant for the mineralisation t = time in days				
Two-compartment first order (c_1+c2<100)	$P = c_1(1 - e^{-k_1 t}) + c_2(1 - e^{-k_2 t})$	(5.3)	**II**-8, **III**-1, **IV**-3	no growth	Scow et al., 1986; Hill & Schaalje, 1985;
	P = amount of pesticide mineralised at time t (% ^{14}C as $^{14}CO_2$) c_1 = total amount of pesticide converted to $^{14}CO_2$ through one first order process c_2 = total amount of pesticide converted to $^{14}CO_2$ through another first order process k_1, k_2 = rate constant for the two first order processes t = time in days				
Two-compartment first order (a+(1-a))=1)	$P = 100((1 - ae^{-tk_1} - (1 - a)e^{-tk_2})$	(5.4)	**II**-9, **III**-2	no growth	**II, III**
	P = amount of pesticide mineralised at time t (% ^{14}C as $^{14}CO_2$) a = fraction of the total amount of pesticide converted to $^{14}CO_2$ through one first order process k_1, k_2 = rate constants for the two first order processes t = time in days (can be replaced with eq. (3) with c_1+c_2=100)				

Model	Equation		Equation no. in paper	Growth/no-growth	References
Three half order without growth	$P = c_0(1 - e^{-k_1 t}) + k_0 t$ P = amount of pesticide mineralised at time t (% ^{14}C as $^{14}CO_2$) c_0 = total amount of pesticide converted to $^{14}CO_2$ through the first order process k_1 = rate constant for the first order process k_0 = rate constant for the zero order process t = time in days	(5.5)	**II**-11, **III**-3, **IV**-4	no growth	Brunner & Focht, 1984; Scow et al., 1986; Knaebel et al., 1994;
Three half order with linear growth	$P = c_0(1 - e^{-k_1 t - (k_2 t^2/2)}) + k_0 t$ P = amount of pesticide mineralised at time t (% ^{14}C as $^{14}CO_2$) c_0 = total amount of pesticide converted to $^{14}CO_2$ through the first order process k_1 = rate constant for the first order process k_0 = rate constant for the zero-order process k_2 = the growth rate constant for the micro-organisms t = time in days	(5.6)	**II**-10, **III**-5	growth	Brunner & Focht, 1984; Scow et al., 1986; Knaebel et al., 1994;
Simple Monod without growth	$-\frac{dc}{dt} = \frac{k_1(c_0 - c)}{k_m + (c_0 - c)}$ c= amount of pesticide at time t c_0= initial amount of the pesticide k_1= rate constant for the degradation k_m= the half saturation constant t = time in days	(5.7)	**II**-12	no growth	Simkins & Alexander, 1984
Logistic growth	$P = c_0 - \frac{c_0 + x_0}{1 + (\frac{x_0}{c_0})e^{k_1(c_0 + x_0)t}}$ P = amount of pesticide mineralised at time t (% ^{14}C as $^{14}CO_2$) c_0 = total amount of pesticide converted to $^{14}CO_2$ through the first order process x_0 = the amount of substrate (pesticide) necessary to produce the initial population density k = rate constant for the mineralisation t = time in days	(5.8)	**II**-14, **III**-6, **IV**-5	growth	Simkins & Alexander, 1984; Albrechtsen & Winding, 1992

Model	Equation		Equation no. in paper	Growth/no-growth	References
Logistic growth + zero order	$$P = c_0 - \frac{c_0 + x_0}{1 + (\frac{x_0}{c_0})e^{k_1(c_0+x_0)t}} + k_0 t$$	(5.9)	**IV**-6	growth	**IV**
	P = amount of pesticide mineralised at time t (% ^{14}C as $^{14}CO_2$) c_0 = total amount of pesticide converted to $^{14}CO_2$ through the first order process x_0 = the amount of substrate (pesticide) necessary to produce the initial population density k = rate constant for the mineralisation k_0 = rate constant for the zero order process t = time in days				
Logarithmic growth	$$P = -x_0(1 - e^{\mu_{max} t})$$	(5.10)	**II**-15	growth	Simkins & Alexander, 1984
	P = amount of pesticide mineralised at time t (% ^{14}C as $^{14}CO_2$) x_0= the amount of substrate (pesticide) necessary to produce the initial population density μ_{max} = maximum specific growth rate t= time in days				
zero order	$$P = k_0 t$$	(5.11)	**II**-13, **IV**-1	no growth	Simkins & Alexander, 1984 Schmidt et al., 1985
	P = amount of pesticide mineralised at time t (measured as % ^{14}C as $^{14}CO_2$) k_0 =rate constant t = time in days				
Logistic growth	$$P = c_0 - c_0(\phi(e^{rt} - 1) + 1)^{-\frac{k}{r}}$$	(5.12)	**II**-16	growth	Schmidt et al., 1985
	P = amount of pesticide mineralised at time t (% ^{14}C as $^{14}CO_2$) c_0 = total amount of pesticide converted to $^{14}CO_2$ through the modelled process k = rate constant ϕ= relation between initial population density and maximum population density r= maximum specific growth rate t= time in days				
Linear growth, low concentration of pesticide	$$P = c_0(1 - e^{-k_1 t - (k_2 t^2/2)})$$	(5.13)	**II**-18, **III**-8	growth	Schmidt et al., 1985
	P = amount of pesticide mineralised at time t (% ^{14}C as $^{14}CO_2$) c_0 = total amount of pesticide converted to $^{14}CO_2$ through the modelled process k_1 =rate constant for the first order process k_2 =linear growth rate constant for micro-organisms				

Model	Equation		Equation no. in paper	Growth/no-growth	References
	t = time in days				
Exponential growth, low concentration of pesticide	$P = c_0 - c_0 e^{-(k/r)(e^{rt}-1)}$	(5.14)	**II**-17, **III**-7, **IV**-9	growth	Schmidt et al., 1985;
	P = amount of pesticide mineralised at time t (% ^{14}C as $^{14}CO_2$) c_0 = total amount of pesticide converted to $^{14}CO_2$ through the modelled process k = rate constant for the exponential mineralisation r = maximum specific growth rate t = time in days				
Exponential growth + zero order, low concentrations of pesticide	$P = c_0 - c_0 e^{-(k/r)(e^{rt}-1)} + k_0 t$	(5.15)	**IV**-10	growth	**IV**
	P = amount of pesticide mineralised at time t (% ^{14}C as $^{14}CO_2$) c_0 = total amount of pesticide converted to $^{14}CO_2$ trough the modelled exponential process k = rate constant for the exponential process k_0 = rate constant for the zero order process r = maximum specific growth rate t = time in days				
Exponential growth, high concentration of pesticide	$P = k\frac{(e^{rt}-1)}{r}$	(5.16)	**IV**-11	growth	Schmidt et al., 1985;
	P = amount of pesticide mineralised at time t (% ^{14}C as $^{14}CO_2$) k = rate constant for the exponential process r = maximum specific growth rate t = time in days				
Exponential growth + zero order, high concentration of pesticide	$P = k\frac{(e^{rt}-1)}{r} + k_0 t$	(5.17)	**IV**-12	growth	**IV**
	P = amount of pesticide mineralised at time t (% ^{14}C as $^{14}CO_2$) k = rate constant for the exponential process k_0 = rate constant for the zero order process r = maximum specific growth rate t = time in days				
Empirical	$P = kt^{1/2} + a$	(5.18)	**II**-20		Stenström, 1988
	P = amount of pesticide mineralised at time t (% ^{14}C as $^{14}CO_2$)				

Model	Equation		Equation no. in paper	Growth/no-growth	References
	k = constant a = constant t = time in days				
Empirical	$P = k_1 t + k_2 t^{½} + a$ P = amount of pesticide mineralised at time t (% ^{14}C as $^{14}CO_2$) k_1 =constant k_2 = constant a = constant t = time in days	(5.19)	**II**-21		Stemström, 1988
Empirical + exponential growth	$P = k_1 t^{½} + \frac{qN_0}{k_2}(e^{k_2 t} - 1)$ P = amount of pesticide mineralised at time t (% ^{14}C as $^{14}CO_2$) k_1 =constant k_2 = rate constant for growth of micro-organisms q = maximum specific rate of metabolism N_0 = initial amount of micro-organisms t = time in days	(5.20)	**II**-21	growth	Stenström, 1988
First order sequential	$P = c_0(1 + \frac{k_1 e^{-k_2 t} - k_2 e^{-k_1 t}}{k_2 - k_1})$ P = amount of pesticide mineralised at time t (% ^{14}C as $^{14}CO_2$) c_0 = total amount of pesticide converted to $^{14}CO_2$ through the first order process k_1, k_2 = rate constant for the two first order processes t = time in days	(5.21)	**II**-23, **III**-4	no growth	Jandell Scientific, 1994
Logistic growth	$P = c_0 - \frac{k_1 c_0}{(k_1 + k_2 c_0)e^{k_1 t} - k_2 c_0}$ P = amount of pesticide mineralised at time t (% ^{14}C as $^{14}CO_2$) c_0 = total amount of pesticide converted to $^{14}CO_2$ through the modelled process k_1 = rate constant k_2 = rate constant t = time in days	(5.22)	**II**-19, **III**-9, **IV**-7	growth	Liu & Zhang, 1986; Liu et al., 1988;

Model	Equation	Equation no. in paper	Growth/no-growth	References
Logistic growth + zero order	$$P = c_0 - \frac{k_1 c_0}{(k_1 + k_2 c_0)e^{k_1 t} - k_2 c_0} + k_0 t \quad (5.23)$$	**IV**-8	growth	**IV**
	P = amount of pesticide mineralised at time t (% ^{14}C as $^{14}CO_2$) c_0 = total amount of pesticide converted to $^{14}CO_2$ by the modelled process k_1 = rate constant k_2 = rate constant k_0 = rate constant for the zero order process t = time in days			
Logistic growth + first order ($c_n + c_b = 100$)	$$P = c_n - \frac{k_1 c_n}{(k_1 + k_2 c_n)e^{k_1 t} - k_2 c_n} + c_b(1 - e^{-k_3 t}) \quad (5.24)$$	**V**-1,2,3, **VI**-2,3,4	growth	**V,VI**
	P = total amount of mineralisation product ($^{14}CO_2$), equivalent to the total amount of mineralised ^{14}C-pesticide at time t (measured as % ^{14}C evolved as $^{14}CO_2$) c_n = total % ^{14}C-pesticide converted to $^{14}CO_2$ according to the Liu & Zhang-model c_b = total % ^{14}C-pesticid converted to $^{14}CO_2$ according to the first order model k_1, k_2 =rate constants $k_1 = k(m_0 + \lambda c_n)$ $k_2 = -k\lambda$ k_3 = rate constants for the first order process λ = growth rate of micro-organisms m_0= initial amount of degrading micro-organisms			
Logistic growth + first order $c_n + c_b < 100$	$$P = c_n - \frac{k_1 c_n}{(k_1 + k_2 c_n)e^{k_1 t} - k_2 c_n} + c_b(1 - e^{-k_3 t}) \quad (5.25)$$	**V**-1,2,3, **VI**-2,3,4	growth	**V,VI**
	P = total amount of mineralisation product ($^{14}CO_2$), equivalent to the total amount of mineralised ^{14}C-pesticide at time t (measured as % ^{14}C evolved as $^{14}CO_2$) c_n = total % ^{14}C-pesticide converted to $^{14}CO_2$ according to the Liu & Zhang-model c_b = total % ^{14}C-pesticid converted to $^{14}CO_2$ according to the first order model k_1, k_2 =rate constants $k_1 = k(m_0 + \lambda c_n)$ $k_2 = -k\lambda$ k_3 = rate constants for the first order process λ = growth rate of micro-organisms m_0= initial amount of degrading micro-organisms			

Brunner & Focht (1984), Scow et al. (1986), Stenström (1988), Knaebel et al. (1994) and Simon et al. (1992) developed and/or used their mathematical models for $^{14}CO_2$ mineralisation curves, the rest of the mathematical expressions in Table 5.1 are my conversions of the mathematical expressions that originally were presented by the authors for the description of degradation curves, in which residual concentrations were measured. The models were fitted with non-linear regression using the procedure NLIN from SAS (SAS, 1989; SAS, 1990; SAS, 1996). In some cases the fit resulted in asymptotically correlation coefficients between parameter estimates which were so high that it was impossible to estimate the parameters of the model. In other cases, parameters, which would only have meaning with positive estimates, turned out with negative estimates. Both situations lead to the conclusion that the model could not be employed. Consequently no mean square values are shown in the Summary tables in papers **II, III** and **IV**. The comparison of various models, resulting in useable fits for one data set, was carried out by comparing the mean square values. The best model came out with the lowest mean square value.

Table 5.2 shows a summary of all my experiments, giving information about the sampling site, the pesticide, the incubation technique, the concentration of the pesticide, the sampling depth, the soil texture, the incubation temperature and growth/no growth. Growth/no growth indicates whether the non-linear regression resulted in useable fits with models, which include growth of micro-organisms. Furthermore the number of paper, **II**, **III** or **IV**, where the results were presented, is shown in the table. Some of the results from paper **II** were used again in paper **VI**.

5.1.3. Mineralisation kinetics in plough layer at low concentrations

ONLY models not including growth of micro-organisms gave useable fits for ETU (0.07 μg g^{-1}) in all soil samples in plough layer from Fladerne Bæk, Denmark, for bentazon (0.08 μg g^{-1}) and mecoprop (0.04 μg g^{-1}) in plough layer from Italy, Spain and Germany, and for mecoprop (0.04 μg g^{-1}) at 3 out of 5 sampling sites/times in Denmark (Fladerne Bæk) (**II**) (The samples in paper **II** are identified as for example mcfb1_I which means mecoprop, Fladerne Bæk, field 1, time of sampling I). The mecoprop experiments were later used in paper **VI**, in which they were only identified by site/time of sampling, for example FB1_I (Table 5.2). Vinter (1998) counted the number of micro-organisms in soil from varying depths from Fladerne Bæk by staining with acridin orange and found that the number of micro-organism reduced from 10^9 to 10^7, moving from plough layer to 1 meter's depth. In soil from all sites/depths and times of sampling from Fladerne Bæk, I subsequently determined the number of mecoprop-degrading micro-organisms by a ^{14}C-MPN-method (**VI**). No significant difference between depths (0, 45 and 75 cm) was seen for the MPN numbers. Mecoprop has been reported as degradable my metabolism (Lappin et al., 1985). When a cometabolic

Table 5.2. Summary of experiments from papers **II**, **III** and **IV** showing sampling site, the pesticide, the concentration of the pesticide, the incubation technique, the concentration of the pesticide, the soil texture, the incubation temperature and growth/no growth for the applied models.

Paper	Site	Pesticide	Incubation technique	Conc. $\mu g\ g^{-1}$	Soil depth cm	Humus %	Clay %	pH	Inc. temp. °C	No growth	Growth
II	FB1_I	mecoprop	disturbed	0.04	15	3.1	5.0	7.1	10	+	
II	FB1_II	mecoprop	disturbed	0.04	15	2.8	3.6	6.9	10		+
II	FB3_I	mecoprop	disturbed	0.04	15	2.7	3.2	6.6	10	+	
II	FB3_II	mecoprop	disturbed	0.04	15	2.8	4.0	6.7	10		+
II	FB4-I	mecoprop	disturbed	0.04	15	4.7	4.6	5.2	10	+	
II	FB1_I	mecoprop	undisturbed	0.04	45	0.9	3.0	6.2	10		+
II	FB1_II	mecoprop	undisturbed	0.04	45	0.3	2.5	6.3	10		+
II	FB3_I	mecoprop	undisturbed	0.04	45	0.8	2.3	6.1	10		+
II	FB3_II	mecoprop	undisturbed	0.04	45	0.9	3.5	5.6	10		+
II	FB4-I	mecoprop	undisturbed	0.04	45	5.1	3.6	5.2	10		+
II	FB1_I	mecoprop	undisturbed	0.04	75	0.2	2.5	5.9	10		+
II	FB1_II	mecoprop	undisturbed	0.04	75	0.1	2.1	6.4	10		+
II	FB3_I	mecoprop	undisturbed	0.04	75	0.2	1.4	6.1	10	+	
II	FB3_II	mecoprop	undisturbed	0.04	75	0.3	3.0	5.5	10		+
II	FB4-I	mecoprop	undisturbed	0.04	75	0.5	2.1	5.6	10		+
II	Italy	mecoprop	disturbed	0.04	0	3.6	16.6	7.2	20	+	
II	Spain	mecoprop	disturbed	0.04	0	3.5	30.5	8.1	20	+	
II	Germany	mecoprop	disturbed	0.04	0	2.1	7.9	7.4	20	+	
II	Italy	mecoprop	undisturbed	0.04	50	0.6	20.9	7.5	15		+
II	Italy	mecoprop	undisturbed	0.04	50	0.6	21.1	7.1	15		+
II	Spain	mecoprop	undisturbed	0.04	45	3.7	30.1	8.2	15	+	
II	Spain	mecoprop	undisturbed	0.04	45	*			15	+	
II	Germany	mecoprop	undisturbed	0.04	75	0.2	9.7	6.6	10		+
II	Germany	mecoprop	undisturbed	0.04	75	0.1	6.9	7.1	10		+
II	Italy	bentazon	disturbed	0.08	0	3.6	16.6	7.2	20	+	
II	Spain	bentazon	disturbed	0.08	0	3.5	30.5	8.1	20	+	
II	Germany	bentazon	disturbed	0.08	0	2.1	7.9	7.4	20	+	
II	Italy	bentazon	undisturbed	0.08	50	0.6	20.9	7.5	15		+
II	Italy	bentazon	undisturbed	0.08	50	0.6	21.1	7.1	15		+
II	Spain	bentazon	undisturbed	0.08	45	3.7	30.1	8.2	15		+
II	Spain	bentazon	undisturbed	0.08	45	*			15		+
II	Germany	bentazon	undisturbed	0.08	75	0.2	9.7	6.6	10		+
II	Germany	bentazon	undisturbed	0.08	75	0.1	6.9	7.1	10		+
II	FB1_II	ETU	disturbed	0.04	15	2.8	3.6	6.9	10	+	
II	FB3_II	ETU	disturbed	0.04	15	2.8	4.0	6.7	10	+	
II	FB1_II	ETU	undisturbed	0.04	45	0.3	2.5	6.3	10		+
II	FB3_II	ETU	undisturbed	0.04	45	0.9	3.5	5.6	10		+
II	FB1_II	ETU	undisturbed	0.04	75	0.1	2.1	6.4	10		+
II	FB3_II	ETU	undisturbed	0.04	75	0.3	3.0	5.5	10		+
III	Nicaragua	maneb	dist. sed.	0.08	0-10	0.2	1.7	9.0	25		+
III	Nicaragua	maneb	dist. sed.	0.08	0-10	1.1	1.7	8.9	25		+
III	Nicaragua	maneb	dist. sed.	0.08	0-10	1.7	6.9	7.6	25		+
III	Nicaragua	maneb	dist. sed.	0.08	0-10	0.9	4.7	7.7	25		+
III	Nicaragua	maneb	dist. sed.	0.08	0-10	0.4	4.7	7.9	25		+
IV	Flakkebjerg	mecoprop	disturbed	0.0005	0-30	2.9	14.3	6.1	15	+	
IV	Flakkebjerg	mecoprop	disturbed	5.0	0-30	2.9	14.3	6.1	15		+
IV	Flakkebjerg	mecoprop	disturbed	50	0-30	2.9	14.3	6.1	15		+
IV	Flakkebjerg	mecoprop	disturbed	5000	0-30	2.9	14.3	6.1	15	no fit	no fit

Paper	Site	Pesticide	Incubation technique	Conc. $\mu g\ g^{-1}$	Soil depth cm	Humus %	Clay %	pH	Inc. temp. °C	No growth	Growth
IV	Flakkebjerg	mecoprop	disturbed	0.0005	30-60	0.3	22.9	6.5	15	+	
IV	Flakkebjerg	mecoprop	disturbed	5.0	30-60	0.3	22.9	6.5	15		+
IV	Flakkebjerg	mecoprop	disturbed	50	30-60	0.3	22.9	6.5	15	no fit	no fit
IV	Flakkebjerg	mecoprop	disturbed	500	30-60	0.3	22.9	6.5	15	no fit	no fit
IV	Flakkebjerg	isoproturon	disturbed	0.001	0-30	2.9	14.3	6.1	15	+	
IV	Flakkebjerg	isoproturon	disturbed	5.0	0-30	2.9	14.3	6.1	15	+	
IV	Flakkebjerg	isoproturon	disturbed	50	0-30	2.9	14.3	6.1	15	+	
IV	Flakkebjerg	isoproturon	disturbed	5000	0-30	2.9	14.3	6.1	15	+	
IV	Flakkebjerg	isoproturon	disturbed	0.001	30-60	0.3	22.9	6.5	15	+	
IV	Flakkebjerg	isoproturon	disturbed	5.0	30-60	0.3	22.9	6.5	15	+	
IV	Flakkebjerg	isoproturon	disturbed	50	30-60	0.3	22.9	6.5	15	+	
IV	Flakkebjerg	isoproturon	disturbed	5000	30-60	0.3	22.9	6.5	15	+	

degradation of mecoprop at low concentrations in plough layer was seen in this study, the explanation must be that the high content of other organic material served as a nutrient for the micro-organisms which degraded the mecoprop cometabolically. The only useful models without growth were 1) a two-compartment first order model in two versions a) eq. (5.3) ($c_1+c_2<100$) and b) eq. (5.4) ($c_1+c_2=100$) and 2) a three half order model without growth (eq. (5.5). The three half order model consists of a first order term and a zero order term, so only two compartment models were useful. A mathematical description of such a complex matter, as is the pesticide mineralisation kinetics in soil, will only be able to include the most dominating processes. In all the cases in which both eq. (5.4) and (5.5) fitted, the process was considered as taking place in two compartments.

Table 5.3 shows selected examples of parameter-estimates according to the two models eq. (5.4) and (5.5). The highest estimates of the first order rate constants k_1 and the amount of pesticide c_1 mineralised according to eq. (5.4) are almost equal to the estimates of the first order rate constant k_1 and the amount of mineralised pesticide c_0 according to eq. (5.5). The second compartment, which in eq. (5.4) is a first order process and in eq. (5.5) a zero order process can thus be described in both ways. The second compartment (which in the curves is shown as the flat part – 0 cm days 300-500 (Figure 5.4.)) is obviously a slow mineralisation of ^{14}C-labelled organic material, which was formed through the transformation of part of the ^{14}C-pesticide. Brunner & Focht (1984) and Scow et al. (1986) came to equal conclusions. The transformation of this ^{14}C-labelled organic material was probably so slow that the concentration can be considered as being constant. Hence, the first order integrated expression for mineralisation:

Table 5.3. Selected parameter estimates ± S.D. and mean square in soil from plough layer determined according to eq. (5.4) and (5.5).

Sample	k_1 acc. to eq. (5.4)	c_1 acc. to eq. (5.4) (c_1 =a x 100)	mean square acc. to eq. (5.4)	k_1 acc. To eq. (5.5)	c_0 acc. to eq. (5.5)	mean square acc. to eq. (5.5)
mcfb 1_I a 15 cm	0.02638 ± 0.00092	34.29 ± 0.61	0.9881	0.02598±0.00084	34.86±0.54	0.9704
mcit 1+2 a 0 cm	0.2803±0.0159	38.86±0.58	2.521	0.2673±0.0158	39.63±0.59	3.068
beit 1+2 a 0 cm	0.02423±0.00067	15.18±0.18	0.0635	0.02331±0.000635	15.69±0.17	0.06967
etfb 1 a 15 cm	0.2060±0.0169	29.67±0.74	3.328	0.1903±0.0163	30.89±0.75	4.231

$$P = c_0(1 - e^{-kt}) \qquad (5.1)$$

changed to a zero order expression

$$P = k_0 t \qquad (5.11)$$

since the corresponding expressions for degradation are

$$c = c_0 e^{-kt} \qquad (5.26)$$

for a first order process, and

$$c = c_0 - k_0 t \qquad (5.27)$$

for a zero order process.

In most cases approximately the same mean square values were obtained for the two models. However, in the cases mcit and etfb a significantly lower mean square value shows was obtained with the two-compartment first order model (eq. (5.4)).

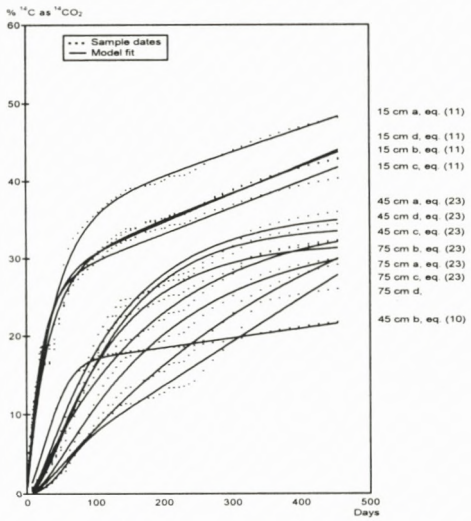


Figure 5.4. Mineralisation of 0.04 µg g^{-1} ^{14}C-mecoprop in Danish soil (FB1_I). Depth (15, 45 and 75 cm), replicate and equation number from paper **II** shown at the end of each curve. (Figure 1a from **II**).

5.1.4. Mineralisation kinetics in subsoil at low concentrations

Mineralisation with growth of microorganisms was the dominating process in the mineralisation studies with ETU, bentazon and mecoprop in subsoil. However, in the studies with mecoprop at 75 cm depth in soil from Fladerne Bæk, field FB3_I (sample mcfb3_I) and in soil from Spain at 45 cm depth, a fit could only be obtained with non-growth models. In many cases, the growth of micro-organisms was so diminutive that both growth-models and non-growth models could fit. The tested models are presented in Table 5.1. Only the models eq. (5.6) (linear growth), eq. (5.13) linear growth, eq. (5.8) (logistic growth), eq. (5.22) (logistic growth) and eq. (5.14) (exponential growth) were applicable (**II**). The only difference between eq. (5.6) and (5.13) is that the zero order term in eq. (5.6) is omitted in eq. (5.13). They are incidentally developed from different theoretical backgrounds. Brunner & Focht (1984) developed eq. (5.6) for the description of a mineralisation in which a linear growth of micro-organisms on the basis of the added substrate (a pesticide or another xenobiotic compound) was included. Schmidt et al. (1985) developed eq. (5.13) for the description of the mineralisation of low concentrations of xenobiotic compounds, in which the growth of micro-organisms occurred on the basis of another substrate. In my mineralisation studies no other substrate than the pesticide was added. Therefore the growth of micro-organisms that occurred (seen by the fit of growth models) could not be due to any other substrate than the pesticide. If the fact that the samples were removed from their natural environment and placed in the laboratory with a flow of atmospheric air, could enhance the growth of the micro-organisms on the basis of the humus in the soil, then the same should have happened in soil from the plough layer. The exponential growth, described with eq. (5.14), must therefore, too, be due to the addition of pesticide, in spite of the theoretical background on which Schmidt et al. (1985) developed their model. For most of the experiments, low mean square values were seen both for equations with linear, logistic and exponential growth (**II**). Examples of mean square values are shown in Table 5.4, in which the mean square values are almost equal for the three models, which include different types of growth. The best fits were obtained for replicates of samples from mcfb1_II, in which the mean square ≤ 0.2057. Inferior fits were obtained for the two replicates of samples from mcfb1_I, in which the mean square values resulted from 1.821-2.431. It is not possible to distinguish between type of growth in these experiments. High variations between the four replicates were seen in many cases.

Table 5.4. Selected examples of mean square values obtained by fitting models with linear, logistic and exponential growth to mineralisation data from subsoil. (Extract from Table **II**-3).

Sample	model with exponential growth (eq. (14))	model with linear growth (eq. (13))	model with logistic growth (eq. (22))
mcfb 1_I c 45 cm	2.103	1.994	1.821
mcfb 1_I d 45 cm	2.431	2.037	1.840
mcfb 1_II a 45 cm	.2057	.1487	.1824
mcfb 1_II b 45 cm	.09277	.08496	.09061
mcfb 1_II c 45 cm	.1824	.1540	.1712

The soil depth was the crucial factor, determining whether the mineralisation of the added pesticide occurred through a process with or without growth of micro-organisms for the low concentration experiments in paper **II**. Other differences between depths registered was the biological activity (measured for the soils from Fladerne Bæk in paper **VI)** and the amount of humus. Both decreased with increasing depth. The biological activity was measured as the capability of the micro-organisms for degrading ^{14}C-Na-acetat (5 μg g^{-1} dry soil) and expressed by means of the rate constant for the mineralisation process according to a later developed model (paper **VI**). ^{14}C-Na-acetat was chosen because it is a compound that form part of the natural metabolism of the micro-organisms. Differences between types of micro-organisms probably played a role, too, when cometabolic mineralisation in plough layer and metabolic mineralisation in subsoil was the general tendency. The occurrence of small oligotroph bacteria in a dormant state in deeper layers could be the reason. In the lag-phase they developed the enzymes necessary for the mineralisation. The temperature could have influenced on the shift between cometabolic and metabolic mineralisation. In Italian, Spanish and German soil from plough layer (**II**), incubated at 20°C, metabolic mineralisation did not occur in any samples. In Danish soil from plough layer, incubated at 10°C, metabolic mineralisation occurred in some of the samples. Higher temperature probably increases the capability of the micro-organisms of using the other organic material. Recently, Wagner et al. (1996) reported that bentazon could be degraded metabolically. In several papers it was stated that ETU was not able to give growth of micro-organisms (Johannesen et al., 1996, Miles & Doerge, 1991), while Vinter (1998) demonstrated, that growth of micro-organisms using ETU as the only carbon source, was possible. However, the growth on the basis of bentazon and ETU, could also account to the formation of metabolically degradable metabolites, since the total mineralisation was measured.

5.1.5. Extended kinetic models used in experiments from tropical climate

The fungicide maneb is extensively used in Nicaragua and with soil erosion it can be transported to river deltas. To assure that the mineralisation models useful under temperate climate also were useful in studies from tropical climate, mineralisation experiments with ^{14}C-maneb in sediment from an estuary in Nicaragua were carried out.

The kinetic models in paper **III** were selected from paper **II**, in which only some of the analysed models were useful. The fit of the models was again performed for each replicate of the samples, since we wanted to know the variations between replicates. The experiments were performed with 0.08 μg ^{14}C-maneb g^{-1} sediment (dry weight) in both July and September 1994. The fits of four models without growth and five models with growth showed that in all samples, metabolic mineralisation was seen (Table 5.2) (**III**). In few of the samples from July and in three of five samples from September, no-growth models were useful, too. The rainy season in Nicaragua runs from May to November which means that more organic material and more micro-organisms will have been carried out in the estuary in September.

Maneb is often assumed to have hydrolysed rapidly and through a chemical pathway. The mineralisation curves can therefore be looked at as mineralisation curves for ETU. The negative aspect when only development of $^{14}CO_2$ is measured is that it cannot be assured, whether the growth of micro-organisms occurred on the basis of the parent compound or on eventual metabolites. On the other hand, the advantage by using ^{14}C-labelled pesticides and measure the evolution of $^{14}CO_2$ is that it is assured that both the parent compound and eventual metabolite are all mineralised, when the development of the mineralisation curves reaches the flat part. The other advantage as mentioned before is, that the mineralisation experiments can be performed in very low concentrations and that the mineralisation can be followed during time in each single sample. The model

$$P = c_0 - \frac{k_1 c_0}{(k_1 + k_2 c_0)e^{k_1 t} - k_2 c_0} \qquad (5.22)$$

where

P = amount of pesticide mineralised at time t (% ^{14}C as $^{14}CO_2$)
c_0 = total amount of pesticide converted to $^{14}CO_2$ according to the modelled process
k_1 = rate constant
k_2 = rate constant
t = time in days

could fit to all data except three replicates (**III**). Of these three replicates a very special development was seen in two of them (Figure 5.5 A site 5 replicate c and Figure 5.5 B site 5 replicate b), which probably was due to an error during the incubation, since they show periods with absolutely no development of $^{14}CO_2$. Comparing samples from September mutually, the lowest mean square was seen for the sites 1 and 3. Inspecting the curves in Figure 5.5.B a deviation between data points and fitted curve is seen after 140 days for samples from the sites 2, 4 and 5, which are the sites where the longest flat part of the curve was seen. These deviations lead to higher mean square values.

5.1.6. Extended kinetic models used in experiments with varying pesticide concentrations

In paper **II**, the no-growth mineralisation models were all two-compartment models. In paper **III**, the deviations in the final part of the most developed samples caused a high mean square. A combination of those two observations made me add a second term to the growth models in paper **IV**, in which the intention with the second term was to describe the flat part of the curve – the part in which a slow mineralisation of the ^{14}C-organic material could be expected like it was seen in the no-growth models. It is important to notice, that even if the existence of a long flat part in the curve, describing the mineralisation of ^{14}C-organic material, was the reason for adding the second term the mineralisation of ^{14}C-organic material did not start until the

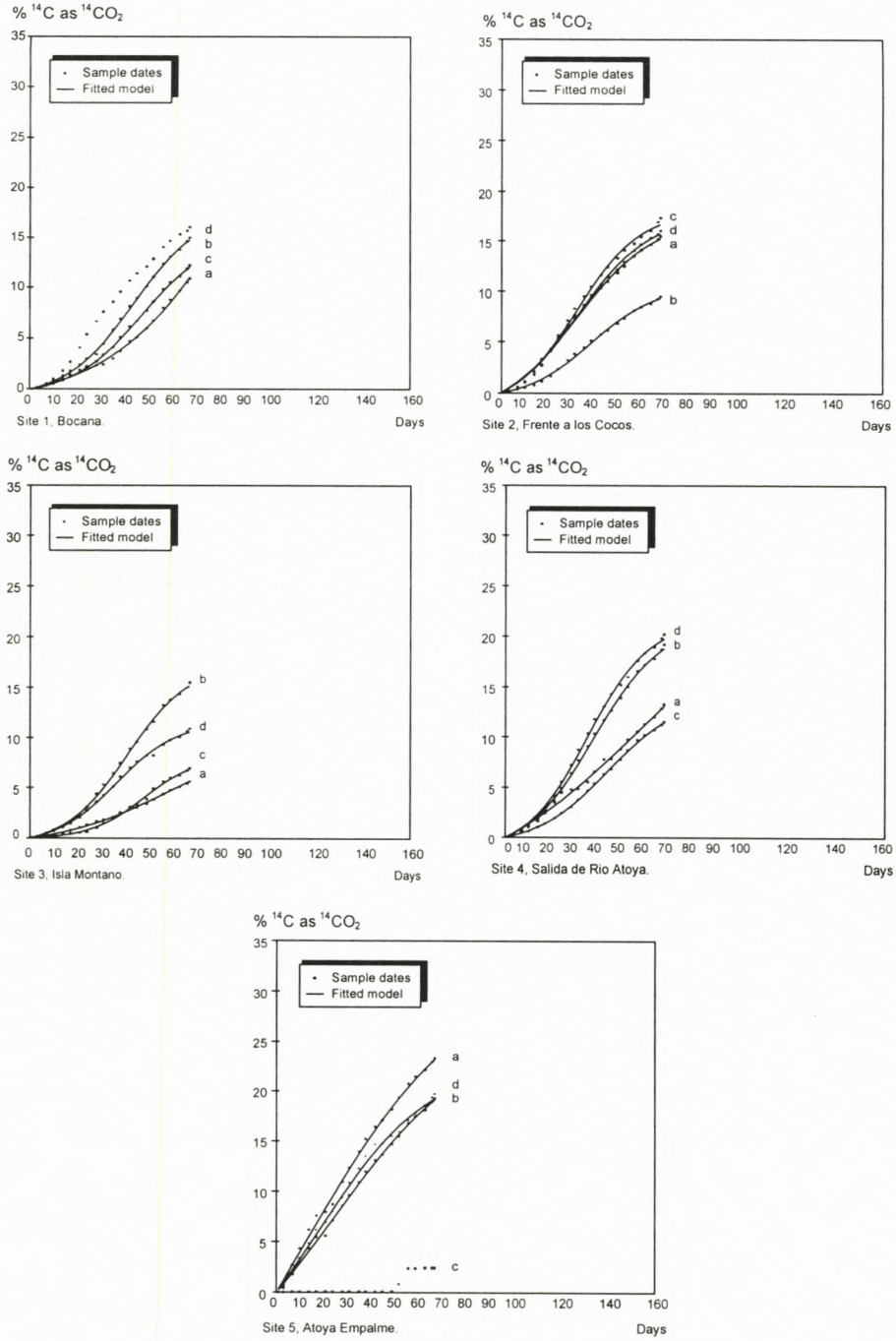


Figure 5.5. Mineralisation of 0.07 µg g^{-1} maneb in sediment from the Nicaraguan estuary "El Naranjo". A. July 1994. (Figure 2-6 from **III**).

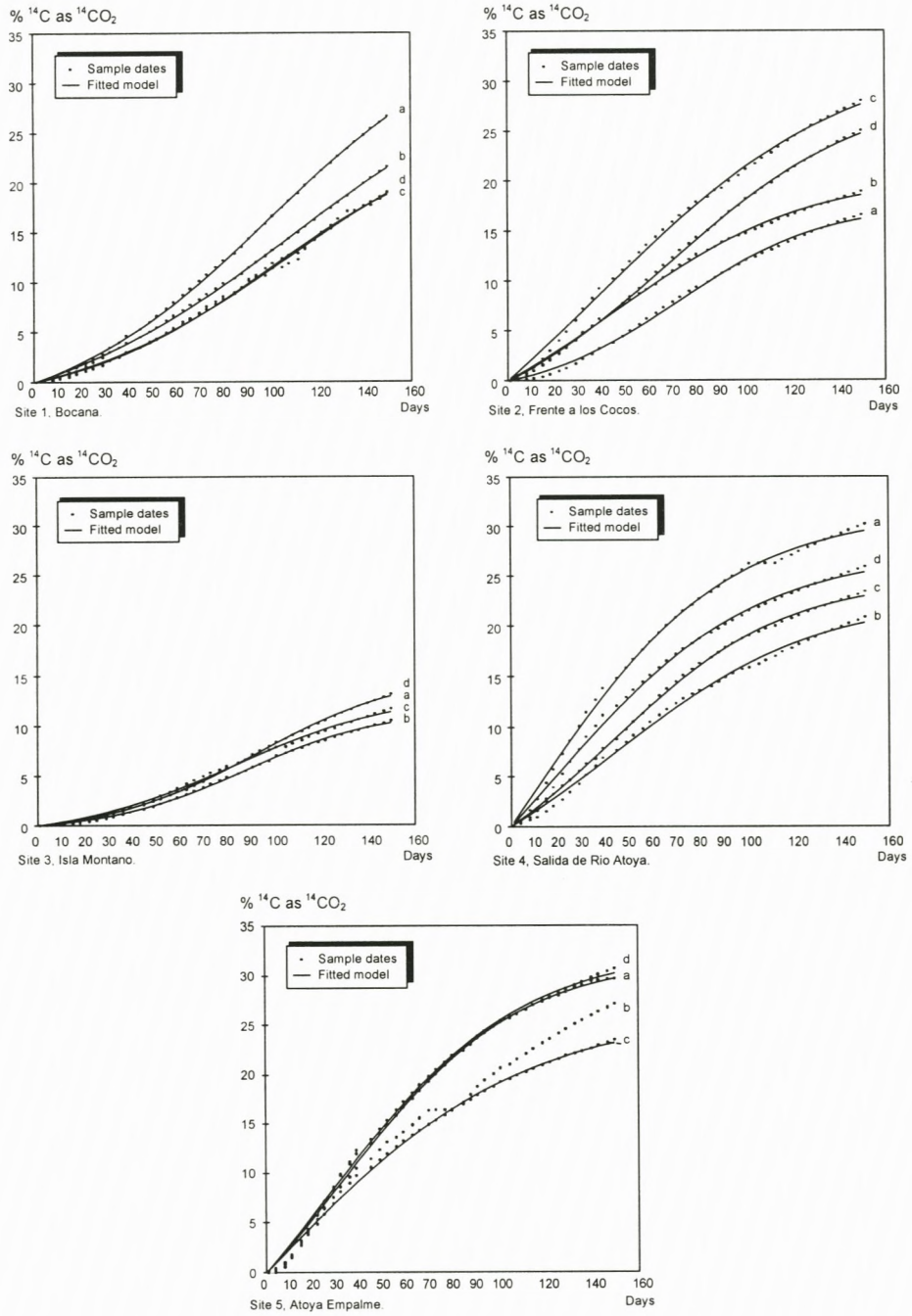


Figure 5.5 continued. Mineralisation of 0.07 μg g^{-1} maneb in sediment from the Nicaraguan estuary "El Naranjo". B. September 1994. (Figure 7-11 from **III**).

curve went flat. The mineralisation of ^{14}C-organic material starts as soon as ^{14}C-organic material has been formed, but at the last and flat part of the curve, it is the dominating and lastly the only process, thus there is no abrupt change from the process described by the first term to the process described by the second term (from one compartment to another). Therefore it is important to be able to describe the whole curve with one model, instead of dividing the curve into parts. Often when the incubation was stopped early in the process, the influence of the second part of the mineralisation cannot be seen. The curves in paper **IV** (Figure 5.6 and Figure 5.7) varied a lot because the experiments were performed in wide range of concentrations. Therefore it was important to include a term in the models, which could describe an eventual mineralisation of ^{14}C-organic material. In paper **II**, I demonstrated

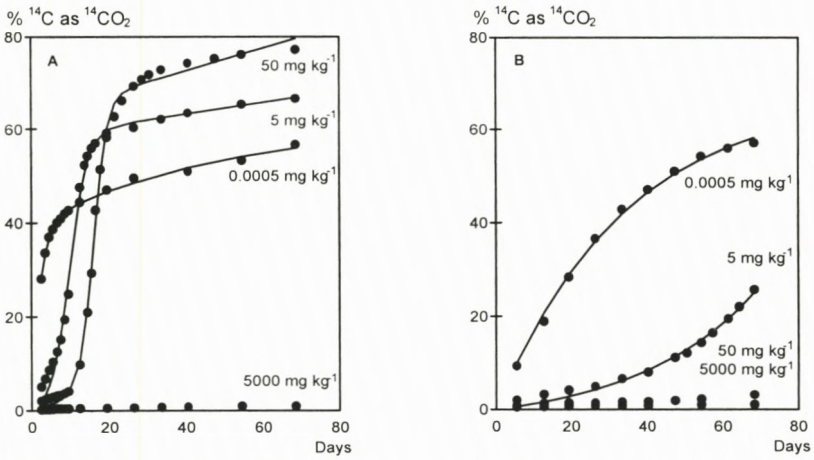


Figure 5.6. Mineralisation of 14C-mecoprop in soil at different concentrations. A. Plough layer. B. Soil from 40-60 cm's depth. (Figure 4 from **IV**).

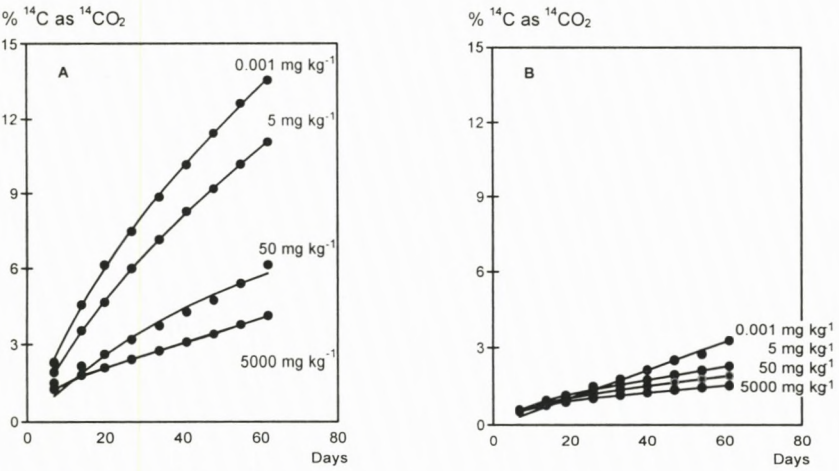


Figure 5.7. Mineralisation of ^{14}C-isoproturon in soil at varying concentrations. A. Plough layer. B. Soil from 40-60 cm depth. (Figure 5 from **IV**).

that the second term of the mineralisation model could either be a first order term (eq. (5.4)) or a zero order term (eq. (5.5)), a first order being the preferred. By adding a first order term to many of the growth models, which already had three parameters, entailed that five parameters should be estimated at the same time. This led to correlation between many of the parameter estimates. I therefore added a zero order term, which has only one parameter to be determined to eq. (5.8), (5.14), (5.16), and (5.22), by which eq. (5.9), (5.15), (5.17) and (5.23) were generated. Furthermore, four no-growth models (eq. (5.1), (5.3), (5.5) and (5.11)) were used.

Table 5.2 and Figure 5.6 show that for mecoprop cometabolic mineralisation (no-growth) was seen at the lowest concentration 0.0005 $\mu g\ g^{-1}$ both in plough layer and in subsoil (40-60 cm). In the plough layer the two- compartment first order model (eq. (5.3)) was used. In subsoil in which the development was much slower and the mineralisation therefore had not yet come to the point where a substantial part of ^{14}C-organic matter was mineralised, I used the simple first order model (eq. (5.2)). At the concentrations 5 and 50 $\mu g\ g^{-1}$ in plough layer, kinetics with growth was seen. At the concentration 5000 $\mu g\ g^{-1}$ the development of the curve was very slow (probably because of the toxicity of the compound towards the micro-organisms) and no model could therefore be fitted. In subsoil at the concentrations 5 $\mu g\ g^{-1}$, kinetic with growth was also seen. At the concentrations 50 and 500 $\mu g\ g^{-1}$ the development of the curves were too slow to fit any model. Table 5.2, which sums up the results of all my kinetic studies of pesticide mineralisation/degradation, includes my mecoprop experiments from paper **II** and **IV**. Beyond my former mentioned conclusion that the soil depth and the biological activity are crucial factors for the mineralisation being cometabolic or metabolic, I must add that the initial concentration of the pesticide also is of great importance for the kinetics according to which the pesticide is mineralised. At low concentrations the mineralisation often turns cometabolic, while at higher concentrations it turns metabolic. The mecoprop data presented in paper **IV** are extracted from a paper presented by Reffstrup et al. (1998). Table 5.5 is a summary of studies, presented in the literature, in which the kinetics either were discussed by the author or was concluded by me after my inspection of the curves in the studies. The data from Reffstrup et al. (1998) is included in Table 5.5. Reffstrup et al. (1998) used a linear or an exponential version of Brunner & Focht's (1984) three half order model to describe the curves with growth, and a first order model to describe the curves without growth and reached the conclusions as I did, for the data included in both publications: Mineralisation occurred without growth at 0.0005 $\mu g\ g^{-1}$ and with growth at 5 $\mu g\ g^{-1}$ or higher concentrations. A comparison of my results for mecoprop mineralisation with the results from Table 5.5 led to a similar conclusion: At a concentration of 2 $\mu g\ g^{-1}$ in plough layer, Helweg (1993) found kinetics with growth at both 5, 10 and 20°C, while at a concentration of 0.05 $\mu g\ g^{-1}$ he found kinetics without growth in the plough layer. The amount of organic material beyond the pesticide had an influence on the mineralisation kinetics, which can be described as if "The concentration of added pesticide/the amount of other organic matter" is very small, the

Table 5.5. Mineralisation kinetics for mecoprop, ETU, maneb, and isoproturon in experiments presented in the literature.

Reference	Compound	Measured degradation/mineralisation	Incubation technique	Conc. $\mu g\ g^{-1}$	Soil depth cm	Humus %	Clay %	pH	Inc. temp. °C	No growth	Growth
Reffstrup et al., 1998	mecoprop	Mineralisation	disturbed soil	0.0005	0-30	2.9	14.3	6.1	15	+	
Reffstrup et al., 1998	mecoprop	Mineralisation	disturbed soil	0.05	0-30	2.9	14.3	6.1	15	+	
Reffstrup et al., 1998	mecoprop	Mineralisation	disturbed soil	0.5	0-30	2.9	14.3	6.1	15		+
Reffstrup et al., 1998	mecoprop	Mineralisation	disturbed soil	5	0-30	2.9	14.3	6.1	15		+
Reffstrup et al., 1998	mecoprop	Mineralisation	disturbed soil	50	0-30	2.9	14.3	6.1	15		+
Reffstrup et al., 1998	mecoprop	Mineralisation	disturbed soil	500	0-30	2.9	14.3	6.1	15		+
Reffstrup et al., 1998	mecoprop	Mineralisation	disturbed soil	5000	0-30	2.9	14.3	6.1	15	no fit	no fit
Reffstrup et al., 1998	mecoprop	Mineralisation	disturbed soil	0.0005	30-60	0.3	22.9	6.5	15	+	
Reffstrup et al., 1998	mecoprop	Mineralisation	disturbed soil	0.05	30-60	0.3	22.9	6.5	15	+	
Reffstrup et al., 1998	mecoprop	Mineralisation	disturbed soil	0.5	30-60	0.3	22.9	6.5	15		+
Reffstrup et al., 1998	mecoprop	Mineralisation	disturbed soil	5	30-60	0.3	22.9	6.5	15		+
Reffstrup et al., 1998	mecoprop	Mineralisation	disturbed soil	50	30-60	0.3	22.9	6.5	15		+
Reffstrup et al., 1998	mecoprop	Mineralisation	disturbed soil	500	30-60	0.3	22.9	6.5	15		+
Helweg, 1993	mecoprop	Mineralisation	disturbed soil	2	0-30	2.6	13.2	6.7	5		+
Helweg, 1993	mecoprop	Mineralisation	disturbed soil	2	0-30	2.6	13.2	6.7	10		+
Helweg, 1993	mecoprop	Mineralisation	disturbed soil	2	0-30	2.6	13.2	6.7	20		+
Helweg, 1993	mecoprop	Mineralisation	disturbed soil	0.05	0-33	2.4	4.0	6.6	10	+	
Helweg, 1993	mecoprop	Mineralisation	undisturbed soil	0.05	33-66*	1.0	3.1	6.8	10		+
Helweg, 1993	mecoprop	Mineralisation	undisturbed soil	0.05	66-99*	0.3	3.1	5.9	10	+	
Johannesen et al., 1996	ETU	Mineralisation	disturbed soil	0.07	0-35	2.7	3.2	6.6	21		+
Johannesen et al., 1996	ETU	Mineralisation	undisturbed soil	0.07	60	0.2	1.4	6.1	10		+
Johannesen et al., 1996	ETU	Mineralisation	undisturbed soil	0.07	100	0.4	1.5	6.1	10		+
Cox et al., 1996	isoproturon	Degradation	disturbed soil	10	0-10	1.9		7.8	15		+
Cox et al., 1996	isoproturon	Degradation	disturbed soil	10	0-10	2.6		7.3	15		+
Cox et al., 1996	isoproturon	Degradation	disturbed soil	10	0-10	2.2		6.8	15		+
Cox et al., 1996	isoproturon	Degradation	disturbed soil	10	0-10	2.6		5.2	15		+
Cox et al., 1996	isoproturon	Degradation	disturbed soil	10	0-10	2.1		7.0	15		+

* Texture at soil depths 25-50 cm and 75-100 cm

pesticide mineralisation will be cometabolic. If the relation is big, the mineralisation will be metabolic. The variation down through the soil layers, where Helweg found growth-kinetics at 33-66 cm and no-growth kinetics at 66-99 cm, is parallel to one of my experiments from (FB3_I), in which I found the same pattern. At the concentration 0.04-0.05 μg g^{-1} the kinetics could be metabolic as well as cometabolic. Reffstrup et al. (1998) found no-growth kinetics at 0.05 μg g^{-1} mecoprop both in plough layer and in subsoil. Reffstrup et al. (1998) performed all their experiments with disturbed soil samples. Helweg (1993), **II** and **VI** used disturbed (partly dried and homogenised) samples from plough layer and undisturbed samples from subsoil. Comparing the Tables 5.2 and 5.5 makes it obvious to ask if the incubation technique (disturbed or undisturbed samples) influenced the mineralisation kinetics. Johannesen et al. (1996) compared the mineralisation of ETU in disturbed and undisturbed samples and did not find significant differences when they compared the amount of $^{14}CO_2$ evolved after a number of days, or when they compared the mineralisation kinetics resulting in the two methods. However, it was very clear in the subsoil experiments by Johannesen et al. (1996) (Figure 5.8) that the lag-phase was longer in disturbed samples than in undisturbed samples. The latter supports the idea that mineralisation studies in subsoil should be performed in undisturbed samples. Under normal agricultural practice, the subsoil will never be undisturbed by anything but percolated water. The temperature is another factor which probably had influence on whether the mineralisation was metabolic or cometabolic. In soil from Denmark (paper **II** and **VI**), incubated at 10°C, I found that some of the plough layer samples showed cometabolic and others showed metabolic mineralisation of 0.04 μg g^{-1} mecoprop, while the German, Spanish and Italian plough layer samples, incubated at 20°C, only showed cometabolic mineralisation of 0.04 μg g^{-1} mecoprop. Reffstrup et al. (1996) and **IV** incubated the samples at 15°C and found cometabolic mineralisation of 0.05 μg g^{-1} mecoprop.

As regards isoproturon, kinetics without growth was seen in both plough layer and subsoil and in all concentrations (Table 5.2 and Figure 5.7). This opposes the statement in several publications, in which it was shown that isoproturon could act as the only carbon source for the growth of micro-organisms (Kubiak et al., 1995; Cox et al., 1996) (Table 5.5). Apparently no influence occurred on for instance dormant oligotroph micro-organisms either, which was one the possible explanations for the growth kinetics seen in subsoil for bentazon and ETU. Metabolites which could have been the source for growth was apparently not formed either. However, it is important to notice that the mineralisation of isoproturon proceeded so slowly that after 60 days of incubation very little $^{14}CO_2$ was formed. A sigmoidal rise in the curve COULD theoretically occur if the incubation had continued for a longer time. As mentioned earlier a prolonged lag-phase was seen in the subsoil experiments by Johannesen et al. (1996), when disturbed samples were used. The same could be the case here. One of the conclusions of these experiments was thus that the incubation of mineralisation experiments should continue until after the mineralisation curve has turned flat.

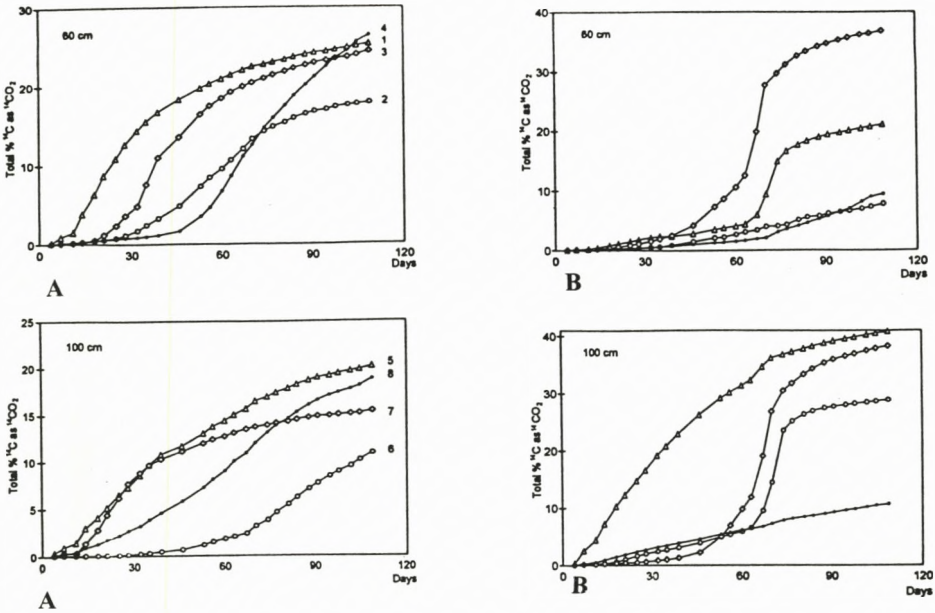


Figure 5.8. Mineralisation of 0.07 μg g^{-1} 14C-ETU in soil from 60 cm and 100 cm's depth incubated at 10°C. A. Undisturbed samples. B. Disturbed samples (Johannesen et al., 1996). (Copyright Elsevier Science. Reproduced with permission).

5.1.7. The development of a general mineralisation model

Liu % Zhang (1986) developed their pesticide degradation model on the basis of

$$m = m_0 + \lambda(c_n - c) \tag{5.28}$$

where

c_n = the initial amount of pesticide (called x_0 by Liu & Zhang (1986))

c = amount of pesticide at time t (called x by Liu & Zhang (1986))

m_0 = the initial amount of micro-organisms, involved in the degradation

m = the amount of micro-organisms, involved in the degradation at time t

λ = growth rate for the micro-organisms

and

$$-\frac{dc}{dt} = kcm \tag{5.29}$$

k being the rate constant.

Liu & Zhang (1986) compiled the equations (5.28) and (5.29) to

$$-\frac{dc}{dt} = k(m_0 + \lambda c_n)c - k\lambda c^2 \qquad (5.30)$$

Introducing the following definitions for k_1 and k_2

$$k_1 = k(m_0 + \lambda c_n) \qquad (5.31)$$

and

$$k_2 = -k\lambda \qquad (5.32)$$

eq. (5.30) was expressed by Liu & Zhang (1986) as

$$-\frac{dx}{dt} = k_1 c + k_2 c^2 \qquad (5.33)$$

Integration of eq. (5.33) led to the following expression according to Liu & Zhang (1986)

$$c = \frac{k_1 c_n}{(k_1 + k_2 c_n)e^{k_1 t} - k_2 c_n} \qquad (5.34)$$

In **II**, I converted eq. (5.34), which describes the degradation of a pesticide, to

$$P = c_n - \frac{k_1 c_n}{(k_1 + k_2 c_n)e^{k_1 t} - k_2 c_n} \qquad (5.22)$$

which describes the mineralisation.

Liu & Zhang (1986) and Liu et al. (1988) stated that their model was useful both when an inflection point was seen on the curve (i.e. the curve had a sigmoidal form), in which case k_2 would be negative and when no inflection point was seen, in which case k_2 would be zero and the model would change to a first order model. I used my conversion of Liu & Zhang's model to a mineralisation model as presented in paper **II** and found that the model could not be used in all cases in which a sigmoidal form was seen, due to an often negative estimate of k_1 or a positive estimate of k_2. Still, the addition of a second term to the model (a second compartment) in the form of a zero order term, as showed in paper **IV**, did not make the model useful.

In paper **V**, I subsequently showed that the converted Liu & Zhang model with the addition of a first order term

$$P = c_n - \frac{k_1 c_n}{(k_1 + k_2 c_n)e^{k_1 t} - k_2 c_n} + c_b(1 - e^{-k_3 t}) \qquad (5.24/5.25)$$

where

P = total amount of evolved mineralisation product ($^{14}CO_2$), equivalent to the total amount of mineralised ^{14}C-pesticide at time t (measured as % ^{14}C evolved as $^{14}CO_2$)

c_n = total % ^{14}C-pesticide converted to $^{14}CO_2$ according to the Liu & Zhang-model (Liu & Zhang, 1986)

c_b = total % ^{14}C-pesticid converted to $^{14}CO_2$ according to the first order model

k_1, k_2 =rate constants

$k_1 = k(m_0 + \lambda c_n)$

$k_2 = -k\lambda$

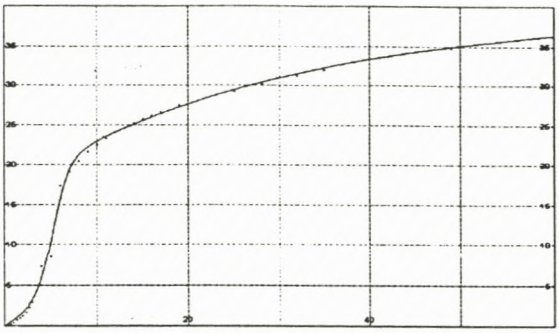
k_3 = rate constant for the first order process

λ = growth rate of the micro-organisms

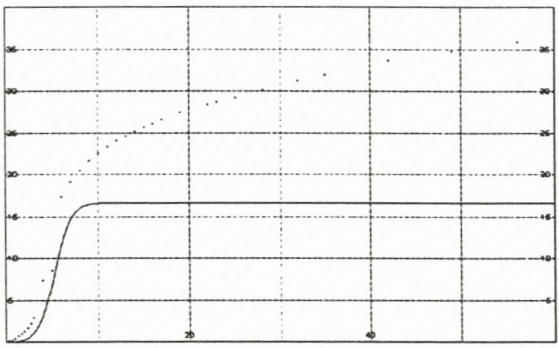
m_0 = initial amount of degradation micro-organisms

could be used and provide well estimated parameters on two conditions, being: 1) good initial estimates were generated through non-linear regression of simplified models with either k_2 or k_3 being 0 and 2) the estimates from the non-linear regression of the simplified models were used as initial estimates for the final non-linear regression with eq. (5.24/5.25) and 3) that parameter values were estimated for two versions of the model, both $c_1 + c_2 = 100$ and $c_1 + c_2 < 100$. An illustration of the mineralisation in the two compartments of the model is seen in Figure 5.9. Data from **V,** sample 24 is shown with points. A. Shows the combined model (eq. 5.24/5.25), B shows the first term of the combined model, corresponding to eq. (5.22)

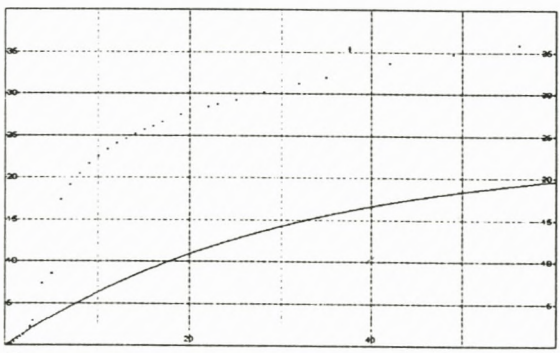
$$P = c_n - \frac{k_1 c_n}{(k_1 + k_2 c_n)e^{k_1 t} - k_2 c_n} \qquad (5.22)$$



A



B



C

Figure 5.9. Mineralisation of 2.0 µg g^{-1} ^{14}C-ETU in plough layer soil (Data from sample no. 24, **V**).

A. Data points ····· and the model ——: $P = c_n - \frac{k_1 c_n}{(k_1 + k_2 c_n)e^{k_1 t} - k_2 c_n} + c_b(1 - e^{-k_3 t})$ (5.24/5.25)

B. Data points ····· and the first term of the model ——: $P = c_n - \frac{k_1 c_n}{(k_1 + k_2 c_n)e^{k_1 t} - k_2 c_n}$ (5.22)

C. Data points ····· and the second term of the model ——: $P = c_b(1 - e^{-k_3 t})$ (5.1)

and C shows the second part of the combined model, the first order mineralisation, corresponding to eq. (5.1):

$$P = c_b(1 - e^{-k_3 t}) \qquad (5.1)$$

If the mineralisation curve is not developed far enough to reach the flat part of the curve, k_3 results = 0 and the model eq (5.24/5.25) is reduced to eq. (5.22) again.

If there is no growth of micro-organisms λ must be 0, and k_2 becomes 0. That reduces eq. (5.22) to

$$P = c_n - \frac{k_1 c_n}{k_1 e^{k_1 t}} \qquad (5.35)$$

or

$$P = c_n(1 - e^{-k_1 t}) \qquad (5.36)$$

When no growth of micro-organisms is seen and k_2 therefore becomes 0, the combined model

$$P = c_n - \frac{k_1 c_n}{(k_1 + k_2 c_n)e^{k_1 t} - k_2 c_n} + c_b(1 - e^{-k_3 t}) \qquad (5.24/5.25)$$

is reduced to

$$P = c_n(1 - e^{-k_1 t}) - + c_b(1 - e^{-k_3 t}) \qquad (5.37)$$

which is identical to eq. (5.3) in Table 5.1.

This way, the goal was reached, to develop a mathematical model, which could fit to all data from the quite complex study in **V**. The model fitted to data both whether the curve had a sigmoidal form, and whether the mineralisation was followed throughout a long time or only until the turning of the curve. Henceforward it will be possible to compare mineralisation rates, even if the curves develop differently, because the resulting parameters k_1 and c_n can be compared.

5.1.8. The application of the general kinetic model

In paper **VI,** I repeated the non-linear regression of the mecoprop mineralisation data from paper **II** with the model, developed in paper **V**. The model fitted all the experiments. In the cases where no growth of micro-organisms was seen, k_2 became 0, and the model became a two compartment first order model. In the cases, where growth was seen and the curve ended with a long flat part, the model fitted in all cases, too, because of the addition of the second term (the first order mineralisation of ^{14}C-organic matter). It is reasonable to believe that a mineralisation model has been developed capable of describing the mineralisation of many other compounds than pesticides. To check the applicability of the model, I fitted the model to the mecoprop data from **IV** supplemented with data from Reffstrup et al. (1996). These data

resulted in very varying mineralisation curves and at the time of the publication of paper **IV** it still had not been possible to find or to develop a model that could fit all the curves. The model eq. 5.24/5.25 showed to fit all these data. The parameter estimates are shown in Table 5.6 and further discussed in chapter 5.2.

However, this model could not describe the curves from the isoproturon experiments with satisfactory precision, because they were only developed over a short time. The model was also tested with bentazon and ETU mineralisation experiments from paper **II**, performed in low concentrations soil samples from plough layer and subsoil. The model resulted in useable fits, and the conclusions concerning cometabolic/metabolic mineralisation were the same as already concluded in paper **II**.

In paper **VI** k_2 was only estimated to 0 in one of the plough layer soils (FB1_I), while in the other plough layer soils (FB1_II, FB3_I, FB3_II, FB4_I) k_2 resulted negative, which means that growth of micro-organisms occurred. However, the microbial growth on basis of the added mecoprop was so small for the samples from FB3_I and FB4_I that none of the growth models in paper **II** could fit. These samples were therefore marked in Table 5.2 as samples not causing growth of micro-organisms by incubation with mecoprop. With the eq. (5.24/5.25) even tendencies of sigmoidal form were clear, which was not the case with the models presented in paper **II**.

Table 5.6. Parameter estimates for data from paper **IV** estimated with model eq. 24/25.

Compound	Depth cm	Conc. µg g^{-1}	Repl.	c_n	k_1	k_2	k_3	c_b
Mecoprop	0-30	0.0005	1	36.9	0.5142	0	0.0372	17.7
Mecoprop	0-30	0.0005	2	41.4	0.4439	0	0.0068	46.8
Mecoprop	0-30	0.05*	1	52.0	0.5025	0	0.0241	17.0
Mecoprop	0-30	0.05*	2	38.1	0.4182	0	0.0148	19.0
Mecoprop	0-30	0.5*	1	38.1	0.4665	-0.0095	0.0203	18.9
Mecoprop	0-30	0.5*	2	32.7	0.4303	-0.0094	0.0236	18.3
Mecoprop	0-30	5.0	1	41.3	0.6182	-0.0193	0.0740	24.9
Mecoprop	0-30	5.0	2	40.7	0.6582	-0.0161	0.0750	23.5
Mecoprop	0-30	50	1	59.7	0.4508	-0.0076	0.0057	40.3
Mecoprop	0-30	50	2	70.0	0.4593	-0.0066	0.0086	30.0
Mecoprop	0-30	500*	1	68.3	0.0978	-0.0014	0.0016	31.7
Mecoprop	0-30	500*	2	64.6	0.0453	-0.0007	0	35.4
Mecoprop	30-60	0.0005	1	54.0	0.0401	0	0.0000	46.0
Mecoprop	30-60	0.05*	1	40.0	0.0693	0	0.0009	60.0
Mecoprop	30-60	0.05*	2	41.5	0.0695	0	0.0009	58.5
Mecoprop	30-60	0.5*	1	55.7	0.0331	-0.0004	0.0007	44.2
Mecoprop	30-60	0.5*	2	55.6	0.0374	-0.0004	0.0007	44.4
Mecoprop	30-60	5.0	1	53.3	0.0841	-0.0016	0.0050	46.7
Mecoprop	30-60	5.0	2	53.6	0.0641	-0.0012	0.0050	32.9
Mecoprop	30-60	50	1	78.8	0.0405	-0.0005	0	21.2
Mecoprop	30-60	50	2	66.9	0.0595	-0.0009	0	33.1

*Supplemental values from Reffstrup et al. (1996)

In paper **VI,** the purpose was to relate the mineralisation rate of mecoprop to a number of soil specific factors, including biological activity. As a measure of the biological activity, I decided to use the capability of the micro-organisms of mineralising ^{14}C-Na-acetat. Modelling this mineralisation was therefore also necessary. The model described above was useful for the description of the mineralisation of ^{14}C-Na-acetat.

Discussing mineralisation of pesticides with and without growth of micro-organisms makes it absolutely necessary to take a step back and analyse if I – and other authors who reported to have found mineralisation with growth – can be sure that the sigmoidal form of the mineralisation curve really expresses growth of micro-organisms. Already in paper **II**, I observed that for some data sets both models including growth of micro-organisms and models describing first order sequential mineralisation fit. The amount of micro-organisms could not be measured continuously, since I worked with undisturbed soil samples. However, other authors (Focht and Brunner (1985), Jacobsen and Pedersen (1992) measured an increased amount of micro-organisms coincident with ascertainments of growth of micro-organisms in the mineralisation curves. It is not very probable that a sequential first order mineralisation should have occurred in subsoil because of sorption/desorption to humus, since the amount of humus is much higher in plough layer in which the sequential first order mineralisation model did not fit. Moreover it is clear that the cases in which fits were obtained both with growth models and the sequential first order model (Figure 5.10 A), were cases where the sigmoidal form was not very pronounced. When the sigmoidal form was more pronounced (Figure 5.10 B) only models with growth fitted. Table 5.7 shows selected examples of mean square values obtained with first order sequential model and the logistic growth model, respectively. Thus it is probable that growth of micro-organisms **was** the reason for the sigmoidal form of the mineralisation curve.

Other studies reported in the literature also reported growth/no-growth cases, either when the degradation of the pesticide or the formation of a mineralisation product was measured. Vink et al. (1994) (Figure 5.11) measured the degradation of 1,3-D (1,3-dichlorpropene) in soil and found that the degradation followed first order kinetics at the concentration 0.03 mg kg^{-1} at 50 cm' depth and 0.3 mg kg^{-1} at 70 cm's depth. At the concentration 5 mg kg^{-1} in 30 cm's depth and 15 mg kg^{-1} in 10 cm's depth a sigmoidal form of the degradation curve was seen, which indicated growth of micro-organisms.

Vink and van der Zee (1996) did a similar study with metamitron and found first order degradation at the concentrations 0.5 and 2 mg kg^{-1} and degradation with growth at 4 and 10 mg kg^{-1}.

The lack, until now, of a mineralisation model capable of describing all types of mineralisation curves, is probably the reason why even new publications have reported mineralisation rates as "% $^{14}CO_2$ developed after a certain number of days"

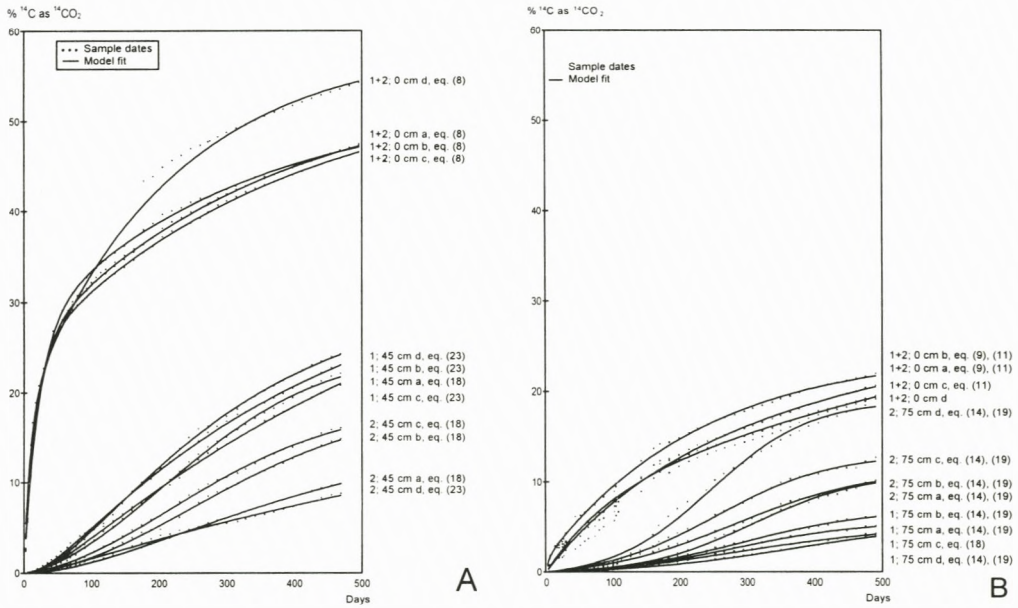


Figure 5.10. Mineralisation of 0.08 μg g^{-1} bentazon in soil. A. The Spanish soil samples show only tendencies of sigmoidal form in subsoil. B. The German soil samples show a more pronounced sigmoidal form in subsoil. (Figure 2b and 2c from **II**).

Table 5.7. Selected examples of mean square values obtained by fitting models describing sequential first order mineralisation and logistic growth mineralisation, respectively, to bentazon data in Spanish (besp) and in German soil (bety). (From Table 3 in **II**).

Sample	Sequential 1.orden	Logistic growth
besp 1 b 45 cm	.06221	.08777
besp 1 c 45 cm	.02816	.06996
besp 1 d 45 cm	.03143	.1729
besp 2 a 45 cm		.01312
besp 2 b 45 cm		.05125
besp 2 c 45 cm		.07655
besp 2 d 45 cm	.03321	.06359
bety 1 a 75 cm		.01496
bety 1 b 75 cm		.01408
bety 1 c 75 cm		.01031
bety 1 d 75 cm		.001935
bety 2 a 75 cm		.03947
bety 2 b 75 cm		.03632
bety 2 c 75 cm		.05830
bety 2 d 75 cm		.4058

(Johannesen et al., 1996; Pieuchot et al., 1996; Lehr et al., 1996, Helweg, 1993). The model developed here is up to now the most advantageous model for the description of the mineralisation of xenobiotic compounds in soil. It would be reasonable to expect that the model also would be useful for the description of mineralisation of xenobiotic compounds in i.e. water.

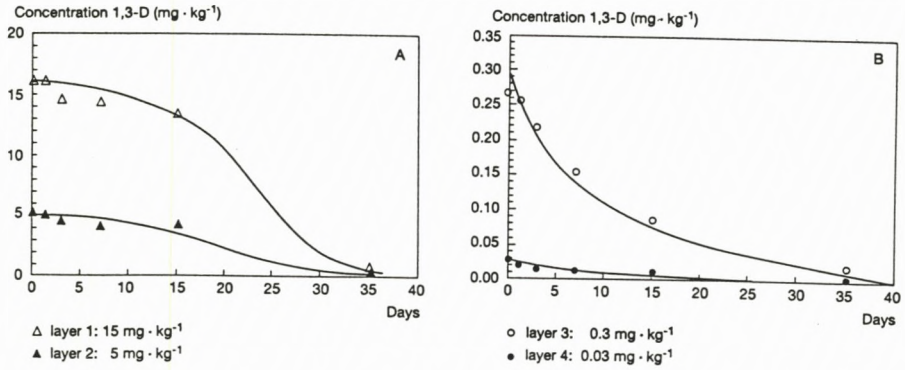


Figure 5.11. Degradation of 1.3-D at varying concentrations. Data point ····· and fitted model ———. A. High concentrations, model with growth. B. Low concentrations, model without growth (Vink et al., 1994). (Copyright Society of Chemical Industry. Reproduced with permission. Permission is granted by John Wiley & Sons Ltd on behalf of the SCI.).

On the basis of the developed model, which can describe all types of mineralisation curves with the same – and therefore comparable – parameters, more trustworthy comparisons of mineralisation rates can hereafter be performed.

5.2. The mineralisation rate in relation to geo-environmental factors

5.2.1. The mineralisation rate in relation to varying pesticide concentrations and soil depth

In paper **IV**, a number of mineralisation experiments of mecoprop and isoproturon at concentrations from 0.0005 $\mu g\ g^{-1}$ to 500 $\mu g\ g^{-1}$ were performed. The comparison of mineralisation rates was only made for some of the concentrations in the paper because at the time of publishing of the paper, it had still not been possible to find a mathematical model that could describe all tested types of mineralisation curve. The model that was developed in the papers **V** and **VI** (eq. (5.24/5.25)) was applied to the data from paper **IV**. As already mentioned in chapter 5.1.8, the model fitted all the data. The results of the parameter estimates are shown in Table 5.6.

Table 5.6 shows that in the two lowest concentrations, mineralisation without growth occurred ($k_2 = 0$) in both plough layer and subsoil, as already concluded in paper **IV** and by Reffstrup et al. (1996). Since the same model now was used for all the concentrations, a direct comparison of the parameter estimates for all concentrations and thus an evaluation of the dependency of the parameter estimates on the initial concentration, can be done. At the concentration 5000 μg g^{-1} in plough layer and 500 and 5000 μg g^{-1} in subsoil no significant development of $^{14}CO_2$ was seen – thus the data could not be modelled. This was probably due to a toxic effect of the pesticides on the micro-organisms. It is possible that after a longer incubation a mineralisation would start.

The rate constant k_1 was not significantly different for the concentrations 0.0005 to 50 μg g^{-1} in each soil layer. However, k_1 was 10 times higher in plough layers than in subsoil. The percentage of the pesticide, transformed according the first term of the model (c_n) increased, when the concentration of the pesticides reached 50 μg g^{-1} both in plough layers and subsoil.

A simple correlation between mineralisation rates for mecoprop and the initial concentration of the compound could therefore not be seen.

5.2.2. The mineralisation rate in relation to temperature, concentration, soil depth and content of organic matter

Many studies of degradation rates of pesticides and their correlation to the dominating factors: temperature, concentration, soil depth and content of organic matter have been performed for each single factor at a time. In paper **V**, I designed a controlled factor study, in which the concurrent effects of two temperatures, two concentrations, two soil depths and two different amounts of added organic matter (the latter called: suspension: water or extract) on the mineralisation of ^{14}C-ETU were investigated.

The mineralisation was described with the model already discussed in chapter 5.1.7:

$$P = c_n - \frac{k_1 c_n}{(k_1 + k_2 c_n)e^{k_1 t} - k_2 c_n} + c_b(1 - e^{-k_3 t}) \qquad (5.24/5.25)$$

where

P = total amount of evolved mineralisation product ($^{14}CO_2$), equivalent to the total amount of mineralised ^{14}C-pesticide at time t (measured as % ^{14}C evolved as $^{14}CO_2$)
c_n = total % ^{14}C-pesticide converted to $^{14}CO_2$ according to the Liu & Zhang-model (Liu & Zhang, 1986)
c_b = total % ^{14}C-pesticid converted to $^{14}CO_2$ according to the first order model
k_1, k_2 =rate constants
$k_1 = k(m_0 + \lambda c_n)$

$k_2 = -k\lambda$
k_3 = rate constant for the first order process
λ = growth rate of the micro-organisms
m_0 = initial amount of degradation micro-organisms

A summary showing both the design of the study, the data points and the fitted model is presented in Figure 5.12. The mineralisation depended on the included factors in a complex – and not always explainable way. A three-way interaction effect depth*concentration *temperature was found for both c_n, k_1, k_2 and$\lambda/_{m0}$. The two-way interaction effect between two of the factors thus depends on the third factor. A three-way interaction effect depth *concentration*suspension was only seen for c_n, while two-way interaction effects were seen for k_1 and k_2. The interaction effects are shown in Figure 5.13 and Figure 5.14. It must be interposed that the fits of the data from the experimental combinations K and L (mineralisation at 5°C in subsoil at the concentration 2.0 µg g^{-1}) (Figure 5.12) must have a high unreliability. $\lambda/_{m0}$, the growth rate of the micro-organisms/the initial amount of involved micro-organisms, and k_2 are parameters which in the present use of the conversion of Liu & Zhang (1987)'s model (conversion to measurement of mineralisation product in %) only can be used to analyse the interaction effects of varying factors. The size of estimates of $\lambda/_{m0}$ and k_2 can be compared for certain factors, but not for the factor concentration. At 0.07 µg g^{-1} the rate constant k_1 was the same at the temperatures 5 and 20°C in plough layer, while k_1 was higher at 20°C than at 5°C in 75 cm's depth. The overall difference between the estimates of k_1 in plough layer and subsoil was 10 times higher than the difference between k_1 at different temperatures in the same soil layer. When the concentration of pesticide increased, k_1 kept constant at 20°C. An increased concentration at 75 cm's depth at 20°C reduced k_1. A concentration of 2.0 µg g^{-1} in subsoil could have a small toxic effect on the micro-organisms. c_n was higher at 20°C than at 5°C at the low concentration in plough layer and the same for different temperatures at the low concentration in subsoil.

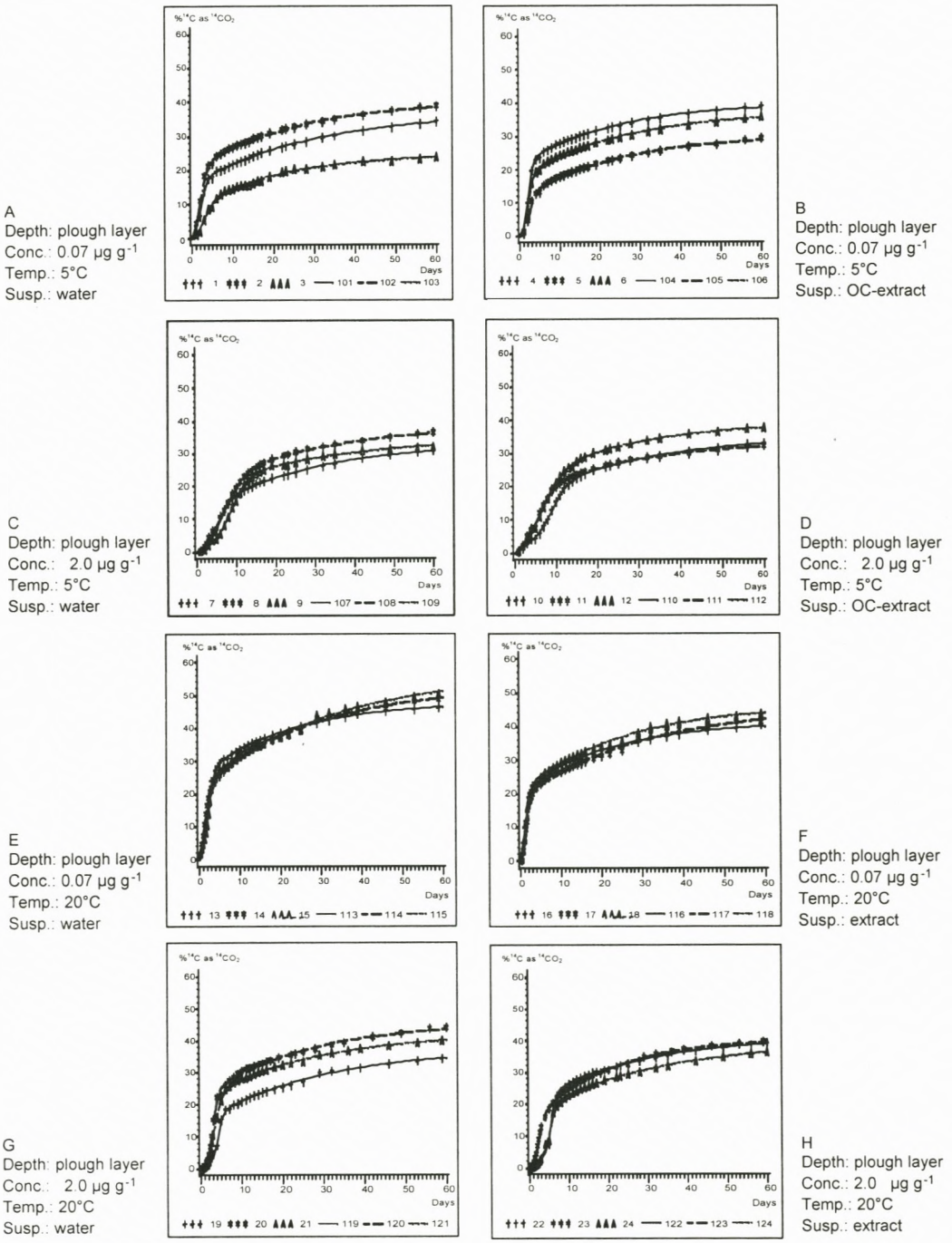


Figure 5.12. Mineralisation of ^{14}C-ETU in soil at varying depths, concentrations, temperatures and content of organic matter. Data points are shown with symbols, the fits of the model are shown as solid and broken lines. (Figures 2-9 from **V**).

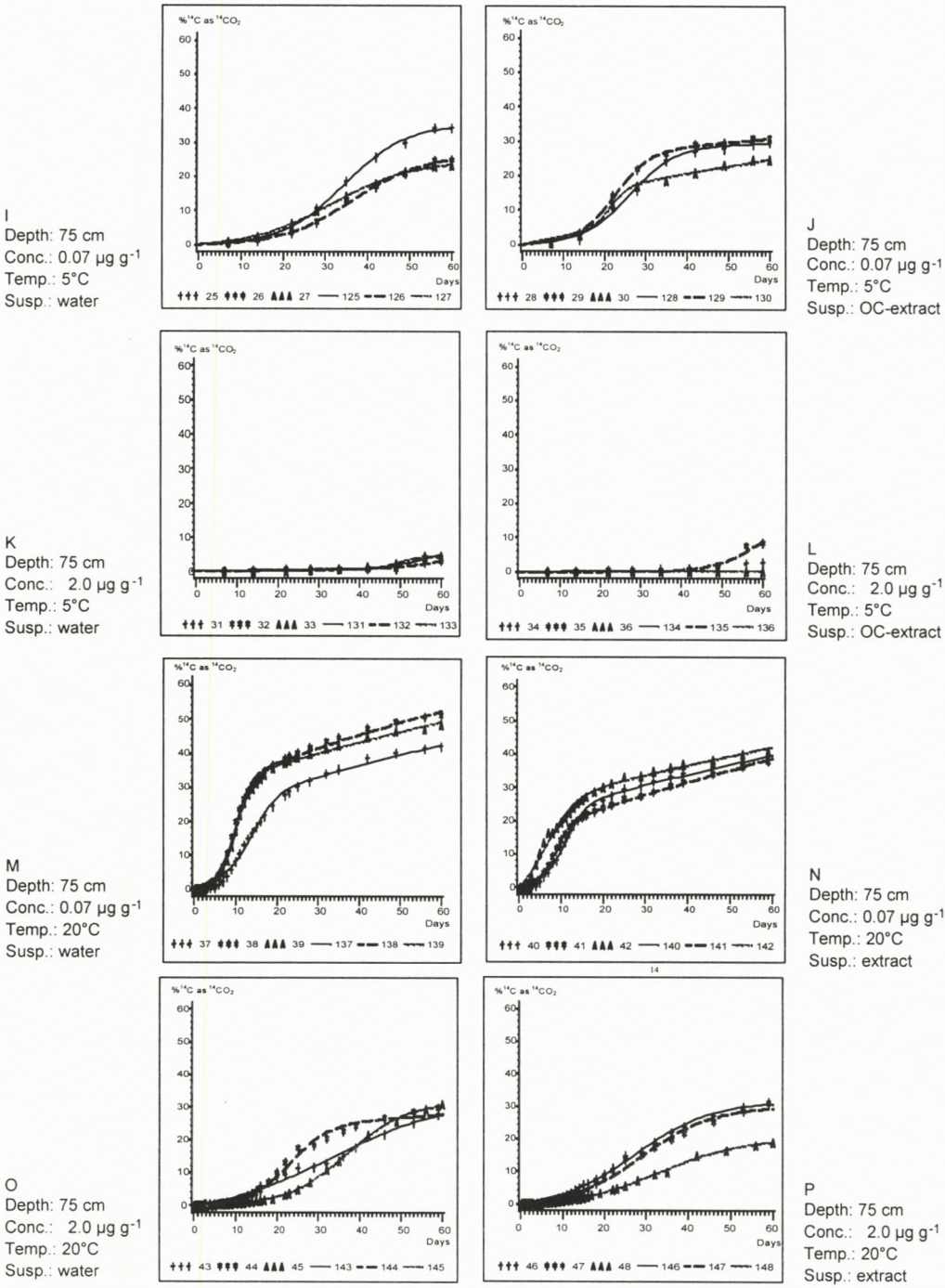


Figure 5.12 continued. Mineralisation of ^{14}C-ETU in soil at varying depths, concentrations, temperatures and content of organic matter. Data points are shown with symbols, the fits of the model are shown as solid and broken lines. (Figures 10-17 from **V**).

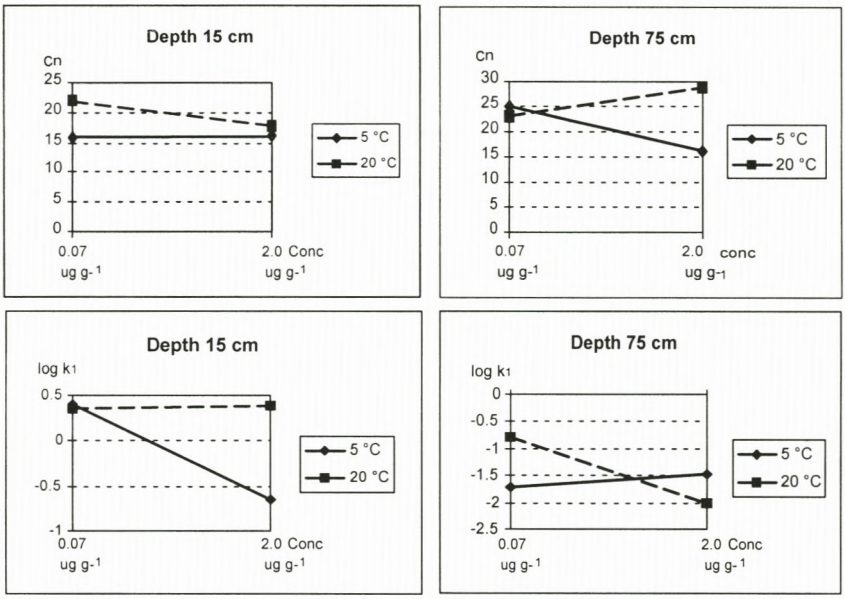


Figure 5.13. Mineralisation of ^{14}C-ETU in soil. Three-way interaction effects of depth, concentration and temperature for the coefficients c_n and k_1. (Figure 18 from **V**).

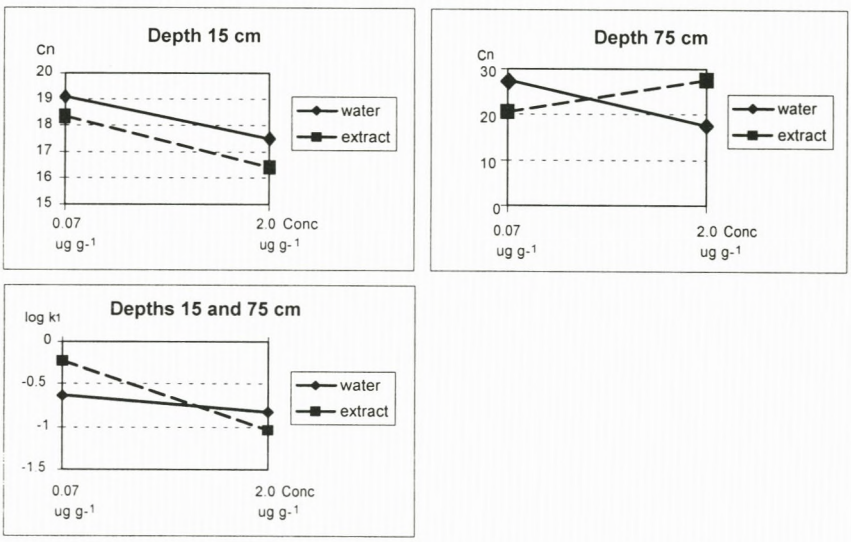


Figure 5.14. Mineralisation of ^{14}C-ETU in soil. The combined interaction effects of depth, concentration and organic matter for coefficients c_n and k_1. (Figure 20 from **V**).

The addition of a solution of dissolved organic carbon compared with the addition of pure water to 50% WHC (suspension: water or extract in the paper) also resulted in interaction effects. Three-way interaction effects depth*concentration*suspension was seen for c_n, two-way interaction effects concentration*suspension for k_1 and k_2 and no effect for λ/m_0. When the organic extract was added, k_1 increased at the low concentration of ^{14}C-ETU, - the presence of the extract increased the capability of the micro-organisms for mineralising ^{14}C-ETU. When a higher amount of ^{14}C-ETU was present (2.0 μg g^{-1}), k_1 was almost the same with and without extract. The effect on c_n of water or extract is the same in plough layer, but not very high, since c_n only reduced from about 19 to 17.5% and from 18 to 16.5%. In subsoil, the effect of added extract on c_n was very significant, the addition of extract increased c_n at 2.0 μg g^{-1} ^{14}C-ETU.

Such complex interaction effect between factors influencing the mineralisation of ^{14}C-ETU has not been shown formerly. However, Vink et al. (1994) modelled the degradation of 1,3-D in soil and showed that the influence of the temperature on the degradation could not be described with a classical Arrhenius-function and moreover, concluded that the degradation had a complex dependence on microbial activity, concentration of pesticide, depth and physical parameters of the soil. In a study of the degradation of metamitron, Vink & van der Zee (1996) found a special low degradation at a combination of low temperature, low concentration of pesticide and high sorption.

5.2.3. A model describing the mineralisation rate in relation to microbial activity, depth, content of organic matter and soil texture

In all the mecoprop mineralisation experiments from Fladerne Bæk (paper **II** and **VI**) the same incubation temperature, water content and concentration of mecoprop were used. For each site and depth and time of sampling the experiments were performed with four replicates. Furthermore, I determined the biological activity, the MPN-number of mecoprop-degrading bacteria, the soil texture, the content of nutrient salts (NO_3-N and NH_4-N), pH and soluble organic carbon. The purpose was to determine the influence of the last-mentioned factors on the parameter estimates k_{1_meco}, k_{2_meco}, k_{3_meco}, c_{n_meco}, c_{b_meco}, determined with eq. (5.24/5.25), since

$$P = c_n - \frac{k_{1\_meco} c_{n\_meco}}{(k_{1\_meco} + k_{2\_meco} c_{n\_meco}) e^{k_{meco} t} - k_{2\_meco} c_{n\_meco}} + c_{b\_meco}(1 - e^{-k_{3\_meco} t}) \quad (5.24/5.25)$$

where

P = total amount of evolved mineralisation product ($^{14}CO_2$), equivalent to the total amount of mineralised ^{14}C-mecoprop at time t (measured as % ^{14}C evolved as $^{14}CO_2$)
c_{n_meco} = total % ^{14}C-mecoprop converted to $^{14}CO_2$ according to the Liu & Zhang-model (Liu & Zhang, 1986)
c_{b_meco} = total % ^{14}C-mecoprop converted to $^{14}CO_2$ according to the first order model

k_{1_meco}, k_{2_meco} = *rate constants*

$k_{1\_meco} = k_{\_meco}(m_0 + \lambda_{\_meco} c_{n\_meco})$

$k_{2\_meco} = -k_{\_meco}\lambda_{\_meco}$

k_{3_meco} = rate constant for the first order process

$\lambda_{\_meco}$ = growth rate of the micro-organisms

$m_{0\ \_meco}$ = initial amount of degradation micro-organisms

First of all, linear regressions between the parameter estimates and the factors: biological activity, MPN-number, % humus, % clay, % sand, % silt, pH, SOC (soluble organic carbon), NO_3-N, NH_4-N, K_d-value and depth were performed. A plot of the residuals showed a lack of homogeneity among the variances. Thus linear regressions between $\log_e k_{1\_meco}$, k_{2_meco}, k_{3_meco}, $R(c_{n\_meco})$, $R(c_{b\_meco})$ and the above-mentioned factors plus R(% humus), R(% clay), R(% sand) and R(% silt) were performed. Here

$$R(x) = \log_e \frac{x}{100 - x} \qquad (5.38)$$

was applied to improve the linear correlations in which %-values entered, since parameters expressed as % will never have a continued distribution near 0 and 100 %. In addition the variance, which generally is less close to 0 and 100 %, were made more homogeneous, and predicted values below 0 or above 100 % were avoided.

On the basis of the best correlations between the variables mentioned above, the final model was

$$P = c_n - \frac{k_1 c_n}{(k_1 + k_2 c_n)e^{k_1 t} - k_2 c_n} + c_b(1 - e^{-k_3 t}) \qquad (5.24/5.25)$$

where

$$\log_e k_1 = \alpha_1 + \beta_1 \cdot \log_e \frac{\%humus}{100 - \%humus} + \beta_2 \cdot ploughlayer \qquad (5.39)$$

$$k_2 = \alpha_2 + \beta_3 \cdot ploughlayer \qquad (5.40)$$

$$k_3 = \alpha_3 + \beta_4 \cdot ploughlayer \qquad (5.41)$$

$$\log_e \frac{c_n}{100 - c_n} = \alpha_n + \beta_5 \cdot \log_e k_1\_naac \qquad (5.42)$$

$$\log_e \frac{c_b}{100 - c_b} = \alpha_b + \beta_6 \log_e \frac{\%clay}{100 - \%clay} + \beta_7 \cdot ploughlayer \qquad (5.43)$$

"Plough layer" was given the value 1 for plough layer soil samples and the value 0 for soil from 45 and 75 cm's depth.

The resulting model together with the data points is shown in Figure 5.15.

5.2.4. The causality of the mineralisation model

The parameter estimates of k_{1_naac} were used as measurements of biological activity. The determination of biological activity/biomass has been made in a number of ways according to the literature, and one single method cannot be considered standard method. Three methods which very often have been used are the fumigation-incubation method (Jenkinson & Powlson, 1976), the fumigation-extraction method (Voroney & Paul, 1984; Vance et al., 1987) and the substrate-induced respiration (Anderson & Domsch, 1978). ATP-methods (Tate & Jenkinson, 1982; Eiland, 1983; Bai et al., 1988), staining followed by direct counting (Söderström, 1977) and determination of biomass by means of determinations of the fatty acid pattern (Zelles et al., 1994) are other relevant methods. Martens (1995) concluded that precise determinations of transformation factor between the methods could not be determined.

In the substrate-induced respiration method (Anderson & Domsch, 1978) glucose is added to the soil and the development of CO_2 is followed every hour. I chose to use the mineralisation of ^{14}C-Na-acetat as a measurement of biological activity, since Na-acetate is a natural substance in the metabolism of the micro-organisms (Dictor et al., 1992). The evolved $^{14}CO_2$ from Na-acetate could then be measured by scintillation counting in the same way as was done in the pesticide incubation experiments. As a parallel to the substrate-induced respiration method, using the developed amount of CO_2 at the time of maximum response, I tested the use of $\%^{14}CO_2$ developed from ^{14}C-Na-acetate after two hours and after four hours, respectively, as a measurement of biological activity. The parameter estimates obtained with eq. (5.24/5.25) were tested for correlation with the values of $\%^{14}CO_2$ after 2 and 4 hours. The correlations found were low. The ^{14}C-Na-acetate mineralisation curves (**VI**) were then described with the model eq. (5.24/5.25) using non-linear regression. The rate constant k_{1_naac} was hereafter used as a measurement of the biological activity.

In chapter 5.1.7 the development of the kinetic model (eq. (5.24/5.25) was described, and the mutual relation between the parameters, c_n, k_1, k_2, k_3, and c_b was explained. The model was applicable to all types of data and gave well-estimated parameters on condition that: 1) good initial estimates were obtained by means of a non-linear regression of simplified models where either k_2 or k_3 were given the value 0, and 2) the estimates from the simplified models were used as initial estimates for the final non-linear regression with eq. (5.24/5.25), and 3) parameter estimates for two versions of the model A: $c_1+c_2=100$ and B: $c_1+c_2<100$, were obtained.

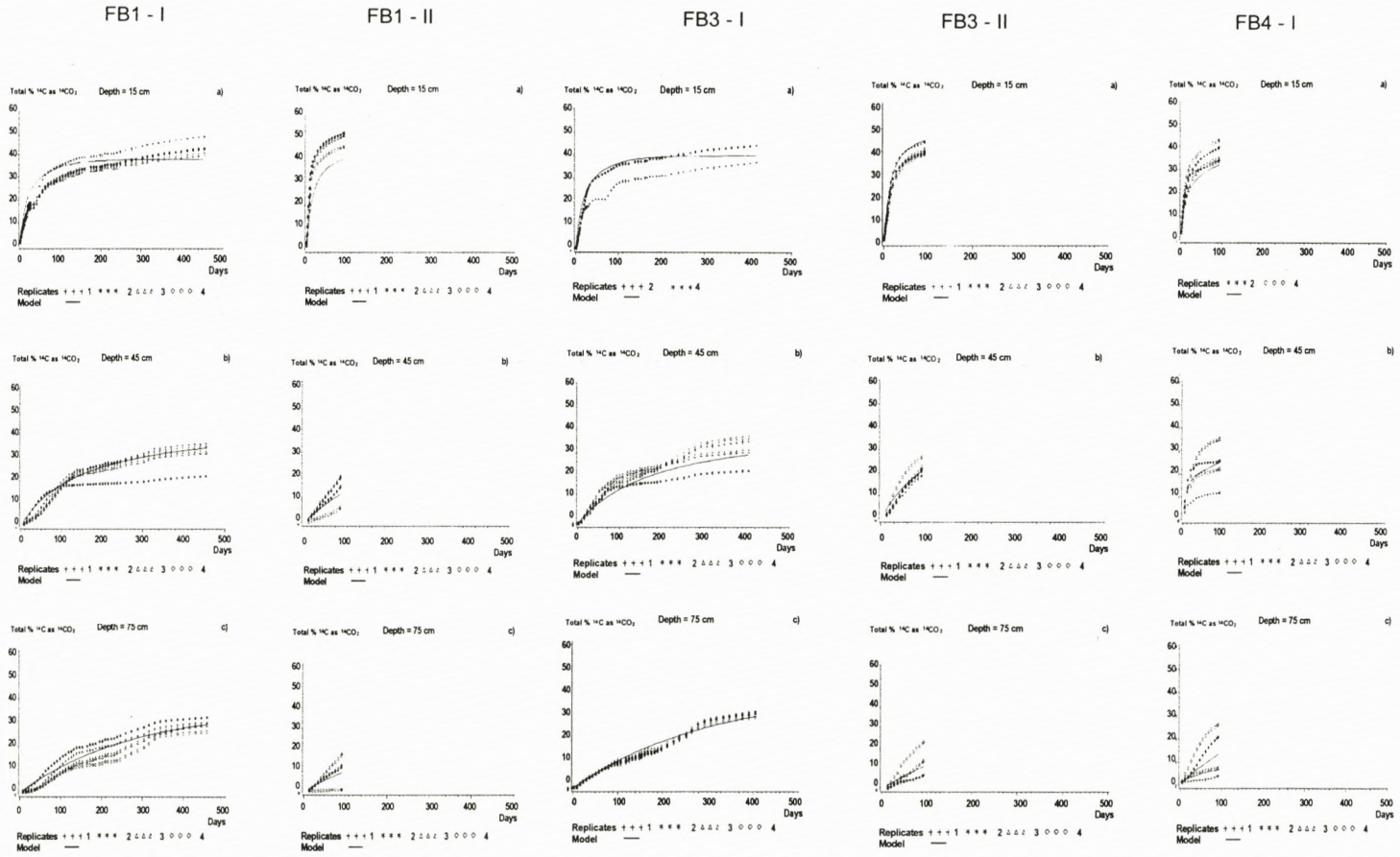


Figure 5.15. Mineralisation of 0.04 µg g^{-1} ^{14}C-mecoprop in Danish soil from 15, 45 and 75 cm's depth. Incubated at 10°C. The data points for the four replicates are shown with symbols. The model developed on the basis of % humus, % clay, biological activity and soil depth is shown with solid lines.

If the mineralisation curve was not developed far enough to include the flat part of the curve, k_3 became 0, and if no growth of micro-organisms happened, k_2 became 0. The general pattern for the pesticide mineralisation, as discussed in 5.1.3-5.1.8, was that pesticides in low concentrations were mineralised with kinetics without growth in plough layer and with kinetics with growth in subsoil. However, during the mineralisation of mecoprop in Danish plough layer soil (**II** and **VI**) (concentration 0.04 μg g^{-1}, incubation temperature 10°C) growth of micro-organisms was seen in all experiments except one. In German, Spanish and Italian soil (concentration 0.04 μg g^{-1}, incubation temperature 20°C) only cometabolic mineralisation was seen. In **IV** and in Reffstrup et al. (1996), mineralisation without growth (k_2=0) was seen at the concentration of 0.05 μg g^{-1} and only in concentrations above this, growth was seen. Thus, temperature and concentration of pesticide are factors that should be included in the composed model in the future.

The composed model eq. (5.24/5.25) and (5.39)-(5.43), in which the estimated values were α_1= 0.98211; β_1=1.04619; β_2=0.42678; α_2=-0.00025405; β_3=-0.00063262; α_3=0.0040430; β_4=0.014518; α_n=-1.23350; β_5=0.92952; α_b=-1.18940; β_6=-0.075358; β_7=-0.42003 described the relation between the parameters c_n, k_1, k_2, k_3, c_b and the geo-environmental factors which influenced the mineralisation.

The relation between the parameters in the mineralisation model and the influencing factors was not directly comparable to similar relations in other published studies, in which other models were used to describe the mineralisation/degradation curves. Rate constants are defined differently in different models. However, the relation between the rate constant k_1 in my model and the influencing geo-environmental factors should have certain similarities to other presented relations between degradation/mineralisation rate constants and geo-environmental factors. Mueller et al. (1992) showed a positive linear correlation between the first order degradation rate constant for fluometuron and the soil's content of organic matter and between the pseudo first order rate constant and the microbial biomass. The correlation of the pseudo first order rate constant with soil depth was clearly negative (Figure 5.16).

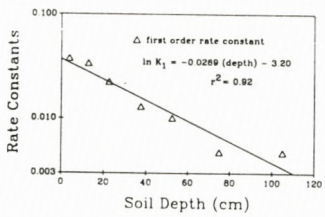


Figure 5.16. The correlation between the pseudo first order degradation rate constant for fluometuron and soil depth (Mueller et al., 1992). (Copyright American Chemical Society. Reproduced with permission).

Contrary to this, Simon et al. (1992) stated that it was not possible to find a good correlation neither between the first order degradation rate constant for fenamiphos and the biomass of the soil or the soil's content of organic matter, respectively, nor between the first order mineralisation constant and the biomass of the soil or the soil's content of organic matter, respectively. However, Simon et al. (1992) found a good correlation between the mineralisation rate and $\%C_{mic}/C_{org}$ (Figure 5.17). Veeh et al. (1996) carried out degradation experiments with 2,4-D and showed a negative correlation between the half-life time and the amount of organic matter. The amount of organic matter decreased down through the soil profile. At the same time, the amount of organic matter was correlated to the number of micro-organisms, counted by plating. Veeh et al. (1996) concluded that for compounds, which have a low sorption to soil organic matter, such correlations should never be used to predict the degradation rates of pesticides. Torstensson & Stenström (1986) developed a method for the determination of basic respiration rates and correlated the respiration rate with the degradation rate constant for linuron and glyphosate. Nevertheless, a correlation to the metabolically degraded 2,4-D could not be shown. Walker et al. (1983) found that the first order degradation rate constant for simazine was significantly correlated to the clay content, the content of organic carbon and pH.

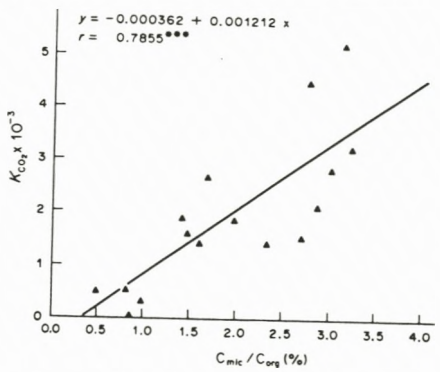


Figure 5.17. The correlation between the first order mineralisation rate constant and $\%C_{mic}/C_{org}$ in 16 soils at 22°C (Simon et al., 1992). (Copyright Elsevier Science. Reproduced with permission).

The fact that the soil depth, the biological activity and the soil's content of organic matter and clay were the factors which showed to be the most important for the mineralisation in my experiments, is thus coherent with the conclusions in the above mentioned papers. However, when modelling the mineralisation is the case, it is not sufficient to test the correlation of the

rate constant with external factors. The influence of external factors on the amount of pesticide transformed in the modelled process (c_n) must also be taken into account.

The soil depth affected all parameters except c_n, % humus together with the soil depth affected k_1, the biological activity affected c_n, and the amount of clay together with the soil depth affected c_b. The effect of the factor depth, was only seen as the model for plough layer differed from the other soil layers. No effect from depth to depth in subsoil was seen. The rate constant k_1 for the mineralisation of mecoprop is higher in plough layer than in subsoil, not only because of the differences in depth but also because of the different amount of humus, present in the different layers. The parameter c_n, the amount of pesticide converted directly to CO_2, increased with increased biological activity. The parameter k_2 always will have the value 0, when no growth of micro-organisms occurs and a negative value when growth occurs. The parameter c_b, the amount of ^{14}C originating from the pesticide which was firstly built into the organic matter and secondly was mineralised to $^{14}CO_2$, increased slightly with decreasing amount of clay. The organic matter was probably sorbed to the surface of the clay minerals and thus became less available for the micro-organisms. Nevertheless, c_b was substantially higher in subsoil than in plough layer. The explanation for this, could be that the parameter estimates of the second compartment of the kinetic model– the first order term – was determined with minor precision in subsoil. The incubations were not continued long time enough to make it possible to determine the parameters in the second compartment with model version B ($c_n + c_b < 100$), thus they were determined with model version A ($c_n + c_b = 100$). For the same reason k_3 was higher in plough layer than in subsoil.

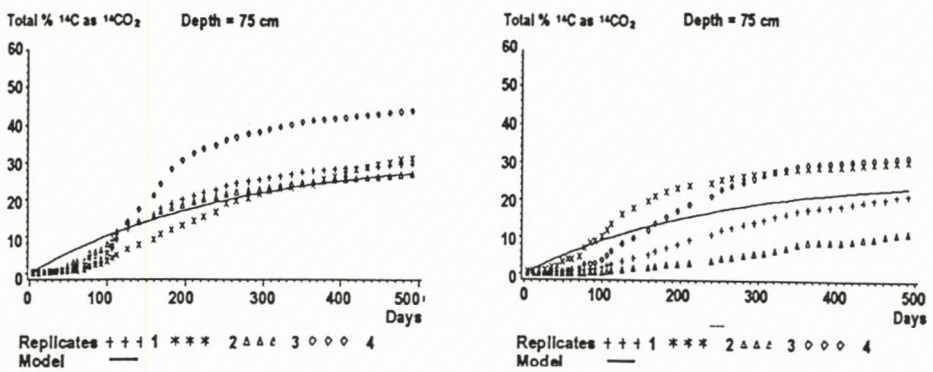


Figure 5.18. Mineralisation of 0.04 μg g^{-1} ^{14}C-mecoprop in German soil from the depth 75 cm. Incubated at 10°C. Data points for the four replicates are shown with symbols. The solid line shows the mineralisation estimated on the basis of % humus, % clay, biological activity and soil depth in German soil, applying the model, developed on the basis of experiments in Danish soil. (Figures 11-12 from **VI**).

The model was developed in paper **VI** on the basis of a number of mecoprop mineralisation studies in Danish soil, incubated at 10°C. As explained earlier, the mecoprop experiments formed part of a higher number of experiments, presented in paper **II**. Two of the rest of the experiments, mineralisation of mecoprop in German soil from 75 cm's depth, were performed at the same temperature and concentration as the mecoprop experiments in Danish soil. These two experiments were used to validate the model. The expected mineralisation of mecoprop in German soil samples was estimated using the model on the basis of the measured values for humus, biological activity, clay and soil depth of the German soil samples. The estimated mineralisation is shown in Figure 5.18. The model did not estimate the initial part of the mineralisation curve very well, but it can without doubt be used for the estimation of c_n, and of the time needed for a total mineralisation of the added ^{14}C-mecoprop.

5.2.5. The future development of the model

A model for the mineralisation of mecoprop, in which the concurrent effect of soil depth, biological activity content of organic matter and texture is described, have not formerly been presented in the literature. The experiments used for the development of the model were limited, since the factors: temperature and initial pesticide concentrations were not included. Obviously, the model should be amplified to include these factors, too. The Arrhenius equation,

$$k = A_0 \exp [-(E_a/RT)] \qquad (5.44)$$

and

$$Q_{10} = \exp [E_a/68627] \qquad (5.45)$$

in which k = rate constant for the degradation (dag^{-1}), E_a = activation energy ($J\ mol^{-1}$), R = gas constant ($J\ mol^{-1}\ K^{-1}$), T = temperature (°K) and A_0 is a constant (Walker et al., 1996), calculatesQ_{10}, as the factor by which the first order degradation rate constant must be corrected, when the temperature is changed by 10°C. The effect of a change in temperature on the parameters c_n, k_1, k_2, k_3 and c_b in my model cannot be concluded directly from the Arrhenius equation. Further studies on the effect of the temperature are therefore needed. For the mineralisation of ETU, I showed an interaction effect between the factors: temperature and concentration among others. It is therefore doubtful that a correction for only one of the factors could be included in the mecoprop mineralisation model.

In the future, the model should obviously be amplified to include sorption and should be validated with other compounds with higher sorption than mecoprop.

6. Synopsis of the conclusions

A kinetic model for the description of mineralisation was developed

$$P = c_n - \frac{k_1 c_n}{(k_1 + k_2 c_n)e^{k_1 t} - k_2 c_n} + c_b(1 - e^{-k_3 t}) \qquad (6.1/6.2)$$

where

P = total amount of evolved mineralisation product ($^{14}CO_2$), equivalent to the total amount of mineralised ^{14}C-pesticide at time t (measured as % ^{14}C evolved as $^{14}CO_2$)

c_n = total % ^{14}C-pesticide converted to $^{14}CO_2$ according to the Liu & Zhang-model (Liu & Zhang, 1986)

c_b = total % ^{14}C-pesticid converted to $^{14}CO_2$ according to the first order model

k_1, k_2 =rate constants

$k_1 = k(m_0 + \lambda c_n)$

$k_2 = -k\lambda$

k_3 = rate constant for the first order process

λ = growth rate of the micro-organisms

m_0 = initial amount of degradation micro-organisms

$c_n + c_b = 100$ for eq. (6.1)

$c_n + c_b < 100$ for eq. (6.2)

The model could describe both the metabolic (with growth of micro-organisms) and the cometabolic (without growth of micro-organisms) mineralisation of pesticides. When no growth occurred $\lambda = 0$ and $k_2 = 0$, and the expression reduced to

$$P = c_n(1 - e^{-k_1 t}) + c_b(1 - e^{-k_3 t}) \qquad (6.3)$$

which is a two-compartment first order model.

The model was based on the relation

$$m = m_0 + \lambda(c_n - c) \qquad (6.4)$$

where

c_n = the initial amount of pesticide

c = amount of pesticide at time t

m_0 = the initial amount of micro-organisms, involved in the degradation

m = the amount of micro-organisms, involved in the degradation at time t

λ = growth rate for the micro-organisms

and

$$-\frac{dc}{dt} = kcm \qquad (6.5)$$

where k is the rate constant.

$$-\frac{dc}{dt} = k(m_0 + \lambda c_n)c - k\lambda c^2 \qquad (6.6)$$

$$-\frac{dx}{dt} = k_1 c + k_2 c^2 \qquad (6.7)$$

Eq. (6.5) is equal to the law of mass action, and eq. (6.7) is equal to the rate equation for autocatalytic reactions.

The developed kinetic model (6.1/6.2) was tested with mineralisation experiments for a number of compounds under very varying conditions. It was tested with mineralisation experiments for mecoprop, ETU and bentazon in low concentrations, for mecoprop and isoproturon in concentrations from 0.0005 – 500 μg g^{-1} both in plough layer and subsoil, and for maneb in low concentrations in river sediment. For all compounds (except isoproturon because the experiments were not fully developed to fit any model) in all conditions, without regard to the kinetic process, the model gave well-estimated parameters. It is thus reasonable to believe that a mineralisation model, which is generally applicable for the description of the mineralisation of xenobiotic compounds is soil, was developed. Such a model was never described in the literature before as far as I know. The model would probably be applicable to mineralisation experiments in i.e. water.

The relation between the parameters from the kinetic model and interaction geo-environmental factors: % humus, % clay, soil depth and biological activity was described in a composed mathematical model, which could predict the mineralisation:

$$P = c_n - \frac{k_1 c_n}{(k_1 + k_2 c_n)e^{k_1 t} - k_2 c_n} + c_b(1 - e^{-k_3 t}) \qquad (6.1/6.2)$$

where

$$\log_e k_1 = \alpha_1 + \beta_1 \cdot \log_e \frac{\%humus}{100 - \%humus} + \beta_2 \cdot ploughlayer \qquad (6.8)$$

$$k_2 = \alpha_2 + \beta_3 \cdot ploughlayer \qquad (6.9)$$

$$k_3 = \alpha_3 + \beta_4 \cdot ploughlayer \qquad (6.10)$$

$$\log_e \frac{c_n}{100 - c_n} = \alpha_n + \beta_5 \cdot \log_e k_1\_naac \qquad (6.11)$$

$$\log_e \frac{c_b}{100 - c_b} = \alpha_b + \beta_6 \log_e \frac{\%clay}{100 - \%clay} + \beta_7 \cdot ploughlayer \qquad (6.12)$$

The composed mineralisation model was developed from the best correlation between model parameters and concurrently influencing factors and was tested with mecoprop mineralisation experiments in a low concentration (0.04 μg g^{-1}). It showed to be useful for the prediction of the mineralisation of mecoprop in soil.

The influence of external factors on the degradation of xenobiotic compounds in soil was mostly described for one factor at a time in other published studies. A complex model describing the concurrent influence of several factors on the mineralisation of mecoprop was not described in any other studies as far as I know.

The application of the first part of the mineralisation model (6.1/6.2) to a number of experiments resulted in the following conclusions:

The mineralisation developed according to this simplified summary:

Soil depth	Compound	Low conc.	High conc.
Plough layer	mecoprop	wo/w growth	w growth
Plough layer	ETU	wo/w growth	w growth
Plough layer	bentazon	wo growth	
Plough layer	isoproturon	wo growth	*
Subsoil	mecoprop	wo/w growth	w growth
Subsoil	ETU	w growth	
Subsoil	bentazon	w growth	
Subsoil	isoproturon	*	*
River sediment	maneb	w growth	

* Not developed far enough to model

The mineralisation of mecoprop, ETU and bentazon in the concentrations 0.04 μg g^{-1}, 0.07 μg g^{-1} and 0.08 μg g^{-1} generally followed kinetics without growth (cometabolic mineralisation) in plough layer soil. In subsoil, down to 75 cm, the mineralisation generally followed kinetics with growth of micro-organisms (metabolic mineralisation).

The mineralisation of maneb in river sediment followed kinetics with growth. The rate constant for the process did not depend on external factors, but the amount of mineralised pesticide varied under the influence of external factors. The highest amount of pesticide was mineralised to CO_2 at the stations with supposed highest biological activity.

For mecoprop and isoproturon in the wide range of concentrations from 0.0005 μg g^{-1} to 5000 μg g^{-1} great variation was seen, in kinetic processes as well as in mineralisation rate. Mecoprop was mineralised according to kinetics without growth in the lowest concentrations. In higher concentrations kinetic with growth was seen. For each soil depth the rate constant k_1

did not differ between the concentrations from 0.0005 to 50 μg g^{-1}. The rate constant was about 10 times higher in plough layer than in subsoil. Isoproturon apparently was mineralised according to kinetics without growth in all concentrations. However, the mineralisation of isoproturon developed very slow and was not developed far enough to model with certainty. An eventual growth phase could have appeared later in the mineralisation curves.

In a 2^k factor study, the influence of the external factors: depth, organic matter, temperature and initial concentration on the mineralisation of ^{14}C-ETU showed to be very complex. A significant three-way interaction effect depth*concentration*temperature was seen for all parameters in the kinetic model, c_n, k_1, k_2 and λ/m_0. A three-way interaction depth*concentration*suspension was seen for the parameter c_n, and two-way interaction effects concentration*suspension for k_1 and k_2.

The mineralisation kinetics for pesticides in soil as well as the mineralisation rate varied thus under the influence of a number of geo-environmental factors.

The composed mineralisation model (6.1-6.12), which describes the effect of the external geo-environmental factors: depth, biological activity, texture and content of humus on the mineralisation rate of mecoprop, should be amplified in the future to include the effect of factors: temperature and initial concentration. Furthermore it should be amplified to include sorption of pesticides before it can be used for compounds with a higher sorption to soil organic matter than is the case for mecoprop.

Future research concerning degradation of pesticides in soil should intend to describe the concurrent effect of the factors which have a significant influence on the degradation. Moreover, a further development of mathematical models, capable of predicting mineralisation, degradation and formation of metabolites on the basis of geo-environmental factors is needed.

7. Resumé (dansk)

De mange fund af pesticidrester i grundvand, der er gjort indenfor det sidste årti, har øget behovet for at undersøge pesticiders skæbne i jorden. Formålet med dette projekt var at udbygge kendskabet til pesticiders mineralisering i jord, idet der blev fokuseret på undersøgelser af, hvilken kinetik en række pesticider blev nedbrudt efter, samt hvilke faktorer der påvirkede nedbrydningshastigheden. Mange publikationer har beskrevet vanskelighederne ved at finde en brugbar matematisk model til beskrivelse af mineralisering af pesticider. Her udvikledes en matematisk model, som var anvendelig til at beskrive såvel cometabolisk mineralisering som metabolisk mineralisering.

Modellen kunne beskrive mineraliseringen af mecoprop, ETU og bentazon under meget varierende forhold. Modellen vil formodentlig kunne anvendes til at beskrive mineraliseringen for andre xenobiotiske stoffer i jord. Anvendelsen af én og samme model til beskrivelsen af varierende forsøg forbedrer muligheden for at sammenligne mineraliseringshastigheder og undersøge jordmiljøfaktorernes indflydelse på hastigheden.

Den udviklede kinetiske model blev anvendt til at beskrive a) betydningen af den initiale concentration og jorddybden for nedbrydningen af mecoprop, b) vekselvirkningseffekten af temperature, jorddybde, OC indhold og initial concentration på mineraliseringen af ETU og c) den samtidige effekt af mikrobiel aktivitet, OC indhold, tekstur og jorddybde på mineraliseringen af mecoprop. a) viste, at ved concentrationer på 0.0005 – 0.05 $\mu g\ g^{-1}$ sås ingen vækst af mikroorganismer, mens der sås vækst af mikroorganismer ved koncentrationer af mecoprop fra 0.5 – 50 $\mu g\ g^{-1}$. b) viste, at der var trevejsvekselvirkninger mellem depth*temperature*concentration for mineraliseringshastigheden af ETU. Undersøgelse af vekselvirkninger mellem de faktorer, der påvirker mineraliseringshastigheden, er således af betydning for at beskrive ETU's mineraliseringshastighed i stedet for at undersøge faktorernes indflydelse én ad gangen. c) viste, at mineraliseringshastigheden for mecoprop i jord var påvirket af den biologiske aktivitet, jordens tekstur, indholdet af humus og jorddybden.

På baggrund af mecopropmineraliseringsforsøg i danske jorde blev der udviklet en prediktiv model, som beskrev mineraliseringen som funktion af biologisk aktivitet, jordens tekstur, indholdet af humus og jorddybden.

Modellen blev valideret på mecopropmineraliseringsforsøg i tyske jorde og viste sig yderst anvendelig til at forudsige tiden for den totale mineralisering af mecoprop.

8. Abstract (English)

The high number of cases where pesticide residues have been found in groundwater during the last decade has enhanced the need for more knowledge about fate of pesticides in soil. The purpose of the present project was to extend the knowledge of pesticide mineralisation in soil. The project focused on studies of the kinetics according to which the pesticides were degraded and on studies of the factors that affected the degradation rate. Many publications have described the difficulties of finding a useful mathematical model for the description of pesticide mineralisation. In the present project a mathematical model was developed, which was useful for describing cometabolic mineralisation as well as metabolic mineralisation.

The kinetic model showed to be able to describe the mineralisation of mecoprop, ETU and bentazone under highly varying conditions. The model will presumably be useful for the description of mineralisation of other xenobiotic compounds in soil. The application of the very same model to describe a variety of mineralisation experiments, enhances the possibilities of comparing mineralisation rates and of investigating the influence of soil environmental factors on the mineralisation rate.

The developed kinetic model was used to describe a) the influence of initial concentration and soil depth on degradation of mecoprop, b) combined interaction effects of temperature, soil depth, organic carbon content and initial concentration on the mineralisation of ETU and c) the simultaneous effects of microbial activity, organic carbon content, texture and soil depth on the mineralisation of mecoprop. a) showed that no growth of microorganisms was seen at concentrations from 0.0005 to 0.05 $\mu g\ g^{-1}$, while growth of microorganisms was seen at concentrations from 0.5 to 50 $\mu g\ g^{-1}$. b) showed three-way interaction effects between depth, temperature and concentration for the mineralisation rate of ETU. Investigations of interaction effects between factors influencing mineralisation rates should thus be preferred for the description of mineralisation of ETU instead of investigating one factor at a time. c) showed that the mineralisation rate of mecoprop was influenced by the microbial activity, soil texture, humus content and soil depth.

On the basis of mineralisation studies of mecoprop in Danish soils, a predictive model, which described the mineralisation as a function of microbial activity, soil texture, humus content and soil depth, was developed.

The model was validated against mecoprop mineralisation studies in German soils and showed to be very useful for the prediction of time for total mineralisation of mecoprop.

9. Resumen (español)

Los múltiples descubrimientos de residuos de plaguicidas en el agua subterránea, encontrados durante el último decenio, han aumentado la necesidad de investigar el destino de los plaguicidas en el suelo. El objetivo de este proyecto fue desarrollar el conocimiento de la mineralización de los plagucidas en suelo, teniendo como enfoque los estudios sobre qué tipo de cinética fue basada la degradación de una serie de plagucidas, además cuáles fueron los factores que influyeron en la velocidad de degradación. Son muchas las publicaciones las que han descrito las dificultades en encontrar un modelo matemático aplicable para la descripción de la mineralización de plaguicidas. En este proyecto se desarrolló un modelo matemático el cual fue útil para describir tanto la mineralización cometabólica como la mineralización metabólica.

El modelo cinético podía describir la mineralización de mecoprop, ETU y bentazón bajo condiciones muy variadas. Probablemente el modelo pueda ser utilizado para describir la mineralización de otros productos cenobióticos en suelo. El uso de un mismo modelo para la descripción de diferentes experimientos mejorará la posibilidad de comparar la velocidad de mineralización y estudiar la influencia de los factores del ambiente del suelo en cuanto a la velocidad.

El modelo cinético desarrollado fue utilizado para describir a) el significado de la concentración inicial y la profundidad del suelo para la degradación de mecoprop, b) el efecto de la acción recíproca de temperatura, profundidad del suelo, contenido de CO, textura y la concentración inicial en la mineralización de ETU y c) el efecto simultáneo por actividad microbiana, contenido de CO, textura y profundidad del suelo en la mineralización de mecoprop. a) mostró que con una concentración de 0.0005 – 0.05 $\mu g\ g^{-1}$ no se dió un crecimiento de los microorganismos, mientras que con una concentración de 0.5 – 50 $\mu g\ g^{-1}$ de mecoprop se dió crecimiento de microorganismos. b) mostró que habian efectos de acciónes recíprocas de triple vía entre profundidad*temperatura*concentración en la velocidad de mineralización de ETU. De este modo es de importancia un estudio/una investigación de los efectos de correlaciones/acciones recíprocas entre los factores que influyen en la velocidad de mineralización en lugar de estudiar la influencia de cada uno de los factores por separado. c) mostró que la velocidad de mineralización de mecoprop en suelo estaba influenciada por actividad microbiana, textura del suelo, contenido de humus y profundidad del suelo.

A base de estudios de mineralización de mecoprop en suelos daneses un modelo predicativo fue desarrollado, el cual describió la mineralización como resultado de actividad biológica, la textura del suelo, el contenido de humus y la profundidad del suelo.

El modelo fue validado en estudios de mineralización de mecoprop en suelos alemanes y resultó muy útil para pronosticar el tiempo de la mineralización total de mecoprop.

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DEGRADATION OF PESTICIDES IN SUBSURFACE SOILS, UNSATURATED ZONE - A REVIEW OF METHODS AND RESULTS

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Methods and results from degradation studies in subsoils, unsaturated zone, were reviewed for mecoprop, 2,4-D, atrazine, alachlor, aldicarb, carbofuran, linuron, oxamyl, methomyl, MCPA, dichlorprop, monochlorprop, dichlorphenol, TCA, parathion, metribuzin, metolachlor and fenamiphos.

Most of the investigations were laboratory studies where small soil samples were sieved and pesticides were added in concentrations from 0.5–5 $\mu g \cdot g^{-1}$. A few of the studies mentioned the importance of working with undisturbed samples; another few studies used isotope-labelled pesticides which made it possible to work with concentrations as low as 0.02 $\mu g \cdot g^{-1}$.

Subsoil samples were characterized according to factors as microbial activity, soil temperature, water content, oxygen content, concentration of pesticide, pretreatment of the soil and soil type, factors considered to have influence on degradation of pesticides. Chemical hydrolysis was considered to be the most dominant pathway in the degradation of aldicarb in subsoil in one of the published papers; all other investigations considered the degradation of pesticides in subsoil to be primarily microbiological. Only a few of the investigations measured the biomass or biological activity of the subsoil samples.

KEY WORDS: Subsurface soil, unsaturated zone, pesticides, degradation, methods, review.

INTRODUCTION

During the last decade pesticides have been detected in ground water and drain water in many European countries as well as in North America.[1,2] Nygaard[3] presented the results from a monitoring of Danish ground water quality, 1989–1991, covering analysis of dichlorprop, mecoprop, MCPA, dinoseb, atrazine and simazine. Pesticides were detected in 36 out of 528 wells. In half of the 36 samples the concentration exceeded 0.1 $\mu g \cdot l^{-1}$. It is not known whether detection of pesticides in ground water at concentrations above the residue limit (0.1 $\mu g \cdot l^{-1}$) are caused by point source pollution or the use of these chemicals in agriculture.

Until recently most of the published degradation studies focused on soil from the upper layer. Persistence criteria for registration of pesticides normally refer to half-lives of pesticides in different soil types and at different application rates,—but not in soil from the subsurface. Nevertheless, the finding of pesticides in ground water has increased the

importance of elucidating degradation rates of these compounds in the subsurface environment. Moreover, information about the kinetics of pesticide biodegradation in subsoils is required for the development and validation of mathematical models used to predict the fate of pesticides in the environment.

The present study reviews the methodology and results in published pesticide degradation studies in subsoils, mainly from the unsaturated zone (the zone above the water table): Alachlor[4,5], aldicarb[6–10], aldicarb sulphoxide[7,9,11,12], aldoxycarb[6,7,9,12,13], atrazine[14–18], carbofuran[19], 2,4-D[18,19], dichlorphenol[20], dichlorprop + monochlorprop[20], fenamiphos[21], linuron[22], MCPA[23], mecoprop[24], methomyl[25], metolachlor[14], metribuzin[5,22,26], oxamyl[12], parathion[20], TCA[20]. Based on the reviewed papers, general recommendations for a methodology for degradation studies are given.

DEGRADATION MECHANISMS IN SUBSOIL

General

Several factors are responsible for the dissipation of pesticide residues from soil, factors such as surface run-off, volatilization, plant uptake, transport through soil and degradation. Pesticides in soil are degraded by photochemical, chemical and microbiological processes. The photochemical degradation (induced by sunlight) is only occurring in surface soil.

Degradation of a pesticide is a series of stepwise processes leading to various end products. If the pesticide is totally mineralized, CO_2 is formed. A part of the pesticide-carbon is built into humus and soil microorganisms. Stable degradation products can be produced, too, and may end up as residues bound in the organic fraction of the soil. Figure 1 illustrates the degradation of a pesticide. Degradation of pesticides in subsoil follows a microbial or chemical pathway or a combination of both.[27]

Microbial degradation

The important role of microorganisms in the degradation of pesticide residues in soil was described by Torstensson[28]. Helweg[29] reviewed degradation studies in soil of 230 pesticides. Microbial degradation was reported in 80 cases and chemical degradation only in 13 cases. Microbial decomposition of pesticides can occur by metabolism or by cometabolism.

The number of microorganisms found in subsoil often is up to 100 times smaller than in soil from the upper layer (Table 1). In Danish subsurface soil Eiland[30] found up to 10^9 bacteria per gram soil at a depth of 1 meter and 10^7 at 5–6 meter depth.

Table 1 Microorganisms in soil determined by direct counting

	Bacteria (mill/g)	Fungi (meter/g)
Plough layer	500–1000	200–2000
Below root zone	1–10	only few

Sinclair and Lee[17] compared degradation rates of atrazine in active (non-sterile) and sterile (autoclaved) subsoil samples. The reason for the lack of degradation in the active soil was said to be due to the small bacterial population.

The addition of nutrients increased the transformation of alachlor, which indicated that the degradation was microbiological and cometabolic.[4] No relation was found between degradation rate (determined during 161 days) and microbial number determined by plate counting on PTYG medium. Viable cell counts often give lower and more variable results than total cell counts.[31]

Degradation of atrazine occurred more rapidly at the surface than at deeper levels. This was explained by the lower number of microorganisms and the lower temperature at lower depths.[18] The reason why the lower number of microorganisms measured at lower depths did not affect the aerobic degradation rate of 2,4-D was not explained.

The faster dissipation rate in the field than in the laboratory of metribuzin[5] was suggested to be due to the treatment of the laboratory sample—a possible decrease in microbial activity during the drying period and a lack of natural cracks and channels in the dried and sieved soil.

The mineralization of carbofuran and the microbial biomass content decreased with depth except in one zone where both were higher.[19] The microbial population present in these subsurface soils seemed to be ineffective in the degradation of 2,4-D.[19]

Chemical degradation

Chemical degradation does not appear to have much importance in the total degradation of pesticides in subsoil. In some cases chemical hydrolysis as one of the degradation steps is mentioned. The degradation rate of aldicarb[8] did not change significantly with depth, and, taking into account that the amount of microorganisms in deeper soil layers normally diminishes, Jones[8] concluded that chemical hydrolysis was an important degradation pathway for aldicarb in subsoil. Microbiological activity was not determined.

Degradation of aldicarb decreased with increasing depth, but total carbamate residues were not influenced by depth. Aerobic degradation of aldicarb in upper soil layers was caused by microbial oxidation and in deep subsurface samples by chemical hydrolysis.[9]

For sterilized (autoclaved) unsaturated subsoil half-life for aldicarb sulphoxide, aldoxycarb and oxamyl increased 3–4 times compared to unsterilized soil. The fact that there was a conversion of pesticides in sterilized soil showed that at least the first stage of degradation was not purely microbiological.[12]

ESTIMATION OF DEGRADATION RATES (DEGRADATION KINETICS)

Pesticides like the phenoxyherbicides (MCPA, mecoprop and 2,4-D) are known to be decomposed metabolically[32] while most other pesticides are decomposed through a cometabolic process.[33] Cases can be seen, where different processes are followed during the step-wise degradation of a pesticide.

Table 2 Summary of degradation rates of pesticides in subsoil from the unsaturated zone (below 30 cm) calculated on basis of residues of parent compound.

Compound	*Soil type*	*% OC*	*Conc* $\mu g \cdot g^{-1}$	*Temp*	*Method*	*Half-life* $T_{1/2}$	*Ref*
Alachlor	loamy sand	0.08–0.14	1.5	20°C	lab.study of composited samp, aerobic incub.	22–285 days	4
Alachlor	loamy sand	0.08–0.14	1.5	20°C	lab.study of composited samp, anaerobic incub.	53–148 days	4
Alachlor	coarse sand	0.04–0.24	appl♠/ 1–4	23°C	field, enclosed samples/lab, dried and sieved soil	34–39 days	5
Aldicarb	sand-clay loam	0.0–2.0	appl	nat°	field, normal application	0.5–2 months	6
Aldicarb	sand	< 0.02 –0.16	appl	nat	field, normal application	11–23 days	7
Aldicarb			appl	nat	field, normal application	0.5–3 months	8
Aldicarb/ sulphox/ Aldoxycarb	sandy	0.01–0.16	4	23°C	lab, moist soil, aerobic incub.	61–178 days	9
Aldicarb/ sulphox/ Aldoxycarb	sandy	0.01–0.16	4	23°C	lab, moist soil, anaerobic incub.	52–105 days	9
Aldoxycarb	sand-clay loam	0.0–2.0	metabo lite	nat	field, metabolite	0.5–2 months	6
Aldoxycarb	sand	< 0.02 –0.16	metabo lite	nat	field, metabolite	69 days	7
Aldoxycarb	silt	0.7	5	15°C	lab, moisture content of soil adjusted	46 days	13
Aldoxycarb	sand	0.5	5	15°C	lab, moisture content of soil adjusted	slow degr.	13
Aldoxycarb	sand	0.8	3	10°C	lab, moisture content of soil adjusted	82 days	12
Aldoxycarb	loamy fine sand	1.2	3	10°C	lab, moisture content of soil adjusted	116 days	12
Aldoxycarb	fine sand	0.4	3	10°C	lab, moisture content of soil adjusted	1100 days	12
Aldicarb sulphoxide	sand	< 0.02 –0.16	metabo lite	nat	field metabolite	69 days	7
Aldicarb sulphoxide	silt	0.7	5	15°C	lab, moisture content of soil adjusted	53 days	11
Aldicarb sulphoxide	sand	0.5	5	15°C	lab, moisture content of soil adjusted	very slow degr.	11
Aldicarb sulphoxide	sand	0.8	3	10°C	lab, moisture content of soil adjusted	84 days	12
Aldicarb sulphoxide	loamy fine sand	1.2	3	10°C	lab, air-dried soil	194 days	12

Table 2 *continued*

Compound	*Soil type*	*% OC*	*Conc* $\mu g \cdot g^{-1}$	*Temp*	*Method*	*Half-life* $T_{1/2}$	*Ref*
Aldicarb sulphoxide	fine sand	0.4	3	10°C	lab, air-dried soil	410 days	12
Atrazine	silty clay/ sandy loam	0.1–1.3	0.5–2	nat	field, enclosed samples	meas. phytotoxicity	18
2,4-D	silty clay/ sandy loam	0.1–1.3	0.5–2	nat	field, enclosed samples	meas. phytotoxicity	18
Fenamiphos	sandy-clay loam	0.16–0.40	appl	nat	field, normal application	7–10 days	21
Linuron		0.6–1.2	2	10°C	lab, 6–30% adjusted moisture	17–39 weeks	22
Linuron		0.6–1.2	2	22°C	lab, 6–30% adjusted moisture	3–8.8 weeks	22
Linuron		0.8	2	10°C	lab, 6–30% adjusted moisture	12–20 weeks	22
Linuron		0.8	2	22°C	lab, 6–30% adjusted moisture	7.2–9.5 weeks	22
Mecoprop	sandy soil	0.2–0.5	0.05	10°C	lab, undisturbed soil cores	34–70 days♦	24
Methomyl	loamy-fine sand	0.1–0.9	appl	nat	field, normal application	0.5–1.6 months	25
Metribuzin	coarse sand	0.04–0.24	appl/ 1–4	23°C	field, enclosed samples/lab, soil dried and sieved	27–69 days	5
Metribuzin		0.6–1.2	2	10°C	lab, 10–60% moisture adjusted	11 weeks	22
Metribuzin		0.6–1.2	2	22°C	lab, 10–60% moisture adjusted	6.5 weeks	22
Metribuzin		0.8	2	10°C	lab, 10–60% moisture adjusted	2 weeks	22
Metribuzin		0.8	2	22°C	lab, 10–60°C moisture adjusted	8.8 weeks	22
Metribuzin		48.3	2	10°C	lab, 10–60% moisture adjusted	43 weeks	22
Metribuzin		48.3	2	22°C	lab, 10–60% moisture adjusted	9.4 weeks	22
Oxamyl	sand	0.8	3	10°C	lab, air-dried soil	26 days	12
Oxamyl	loamy fine sand	1.2	3	10°C	lab, air-dried soil	92 days	12
Oxamyl	fine sand	0.4	3	10°C	lab, air-dried soil	415 days	12

♠ applied as in normal agricultural practice
° natural circumstances
♦ half-life of mecoprop based on correlation between evolution of CO_2 and residues of mecoprop

Generally first-order reaction kinetics are presumed for the degradation process, sometimes even for pesticides which are decomposed by metabolic degradation. In a first-order reaction

$$dc/dt = -k \cdot c(t)$$

If ln c(t) is plotted versus time, the degradation curve turns out to be a straight line with slope $-k$.[34]

Degradation of aldoxycarb in silty subsoil[13], aldicarb sulphoxide in silty and sandy subsoil[11], aldoxycarb, aldicarb sulphoxide and oxamyl in sandy subsoil[12] and alachlor in subsoil both under aerobic and anerobic conditions was reported to follow a first-order reaction. Stenström[35] checked the equation for first-order kinetics against experimental data on degradation of herbicides. The first-order rate constant proved to be dependent on initial concentration. Applying an empirical equation $c = c_0 - k \cdot t^{1/2}$ to the degradation experiments, a high correlation was found between the rate constant k and biological activity. This could be valid for subsoils, too. The order of reaction for linuron and metribuzin degradation in subsoil varied from 1.36 to 6.26.[22] Metribuzin degradation in subsoil was a half-order process.[26]

Some authors calculated degradation half-lives assuming first-order kinetics.[5–10,21,25] In some cases, where field studies with normal application of the pesticide were carried out, the reported half-lives should be seen as dissipation rates, since surface losses via pathways such as volatilization and plant uptake would influence the concentrations found.[5,6,7,21,25]

Having analyzed the changes of concentration of parent pesticide with time, half-life can be calculated as $T_{1/2} = \ln 2/k$, assuming first-order kinetics. Reported half-lives in different soil types, at different temperature, concentration and OC content and with different methods are summarized in Table 2.

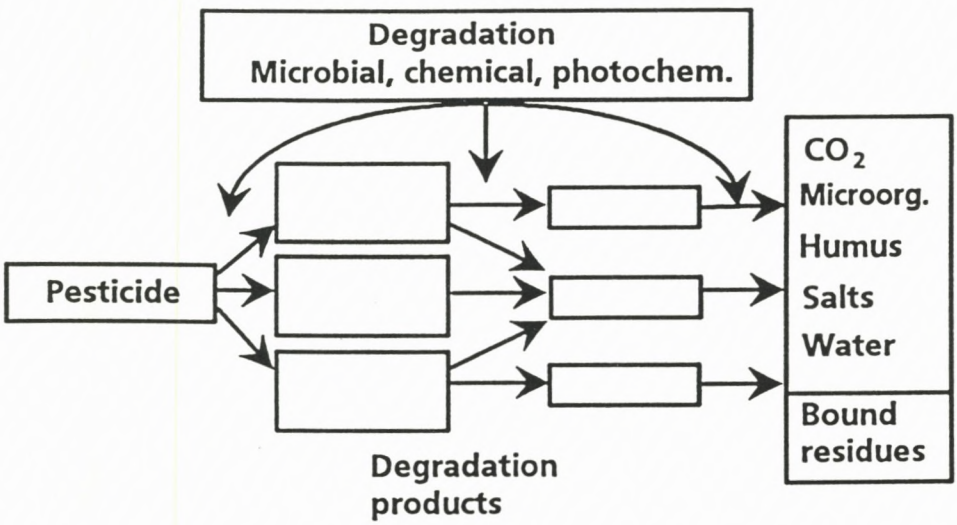

Figure 1 Diagram showing degradation of pesticides.[27]

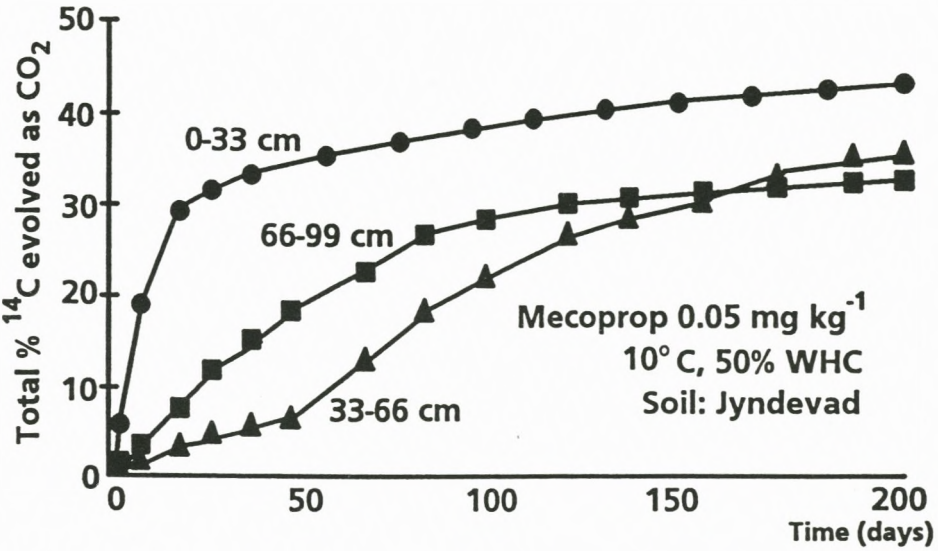

Figure 2 Degradation of ^{14}C-mecoprop (0.05 $\mu g \cdot g^{-1}$) in a soil profile.[24]

Other authors made degradation experiments following the evolution of $^{14}CO_2$ from ^{14}C-labelled pesticide. As seen in Figure 1., only part of the pesticide turns into CO_2. For that reason the evolution of $^{14}CO_2$ cannot be used to calculate half-lives. A typical pattern for the evolution of $^{14}CO_2$ from a pesticide is seen in Figure 2. When the rate of evolution of $^{14}CO_2$ decreases, the remaining ^{14}C has been built into stable organic compounds in the soil. Further evolution of $^{14}CO_2$ (the "flat" part of the curve) is a result of turn-over of biomass and other organic residues of the soil. Reported results from studies where the degradation was measured through evolution of $^{14}CO_2$ are summarized in Table 3.

Helweg[24] found a correlation between the amount of evolved $^{14}CO_2$ and the corresponding amounts of decomposed ^{14}C-mecoprop. Only on the basis of such a correlation, $^{14}CO_2$ evolution can be used to calculate half-lives.

FACTORS INFLUENCING DEGRADATION RATES

In almost all the reviewed papers a decrease in degradation rate with increasing depth was seen. The factors that were mentioned to be of importance for the degradation rate of a pesticide in subsoil were: microbial activity, soil temperature, water content, oxygen content, concentration of pesticide, repeated treatment of the soil and soil type. Reported degradation rates for pesticides in subsoil at varying conditions are summarized in Tables 2 and 3.

Table 3 Summary of degradation rates of pesticides in subsoil from the unsaturated zone (below 30 cm). Degradation rates reported as number of days for evolution of a certain amount of CO_2 from ^{14}C-labelled pesticide.

Compound	*Soil type*	*% OC*	*Conc* $\mu g \cdot g^{-1}$	*Temp*	*Method*	*Degr. rate*	*Ref*
Aldicarb	sand	0.02	5	23°C	lab, moist soil, aerobic incub.	15.8% in 63 days	10
Aldicarb	sand	0.52	5	23°C	lab, moist soil, aerobic incub.	16.9% in 63 days	10
Aldicarb	sandy loam	0.15	5	23°C	lab, moist soil, aerobic incub.	26.7% in 63 days	10
Aldicarb	loamy sand	0.18	5	23°C	lab, moist soil, aerobic incub.	16.9% in 63 days	10
Aldicarb	sand	0.02	5	23°C	lab, moist soil, anaerobic incub.	4.8% in 63 days	10
Aldicarb	sand	0.52	5	23°C	lab, moist soil, anaerobic incub.	12.6% in 63 days	10
Aldicarb	sandy loam	0.15	5	23°C	lab, moist soil, anaerobic incub.	17.2% in 63 days	10
Aldicarb	loamy sand	0.18	5	23°C	lab, moist soil, anaerobic incub.	12.9% in 63 days	10
Atrazine	sand/silt/clay	0.05–0.37	10	12°C	lab, moist soil, saturating with ground water	no degr.	14
Atrazine	coarse sandy	0.1	2	10°C	lab, moist soil, soil formerly treated with manure	21% in 500 days	15
Atrazine	clay	0.1	2	10°C	lab, moist soil	0.4% in 500 days	15
Atrazine	coarse sandy	0.1	0.02	10°C	lab, moist soil, soil formerly treated with manure	11–14% in 535 days	15
Atrazine	clay	0.1	0.02	10°C	lab, moist soil	11–14% in 535 days	15
Atrazine	coarse sandy	0.1	0.1	10°C	lab, moist soil, soil formerly treated with manure	11–14% in 535 days	15
Atrazine	clay	0.1	0.1	10°C	lab, moist soil	11–14% in 535 days	15
Atrazine	coarse sand		0.02	10°C	lab, undisturbed soil cores, N_2-atmosphere	5–22% in 626 days	16
Atrazine	coarse sand		0.1	10°C	lab, undisturbed soil cores, N_2-atmosphere	5–33% in 626 days	16
Atrazine	clayey sandy soil	0.01–0.03	0.1	22°C	lab, moist soil, aerobic incub.	no degr.	17
Atrazine	clayey sandy soil	0.01–0.03	0.1	22°C	lab, moist soil, anaerobic incub.	slow degr.	17
carbofuran		0.00–0.25	0.033		lab, moist soil	23–45% in 12 weeks	19
2,4-D		0-00–0.25	0.033		lab, moist soil	< 10–58% in 12 weeks	19

Table 3 *continued*

Compound	*Soil type*	*% OC*	*Conc* $\mu g \cdot g^{-1}$	*Temp*	*Method*	*Degr.rate*	*Ref*
Dichlorphenol	sand	0.05	0.05	10°C	lab, undisturbed soil cores, N_2-atmosphere	11–15% in 359 days	20
Dichlorphenol	moraine sand	1	0.05	10°C	lab, undisturbed soil cores, N_2-atmosphere	10% in 359 days	20
Dichlorphenol	sand	0.05	5	10°C	lab, undisturbed soil cores, N_2-atmosphere	5–10% in 359 days	20
Dichlorphenol	moraine sand	1	5	10°C	lab, undisturbed soil cores, N_2-atmosphere	1–2% in 359 days	20
Dichlorprop + monochlorprop	sand	0.05	0.05	10°C	lab, undisturbed soil cores, N_2-atmosphere	10–16% in 447 days	20
Dichlorprop + monochlorprop	moraine sand	1	0.05	10°C	lab, undisturbed soil cores, N_2-atmosphere	12–15% in 447 days	20
Dichlorprop- + monochlorprop	sand	0.05	5	10°C	lab, undisturbed soil cores, N_2-atmosphere	12–17% in 447 days	20
Dichlorprop + monochlorprop	moraine sand	1	5	10°C	lab, undisturbed soil cores, N_2-atmosphere	2% in 447 days	20
MCPA	clayey sandy soil	0.1	5	10°C	lab, undisturbed soil cores, MCPA formerly used	40% in 80 days	23
MCPA	sand	0.1	5	10°C	lab, undisturbed soil cores, MCPA formerly used	20% in 240 days	23
MCPA	clayey sandy soil	0.1	5	10°C	lab. undisturbed soil cores	3% in 80 days	23
MCPA	sand	0.1	5	10°C	lab, undisturbed soil cores	13% in 240 days	23
Mecoprop	sandy soil	0.2–0.5	0.05	10°C	lab, undisturbed soil cores	36% in 227 days	24
Metolachlor	sand/silt/clay	0.05–0.37	10–20	12°C	lab, moist soil, saturating with ground water	no degr.	14
Metribuzin	silty clay loam		0.1–1	25°C	lab, moist soil	5% in 91 days	26
Parathion	sand	0.05	0.05	10°C	lab, undisturbed soil cores, N_2-atmosphere	3–6% in 419 days	20
Parathion	moraine sand	1	0.05	10°C	lab, undisturbed soil cores, N_2-atmosphere	7–14% in 419 days	20

Table 3 *continued*

Compound	*Soil type*	*% OC*	*Conc* $\mu g \cdot g^{-1}$	*Temp*	*Method*	*Degr.rate*	*Ref*
Parathion	sand	0.05	5	10°C	lab, undisturbed soil cores, N_2-atmosphere	12–14% in 438 days	20
Parathion	moraine sand	1	5	10°C	lab, undisturbed soil cores, N_2-atmosphere	16–20% in 438 days	20
TCA	sand	0.05	0.05	10°C	lab, undisturbed soil cores, N_2-atmosphere	35–40% in 833 days	20
TCA	moraine sand	0.1	0.05	10°C	lab, undisturbed soil cores, N_2-atmosphere	22% in 833 days	20
TCA	sand	0.05	5	10°C	lab, undisturbed soil cores, N_2-atmosphere	8–31% in 833 days	20
TCA	moraine sand	0.1	5	10°C	lab, undisturbed soil cores, N_2-atmosphere	2–3% in 833 days	20

♠ applied as in normal agricultural practice
° natural circumstances

Microbial activity

As mentioned above, the degradation of a pesticide in soil is considered to be merely microbial.[4,17,19,23,26] However, no direct correlation between degradation rate and microbial activity could be shown. The microbial activity depends on number of microorganisms present, soil temperature, moisture, presence of oxygen and composition of soil (pH, OC content and nutrients).

Soil temperature

Degradation rate of aldicarb increased with higher temperature.[8] Degradation of atrazine occurred more rapidly at the surface than at deeper levels.[18] This was explained by the lower number of microorganisms *and* the lower temperature at lower depths.

Water content

Moisture is essential for microbial activity and for pesticide transport. In dry soils microbial activity diminishes, and in water saturated soils anaerobic conditions may prevail, which will impede the activity of all aerobic and microaerophilic bacteria. The content of water will generally not be a limiting factor for degradation in subsoil from the unsaturated zone, since downward and upward movement of water will prevent the soil from drying out.

High soil moisture content was one of the factors that tended to increase the degradation rate of aldicarb.[8] Ou *et al.*[10] showed an increasing degradation rate of aldicarb with increasing water content in subsoil in one case, in the other there was no significant difference. Konopka and Turco[14] showed no degradation of atrazine and metolachlor in water saturated soil from

the unsaturated zone. Kempson-Jones and Hance[22] found shorter half-lives of linuron and metribuzin at higher temperature and moisture levels in subsoil.

Oxygen content

The unsaturated zone is normally aerobic and the oxygen content in the soil atmosphere is often close to oxygen content in atmospheric air.

Sinclair and Lee[17] found that atrazine was slowly degraded in anaerobic subsoil. With aerobic incubation no degradation was seen. The degradation of 2,4-D was slower under anaerobic conditions, but for atrazine no difference was seen.[18] Alachlor had a half-life of 22–285 days under aerobic conditions and 53–148 days under anaerobic comditons.[4] Ou *et al.*[9] found an aerobic half-life for total carbamate residues (aldicarb, aldicarb sulphoxide and aldoxycarb) of 61–178 days and an anaerobic half-life of 52–105 days. In loamy sand and sandy loam the aerobic degradation was significantly more rapid than the anaerobic. No significant difference was shown in sandy samples.[10]

Concentration of pesticide

Few investigations were made comparing degradation rates in subsoil of pesticides at varying concentrations.

The degradation rate of dichlorprop and dichlorphenol was significantly slower at 5 $\mu g \cdot g^{-1}$ than at 0.05 $\mu g \cdot g^{-1}$ in moraine sand.[20] For parathion and TCA no significant difference at varying concentrations was shown.[20]

Extrapolating degradation rate results from laboratory studies at high concentrations to nature, where the pesticides often are found at very low concentrations, can lead to erroneous conclusions of the fate of these compounds.[36]

Repeated treatments

Treatment of soil with pesticides can result in a build up of microorganisms capable of degrading the pesticide.

Zeuthen *et al.*[23] reported a significantly higher degradation rate of MCPA in subsoil taken 1 m below a barley field treated with phenoxyacids for 10 years than in subsoil taken below an uncropped field. Also the number of MCPA degraders determined by a ^{14}C-MPN method was significantly higher in subsoil below the field where MCPA had been used.

Soil type (OC content, pH)

Overall microbial activity often depends upon pH and upon content of organic material in the soil. These parameters may also influence adsorption of the pesticide and chemical hydrolysis. Smelt *et al.*[11, 12, 13] found slower degradation rates of aldoxycarb, aldicarb

sulphoxide and oxamyl in sandy subsoil than in silty subsoil. The low pH of the sandy subsoil could be the reason for this. At high concentrations (5 $\mu g \cdot g^{-1}$) Helweg[20] found a significant lower degradation rate of dichlorprop + monochlorprop in moraine sand (1% OC) than in sand (0.05% OC).

METHODS

Environmental factors that influence degradation rates of pesticides are all closely interrelated and it is difficult to investigate only one factor at a time. Moreover, it is difficult to compare degradation rates from different published studies because of the variation between employed methods.

Comparing degradation rates for example for atrazine (Table 3) in different studies, it is seen that these vary from a degradation to CO_2 of 21% in 500 days to no degradation at all. These differences could—to some extent—be the result of differences between employed methods.

One important methodological difference is the way of reporting degradation rates. In Table 2 half-lives are calculated assuming first-order kinetics on basis of residues of parent compound. In Table 3 degradation rates are reported as number of days for the evolution of a certain percentage of CO_2. Another important difference is, whether the investigation is made in the field or in the laboratory.

Field studies

In field studies performed after normal agricultural application of the pesticide it is difficult to distinguish between degradation and transport. Dissipation rates may include both degradation, movement, volatilization and plant uptake.

Hornsby *et al.*[7] discussed the contrast between sampling protocols designed to maximize the possibility of finding the applied pesticide and protocols designed to obtain "representative soil samples". With the sampling design used, they computed reliable "field-average concentrations".

Lavy *et al.*[18] eliminated leaching as a dissipation factor in their degradation study of 2,4-D and atrazine. Sieved soil samples with added pesticide (from 0.5 to 2 $\mu g \cdot g^{-1}$ to match the soil adsorption capacity) were buried in jars in the soil profile for up to 41 months in order to incubate the samples as closely as possible to natural conditions.

Jones *et al.*[5] carried out a comparative study of dissipation by depth of alachlor and metribuzin both in the field and in the laboratory. Statistical comparison was made when possible. The field study was made with soil columns enclosed in steel tubes and with injection of the pesticide to eliminate leaching as a dissipation factor. At the lowest depth, metribuzin dissipated significantly faster in the field than in the laboratory. This was most likely due to the treatment of the laboratory sample—a possible decrease in microbial activity during the drying period and a lack of natural cracks and channels in the dried and sieved soil.

In most field studies, considerable variability is found in pesticide residue concentrations in soil samples. Jones *et al.*[6] found a CV % (rel.std.dev) of replicate samples of 86–223% in their aldicarb study. Minton *et al.*[21] reported a CV % as high as 400% in field degradation studies of fenamiphos. Jones[8] collected and analyzed 3100 soil samples for one published field degradation study to be able to assess the effect of spatial variability on the measurements.

Laboratory studies

Few unsaturated zone field studies have been undertaken and/or published because of the high number of soil samples needed to reduce the influence of variability on the results and the expense associated with the collection and analysis of such a high number of samples. Most of the published data on degradation of pesticides in subsoils were generated in laboratories.

Most of the laboratory studies with subsoil samples were made with dried and sieved samples where pesticide was added and the samples then given a water content close to field capacity. Helweg[24] worked with undisturbed subsoil core samples injecting the pesticide and adjusting the water content. Jones *et al.*[5] used undisturbed subsoil cores in their field studies comparing the results with laboratory studies with dried and sieved samples. In most of the studies the concentrations of added pesticide ranged from 0.5–5 $\mu g \cdot g^{-1}$, corresponding to concentrations in the plough layer after normal field application; in a few studies[15,16] where ^{14}C-labelled pesticides were used, it was possible to work with concentrations as low as 0.02 $\mu g \cdot g^{-1}$.

Helweg[24] determined the degradation rate of ^{14}C-ring-labelled mecoprop. In subsurface soil the CV % of four replicates was 30–38%.

Helweg[27] described in detail a system for laboratory studies of undisturbed soil samples using ^{14}C-labelled compounds.

DISCUSSION AND RECOMMENDATIONS

The complex structure of soil, the close interrelationship between factors that influence degradation, and the difficulties in maintaining the environment of the microorganisms natural during the investigations make subsoil degradation studies complicated. The interrelation between factors that influence on degradation was described by Anderson.[37] The factors were a) The structure of the pesticide, b) The availability of the pesticide to enzymes or microbial cells (mobility of pesticide in soil, amount of water in soil, total amount of pesticide present in the soil), c) The quantities of enzymes or cells that can degrade the pesticide d) The activity of these enzymes or cells (depending on soil temperature, soil moisture composition of soil atmosphere, nutrients available and soil pH).[37]

Field studies such as the ones by Lavy *et al.*[18] and Jones *et al.*[5] where leaching, volatilization and plant uptake as dissipation factors are eliminated or laboratory studies are the easiest type of degradation studies. The modern use of simulation models to predict the

environmental fate of pesticides and to evaluate the threat of these pesticides to ground-water also need precise, reliable data sets for—among many factors—degradation rates at all levels of the unsaturated zone.

Laboratory studies such as those described by Helweg[16, 24, 27] and Zeuthen *et al.*[23] are to be recommended for subsoil degradation studies because they leave the soil samples undisturbed. Drying and sieving of subsoil affect the microbial activity. Variations between replicates of undisturbed soil samples are expected to be higher than in dried and sieved samples because of the greater heterogeneity of the undisturbed soil. This must be taken into account, working with a sufficient number of replicates, calculating standard deviations and making statistical comparisons of the results. Furthermore it is important to ensure that the subsurface samples are not contaminated with surface soil. The influence of microorganisms on degradation can be determined by incubation of sterilized soil samples. Saltzman and Mingelgrin[38] showed that sterilization with KN_3, ethylene oxide and by autoclaving resulted in changes in the soil properties which affected the degradation capacity of reinoculated soil. Sterilization by irradiation is a possible alternative. However, sterilization cannot assure us, that degradation is not carried out by microbial extracelluar enzymes, produced before the sterilization.

A disadvantage in laboratory studies could be a possible lack of nutrients in the enclosed soil samples as the incubation proceeds.

If only residues of parent compound are measured, one cannot be sure that no toxic residues are formed. In the studies of aldicarb[6,9,12] and fenamiphos[21] the toxic metabolites were known and measured, too. If only CO_2-evolution is measured, half-life cannot be calculated and it is difficult to know, when there is nothing left of the parent compound. Both residues of parent compound and CO_2-evolution should be measured.

Laboratory degradation studies should be performed at concentrations as close to the naturally occurring residue concentrations as possible. It is suggested that subsoil degradation studies include characterization not only of the physical composition of the soil, but especially investigations of the relation between degradation rate and microbial biomass and activity as described by Anderson.[37,39,40]

It is clearly to be recommended that standardized laboratory studies on degradation of pesticides are performed,—but it is absolutely necessary to validate results in field experiments. Results obtained in studies where the above methodological recommendations were followed, will be published in the near future.

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Modelling the mineralization kinetics for low concentrations of pesticides in surface and subsurface soil

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Abstract

A number of mathematical models were fitted to mineralization results of low concentrations (004–0.08 $\mu g \cdot g^{-1}$) of mecoprop, bentazon and ethylene thiourea (ETU) in surface (ploughed layer) and subsurface soil in different soil types and at different temperatures. It was shown that surface soil kinetics generally could be described with models not including growth of microorganisms and subsurface soil kinetics could best be described with models taking the growth of microorganisms in account. We recommend the use of such kinetic models when pesticide fate in soil is to be predicted. © 1997 Elsevier Science B.V.

Keywords: Mecoprop; Bentazon; Ethylene thiourea; Degradation; Metabolic; Cometabolic

1. Introduction

Degradation studies of many pesticides in soil have been reported in large numbers during the last decade. Smith (1989) summarised the results of 96 phenoxyalcanoic acid degradation studies, Roeth (1986) reviewed enhanced herbicide degradation in soil with repeated application and Fomsgaard (1995) reviewed results and methods from subsoil degradation studies for a variety of pesticides. Of all the reviewed subsoil studies only a few of them took into account the vulnerability of microorganisms to changes in their environment caused by actions such as sieving and drying the soil.

Degradation of pesticides can follow a chemical or a microbial pathway or a combination of both. However, microbial degradation is the most important pathway. Microbial decomposition can occur by metabolism, where the microorganisms can derive energy from the degradation process, or by cometabolism, where microorganisms obtain energy from other sources. Degradation of a pesticide is a series of stepwise processes leading to various end products. If the pesticide is totally mineralised, CO_2 is formed and a part of the pesticide-carbon is built into humus and soil microorganisms. Each degradation process can be either metabolic or cometabolic and a number of

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different microorganisms can be involved in the degradation. Soil is a heterogeneous matrix, where the microbial degradation is considered mainly to take place in the soil solution where contact between microorganisms and the pesticide can be easily obtained. If some of the pesticide is adsorbed on soil solid matter another process which has to be then considered in the overall view on degradation is the desorption process. An overall view on the degradation process must then be a very complex matter.

Pesticide degradation studies in soil are often performed at higher concentrations of pesticides than the concentrations actually present in soil after leaching through normal agricultural use. Degradation rates from these studies cannot be extrapolated to lower concentrations. Thus degradation studies at low concentrations are needed.

A number of kinetic studies on mineralization of xenobiotic compounds in aquatic environments have been reported (Paris et al., 1981; Robinson and Tiedje, 1983; Simkins and Alexander, 1984; Schmidt et al., 1985; Hoover et al., 1986; Jones and Alexander, 1986; Jørgensen et al., 1995). Kinetic studies of mineralization of easy degradable organic compounds in soil were presented by Brunner and Focht (1984) (with 42 days of incubation) and Scow et al. (1986) (with 60 h of incubation). Pesticides at low concentrations which degrade much slower than the other organic compounds treated, were not included in these studies. A number of kinetic studies of mineralization of pesticides in soil at $\mu g \cdot g^{-1}$ level have been reported using simple first order or Michaelis-Menten kinetics (Hamaker, 1972; Parker and Doxtader, 1982; Simon et al., 1992). Hance and Haynes (1981) used a power-rate model for describing the kinetics of linuron and metribuzin at 5 $\mu g \cdot g^{-1}$.

Hill and Schaalje (1985) described a two-compartment model for the dissipation of deltamethrin in soil (field experiment with normal application) and Gustafson and Holden (1990) developed a multi-compartment model which was applied to a number of previously published studies. Liu and Zhang (1986) and Liu et al. (1988) applied their model to studies of BHC and DDT degradation at the $\mu g \cdot g^{-1}$ level.

Only a few kinetic studies on pesticide degradation in soil at concentrations as low as 0.04–0.08 $\mu g \cdot g^{-1}$ has been published.

Stenström (1988) developed an empirical model for pesticide degradation at low concentrations and Mueller et al. (1992) used first order kinetics for describing degradation of fluometuron at 0.08 $\mu g \cdot g^{-1}$. Vink et al. (1994) modelled the breakdown of 1,3-dichlorpropene at varying concentrations down to 0.03 $\mu g \cdot g^{-1}$.

Mecoprop degradation studies in subsoil were reported by Helweg (1993), whereas no degradation studies in subsoil have been reported for bentazon and ethylene thiourea (ETU) (Fomsgaard, 1995). No kinetic mineralization models of the three compounds have been reported formerly. Mecoprop and bentazon are commonly used herbicides, applied to a variety of crops in Denmark. ETU is a metabolite of the fungicides maneb, zineb and mancozeb. All these three compounds show low sorption to soil, thus they could be considered a threat to ground water. Degradation studies of the compounds at low concentrations both in the ploughed layer and subsoil are needed urgently. FOCUS (1995) (Forum for the Coordination of pesticide fate models and their USe, a work group of the European Commision) compared and evaluated nine dynamic pesticide fate models. Eight of the nine models used first order kinetics for describing pesticide degradation. The group concluded that one of the improvements needed was a better description of the degradation processes in soil.

The purpose of the present study was to compare the applicability of a number of mathematical kinetic models, most of them developed for other sample types, to the mineralization of mecoprop, bentazon and ETU in surface and subsurface soil at low concentrations in different soil types. Empirical models, used by other authors to describe degradation, as well as models based on theoretical considerations about the soil system and microbial activity were taken into consideration. All the models used were degradation models, and based on the fits that result from each model, the underlying degradation process was discussed. The degradation kinetics of pesticides in soil were thus elucidated through the mathe-

matical models that fit. The experiments were performed with undisturbed subsoil samples at mean subsurface temperatures to simulate natural conditions.

2. Materials and methods

2.1. Soils

At a number of sites in Denmark, Germany, Italy and Spain soil samples with different soil textures were taken. Four replicate samples were taken at each site and depth (ploughed layer 15 cm, subsoil 45 and 75 cm) for the degradation experiments on mecoprop and ETU in Danish soils. For bentazon and mecoprop in Spanish, German and Italian soil, replicate samples of subsoil (45 cm, 75 cm and 50 cm respectively) were taken at two sites and a composite sample was taken in the ploughed layer (0 cm) but incubated as four replicates. A composite sample was taken for determination of texture. Stainless steel tubes were forced into the soil in a vertical position while maintaining aseptic conditions. The samples were stored at 5°C until incubation. The ploughed layer samples (0–15 cm) were sieved (2 mm) to remove roots and plant material and the subsoil samples (45–75 cm) were kept undisturbed. For the determination of sorption, the samples were sterilised with electron beam radiation of 2×11 kGy.

2.2. Chemicals

Ring ^{14}C-labelled mecoprop (2-(4-chloro-2-methylphenoxy)propanoic acid) with a specific activity of 24 $\mu Ci \cdot mg^{-1}$ and a radiochemical purity of 99%, ring ^{14}C-labelled bentazon (3-isopropyl-1*H*-2,1,3-benzothiadiazine-4(3*H*)-one-2,2-dioxide) with a specific activity of 17.4 $\mu Ci \cdot mg^{-1}$ and a radiochemical purity of 100%, and ring ^{14}C-labelled ETU with a specific activity of 81 $\mu Ci \cdot mg^{-1}$ and a radiochemical purity of 95% was obtained from Amersham. Unlabelled bentazon with a purity of 99.5% was obtained from Merck.

2.3. Degradation experiments

The incubation experiments were performed at the lowest possible concentrations based on the specific activity (mecoprop 0.04 $\mu g \cdot g^{-1}$, ETU 0.07 $\mu g \cdot g^{-1}$ and bentazon 0.08 $\mu g \cdot g^{-1}$). The ^{14}C-labelled pesticide or a mixture of unlabelled and ^{14}C-labelled pesticide was added individually for each compound to the ploughed layer soil samples by mixing in an Erlenmeyer flask, and to the subsoil samples by injection with a long needle into the undisturbed soil column to maintain incubation conditions as close to natural conditions as possible. Water content was adjusted to approximately 50% of the water holding capacity. Incubation temperatures are shown in Table 1. Evolved $^{14}CO_2$ was absorbed in traps of KOH according to Helweg (1993) and quantified by liquid scintillation counting to follow the mineralization of the compounds.

2.4. Determination of sorption

Sorption (K_d) was determined according to OECD (1981). Five g of dried, sieved and sterilised soil was shaken for 16 h in 25 ml 0.01 M $CaCl_2$ with isotope-labelled pesticide (5 $\mu g \cdot g^{-1}$). The K_d-value was calculated as

$$\frac{\mu g \cdot g^{-1} \text{ soil}}{\mu g \cdot ml^{-1} \text{ solution}} \quad (1)$$

3. Data analysis

Accumulated amounts of evolved $^{14}CO_2$, calculated as percentage radioactivity of the total amount of added radioactivity were described as a function of incubation time, $^{14}CO_2$ then corresponding to the amount of mineralised pesticide. A number of non-linear models were fit to the curves to evaluate the differences in the kinetics of mineralization.

3.1. Models

A number of models used by other authors for modelling degradation of xenobiotic compounds

Table 1
Texture and pH of soil samples from Fladerne Bæk (Denmark), Italy, Spain and Germany, sampling date and place, incubation temperature and name given to incubated samples

Site and depth	Humus (%)	Clay (%)	Silt (%)	Sand (%)	pH	Incubation temperature	Mecoprop incubations	ETU incubations	Bentazone incubations	K_d mecoprop $\mu g \cdot g^{-1}/\mu g \cdot ml^{-1}$	K_d ETU $\mu g \cdot g^{-1}/\mu g \cdot ml^{-1}$	K_d bentazone $\mu g \cdot g^{-1}/\mu g \cdot ml^{-1}$
Denmark (FB 1_I) 15 cm Jan 93	3.1	4.0	3.9	89.0	7.1	10°C	mcfb 1_I 15 cm			0.77		
Denmark (FB 1_I) 45 cm Jan 93	0.9	3.0	2.4	93.6	6.2	10°C	mcfb 1_I 45 cm			0.66		
Denmark (FB 1_I) 75 cm Jan 93	0.2	2.5	1.9	95.3	5.9	10°C	mcfb 1_I 75 cm			0.26		
Denmark (FB 1_II) 15 cm March 94	2.8	3.6	2.8	90.8	6.9	10°C	mcfb 1_II 15 cm	etfb 1 15 cm		0.78	0.17	
Denmark (FB 1_II) 45 cm March 94	0.3	2.5	1.4	95.7	6.3	10°C	mcfb 1_II 45 cm	etfb 1 45 cm		0.39	0.13	
Denmark (FB 1_II) 75 cm March 94	0.1	2.1	1.4	96.3	6.4	10°C	mcfb 1_II 75 cm	etfb 1 75 cm		0.20	0.06	
Denmark (FB 3_I) 15 cm March 93	2.7	3.2	2.8	91.2	6.6	10°C	mcfb 3_I 15 cm			0.68		
Denmark (FB 3_I) 45 cm March 93	0.8	2.3	1.2	95.7	6.1	10°C	mcfb 3_I 45 cm			0.55		
Denmark (FB 3_I) 75 cm March 93	0.2	1.4	1.2	97.3	6.1	10°C	mcfb 3_I 75 cm			0.00		
Denmark (FB 3_II) 15 cm March 94	2.8	4.0	2.9	90.3	6.7	10°C	mcfb 3_II 15 cm	etfb 3 15 cm		0.73	0.20	

Denmark (FB 3_II) 45 cm March 94	0.9	3.5	2.4	93.1	5.6	10°C	mcfb 3_II 45 cm	etfb 3 45 cm		0.46	0.04	
Denmark (FB 3_II) 75 cm March 94	0.3	3.0	1.4	95.2	5.5	10°C	mcfb 3_II 75 cm	etfb 3 75 cm		0.20	0.08	
Denmark (FB 4_I) 15 cm Jan 95	4.7	4.6	3.8	87.0	5.2	10°C	mcfb 4_I 15 cm			2.79		
Denmark (FB 4_I) 45 cm Jan 95	5.1	3.6	1.9	89.3	5.2	10°C	mcfb 4_I 45 cm			2.64		
Denmark (FB 4_I) 75 cm Jan 95	0.5	2.1	2.8	94.6	5.6	10°C	mcfb 4_I 75 cm			0.14		
Italy 1+2 0 cm April 93	3.6	16.6	48.9	30.8	7.2	20°C	mcit 1+2 0 cm		beit 1+2 0 cm	0.71		0.23
Italy 1 50 cm April 93	0.6	20.9	48.3	30.0	7.5	15°C	mcit 1 50 cm		beit 1 50 cm	0.24		0.04
Italy 2 50 cm April 93	0.6	21.1	52.7	25.6	7.1	15°C	mcit 2 50 cm		beit 2 50 cm	0.16		0.00
Spain 1+2 0 cm Dec 93	3.5	30.5	29.0	2.0	8.1	20°C	mcsp 1+2 0 cm		besp 1+2 0 cm	0.44		0.04
Spain 1 45 cm Dec 93	3.7	30.1	30.3	1.2	8.2	15°C	mcsp 1 45 cm		besp 1 45 cm	0.45		0.04
Spain 2 45 cm Dec 93	[a]					15°C	mcsp 2 45 cm		besp 2 45 cm	0.20		0.00
Germany 1+2 0 cm April 93	2.1	7.9	33.7	57.4	7.4	20°C	mcty 1+2 0 cm		bety 1+2 0 cm	0.36		0.17
Germany 1 75 cm April 93	0.2	9.7	5.7	84.4	6.6	10°C	mcty 1 75 cm		bety 1 75 cm	0.28		0.14
Germany 2 75 cm April 93	0.1	6.9	1.9	91.1	7.1	10°C	mcty 2 75 cm		bety 2 75 cm	0.06		0.00

[a] Sample lost.

are presented below, converted to be used in the present study, where the mineralization product as a percentage of added pesticide was measured. The models then describe the percentage of pesticide mineralised at a given time t. Since the concentration of mineralization product was measured, the model:

$$P = c_0 - c(t) \tag{2}$$

was used as a basis for all the models in integrated form, where P is the concentration of mineralization product at time t equal to pesticide mineralised at time t (measured as $\%^{14}C$ in $^{14}CO_2$ coming from ^{14}C labelled pesticide), c_0 is the total concentration of the pesticide converted by the process to $^{14}CO_2$, and $c(t)$ is the concentration of the pesticide at time t.

In many cases, first order reaction kinetics, where the rate of degradation is proportional to the residue remaining, was presumed for pesticide degradation processes (Ou et al., 1988; Jones et al., 1990, 1986; Hornsby et al., 1990; Minton et al., 1990), described by the model

$$\frac{dc}{dt} = -k \cdot c \tag{3}$$

or in integrated form

$$c(t) = c_0 \cdot e^{-k \cdot t} \tag{4}$$

where $c(t)$ is the concentration of the pesticide at time t, c_0 is the initial concentration of the pesticide, k is the degradation rate constant, and t is the time in days.

If the degradation follows the above first order kinetics, and the changes in concentration of parent pesticide with time have been analyzed, the half-life of the pesticide can be calculated as:

$$T_{\frac{1}{2}} = \ln 2/k \tag{5}$$

The increase in $^{14}CO_2$ production from ^{14}C labelled pesticide following a first order process can then be described by the model

$$P = c_0 \cdot (1 - e^{-kt}) \tag{6}$$

where P is the concentration of the pesticide mineralised at time t (measured as $\%^{14}C$ in $^{14}CO_2$), c_0 is the total concentration of the pesticide converted by the process to $^{14}CO_2$, k is the degradation rate constant, and t is the time in days (Simon et al., 1992; Mueller et al., 1992; Knaebel et al., 1994).

If the total amount of pesticide added to the soil is converted to $^{14}CO_2$ by first order metabolism, then $c_0 = 100$, the degradation rate constant k is the only parameter to be estimated and the model becomes:

$$P = 100(1 - e^{-kt}) \tag{7}$$

Scow et al. (1986) and Hill and Schaalje (1985) proposed a two-compartment model consisting of two simultaneously occurring first order processes as a useful model for describing pesticide mineralization:

$$P = c_1(1 - e^{-k_1 t}) + c_2(1 - e^{-k_2 t}) \tag{8}$$

where P is the concentration of pesticide mineralised at time t (% ^{14}C as $^{14}CO_2$), c_1 is the total concentration of pesticide converted to $^{14}CO_2$ by one first order metabolism, c_2 is the total concentration of pesticide converted to $^{14}CO_2$ by another first order metabolism, k_1, k_2 are the degradation rate constants for the two first order processes, and t is the time in days.

Hill and Schaalje (1985) considered one compartment as representing the surface soil layer, where the dissipation is more rapid, and from where the pesticide moves into the second compartment, the deeper soil layer, where slower dissipation kinetics is found. Scow et al. (1986) considered the first compartment as being the sorbed pesticide and the other the dissolved pesticide and dissipation was assumed to occur in both compartments at different rates.

If the total amount of pesticide added to the soil is converted to $^{14}CO_2$ by the two simultaneous first order processes, the model is as follows:

$$P = 100((1 - ae^{-tk_1} - (1 - a)e^{-tk_2}) \tag{9}$$

where P is the concentration of pesticide mineralised at time t (% ^{14}C as $^{14}CO_2$), k_1, k_2 are the degradation rate constants for the two first order processes, t is the time in days, and a is the fraction of total amount of pesticide converted to $^{14}CO_2$ by one first order process.

A deterministic three-half-order kinetic model was used by Brunner and Focht (1984), Scow et

Table 2
Residual mean for all fitted equations for ploughlayer soils

Sample	Equations without growth				Equations with growth of microorganisms					Figure reference
	Eq. (8)	Eq. (9)	Eq. (11)	Eq. (23)	Eq. (10)	Eq. (14)	Eq. (17)	Eq. (18)	Eq. (19)	
mcfb 1_I a 15 cm		0.9881	0.9704							Fig. 1a
mcfb 1_I b 15 cm	0.7324	0.8875	0.9382							
mcfb 1_I c 15 cm	0.6078	1.361	1.477							
mcfb 1_I d 15 cm	1.061	1.482	1.576							
mcfb 1_II a 15 cm		2.788	2.775		0.8898					Fig. 1b
mcfb 1_II b 15 cm		2.673	2.662		1.111					
mcfb 1_II c 15 cm		2.891	2.885		0.6978	2.985			2.984	
mcfb 1_II d 15 cm		2.796	2.792		0.6303	2.735			2.734	
mcfb 3_I a 15 cm										Fig. 1c
mcfb 3_I b 15 cm	0.9921	1.397	1.465							
mcfb 3_I c 15 cm										
mcfb 3_I d 15 cm		1.882	1.876							
mcfb 3_II a 15 cm					0.2188	0.9978	1.403	1.266	0.9978	Fig. 1d
mcfb 3_II b 15 cm					0.1826	0.6199	0.7877		0.6199	
mcfb 3_II c 15 cm					0.1302	0.3968	0.4608	0.4545	0.3968	
mcfb 3_II d 15 cm					0.1647	0.3960	0.5077	0.4832	0.3960	
mcfb 4_I a 15 cm		0.4511	0.4472		0.3634					Fig. 1e
mcfb 4_I b 15 cm	0.3652	0.6980	0.7421							
mcfb 4_I c 15 cm	0.3317	0.9975	1.039							
mcfb 4_I d 15 cm	0.1431	0.1464	0.1531							
mcit 1+2 a 0 cm	0.8773	2.521	3.068							Fig. 1f
mcit 1+2 b 0 cm	0.6854	2.471	3.089							
mcit 1+2 c 0 cm	0.9938	2.845	3.412							
mcit 1+2 d 0 cm	0.7417	2.648	3.236							
mcsp 1+2 a 0 cm	0.5767	2.254	2.788							Fig. 1g
mcsp 1+2 b 0 cm	0.4358	1.993	2.496							
mcsp 1+2 c 0 cm	0.5253	1.825	2.265							
mcsp 1+2 d 0 cm	0.4121	1.940	2.429							
mcty 1+2 a 0 cm	0.1862	1.093	1.598							Fig. 1h
mcty 1+2 b 0 cm	0.2664	1.181	1.567							
mcty 1+2 c 0 cm	0.1365	0.7275	1.036							
mcty 1+2 d 0 cm	0.1784	1.604	2.163							
beit 1+2 a 0 cm	0.06165	0.0635	0.06967							Fig. 2a
beit 1+2 b 0 cm		0.0298	0.02865							
beit 1+2 c 0 cm	0.03848	0.0375	0.04076							
beit 1+2 d 0 cm		0.0530	0.04830							
besp 1+2 a 0 cm	0.1609	0.4410	0.5762							Fig. 2b
besp 1+2 b 0 cm	0.1392	0.4667	0.6916							
besp 1+2 c 0 cm	0.1753	0.4644	0.6921							
besp 1+2 d 0 cm	1.730	4.558	6.323							
bety 1+2 a 0 cm		0.3778	0.3779							Fig. 2c
bety 1+2 b 0 cm		0.1758	0.1841							
bety 1+2 c 0 cm		0.1638	0.1753							
bety 1+2 d 0 cm										
etfb 1 a 15 cm	0.8888	3.328	4.231							Fig. 3a
etfb 1 b 15 cm	1.202	3.034	3.817							
etfb 1 c 15 cm	1.265	1.850	2.232							
etfb 1 d 15 cm	0.7992	3.127	3.678							
etfb 3 a 15 cm	0.2011	0.7038	0.8351							Fig. 3b
etfb 3 b 15 cm	0.8827	2.340	2.675							
etfb 3 c 15 cm	0.9065	2.647	3.101							
etfb 3 d 15 cm	0.8148	1.856	2.203							

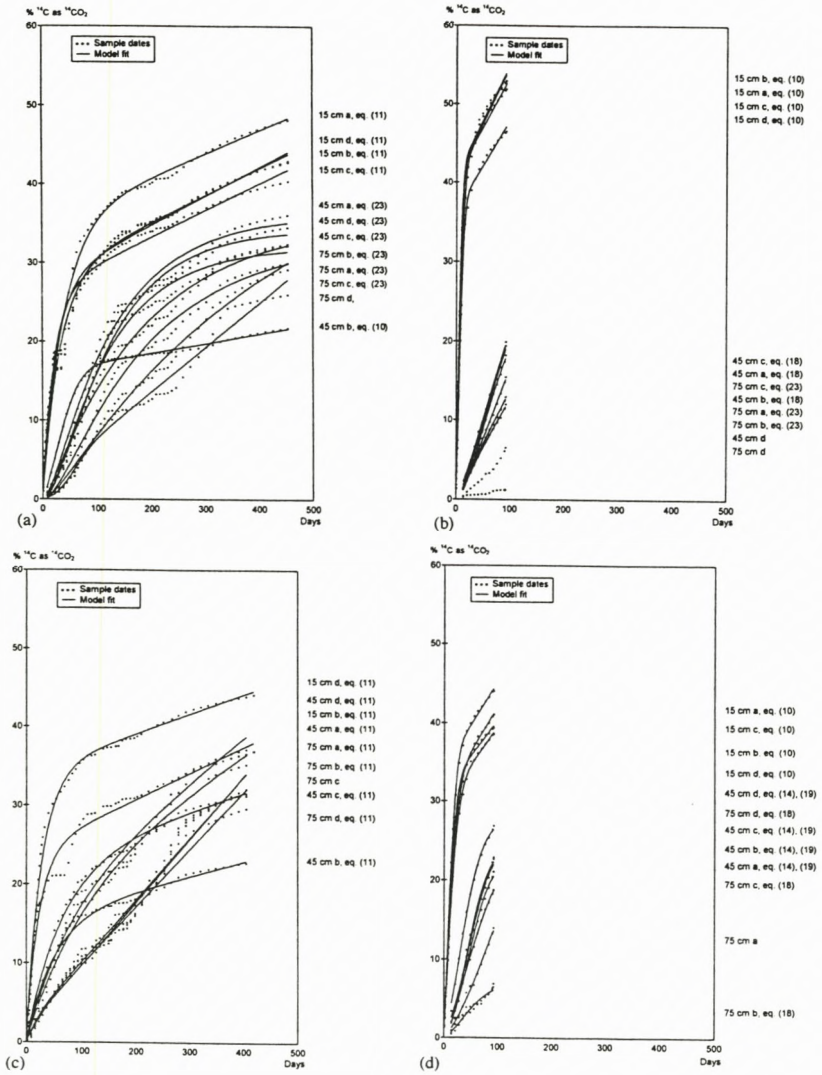
% ^{14}C as $^{14}CO_2$
Sample dates
Model fit
15 cm a, eq. (11)
15 cm d, eq. (11)
15 cm b, eq. (11)
15 cm c, eq. (11)
45 cm a, eq. (23)
45 cm d, eq. (23)
45 cm c, eq. (23)
75 cm b, eq. (23)
75 cm a, eq. (23)
75 cm c, eq. (23)
75 cm d,
45 cm b, eq. (10)
(a)
Days
15 cm b, eq. (10)
15 cm a, eq. (10)
15 cm c, eq. (10)
15 cm d, eq. (10)
45 cm c, eq. (18)
45 cm a, eq. (18)
75 cm c, eq. (23)
45 cm b, eq. (18)
75 cm a, eq. (23)
75 cm b, eq. (23)
45 cm d
75 cm d
(b)
15 cm d, eq. (11)
45 cm d, eq. (11)
15 cm b, eq. (11)
45 cm a, eq. (11)
75 cm a, eq. (11)
75 cm b, eq. (11)
75 cm c
45 cm c, eq. (11)
75 cm d, eq. (11)
45 cm b, eq. (11)
(c)
15 cm a, eq. (10)
15 cm c, eq. (10)
15 cm b, eq. (10)
15 cm d, eq. (10)
45 cm d, eq. (14), (19)
75 cm d, eq. (18)
45 cm c, eq. (14), (19)
45 cm b, eq. (14), (19)
45 cm a, eq. (14), (19)
75 cm c, eq. (18)
75 cm a
75 cm b, eq. (18)
(d)

Fig. 1.

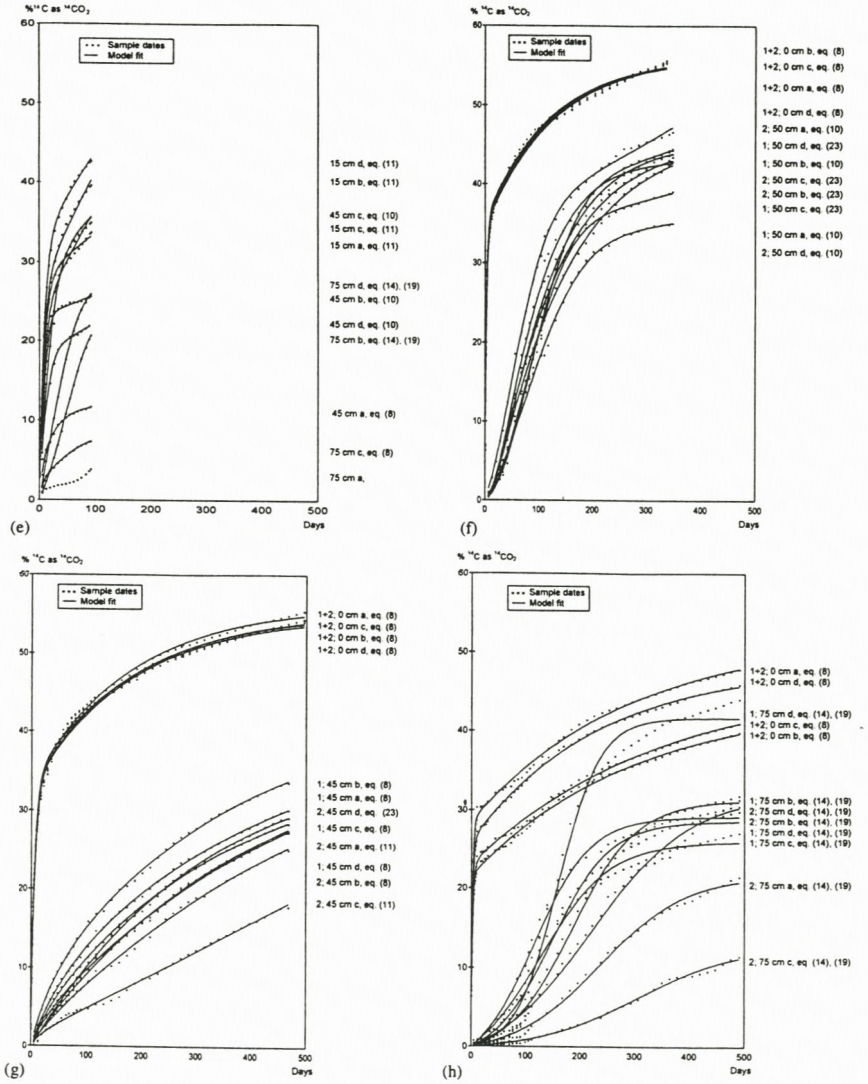

Fig. 1. (*Continued*)

al. (1986) and Knaebel et al. (1994) to describe degradation of xenobiotic compounds in soil. The three-half-order model with linear growth of the degrading microorganisms was expressed as

$$P = c_0(1 - e^{-k_1 t - (k_2 t^2/2)}) + k_0 t. \quad (10)$$

where P is the concentration of pesticide mineralised at time t (% ^{14}C as $^{14}CO_2$), c_0 is the total concentration of pesticide converted to $^{14}CO_2$ by first order metabolism, k_1 is the degradation rate constant for the first order process, k_2 is the linear growth rate term describing growth of microorganisms, and k_0 is the degradation rate constant for the zero order process.

When there is no growth of microorganisms, k_2 becomes zero, and Eq. (10) is simplified to a first order model plus a zero order linear term

$$P = c_0(1 - e^{-k_1 t}) + k_0 t \quad (11)$$

where P is the concentration of pesticide mineralised at time t (% ^{14}C as $^{14}CO_2$), c_0 is the total concentration of pesticide converted to $^{14}CO_2$ by first order metabolism, k_1 is the degradation rate constant for the first order process, and k_0 is the degradation rate constant for the zero order process.

Simkins and Alexander (1984) used Monod kinetics to describe the relationship between growth rate and substrate concentration where the growth dynamics were limited only by the concentration of one substrate (metabolic degradation) as a basis. They developed six models describing mineralization kinetics, zero order, Monod without growth, first order, logistic, Monod with growth and logarithmic. The Monod model with growth has too many parameters to be estimated without correlation between parameters. The simple Monod model without growth must be fitted in its differential form, considering that mineralization product is measured:

$$-\frac{dc}{dt} = \frac{k_1(c_0 - c)}{k_m + (c_0 - c)} \quad (12)$$

The first order model be Simkins and Alexander (1984) is exactly like the first order model already described Eqs. (4) and (6).

The zero order model (Simkins and Alexander, 1984) in its integrated form, is as follows:

$$P = kt \quad (13)$$

where P is the concentration of pesticide mineralised at time t (% ^{14}C as $^{14}CO_2$), k is the degradation rate constant, and t is the time in days.

The logistic model according to Simkins and Alexander (1984) based on the assumption that one substrate (here: the pesticide) is the limiting factor:

$$P = c_0 - \frac{c_0 + x_0}{1 + \left(\frac{x_0}{c_0}\right) e^{k_1(c_0 + x_0)t}} \quad (14)$$

where P is the concentration of pesticide mineralised at time t (% ^{14}C as $^{14}CO_2$), c_0 is the total concentration of pesticide converted to $^{14}CO_2$ by first order metabolism, x_0 is the amount of substrate (pesticide) required to produce the initial

Fig. 1. (a) Mineralization of 0.04 $\mu g \cdot g^{-1}$ mecoprop in Danish soil, FB 1_I, Jan 93 (mcfb 1_I). Depth (15, 45 and 75 cm), replicate number and model equation shown at the end of each data curve. (b) Mineralization of 0.04 $\mu g \cdot g^{-1}$ mecoprop in Danish soil, FB 1_II, March 94 (mcfb 1_II). Depth (15, 45 and 75 cm), replicate number and model equation shown at the end of each data curve. (c) Mineralization of 0.04 $\mu g \cdot g^{-1}$ mecoprop in Danish soil, FB 3_I, March 93 (mcfb 3_I). Depth (15, 45 and 75 cm), replicate number and model equation shown at the end of each data curve. (d) Mineralization of 0.04 $\mu g \cdot g^{-1}$ mecoprop in Danish soil, FB 3_II, March 94 (mcfb 3_II). Depth (15, 45 and 75 cm), replicate number and model equation shown at the end of each data curve. (e) Mineralization of 0.04 $\mu g \cdot g^{-1}$ mecoprop in Danish soil, FB 4_I, Jan 95 (mcfb 4_I). Depth (15, 45 and 75 cm), replicate number and model equation shown at the end of each data curve. (f) Mineralization of 0.04 $\mu g \cdot g^{-1}$ mecoprop in Italian soil, April 93 (mcit). Ploughed layer samples (0 cm) incubated as composite samples from hole 1 + 2. Subsoil samples (50 cm) incubated individually, each replicate from each hole. Hole number, depth, replicate number and model equation shown at the end of each data curve. (g) Mineralization of 0.04 $\mu g \cdot g^{-1}$ mecoprop in Spanish soil, Dec. 93 (mcsp). Ploughed layer samples (0 cm) incubated as composite samples from hole 1 + 2. Subsoil samples (45 cm) incubated individually, each replicate from each hole. Hole number, depth, replicate number and model equation shown at the end of each data curve. (h) Mineralization of 0.04 $\mu g \cdot g^{-1}$ mecoprop in German soil, April 93 (mcty). Ploughed layer samples (0 cm) incubated as composite samples from hole 1 + 2. Subsoil samples (75 cm) incubated individually, each replicate from each hole. Hole number, depth, replicate number and model equation shown at the end of each data curve.

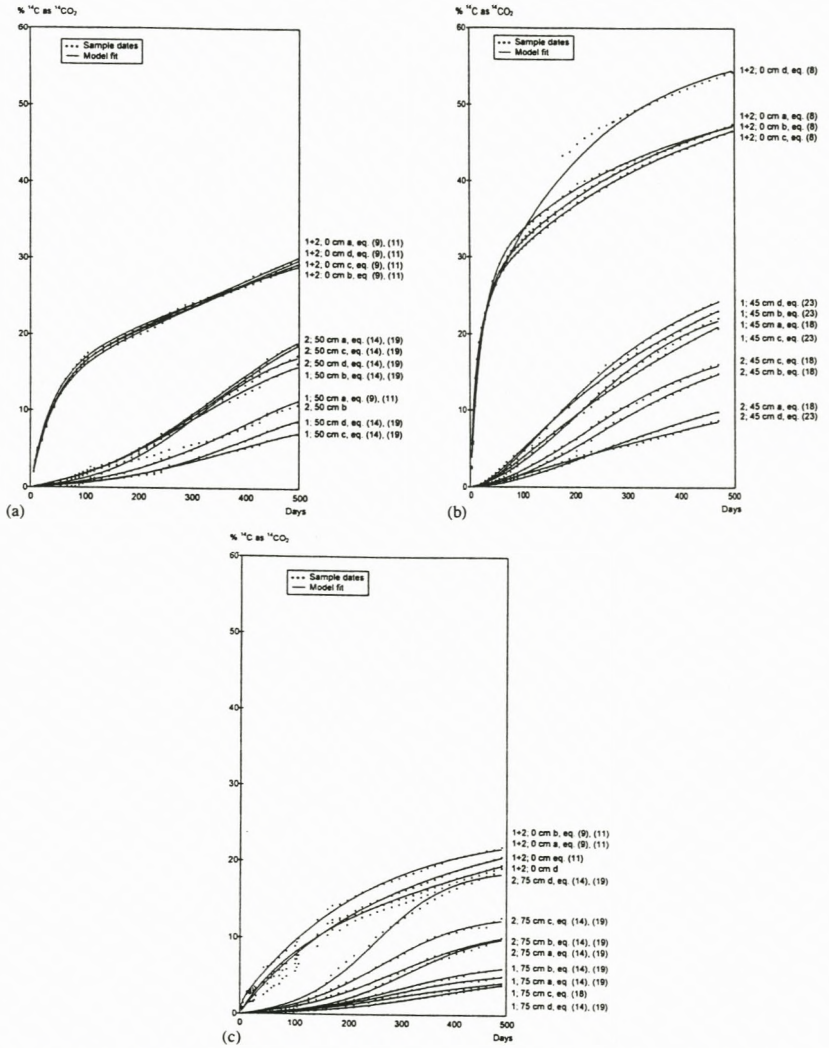

Fig. 2. (a) Mineralization of 0.08 $\mu g \cdot g^{-1}$ bentazon in Italian soil, April 93 (beit). Ploughed layer samples (0 cm) incubated as composite samples from hole 1 + 2. Subsoil samples (50 cm) incubated individually, each replicate from each hole. Hole number, depth, replicate number and model equation shown at the end of each data curve. (b) Mineralization of 0.08 $\mu g \cdot g^{-1}$ bentazon in Spanish soil, Dec. 93 (besp). Ploughed layer samples (0 cm) incubated as composite samples from hole 1 + 2. Subsoil samples (45 cm) incubated individually, each replicate from each hole. Hole number, depth, replicate number and model equation shown at the end of each data curve. (c) Mineralization of 0.08 $\mu g \cdot g^{-1}$ bentazon in German soil, April 93 (bety). Ploughed layer samples (0 cm) incubated as composite samples from hole 1 + 2. Subsoil samples (75 cm) incubated individually, each replicate from each hole. Hole number, depth, replicate number and model equation shown at the end of each data curve.

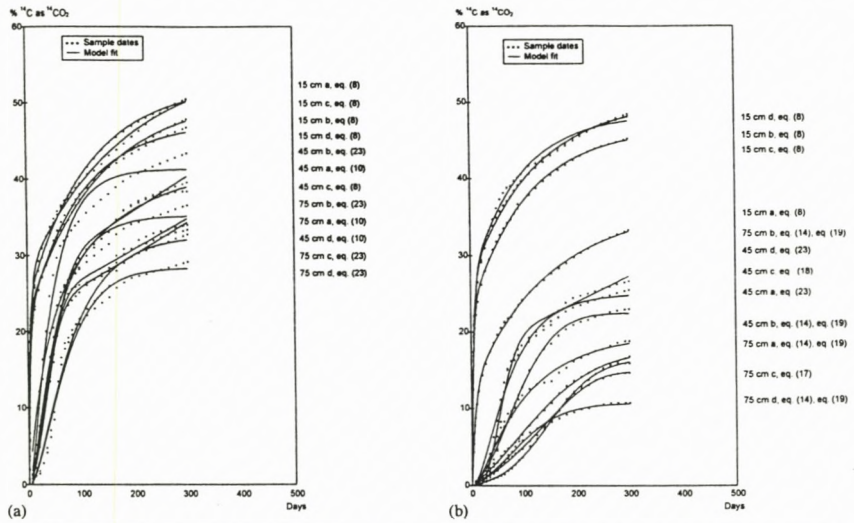

Fig. 3. (a) Mineralization of 0.07 $\mu g \cdot g^{-1}$ ETU in Danish soil, FB 1_II, March 94 (etfb 1). Depth (15, 45 and 75 cm), replicate number and model equation shown at the end of each data curve. (b) Mineralization of 0.07 $\mu g \cdot g^{-1}$ ETU in Danish soil, FB 3_II, March 94 (etfb 3). Depth (15, 45 and 75 cm), replicate number and model equation shown at the end of each data curve.

population density, k is the degradation rate constant, and t is the time in days.

The logarithmic model based on the same assumption:

$$P = -X_0(1 - e^{\mu_{max} t}) \quad (15)$$

where P is the concentration of pesticide mineralised at time t (% ^{14}C as $^{14}CO_2$), X_0 is the amount of substrate (pesticide) required to, produce the initial population density, t is the time in days, and μ_{max} is the maximum specific growth rate.

Schmidt et al. (1985) developed 12 kinetic models to describe the metabolism of organic substrates that are not supporting growth, because the degradation is cometabolic (where the energy for growth derives from another substrate), or because the substrate of interest is present at a very low concentration and therefore not important in determining the growth rate of the active organisms. The models combined logistic growth (when there is an upper limit to population density), exponential growth, linear growth and no growth with low, intermediate and high concentrations of the test substrate. Here, only the models for low concentrations are considered. No growth and low concentration of test substrate result in a first order model, which has already been described.

Logistic growth and low concentration of test substrate (Schmidt et al., 1985):

$$P = c_0 - c_0(\Phi(e^{rt} - 1) + 1)^{-k/r} \quad (16)$$

where P is the concentration of pesticide mineralised at time t (% ^{14}C as $^{14}CO_2$), c_0 is the total concentration of pesticide converted to $^{14}CO_2$ by the process, Φ is the relation between initial population density and maximum achievable population density, k is the degradation rate constant, r is the maximum specific growth rate, and t is the time.

Exponential growth and low concentrations of test substrate (Schmidt et al., 1985):

$$P = c_0 - c_0 e^{-(k/r)(e^{rt} - 1)} \quad (17)$$

where P is the concentration of pesticide mineralised at time t (% ^{14}C as $^{14}CO_2$), c_0 is the total concentration of pesticide converted to $^{14}CO_2$ by the modelled process, k is the degradation rate constant, r is the maximum specific growth rate, and t is the time.

Linear growth and low concentration of test substrate (Schmidt et al., 1985):

$$P = c_0(1 - e^{-k_1 t - (k_2 t^2/2)}) \quad (18)$$

where P is the concentration of pesticide mineralised at time t (% ^{14}C as $^{14}CO_2$), c_0 is the total concentration of pesticide converted to $^{14}CO_2$ by the modelled process, k is the degradation rate constant, and t is the time.

This model is the same as Eq. (10) without the zero order term.

Liu and Zhang (1986) and Liu et al. (1988) assumed that the degradative processes of pesticides in soil involves microbial utilisation of pesticides as an energy source (metabolic degradation) and developed a model able to describe degradation curves no matter whether the degradation curve has an inflection point or not. The model is:

$$P = c_0 - \frac{k_1 c_0}{(k_1 + k_2 c_0)e^{k_1 t} - k_2 c_0} \quad (19)$$

where P is the concentration of pesticide mineralised at time t (% ^{14}C as $^{14}CO_2$), c_0 is the total concentration of pesticide converted to $^{14}CO_2$ by the modelled process, k_1 is the rate constant, and k_2 is the rate constant.

Stenström (1988) used the following empirical model:

$$P = kt^{1/2} + a \quad (20)$$

where P is the concentration of pesticide mineralised at time t (% ^{14}C as $^{14}CO_2$), k is the degradation rate constant, t is the time, and a is a constant.

In an experiment with ^{14}C labelled linuron, Stenström (1988) used a zero order model for one part of the curve and the empirical model Eq. (20) for another part of the curve. In the present study, a combination of the two was used:

$$k_1 t + k_2 t^{1/2} + a \quad (21)$$

where P is the concentration of pesticide mineralised at time t (% ^{14}C as $^{14}CO_2$), k_1 is the degradation rate constant, k_2 is the degradation rate constant, and t is the time.

Stenström (1988) proposed a combination of his empirical model with a model including the exponential growth of microorganisms (Hoover et al., 1986) for treatment of sigmoidal curves, i.e. curves with an initial phase with an increasing degradation rate followed by a phase with a decreasing degradation rate

$$P = k_1 t^{1/2} + \frac{qN_0}{k_2}(e^{k_2 t} - 1) \quad (22)$$

where P is the concentration of pesticide mineralised at time t (% ^{14}C as $^{14}CO_2$), k_1 is the degradation rate constant, q is the maximum specific metabolic rate, N_0 is the initial amount of microorganisms, k_2 is the rate constant for growth of the microorganisms, and t is the time.

Since the inspection of especially subsoil mineralization curves gave an impression of two sequences in the evolution of CO_2, a model expressing first order sequential mineralization was included:

$$P = c_0\left(1 + \frac{k_1 e^{-k_2 t} - k_2 e^{-k_1 t}}{k_2 - k_1}\right) \quad (23)$$

where P is the concentration of pesticide mineralised at time t (% ^{14}C as $^{14}CO_2$), c_0 is the total concentration of pesticide converted to $^{14}CO_2$ by first order metabolism, k_1, k_2 is the degradation rate constants for the two first order processes, and t is the time in days.

Models 6–23 were all fitted to the curves showing accumulated data for $^{14}CO_2$ production.

3.2. Random variation

All the models above may be written generally as:

$$P = F(\theta, t)$$

where P is the concentration of pesticide mineralised at time t (% ^{14}C as $^{14}CO_2$), θ is the parameters of the model, e.g. C_0, k_1, k_2, t is the time in days, and $F(\theta, t)$ is the non-linear model.

Table 3
Residual mean for all fitted equations for subsurface soils

Sample	Equations without growth				Equations with growth of microorganisms					Figure reference
	Eq. (8)	Eq. (9)	Eq. (11)	Eq. (23)	Eq. (10)	Eq. (14)	Eq. (17)	Eq. (18)	Eq. (19)	
mcfb 1_I a 45 cm				1.231				2.049		Fig. 1a
mcfb 1_I b 45 cm			0.4728	0.7348	0.03665					
mcfb 1_I c 45 cm				1.099		1.821	2.103	1.994	1.821	
mcfb 1_I d 45 cm				1.148		1.840	2.431	2.037	1.840	
mcfb 1_II a 45 cm						0.1824	0.2057	0.1487	0.1824	Fig. 1b
mcfb 1_II b 45 cm						0.09043	0.09277	0.08496	0.09061	
mcfb 1_II c 45 cm						0.1712	0.1824	0.1540	0.1712	
mcfb 1_II d 45 cm										
mcfb 3_I a 45 cm			1.101							Fig. 1c
mcfb 3_I b 45 cm			0.4850		0.1288					
mcfb 3_I c 45 cm			0.5274		0.2321					
mcfb 3_I d 45 cm			1.607							
mcfb 3_II a 45 cm				0.06472		0.09875	0.1039	0.09322	0.09875	Fig. 1d
mcfb 3_II b 45 cm						0.3310	0.4841	0.2601	0.3310	
mcfb 3_II c 45 cm				0.06003		0.1943	0.2929	0.1371	0.1943	
mcfb 3_II d 45 cm				0.03467		0.1992	0.3575	0.1722	0.1992	
mcfb 4_I a 45 cm	0.03399		0.04627							Fig. 1e
mcfb 4_I b 45 cm					0.1715			0.3201		
mcfb 4_I c 45 cm					0.07739					
mcfb 4_I d 45 cm			0.1820		0.1708					
mcfb 1_I a 75 cm				1.026		1.724	1.858	1.750	1.724	Fig. 1a
mcfb 1_I b 75 cm				0.6393						
mcfb 1_I c 75 cm				0.6063		0.7716	0.7707	0.7666	0.7717	
mcfb 1_I d 75 cm										
mcfb 1_II a 75 cm				0.02420			0.04452	0.03818		Fig. 1b
mcfb 1_II b 75 cm				0.02645		0.04636	0.05036	0.04309	0.04636	
mcfb 1_II c 75 cm				0.04128		0.08378	0.1215	0.05151	0.08378	
mcfb 1_II d 75 cm										
mcfb 3_I a 75 cm			1.115							Fig. 1c
mcfb 3_I b 75 cm			1.194							
mcfb 3_I c 75 cm										
mcfb 3_I d 75 cm			1.021							
mcfb 3_II a 75 cm										Fig. 1d
mcfb 3_II b 75 cm						0.2311	0.2429	0.2205	0.2311	
mcfb 3_II c 75 cm						0.2315	0.2374	0.2106	0.2315	
mcfb 3_II d 75 cm						0.4878	0.6931	0.3283	0.4878	
mcfb 4_I a 75 cm										Fig. 1e
mcfb 4_I b 75 cm						0.01513	0.01372	0.02516	0.01513	
mcfb 4_I c 75 cm	0.005961		0.01693							
mcfb 4_I d 75 cm				0.1046		0.01185	0.02049	0.01224	0.01185	
mcit 1 a 50 cm					0.4968	0.64831	0.201	0.6558	0.6483	Fig. 1f
mcit 1 b 50 cm					0.1408	0.3611	1.233	0.2993	0.3611	
mcit 1 c 50 cm				0.6730	0.8982	1.575	1.816	1.640	1.575	
mcit 1 d 50 cm				0.5521		1.663	2.513	1.783	1.663	
mcit 2 a 50 cm				0.2969	0.2633	1.022	1.664	1.286	1.022	Fig. 1f
mcit 2 b 50 cm				0.3122	0.4812	1.099	1.491	1.200	1.099	
mcit 2 c 50 cm				1.078		2.073	3.478	2.208	2.073	
mcit 2 d 50 cm					0.6493	0.6950			0.6950	

Table 3 (continued)

Sample	Equations without growth				Equations with growth of microorganisms					Figure reference
	Eq. (8)	Eq. (9)	Eq. (11)	Eq. (23)	Eq. (10)	Eq. (14)	Eq. (17)	Eq. (18)	Eq. (19)	
mcsp 1 a 45 cm	0.08272		0.09951							Fig. 1g
mcsp 1 b 45 cm	0.08668		0.1942							
mcsp 1 c 45 cm	0.02961		0.05533							
mcsp 1 d 45 cm	0.03499		0.06115							
mcsp 2 a 45 cm			0.07347							Fig. 1g
mcsp 2 b 45 cm	0.1191		0.1343							
mcsp 2 c 45 cm			0.1006							
mcsp 2 d 45 cm				0.04226	0.04819					
mcty 1 a 75 cm						1.054	1.972		1.054	Fig. 1h
mcty 1 b 75 cm						0.8722	1.349	0.5408	0.8722	
mcty 1 c 75 cm						1.015	1.622	0.8965	1.015	
mcty 1 d 75 cm						2.264	4.546	2.264		
mcty 2 a 75 cm						0.2482	0.5007		0.2482	Fig. 1h
mcty 2 b 75 cm						0.8498	1.634	0.7911	0.8498	
mcty 2 c 75 cm						0.06719	0.09636		0.06719	
mcty 2 d 75 cm						0.4986	1.213		0.4986	
beit 1 a 50 cm						0.009421	0.01174		0.009421	Fig. 2a
beit 1 b 50 cm						0.09205	0.1216	0.05115	0.09205	
beit 1 c 50 cm						0.02215	0.02268	0.02617	0.02215	
beit 1 d 50 cm						0.009770	0.008935	0.02312		
beit 2 a 50 cm						0.04525	0.03973	0.07629	0.04525	Fig. 2a
beit 2 b 50 cm										
beit 2 c 50 cm						0.01895	0.02756	0.01668	0.01895	
beit 2 d 50 cm						0.1357	0.2062		0.1357	
besp 1 a 45 cm						0.1241	0.2196	0.05737	0.1242	Fig. 2b
besp 1 b 45 cm				0.06221		0.08777	0.1386	0.07266	0.08777	
besp 1 c 45 cm				0.02816		0.06996	0.09675	0.04979	0.06996	
besp 1 d 45 cm				0.03143		0.1729	0.2936	0.1079	0.1729	
besp 2 a 45 cm						0.01312	0.02201	0.004299	0.01312	Fig. 2b
besp 2 b 45 cm						0.05125	0.07938	0.02659	0.05125	
besp 2 c 45 cm						0.07655	0.1221	0.03618	0.07655	
besp 2 d 45 cm				0.03321		0.06359	0.06746	0.05856	0.06359	
bety 1 a 75 cm						0.01496			0.01496	Fig. 2c
bety 1 b 75 cm						0.01408	0.02251		0.01407	
bety 1 c 75 cm						0.01031		0.006250	0.01031	
bety 1 d 75 cm						0.001935	0.6767		0.001935	
bety 2 a 75 cm						0.03947	0.05423	0.02197		Fig. 2c
bety 2 b 75 cm						0.03632	0.07271		0.03632	
bety 2 c 75 cm						0.05830	0.1036		0.05830	
bety 2 d 75 cm						0.4058	0.6767		0.4059	
etfb 1 a 45 cm				1.193	0.4490	2.107	2.731	2.459	2.107	Fig. 3a
etfb 1 b 45 cm				2.836		4.289			4.289	
etfb 1 c 45 cm	1.258		1.318							
etfb 1 d 45 cm				1.064	0.2949					
etfb 3 a 45 cm				0.3288		0.6172	0.6717	0.6462	0.6172	Fig. 3b
etfb 3 b 45 cm				0.09130		0.0303	0.03498	0.03181	0.0303	
etfb 3 c 45 cm						0.3247	0.6004	0.2697	0.3247	
etfb 3 d 45 cm				0.9219		1.566	2.113	1.645	1.566	
etfb 1 a 75 cm				1.767	0.5732	2.410			2.410	Fig. 3a

Table 3 (continued)

Sample	Equations without growth				Equations with growth of microorganisms					Figure reference
	Eq. (8)	Eq. (9)	Eq. (11)	Eq. (23)	Eq. (10)	Eq. (14)	Eq. (17)	Eq. (18)	Eq. (19)	
etfb 1 b 75 cm				1.433		2.580	3.095	2.950	2.580	
etfb 1 c 75 cm				1.629		2.693	3.337	2.833	2.693	
etfb 1 d 75 cm				1.515		1.988	3.002	1.968	1.988	
etfb 3 a 75 cm						0.01757	0.05789		0.01757	Fig. 3b
etfb 3 b 75 cm						2.961	4.250		2.961	
etfb 3 c 75 cm						0.08756	0.05172	0.1163	0.08756	
etfb 3 d 75 cm						0.09787	0.1603	0.08561	0.09787	

The records of the concentration of mineralization product formed at time t include some random noise, thus the model for the records may be written as:

$$P^* = F(\theta, t) + \varepsilon$$

where P^* is our records of the concentration of pesticide mineralised at time t (% ^{14}C as $^{14}CO_2$), ε is the random effect which we assume to be independently distributed with zero mean and constant variance, σ^2 say, t is the time in days, and $F(\theta, t)$ is the non-linear model.

3.3. Non-linear regression

If we assume the random effects to be distributed this way for all the models, we may estimate the parameters of the models by non-linear regression analysis (Draper and Smith, 1981). It must be possible to solve the models analytically for the dependent variable, and the procedure comes out with estimates, S.D. of the estimates, residual sum of squares and the asymptotic correlation matrix. Non-linear regression analysis requires initial estimates of the parameters. These initial estimates may be found in various ways. If the model can be linearized through a transformation, the initial parameters can be found on the basis of this linearization (i.e. a plot or a fit). In cases as most of the models treated in the present work where the model is intrinsically non-linear, linearization is not possible, and initial parameters must be chosen on the basis of experience or various plots.

Two methods of estimating the parameters which minimise the residual sum of squares in non-linear models were used. The method of Marquardt (Marquardt, 1963) is a compromise between the method of steepest descent and the method of linearization by a Taylor series (also named the Gauss-Newton method). The implementation used here requires the derivatives $(\partial F(\theta_i, t)/\partial\theta_i)$ to be solved analytically—as well as the model. The method of multivariate secant (Ralston and Jennrich, 1978) is based on the method of linearization by a Taylor series but the derivatives are estimated from the history of the iterations (i.e. the data) and thus they do not need to be solved analytically. For further details on the methods see Draper and Smith (1981) or Bates and Watts (1988). The calculations were performed by the procedure NLIN of SAS (SAS, 1989).

Some of the model fits were performed with Marquardt as well as with the multivariate secant method. Giving the same results, the multivariate secant method was chosen for the rest of the model fits.

4. Results and discussion

Date of sampling, soil depth, texture of soil, incubation temperature, pH and K_d values are shown in Table 1. Figs. 1–3 show the mineralization curves for mecoprop, bentazon and ETU in the ploughed layer and subsoil and one example of a fitted model in each case.

Table 4
Parameters estimated ± S.D. according to Eq. (8) for mecoprop, bentazone and ETU in ploughed layer soils from Denmark, Italy, Spain and Germany

Site	c_1	k_1	c_2	k_2
mcfb 1_I b 15 cm	21.42 ± 1.39	0.04327 ± 0.00327	24.53 ± 0.87	0.004234 ± 0.000743
mcfb 1_I c 15 cm	17.06 ± 0.82	0.08581 ± 0.00645	23.42 ± 0.62	0.006758 ± 0.000531
mcfb 1_I d 15 cm	18.10 ± 1.20	0.07053 ± 0.00672	25.46 ± 0.84	0.005892 ± 0.000682
mcfb 3_I b 15 cm	17.27 ± 1.31	0.06585 ± 0.00683	20.80 ± 0.87	0.005855 ± 0.000954
mcfb 4_I b 15 cm	15.79 ± 2.00	0.2099 ± 0.0292	25.15 ± 1.41	0.02875 ± 0.00438
mcfb 4_I c 15 cm	9.893 ± 1.35	40.2943 ± 0.0541	25.77 ± 1.04	0.03477 ± 0.00342
mcfb 4_I d 15 cm	30.58 ± 1.66	0.1179 ± 0.0060	22.52 ± 7.61	0.008358 ± 0.005721
mcit 1+2 a 0 cm	34.99 ± 0.56	0.3544 ± 0.0174	21.26 ± 0.68	0.007665 ± 0.000781
mcit 1+2 b 0 cm	34.36 ± 0.49	0.3655 ± 0.0162	22.04 ± 0.60	0.007660 ± 0.000661
mcit 1+2 c 0 cm	35.22 ± 0.60	0.3664 ± 0.0192	20.89 ± 0.68	0.008163 ± 0.000848
mcit 1+2 d 0 cm	35.01 ± 0.51	0.3687 ± 0.0168	20.95 ± 0.59	0.008085 ± 0.000728
mcsp 1+2 a 0 cm	33.24 ± 0.50	0.1676 ± 0.0059	23.05 ± 0.45	0.005487 ± 0.000388
mcsp 1+2 b 0 cm	32.89 ± 0.44	0.1668 ± 0.0051	22.30 ± 0.39	0.005516 ± 0.000349
mcsp 1+2 c 0 cm	33.66 ± 0.49	0.1544 ± 0.0050	21.83 ± 0.45	0.005203 ± 0.000393
mcsp 1+2 d 0 cm	32.63 ± 0.43	0.1613 ± 0.0049	22.30 ± 0.38	0.005512 ± 0.000343
mcty 1+2 a 0 cm	28.55 ± 0.17	0.4386 ± 0.0112	23.92 ± 0.56	0.003352 ± 0.000181
mcty 1+2 b 0 cm	21.45 ± 0.19	0.5496 ± 0.0238	22.36 ± 0.63	0.003414 ± 0.000223
mcty 1+2 c 0 cm	22.93 ± 0.15	0.3773 ± 0.0097	23.58 ± 0.63	0.002932 ± 0.000167
mcty 1+2 d 0 cm	26.02 ± 0.18	0.3935 ± 0.0106	22.43 ± 0.35	0.004221 ± 0.000187
beit 1+2 a 0 cm	14.43 ± 0.54	0.2567 ± 0.001277	40.93 ± 11.72	0.0008767 ± 0.0003461
beit 1+2 c 0 cm	13.47 ± 0.41	0.02492 ± 0.001031	85.25 ± 44.46	0.000412 ± 0.000248
besp 1+2 a 0 cm	26.68 ± 0.50	0.04687 ± 0.00144	25.96 ± 0.63	0.003140 ± 0.000273
besp 1+2 b 0 cm	24.65 ± 0.38	0.05497 ± 0.00159	30.08 ± 0.78	0.002815 ± 0.000206
besp 1+2 c 0 cm	24.10 ± 0.40	0.05822 ± 0.00188	31.08 ± 1.06	0.002597 ± 0.000222
besp 1+2 d 0 cm	18.20 ± 1.07	0.1024 ± 0.0144	40.33 ± 0.94	0.004614 ± 0.000427
etfb 1 a 15 cm	23.74 ± 0.73	0.3061 ± 0.0226	28.39 ± 0.71	0.008758 ± 0.000759
etfb 1 b 15 cm	22.19 ± 0.82	0.3027 ± 0.0272	28.25 ± 0.97	0.007532 ± 0.000876
etfb 1 c 15 cm	27.30 ± 0.91	0.2129 ± 0.0152	27.93 ± 1.76	0.005600 ± 0.001013
etfb 1 d 15 cm	21.13 ± 0.84	0.2433 ± 0.0197	26.15 ± 0.69	0.01021 ± 0.00089
etfb 3 a 15 cm	12.62 ± 0.50	0.1311 ± 0.0088	23.80 ± 0.50	0.006553 ± 0.000540
etfb 3 b 15 cm	26.49 ± 0.99	0.2036 ± 0.0145	21.95 ± 0.79	0.01045 ± 0.00116
etfb 3 c 15 cm	22.80 ± 0.82	0.2564 ± 0.0200	23.75 ± 0.72	0.009016 ± 0.000968
etfb 3 d 15 cm	26.85 ± 0.84	0.1998 ± 0.0123	23.49 ± 0.77	0.007767 ± 0.000970

Many published pesticide degradation studies in soil analysed the changes of concentration of parent pesticide and calculated half-lives assuming first order kinetics (Jones et al., 1986, 1989, 1990; Ou et al., 1988; Hornsby et al., 1990; Minton et al., 1990). In some cases, where field studies were performed, reported half-lives should be seen as dissipation rates, since surface losses via pathways such as volatilisation and plant uptake would influence the concentrations found (Jones et al., 1990, 1989, 1986; Hornsby et al., 1990; Minton et al., 1990). Other authors made degradation experiments following the evolution of $^{14}CO_2$ from ^{14}C-labelled pesticide (Ou et al., 1985; Konopka and Turco, 1991; Sinclair and Lee, 1992; Dictor et al., 1992; Helweg, 1993). Such degradation experiments were mineralization experiments. Most published laboratory studies of the fate of pesticides in water analysed the changes of concentration of the parent pesticide with time in aliquots of the sample and calculated the half-life. In soil degradation studies in the laboratory, it is not possible to take out aliquots of the sample, so if the degradation process is to be followed with time in the same sample, degradation studies in soil must be performed quantifying the amount of

Table 5
Parameters estimated ± S.D. according to Eq. (9) for mecoprop, bentazone and ETU in ploughed layer soils from Denmark, Italy, Spain and Germany

Site	k_1	k_2	a
mcfb 1_I a 15 cm	0.02638 ± 0.00092	0.0005212 ± 0.0000329	0.3429 ± 0.0061
mcfb 1_I b 15 cm	0.03296 ± 0.00126	0.0005570 ± 0.0000252	0.2734 ± 0.0048
mcfb 1_I c 15 cm	0.04832 ± 0.00223	0.0005325 ± 0.0000261	0.2568 ± 0.0047
mcfb 1_I d 15 cm	0.04231 ± 0.00203	0.0005915 ± 0.0000293	0.2651 ± 0.0054
mcfb 1_II a 15 cm	0.1129 ± 0.0082	0.001635 ± 0.000516	0.4467 ± 0.0191
mcfb 1_II b 15 cm	0.1135 ± 0.0080	0.001606 ± 0.000506	0.4512 ± 0.0186
mcfb 1_II c 15 cm	0.08298 ± 0.00678	0.0009720 ± 0.0006664	0.4666 ± 0.0257
mcfb 1_II d 15 cm	0.08451 ± 0.00742	0.0007644 ± 0.0005839	0.4196 ± 0.0244
mcfb 3_I b 15 cm	0.04520 ± 0.00238	0.0004976 ± 0.0000300	0.2347 ± 0.0054
mcfb 3_I d 15 cm	0.03698 ± 0.00163	0.0004195 ± 0.0000430	0.3373 ± 0.0069
mcfb 4_I a 15 cm	0.1127 ± 0.0050	0.0007947 ± 0.0001491	0.2798 ± 0.0072
mcfb 4_I b 15 cm	0.1253 ± 0.0073	0.002215 ± 0.000185	0.2646 ± 0.0088
mcfb 4_I c 15 cm	0.1165 ± 0.0093	0.001885 ± 0.000218	0.2330 ± 0.0111
mcfb 4_I d 15 cm	0.1125 ± 0.0025	0.001850 ± 0.000097	0.3221 ± 0.0044
mcit 1+2 a 0 cm	0.2803 ± 0.0159	0.001053 ± 0.000057	0.3886 ± 0.0058
mcit 1+2 b 0 cm	0.2858 ± 0.0164	0.001092 ± 0.000056	0.3831 ± 0.0057
mcit 1+2 c 0 cm	0.2856 ± 0.0171	0.001047 ± 0.000060	0.3932 ± 0.0061
mcit 1+2 d 0 cm	0.2883 ± 0.0168	0.001047 ± 0.000060	0.3905 ± 0.0059
mcsp 1+2 a 0 cm	0.1303 ± 0.0057	0.0007421 ± 0.0000312	0.3827 ± 0.0048
mcsp 1+2 b 0 cm	0.1294 ± 0.0054	0.0007046 ± 0.0000286	0.3785 ± 0.0045
mcsp 1+2 c 0 cm	0.1234 ± 0.0048	0.0006885 ± 0.0000276	0.3823 ± 0.0044
mcsp 1+2 d 0 cm	0.1251 ± 0.0052	0.0006986 ± 0.0000282	0.3763 ± 0.0045
mcty 1+2 a 0 cm	0.3740 ± 0.0183	0.0006579 ± 0.0000170	0.3041 ± 0.0028
mcty 1+2 b 0 cm	0.4390 ± 0.0309	0.0005488 ± 0.0000150	0.2325 ± 0.0028
mcty 1+2 c 0 cm	0.3209 ± 0.0149	0.0005494 ± 0.0000126	0.2453 ± 0.0023
mcty 1+2 d 0 cm	0.3121 ± 0.0335	0.0006366 ± 0.0000203	0.2868 ± 0.0035
beit 1+2 a 0 cm	0.02423 ± 0.00067	0.0003583 ± 0.000006	0.1518 ± 0.0018
beit 1+2 b 0 cm	0.02507 ± 0.00052	0.0004163 ± 0.0000042	0.1380 ± 0.0012
beit 1+2 c 0 cm	0.02490 ± 0.00059	0.0004057 ± 0.0000047	0.1349 ± 0.0014
beit 1+2 d 0 cm	0.02439 ± 0.00067	0.0003989 ± 0.0000057	0.1398 ± 0.0016
besp 1+2 a 0 cm	0.03882 ± 0.00117	0.0005886 ± 0.0000160	0.3063 ± 0.0032
besp 1+2 b 0 cm	0.04571 ± 0.00155	0.0006708 ± 0.0000148	0.2801 ± 0.0031
besp 1+2 c 0 cm	0.04923 ± 0.00173	0.0006701 ± 0.0000141	0.2699 ± 0.0029
besp 1+2 d 0 cm	0.05836 ± 0.00688	0.001115 ± 0.000047	0.2579 ± 0.0092
bety 1+2 a 0 cm	0.005343 ± 0.001386	0.0001545 ± 0.0000916	0.1544 ± 0.0478
bety 1+2 b 0 cm	0.009468 ± 0.000848	0.0002233 ± 0.0002804	0.1287 ± 0.0099
bety 1+2 c 0 cm	0.009505 ± 0.00110	0.0002312 ± 0.0000265	0.09691 ± 0.0097
etfb 1 a 15 cm	0.2060 ± 0.0169	0.001341 ± 0.000071	0.2967 ± 0.00743
etfb 1 b 15 cm	0.2178 ± 0.0189	0.001270 ± 0.000064	0.2686 ± 0.0069
etfb 1 c 15 cm	0.1779 ± 0.0105	0.001213 ± 0.0000531	0.3046 ± 0.0056
etfb 1 d 15 cm	0.1487 ± 0.0121	0.001112 ± 0.000067	0.2856 ± 0.0075
etfb 3 a 15 cm	0.08228 ± 0.00560	0.0007782 ± 0.0000291	0.1732 ± 0.0041
etfb 3 b 15 cm	0.1435 ± 0.0088	0.0009567 ± 0.0000614	0.3315 ± 0.0065
etfb 3 c 15 cm	0.1773 ± 0.0134	0.001026 ± 0.000059	0.2762 ± 0.0083
etfb 3 d 15 cm	0.1551 ± 0.0088	0.001051 ± 0.0000534	0.3155 ± 0.0057

CO_2 evolved through the total mineralization, and this can only be done with the use of isotope-labelled pesticide measuring the formation of evolved $^{14}CO_2$. The use of isotope-labelled compounds makes it possible to perform degradation experiments in the low concentrations that are most relevant when pesticides have been used in normal agricultural practice. However, the evolu-

Table 6
Parameters estimated ± S.D. according to Eq. (10) for mecoprop, bentazone and ETU in ploughed layer soils from Denmark, Italy, Spain and Germany

Site	c_0	k_1	k_2	k_0
mcfb 1_II a 15 cm	40.34 ± 0.71	0.07101 ± 0.00799	0.01693 ± 0.00275	0.1426 ± 0.0112
mcfb 1_II b 15 cm	40.76 ± 0.80	0.07468 ± 0.00886	0.01615 ± 0.00304	0.1410 ± 0.0127
mcfb 1_II c 15 cm	40.59 ± 0.74	0.05462 ± 0.00521	0.01033 ± 0.00150	0.1271 ± 0.0113
mcfb 1_II d 15 cm	36.40 ± 0.68	0.05101 ± 0.00575	0.01194 ± 0.00169	0.1141 ± 0.0104
mcfb 3_II a 15 cm	34.38 ± 0.56	0.03674 ± 0.00215	0.004772 ± 0.000481	0.1069 ± 0.00809
mcfb 3_II b 15 cm	31.19 ± 0.56	0.04468 ± 0.00209	0.003664 ± 0.000478	0.09080 ± 0.00796
mcfb 3_II c 15 cm	30.85 ± 0.63	0.04339 ± 0.00145	0.002228 ± 0.000311	0.1109 ± 0.00842
mcfb 3_II d 15 cm	29.76 ± 0.70	0.04037 ± 0.00167	0.002377 ± 0.000349	0.09663 ± 0.00936
mcfb 4_I a 15 cm	27.04 ± 0.65	0.1053 ± 0.0055	0.003143 ± 0.001663	0.06737 ± 0.00947

tion of $^{14}CO_2$ is not an expression of the disappearance of the parent compound, but shows the total mineralization.

The measurement of sorption was made through K_d values which are based on linear equilibrium sorption processes. The K_d values were used to give an overall picture of the soils capability for sorption of the pesticide. Sorption can be the reason for only some pesticide turning into $^{14}CO_2$ and it can influence the degradation process. In the present study evolved $^{14}CO_2$—coming from the degradation of the ^{14}C-pesticide—was measured so only degradation models have been considered.

To be able to compare mineralization rates in different soil types and under varying circumstances, a mathematical description of mineralization kinetics of pesticides in soil is needed. In the present study mecoprop, bentazon and ETU mineralization was investigated in soils from Denmark, Germany, Italy and Spain. The incubation temperatures, which can be seen in Table 1, were held as close to natural conditions as possible. The incubation time varied because the purpose of the study also was to identify residues of the parent pesticide and/or metabolites after a certain time. Results will be published in a later paper. The models, which were described in Section 3 were chosen on the assumption that they should be simple enough so that all the parameters could be estimated. The models published by Vink et al. (1994) and Jørgensen et al. (1995) had so many parameters that they could only be used if one or more of the parameters was given a fixed value. The same was the case for an exponential model by Brunner and Focht (1984). Models which were designed for high concentrations of pesticides were not included either.

An overall view on the depicted curves show a general difference in progress of ploughed layer curves and subsoil curves. Almost all the curves from the subsoil show an increase in the rate of formation of $^{14}CO_2$ at the beginning of the incubation, whereupon the formation of $^{14}CO_2$ becomes stable or decreases, resulting in sigmoidal curves. Most of the ploughed layer curves show only a decrease in formation of the mineralization product $^{14}CO_2$.

Because of considerable variations between some of the replicates due to the heterogeneity of the soil, all the curves were treated individually, and an attempt was made to fit each model to each of the mineralization curves. It is not possible to compare models of different families with an F test but still the residual sum of squares serves as a measure for the goodness of fit. The degree of correlation between parameters and how realistic the parameters were also taken into account.

When the curve fit came out with the result 'Jacobian singular' (the asymptotic correlation is too high to estimate the parameters) or when parameters were determined with a value of zero or with negative value, the corresponding fits were not included in the tables. When a model did not fit to any sample at all, no table is shown.

Table 7
Parameters estimated ± S.D. according to Eq. (11) for mecoprop, bentazone and ETU in ploughed layer soils from Denmark, Italy, Spain and Germany

Site	c_0	k_1	k_0
mcfb 1_I a 15 cm	34.86 ± 0.54	0.02598 ± 0.00084	0.02962 ± 0.00185
mcfb 1_I b 15 cm	28.11 ± 0.45	0.03184 ± 0.00118	0.03436 ± 0.00162
mcfb 1_I c 15 cm	26.36 ± 0.46	0.04639 ± 0.00212	0.03390 ± 0.00176
mcfb 1_I d 15 cm	27.33 ± 0.51	0.04049 ± 0.00189	0.03667 ± 0.00190
mcfb 1_II a 15 cm	44.87 ± 1.75	0.1126 ± 0.0079	0.08237 ± 0.02570
mcfb 1_II b 15 cm	45.32 ± 1.71	0.1131 ± 0.0076	0.08030 ± 0.02510
mcfb 1_II c 15 cm	46.71 ± 2.41	0.08297 ± 0.00657	0.04935 ± 0.03320
mcfb 1_II d 15 cm	41.99 ± 2.33	0.08450 ± 0.00724	0.04272 ± 0.03212
mcfb 3_I b 15 cm	24.01 ± 0.51	0.04376 ± 0.00224	0.03328 ± 0.00209
mcfb 3_I d 15 cm	34.01 ± 0.64	0.03668 ± 0.00156	0.02510 ± 0.00253
mcfb 4_I a 15 cm	28.04 ± 0.69	0.1126 ± 0.0048	0.05480 ± 0.01017
mcfb 4_I b 15 cm	27.16 ± 0.84	0.1217 ± 0.0068	0.1399 ± 0.0125
mcfb 4_I c 15 cm	23.95 ± 1.05	0.1128 ± 0.0087	0.1255 ± 0.0155
mcfb 4_I d 15 cm	32.63 ± 0.41	0.1112 ± 0.0024	0.1112 ± 0.0060
mcit 1+2 a 0 cm	39.63 ± 0.59	0.2673 ± 0.0158	0.05259 ± 0.00316
mcit 1+2 b 0 cm	39.14 ± 0.59	0.2710 ± 0.0162	0.05465 ± 0.00316
mcit 1+2 c 0 cm	40.09 ± 0.62	0.2722 ± 0.0168	0.05188 ± 0.00332
mcit 1+2 d 0 cm	39.83 ± 0.61	0.2747 ± 0.0167	0.05211 ± 0.00324
mcsp 1+2 a 0 cm	39.14 ± 0.49	0.1246 ± 0.0058	0.03708 ± 0.00173
mcsp 1+2 b 0 cm	38.66 ± 0.46	0.1242 ± 0.0055	0.03579 ± 0.00164
mcsp 1+2 c 0 cm	38.99 ± 0.45	0.1189 ± 0.0050	0.03495 ± 0.00157
mcsp 1+2 d 0 cm	38.43 ± 0.46	0.1201 ± 0.0053	0.03566 ± 0.00162
mcty 1+2 a 0 cm	30.97 ± 0.32	0.3562 ± 0.0200	0.03862 ± 0.00121
mcty 1+2 b 0 cm	23.69 ± 0.31	0.4148 ± 0.0321	0.03654 ± 0.00116
mcty 1+2 c 0 cm	24.97 ± 0.26	0.3071 ± 0.0163	0.03597 ± 0.00098
mcty 1+2 d 0 cm	29.28 ± 0.38	0.2966 ± 0.0191	0.03832 ± 0.00143
beit 1+2 a 0 cm	15.69 ± 0.17	0.02331 ± 0.000635	0.02698 ± 0.000511
beit 1+2 b 0 cm	14.45 ± 0.11	0.02379 ± 0.000450	0.03136 ± 0.000323
beit 1+2 c 0 cm	14.11 ± 0.13	0.02362 ± 0.000545	0.03075 ± 0.000386
beit 1+2 d 0 cm	14.58 ± 0.15	0.02329 ± 0.000568	0.03015 ± 0.000426
besp 1+2 a 0 cm	31.53 ± 0.34	0.03709 ± 0.00121	0.03392 ± 0.00106
besp 1+2 b 0 cm	29.09 ± 0.34	0.04291 ± 0.00165	0.03938 ± 0.00109
besp 1+2 c 0 cm	28.03 ± 0.33	0.04617 ± 0.0018	0.04007 ± 0.00106
besp 1+2 d 0 cm	28.98 ± 1.00	0.04541 ± 0.0053	0.05774 ± 0.00323
bety 1+2 a 0 cm	15.81 ± 4.39	0.005286 ± 0.001284	0.01195 ± 0.00716
bety 1+2 b 0 cm	13.38 ± 0.96	0.009221 ± 0.0007788	0.01740 ± 0.00212
bety 1+2 c 0 cm	10.18 ± 0.92	0.009221 ± 0.000999	0.01869 ± 0.00222
etfb 1 a 15 cm	30.89 ± 0.75	0.1903 ± 0.0163	0.07448 ± 0.00447
etfb 1 b 15 cm	27.96 ± 0.71	0.2018 ± 0.0183	0.07461 ± 0.00422
etfb 1 c 15 cm	31.37 ± 0.56	0.1688 ± 0.0103	0.06881 ± 0.00329
etfb 1 d 15 cm	29.54 ± 0.74	0.1396 ± 0.0117	0.06482 ± 0.00433
etfb 3 a 15 cm	17.96 ± 0.42	0.07729 ± 0.00547	0.05562 ± 0.00232
etfb 3 b 15 cm	33.82 ± 0.63	0.1385 ± 0.0086	0.05378 ± 0.00350
etfb 3 c 15 cm	29.19 ± 0.66	0.1685 ± 0.0131	0.06120 ± 0.00388
etfb 3 d 15 cm	32.31 ± 0.57	0.1488 ± 0.0088	0.05986 ± 0.003323

Models expressed by Eqs. (6), (7), (12), (13), (15), (16) and (20)–(22) did not give usable fits in any of the cases; parameter results and residual means from these equations are therefore not presented in any table or figure. Residual means for all fitted models for the ploughed layer soils

are shown in Table 2 and for subsurface soil in Table 3.

4.1. Surface soil (ploughed layer) mineralization

Only in a few cases, Eqs. (10), (14) and (17)–(19), that include growth of microorganisms were useful for modelling the ploughed layer degradation:

The three models that showed to be generally useful in modelling the mineralization of mecoprop, bentazon and ETU in the ploughed layer were Eqs. (8), (9) and (11) where growth of microorganisms is not taken into account:

Parameter estimates ± S.D. for mecoprop, bentazon and ETU in ploughed layer soil samples modelled with Eqs. (8)–(11), (14) and (17)–(19) are presented in Tables 4–11.

Mcfb 1_II, mcfb 3_II and mcfb 4_I (mecoprop in Danish soil) were only incubated for 100 days. All other samples were incubated during 3–500 days and there is no doubt that after such a long incubation time, more than one important process must have taken place in the evolution of $^{14}CO_2$. This can clearly be seen from the curves of $^{14}CO_2$-development (Fig. 1a, c, f, g, h; Fig. 2a, b, c; Fig. 3 a, b), where the ploughed layer curves are rising quite steeply at the beginning then they curve and in the last part they flatten out.

Even if mcfb 1_II, mcfb 3_II and mcfb 4_I were only incubated for 100 days, a curvature is clearly seen in the ploughed layer curves (Fig. 1b, d, e) that would be followed by a flat part, if the incubation were continued. More than one process most then be taken into account when modelling these curves, too.

In Eq. (9) the mineralization of the total amount of pesticide ($c_0 = 100$) is modelled as being two first order processes, in Eq. (11) as one first order and one zero order process and in Eq. (10) as one first order and one zero order process, where a term describing linear growth of microorganisms is included. Brunner and Focht (1984) (Eqs. (10) and (11)) assumed that the zero order process represents the conversion of organic material, where ^{14}C had been built in, to $^{14}CO_2$ (i.e. the 'flat' part of the curve). It is worth considering, if the zero order process could be the process that dominates in the beginning of the degradation. Even if the K_d values for all the pesticides are low, there will be a certain amount adsorbed onto the soil particles because of the relative low amount of soil water and if the desorption of the adsorbed pesticide is rapid, there will be a constant concentration of pesticide in the soil water at the beginning of the degradation which could make the first part of the process a zero order process. In such a case it could be relevant to treat the curve part by part.

Surprisingly, all the k_1 values from Eq. (9) (Table 5) were equal to all the k_1 values in Eq. (11) (Table 7) and so were the amounts degraded in one of the first order processes in Eq. (9) ($a \cdot 100$) (Table 5) and the amounts degraded in the first order process (c_0) in Eq. (11)) (Table 7). The most important processes involved in the mineralization of mecoprop, bentazon and ETU in ploughed layer soil can then be considered two first order processes as well as one first order + one zero order process. If zero order ($-dC/dt = k_1$) and first order kinetics ($-dC/dt = k_1 \cdot C$) are considered as extremes based on Monod kinetics ($-dC/dt = k_1 \cdot C/(k_s + C)$), the interchangeability between zero order ($C_0 \gg k_s$) and first order ($C_0 \ll k_s$) has no meaning. This could be due to the fact, that half-saturation constants as they appear in Monod equations are not important in such a complex system as is the soil environment, where adsorption–desorption processes may have more importance. The interchangeability between zero order and first order kinetics is better understood from a power–rate point of view, where first order changes to zero order when the amount of substrate (C) is constant. The amount of organic material with ^{14}C built into it, probably changes so slowly, that it can be considered a constant value. Thus the most rapid first order process in Eq. (9) and the first order process in Eq. (11) probably had to do with transformation of the parent compound, and the other slower first order process in Eq. (9) and the zero order process in Eq. (11) expresses the transformation of organic material where ^{14}C was built-in.

The model according to Eq. (8) consists of two first order processes, too, but estimates the amount of pesticide mineralised according to each

Table 8
Parameters estimated ± S.D. according to Eq. (14) for mecoprop, bentazone and ETU in ploughed layer soils from Denmark, Italy, Spain and Germany

Site	c_0	x_0	k_1
mcfb 1_II c 15 cm	49.63 ± 0.62	105.1 ± 76.5	0.0006446 ± 0.0004175
mcfb 1_II d 15 cm	44.34 ± 0.57	65.32 ± 39.82	0.001016 ± 0.000527
mcfb 3_II a 15 cm	42.62 ± 0.41	42.93 ± 12.53	0.0009609 ± 0.0002307
mcfb 3_II b 15 cm	38.39 ± 0.35	65.15 ± 23.20	0.0007032 ± 0.0002210
mcfb 3_II c 15 cm	40.49 ± 0.39	129.9 ± 59.5	0.0003134 ± 0.0001350
mcfb 3_II d 15 cm	37.97 ± 0.347	0.23 ± 21.87	0.0005524 ± 0.0001547

Table 9
Parameters estimated ± S.D. according to Eq. (17) for mecoprop, bentazone and ETU in ploughed layer soils from Denmark, Italy, Spain and Germany

Site	c_0	k	r
mcfb 3_II a 15 cm	42.66 ± 0.51	0.04642 ± 0.00234	00.01519 ± 0.00549
mcfb 3_II b 15 cm	38.48 ± 0.44	0.04979 ± 0.00196	0.008636 ± 0.00458
mcfb 3_II c 15 cm	40.57 ± 0.54	0.04256 ± 0.00115	0.004246 ± 0.00322
mcfb 3_II d 15 cm	37.91 ± 0.43	0.04145 ± 0.00132	0.008462 ± 0.00336

Table 10
Parameters estimated ± S.D. according to Eq. (18) for mecoprop, bentazone and ETU in ploughed layer soils from Denmark, Italy, Spain and Germany

Site	c_0	k_1	k_2
mcfb 3_II a 15 cm	42.50 ± 0.45	0.04386 ± 0.00272	0.001184 ± 0.000366
mcfb 3_II c 15 cm	40.51 ± 0.49	0.04231 ± 0.00123	0.0002251 ± 0.0001506
mcfb 3_II d 15 cm	37.85 ± 0.39	0.04073 ± 0.00146	0.0004706 ± 0.000171

Table 11
Parameters estimated ± S.D. according to Eq. (19) for mecoprop, bentazone and ETU in ploughed layer soils from Denmark, Italy, Spain and Germany

Site	c_0	k_1	k_2
mcfb 1_II c 15 cm	49.63 ± 0.62	0.09978 ± 0.01545	−0.0006446 ± 0.0004174
mcfb 1_II d 15 cm	44.34 ± 0.57	0.1114 ± 0.0175	−0.001016 ± 0.000527
mcfb 3_II a 15 cm	42.62 ± 0.41	0.08220 ± 0.00764	−0.0009608 ± 0.0002307
mcfb 3_II b 15 cm	38.39 ± 0.35	0.07281 ± 0.00657	−0.007032 ± 0.000221
mcfb 3_II c 15 cm	40.49 ± 0.39	0.05342 ± 0.00437	−0.0003134 ± 0.0001350
mcfb 3_II d 15 cm	37.97 ± 0.34	0.05977 ± 0.004637	−0.0005524 ± 0.0001547

process. In some cases where the fit was good (mcit, mcsp, mcty, besp), there seemed to be two underlying first order processes long before reaching the mineralization of the total amount of added ^{14}C ($c_1 + c_2 < 100$). This could be due to the fact that two first order processes were involved only in the mineralization of mecoprop. Such processes could be (a) mineralization of mecoprop by different strains at different rates, (b) mineralization of available mecoprop + slower

Table 12
Parameters ± S.D. estimated according to Eq. (8) for mecoprop, bentazone and ETU in subsurface soils from Denmark, Italy and Spain

Site	c_1	k_1	c_2	k_2
mcfb 4_I a 45 cm	4.114 ± 1.649	0.1482 ± 0.0529	7.906 ± 1.376	0.03132 ± 0.00775
mcfb 4_I c 75 cm	1.936 ± 0.256	0.1789 ± 0.0357	8.075 ± 0.514	0.01222 ± 0.00222
mcsp 1 a 45 cm	5.737 ± 1.733	0.01897 ± 0.00464	36.42 ± 1.76	0.002371 ± 0.000475
mcsp 1 b 45 cm	5.989 ± 0.843	0.02884 ± 0.00459	37.83 ± 0.88	0.002835 ± 0.000266
mcsp 1 c 45 cm	2.715 ± 0.569	0.02652 ± 0.00584	35.32 ± 0.56	0.002784 ± 0.000185
mcsp 1 d 45 cm	2.358 ± 0.455	0.02904 ± 0.00688	41.38 ± 1.59	0.001981 ± 0.000170
mcsp 2 b 45 cm	45.06 ± 4.37	0.001653 ± 0.000296	0.9940 ± 0.7207	0.03088 ± 0.02985
etfb 1 c 45 cm	18.99 ± 10.52	0.03174 ± 0.01239	22.74 ± 6.95	0.0006869 ± 0.0004995

mineralization of desorbing mecoprop, and (c) mineralization of mecoprop + mineralization of an intermediately formed metabolite. If it had been possible to estimate the parameters of three first order processes at a time, maybe two first order processes for the mineralization of mecoprop and a third first order process for the mineralization of organic matter would have shown up. Gustafson and Holden (1990) estimated the number of first order processes occurring—but to do that, they assumed that they all had the same rate constant. I assumed that the rate constants were different and estimated the value of each.

The samples where the curve rises very steeply (mcfb 1_II and mcfb 3_II, Fig. 1b, d) were the only ploughed layer samples where Eq. (10) which includes the linear growth term, gave useful fits, and without doubt they gave the best fit (low residual means, Table 2). Eq. (18), which includes the linear growth term, too, but not the zero order term, was useful for three of the mcfb 3_II samples, but Eq. (10) gave better fits. The determined amount of c_0 fits close to the point where the curve bends. This indicates that the first order process dominated in the beginning and the zero order must be the conversion of organic material to $^{14}CO_2$ as Brunner and Focht (1984) assumed. A

Table 13
Parameters ± S.D. estimated according to Eq. (10) for mecoprop, bentazone and ETU in subsurface soils from Denmark, Italy and Spain

Site	c_0	k_1	k_2	k_0
mcfb 1_I b 45 cm	15.99 ± 0.08	0.009572 ± 0.000716	0.0006319 ± 0.0000374	0.01235 ± 0.0003124
mcfb 3_I b 45 cm	13.67 ± 0.19	0.008224 ± 0.001508	0.0006343 ± 0.0000796	0.02389 ± 0.0007434
mcfb 3_I c 45 cm	18.19 ± 0.29	0.009217 ± 0.001213	0.0003968 ± 0.0000559	0.03518 ± 0.00112
mcfb 4_I b 45 cm	22.91 ± 0.45	0.07326 ± 0.00827	0.005648 ± 0.001754	0.02932 ± 0.00665
mcfb 4_I c 45 cm	28.59 ± 1.20	0.04553 ± 0.00162	0.0007668 ± 0.0002932	0.07524 ± 0.01425
mcfb 4_I d 45 cm	18.18 ± 0.92	0.06765 ± 0.00628	0.001406 ± 0.001170	0.04023 ± 0.01281
mcit 1 a 50 cm	32.46 ± 1.52	0.0008961 ± 0.0005129	0.0001572 ± 0.0000016	0.01834 ± 0.00520
mcit 1 b 50 cm	36.40 ± 1.01	0.0002911 ± 0.0002408	0.0001370 ± 0.0000007	0.02281 ± 0.00337
mcit 1 c 50 cm	20.46 ± 1.35	0.001112 ± 0.001256	0.0002343 ± 0.0000043	0.06652 ± 0.00491
mcit 2 a 50 cm	33.23 ± 0.68	0.004374 ± 0.000458	0.0002274 ± 0.0000018	0.03996 ± 0.00251
mcit 2 b 50 cm	25.46 ± 1.12	0.002324 ± 0.000709	0.0001948 ± 0.0000244	0.05326 ± 0.00398
mcit 2 d 50 cm	31.21 ± 3.01	0.002638 ± 0.000529	0.000009711 ± 0.000001801	0.01108 ± 0.00955
mcsp 2 d 45 cm	17.43 ± 3.88	0.004910 ± 0.0005698	0.0000115 ± 0.0000076	0.02649 ± 0.00715
etfb 1 a 45 cm	26.84 ± 0.64	0.002252 ± 0.001219	0.0006226 ± 0.0000641	0.04459 ± 0.00292
etfb 1 d 45 cm	21.78 ± 0.41	0.003591 ± 0.001616	0.0009116 ± 0.0000993	0.04132 ± 0.00199
etfb 1 a 75 cm	22.96 ± 0.58	0.003391 ± 0.002097	0.0008911 ± 0.0001277	0.03900 ± 0.00280

Table 14
Parameters ± S.D. estimated according to Eq. (11) for mecoprop, bentazone and ETU in subsurface soils from Denmark, Italy and Spain

Site	c_0	k_1	k_0
mcfb 1_I b 45 cm	17.24 ± 0.46	0.02227 ± 0.00132	0.008839 ± 0.001495
mcfb 3_I a 45 cm	18.49 ± 3.22	0.009896 ± 0.001911	0.04507 ± 0.00818
mcfb 3_I b 45 cm	15.41 ± 0.65	0.01988 ± 0.00156	0.01859 ± 0.00219
mcfb 3_I c 45 cm	22.09 ± 0.98	0.01547 ± 0.00105	0.02372 ± 0.00302
mcfb 3_I d 45 cm	15.94 ± 2.26	0.01311 ± 0.00251	0.05652 ± 0.00652
mcfb 4_I a 45 cm	8.997 ± 0.382	0.07818 ± 0.00624	0.02955 ± 0.00510
mcfb 4_I d 45 cm	19.66 ± 0.97	0.07027 ± 0.00575	0.02234 ± 0.01311
mcfb 3_I a 75 cm	1.657 ± 0.412	0.06285 ± 0.06621	0.07992 ± 0.00178
mcfb 3_I b 75 cm	2.353 ± 0.533	0.03983 ± 0.02817	0.07805 ± 0.00216
mcfb 3_I d 75 cm	2.542 ± 0.613	0.02956 ± 0.01799	0.07278 ± 0.00233
mcfb 4_I c 75 cm	3.326 ± 0.183	0.09463 ± 0.01137	0.04534 ± 0.00256
mcsp 1 a 45 cm	14.74 ± 0.72	0.009916 ± 0.000585	0.03381 ± 0.00169
mcsp 1 b 45 cm	15.98 ± 0.69	0.01236 ± 0.00074	0.03925 ± 0.00177
mcsp 1 c 45 cm	14.86 ± 0.84	0.007994 ± 0.000471	0.03031 ± 0.00177
mcsp 1 d 45 cm	10.23 ± 0.70	0.008887 ± 0.001558	0.03752 ± 0.00156
mcsp 2 a 45 cm	22.77 ± 5.34	0.003833 ± 0.000661	0.01851 ± 0.00723
mcsp 2 b 45 cm	11.33 ± 3.30	0.005244 ± 0.001279	0.03201 ± 0.00551
mcsp 2 c 45 cm	1.175 ± 0.143	0.04748 ± 0.02043	0.03637 ± 0.00049
etfb 1 c 45 cm	29.29 ± 1.61	0.02318 ± 0.00208	0.03319 ± 0.00672

specific point on the curve where the process shifted from first order to zero order cannot be indicated. It is most likely that both processes occurred at the same time because as soon as a small amount of ^{14}C is built into organic matter the slow turnover can take place. For that reason, dividing the curve into pieces and modelling one piece at a time is not recommendable.

Mecoprop and bentazon in Italian, Spanish and German soils (mcit 1 + 2, mcsp 1 + 2, mcty 1 + 2) from the ploughed layer were incubated as four replicates taken from one homogenised sample from each place. As expected, the variation between the resulting curves was small. The high variations between replicates of other samples not homogenised is thus shown to be due to the heterogeneity of the soil. Only one replicate of bentazon degradation in Spanish soil (besp 1 + 2 d) showed a strange variation from the rest, probably due to problems with incubation.

The few cases in all the ploughed layer incubations, where the model including the linear growth term fits (mcfb 1_II and mcfb 3_II) are the same where other models (Eqs. (14), (17) and (23)) which take growth of microorganisms into account, can be used. In no other ploughed layer samples, either for mecoprop, bentazon or ETU in varying types of soil, can models taking growth into account be used. The ploughed layer degradation is then shown to be highly dominated by cometabolic degradation processes, even if mecoprop is a compound where metabolic degradation is well-known. The reason for the cometabolic degradation dominating in the ploughed layer could be due to the presence of a high number of varying microbial species and the presence of organic material, which serves as nutrients for the cometabolic degrading bacteria, which then makes the cometabolic degradation of pesticides the most dominating process.

To assure that the good fits of the growth models to the ploughed layer samples with the steepest rising curve does not relate to the same samples being only incubated for 90 days all the long-time incubated samples were modelled after 90 days too. None of them fitted to the growth models.

Table 15
Parameters ± S.D. estimated according to Eq. (14) for mecoprop, bentazone and ETU in subsurface soils from Denmark, Italy and Spain

Site	c_0	x_0	k_1
mcfb 1_I c 45 cm	31.39 ± 0.58	23.24 ± 7.92	0.0002225 ± 0.0000596
mcfb 1_I d 45 cm	33.08 ± 0.50	12.89 ± 3.17	0.0003148 ± 0.0000522
mcfb 1_II a 45 cm	27.92 ± 4.16	4.772 ± 1.652	0.0009002 ± 0.0003401
mcfb 1_II b 45 cm	33.32 ± 15.75	8.822 ± 5.151	0.0004097 ± 0.0003928
mcfb 1_II c 45 cm	31.78 ± 6.74	7.757 ± 3.265	0.0006010 ± 0.0003170
mcfb 3_II a 45 cm	24.92 ± 2.22	7.867 ± 2.364	0.0008490 ± 0.0002621
mcfb 3_II b 45 cm	22.84 ± 1.13	2.399 ± 0.724	0.001920 ± 0.000368
mcfb 3_II c 45 cm	25.48 ± 1.32	4.047 ± 0.982	0.001404 ± 0.000265
mcfb 3_II d 45 cm	27.97 ± 0.59	6.561 ± 1.276	0.001395 ± 0.000185
mcfb 1_I a 75 cm	31.22 ± 1.18	19.27 ± 7.22	0.000171 ± 0.000054
mcfb 1_I c 75 cm	37.17 ± 3.14	25.31 ± 9.99	0.0000845 ± 0.0000351
mcfb 1_II b 75 cm	17.82 ± 2.92	5.833 ± 2.336	0.0009803 ± 0.0004660
mcfb 1_II c 75 cm	22.51 ± 1.25	2.369 ± 0.458	0.001596 ± 0.000252
mcfb 3_II b 75 cm	7.219 ± 1.580	1.678 ± 1.771	0.004099 ± 0.003633
mcfb 3_II c 75 cm	24.99 ± 10.85	3.755 ± 2.357	0.0008684 ± 0.0007598
mcfb 3_II d 75 cm	24.41 ± 1.19	2.612 ± 0.850	0.001861 ± 0.000376
mcfb 4_I b 75 cm	25.71 ± 0.54	5.356 ± 0.434	0.001120 ± 0.000081
mcfb 4_I d 75 cm	28.52 ± 0.23	15.01 ± 1.02	0.0008464 ± 0.0000491
mcit 1 a 50 cm	38.08 ± 0.29	5.222 ± 0.697	0.0005452 ± 0.0000365
mcit 1 b 50 cm	43.61 ± 0.24	5.256 ± 0.447	0.0004541 ± 0.0000193
mcit 1 c 50 cm	43.79 ± 1.14	27.68 ± 8.57	0.0001649 ± 0.0000423
mcit 1 d 50 cm	43.49 ± 0.63	14.21 ± 3.13	0.0002987 ± 0.0000437
mcit 2 a 50 cm	45.51 ± 0.41	24.29 ± 4.66	0.0002628 ± 0.0000360
mcit 2 b 50 cm	43.29 ± 0.66	21.90 ± 4.77	0.0002187 ± 0.0000361
mcit 2 c 50 cm	41.83 ± 0.50	9.022 ± 2.079	0.0004557 ± 0.0000586
mcit 2 d 50 cm	35.13 ± 0.39	8.038 ± 1.289	0.0004268 ± 0.0000417
mcty 1 a 75 cm	28.56 ± 0.30	1.793 ± 0.316	0.0006113 ± 0.0000443
mcty 1 b 75 cm	32.10 ± 0.73	2.913 ± 0.478	0.0003090 ± 0.0000292
mcty 1 c 75 cm	25.94 ± 0.33	3.703 ± 0.702	0.0005202 ± 0.0000523
mcty 1 d 75 cm	41.59 ± 0.38	0.6565 ± 0.1435	0.0006134 ± 0.0000365
mcty 2 a 75 cm	21.63 ± 0.32	1.105 ± 0.144	0.0005546 ± 0.0000341
mcty 2 b 75 cm	29.14 ± 0.25	3.187 ± 0.500	0.0005886 ± 0.0000441
mcty 2 c 75 cm	12.73 ± 0.34	0.4679 ± 0.0674	0.0008231 ± 0.0000638
mcty 2 d 75 cm	31.31 ± 0.26	1.398 ± 0.164	0.0005095 ± 0.0000235
beit 1 a 50 cm	16.61 ± 0.47	0.8153 ± 0.0497	0.0004441 ± 0.0000249
beit 1 b 50 cm	20.02 ± 0.81	1.963 ± 0.252	0.0003440 ± 0.0000373
beit 1 c 50 cm	10.20 ± 0.65	0.6209 ± 0.0911	0.0006937 ± 0.0000943
beit 1 d 50 cm	12.55 ± 0.42	0.4424 ± 0.0354	0.0006557 ± 0.0000435
beit 2 a 50 cm	25.90 ± 0.78	1.863 ± 0.144	0.0002708 ± 0.0000186
beit 2 c 50 cm	25.89 ± 0.56	1.897 ± 0.0986	0.0002635 ± 0.0000127
beit 2 d 50 cm	19.53 ± 0.53	0.7236 ± 0.0979	0.0005206 ± 0.0000395
besp 1 a 45 cm	23.86 ± 0.46	2.695 ± 0.278	0.0003667 ± 0.0000257
besp 1 b 45 cm	25.38 ± 0.47	6.044 ± 0.580	0.0002557 ± 0.0000201
besp 1 c 45 cm	24.18 ± 0.56	4.753 ± 0.456	0.0002644 ± 0.0000217
besp 1 d 45 cm	25.65 ± 0.46	4.022 ± 0.445	0.0003250 ± 0.0000251
besp 2 a 45 cm	11.65 ± 0.26	1.109 ± 0.0898	0.0006815 ± 0.0000429
besp 2 b 45 cm	16.94 ± 0.41	1.385 ± 0.142	0.0005156 ± 0.0000372
besp 2 c 45 cm	17.75 ± 0.42	1.847 ± 0.209	0.0004834 ± 0.0000383
besp 2 d 45 cm	11.98 ± 1.55	3.582 ± 1.181	0.0003364 ± 0.0001271
bety 1 a 75 cm	5.642 ± 0.156	0.3165 ± 0.0460	0.001705 ± 0.000146
bety 1 b 75 cm	6.930 ± 0.162	0.3406 ± 0.0404	0.001406 ± 0.000098
bety 1 c 75 cm	4.769 ± 0.156	0.3135 ± 0.0468	0.001854 ± 0.000180
bety 1 d 75 cm	5.381 ± 0.160	0.2667 ± 0.0206	0.001452 ± 0.000093

Table 15 (continued)

Site	c_0	x_0	k_1
bety 2 a 75 cm	11.39 ± 0.27	0.6950 ± 0.0821	0.0008171 ± 0.0000592
bety 2 b 75 cm	10.93 ± 0.21	0.2480 ± 0.0315	0.001094 ± 0.000063
bety 2 c 75 cm	13.15 ± 0.20	0.7057 ± 0.0078	0.0008307 ± 0.0000469
bety 2 d 75 cm	18.97 ± 0.39	0.6729 ± 0.1326	0.0006894 ± 0.0000572
etfb 1 a 45 cm	37.93 ± 0.55	22.23 ± 7.74	0.0004003 ± 0.0001003
etfb 1 b 45 cm	41.19 ± 0.69	58.38 ± 43.20	0.0002628 ± 0.0001609
etfb 3 a 45 cm	19.09 ± 0.69	16.77 ± 8.80	0.0003820 ± 0.0001724
etfb 3 b 45 cm	17.96 ± 0.22	5.128 ± 0.430	0.0005879 ± 0.0000390
etfb 3 c 45 cm	22.57 ± 0.25	3.544 ± 0.565	0.0009279 ± 0.0000816
etfb 3 d 45 cm	24.14 ± 0.45	6.156 ± 2.275	0.0009107 ± 0.0002006
etfb 1 a 75 cm	32.79 ± 0.64	53.24 ± 40.96	0.0002432 ± 0.0001603
etfb 1 b 75 cm	34.73 ± 0.54	24.48 ± 11.21	0.0004579 ± 0.0001538
etfb 1 c 75 cm	31.51 ± 0.69	10.64 ± 4.19	0.0005457 ± 0.0001405
etfb 1 d 75 cm	27.48 ± 0.44	3.701 ± 1.231	0.001138 ± 0.000181
etfb 3 a 75 cm	16.69 ± 0.10	0.6873 ± 0.0368	0.001244 ± 0.000030
etfb 3 b 75 cm	24.62 ± 0.51	1.485 ± 0.679	0.001610 ± 0.000279
etfb 3 c 75 cm	16.01 ± 0.33	2.180 ± 0.289	0.0008785 ± 0.0000781
etfb 3 d 75 cm	10.68 ± 0.16	1.896 ± 0.358	0.001734 ± 0.000192

4.2. Subsoil mineralization

The only models shown to be relevant in modelling the mineralization kinetics of the pesticides in subsoil were Eqs. (8), (10), (11), (14), (17)–(19) and (23)).

Parameter estimates ± S.D. according to models Eqs. (8), (10), (11), (14), (17)–(19) and (23) are shown in Tables 12–19.

Eqs. (8), (11) and (23) are models that do not involve microbial growth. Eqs. (10) and (18) treats the growth as linear growth, where Eq. (10) includes a zero order term, too, to model the turnover of ^{14}C built into humus. The zero order term is excluded in Eq. (18) and the choice between those two model is clear. Eq. (10) is best, where the $^{14}CO_2$ evolution curve has flattened (Table 3, mcit). Eq. (10)/Eq. (18) will therefore be considered the same.

Simkins and Alexander (1984) showed that Eq. (14) was useful to model low concentrations of benzoate mineralization in sewage and Albrechtsen and Winding (1992) used the same model for modelling ^{14}C-uptake in microorganisms from ^{14}-acetate and ^{14}-phenol. The model is useful and gives low residuals for many of the subsoil samples. Liu and Zhang (1986) and Liu et al. (1988) (Eq. (19)) developed their model taking into account an increment in the number of microorganisms but considering the amount of substrate as the limiting factor. They concluded that the model could always be used for describing degradation of pesticides in soil, whether the curve has an inflection point or not. In the present study the model could not be used for most of the ploughed layer samples where no inflection is seen, but it was useful for many of the subsoil samples. According to Liu and Zhang (1986) the model will always give negative k_2 values when an inflection point is present and should give k_2 values = 0, where no inflection point is present. In the cases, where Eq. (19) was considered not useful, negative k_1 values or positive k_2 values were seen.

Comparing Eqs. (14) and (19) they give equal results. They give the same residual residual means (Table 3), the value for c_0 is the same according to both models and the numeric value for k_2 in Eq. (19)) and k_1 in Eq. (14) are exactly the same (Tables 15 and 18). Eq. (14)/Eq. (19) are therefore considered together as models taking logistic growth of microorganisms into account where the pesticide is the growth limiting factor (metabolic degradation).

Table 16
Parameters ± S.D. estimated according to Eq. (17) for mecoprop, bentazone and ETU in subsurface soils from Denmark, Italy and Spain

Site	c_0	k	r
mcfb 1_I c 45 cm	31.72 ± 0.81	0.006122 ± 0.000353	0.002413 ± 0.001022
mcfb 1_I d 45 cm	33.24 ± 0.61	0.005253 ± 0.000351	0.004177 ± 0.0009371
mcfb 1_II a 45 cm	23.95 ± 3.55	0.005405 ± 0.000474	0.02117 ± 0.00458
mcfb 1_II b 45 cm	27.40 ± 14.43	0.004509 ± 0.002035	0.01282 ± 0.00633
mcfb 1_II c 45 cm	26.80 ± 5.66	0.005787 ± 0.000828	0.01691 ± 0.00496
mcfb 3_II a 45 cm	21.57 ± 1.57	0.008083 ± 0.000367	0.01833 ± 0.00324
mcfb 3_II b 45 cm	21.19 ± 0.98	0.006200 ± 0.000735	0.03090 ± 0.00471
mcfb 3_II c 45 cm	23.37 ± 1.22	0.007237 ± 0.000512	0.02610 ± 0.003756
mcfb 3_II d 45 cm	26.73 ± 0.60	0.01151 ± 0.00077	0.02475 ± 0.00326
mcfb 1_I a 75 cm	30.55 ± 1.30	0.003759 ± 0.000206	0.003048 ± 0.000948
mcfb 1_I c 75 cm	33.15 ± 2.36	0.002452 ± 0.000113	0.002825 ± 0.000715
mcfb 1_II a 75 cm	17.53 ± 3.17	0.006618 ± 0.000851	0.01424 ± 0.00414
mcfb 1_II b 75 cm	15.46 ± 2.65	0.006904 ± 0.000794	0.01507 ± 0.00449
mcfb 1_II c 75 cm	19.63 ± 1.00	0.004933 ± 0.000282	0.02896 ± 0.00295
mcfb 3_II b 75 cm	7.309 ± 2.800	0.008298 ± 0.001903	0.01695 ± 0.01571
mcfb 3_II c 75 cm	20.33 ± 8.70	0.004203 ± 0.001273	0.01963 ± 0.00801
mcfb 3_II d 75 cm	22.89 ± 1.03	0.006610 ± 0.000803	0.03101 ± 0.00499
mcfb 4_I b 75 cm	22.35 ± 0.31	0.007374 ± 0.000111	0.02422 ± 0.00090
mcfb 4_I d 75 cm	26.77 ± 0.23	0.01451 ± 0.00020	0.01753 ± 0.00092
mcit 1 a 50 cm	37.42 ± 0.34	0.003814 ± 0.000263	0.01263 ± 0.00104
mcit 1 b 50 cm	42.83 ± 0.37	0.003330 ± 0.000194	0.01172 ± 0.00081
mcit 1 c 50 cm	42.81 ± 1.21	0.005222 ± 0.000255	0.004061 ± 0.001014
mcit 1 d 50 cm	43.14 ± 0.72	0.005429 ± 0.000356	0.006230 ± 0.001096
mcit 2 a 50 cm	45.27 ± 0.51	0.007770 ± 0.000399	0.005285 ± 0.000999
mcit 2 b 50 cm	42.63 ± 0.72	0.005677 ± 0.000271	0.004913 ± 0.000877
mcit 2 c 50 cm	41.53 ± 0.60	0.005655 ± 0.000534	0.009403 ± 0.001608
mcty 1 a 75 cm	28.09 ± 0.36	0.001648 ± 0.000182	0.01082 ± 0.00097
mcty 1 b 75 cm	30.69 ± 0.70	0.001254 ± 0.000090	0.006491 ± 0.000528
mcty 1 c 75 cm	25.62 ± 0.36	0.002664 ± 0.000233	0.007434 ± 0.000869
mcty 1 d 75 cm	40.91 ± 0.49	0.0006781 ± 0.0001208	0.01815 ± 0.00143
mcty 2 a 75 cm	20.57 ± 0.32	0.0009062 ± 0.0000713	0.008218 ± 0.000491
mcty 2 b 75 cm	28.72 ± 0.31	0.002553 ± 0.000234	0.01053 ± 0.00104
mcty 2 c 75 cm	11.43 ± 0.25	0.0005573 ± 0.0000393	0.008137 ± 0.000413
mcty 2 d 75 cm	30.59 ± 0.33	0.001099 ± 0.000094	0.01035 ± 0.00061
beit 1 a 50 cm	13.18 ± 0.28	0.0004944 ± 0.0000098	0.006641 ± 0.000167
beit 1 b 50 cm	17.52 ± 0.65	0.0008766 ± 0.0000342	0.005555 ± 0.000365
beit 1 c 50 cm	8.188 ± 0.366	0.0005804 ± 0.0000243	0.006321 ± 0.000363
beit 1 d 50 cm	9.889 ± 0.203	0.0004014 ± 0.0000102	0.007447 ± 0.000186
beit 2 a 50 cm	21.03 ± 0.38	0.0006665 ± 0.0000137	0.006260 ± 0.000174
beit 2 c 50 cm	21.15 ± 0.39	0.0006640 ± 0.0000130	0.006012 ± 0.000153
beit 2 d 50 cm	17.48 ± 0.40	0.0005388 ± 0.0000361	0.007949 ± 0.000399
besp 1 1 45 cm	21.98 ± 0.42	0.001290 ± 0.000054	0.006364 ± 0.000366
besp 1 2 45 cm	23.39 ± 0.43	0.001877 ± 0.000050	0.004766 ± 0.000305
besp 1 3 45 cm	21.67 ± 0.45	0.001544 ± 0.000039	0.005028 ± 0.000283
besp 1 4 45 cm	24.02 ± 0.44	0.001663 ± 0.000069	0.005801 ± 0.000388
besp 2 a 45 cm	10.23 ± 0.21	0.0009839 ± 0.0000295	0.006363 ± 0.000274
besp 2 b 45 cm	15.09 ± 0.33	0.0009462 ± 0.0000381	0.006829 ± 0.000334
besp 2 c 45 cm	16.19 ± 0.36	0.001169 ± 0.000051	0.006402 ± 0.000379
besp 2 d 45 cm	10.93 ± 1.70	0.001413 ± 0.000155	0.003191 ± 0.000835
bety 1 b 75 cm	6.257 ± 0.130	0.0006704 ± 0.0000385	0.007480 ± 0.000365
bety 1 d 75 cm	18.14 ± 0.36	0.0007327 ± 0.0000828	0.008930 ± 0.000659
bety 2 a 75 cm	10.22 ± 0.19	0.0007665 ± 0.0000399	0.007254 ± 0.000340

Table 16 (continued)

Site	c_0	k	r
bety 2 b 75 cm	9.975 ± 0.190	0.0004277 ± 0.0000336	0.009073 ± 0.000416
bety 2 c 75 cm	12.22 ± 0.17	0.0008237 ± 0.0000504	0.008001 ± 0.000376
bety 2 d 75 cm	18.14 ± 0.36	0.0007327 ± 0.0000828	0.008930 ± 0.000659
etfb 1 a 45 cm	38.21 ± 0.70	0.01119 ± 0.00008	0.004771 ± 0.002011
etfb 3 a 45 cm	19.03 ± 0.92	0.007175 ± 0.000459	0.003362 ± 0.001885
etfb 3 b 45 cm	16.75 ± 0.16	0.003562 ± 0.000073	0.007696 ± 0.000351
etfb 3 c 45 cm	22.20 ± 0.29	0.004396 ± 0.000328	0.01211 ± 0.00127
etfb 3 d 45 cm	24.33 ± 0.55	0.007955 ± 0.000904	0.008188 ± 0.002520
etfb 1 b 75 cm	35.19 ± 0.73	0.01423 ± 0.00125	0.003427 ± 0.002660
etfb 1 c 75 cm	31.61 ± 0.79	0.007625 ± 0.000804	0.007012 ± 0.002273
etfb 1 d 75 cm	27.38 ± 0.55	0.006321 ± 0.000966	0.01566 ± 0.00334
etfb 3 a 75 cm	15.75 ± 0.12	0.001239 ± 0.000054	0.01501 ± 0.00044
etfb 3 b 75 cm	24.35 ± 0.58	0.003691 ± 0.000957	0.02458 ± 0.00514
etfb 3 c 75 cm	14.77 ± 0.15	0.002346 ± 0.000079	0.01072 ± 0.00044
etfb 3 d 75 cm	10.52 ± 0.17	0.004393 ± 0.000334	0.01023 ± 0.00126

Eq. (10)/Eq. (18) include linear growth and Eq. (17) include exponential growth of microorganisms. In both cases the microorganisms are deriving energy for growth from another substrate rather than the pesticide. Eq. (14)/Eq. (19) include logistic growth of microorganisms, deriving energy from the pesticide. In many of the cases in the present studies of subsoil, where Eq. (14)/Eq. (19) can be used, Eq. (17) or Eq. (10)/Eq. (18) can also be used (Table 3), so it seems that it is not easy to distinguish between the types of growth going on. In the present study no other substrate was added, so microorganisms growing must derive energy from the pesticide. The fact that the samples were taken out of their natural environment (even if they were kept undisturbed) and a flow of atmospheric air passed through maybe could have caused the use of the small amounts of other organic compounds present by the specialised subsoil bacteria and for that reason, Eq. (10)/Eq. (18) and Eq. (17) gave usable fits. However, such an effect was not seen in the ploughed layer samples even if much more organic material was present than in subsoil samples.

For few samples, it was not possible to find any model able to fit (mcfb 1_II 45 cm d, mcfb 1_I 75 cm d, mcfb 1_II 75 cm d, mcfb 3_II 75 cm a, mcsp 2 d, and beit 2 b).

Where none of the models including growth of microorganisms fitted, Eq. (11) (first order + zero order) was the model to use. The heterogeneity of the soil is very clear where some replicates from the same site mineralise pesticide with a process including growth of microorganisms, while in other replicates from the same site, the cometabolic processes dominate.

It is interesting that Eq. (23) (two sequential first order processes) does fit in some of the same subsoil cases where the growth models fit, too, and in the ploughed layer where growth models did not fit, Eq. (23) did not fit either. The reason must be that a sequential mineralization is easier seen in the subsoil where the amount of substrate is very small. It is worth considering, if the general picture of the subsoil curves (sigmoidal curves) could be due to other factors than growth of microorganisms, and the fit of the growth models is a causality, if for example the conditions in the subsoil promote a slow mineralization of the parent pesticide and then a faster mineralization of a metabolite. However, if that was the case, the sequential model (Eq. (23)) should fit for all the subsoil samples which it does not.

Kinetic processes with the growth of microorganisms were dominating for bentazon degradation in Italian, Spanish and German subsoil. In Spanish subsoil, where the amount of organic carbon was high, this apparently changes mecoprop mineralization to be mainly cometabolic. ETU degradation in Danish subsoil was domi-

Table 17
Parameters ± S.D. estimated according to Eq. (18) for mecoprop, bentazone and ETU in subsurface soils from Denmark, Italy and Spain

Site	c_0	k_1	k_2
mcfb 1_I a 45 cm	36.19 ± 1.17	0.006218 ± 0.000328	0.000009112 ± 0.000007733
mcfb 1_I c 45 cm	31.28 ± 0.59	0.005582 ± 0.000469	0.00002812 ± 0.00000893
mcfb 1_I d 45 cm	32.81 ± 0.47	0.004064 ± 0.000483	0.00004903 ± 0.00000879
mcfb 1_II a 45 cm	28.81 ± 4.03	0.003329 ± 0.000390	0.0001758 ± 0.0000610
mcfb 1_II b 45 cm	33.61 ± 14.32	0.003360 ± 0.000107	0.00006598 ± 0.00005691
mcfb 1_II c 45 cm	31.89 ± 6.15	0.004173 ± 0.000426	0.0001289 ± 0.0000621
mcfb 3_II a 45 cm	24.48 ± 1.99	0.006341 ± 0.000415	0.0001944 ± 0.0000572
mcfb 3_II b 45 cm	23.12 ± 1.06	0.001995 ± 0.001168	0.0004623 ± 0.0000827
mcfb 3_II c 45 cm	25.43 ± 1.09	0.003973 ± 0.000729	0.0003813 ± 0.0000610
mcfb 3_II d 45 cm	27.46 ± 0.47	0.007714 ± 0.000999	0.0005781 ± 0.0000704
mcfb 4_I b 45 cm	25.03 ± 0.22	0.08643 ± 0.00712	0.0007139 ± 0.0009532
mcfb 1_I a 75 cm	30.44 ± 0.99	0.003347 ± 0.000302	0.0000196 ± 0.0000058
mcfb 1_I c 75 cm	35.42 ± 2.55	0.002213 ± 0.000105	0.00000804 ± 0.00000298
mcfb 1_II a 75 cm	20.13 ± 3.13	0.005258 ± 0.000459	0.0001157 ± 0.0000507
mcfb 1_II b 75 cm	17.47 ± 2.55	0.005469 ± 0.000427	0.0001361 ± 0.0000592
mcfb 1_II c 75 cm	24.09 ± 1.28	0.002038 ± 0.000391	0.0002671 ± 0.0000376
mcfb 3_II b 75 cm	6.789 ± 1.020	0.004903 ± 0.003621	0.0004096 ± 0.0002797
mcfb 3_II c 75 cm	26.35 ± 11.08	0.002444 ± 0.000529	0.0001153 ± 0.0000943
mcfb 3_II d 75 cm	24.30 ± 0.92	0.001548 ± 0.001309	0.0005419 ± 0.0000884
mcfb 4_I b 75 cm	26.28 ± 0.78	0.005267 ± 0.000226	0.0002513 ± 0.0000258
mcfb 4_I d 75 cm	27.75 ± 0.20	0.01294 ± 0.00023	0.0003547 ± 0.0000201
mcit 1 a 50 cm	37.92 ± 0.27	0.001819 ± 0.000368	0.0001193 ± 0.0000084
mcit 1 b 50 cm	43.45 ± 0.20	0.001262 ± 0.000191	0.0001033 ± 0.0000042
mcit 1 c 50 cm	42.89 ± 0.99	0.004688 ± 0.000360	0.00003472 ± 0.00000873
mcit 1 d 50 cm	42.96 ± 0.56	0.003985 ± 0.000491	0.00007368 ± 0.00001109
mcit 2 a 50 cm	45.14 ± 0.42	0.006659 ± 0.000499	0.00007582 ± 0.00001212
mcit 2 b 50 cm	42.64 ± 0.59	0.004872 ± 0.000367	0.00004974 ± 0.00000844
mcit 2 c 50 cm	41.50 ± 0.47	0.003206 ± 0.000716	0.0001309 ± 0.0000174
mcty 1 b 75 cm	32.23 ± 0.56	0.0002035 ± 0.0001217	0.0000232 ± 0.0000017
mcty 1 c 75 cm	25.72 ± 0.28	0.0009965 ± 0.0003145	0.0000556 ± 0.0000053
mcty 2 b 75 cm	29.06 ± 0.23	0.0007446 ± 0.0003094	0.0000758 ± 0.0000058
beit 1 b 50 cm	21.53 ± 0.79	0.0003391 ± 0.0000425	0.000009432 ± 0.000000842
beit 1 c 50 cm	16.96 ± 3.37	0.0001635 ± 0.0000245	0.000003837 ± 0.000001645
beit 1 d 50 cm	34.37 ± 10.12	0.00003482 ± 0.00001161	0.000002286 ± 0.000000838
beit 2 a 50 cm	35.90 ± 3.03	0.0002410 ± 0.0000224	0.000005220 ± 0.000000779
beit 2 c 50 cm	34.69 ± 1.36	0.0002248 ± 0.0000112	0.000005361 ± 0.000000373
besp 1 1 45 cm	24.21 ± 0.33	0.0005277 ± 0.0000557	0.00001862 ± 0.000000899
besp 1 2 45 cm	25.07 ± 0.39	0.001372 ± 0.000059	0.00001542 ± 0.00000109
besp 1 3 45 cm	24.15 ± 0.46	0.001046 ± 0.000045	0.00001307 ± 0.000000907
besp 1 4 45 cm	25.48 ± 0.34	0.0009156 ± 0.0000775	0.00002084 ± 0.00000124
besp 2 a 45 cm	12.55 ± 0.19	0.0003829 ± 0.0000250	0.00001222 ± 0.000000492
besp 2 b 45 cm	18.20 ± 0.39	0.0002698 ± 0.0000459	0.00001401 ± 0.000000815
besp 2 c 45 cm	18.21 ± 0.31	0.0004326 ± 0.0000566	0.00001686 ± 0.000000942
besp 2 d 45 cm	11.29 ± 1.11	0.001130 ± 0.000084	0.000007808 ± 0.000002352
bety 1 c 75 cm	5.182 ± 0.169	0.00008865 ± 0.00006991	0.00001301 ± 0.00000110
bety 2 a 75 cm	12.24 ± 0.27	0.00003872 ± 0.00005691	0.00001432 ± 0.00000086
etfb 1 a 45 cm	37.77 ± 0.56	0.009544 ± 0.001135	0.0001188 ± 0.0000359
etfb 3 a 45 cm	18.85 ± 0.66	0.006730 ± 0.000625	0.00004012 ± 0.00001921
etfb 3 b 45 cm	17.66 ± 0.21	0.002844 ± 0.000110	0.00004488 ± 0.000003087
etfb 3 c 45 cm	22.39 ± 0.21	0.002101 ± 0.000414	0.0001354 ± 0.0000111
etfb 3 d 45 cm	23.89 ± 0.41	0.004259 ± 0.001415	0.0002050 ± 0.0000455
etfb 1 b 75 cm	34.70 ± 0.56	0.01241 ± 0.00164	0.0001285 ± 0.0000561
etfb 1 c 75 cm	31.20 ± 0.62	0.005362 ± 0.001222	0.0001333 ± 0.0000353
etfb 1 d 75 cm	27.33 ± 0.41	0.001597 ± 0.001439	0.00003000 ± 0.00004801
etfb 3 c 75 cm	16.15 ± 0.41	0.001433 ± 0.000221	0.00005022 ± 0.00000571
etfb 3 d 75 cm	10.57 ± 0.13	0.002309 ± 0.000452	0.0001128 ± 0.0000118

Table 18
Parameters ± S.D. estimated according to Eq. (19) for mecoprop, bentazone and ETU in subsurface soils from Denmark, Italy and Spain

Site	c_0	k_1	k_2
mcfb 1_I c 45 cm	31.39 ± 0.58	0.01215 ± 0.00142	−0.0002225 ± 0.0000596
mcfb 1_I d 45 cm	33.08 ± 0.50	0.01448 ± 0.00131	−0.0003148 ± 0.0000521
mcfb 1_II a 45 cm	27.92 ± 4.16	0.02942 ± 0.00598	−0.0009002 ± 0.0003401
mcfb 1_II b 45 cm	35.89 ± 20.48	0.01608 ± 0.00856	−0.0003533 ± 0.0003939
mcfb 1_II c 45 cm	31.78 ± 6.74	0.02376 ± 0.00660	−0.0006010 ± 0.0003170
mcfb 3_II a 45 cm	24.92 ± 2.22	0.02784 ± 0.00477	−0.0008491 ± 0.0002621
mcfb 3_II b 45 cm	22.84 ± 1.13	0.04846 ± 0.00596	−0.001920 ± 0.000368
mcfb 3_II c 45 cm	25.48 ± 1.32	0.04146 ± 0.00472	−0.001404 ± 0.000265
mcfb 3_II d 45 cm	27.97 ± 0.59	0.04816 ± 0.00391	−0.001395 ± 0.000185
mcfb 1_I a 75 cm	31.22 ± 1.18	0.008644 ± 0.001349	−0.0001712 ± 0.0000544
mcfb 1_I c 75 cm	37.17 ± 3.14	0.005279 ± 0.001095	−0.0000845 ± 0.0000351
mcfb 1_II b 75 cm	17.82 ± 2.92	0.02319 ± 0.00593	−0.0009803 ± 0.0004660
mcfb 1_II c 75 cm	22.51 ± 1.25	0.03972 ± 0.00364	−0.001596 ± 0.000252
mcfb 3_II b 75 cm	7.219 ± 1.580	0.03647 ± 0.01900	−0.004099 ± 0.003633
mcfb 3_II c 75 cm	24.99 ± 10.85	0.05027 ± 0.00661	−0.0008684 ± 0.0007598
mcfb 3_II d 75 cm	24.41 ± 1.19	0.05027 ± 0.00661	−0.001860 ± 0.000376
mcfb 4_I b 75 cm	25.71 ± 0.54	0.03480 ± 0.00147	−0.001121 ± 0.000081
mcfb 4_I d 75 cm	28.52 ± 0.23	0.03684 ± 0.00110	−0.0008464 ± 0.0000491
mcit 1 a 50 cm	38.08 ± 0.29	0.02361 ± 0.001114	−0.0005452 ± 0.0000365
mcit 1 b 50 cm	43.61 ± 0.24	0.02219 ± 0.00067	−0.0004541 ± 0.0000193
mcit 1 c 50 cm	43.79 ± 1.14	0.01179 ± 0.00146	−0.0001649 ± 0.0000423
mcit 1 d 50 cm	43.49 ± 0.63	0.01723 ± 0.00147	−0.0002987 ± 0.0000437
mcit 2 a 50 cm	45.51 ± 0.41	0.01835 ± 0.00123	−0.0002628 ± 0.0000360
mcit 2 b 50 cm	43.29 ± 0.66	0.01426 ± 0.00121	−0.0002187 ± 0.0000361
mcit 2 c 50 cm	41.83 ± 0.50	0.02317 ± 0.00192	−0.0004557 ± 0.0000586
mcit 2 d 50 cm	35.12 ± 0.39	0.01843 ± 0.00114	−0.0004268 ± 0.0000417
mcty 1 a 75 cm	28.56 ± 0.30	0.01856 ± 0.00104	−0.0006113 ± 0.0000442
mcty 1 b 75 cm	32.10 ± 0.73	0.01082 ± 0.00068	−0.0003090 ± 0.0000292
mcty 1 c 75 cm	25.94 ± 0.33	0.01542 ± 0.00109	−0.0005202 ± 0.0000523
mcty 1 d 75 cm	41.59 ± 0.38	0.02591 ± 0.00135	−0.0006134 ± 0.0000365
mcty 2 a 75 cm	21.63 ± 0.32	0.01261 ± 0.00055	−0.0005546 ± 0.0000341
mcty 2 b 75 cm	29.14 ± 0.25	0.01903 ± 0.00106	−0.005886 ± 0.000044
mcty 2 c 75 cm	12.73 ± 0.34	0.01086 ± 0.00053	−0.0008231 ± 0.0000639
mcty 2 d 75 cm	31.31 ± 0.26	0.01667 ± 0.00060	−0.0005095 ± 0.0000235
beit 1 a 50 cm	16.61 ± 0.47	0.0007737 ± 0.0002133	−0.0004441 ± 0.0000249
beit 1 b 50 cm	20.02 ± 0.81	0.007563 ± 0.000467	−0.0003440 ± 0.0000373
beit 1 c 50 cm	10.20 ± 0.65	0.007504 ± 0.000518	−0.0006937 ± 0.0000943
beit 2 a 50 cm	25.91 ± 0.78	0.007521 ± 0.000275	−0.0002708 ± 0.0000186
beit 2 c 50 cm	25.89 ± 0.56	0.007321 ± 0.000185	−0.0002635 ± 0.0000127
beit 2 d 50 cm	19.52 ± 0.53	0.01054 ± 0.00049	−0.0005206 ± 0.0000395
besp 1 a 45 cm	23.86 ± 0.46	0.009736 ± 0.000431	−0.0003667 ± 0.0000257
besp 1 b 45 cm	25.38 ± 0.47	0.008034 ± 0.000375	−0.0002557 ± 0.0000201
besp 1 c 45 cm	24.18 ± 0.56	0.007651 ± 0.000370	−0.0002644 ± 0.0000217
besp 1 d 45 cm	25.65 ± 0.46	0.009645 ± 0.000469	−0.0003250 ± 0.0000251
besp 2 a 45 cm	11.65 ± 0.26	0.008693 ± 0.000322	−0.0006815 ± 0.0000428
besp 2 b 45 cm	16.95 ± 0.41	0.009446 ± 0.000413	−0.0005156 ± 0.0000372
besp 2 c 45 cm	17.75 ± 0.42	0.009475 ± 0.000466	−0.0004834 ± 0.0000383
besp 2 d 45 cm	11.98 ± 1.55	0.005237 ± 0.001075	−0.0003364 ± 0.0001271
bety 1 a 75 cm	5.642 ± 0.156	0.01016 ± 0.00055	−0.001705 ± 0.000146
bety 1 b 75 cm	6.930 ± 0.162	0.01022 ± 0.00044	−0.001406 ± 0.000096
bety 1 c 75 cm	4.769 ± 0.156	0.009420 ± 0.000563	−0.001854 ± 0.000180
bety 1 d 75 cm	5.381 ± 0.160	0.008199 ± 0.000274	−0.001452 ± 0.000093

Table 18 (continued)

Site	c_0	k_1	k_2
bety 2 b 75 cm	10.93 ± 0.21	0.01223 ± 0.00046	−0.001094 ± 0.000063
bety 2 c 75 cm	13.15 ± 0.20	0.01151 ± 0.00044	−0.0008308 ± 0.0000469
bety 2 d 75 cm	18.97 ± 0.39	0.01355 ± 0.00081	−0.0006895 ± 0.0000571
etfb 1 a 45 cm	37.93 ± 0.55	0.02408 ± 0.00286	−0.0004003 ± 0.000100
etfb 1 b 45 cm	41.19 ± 0.69	0.02617 ± 0.00468	−0.0002628 ± 0.0001609
etfb 3 a 45 cm	19.08 ± 0.69	0.01370 ± 0.00263	−0.0003820 ± 0.0001724
etfb 3 b 45 cm	17.96 ± 0.22	0.01357 ± 0.00053	−0.0005879 ± 0.0000390
etfb 3 c 45 cm	22.57 ± 0.25	0.02423 ± 0.00146	−0.0009279 ± 0.0000816
etfb 3 d 45 cm	24.14 ± 0.45	0.02759 ± 0.00381	−0.0009107 ± 0.0002006
etfb 1 a 75 cm	32.79 ± 0.64	0.02092 ± 0.00382	−0.0002431 ± 0.0001603
etfb 1 b 75 cm	34.73 ± 0.54	0.02711 ± 0.00392	−0.0004579 ± 0.0001538
etfb 1 c 75 cm	31.51 ± 0.69	0.02300 ± 0.00343	−0.0005457 ± 0.0001405
etfb 1 d 75 cm	27.48 ± 0.44	0.03550 ± 0.00405	−0.001138 ± 0.000182
etfb 3 a 75 cm	16.69 ± 0.10	0.02161 ± 0.00038	−0.001244 ± 0.000030
etfb 3 b 75 cm	24.62 ± 0.51	0.04204 ± 0.00585	−0.001610 ± 0.000279
etfb 3 c 75 cm	16.01 ± 0.33	0.01598 ± 0.00092	−0.0008785 ± 0.0000871
etfb 3 d 75 cm	10.67 ± 0.16	0.02181 ± 0.00161	−0.001734 ± 0.000192

nated by sequential first order processes in the case where the degradation was fastest, but followed kinetics with growth where the degradations was slowest. Neither bentazon nor ETU have ever been reported to be metabolically degradable. Formation of intermediate metabolites which could serve as nutrients for microorganisms could be an explanation for this. Another explanation could be that degradation of low concentrations of bentazon and ETU do follow kinetics with grǒwth of microorganisms because of the special living conditions for microorganisms in subsoil (e.g. presence of dormant microorganisms). The factors that determine the changes from processes without growth to processes with growth as the most dominant for mecoprop degradation in Danish subsoil are not easily determined. More studies investigating the influence of nutrients present are needed.

5. Conclusion

The mineralization of mecoprop, bentazon and ETU consist of a large number of processes, a pathway through formation of metabolites, degradation of the pesticide present in soil water followed by desorption and degradation of the primarily adsorbed pesticide, building in of pesticide-carbon in soil organic matter followed by a slow degradation of organic matter to CO_2. The description of such complicated processes will always express the dominant processes. It was not expected to find one model being the one and only giving good fits. Such a heterogeneous system soil will always cause difficulties in describing the kinetics of a biodegradation process and the process will certainly consist of a number of processes that cannot be modelled for all of them. So choosing a specific model for describing the kinetics means ‚choosing the model which describes the dominant processes.

The present study showed that a number of mathematical models used for modelling degradation of xenobiotic compounds in other studies can be used for modelling mineralization of low concentrations of pesticides in soil. It was also showed which of these models cannot be applied.

It was clearly shown, that degradation of low concentrations (0.04–0.08 $\mu g \cdot g^{-1}$) of mecoprop, bentazon and ETU follow different kinetics in the ploughed layer and in the subsoil. The kinetics that dominate in the ploughed layer are de-

Table 19
Parameters ± S.D. estimated according to Eq. (23) for mecoprop, bentazone and ETU in subsurface soils from Denmark, Italy and Spain

Site	c_0	k_1	k_2
mcfb 1_I a 45 cm	36.02 ± 0.58	0.008080 ± 0.000514	0.06843 ± 0.01528
mcfb 1_I b 45 cm	19.76 ± 0.19	0.01986 ± 0.00137	0.2128 ± 0.0991
mcfb 1_I c 45 cm	32.01 ± 0.52	0.009039 ± 0.000702	0.04790 ± 0.00944
mcfb 1_I d 45 cm	34.20 ± 0.57	0.009472 ± 0.000914	0.03375 ± 0.00627
mcfb 3_II a 45 cm	47.30 ± 9.76	0.005838 ± 0.001629	0.1526 ± 0.0383
mcfb 3_II c 45 cm	34.46 ± 4.70	0.01427 ± 0.00452	0.05643 ± 0.01480
mcfb 3_II d 45 cm	30.33 ± 0.71	0.02813 ± 0.00325	0.06862 ± 0.00930
mcfb 1_I a 75 cm	33.11 ± 1.18	0.005442 ± 0.000584	0.03862 ± 0.00866
mcfb 1_I b 75 cm	35.24 ± 0.67	0.005446 ± 0.000277	0.1465 ± 0.0524
mcfb 1_I c 75 cm	47.17 ± 3.71	0.002308 ± 0.000297	0.05841 ± 0.01558
mcfb 1_II a 75 cm	62.09 ± 27.38	0.002609 ± 0.001317	0.1813 ± 0.0482
mcfb 1_II b 75 cm	45.44 ± 17.57	0.003418 ± 0.001578	0.1626 ± 0.0456
mcfb 1_II c 75 cm	31.61 ± 10.03	0.01853 ± 0.06531	0.02253 ± 0.07421
mcfb 4_I d 75 cm	33.72 ± 1.21	0.01706 ± 0.00134	0.2528 ± 0.0535
mcit 1 c 50 cm	46.57 ± 0.98	0.007300 ± 0.000497	0.05789 ± 0.00973
mcit 1 d 50 cm	45.43 ± 0.63	0.01059 ± 0.00082	0.03615 ± 0.00485
mcit 2 a 50 cm	46.69 ± 0.28	0.01236 ± 0.00042	0.06260 ± 0.00555
mcit 2 b 50 cm	45.50 ± 0.51	0.008857 ± 0.000403	0.05116 ± 0.00529
mcit 2 c 50 cm	43.09 ± 0.06	0.01508 ± 0.00257	0.02994 ± 0.00721
mcsp 2 d 45 cm	38.19 ± 0.44	0.003103 ± 0.000068	0.5020 ± 0.2057
besp 1 b 45 cm	35.99 ± 1.75	0.002410 ± 0.000210	0.02382 ± 0.00225
besp 1 c 45 cm	42.55 ± 2.80	0.001619 ± 0.000160	0.02145 ± 0.00165
besp 1 d 45 cm	31.20 ± 0.98	0.004055 ± 0.000408	0.01203 ± 0.00118
besp 2 d 45 cm	25.42 ± 7.01	0.0009495 ± 0.0003248	0.02986 ± 0.00651
etfb 1 a 45 cm	38.59 ± 0.49	0.01713 ± 0.00140	0.08036 ± 0.01718
etfb 1 b 45 cm	41.22 ± 0.55	0.02337 ± 0.00223	0.1347 ± 0.0460
etfb 1 d 45 cm	32.27 ± 0.41	0.01760 ± 0.00122	0.1461 ± 0.0445
etfb 3 a 45 cm	19.65 ± 0.53	0.009926 ± 0.000987	0.07584 ± 0.02004
etfb 3 b 45 cm	21.85 ± 1.12	0.005465 ± 0.000646	0.04025 ± 0.00649
etfb 3 d 45 cm	24.93 ± 0.54	0.01696 ± 0.00324	0.04515 ± 0.01419
etfb 1 a 75 cm	32.86 ± 0.52	0.01850 ± 0.00169	0.1329 ± 0.0478
etfb 1 b 75 cm	35.09 ± 0.44	0.02054 ± 0.00185	0.09842 ± 0.02573
etfb 1 c 75 cm	32.46 ± 0.82	0.01503 ± 0.00270	0.04584 ± 0.01430
etfb 1 d 75 cm	28.36 ± 0.63	0.02521 ± 0.05874	0.02766 ± 0.06758

scribed with models without the growth of microorganisms, and the kinetics that dominate in the subsoil are described with models which include the growth of microorganisms for varying soil types and at varying incubation temperatures. It is highly recommended to consider kinetic models taking the growth of microorganisms in account when dynamic pesticide fate models for soil are to be further developed.

Kinetic modelling studies of other pesticides at different concentrations and under varying conditions are still needed.

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DEGRADATION OF ^{14}C-MANEB IN SEDIMENT FROM A NICARAGUAN ESTUARY

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Sediment samples were collected at 5 sites in the Nicaraguan estuary "El Naranjo" in July 94 and September 94. The samples were incubated with ^{14}C-maneb (0.08 $\mu g \cdot g^{-1}$dw sediment), and evolved $^{14}CO_2$ and residual ^{14}C-ETU in soil were measured. Mineralization kinetics of ^{14}C-maneb was best described with kinetic models which include growth of microorganisms. The amounts of ^{14}C-maneb mineralized were highest at the sites closest to the mouth of the river. No significant differences in degradation between July and September were seen. After 67 days between 9.73 and 16.18% of added ^{14}C had evolved as $^{14}CO_2$ in the July samples and after 150 days between 11.18 and 27.37% of added ^{14}C had evolved as $^{14}CO_2$ from the September samples. When 4.61–8.20% of added ^{14}C was found in the soil extract, 0.00–2.72% was ^{14}C-ETU.

Keywords: ^{14}C-maneb; degradation rate; kinetics; sediment; Nicaragua; ETU

INTRODUCTION

The problems concerning use of pesticides in the agricultural system of Nicaragua is a main topic that requires research, not only regarding pesticide residues in crop, food, freshwater, drinking water etc., but also research in reference to the fate of pesticides in the environment of Nicaragua.

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Until now, research in the fate of pesticides in Nicaragua mostly has considered determination of residues of organochlorine and organophosphorous pesticides in aquatic environments. The use of pesticides demanded such investigations. Besides this kind of studies, research in fate of modern pesticides in Nicaragua is also important.

The ethylene bisdithiocarbamate (EBDC) fungicide maneb (Trimangol 80, Poligram M, Plantineb 80 PM) is used in Nicaragua in cultures of onion, beans, maize, tobacco and tomato.

Most studies concerning transformation of EBDC fungicides focus on the formation of ETU as a degradation product because of its specific toxicity and high water solubility ($20\,g\,l^{-1}$) [1]. ETU has been shown to be carcinogenic in laboratory animals [2].

Neither studies on degradation of maneb in sediment with subsequent formation of ETU nor studies on fate of maneb in sediment from tropical climate have ever been reported.

The purpose of the present investigation was to study the mineralization kinetics and velocity of maneb in sediment from a Nicaraguan estuary (Estero "El Naranjo"), situated at the west coast of Nicaragua (Fig. 1). The estuary receives the run-off from an extensive area, intensively cultivated with a number of crops including bean, maize and cotton. Knowledge about degradation of pesticides in the estuary is therefore highly needed.

MATERIALS AND METHODS

Sediment Samples

The sediment was sampled with an Ekman dredge in the month of July 1994 and September 1994 at 5 sites in the esturarine-coastal lagoon system of the Atoya River (Estuary "El Naranjo"), located at the Pacific Coast of Nicaragua (Fig. 1). The texture of the dried sediment taken in July 1994 is shown in Table I. The samples were stored at 5°C until incubation.

Chemicals

Ring ^{14}C-labelled maneb with a specific activity of 105 $mCi \cdot g^{-1}$ and a radiochemical purity of 98.4% and ring ^{14}C-labelled ETU (ethylene

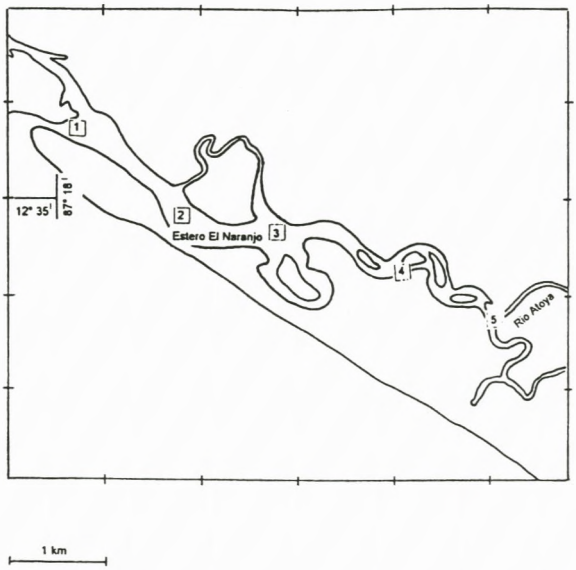


FIGURE 1 Sediment sampling sites, estuary "El Naranjo", July and September 1994.

thiourea) with a specific activity of 81 $mCi \cdot g^{-1}$ and a radiochemical purity of 95% were obtained from Amersham.

Degradation Experiments

The incubation experiments were performed at as low concentrations as possible (considering detection limits and specific activity of the standard) (0.08 $\mu g \cdot g^{-1}$dry weight (dw)). Water from each sampling site was added to cover the sediment. The ^{14}C-labelled maneb was added to the sediment samples by mixing in a 100 ml Duran flask under N_2-flushing. Samples were incubated at 25°C to simulate natural conditions. A gentle stream of atmospheric air was passed through the samples twice a week to collect evolved $^{14}CO_2$ in two 0.1 N KOH solutions and eventual volatile organic compounds in glycerol. $^{14}CO_2$ was measured in a liquid scintillation counter according to Helweg [3] and used to describe the mineralization of the added ^{14}C-maneb. After the incubation period the sediment samples from July 1994 were

TABLE I Texture of sediment samples. Estuary "El Naranjo", Nicaragua, July 1994

	$CaCO_3$ %	*Fine sand %*	*Coarse sand %*	*Coarse silt %*	*Humus % (= % OC × 1.72)*	*Clay %*	*Silt %*	*pH**
Site 1. Bocana	16	18.6	60.3	1.3	0.2	1.7	1.9	9.0
Site 2. Frente a los Cocos	5.2	72.9	12.4	4.9	1.1	1.7	1.9	8.9
Site 3. Isla Montano	–	28.8	53.8	2.7	1.7	6.9	6.1	7.6
Site 4. Salida de Rio Atoya	–	10.9	79.7	1	0.9	4.7	2.8	7.7
Site 5. Atoya Empalme	–	2.2	90.0	1	0.4	4.7	1.7	7.9

* in water; Particle size: Clay < 0.002 mm; slit 0.002–0.02 mm; coarse silt 0.02–0.063 mm; fine sand 0.063–0.2 mm; coarse sand 0.2–2 mm.

extracted and the sediment was combusted. ^{14}C-content of extract as well as ^{14}C-content remaining in sediment were quantified by liquid scintillation counting. The sediment samples from September 1994 were treated the same way, but additionally the amount of ^{14}C-ETU present in the extract was quantified.

50 g sediment (calculated as dw) was extracted with 200 ml 0.01 M $CaCl_2$. The extract was centifuged and filtered. The amount of extract was measured and 2 aliquots of 1 ml was counted in a liquid scintillation counter.

Determination of ETU in Extract

150 ml of the extract was transferred to a roundbottom flask of at least 300 ml and freezed during rotation to obtain a thin shell of thoroughly frozen extract at the inner wall of the flask. Immediately after freezing, the flask was transferred to a lyofilizing apparatus and lyofilized under vacuum. The samples must be kept frozen during the whole process. The lyofilization process was stopped, when no more ice was left in the flask and the concentrated sample was dissolved in 10 ml methanol. 2×1 ml was counted by liquid scintillation counting and 100 ml was used for TLC.

Quantification of ^{14}C-ETU was intended with HPLC, – but the separation between ETU and EU could not be confirmed because EU does not absorb light in the useable UV-area, so a TLC-method has to be developed.

100 ml methanol extract was applied to a 20×20 cm Kieselgel plate 60–254 and developed in buthanol:acetic acid:water 12:3:5. To avoid decomposition of ^{14}C-ETU on the TLC-plate, thiourea was applied as a preservative together with the sample spots [4].

To identify the separated ETU and EU, both compounds (without ^{14}C-labelling) were applied in all spots and after development the plate was sprayed with Ehrlichs reagent (10% 4(dimethylamino)benzaldehyde in HCl:acetone 1:4) which gives a yellow easy identifiable colour for ETU and EU. The TLC plate was cut into pieces and ^{14}C-ETU was extracted with water and quantified in a liquid scintillation counter. The recovery of ETU was > 82%. The amount of ^{14}C present in sediment after extraction (strongly absorbed or built into organic material) was determined by combustion of the sediment.

RESULTS AND DISCUSSION

Sorption of EBDC fungicides to soil varies depending on soil type [5]. Taking out representative aliquots of the incubated samples to determine residues of maneb would therefore result difficult. Moreover, no specific analytic method for determination of maneb is known; – destructive methods where maneb is quantified through the formation of CS_2 after acid hydrolysis [6] would not be recommendable at the actual low concentrations. Use of realistic low concentration in simulation degradation experiments is important, because mineralization kinetics at high and low concentrations can differ. [7, 8] Stenström and Torstensson [9] suggested, that variations in reported half-lives of EBDC fungicides and ETU may be due to the degradation kinetics not following first order kinetics, and that at low concentrations ($< 1 \mu g \cdot g^{-1}$) the compounds may have high presistence.

To follow the mineralization process with time in the same sample, the amount of CO_2 evolved through the mineralization process was quantified using ^{14}C-maneb and following the evolution of $^{14}CO_2$. Accumulated amounts of evolved $^{14}CO_2$, calculated as percentage radioactivity of total amount of added radioactivity were described as a function of incubation time. $^{14}CO_2$ then corresponds to the total amount mineralized pesticide. The mineralization curves are shown in Figures 2–11. The curves showed generally the same form, with an increment in $^{14}CO_2$ production at the beginning, so a number of non-linear models were fit to the curves to evaluate the kinetics of mineralization of each replicate. The curves resembled curves from former subsoil mineralization experiments, so the fitted models which are presented in Table II, as well as the principle for choosing useable models, were chosen according to Fomsgaard [10]. Eqs. (1)–(4) (Tab. II) are models which do not include growth of micorooganisms. whereas Eqs. (5)–(9) include linear or logistic growth of microorganisms. It is reasonable to think, that half–saturation constants as they appear in the Monod equations [11] are not important in the present low concentration experiments, where adsorption–desorption processes may have more importance. The software used was Jandel Scientific Tablecurve 2D [15]. From Table III where the residual sum of squares for all the fitted models are presented, it is seen, that models which include growth gave the best fit to all the mineralization curves

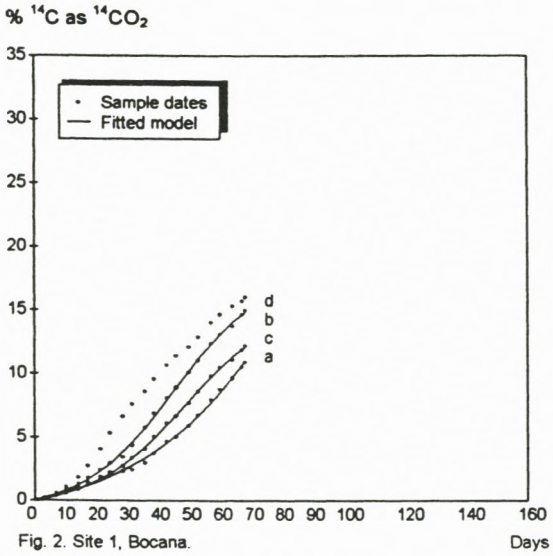


FIGURE 2 Mineralization of 0.08 $\mu g \cdot g^{-1}$ ^{14}C-maneb in sediment from a Nicaraguan Estuary "El Naranjo". July 1994.

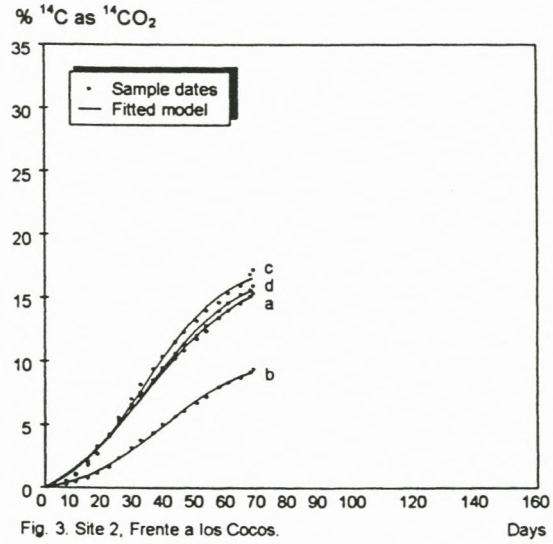


FIGURE 3 Mineralization of 0.08 $\mu g \cdot g^{-1}$ ^{14}C-maneb in sediment from a Nicaraguan Estuary "El Naranjo". July 1994.

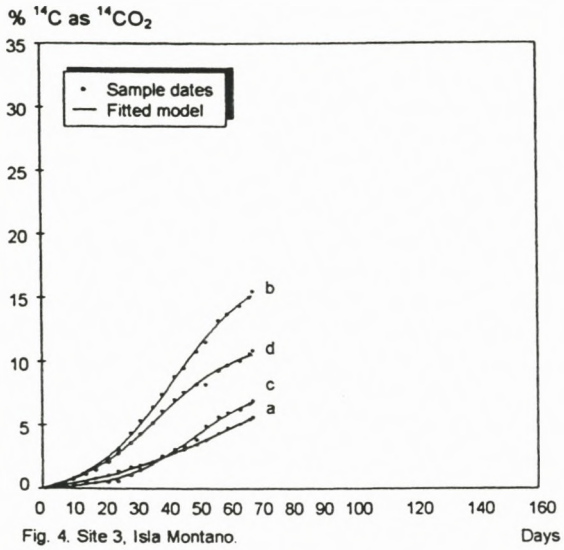


FIGURE 4 Mineralization of 0.08 $\mu g \cdot g^{-1}$ ^{14}C-maneb in sediment from a Nicaraguan Estuary "El Naranjo". July 1994.

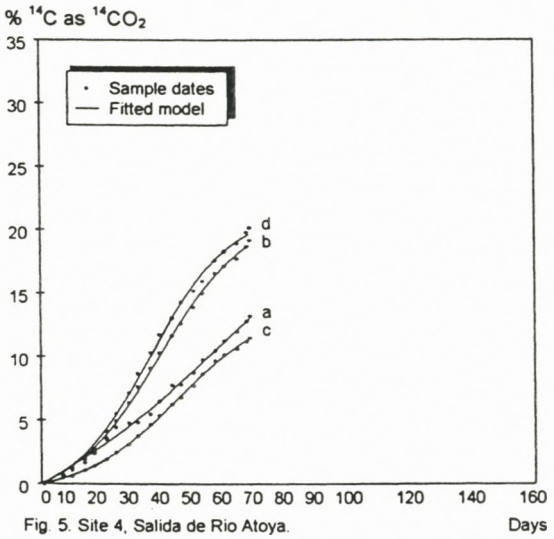


FIGURE 5 Mineralization of 0.08 $\mu g \cdot g^{-1}$ ^{14}C-maneb in sediment from a Nicaraguan Estuary "El Naranjo". July 1994.

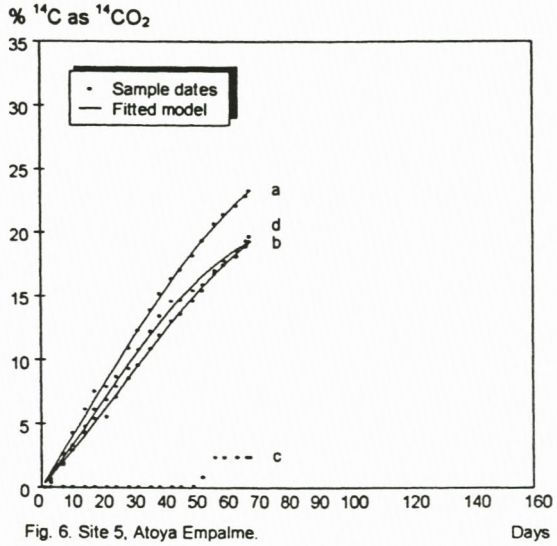


FIGURE 6 Mineralization of 0.08 $\mu g \cdot g^{-1}$ ^{14}C-maneb in sediment from a Nicaraguan Estuary "El Naranjo". July 1994.

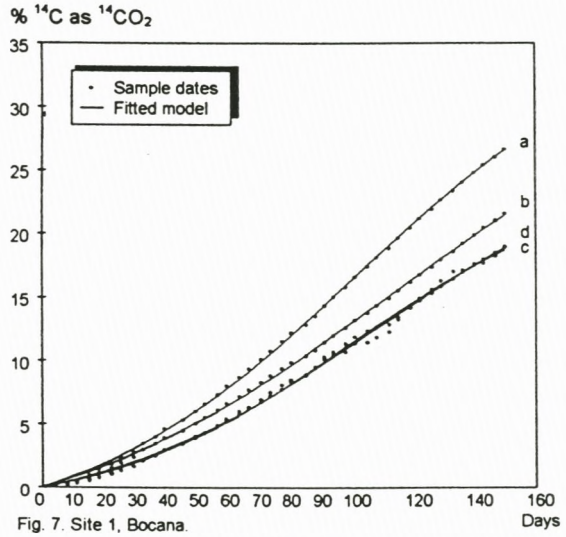


FIGURE 7 Mineralization of 0.08 $\mu g \cdot g^{-1}$ ^{14}C-maneb in sediment from a Nicaraguan Estuary "El Naranjo". September 1994.

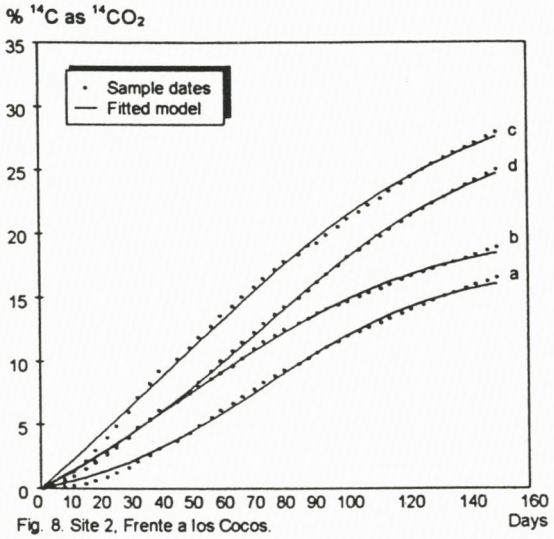


FIGURE 8 Mineralization of 0.08 $\mu g \cdot g^{-1}$ ^{14}C-maneb in sediment from a Nicaraguan Estuary "El Naranjo". September 1994.

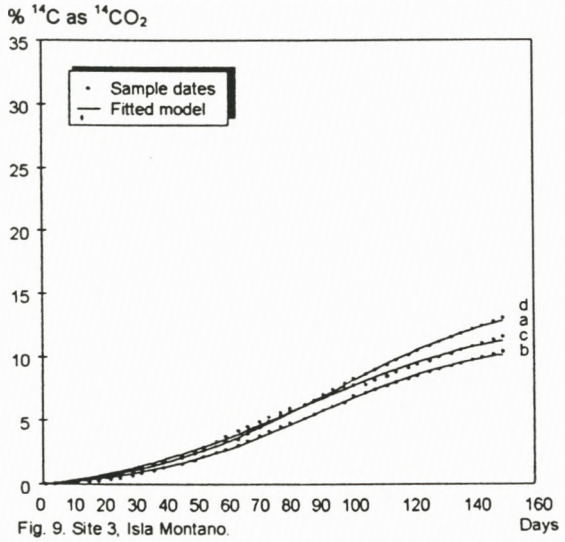


FIGURE 9 Mineralization of 0.08 $\mu g \cdot g^{-1}$ ^{14}C-maneb in sediment from a Nicaraguan Estuary "El Naranjo". September 1994.

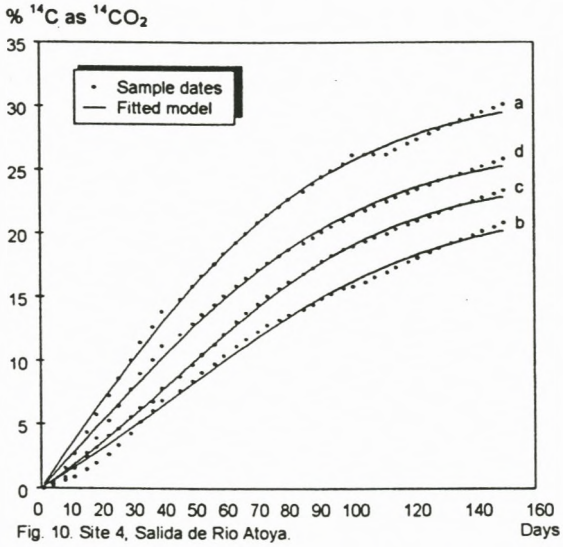


FIGURE 10 Mineralization of 0.08 $\mu g \cdot g^{-1}$ ^{14}C-maneb in sediment from a Nicaraguan Estuary "El Naranjo". September 1994.

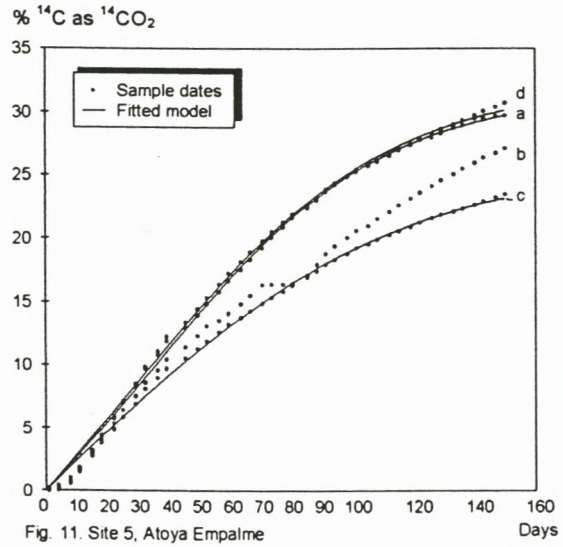
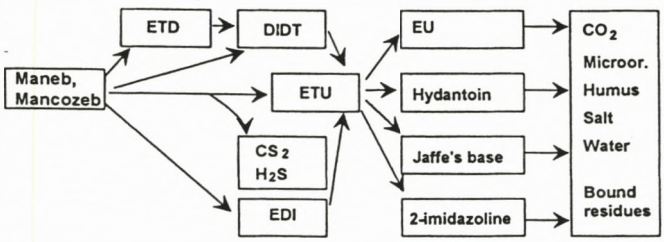


FIGURE 11 Mineralization of 0.08 $\mu g \cdot g^{-1}$ ^{14}C-maneb in sediment from a Nicaraguan Estuary "El Naranjo". September 1994.

even if there was some variation in the texture of the sediment between sites. Maneb hydrolyses rapidly in contact with moisture [21] and direct microbial degradation is not known. The metabolite ETU has not been shown to produce growth of microorganism. [4, 22, 23]. Thus the growth of microorganisms based on the addition of ^{14}C-maneb probably was due to the capability of the microorganisms to exploit energy from one of the other possible metabolites, for instance EU, since the curves depict the total mineralization. Figure 12 shows possible metabolites of maneb. The degradation of ETU to EU has been reported to be primarily chemical, whereas mineralization of EU to CO_2 was microbial [24].

The model fit according to Eq. (9) [19, 20] (Tab. II) is presented as the solid line in Figures 2–11. The resulting parameters of the model expressed with eq. (9), estimated after 67 days, were chosen to compare degradation rates between samples (Tab. IV). A two-way Analysis of Variance ($\alpha = 0.05$) showed a significant difference between the amounts of ^{14}C-maneb mineralized through the process (c_0) at different sites after 67 days, but no significant difference between degradation rates (k_1). The amounts of ^{14}C-maneb mineralized were highest at site 4 and 5 both in July and September. Many pesticide degradation studies in soil show correlation between degradation rates of pesticides and amount of humus present. No such correlation is found in this case (Tabs. I and IV). The explanation of the higher



ETU *ethylenethiourea*
DIDT *5,6-dihydro-3H-imidazo(2,1-C)-1,2,4-dithiazole-3-thione*
EU *ethyleneurea*
ETD *ethylenethiuram disulfide*
EDI *ethylene diisothiocyanate*

FIGURE 12 Degradation pathways for EBDC fungicides in soil. Adapted from WHO (1988) and IUPAC (1977).

TABLE II Models used for describing mineralization kinetics of ^{14}C-maneb

Equation	*Reference*	*Equation no.*
$P = c_1(1 - e^{-k_1 t}) + c_2(1 - e^{-k_2 t})$	[11, 12]	eq. 1
P = concentration of pesticide mineralised at time t (% ^{14}C as $^{14}CO_2$) c_1 = total concentration of pesticide converted to $^{14}CO_2$ by one first-order metabolism c_2 = total concentration of pesticide converted to $^{14}CO_2$ by another first-order metabolism k_1, k_2 = degradation rate constants for the two first-order processes t = time in days		
$P = 100((1 - ae^{-tk_1} - (1 - a)e^{-tk_2})$	[10]	eq. 2
P = concentration of pesticide mineralised at time t (% ^{14}C as $^{14}CO_2$) k_1, k_2 = degradation rate constants for the two first-order processes t = time in days a = fraction of total amount of pesticide converted to $^{14}CO_2$ by one first-order process		
$P = c_0(1 - e^{-k_1 t}) + k_0 t$	[11, 13, 14]	eq. 3
P = concentration of pesticide mineralised at time t (% ^{14}C as $^{14}CO_2$) c_0 = total concentration of pesticide converted to $^{14}CO_2$ by first-order metabolism k_1 = degradation rate constant for the first-order process k_0 = degradation rate constant for the zero-order process		
$P = c_0(1 + (k_1 e^{-k_2 t} - k_2 e^{-k_1 t})/(k_2 - k_1))$	[15]	eq. 4
P = concentration of pesticide mineralised at time t (% ^{14}C as $^{14}CO_2$) c_0 = total concentration of pesticide converted to $^{14}CO_2$ by first-order metabolism k_1, k_2 = degradation rate constants for the two first-order processes t = time in days		
$P = c_0(1 - e^{-k_1 t}(k_2 t^2/2)) + k_0 t$	[11, 13, 14]	eq. 5
P = concentration of pesticide mineralised at time t (% ^{14}C as $^{14}CO_2$) c_0 = total concentration of pesticide converted to $^{14}CO_2$ by first-order metabolism k_1 = degradation rate constant for the first-order process		

TABLE II (Continued)

Equation	*Reference*	*Equation no.*
k_2 = linear growth rate term describing growth on micro-organisms k_0 = degradation rate constant for the zero-order process $P = c_0 - (c_0 + x_0/1 + ((x_0)/((c_0))e^{k_1(c_0+x_0)t})$ P = concentration of pesticide mineralised at time t (% ^{14}C as $^{14}CO_2$) c_0 = total concentration of pesticide converted to $^{14}CO_2$ by first-order metabolism x_0 = the amount of substrate (pesticide) required to produce the initial population density k = degradation rate constant t = time in days	[16, 17]	eq. 6
$P = c_0 - c_0 e^{-(k/r)(e^{rt}-1)}$ P = Concentration of pesticide mineralised at time t (% ^{14}C as $^{14}CO_2$) c_0 = Total concentration of pesticide converted to $^{14}CO_2$ by the modelled process k = degradation rate constant r = the maximum specific growth rate t = time	[18]	eq. 7
$P = c_0(1 - e^{-k_1 t} - (k_2 t^2/2))$ P = Concentration of pesticide mineralised at time t (% ^{14}C as $^{14}CO_2$) c_0 = Total concentration of pesticide converted to $^{14}CO_2$ by the modelled process k = degradation rate constant t = times	[18]	eq. 8
$P = c_0 - (k_1 c_0/(k_1 + k_2 c_0)e^{k_1 t} - k_2 c_0)$ P = Concentration of pesticide mineralised at time t (% ^{14}C as $^{14}CO_2$) c_0 = total concentration of pesticide to $^{14}CO_2$ by the modelled process k_1 = first order degradation rate constant k_2 = second order degradation rate constant	[19, 20]	eq. 9

TABLE III Residual mean for all fitted equations for mineralization of ^{14}C-maneb in sediment from a Nicarguan estuary, "EI Naranjo"

	Models without growth of microorganisms					*Models with growth of microorganisms*			
	Eq. (1)	*Eq.* (2)	*Eq.* (3)	*Eq.* (4)	*Eq.* (5)	*Eq.* (6)	*Eq.* (7)	*Eq.* (8)	*Eq.* (9)
Site 1, July 94, a	–	–	–	–	–	0.1027	0.04004	0.06329	0.1027
Site 1, July 94, b	–	–	–	–	–	0.02579	0.03741	0.05052	0.02579
Site 1, July 94, c	–	–	–	–	–	0.01019	0.01523	–	0.01019
Site 1, July 94, d	–	–	–	–	–	–	–	–	–
Site 2, July 94, a	–	–	–	0.01351	–	0.06887	0.1042	0.03862	0.06887
Site 2, July 94, b	–	–	–	–	–	0.03181	0.05123	–	0.03181
Site 2, July 94, c	–	–	–	–	–	0.1656	0.2752	–	0.1656
Site 2, July 94, d	–	–	–	–	–	0.1148	0.1726	0.06291	0.1148
Site 3, July 94, a	–	–	–	–	–	0.008894	0.009306	0.007051	0.008892
Site 3, July 94, b	–	–	–	–	–	0.04683	0.08061	–	0.04683
Site 3, July 94, c	–	–	–	–	–	0.02634	0.02823	–	0.02634
Site 3, July 94, d	–	–	–	–	–	0.03709	0.06824	–	0.03709
Site 4, July 94, a	–	–	–	–	–	0.09124	0.09225	0.08816	0.09133
Site 4, July 94, b	–	–	–	–	–	0.06070	0.1121	0.03291	0.06073
Site 4, July 94, c	–	–	–	–	–	0.01129	0.01399	0.01859	0.11129
Site 4, July 94, d	–	–	–	–	–	0.1212	0.2430	–	0.1213
Site 5, July 94, a	–	–	–	0.1605	0.1724	0.1625	0.1612	0.1627	0.1625
Site 5, July 94, b	–	–	–	–	–	0.06070	0.1121	0.03291	0.07593
Site 5, July 94, c	–	–	–	–	–	–	–	–	–
Site 5, July 94, d	–	–	–	0.1257	0.1297	0.1597	0.1790	0.1583	0.1597
Site 1, Sept. 94, a	–	–	–	–	–	0.03882	0.05258	0.01891	0.03882
Site 1, Sept. 94, b	–	–	–	–	–	0.04488	0.05190	0.02945	0.04488
Site 1, Sept. 94, c	–	–	–	–	–	0.04848	0.06705	0.02402	0.04848
Site 1, Sept. 94, d	–	–	–	–	–	0.1188	0.1257	0.09824	0.1188
Site 2, Sept. 94, a	–	–	–	–	–	0.08335	0.1517	0.03204	0.08335
Site 2, Sept. 94, b	–	–	–	0.01652	0.01915	0.05484	0.08443	0.04968	0.05484
Site 2, Sept. 94, c	–	–	–	0.04466	–	0.2054	0.2376	0.1982	0.2054
Site 2, Sept. 94, d	–	–	–	0.01873	–	0.05381	0.08731	0.03311	0.05381

TABLE III (Continued)

	Models without growth of microorganisms					*Models with growth of microorganisms*			
	Eq. (1)	*Eq.* (2)	*Eq.* (3)	*Eq.* (4)	*Eq.* (5)	*Eq.* (6)	*Eq.* (7)	*Eq.* (8)	*Eq.* (9)
Site 3, Sept. 94, a	–	–	–	–	–	0.04590	0.06940	0.002283	0.04587
Site 3, Sept. 94, b	–	–	–	–	–	0.03313	0.04606	0.01655	0.03313
Site 3, Sept. 94, c	–	–	–	–	–	0.01455	0.03359	–	0.01455
Site 3, Sept. 94, d	–	–	–	–	–	0.01039	0.02475	0.01136	0.01039
Site 4, Sept. 94, a	–	–	–	0.07490	0.1347	0.2218	0.2686	0.2401	0.2218
Site 4, Sept. 94, b	–	–	–	0.04915	–	0.1827	0.2321	0.1730	0.1827
Site 4, Sept. 94, c	–	–	–	0.05343	0.02633	0.05545	0.1043	0.05420	0.05545
Site 4, Sept. 94, d	–	–	–	0.05885	0.08826	0.2485	0.2979	0.2607	0.2485
Site 5, Sept. 94, a	–	–	–	0.08612	0.1831	0.2086	0.2574	0.2025	0.2086
Site 5, Sept. 94, b	–	–	–	–	–	–	–	–	–
Site 5, Sept. 94, c	–	–	–	0.04638	0.09818	0.1540	0.1689	0.1586	0.1542
Site 5, Sept. 94, d	–	–	–	0.03433	0.1022	0.2293	0.3006	0.2356	0.2294

*not fitting.

amount mineralized at sites 4 and 5 could be a higher biological activity at the sites closest to the mouth of River Atoya. No significant difference were seen between the two months July and September. Both of them are in the rainy season.

Particle size distribution (high amount of sand) in most sediment samples indicates high stream velocity (Tab. I), and so it could be expected, that atmospheric air could reach the upper layer of the sediment. For that reason, incubations were performed a gentle stream of atmospheric air passing over the water saturated samples.

Tables V and VI show the total recovery of ^{14}C as $^{14}CO_2$, in glycerol, in the extract and in the combusted sediment in incubation experiments from July and September 1994, respectively. After 67 days between 9.73 and 16.18% of added ^{14}C had evolved as $^{14}CO_2$ in the July samples, and after 150 days between 11.18 and 27.37% of added ^{14}C had evolved as $^{14}CO_2$ from the September samples. Musumeci *et al.* [25] found that after 25 days in laboratory studies of Brasilian soil, 1% of added ^{14}C-maneb (740 $\mu g \cdot g^{-1}$soil) was mineralized to $^{14}CO_2$. In soil under field conditions (climate not reported), Rhodes [26] found that 50% of added ^{14}C in ^{14}C-maneb (2 lb/acre) had disappeared after 4–8 weeks. Rhodes did not find any leaching of neither maneb nor metabolites.

In the first set of samples from July 1994, which was only incubated for 67 days, 6.91–14.89% ^{14}C was present in the extract (Tab. V). The content of ^{14}C-ETU in the extracts was determined in the experiments

TABLE IV Estimated parameter according to Eq. (9) for mineralization studies of ^{14}C-maneb in the Nicaraguan estuary "EI Naranjo". c_0 = Total concentration of pesticide (%) converted to CO_2 by the modelled process after 67 days. k_1 = first order degradation rate constant. k_2 = second order degradation rate constant

	C_0 *mean ± std. dev*	k_1*mean ± std. dev*	k_2*mean ± std. dev*	*repl. no.*
Site 1, July 94	16.01 ± 1.63	0.06789 ± 0.00222	−0.004099 ± 0.003168	3
Site 2, July 94	15.78 ± 3.58	0.06909 ± 0.00595	−0.004244 ± 0.001534	4
Site 3, July 94	11.73 ± 4.05	0.07106 ± 0.01954	−0.006293 ± 0.003090	4
Site 4, July 94	21.20 ± 5.65	0.06031± 0.02221	−0.002977 ± 0.001518	4
Site 5, July 94	25.59 ± 5.90	0.04756 ± 0.02057	−0.001630 ± 0.001285	3
Site 1, Sept. 94	11.43 ± 2.55	−0.04247 ± 0.001136	−0.04995 ± 0.00352	4
Site 2, Sept. 94	14.44 ± 3.85	0.05526 ± 0.00998	−0.003792 ± 0.001746	4
Site 3, Sept. 94	7.419 ± 1.159	0.05096 ± 0.00339	−0.006721 ± 0.001282	4
Site 4, Sept. 94	19.32 ± 4.79	0.05459 ± 0.01745	−0.002684 ± 0.001413	4
Site 5, Sept. 94	18.09 ± 2.67	0.06368 ± 0.00193	−0.003090 ± 0.000528	3

TABLE V Degradation of ^{14}C-maneb (0.08 μg·g^{-1}) in sediment from Estuary "El Nicaragua. July 1994. Mean ± std.dev. Incubated 67 days

	% ^{14}C as $^{14}CO_2$	*%^{14}C in glycerol*	*%^{14}C in extract*	*% ETU in extract*	*% ^{14}C in combusted sediment*	*Mean total recovery of % ^{14}C* [a]
Site 1. Bocana	11.52 ± 2.88	0.00 ± 0.00	14.89 ± 4.60	not analyzed	39.32 ± 2.61	72.33 ± 15.31
Site 2. Frente a los Cocos	14.53 ± 3.50	0.01 ± 0.01	8.63 ± 1.13	–	48.69 ± 7.21	77.65 ± 8.68
Site 3. Isla Montano	9.73 ± 4.44	0.03 ± 0.04	8.46 ± 1.12	–	61.85 ± 5.87	84.61 ± 6.23
Site 4. Salida de Rio Atoya	16.04 ± 4.30	0.01 ± 0.01	7.19 ± 0.52	–	58.12 ± 16.22	87.17 ± 10.42
Site 5. Atoya Empalme	16.18 ± 9.38	3.64 ± 6.99	6.91 ± 0.65	–	44.91 ± 7.21	77.41 ± 18.03

[a] ^{14}C as $^{14}CO_2$ + ^{14}C in extract + ^{14}C in glycerol + ^{14}C in combusted soil.

TABLE VI Degradation of ^{14}C-maneb (0.08 $\mu g \cdot g^{-1}$) in sediment from Estuary "El Nicaragua. Sept. 1994. Mean ± std.dev. Incubated 150 days

	% ^{14}C as $^{14}CO_2$	% ^{14}C in glycerol	% ^{14}C in extract	% ETU in extract	% ^{14}C in combusted sediment	Mean total recovery of % ^{14}C [a]
Site 1. Bocana	21.35 ± 3.65	0.00 ± 0.00	8.20 ± 0.27	0.00 ± 0.00	29.24 ± 3.64	58.80 ± 2.63
Site 2. Frente a los Cocos	21.92 ± 5.28	0.00 ± 0.00	6.11 ± 0.47	0.85 ± 0.51	49.32 ± 6.91	78.19 ± 5.82
Site 3. Isla Montano	11.18 ± 1.43	0.00 ± 0.00	6.92 ± 0.83	2.72 ± 0.43	63.13 ± 1.45	83.95 ± 0.61
Site 4. Salida de Rio Atoya	24.92 ± 3.89	0.00 ± 0.00	5.34 ± 0.61	1.15 ± 0.27	47.16 ± 6.38	78.57 ± 4.72
Site 5. Atoya Empalme	27.37 ± 3.27	0.00 ± 0.00	4.61 ± 0.42	0.49 ± 0.11	36.59 ± 5.62	69.06 ± 4.18

[a] ^{14}C as $^{14}CO_2$ + ^{14}C in extract + ^{14}C in glycerol + ^{14}C in combusted soil.

from September. The extraction method extracts ^{14}C-ETU and other water soluble metabolites. Table IV shows that when 4.61 – 8.20% of added ^{14}C is found in the extract, only a small fraction is ^{14}C-ETU. In a lysimeter study, where the lysimeters were grown with potatoes in temperature climate and treated 6 times with ^{14}C-maneb (2 kg a.i./ha), Fomsgaard & Helweg [27] showed, that the amount of ETU in drain water never exceeded 0.1 $\mu g \cdot l^{-1}$. Kaufmann and Fletscher [22] made degradation studies of ^{14}C-ETU in soil and found that after two days all ^{14}C-ETU was transformed to ^{14}C-EU and after 4 days 43% was mineralized to $^{14}CO_2$. ETU was reported to be degraded to EU in sterile as well as in non-sterile soils, but a total mineralization of ETU to CO_2 did only occur in non-sterils soils, [28, 1].

Identification of other water extractable metabolites in future studies is recommendable. Until now it has been impossible to purchase Jaffe's base and 2-imidizoline. The amount of ^{14}C present in combusted sediment could be strongly adsorbed ^{14}C-maneb as well as ^{14}C built into organic matter. The curves from September 1994 (Figs. 7 – 11) show that a decline in $^{14}CO_2$ formation has begun (the curves have flattened out). Other studies [11, 13] have shown that the "flat" part of a mineralization curve owe to the slow mineralization of ^{14}C which has been built into organic matter.

Degradation studies of ^{14}C-maneb (2 $\mu g \cdot g^{-1}$) in lake sediment from Denmark, incubated at 20°C, followed the same kinetics (Eq. (9)) (Fig. 13) as in the sediment samples from the Nicaraguan estuary (incubated at 25°C) but the degradation was faster in the Danish lake (Tab. VII) where 30.9% ^{14}C was evolved as CO_2 after 70 days. In ploughlayer samples from a Danish sandy soil (^{14}C-maneb, 2 $\mu g \cdot g^{-1}$ 20°C), the degradation followed no-growth kinetics (Eq. (3)) (Fig. 14), but the amount of % ^{14}C evolved as $^{14}CO_2$ (29.8 – 36.2% after 70 days) was higher than in the sediment samples from the Nicaraguan estuary. These differences could owe to various factors not determined (amount of nutrients, amount of organic material, concentration of pesticide, amount of available oxygen, and microbial activity and diversity). The degradation of low concentrations of ^{14}C-maneb in Nicaraguan sediment is slower than in the compared samples from Denmark, but formation of ETU from maneb in the Nicaraguan estuary does not seem to be a theme of much concern.

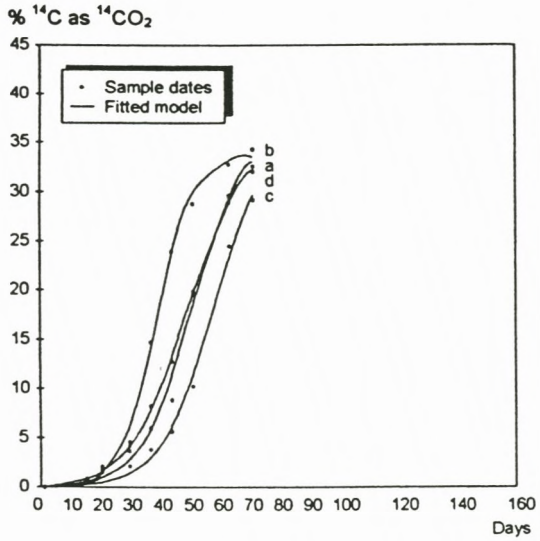


FIGURE 13 Mineralization of 2 $\mu g \cdot g^{-1}$ ^{14}C-maneb in lake sediment, Tuel Sϕ, Denmark.

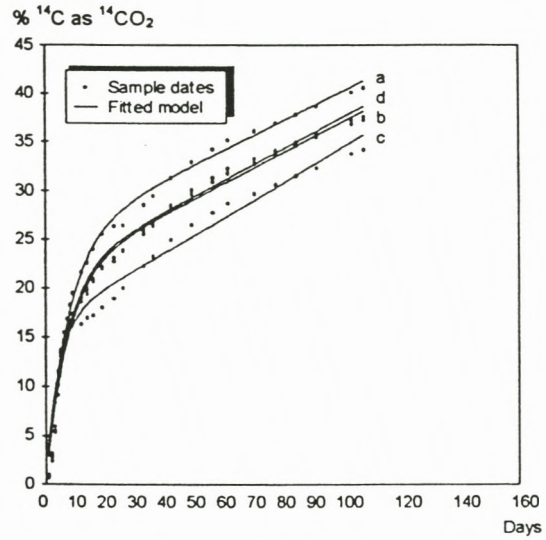


FIGURE 14 Mineralization of 2 $\mu g \cdot g^{-1}$ ^{14}C-maneb in plough layer soil, Fladerne Baek, Denmark.

TABLE VII Degradation of ^{14}C-maneb ($2.0\,\mu g \cdot g^{-1}$) in sediment from lake "Tuelsø", Denmark (incubated 70 days) and in sandy ploughlayer soil, Fladerne Baek, Denmark (in-cubated 108 days). June, 1994. Mean ± std.dev

	% ^{14}C *as* $^{14}CO_2$	% ^{14}C *in extract*	% ^{14}C *in combusted sediment*	*Mean total recovery of* % ^{14}C [a]
Lake Tuelsø	30.9 ± 2.6	4.6 ± 1.0	38.7 ± 4.4	74.3 ± 3.3
Ploughlayer soil	37.7 ± 2.7	0.9 ± 0.1	44.5 ± 3.3	83.1 ± 4.3

[a] ^{14}C in CO_2 + ^{14}C in extract + ^{14}C in combusted soil.

CONCLUSIONS

The best fit of the curves depiciting mineralization of ^{14}C-maneb to $^{14}CO_2$ were equations that include microbial growth, indicating that biological metabolism is involved in the total degradation of maneb to CO_2.

According to the present results, where the incubated concentrations of ^{14}C-maneb resembled possible concentrations of maneb in the estuary after normal agricultural use, the use of this fungicide in the catchment area of the Nicaraguan estuary "El Naranjo" does not seem to cause problems concerning accumulation of ETU in the estuarine sediment. The mineralization of ^{14}C-maneb in the sediment was slow. Investigations about formation of other metabolites than ETU are therefore recommended.

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The skillful assistance of Helle Priess and Marianne Nielsen in the technical laboratory work, of Sonja Graugaard adapting the manuscript and of Henny Rasmussen drawings figures is highly appreciated.

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DEGRADATION OF MECOPROP AND ISOPROTURON IN SOIL INFLUENCE OF INITIAL CONCENTRATION

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Models used to describe rates of degradation are presented and exemplified, and data from mecoprop at 0.0005 to 5000 mg kg^{-1} and isoproturon at 0.001 to 5000 mg kg^{-1} were tested in the models. Degradation was described by evolution of $^{14}CO_2$ from ^{14}C-labelled pesticides incubated in soil sampled in plough layer and in subsurface.

For mecoprop the degradation rate of 0.0005 mg kg^{-1} followed first-order models in both plough layer and in subsoil. At 5 mg kg^{-1} the degradation showed kinetics with exponential growth in both surface and subsoil. At 5000 mg kg^{-1} the degradation was very slow.

The degradation of isoproturon at all concentrations and soil types followed kinetics without growth of microorganisms. The model that gave the best fit for degradation of isoproturon was a three-half order model consisting of one first-order process and one of zero-order.

The rate of degradation for both pesticides and soil types was highest at the low concentrations, whereas at 5000 mg kg^{-1} the degradation was very low. Thus degradation appears even at concentrations near the drinking water limit whereas the degradation at very high concentration e.g. near point sources with pesticides may be very limited or absent.

Keywords: Degradation kinetics; pesticides; mecoprop; isoproturon; concentrations

INTRODUCTION

Pesticides can appear at a wide range of concentrations in soil. Typical initial mean concentrations in the top 10 cm of field soils are from 0.02 mg kg^{-1} for the low dose herbicides to about 1 mg kg^{-1}. The real concentrations in the treated soil on the other hand vary much more, and during degradation and after leaching of pesticides out of the plough layer very low concentrations will appear in

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subsurface. Very high concentrations may appear from point sources like pesticide spillage on sites used for filling of sprayers and on waste disposal sites.

All these ranges of concentrations from several thousands mg kg^{-1} to below 0.0001 mg kg^{-1} have to be decomposed in the soil since even concentrations of 0.0001 mg kg^{-1} are relevant for protection against ground water pollution at the EEC drinking water limit which is 0.0001 mg l^{-1}. It is possible to determine rates of degradation at this wide range of concentrations by the use of evolution of $^{14}CO_2$ from ^{14}C-labelled pesticides.

Figure 1 shows the ranges of pesticide concentrations which can be found in the environment. The high concentration may appear from pesticides disposed on waste disposal sites, total weed control and spill on filling sites. Very low concentrations of pesticides in soil may appear after deposition of pesticides on untreated areas from rainwater, low concentrations of pesticides are also found in the ground water zone and in drain water.

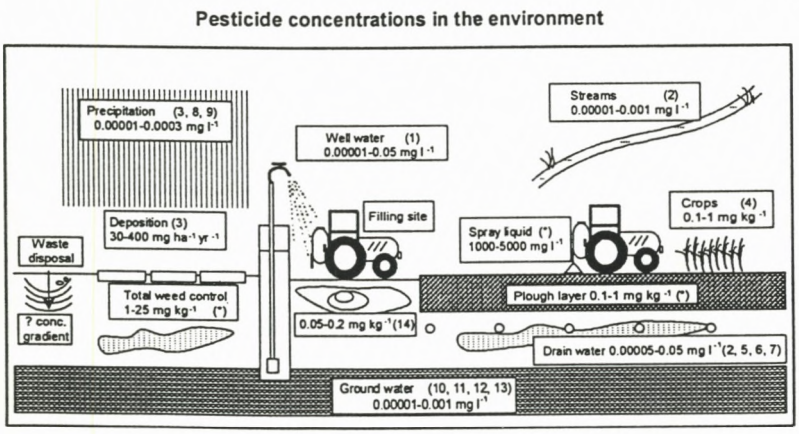


FIGURE 1 Concentration ranges of pesticides identified in the environment in Northern Europe. Numbers on the figure refer to the references. * Calculated from use rates.

Waste disposal sites

Pesticide waste may have been disposed of in large quantities during the past 40 years. Such waste appears when farmers dispose of "empty" containers, and where pesticide residues (such as pesticides destroyed by improper storage, e.g. low temperature) are disposed of. Pesticide waste may also come from effluent from production plants, broken packages etc. Until recently, there was no proper

way to dispose of this waste, and most of it was either buried on farm land and near factories, disposed of on private waste disposals or was brought to municipal land fills. It is difficult to determine the abundance of these pesticide point sources, since little is known about the disposal of chemical waste in earlier days.

Finding of mecoprop and dichlorprop in US municipal landfills[15] have led to the conclusion that "the chlorinated 2-phenoxypropionic herbicides, particular mecoprop, are ubiquitous in municipal landfill leachates from US". Phenoxypropanoic acids have also been identified in leachate from Danish landfill[16].

Total weed control

The application of pesticides is very often much higher for total weed control than for normal treatment on agricultural land. Previous advise has been to use up to 10 to 20 kg a.i./ha of atrazine and simazine, 12 to 30 kg a.i./ha of monuron and diuron and 15 to 20 kg a.i./ha of chlorthiamid and dichlobenil[17]. These sites, which may be road sides, industrial areas, railways and farm yards are often covered by gravel and sand low in organic material like soil sampled in subsurface. The degradation rate on these sites will therefore be very much slower than in field soil[18]. It is not surprising, that very often found pollutants in Danish groundwater are 2,6-dichlorobenzamide (BAM), a mobile metabolite of chlorthiamid and dichlobenil, and metabolites from atrazine[19].

Filling of sprayers

Filling of sprayers and rinsing of spraying equipment will often be performed on the same site year after year. Pesticides from surplus of diluted pesticide solutions, which may contain 1000 to 5000 mg l^{-1} of pesticide, spillage of concentrated chemicals and run off from spray washing may end up here. Jørgensen et al.[14] have found concentrations of mecoprop and dichlorprop of 0.1 to 0.2 mg kg^{-1} 4 meters below such a site.

Deposition from precipitation

Cleemann et al.[8] found a deposition of γ-HCH of 70 to 170 mg per ha per year in Denmark. From Sweden, Kreuger[3] has reported deposition of 30 to 50 mg of phenoxyherbicides per ha per year and in Germany, depositions of about 400 mg ha^{-1} of lindane and up to 200 mg ha^{-1} of isoproturon have been found[9]. The depositions are highest in the spraying season[3]. A deposition of 50 to 100 mg ha^{-1} yr^{-1} may result in a concentration in the top 1 cm of soil of about 0.0003 to

0.001 mg kg^{-1}. It is important, that also these low concentrations can be decomposed.

Ground water and drain water

Findings in ground- and drainwater have shown pesticide contents between 0.00005 and 0.05 mg l^{-1} in drain water[2,5,6,7] and between 0.00001 and 0.001 mg l^{-1} in ground water[10,11,12,13]

The European Community Directive on Drinking water quality (The drinking water directive, DWD) from 1980 stated that pesticides and related products in drinking water should not exceed 0.1 µg l^{-1}(0.0001 mg l^{-1}) for individual pesticides and 0.5µg l^{-1} for total pesticides.

Influence of concentration on degradation

Degradation kinetics has previously been shown to depend on concentrations. For pesticides which are degraded by metabolism, exponential degradation may be found showing proliferation of degrading micro-organisms. At normal field concentrations this has been shown for the herbicide mecoprop[20,21] and for MCPA[22,23].

At low concentrations however, the degradation kinetics may be of first order. This has also previously been shown for very low concentrations of phenol and p-nitrophenol[24] and for 2,4-D[25].

At very high concentrations the degradation rates may be very low. Ou et al.[26] thus found 2,4-D to be very slowly degraded at concentrations of 20000 mg kg^{-1}, either due to toxic effect on the micro-organisms or due to limited availability of supplementary nutrients. This is also found for mecoprop and indicates, that point sources may be very long lasting[20].

The purpose of the present study was to elucidate the mineralization kinetics for pesticides at different concentrations. The kinetics are exemplified by results with mecoprop and isoproturon both in plough layer and in subsurface soil.

MATERIALS AND METHODS

Pesticide degradation was determined by the evolution of $^{14}CO_2$ from ^{14}C-labelled pesticides. Mecoprop (^{14}C-ringlabelled) (Figure 2a), was incubated in a flow-through system, where 50 g soil was incubated in 100 ml Erlenmeyer flasks and moistened CO_2-free air was led through the flask and then through one

absorber with glycerol and two with 1 N KOH to absorb evaporated compounds soluble in oil and $^{14}CO_2$ respectively. For the isoproturon-experiment, 50 g of soil with added ^{14}C-ringlabelled isoproturon (Figure 2b), was incubated in a 100 ml beaker which was stored in a closed 11 glass jar with a 50 ml beaker with 10 ml 1 N KOH to absorb $^{14}CO_2$.

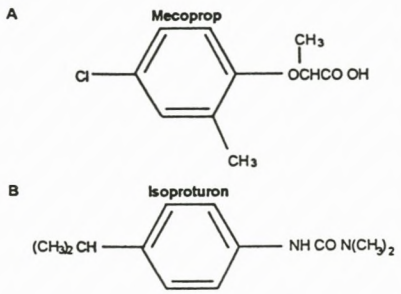


FIGURE 2 A. Structure of the herbicide mecoprop ((±)-2-(4-chloro-2-methylphenoxy)propanoic acid) and B. isoproturon (N,N-dimethyl-N'-[4-(1-methylethyl)phenyl]urea). Both are labelled with ^{14}C in the phenylring

Soil was sampled at Research Centre Flakkebjerg. The soil had not been treated with mecoprop or isoproturon for the last 2 years. Surface soil was collected at a depth of 0–30 cm and subsurface soil at 40–60 cm. After sampling, the soils were dried to about 25% of total water holding capacity (WHC) with frequent mixing to avoid extreme superficial dry-out. The dried soil was sieved to <2 mm and stored at 5°C for not more than 0.5 month before use. Table I shows the composition of the soil.

TABLE I Texture, pH (H_2O) and humus content in the soil

Depth cm	*Clay*	*Silt*	*Sand*	*Humus*	*pH*
0–30	14.3	17.7	65.2	2.9	6.1
40–60	22.9	11.1	65.7	0.3	6.5

Clay: <0.002 mm, Silt: 0.002 – 0.02 mm, Sand 0.02 – 2 mm. Humus: %C × 1.72

Accumulated amounts of evolved $^{14}CO_2$, calculated as percentage radioactivity of the total amount of added radioactivity, were described as a function of incubation time, $^{14}CO_2$ then corresponding to the amount of mineralised pesticide. A number of non-linear models were fit to the curves to evaluate the differences in the kinetics of mineralization.

Table II shows the degradation models which were tested in the present experiments.

TABLE II Models which have been tested for the best fit with the $^{14}CO_2$-evolution data

Model	*Equation*
0. order[27]	$P = k_0 t$ eq.(1)
	P = concentration of pesticide mineralised at time t (measured as % ^{14}C as $^{14}CO_2$)
	k_0 = degradation rate constant
	t = time in days
1. order[28,29,30]	$P = c_0(1 - e^{-kt})$ eq.(2)
	P = concentration of pesticide mineralised at time t (% ^{14}C as $^{14}CO_2$)
	c_0 = total concentration of pesticide converted by the process to $^{14}CO_2$
	k = degradation rate constant
	t = time in days
Two-compartment 1. order[24,31]	$P = c_1(1-e^{-k_1 t}) + c_2(1-e^{-k_2 t})$ eq.(3)
	P = concentration of pesticide mineralised at time t (% ^{14}C as $^{14}CO_2$)
	c_1 = total concentration of pesticide converted to $^{14}CO_2$ by one first-order metabolism
	c_2 = total concentration of pesticide converted to $^{14}CO_2$ by another first-order metabolism
	k_1, k_2 = degradation rate constants for the two first-order processes
	t = time in days
Three half order without growth[32,24,28]	$P = c_0(1-e^{-k_1 t}) + k_0 t$ eq.(4)
	P = concentration of pesticide mineralised at time t (% ^{14}C as $^{14}CO_2$)
	c_0 = total concentration of pesticide converted to $^{14}CO_2$ by first-order metabolism
	k_1 = degradation rate constant for the first-order process
	k_0 = degradation rate constant for the zero-order process
	t = time in days
Logistic growth[33,34]	$P = c_0 - \frac{c_0 + x_0}{1 + \left(\frac{x_0}{c_0}\right) e^{k_1 (c_0 + x_0) t}}$ eq.(5)
	P = concentration of pesticide mineralised at time t (% ^{14}C as $^{14}CO_2$)
	c_0 = total concentration of pesticide converted to $^{14}CO_2$ by first-order metabolism
	x_0 = the amount of substrate (pesticide) required to produce the initial population density
	k = degradation rate constant
	t = time in days

Logistic growth + 0. order

$$P=c_0-\frac{c_0+x_0}{1+\left(\frac{x_0}{c_0}\right)e^{k_1(c_0+x_0)t}}+k_0t \quad \text{eq.(6)}$$

P = concentration of pesticide mineralised at time t (% ^{14}C as $^{14}CO_2$)
c_0 = total concentration of pesticide converted to $^{14}CO_2$ by first-order metabolism
x_0 = the amount of substrate (pesticide) required to produce the initial population density
k = degradation rate constant
k_0 = degradation rate constant for zero order degradation
t = time in days

Logistic growth[35,36]

$$P=c_0-\frac{k_1c_0}{(k_1+k_2c_0)e^{k_1t}-k_2c_0} \quad \text{eq.(7)}$$

P = Concentration of pesticide mineralised at time t (% ^{14}C as $^{14}CO_2$)
c_0 = total concentration of pesticide converted to $^{14}CO_2$ by the modelled process
k_1 = rate constant
k_2= rate constant
t = time in days

Logistic growth + 0. order

$$P=c_0-\frac{k_1c_0}{(k_1+k_2c_0)e^{k_1t}-k_2c_0}+k_0t \quad \text{eq.(8)}$$

P = Concentration of pesticide mineralised at time t (% ^{14}C as $^{14}CO_2$)
c_0 = total concentration of pesticide converted to $^{14}CO_2$ by the modelled process
k_1 = rate constant
k_2= rate constant
k_0 = degradation rate constant for zero order degradation
t = time in days

Exponential growth, low concentration[27]

$$P=c_0-c_0e^{-(k/r)(e^{rt}-1)} \quad \text{eq.(9)}$$

P = Concentration of pesticide mineralised at time t (% ^{14}C as $^{14}CO_2$)
c_0 = total concentration of pesticide converted to $^{14}CO_2$ by the modelled process
k = degradation rate constant
r = the maximum specific growth rate
t = time

Exponential growth + 0. order, low conc.

$$P=c_0-c_0e^{-(k/r)(e^{rt}-1)}+k_0t \quad \text{eq.(10)}$$

P = Concentration of pesticide mineralised at time t (% ^{14}C as $^{14}CO_2$)
c_0 = total concentration of pesticide converted to $^{14}CO_2$ by the modelled process
k = degradation rate constant
k_0 = degradation rate constant for zero order degradation
r = the maximum specific growth rate
t = time

Exponential growth, high concentration[27]

$$P = k\frac{(e^{rt}-1)}{r} \quad \text{eq.(11)}$$

P = Concentration of pesticide mineralised at time t (% ^{14}C as $^{14}CO_2$)
k = degradation rate constant
r = the maximum specific growth rate
t = time

Exponential growth + 0. order, high conc.

$$P = k\frac{(e^{rt}-1)}{r} + k_0 t \quad \text{eq.(12)}$$

P = Concentration of pesticide mineralised at time t (% ^{14}C as $^{14}CO_2$)
k = degradation rate constant
k_0 = degradation rate constant for zero order degradation
r = the maximum specific growth rate
t = time

The software used was Table Curve 2D[37]. The principles for the non-linear regression were previously described by Fomsgaard[38].

RESULTS AND DISCUSSIONS

General description of degradation rates

Degradation may be described by the degradation of parent compound or in some cases even "disappearance" which also may involve evaporation, leaching and sorption in the soil. To use a more sensitive measure of degradation rate, these experiments use evolution of $^{14}CO_2$ from ^{14}C-labelled pesticides. It should be taken into account, that evolution of CO_2 expresses the total mineralization, which is supposed to be "real" degradation. The degradation normally appears via a number of degradation products and finally ending up in CO_2 -evolution with some carbon from the pesticide being built into micro-organisms and in organic compounds in soil. Thus, Helweg[39] showed that when 12% ^{14}C from ^{14}C-labelled mecoprop was evolved as $^{14}CO_2$, only 50% of the applied mecoprop could be recovered in the soil.

Generally 3 different rate models are known to be useful for describing mineralization of pesticides. First-order (degradation rate dependent on concentration), zero-order (constant degradation rate) and models which involve growth of micro-organisms, either with exponential growth or with logistic growth which is limited by availability of substrate.

Figure 3 shows general diagrams for the three different degradation models, both shown by degradation of parent compound and by formation of $^{14}CO_2$.The figures are based on a relation between parent compound degradation and $^{14}CO_2$-formation of 2 to 1 e.g. when 10% of the ^{14}C-labelled parent compound is degraded, 5% of the added ^{14}C is evolved as $^{14}CO_2$.

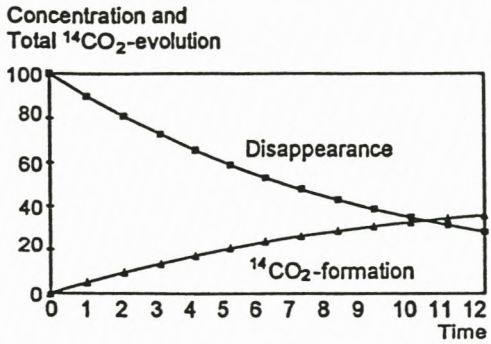
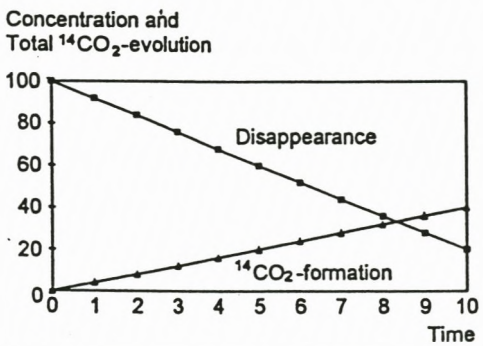


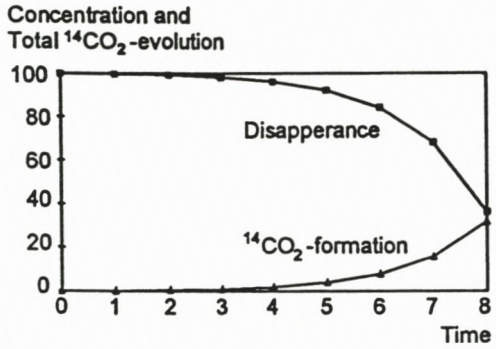
FIGURE 3 General diagramme of models for pesticide degradation.
A. first-order reaction kinetics[30]

Rate equation: $-\frac{dc}{dt}=kc$ Disappearance: $c=c_0e^{-kt}$. Formation: $P=c_0(1-e^{-kt})$.



B. Zero-order reaction kinetics[27]

Rate equation: $-\frac{dc}{dt}=k_0$ Disappearance : $c=c_0-k_0t$. Formation: $P=k_0t$.



C. Degradation with growth

Rate equation, log. growth[33]: $-\frac{dc}{dt}=k_1c(c_0+x_0-c)$

Disappearance, log. growth[33]: $c=\frac{c_0+x_0}{1+\left(\frac{x_0}{c_0}\right)e^{k_1(c_0+x_0)t}}$

Formation, log. growth[33]: $P=c_0-\frac{c_0+x_0}{1+\left(\frac{x_0}{c_0}\right)e^{k_1(c_0+x_0)t}}$

Rate equation, exp. growth[27]: $-\frac{dc}{dt}=ke^{rt}$

Disappearance, exp. growth[27]: $c=c_0-\frac{k(e^{rt}-1)}{r}$

Formation, exp. growth[27]: $P=k\frac{(e^{rt}-1)}{r}$

Modelling results for mecoprop

The models from Table II were tested for the rate of $^{14}CO_2$-evolution from a number of concentrations of mecoprop from 0.0005 mg kg^{-1} to 5000 mg kg^{-1} and for isoproturon from 0.001 to 5000 mg kg^{-1} in plough layer and in subsurface soil.

The residual mean for the fitted equations, as presented inTable III, served as a measure of the goodness of fit. The lowest residual mean gives the best fit. When a model did not fit, no value is shown. Figure 4 shows the mineralization curves for mecoprop, a) plough layer, b) subsurface soil and Figure 5 shows the mineralization curves for isoproturon, a) plough layer, b) subsurface soil. The fit of the best model for each sample is presented as the solid line in Figures 4 and 5, whereas the dots show the actual $^{14}CO_2$ evolution data.

TABLE III Residual mean for all fitted equations. Best fit is in italics

sample	*Equations without growth of micro-organisms*				*Equations with growth of micro-organisms*								
	eq(1)	*eq(2)*	*eq(3)*	*eq(4)*	*eq(5)*	*eq(6)*	*eq(7)*	*eq(8)*	*eq(9)*	*eq(10)*	*eq(11)*	*eq(12)*	*Fig. ref.*
Mecoprop, plough layer													
0.0005 mg.kg^{-1}	-*)	11.25	*0.3517*	0.6185	-	-	-	-	-	-	-	-	5a
5 mg kg^{-1}		80.92	-	-	-	*4.8645*	-	-	-	-	-	-	5a
50 mg kg^{-1}	-	175.93	-	-	6.5725	*2.4824*	-	-	-	-	-	-	5a
5000 mg kg^{-1}	-	-	-	-	-	-	-	-	-	-	-	-	5a
Mecoprop, subsurface													
0.0005 mg kg^{-1}	59.46	*0.9200*	-	-	-	-	-	-	-	-	-	-	5b
5 mg kg^{-1}	8.29	-	-	-	-	-	-	-	-	-	0.7938	-	5b
50 mg kg^{-1}	-	-	-	-	-	-	-	-	-	-	-	-	5b
500 mg kg^{-1}	-	-	-	-	-	-	-	-	-	-	-	-	5b
Isoproturon, plough layer													
0.001 mg kg^{-1}	0.8971	0.02651	-	*0.01209*	-	-	-	-	-	-	-	-	6a
5 mg kg^{-1}	0.4684	0.008999	-	*0.002063*	-	-	-	-	-	-	-	-	6a
50 mg kg^{-1}	0.2337	*0.08047*	-	-	-	-	-	-	-	-	-	-	6a
5000 mg kg^{-1}	0.2628	0.05187	-	*0.0005091*	-	-	-	-	-	-	-	-	6a
Isoproturon, subsurface													
0.001 mg kg^{-1}	0.01791	-	-	-	-	-	-	-	-	-	-	-	6b
5 mg kg^{-1}	0.07791	0.005642	-	*0.0000422*	-	-	-	-	-	-	-	-	6b
50 mg kg^{-1}	0.07259	0.003699	-	*0.0001177*	-	-	-	-	-	-	-	-	6b
5000 mg kg^{-1}	-	-	-	-	-	-	-	-	-	-	-	-	6b

*) – no useable fit

As appears from Table III, mecoprop mineralization in a concentration of 0.0005 mg kg^{-1}, both in plough layer and subsoil followed kinetics without growth.

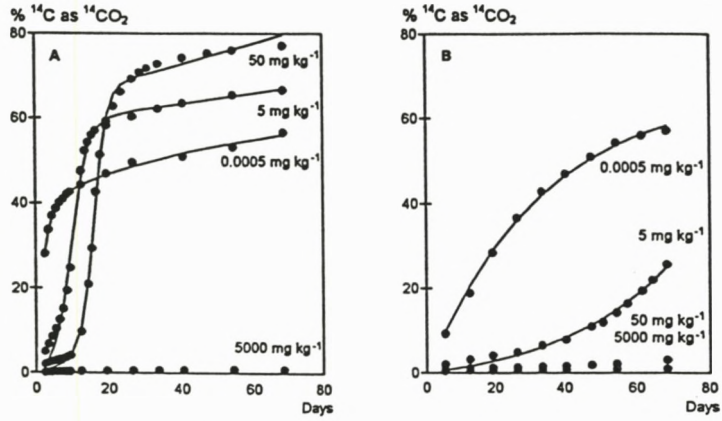


FIGURE 4 Degradation of ^{14}C-labelled mecoprop in soil at different concentrations shown by the evolution of $^{14}CO_2$. Mean of two replicates. Data taken from Reffstrup et al.[20]. Dots: data points, solid line: modelled equation. By courtesy Pesticide Science, SCI.
A. $^{14}CO_2$-evolution in soil from plough layer. 0.0005 mg kg^{-1} no-growth model; 5 mg kg^{-1} growth model; 50 mg kg^{-1} growth model; 5000 mg kg^{-1} no useable fit.
B. $^{14}CO_2$-evolution in subsoil. 0.0005 mg kg^{-1} no-growth model; 5 mg kg^{-1} growth model; 50 mg kg^{-1} no useable fit; 500 mg kg^{-1} no useable fit

The best model fit for mecoprop 0.0005 mg kg^{-1} in plough layer was given by eq. (3), a two-compartment first-order model, which consists of two simultaneously occurring first order processes. Probably one (rapid) first order process (rate constant k_1= 0.47), dominating in the beginning, expresses the mineralization of the pesticide in solution. The other (slower) first order process (the "flat" part of the curve, rate constant k_2 = 0.02), dominating from about 15 days, may express the degradation of slowly released mecoprop or degradation of organic compounds e.g. humus where part of the added ^{14}C had been built in[38]. Former studies[39] showed, that when 40% of ^{14}C-mecoprop has been converted to $^{14}CO_2$, no significant amounts of ^{14}C-mecoprop could be extracted.

Mineralization of 0.0005 mg kg^{-1} mecoprop in subsoil followed eq. (2), a simple 1. order model with a rate constant k=0.02. The rate of degradation at low concentrations of the pesticides may be limited by the rate of diffusion of the substrate to a widely distributed, but very small population of micro-organisms, which are able to metabolise the pesticides. This is even more pronounced in the

subsoil, with the lower biomass. Even if the mineralization rate of mecoprop was faster in plough layer than in subsoil, a higher amount of added ^{14}C-mecoprop was converted to $^{14}CO_2$ in subsoil than in plough layer. The presence of higher amounts of humus in plough layer may favour processes where ^{14}C from mecoprop is built into humus or where mecoprop is made unavailable to degradation.

Mineralization of 5 and 50 mg kg^{-1} mecoprop in plough layer and 5 mg kg^{-1} in subsoil soil followed kinetics with growth (eq. 5 to 12).

The models were based on logistic growth of micro-organisms, where there is a limitation for growth, and on exponential growth, where there is no limitation. Two different models with logistic growth (eq. (5) and eq.(7)), one model with exponential growth and low concentration of substrate (here: pesticide) (eq. (9)) and one with exponential growth and high concentration of substrate (eq. 11) were tested.

For some of the data presented, a very slow zero-order like phase was seen at the end of the experiment. For that reason the logistic and the exponential models were combined with zero order degradation, too, to test the fit (eq. (6),(8),(10),(12)).

The residual means obtained from the fits are shown in Table III.

For both 5 and 50 mg kg^{-1} mecoprop in plough layer the mineralization followed kinetics with logistic growth combined with a zero order process (eq. (6)). Since probably no available ^{14}C-mecoprop was left after 20–30 days, these limitations made kinetics with logistic growth give the best fit.

The mineralization of 5 mg kg^{-1} mecoprop in subsurface followed kinetics with exponential growth, high conc. (eq. 11). At the end of incubation time (70 days) 28 % of the added ^{14}C-mecoprop was mineralised to $^{14}CO_2$, and probably ^{14}C-mecoprop was still left in the soil.

At the very high concentration 5000 mg kg^{-1} in plough layer and 50 and 5000 mg kg^{-1} in subsoil, as they might appear near point sources, the degradation was very slow, and no usable model fits could be found. The toxicity of the pesticide to the micro-organisms may be limiting for the degradation. The rates of degradation during the first week are only about 5 to 10% in subsoil compared to ploughlayer soil for most of the concentrations tested.

Degradation of phenoxyherbicides has previously been reported as taking place through a metabolic process, because enhanced degradation rate appeared at repeated application to microbial communities[40,41]. The present results show that kinetics for mecoprop degradation highly depend on the initial concentration.

Modelling results for isoproturon

Figures 5a and b show the degradation of isoproturon 0.001, 5, 50 and 5000 mg kg^{-1} in ploughlayer and subsurface soil, respectively.

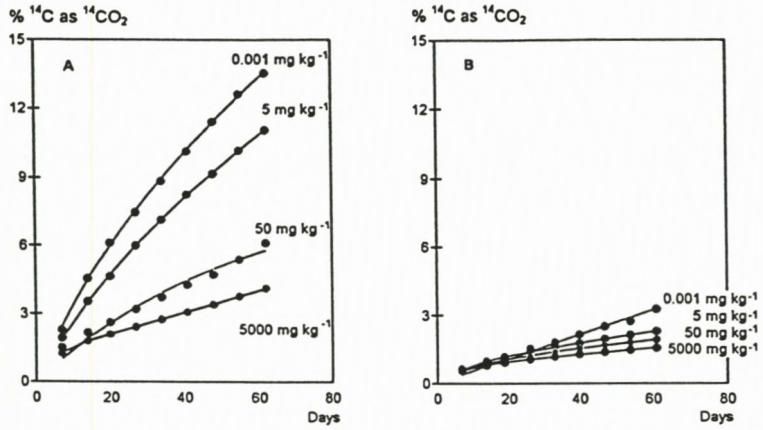


FIGURE 5. Degradation of ^{14}C-labelled isoproturon in soil at different concentrations shown by the evolution of $^{14}CO_2$. Mean of three replicates. Dots: data points, solid line: modelled equation
A. $^{14}CO_2$-evolution in soil from plough layer. All concentrations no-growth model.
B. $^{14}CO_2$-evolution in subsoil. 5000 mg kg^{-1} no useable fit. All other concentrations no growth model

For all concentrations of isoproturon both in plough layer and subsoil (except 5000 mg kg^{-1} in subsoil, where the degradation was too slow to give usable fits) the mineralization followed kinetics without growth of micro-organisms. The model that gave the best fit in most cases was eq. (4) (lowest residual mean, see Table III), a three-half order model, consisting of one first order process and a zero order process.

The explanation given by Brunner and Focht and Scow et al.[32,24] for the fit of the three-half order model to mineralization curves was, that the first order process expressed the mineralization of the chemical in solution and that the zero order process expressed the conversion of humus, where ^{14}C had been built into it may also express the degradation of slowly released isoproturon. The same concept was useful for explaining mineralization kinetics of low concentrations of pesticides, previously analysed by Fomsgaard[38]. In the present case, the kinetics composed of both a first and a zero order process occurred even if only <15 % of ^{14}C isoproturon was converted to $^{14}CO_2$, and the curve still had a steep raise. The adsorption and slow release of isoproturon could be the explanation for this. The three-half order model was also seen by Dörfler et al. (1996)[42] for the degradation of ^{14}C-DEHP in different soil samples.

Degradation rates of isoproturon are obviously slower in subsoil than in plough layer with rates about 20% of ploughlayer (Figures 5a and b). The rate of degradation in subsoil may be limited by the supply of inorganic nutrients, and the lower number of micro-organisms present in the subsoil. As seen for mecoprop the degradation rate of isoproturon is slowest at the high concentrations, though there was not seen a complete stop of the degradation at any concentration of isoproturon.

CONCLUSIONS

- The concentrations of pesticides in the environment vary from concentrated chemicals in waste disposals to trace concentrations near or below the drinking water limit.
- Trace concentrations were degraded fastest and followed first order reaction kinetics for both mecoprop and isoproturon.
- Degradation took place even at concentrations in soil near the drinking water limit.
- Degradation rate at field concentrations showed growth for mecoprop (metabolic degraded pesticide) and first order reaction kinetics for isoproturon (cometabolic degraded).
- High concentrations were degraded relatively slow. Degradation in subsurface soil was most sensitive to high concentrations.
- Degradation in surface and subsurface soil showed identical patterns but the rate in subsurface was only about 5 to 20% of the rate in surface soil.
- Under aerobic condition, trace concentrations of mecoprop and isoproturon can be degraded in both surface and subsurface soil.
- High concentrations of pesticides are degraded so slow, that point sources often will be very long lasting pollution sources.

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ETU MINERALIZATION IN SOIL UNDER INFLUENCE OF ORGANIC CARBON CONTENT, TEMPERATURE, CONCENTRATION, AND DEPTH

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The mineralization of ^{14}C-ETU was measured by the evolution of $^{14}CO_2$ and described with a mathematical model consisting of two terms – one term describing the immediate mineralization of ^{14}C-ETU and another term describing the first order degradation of humus and/or biomass, where ^{14}C had been built in. The influence of pesticide concentration, depth of soil, and incubation temperature showed combined interaction effects on the amount of $^{14}CO_2$ formed during the process and on the degradation rate of the pesticide. With the addition of soil extract, a combined effect between concentration and addition of organic extract was seen for the degradation rate, while a three-way interaction between depth, concentration and organic extract was seen for the formation of $^{14}CO_2$. Degradation of ^{14}C-ETU can thus not be described only through investigations of one single of the mentioned parameters.

Keywords: Ethylene thiourea; plough layer soil; subsoil; mineralization kinetics; degradation rates; organic carbon; temperature; concentration; interaction effects

INTRODUCTION

Fungicides of the EBDC group, maneb, mancozeb and zineb, have been widely used in the cultivation of potatoes, vegetables and berries. In the

* Corresponding author.

year 1995, 251 t maneb and 258 t mancozeb (a.i.) was sold in Denmark for agricultural purposes [1].

ETU (ethylene thiourea) is present as an impurity (up to 10%) in fungicides of the EBDC group, and it is also an important metabolite from the biotic degradation of the fungicides. In the industry, ETU is commonly used as an additive in the production of rubber. ETU is a polar compound with a high water solubility and it is known to be mutagenic and teratogenic in rats [2], for which reason most studies on degradation of EBDC fungicides focus on the formation of ETU [3–8].

Fielding *et al.* [9] reported that ETU was found in Dutch ground water in a range from < 0.1 to 34 $\mu g\,l^{-1}$ and Neil and Williams [10] found ETU in 18 of 40 ground water wells in Maine with a maximum value of 23 $\mu g\,l^{-1}$.

A subject search in the databases Agricola, Environment and Uncover on ETU or ethylene thiourea published in or after 1990 did only result in 4 references treating ETU degradation in soil [11–14] and no references on ETU presence in ground or river water. Only few other ETU degradation studies in soil have been published [15–18]. None of the published degradation studies investigated the simultaneous effects of factors influencing the degradation rate.

The purpose of the present project was to study the interacting influence of temperature, organic carbon content and concentration on the mineralization rate of ETU.

MATERIALS AND METHODS

Soil

Coarse sandy soil was sampled at two depths (15 cm (plough layer) and 75 cm (subsoil)) in November 1995 at a farm in Fladerne Bæk in the western part of Denmark. Soil texture, pH, humus and content of soluble organic carbon are shown in Table I. The water content was 10.1% (g water/100 g wet soil) in the plough layer and 3.8% in the subsoil. EBCC fungicides had been used

TABLE I Texture, pH (H_2O), humus and soluble organic carbon (SOC) content in dry soil

Depth (cm)	*Clay* %	*Silt* %	*Coarse silt* %	*Sand* %	*Coarse sand* %	*Humus* %	*SOC* $\mu g\,g^{-1}$	*pH*
15	3.2	2.2	1.0	16.3	73.9	3.4	339	5.92
75	2.1	0.9	1.0	11.2	84.7	0.2	197	5.54

Clay: $< 2\,\mu m$, Silt: 2–20 μm, Coarse silt: 20–63 μm, Sand: 63–200 μm, Coarse sand: $> 200\,\mu m$, Humus: %C × 1.72.

in the field in 1995, when potatoes were grown and sprayed 6 times with 2.0 kg dithane ha^{-1}.

Undisturbed soil samples were taken in stainless steel tubes with length 8.55 cm, diameter 6.1 cm. The tubes were forced into the soil in a vertical position avoiding cross contamination. All tubes were capped and the samples were stored at approximately 5°C until application of pesticide.

Chemicals

Ring ^{14}C-labelled ETU (N,N'-(1,2-^{14}C) ethylene thiourea) with a specific activity of 3.00 MBq mg^{-1} and a radiochemical purity of 95–96% was obtained from Amersham. 96% ethanol was obtained from Merck and Ultima Gold scintillation liquid from Packard.

Organic carbon extract was prepared shaking 1000 g plough layer soil with 1000 ml tap water during 45 min. A 5 g $CaCO_3$ were added, and the suspension was filtered several times through filterpaper until a clear solution was obtained. The extract was sterilised in 100 ml Duran bottles by autoclaving 30 min at 121°C and a pressure of 2 bar. The extract was added to half of the samples together with ^{14}C-ETU. The TOC (total organic carbon) content of the extract was 650 mg l^{-1}, determined in a Dohrman DX 80 equipment. The chemical composition of the organic carbon compounds in the extract was not determined, but soluble carbon compounds in soil generally will be low molecular humic and fulvic acids [19].

Experimental Design

A 2^4 factor design was used, i.e. each of 4 factors was investigated at two levels: Depth (15 or 75 cm), concentration of ETU (0.07 or 2.0 μg g^{-1}), temperature (5 or 20°C), and suspension (water or extract added) (Table II). For each combination, 3 replicates were made.

A quantity of 10 ml (for plough layer soils) and 20 ml (for subsoils) of a water solution of ^{14}C-ETU or an organic extract solution of ^{14}C-ETU, respectively, was applied to the surface of the undisturbed soil core and allowed to penetrate the soil core by gravity. The weight of plough layer samples was approximately 350 g dry soil and of subsoil samples 335 g. The concentration of the applied solution was calculated to give a final concentration of 0.07 or 2.0 g ^{14}C-ETU g^{-1} soil (dry weight). The final water content in the samples, ready for incubation, was about 12% (g water/100 g wet soil) for plough layer samples and 9% for subsoil samples. The initial content of soluble organic carbon was 339 μg g^{-1} and 197 μg g^{-1} for plough

TABLE II Design of experiment

Treat. id.	*Sample*	*Depth* (cm)	*Concentration* ($\mu g\,g^{-1}$)	*Temperature* (°C)	*Suspension*
A	1,2,3	15	0.07	5	Water
B	4,5,6	15	0.07	5	OC extract added
C	7,8,9	15	2.0	5	Water
D	10,11,12	15	2.0	5	OC extract added
E	13,14,15	15	0.07	20	Water
F	16,17,18	15	0.07	20	OC extract added
G	19,20,21	15	2.0	20	Water
H	22,23,24	15	2.0	20	OC extract added
I	25,26,27	75	0.07	5	Water
J	28,29,30	75	0.07	5	OC extract added
K	31,32,33	75	2.0	5	Water
L	34,35,36	75	2.0	5	OC extract added
M	37,38,39	75	0.07	20	Water
N	40,41,42	75	0.07	20	OC extract added
O	43,44,45	75	2.0	20	Water
P	46,47,48	75	2.0	20	OC extract added

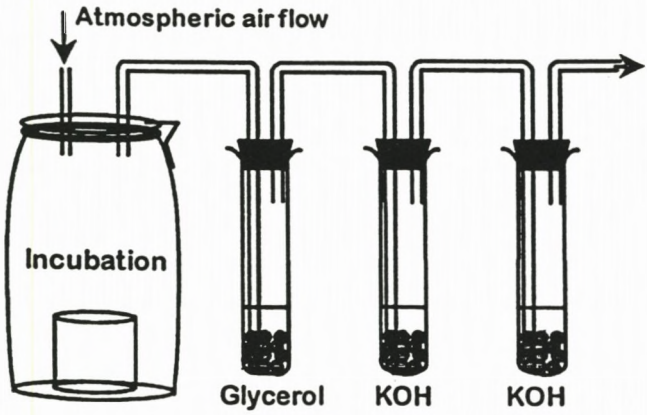

FIGURE 1 Incubation system for pesticide degradation studies.

layer and subsoil, respectively. The final content of soluble organic carbon in the incubated samples was $358\,\mu g\,g^{-1}$ and $236\,\mu g\,g^{-1}$ in plough layer and subsoil (dry soil), respectively. Soluble organic carbon (SOC) was determined shaking 20 g soil with 90 ml water for 30 min, acidifying with 2 ml conc. H_3PO_4 to remove carbonates, neutralising with NaOH and measuring non-volatile organic carbon in a Dohrman DX 80 equipment.

The samples were incubated in a system as shown in Figure 1 with a flow of 6–8 ml atmospheric air min^{-1}. The liberated $^{14}CO_2$, originating from

^{14}C-ETU, was collected in KOH and measured in a liquid scintillation counter. The accumulated amount of $^{14}CO_2$ was depicted as a function of time in days (Figures 2–17).

Data Analysis

In order to analyse the data in the present study, it was assumed that the accumulated $^{14}CO_2$ could be described by a modification of the Liu and Zhang-model [20] (Eqs. (1)–(3)). For the modification it was assumed that only one part of the added ^{14}C-ETU was available for immediate decomposition (Eq. (2)), while another part of the added ^{14}C was built into organic material of the soil which was later degraded through a first order process (Eq. (3)). The model used was thus:

$$C_t = C_m + C_h, \tag{1}$$

$$C_m = c_n - \frac{k_1 c_n}{(k_1 + k_2 c_n)e^{k_1 t} - k_2 c_n}, \tag{2}$$

$$C_h = c_b(1 - e^{-k_3 t}), \tag{3}$$

where C_t = total concentration of mineralization product ($^{14}CO_2$) formed, equivalent to total concentration of ^{14}C-ETU mineralised, at time t (measured as %^{14}C evolved as % of $^{14}CO_2$), C_m = %$^{14}CO_2$ formed, equivalent to %^{14}C-ETU mineralised, at time t according to the Liu and Zhang-model, C_h = %$^{14}CO_2$ formed, equivalent to %^{14}C-ETU mineralised, at time t according to a first order model, c_n = total % of ^{14}C-ETU converted to $^{14}CO_2$ according to the Liu and Zhang-model, c_b = total % of ^{14}C-ETU converted to $^{14}CO_2$ according to the first order model, $k_1 = k(m_0+\lambda)$, $k_2 = -k\lambda$, k_3 = degradation rate constant for the first order process, k = degradation rate constant for the process according to the Liu and Zhang model, m_0 = number of micro-organisms involved in pesticide degradation at start time, λ = growth rate constant for micro-organisms, t = time in days.

Two different versions of this model were estimated:

Model A: $c_n + c_b = 100\%$ – assuming that only two processes took place in the decomposition, the immediate decomposition of ^{14}C-ETU and a first-order mineralization process.

Model B: $c_n + c_b < 100\%$ – assuming that more than two processes could take place in the decomposition, but that this (these) process(es) may be ignored during the first 60 days of the mineralization experiment.

The 5 coefficients of the model were estimated by the method of least squares. As the model is non-linear, an iterative method was used. In order to obtain good initial estimates for the iterative process some simplified models were first estimated. The simplifications were that either k_2 or k_3 was assumed to be zero. The following two restrictions were put on the parameters:

$$c_n + c_b \leq 100, \tag{4}$$

$$c_n \leq -k_1/k_2. \tag{5}$$

The coefficients of both model A and B were estimated for each replicate of 16 different treatment combinations, 48 samples in total. In order to describe how the coefficients depended on the treatments, each coefficient was analysed separately assuming a linear model. Based on this model the significance of main effects and interaction effects were tested. The relevant significant effects were then described by the marginal means of the effect in question.

In order to obtain approximate variance homogeneity the logarithms of the estimated k_1, $-k_2$ and λ/m_0 were analysed instead of the estimates. The parameters were estimated and differences were tested according to the theory of general linear models [21].

The following linear model was used to analyse the estimates of the coefficients for c_n, $\log(k_1)$, $\log(-k_2)$ and $\log(\lambda/m_0)$

$$\begin{aligned} Y_{dctsr} = {} & \mu + \alpha_d + \beta_c + \gamma_t + \delta_s + (\alpha\beta)_{dc} + (\alpha\gamma)_{dt} + (\alpha\delta)_{ds} + (\beta\gamma)_{ct} + (\beta\delta)_{cs} \\ & + (\alpha\beta\gamma)_{dct} + (\alpha\beta\delta)_{dcs} + (\alpha\gamma\delta)_{dts} + (\beta\gamma\delta)_{cts} + \varepsilon_{dctsr} \end{aligned} \tag{6}$$

where Y_{dctsr} = value of the coefficient (or transformed coefficient) for each sample, μ, α, β, γ, δ, $(\alpha\beta)$, $(\alpha\gamma)$, $(\alpha\delta)$, $(\beta\gamma)$, $(\beta\delta)$, $(\gamma\delta)$, $(\alpha\beta\gamma)$, $(\alpha\beta\delta)$, $(\alpha\gamma\delta)$, $(\beta\gamma\delta)$ and $(\alpha\beta\gamma\delta)$ with indices are parameters describing treatment effects, d = depth of soil sample (15 or 75 cm) c = concentration of ETU (0.07 or 2.0 $\mu g\, g^{-1}$), t = temperature (5 or 20°C), s = suspension (water or extract added), r = replicate (1, 2 or 3), ε_{dctsr} is assumed to be normally distributed with mean zero and a variance which is constant within each group. For most coefficients the groups coincide with the depth from which the sample came, i.e. $\varepsilon_{dctsr} \sim N(0, \sigma_i^2)$, where i is the group to which the sample belongs.

All statistical calculations were done using procedures from SAS [22–24].

RESULTS AND DISCUSSION

In soil degradation studies, where undisturbed soil samples are used, it is not possible to take out aliquots of the sample to follow the disappearance of the

parent compound. If pesticide mineralization is to be followed in the same sample, $^{14}CO_2$, formed through the mineralization of ^{14}C-labelled pesticide, must be quantified. For comparison of degradation rates and correlation with other parameters, the curves depicting the $^{14}CO_2$ formation must then be described with a mathematical model. The use of ^{14}C-labelled pesticides for degradation studies following the formation of $^{14}CO_2$ makes it possible to perform degradation studies in very low concentration. When undisturbed soil samples are used, it is a great advantage that no changes in the environment of microcosm are caused.

Liu and Zhang [20] assumed that the degradative processes of pesticides in soil involves microbial utilisation of pesticide as an energy source (metabolic degradation) and stated that their model, which described the decrease in pesticide concentration, was able to describe degradation curves whether they had an inflection point or not. Fomsgaard [14] converted the model to express degradation of ^{14}C-labelled pesticides through the accumulated formation of $^{14}CO_2$ and found that only for cases where an inflection was seen, generally in subsoil samples, the model gave useful fits. The converted Liu and Zhang model was used by Fomsgaard *et al.* [25] to model ^{14}C-maneb mineralization in sediment, and by Fomsgaard *et al.* [26] to model ^{14}C-mecoprop degradation in undisturbed subsoil. The converted Liu and Zhang model was modified by Helweg *et al.* [27], adding a zero order term to describe mineralization of 5 $\mu g\, g^{-1}$ ^{14}C-mecoprop. In the present study a further modification of the Liu and Zhang model, where a first order term is added, was shown to be the most useful model for describing the mineralization of ^{14}C-ETU. To be able to perform the non-linear procedure of the modified model, where 5 parameters were to be estimated, the procedure described in "Data analysis" was followed.

Tables III and IV show the parameter estimates and mean squares according to model A and model B, respectively. A general tendency was seen, that model B ($c_n + c_b < 100\%$) gave the best fit (lowest mean square) for the plough layer samples (samples 1–24) and that model A ($c_n + c_b = 100\%$) gave the best fit for the subsoil samples (sample 25–48). In some cases for subsoil samples, model B could not give any fit at all. The accumulated amounts of $^{14}CO_2$ depicted as a function of time in days and the chosen non-linear model (B for plough layer, A for subsoil) for each data-set are presented in Figures 2–17.

The inclusion of a first order process in the models could lead to an assumption that a part of ^{14}C-ETU *either* at first was adsorbed to the soil particles and then slowly desorbed, where the desorption process or the following degradation to $^{14}CO_2$ was a first order process *or* was relatively fast microbiologically degraded building ^{14}C into organic material with subsequent slow first order degradation of organic material causing evolution of

TABLE III Estimates and mean squares according to model A for all replicates

Treat	*Sample*	*Depth* (cm)	*Conc.* ($\mu g\,g^{-1}$)	*Temp.* (°C)	*Suspen.*	c_n	k_1	k_2	k_3	c_b	λ/m_0	*Mean square*	*Reference figure no.*
A	1	15	0.07	5	normal	19.6875	0.71868	−0.02926	0.0037200	80.3125	0.2051	0.92217	
A	2	15	0.07	5	normal	25.1648	0.55879	−0.01371	0.0037337	74.8352	0.0641	0.80913	
A	3	15	0.07	5	normal	14.7615	0.38851	−0.01996	0.0021143	85.2385	0.2125	0.66533	
B	4	15	0.07	5	extract	25.5696	1.14664	−0.04172	0.0037414	74.4304	0.5226	1.13772	
B	5	15	0.07	5	extract	16.5973	0.48811	−0.02079	0.0030176	83.4027	0.1453	0.73374	
B	6	15	0.07	5	extract	22.0537	0.92921	−0.03775	0.0036434	77.9463	0.3909	0.94947	
C	7	15	2.0	5	normal	17.3718	0.44229	−0.02375	0.0032091	82.6282	0.7973	0.48354	
C	8	15	2.0	5	normal	25.3094	0.29886	−0.01046	0.0028053	74.6906	0.3077	0.17906	
C	9	15	2.0	5	normal	23.4006	0.39925	−0.01658	0.0021894	76.5994	1.4615	0.18712	
D	10	15	2.0	5	extract	21.6939	0.35960	−0.01589	0.0027249	78.3061	1.0621	0.15150	
D	11	15	2.0	5	extract	22.0190	0.36139	−0.01450	0.0024735	77.9810	0.3438	0.33016	
D	12	15	2.0	5	extract	27.2236	0.30027	−0.00974	0.0027614	72.7764	0.2772	0.24935	
F	13	15	0.07	20	normal	30.6790	0.95714	−0.02727	0.0051890	69.3210	0.2263	1.42929	
E	14	15	0.07	20	normal	36.5606	0.02373	0.00890	0.0042370	63.4394	−0.0254	1.46146	
E	15	15	0.07	20	normal	26.4051	1.11594	−0.03886	0.0078121	73.5949	0.4325	1.45576	
F	16	15	0.07	20	extract	26.0202	0.81052	−0.01841	0.0042076	73.9798	0.0555	1.41732	
F	17	15	0.07	20	extract	22.4907	1.25537	−0.04798	0.0057014	77.5093	0.2723	1.43324	
F	18	15	0.07	20	extract	25.0780	0.99990	−0.02929	0.0058453	74.9220	0.1103	1.86548	
G	19	15	2.0	20	normal	18.4386	1.16075	−0.06255	0.0042339	81.5614	8.5397	0.83119	
G	20	15	2.0	20	normal	27.0493	1.12184	−0.04033	0.0050044	72.9507	1.3015	1.34525	
G	21	15	2.0	20	normal	25.8691	0.85594	−0.03164	0.0041055	74.1309	0.8426	0.59494	
H	22	15	2.0	20	extract	24.7631	0.98030	−0.03941	0.0039593	75.2369	9.1157	1.06302	
H	23	15	2.0	20	extract	23.4689	0.42205	−0.01265	0.0046812	76.5311	0.1011	1.25644	
H	24	15	2.0	20	extract	21.1287	0.83035	−0.03883	0.0039548	78.8713	3.9510	0.75680	
I	25	75	0.07	5	normal	35.3530	0.13500	−0.00378	0	64.6470	2.9841	0.83315	10
I	26	75	0.07	5	normal	26.6173	0.11931	−0.00443	0	73.3827	2.9248	0.47151	10
I	27	75	0.07	5	normal	24.7328	0.10408	−0.00406	0	75.2672	1.0723	0.13020	10
J	28	75	0.07	5	extract	28.5330	0.17638	−0.00612	0.0001738	71.4670	3.5739	1.34209	11
J	29	75	0.07	5	extract	24.0359	0.24807	−0.01028	0.0015735	75.9641	11.6824	0.45183	11
J	30	75	0.07	5	extract	11.6779	0.46841	−0.04011	0.0026618	88.3221	2000.0000	0.88713	11
K	31	75	2.0	5	normal	6.6591	0.19319	−0.02901	0.0000818	93.3409	10000.0000	0.22371	12

K	32	75	2.0	5	normal	14.1091	0.14552	−0.01031	0.0001806	85.8909	2500.0000	0.14830	12
K	33	75	2.0	5	normal	3.2785	0.46122	−0.14068	0.0002014	96.7215	—	0.08376	12
L	34	75	2.0	5	extract	1.0001	0.68510	−0.68506	0.0002179	98.9999	—	0.04163	13
L	35	75	2.0	5	extract	18.4627	0.17148	−0.00929	0	81.5373	1666.6670	1.00376	13
L	36	75	2.0	5	extract	29.9997	0.30000	−0.01000	0.0000256	70.0003	—	0.00034	13
M	37	75	0.07	20	normal	22.0415	0.34773	−0.01566	0.0050439	77.9585	6.3694	1.23303	14
M	38	75	0.07	20	normal	28.1078	0.56907	−0.02018	0.0068802	71.8922	11.0011	1.09187	14
M	39	75	0.07	20	normal	28.9604	0.55129	−0.01898	0.0055624	71.0396	11.0497	0.68363	14
N	40	75	0.07	20	extract	20.2297	0.45143	−0.02219	0.0046423	79.7703	8.4817	0.68179	15
N	41	75	0.07	20	extract	15.0281	0.64600	−0.04287	0.0054391	84.9719	23.8095	0.45565	15
N	42	75	0.07	20	extract	24.1145	0.29284	−0.01063	0.0044796	75.8855	0.2920	1.76197	15
O	43	75	2.0	20	normal	54.8662	0.05621	−0.00090	−0.0048074	45.1338	0.3379	0.83927	16
O	44	75	2.0	20	normal	21.8840	0.22490	−0.01022	0.0013103	78.1160	8.8573	1.17127	16
O	45	75	2.0	20	normal	32.6244	0.13454	−0.00410	−0.0001991	67.3756	4.4424	0.06886	16
P	46	75	2.0	20	extract	31.6009	0.10843	−0.00326	0	68.3991	0.6165	0.41672	17
P	47	75	2.0	20	extract	30.2042	0.11660	−0.00373	0	69.7958	0.9640	0.85150	17
P	48	75	2.0	20	extract	29.6608	0.08323	−0.00264	−0.0017451	70.3392	0.5437	0.05052	17

TABLE IV Estimates and mean square according to model B for all replicates

Treat	*Sample*	*Depth* (cm)	*Conc.* ($\mu g\,g^{-1}$)	*Temp.* (°C)	*Suspen.*	c_n	k_1	k_2	k_3	c_b	λ/m_0	*Mean square*	*Reference figure no.*
A	1	15	0.07	5	normal	15.6060	1.44034	−0.08874	0.034848	21.0379	1.5979	0.19458	2
A	2	15	0.07	5	normal	20.0708	1.22539	−0.05562	0.039686	20.3263	0.5103	0.10991	2
A	3	15	0.07	5	normal	8.8372	0.96368	−0.10552	0.046945	15.9122	3.3829	0.18465	2
B	4	15	0.07	5	extract	21.1097	1.94314	−0.09073	0.042963	18.7667	3.2647	0.14914	3
B	5	15	0.07	5	extract	11.1299	1.88302	−0.16761	0.040856	19.4681	9.5328	0.15301	3
B	6	15	0.07	5	extract	17.6606	1.80077	−0.10034	0.039469	19.6471	3.4989	0.11908	3
C	7	15	2.0	5	normal	10.2855	0.93578	−0.09064	0.038885	22.5821	25.7064	0.22660	4
C	8	15	2.0	5	normal	19.2102	0.37904	−0.01848	0.031970	20.0967	0.7720	0.12229	4
C	9	15	2.0	5	normal	18.3398	0.50433	−0.02721	0.033059	16.2433	5.1759	0.10302	4
D	10	15	2.0	5	extract	14.3485	0.54461	−0.03768	0.035759	21.0071	9.6711	0.05541	5
D	11	15	2.0	5	extract	15.0616	0.54375	−0.03465	0.042318	18.3021	1.5827	0.17572	5
D	12	15	2.0	5	extract	19.4299	0.40125	−0.01951	0.038791	20.2550	0.8785	0.17180	5
E	13	15	0.07	20	normal	25.3828	1.33713	−0.04934	0.045019	22.4982	0.5813	0.27689	6
E	14	15	0.07	20	normal	22.2740	0.77854	−0.01970	0.035277	30.7020	0.0579	0.47989	6
E	15	15	0.07	20	normal	22.5060	1.43725	−0.06098	0.030561	34.1313	0.9416	0.53368	6
F	16	15	0.07	20	extract	21.6238	1.42961	−0.05476	0.041658	20.1278	0.2229	0.65542	7
F	17	15	0.07	20	extract	18.7203	1.90462	−0.09556	0.033498	27.1148	0.8261	0.41980	7
F	18	15	0.07	20	extract	19.7163	2.00000	−0.09382	0.041898	26.4400	0.6242	0.38986	7
G	19	15	2.0	20	normal	14.1344	1.82240	−0.12888	0.035105	23.2587	175.4386	0.23324	8
G	20	15	2.0	20	normal	21.3019	1.92776	−0.09018	0.041688	24.3199	13.1578	0.47957	8
G	21	15	2.0	20	normal	21.8376	1.06703	−0.04770	0.035268	20.8450	1.8789	0.14361	8
H	22	15	2.0	20	extract	19.3292	1.70194	−0.08804	0.038635	21.8378	625.0000	0.47748	9
H	23	15	2.0	20	extract	13.5233	1.41057	−0.10154	0.051047	27.4137	2.7107	0.21066	9
H	24	15	2.0	20	extract	16.7031	1.18092	−0.07056	0.032030	23.0531	29.5858	0.38514	9
I	25	75	0.07	5	normal	35.3530	0.13500	−0.00378	0	63.6470	2.9841	1.04144	
I	26	75	0.07	5	normal	26.6173	0.11931	−0.00443	0	72.3827	2.9248	0.58939	
I	27	75	0.07	5	normal	24.7328	0.10408	−0.00406	0	75.2672	1.0723	0.16275	
J	28	75	0.07	5	extract	5	0	0	0	0	—	1.67762	
J	29	75	0.07	5	extract	5	0	0	0	0	—	0.56478	
J	30	75	0.07	5	extract	11.6779	0.46840	−0.04011	0.002665	88.3221	2000.0000	1.11164	
K	31	75	2.0	5	normal	6.1607	0.19622	−0.03185	0.000102	76.8611	10000.000	0.27200	

K	32	75	2.0	5	normal	14.1186	0.14557	−0.01031	0.094632	0.5386	2000	0.14663
K	33	75	2.0	5	normal	3.2785	0.47062	−0.14355	0.000206	95.7775	—	0.10446
L	34	75	2.0	5	extract	1.0001	0.68510	−0.68506	0.000218	98.9999	—	0.05204
L	35	75	2.0	5	extract	18.4627	0.17148	−0.00929	0	81.5373	1666.6666	1.25470
L	36	75	2.0	5	extract	30	0.3	−0.01	0.000026	69.5502	—	0.00043
M	37	75	0.07	20	normal	5	0	0	0	0	—	1.27555
M	38	75	0.07	20	normal	5	0	0	0	0	—	1.12953
M	39	75	0.07	20	normal	5	0	0	0	0	—	0.70720
N	40	75	0.07	20	extract	5	0	0	0	0	—	0.70614
N	41	75	0.07	20	extract	5	0	0	0	0	—	0.47192
N	42	75	0.07	20	extract	16.6619	0.42099	−0.02382	0.027048	30.8263	0.9919	1.71094
O	43	75	2.0	20	normal	32.5007	0.07058	−0.00199	−0.00481	0	0.3370	0.92737
O	44	75	2.0	20	normal	26.8147	0.17282	−0.00635	0.000256	71.8929	2.4691	1.24996
O	45	75	2.0	20	normal	32.6244	0.13454	−0.00410	−0.00020	67.3756	4.4424	0.07123
P	46	75	2.0	20	extract	31.6009	0.10843	−0.00326	0	67.3991	0.6165	0.43160
P	47	75	2.0	20	extract	30.2081	0.11657	−0.00373	0	0	0.9635	0.88191
P	48	75	2.0	20	extract	21.7784	0.10140	−0.00450	−0.00034	78.2216	1.3379	0.05715

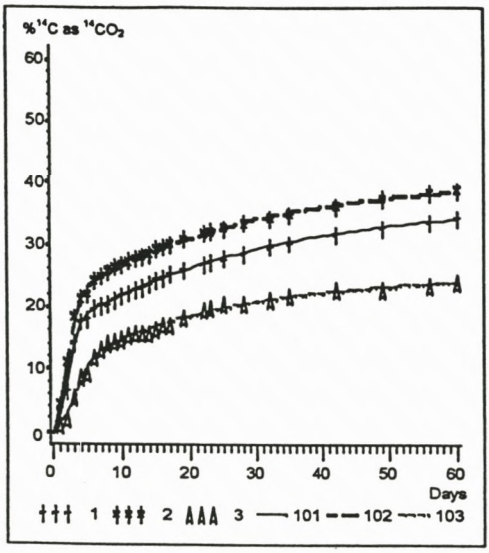

FIGURE 2 Mineralisation of ^{14}C-ETU in soil described as evolution of $\%^{14}C$ as $^{14}CO_2$ in function of time in days. Depth: plough layer; concentration: 0.07 $\mu g\,g^{-1}$; temperature: 5°C; suspension: water. Treat. id. A, samples 1, 2, 3 to match model fits no. 101, 102, 103, respectively.

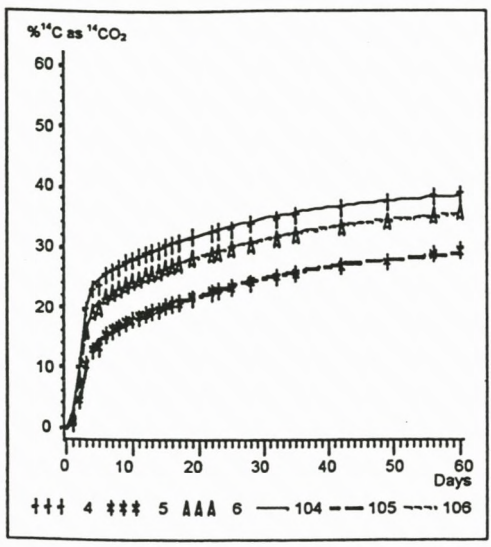

FIGURE 3 Mineralisation of ^{14}C-ETU in soil described as evolution of $\%^{14}C$ as $^{14}CO_2$ in function of time in days. Depth: plough layer; concentration: 0.07 $\mu g\,g^{-1}$; temperature: 5°C; suspension: extract. Treat. id. B, no. 4, 5, 6, to match model fits no. 104, 105, 106, respectively.

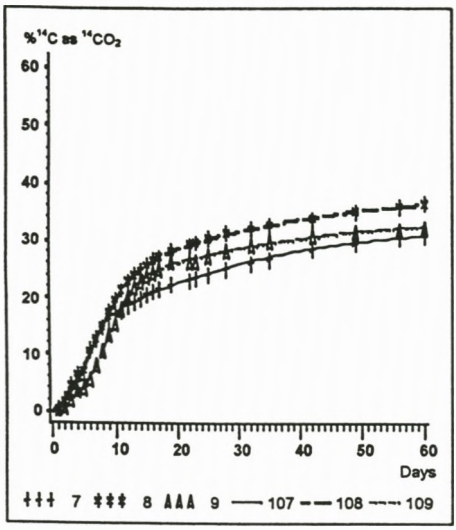

FIGURE 4 Mineralisation of ^{14}C-ETU in soil described as evolution of $\%^{14}C$ as $^{14}CO_2$ in function of time in days. Depth: plough layer; concentration: 2.0 $\mu g\, g^{-1}$; temperature: 5°C; suspension: water. Treat. id. C, no. 7, 8, 9, to match model fits no. 107, 108, 109, respectively.

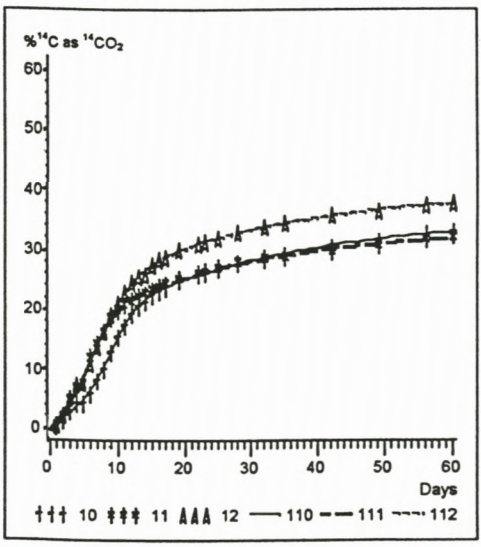

FIGURE 5 Mineralisation of ^{14}C-ETU in soil described as evolution of $\%^{14}C$ as $^{14}CO_2$ in function of time in days. Depth: plough layer; concentration: 2.0 $\mu g\, g^{-1}$; temperature: 5°C; suspension: extract. Treat. id. D, no. 10, 11, 12 to match model fits no. 110, 111, 112, respectively.

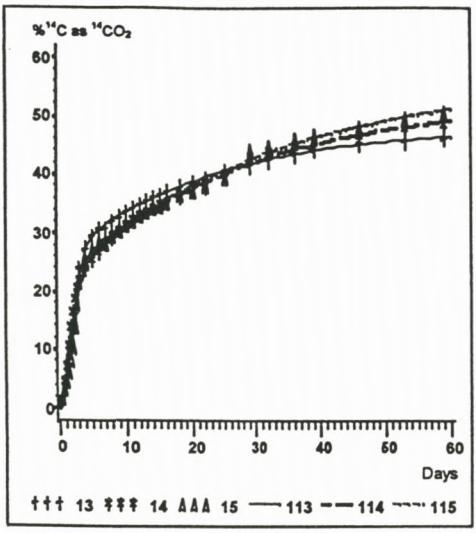

FIGURE 6 Mineralisation of ^{14}C-ETU in soil described as evolution of %^{14}C as $^{14}CO_2$ in function of time in days. Depth: plough layer; concentration: 0.07 µg g^{-1}; temperature: 20°C; suspension: water. Treat. id. E, no. 13, 14, 15, to match model fits no. 113, 114, 115, respectively.

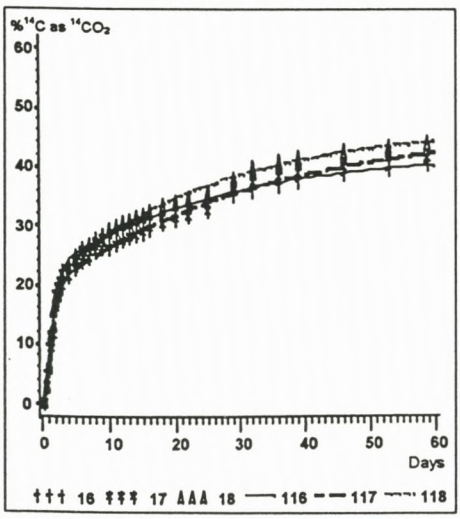

FIGURE 7 Mineralisation of ^{14}C-ETU in soil described as evolution of %^{14}C as $^{14}CO_2$ in function of time in days. Depth: plough layer; concentration: 0.07 µg g^{-1}; temperature: 20°C; suspension: extract. Treat. id. F, no. 16, 17, 18, to match model fits no. 116, 117, 118, respectively.

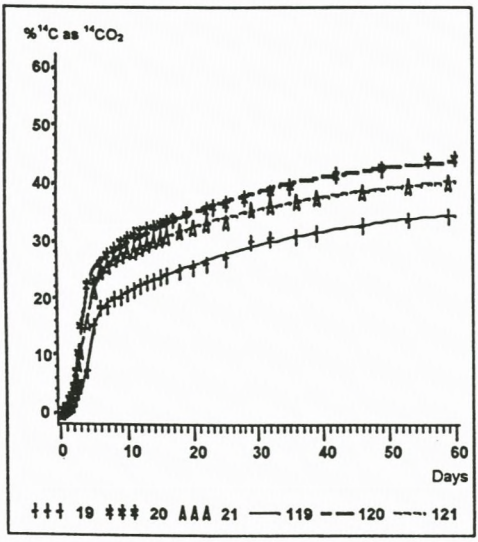

FIGURE 8 Mineralisation of ^{14}C-ETU in soil described as evolution of $\%^{14}C$ as $^{14}CO_2$ in function of time in days. Depth: plough layer; concentration: 2.0 $\mu g\, g^{-1}$; temperature: 20°C; suspension: water. Treat. id. G, no. 19, 20, 21, to match model fits no. 119, 120, 121, respectively.

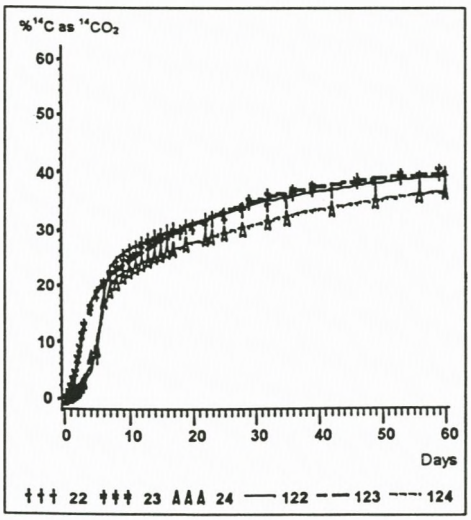

FIGURE 9 Mineralisation of ^{14}C-ETU in soil described as evolution of $\%^{14}C$ as $^{14}CO_2$ in function of time in days. Depth: plough layer; concentration: 2.0 $\mu g\, g^{-1}$; temperature: 20°C; suspension: extract. Treat. id. H, no. 22, 23, 24, to match model fits no. 122, 123, 124, respectively.

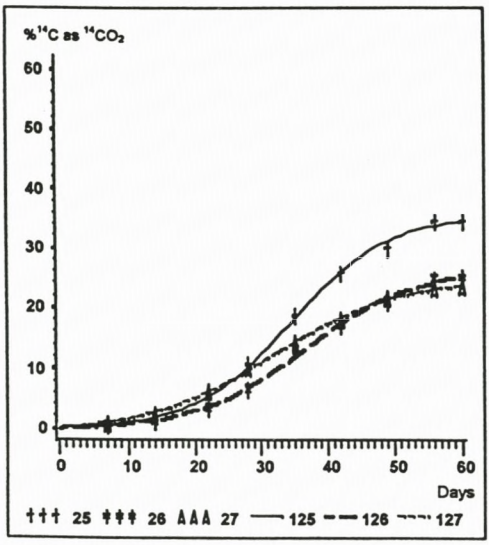

FIGURE 10 Mineralisation of ^{14}C-ETU in soil described as evolution of $\%^{14}C$ as $^{14}CO_2$ in function of time in days. Depth: 75 cm; concentration: 0.07 $\mu g\, g^{-1}$; temperature: 5°C; suspension: water. Treat. id. I, no. 25, 26, 27, to match model fits no. 125, 126, 127, respectively.

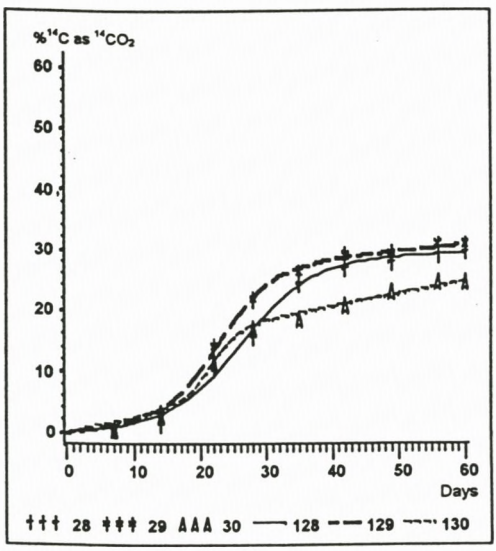

FIGURE 11 Mineralisation of ^{14}C-ETU in soil described as evolution of $\%^{14}C$ as $^{14}CO_2$ in function of time in days. Depth: 75 cm; concentration: 0.07 $\mu g\, g^{-1}$; temperature: 5°C; suspension: extract. Treat. id. J, no. 25, 26, 27, to match model fits no. 125, 126, 127, respectively.

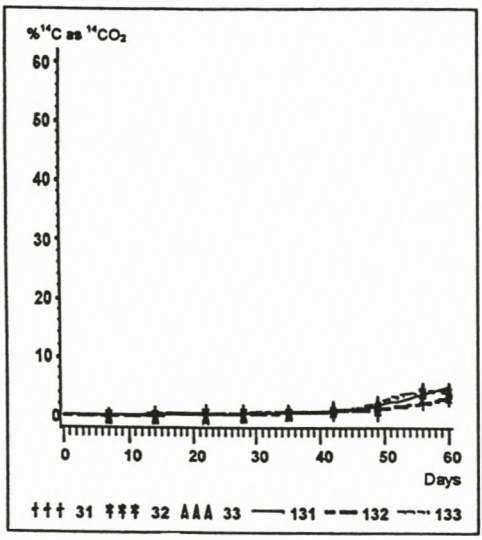

FIGURE 12 Mineralisation of ^{14}C-ETU in soil described as evolution of $\%^{14}C$ as $^{14}CO_2$ in function of time in days. Depth: 75cm; concentration: $2.0\,\mu g\,g^{-1}$; temperature: 5°C; suspension: water. Treat. id. K, no. 31, 32, 33, to match model fits no. 131, 132, 133, respectively.

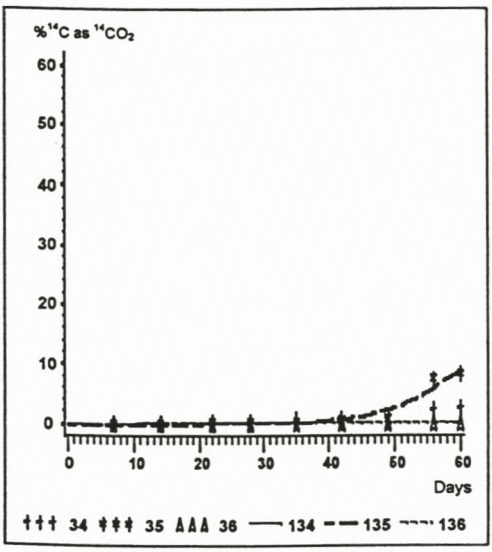

FIGURE 13 Mineralisation of ^{14}C-ETU in soil described as evolution of $\%^{14}C$ as $^{14}CO_2$ in function of time in days. Depth: 75cm; concentration: $2.0\,\mu g\,g^{-1}$; temperature: 5°C; suspension: extract. Treat. id. L, no. 34, 35, 36 to match model fits no. 134, 135, 136, respectively.

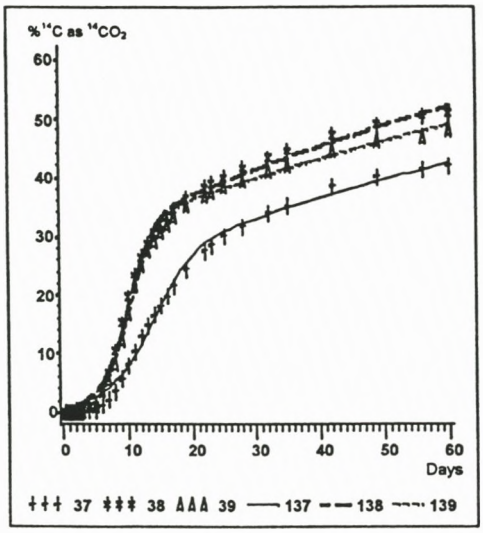

FIGURE 14 Mineralisation of ^{14}C-ETU in soil described as evolution of $\%^{14}C$ as $^{14}CO_2$ in function of time in days. Depth: 75cm; concentration: $0.07\,\mu g\,g^{-1}$; temperature: 20°C; suspension: water. Treat. id. M, no. 37, 38, 39, to match model fits no. 137, 138, 139, respectively.

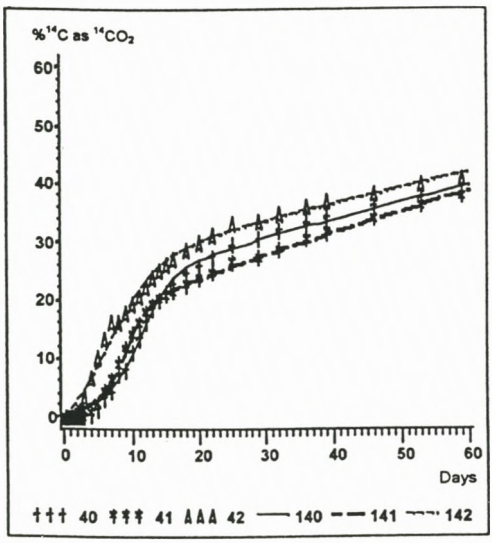

FIGURE 15 Mineralisation of ^{14}C-ETU in soil described as evolution of $\%^{14}C$ as $^{14}CO_2$ in function of time in days. Depth: 75cm; concentration: $0.07\,\mu g\,g^{-1}$; temperature: 20°C; suspension: extract. Treat. id. N, no. 40, 41, 42, to match model fits no. 140, 141, 142, respectively.

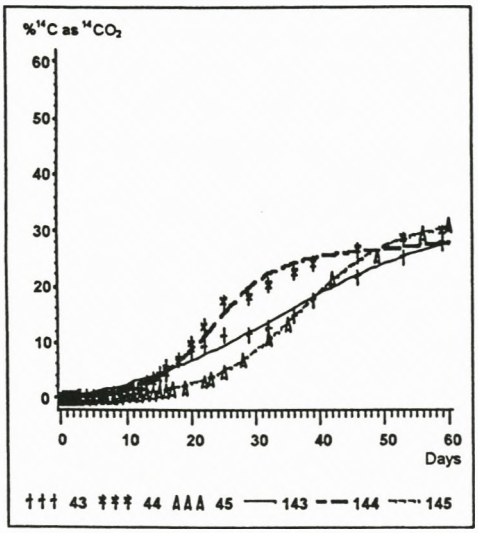

FIGURE 16 Mineralisation of ^{14}C-ETU in soil described as evolution of %^{14}C as $^{14}CO_2$ in function of time in days. Depth: 75 cm; concentration: 2.0 $\mu g\,g^{-1}$; temperature: 20°C; suspension: water. Treat. id. O, no. 44, 45, 46, to match model fits no. 144, 145, 146, respectively.

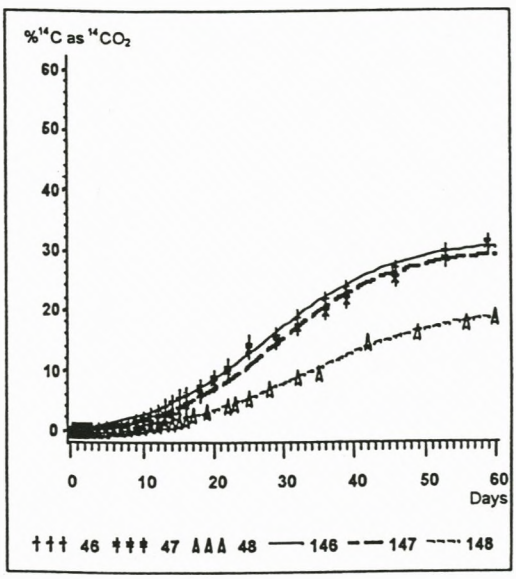

FIGURE 17 Mineralisation of ^{14}C-ETU in soil described as evolution of %^{14}C as $^{14}CO_2$ in function of time in days. Depth: 75 cm; concentration: 2.0 $\mu g\,g^{-1}$; temperature: 20°C; suspension: extract. Treat. id. P, no. 46, 47, 48, to match model fits no. 146, 147, 148, respectively.

$^{14}CO_2$ [28,29]. However, both Johannesen *et al.* [13] and Fomsgaard and Helweg [30] showed that, when ^{14}C-ETU mineralization experiments in soil are performed, no ^{14}C-ETU could be extracted from the soil if the mineralization experiment was stopped at a time, when the $^{14}CO_2$-formation curve had flattened out. Thus, in the present experiment it should not be expected, that any ^{14}C-ETU would be left in the soil except for the samples 31–36. When no ^{14}C-ETU or other ^{14}C-compounds are left in the soil at the time, where the curve flattens out, it must be concluded that the flat part of the curve depicts the slow first order $^{14}CO_2$ formation process coming from degradation of organic material where ^{14}C had been built in.

Sorption and subsequent desorption of ^{14}C-ETU could still take place, but the process was probably not of sufficient importance to influence the results of the modelling process. The reason for model B ($c_n+c_b < 100\%$) giving the best fit for plough layer samples and model A ($c_n + c_b = 100\%$) giving the best fits for subsoil samples was first thought to be a sorption–desorption process taking place in the plough layer. Such a theory, however, had to be rejected owing to the already mentioned results of Johannesen *et al.* [13] and Fomsgaard and Helweg [30]. To elucidate the reason for model B giving best fits in the plough layer, the whole modelling process was performed with data from the plough layer (samples 1–24) after 10 and 20 days, respectively. The estimates for c_n, k_1 and k_2 for these data from both model A and B, determined where the flattening of the $^{14}CO_2$-formation curve began, proved to be very close to the estimates for c_n, k_1 and k_2 from model B, determined after 60 days. (Examples after 10 days are shown in Table V). When the process was modelled after 10 days, model A and B gave similar estimates for k_3 and c_b in the two models, but the estimates for k_3 and c_b were very different from the estimates determined according to model B after 60 days. If the mineralization of ^{14}C-organic material was followed for a longer period, the values for k_3 and c_b changed. Therefore the terms k_3 and c_b were not summarised or compared by the proposed linear model (Eq. 6), while estimates for c_n, k_1 and k_2 determined according to model A in subsoil were compared to estimates for c_n, k_1 and k_2 according to model B in plough layer. λ/m_0 (growth rate for micro-organisms/number of micro-organisms at start time) for each sample was determined as

$$\frac{\lambda}{m_0} = \frac{-k_2}{k_1 + k_2 c_n}. \tag{7}$$

Since m_0 was not determined in the study, only the relation λ/m_0 could be estimated. If possible, m_0 (number of micro-organisms involved in pesticide degradation) should be determined, to be able to estimate λ, as well as k.

TABLE V Examples of estimates obtained according to model A and B after 10 days compared to estimates obtained according to model B after 60 days

Treat.	*Sample*	*Depth* (cm)	*Conc.* ($\mu g\, g^{-1}$)	*Temp.* (°C)	*Suspension*	c_n	k_1	k_2	k_3	c_b	*Mean square*	*Reference figure no.*	*Days*	*Model*
A	1	15	0.07	5	normal	15.6060	1.44034	−0.08874	0.034848	21.0379	0.19458	2	60	B
A	1	15	0.07	5	normal	16.2740	1.38626	−0.08143	0.006722	83.7260	0.69228		10	A
A	1	15	0.07	5	normal	16.2740	1.38626	−0.08143	0.00672	83.7260	0.83073		10	B
F	16	15	0.07	20	extract added	21.6238	1.42961	−0.05476	0.041658	20.1278	0.65542	7	60	B
F	16	15	0.07	20	extract added	20.7455	1.58107	−0.06495	0.011028	79.2545	1.33380		10	A
F	16	15	0.07	20	extract added	20.7455	1.58107	−0.06495	0.011028	79.2545	1.45460		10	B

The probability values for effects (main and interaction effects) of c_n, k_1, k_2 and λ/m_0 obtained using the model in Eq. (6) are shown in Table VI. Effects at 5% level of significance are marked with (*). The three-way interaction effect depth*conc*temp was significant for both c_n, k_1, k_2 and λ/m_0. The interaction between two of those factors (depth*conc, depth*temp, conc*temp) thus depended on the level of the third factor, which is illustrated in Figure 18. Considering degradation rates of ETU as a sole function of temperature, concentration or depth would thus be a simplification. The fit of the treatments K and L (mineralisation at 5°C at 75 cm depth in a concentration of $2.0\,\mu g\,g^{-1}$) must be quite uncertain, since the evolution of $^{14}CO_2$ hardly started during the incubation time.

Since the calculation of transformed ^{14}C-pesticide was transformed to %, it must be emphasized, that the parameters λ/m_0 (growth rate of microorganisms/initial amount of degrading microorganisms) and k_2 only can be used to investigate the interaction effects. Comparison of the sizes of the two parameters can be done, but only for equal concentrations. Figure 18 shows that changes in degradation rate of ^{14}C-ETU, k_1, at varying depths, temperatures and concentrations followed a pattern which showed a negligible increase with increasing concentration of ^{14}C-ETU at depth 15 cm and 20°C, while a considerable decrease in k_1 was seen at 5°C. At 75 cm depth, a slight increase

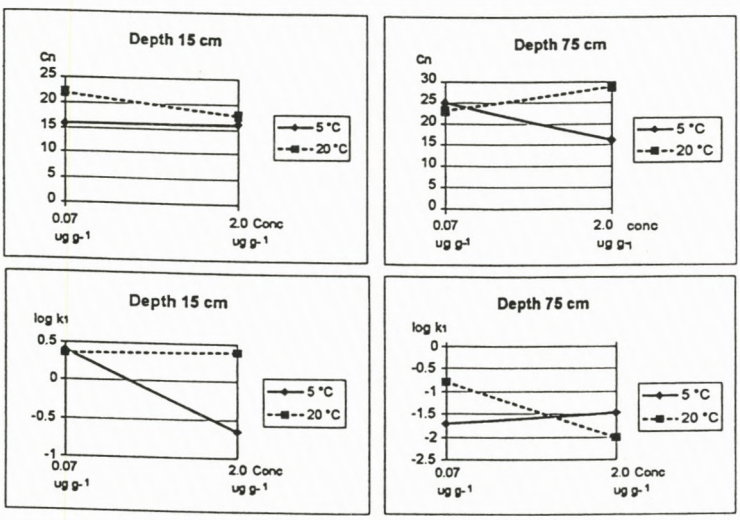

FIGURE 18 The combined interaction effect of depth, concentration and temperature for the coefficients c_n and k_1.

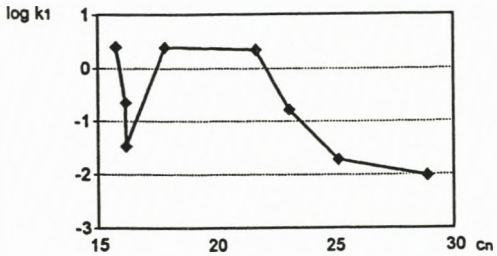

FIGURE 19 k_1 in function of c_n for ^{14}C-ETU degradation studies.

in k_1 was seen at 5°C, when the concentration of added ^{14}C-ETU was higher, while a decrease in k_1 was seen at 20°C with increasing concentration of ^{14}C-ETU. The growth rate of the micro-organisms, λ does not relate directly to k_1, since $k_1 = k(m_0 + \lambda c_n)$. k_2 follows the same pattern as k_1, being

$$k_2 = -\frac{k_1}{m_0 + \lambda c_n}\lambda. \tag{8}$$

k_2 decreases as log $(-k_2)$ increases and vice versa.

The amount (in%) of initially added ^{14}C-ETU which was mineralised to $^{14}CO_2$ by micro-organisms, c_n, was higher at 20°C than at 5°C when 0.07 μg g^{-1} ^{14}C-ETU was added to plough layer soil, and identical at 20°C and 5°C when 2.0 μg g^{-1} were added. A tendency was seen, that with higher k_1, a lower c_n (low% of $^{14}CO_2$ formed) was found (Figure 19). A clear relation could not be seen, however.

Both k_1 and c_n can be read approximately from the Figures 2–17, k_1 being the slope of the initial part of the curve, and c_n being the y-value, where the curve bends. Estimating the values through mathematical modelling, however, is preferred.

From Table VI it is seen, that λ/m_0 was not influenced by suspension, the two-way interaction effect conc*suspension was significant for k_1 and k_2, while the three-way interaction effect depth*conc*suspension was significant only for c_n (Figure 20) shows the combined effects of depth, concentration and suspension. At 15 cm depth the effect of suspension on c_n (% $^{14}CO_2$ formed during the mineralization process) at 0.07 μg g^{-1} and 2.0 μg ^{14}C-ETU g^{-1} with water or with organic extract was the same (higher c_n with water than with extract and higher c_n at 0.07 μg g^{-1} than at 2.0 μg g^{-1}), while at 75 cm depth, the effect of ^{14}C-ETU concentration on c_n increased when organic extract was added and decreased when no organic extract was added. Since no interaction effect between concentration and depth with suspension was seen for k_1,

TABLE VI Probability values (%) from the analysis of variance for four-way factorial ^{14}C-ETU mineralization experiments. Effects at 5% level of significance are shown with (*)

	c_n	k_1	k_2	λ/m_0
Depth	0.09*	0.01*	0.01*	0.01*
Conc.	26.54	0.01*		
Depth*conc	96.31	98.10	89.08	26.90
Temp.	0.45*	0.16*	98.10	0.02*
Depth*temp	61.74	13.76	25.73	0.02*
Conc.*temp.	8.69	32.19	22.80	3.33*
Depth*conc.*temp.	0.32*	0.01*	0.01*	0.01*
Susp.	89.58	29.77	83.47	64.55
Depth*susp.	44.66	55.35	28.93	77.61
Conc.*susp.	0.99*	0.42*	0.29*	25.93
Depth*conc.*suspen.	0.75*	78.83	13.75	61.50
Temp.*suspen.	10.15	8.63	92.97	22.57
Depth*temp.*suspen.	73.93	7.71	46.75	13.72
Conc.*temp.*suspen.	33.71	54.74	29.90	46.55
Depth*conc.*temp.*suspen.	18.59	65.90	23.52	82.63

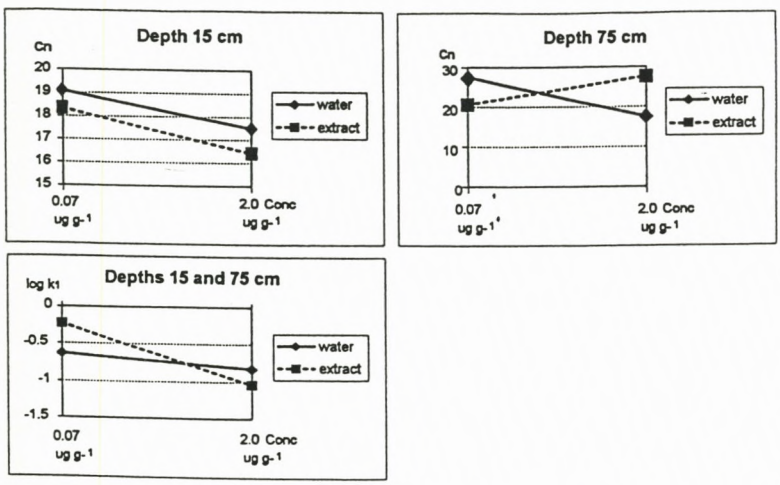

FIGURE 20 The combined interaction effects of depth, concentration and suspension for coefficients c_n and k_1.

Figure 20 only shows the interaction effects of conc*suspension for k_1. The coefficient k_1 decreased more steeply with concentration of ^{14}C-ETU when extract was added, than when only water was present. Since the interaction effects on pesticide degradation of depth, concentration and content of organic material never was elucidated in other published studies, it is not

possible to compare the obtained results with other results. More studies are needed to elucidate the complexity of interaction effects.

CONCLUSIONS

The first 60 days mineralization of ^{14}C-ETU, measured through the evolution of $^{14}CO_2$ can be described with a mathematical model consisting of two terms – one term describing the immediate mineralization of ^{14}C-ETU and another term describing the first order degradation of humus and/or biomass, where ^{14}C had been built in. For a further development of the model, measurements of initial amount of biomass or number of micro-organisms is needed.

The same mathematical model can describe the mineralization, independent of changes in concentration of pesticide, temperature, depth and organic carbon content. The mentioned factors do influence the degradation rate, however. Combined effects of depth, concentration and temperature and of concentration and organic carbon content indicate that future studies of degradation of other pesticides should not be limited to examine the mentioned factors one by one, but that studies should be designed to allow for examination of interaction effects.

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Influence of microbial activity, organic carbon content, soil texture and soil depth on mineralisation rates of low concentrations of ^{14}C-mecoprop—development of a predictive model

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Abstract

The high number of cases where pesticide residues have been found in groundwater during the last decade has enhanced the need for more knowledge about the fate of pesticides in soil. The purpose of the present study was to extend the knowledge of pesticide mineralisation in soil. Many publications have described the difficulties of finding a useful mathematical model for the description of pesticide mineralisation. In the present study a mathematical model is presented, which was useful for describing cometabolic mineralisation as well as metabolic mineralisation. On the basis of mineralisation studies of mecoprop in Danish soils, a predictive model, which described the mineralisation as a function of biological activity, soil texture, humus content and soil depth, was developed. The model was validated against mecoprop mineralisation studies in German soils and was shown to be very useful for the prediction of time for total mineralisation of mecoprop. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Soil; Microbial degradation; Metabolism; Cometabolism; Pesticides; Model

1. Introduction

To evaluate the threat of pesticides to ground water, pesticide degradation studies are performed. The results of the degradation studies, performed under conditions as close to nature as possible, can be evaluated directly and used to decide whether the compounds can be used for agricultural purposes. These results can also be used together with results from other relevant studies as an input in dynamic models to predict pesticide fate.

Pesticide degradation studies are time- and resource-consuming. Dynamic pesticide fate models could benefit from the knowledge of correlation between degradation rates and other more easily measurable factors.

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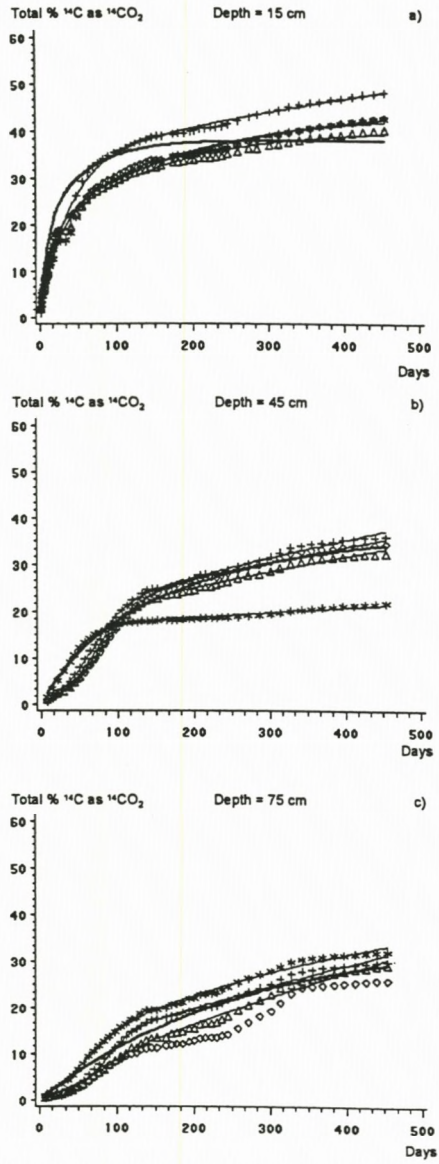

Fig. 1.

Boesten et al. (1995) compared and evaluated nine dynamic pesticide fate models. Of the nine models, eight used first order kinetics for describing pesticide degradation and considered only temperature and depth as having influence on the half-life time DT_{50}.

Mecoprop [2-(2-methyl-4-chlorophenoxy)propionic acid] is a phenoxypropanoic herbicide used widely in many countries. It is used to control broad-leaf weeds in cereal crops and is structurally related to other phenoxyalcanoic acid herbicides, such as 2,4-dichlorophenoxyacetic acid (2,4-D), 2-methyl-4-chlorophenoxyacetic acid (MCPA), and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). Phenoxyalcanoic acid herbicides are known to be degraded microbially (Loos, 1975; Sandmann et al., 1988). Even though recently mecoprop has been used frequently, only a few studies have been undertaken to determine the degradation of mecoprop in soil. And as for many other pesticides, information on persistence of mecoprop in subsoil is lacking. It has been reported that mecoprop is degraded mainly by micro-organisms in the soil (Lindholm et al., 1982; Lappin et al., 1985). Many reports on degradation of the phenoxyacetic acids, 2,4-D and MCPA, have shown that micro-organisms (both in single and mixed cultures) can adapt to repeated applications of these pesticides, which is seen as an enhanced degradation of the pesticide, the degradation being a metabolic process (Fryer and Kirkland, 1970; Torstensson et al., 1975; Torstensson, 1977; Smith and Aubin, 1994; Smith et al., 1994). It could be expected, that the biode-gradation of mecoprop and the other phenoxypropanoic acids would be similar to the degradation of the phenoxyacetic acids because of the similarities between molecular structure. Lappin et al. (1985) described a synergistic microbial community (comprised of five microbial species) that was capable of growing on mecoprop as the only carbon and energy source. When exposed to fresh herbicide additions, the community was able to shorten the lag phase from 30 days to less than 24 h.

Fig. 1. Mineralisation of 0.04 μg g^{-1} ^{14}C-mecoprop for each of four replicates in soil from 15-, 45- and 75-cm depth at site FB1_I. Symbols: data points. Broken lines: non-linear model fits. Bold solid line: composite model.

A number of studies have reported correlations between degradation rates of pesticides and soil properties. Mostly such correlations have been investigated for the soil properties, one at a time. Mueller et al. (1992) showed a positive linear correlation between the pseudo 1. order degradation rate constant for fluometuron and soil organic matter content as well as soil microbial biomass. The degradation of mecoprop was reported to be influenced by temperature, soil moisture content and concentration of pesticide (Smith and Hayden, 1981; Helweg, 1993).

Standard methods for measurement of microbial biomass and/or microbial activity are difficult to develop, since validation of the methods is difficult. The three methods most widely used for measuring microbial biomass-C in soil are fumigation-incubation (Jenkinson and Powlson, 1976), fumigation-extraction (Voroney and Paul, 1984; Vance et al., 1987) and substrate-induced respiration (Anderson and Domsch, 1978). The three methods are referred to in recent publications (Martens 1995; Stenström et al. 1998). ATP methods (Tate and Jenkinson, 1982; Eiland, 1983; Bai et al., 1988), staining followed by direct counting (Söderström, 1977), and determination of biomass through fatty acid patterns (Zelles et al., 1994) are other published methods for the measurement of microbial biomass. Martens (1995) concluded that no general conclusion could be made concerning the reliability of each method, and that exact determination of conversion factors between the methods could not be obtained.

In the substrate-induced respiration method (Anderson and Domsch, 1978) glucose is added to the soil samples and CO_2 development is measured hour by hour. Anderson and Domsch (1978) indicated that 40 mg biomass C respires 1 ml CO_2 h^{-1} at the stage of maximum initial response. In the present study mineralisation of ^{14}C-Na-acetate was used as a measurement of microbial activity. Na-acetate is easily degraded

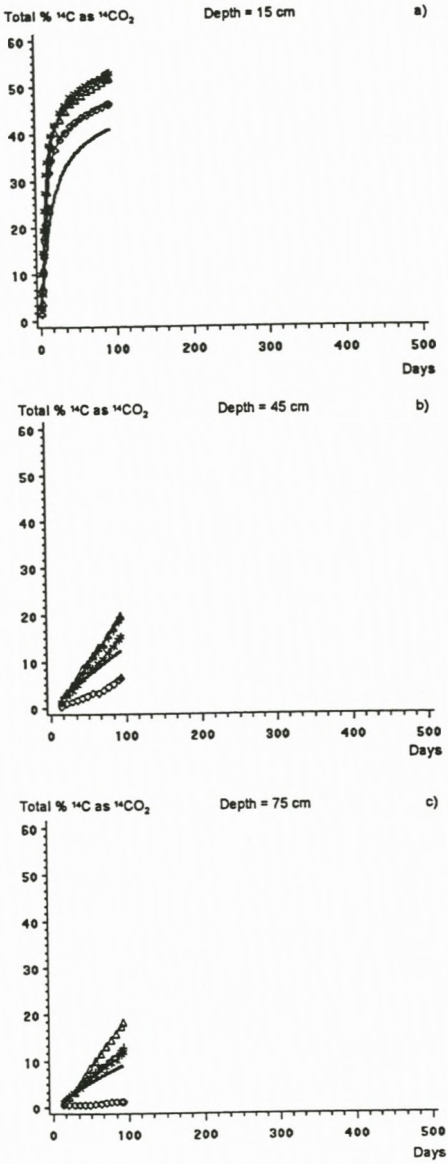

Fig. 2. Mineralisation of 0.04 μg g^{-1} ^{14}C-mecoprop for each of four replicates in soil from 15-, 45- and 75-cm depths at site FB1_II. Symbols: data points. Broken lines: non-linear model fits. Bold solid line: composite model.

by all micro-organisms since it is a natural substance in their metabolism.

The purpose of the present investigation was to compare degradation rates of mecoprop in different soil types at various depths and to develop and use a model to assess the influence of microbial activity, organic carbon and nutrient content on degradation rates of the pesticides.

2. Materials and methods

2.1. Soils

Data of Fomsgaard (1997) were used. Soil samples from Fladerne Bæk, field FB1, field FB3 and garden FB4, taken in January 1993, March 1993, March 1994 and January 1995 were included. Texture of the soil is shown in Table 1. The farmers' cultivation and spraying program for fields FB1 and FB3 is shown in Table 2. In the garden FB4, pesticides had not been used for years, except for Round-up, which had been used for defoliation in summer 1994.

Four replicate samples were taken at each site/time (FB1_I, FB1_II, FB3_I, FB3_II and FB4-I—hereafter we use the term site to denote combinations of site and time of sampling) and at each depth (ploughlayer = 15 cm; subsoil = 45 and 75 cm) for the degradation experiments of mecoprop. Additional samples were taken for sterilisation and subsequent incubation. At each site and depth, four replicate samples were taken for determination of microbial activity, quantified as the capacity of degrading ^{14}C-Na-acetate. Also four replicate samples were taken to determine the most probable number (MPN) of mecoprop degrading bacteria. Composite samples were taken for determination of soil texture and mecoprop sorption. Stainless steel tubes were forced into the soil in a horizontal position using aseptic tools. Plough layer samples (0–15 cm) were sieved (2 mm) to remove roots and plant

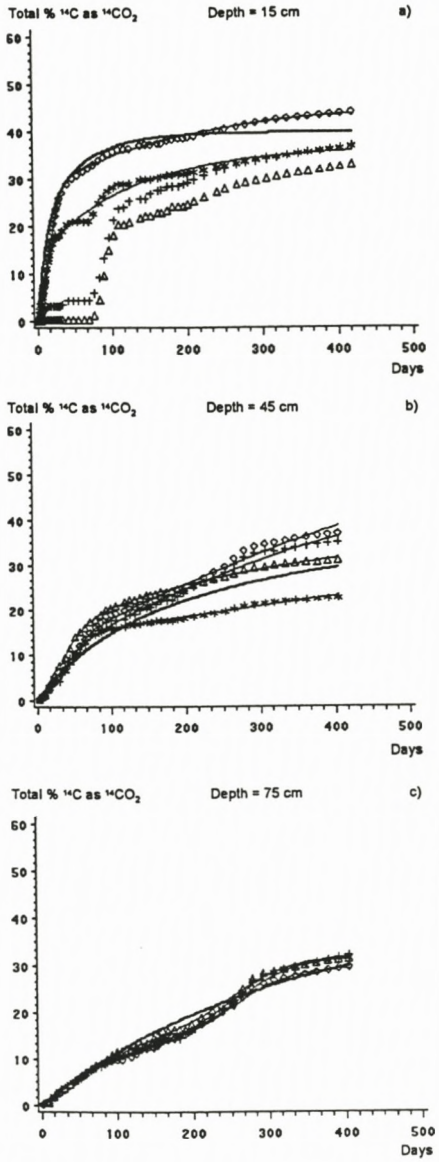

Fig. 3. Mineralisation of 0.04 μg g^{-1} ^{14}C-mecoprop for each of four replicates in soil from 15-, 45- and 75-cm depth at site FB3_I. Symbols: data points. Broken lines: non-linear model fits. Bold solid line: composite model.

Fig. 3.

Table 1
Sampling site, sampling time, depth, texture, pH (H_2O), incubation temperature, humus, soluble organic carbon, NO_3–N, NH_4–N and most probable number (MPN) for mecoprop and Na-acetate mineralisation experiments

Site	Sampling time	Depth (cm)	Humus (%)	Clay (%)	Silt (%)	Sand (%)	pH	Incubation time (days)	K_d mecoprop (l kg^{-1})	Soluble organic C (mg kg^{-1})	NO_3–N (mg kg^{-1})	NH_4–N (mg kg^{-1})	MPN bacteria g^{-1} for mecoprop (mean ± S.D. of four replicates)
FB 1_I	Jan, 93	15	3.1	4	3.9	89	7.1	500	0.77	411.8	4	16	$7.8\times10^{6}\pm3.8\times10^{6}$
FB 1_I	Jan, 93	45	0.9	3	2.4	94	6.2	500	0.66	310.2	0.3	6.1	$3.7\times10^{6}\pm4.9\times10^{6}$
FB 1_I	Jan, 93	75	0.2	2.5	1.9	95	5.9	500	0.26	–	–	–	$9.5\times10^{6}\pm3.9\times10^{6}$
FB 1_II	Mar, 94	15	2.8	3.6	2.8	91	6.9	93	0.79	339.2	24	3	$6.3\times10^{6}\pm7.3\times10^{6}$
FB 1_II	Mar, 94	45	0.3	2.5	1.4	96	6.3	93	0.39	246.2	0.4	1.3	$2.8\times10^{7}\pm3.7\times10^{7}$
FB 1_II	Mar, 94	75	0.1	2.1	1.4	96	6.4	93	0.20	172.5	0	0.9	$1.1\times10^{7}\pm0.8\times10^{7}$
FB 3_I	Mar, 93	15	2.7	3.2	2.8	91	6.6	500	0.69	286.5	2.9	18	$4.2\times10^{6}\pm3.0\times10^{6}$
FB 3_I	Mar, 93	45	0.8	2.3	1.2	96	6.1	500	0.55	–	–	–	$3.2\times10^{6}\pm3.3\times10^{6}$
FB 3_I	Mar, 93	75	0.2	1.4	1.2	97	6.1	500	0.00	159.6	0	0.4	$5.1\times10^{6}\pm4.6\times10^{6}$
FB 3_II	Mar, 94	15	2.8	4	2.9	90	6.7	93	0.73	339.1	3.3	15	$1.8\times10^{7}\pm1.0\times10^{7}$
FB 3_II	Mar, 94	45	0.9	3.5	2.4	93	5.6	93	0.46	329.8	0.6	3.6	$2.2\times10^{6}\pm2.4\times10^{6}$
FB 3_II	Mar, 94	75	0.3	3	1.4	95	5.5	93	0.20	196.7	0.4	0.7	$1.3\times10^{6}\pm0.6\times10^{6}$
FB 4_I	Jan, 95	15	4.7	4.6	3.8	87	5.2	93	2.79	450.5	3.6	8	$2.5\times10^{6}\pm0.5\times10^{6}$
FB 4_I	Jan, 95	45	5.1	3.6	1.9	89	5.2	93	2.64	493.4	1.7	9.3	$4.8\times10^{5}\pm1.3\times10^{5}$
FB 4_I	Jan, 95	75	0.5	2.1	2.8	95	5.6	93	0.14	255.0	0.3	2.1	$4.6\times10^{6}\pm5.7\times10^{6}$

Table 2
Cultivation and spraying program for fields FB1 and FB3

Year	Field FB1		Field FB3	
	Crop	Pesticide, amounts of a.i. per ha	Crop	Pesticide, amounts of a.i. per ha
1988	Fodder beat		Fodder beat	
1989	Pea	Cypermethrin, 50 g; bentazon, 438 g; MCPA, 219 g; cyanazine, 500 g; pirimicarb, 500 g; maneb, 1.8 l	Pea	Cypermethrin, 50 g; bentazon, 438 g; MCPA, 219 g; cyanazine, 500 g; pirimicarb, 500 g; maneb, 1.8 l
1990	Seed potato	Metribuzin, 280 g; mancozeb, 1365 g × 5; diquat-dibromide, 1.24 l	Wholecrop, barley (pea)	Bentazon, 500 g; MCPA 250 g
1991	Spring barley	Tribenuron-methyl, 37 g; fenpropimorph, 150 g; propiconazol, 63 g	Grass for harvest	
1992	Industrial potato	Metribuzin, 280 g; metribuzin, 140 g; fluazifop-*P*-butyl, 188 g; mancozeb, 1500 g × 8	Industrial potato	Metribuzin, 280 g; metribuzin, 140 g; fluazifop-*P*-butyl, 188 g; mancozeb, 1500 g × 8
1993	Spring barley, catchcrop Steffi	MCPA, 94 g; dichlorprop, 74 g; ioxynil, 15 g; bromoxynil, 9 g; tribenuron-methyl, 37 g; fenpropimorph, 75 g; prochloraz, 45 g; fenpropimorph, 113 g; prochloraz, 68 g	Spring barley, catchcrop Steffi	MCPA, 94 g; dichlorprop, 74 g; ioxynil, 15 g; bromoxynil, 9 g; tribenuron-methyl, 37 g; fenpropimorph, 75 g; prochloraz, 45 g; fenpropimorph, 113 g; prochloraz, 68 g

Table 3
Estimates and mean square for model fits to ^{14}C-mecoprop mineralisation data

Depth (cm)	Site	Replicate	c_{n_meco}	k_{1_meco}	k_{2_meco}	k_{3_meco}	c_{b_meco}	$\lambda/m_{0\ \_meco}$	Mean square	Model version
15	FB 1_I	1	34.3	0.0264	0.0000	0.0005	65.7	0.0	1.02	B
15	FB 1_I	2	21.4	0.0433	0.0000	0.0042	24.5		0.73	B
15	FB 1_I	3	17.1	0.0858	0.0000	0.0068	23.4		0.61	B
15	FB 1_I	4	18.1	0.0705	0.0000	0.0059	25.5		1.06	B
45	FB 1_I	1	18.3	0.0437	−0.0023	0.0006	81.7	1.0	0.25	A
45	FB 1_I	2	16.5	0.0398	−0.0016	0.0001	82.5	0.1	0.12	B
45	FB 1_I	3	17.6	0.0451	−0.0025	0.0005	78.0	1.5	0.15	B
45	FB 1_I	4	19.5	0.0405	−0.0020	0.0005	80.0	1.4	0.21	B
75	FB 1_I	1	9.3	0.0664	−0.0072	0.0006	90.7	33.3	0.40	B
75	FB 1_I	2	9.6	0.0390	−0.0037	0.0020	40.2	1.2	0.32	B
75	FB 1_I	3	3.3	0.0566	−0.0171	0.0007	96.7	66.7	0.59	A
75	FB 1_I	4								No fit
15	FB 1_II	1	33.1	0.3599	−0.0089	0.0256	21.3	0.1	0.30	B
15	FB 1_II	2	31.5	0.3858	−0.0102	0.0305	22.4	0.2	0.33	B
15	FB 1_II	3	35.8	0.2424	−0.0053	0.0186	19.4	0.1	0.54	B
15	FB 1_II	4	32.3	0.2577	−0.0064	0.0184	17.2	0.1	0.51	B
45	FB 1_II	1	27.6	0.0301	−0.0009	0.0000	72.4	0.2	0.18	A
45	FB 1_II	2	32.0	0.0180	−0.0004	0.0000	68.0	0.1	0.09	A
45	FB 1_II	3	31.1	0.0241	−0.0006	0.0000	68.9	0.1	0.18	A
45	FB 1_II	4								No fit
75	FB 1_II	1	20.5	0.0215	−0.0008	0.0000	79.5	0.1	0.04	A
75	FB 1_II	2	17.8	0.0232	−0.0010	0.0000	82.2	0.2	0.05	A
75	FB 1_II	3	22.5	0.0397	−0.0016	0.0000	77.5	0.4	0.08	A
75	FB 1_II	4								No fit
15	FB 3_I	1								Omitted
15	FB 3_I	2	13.9	0.2244	−0.0139	0.0077	22.9	0.4	0.61	B
15	FB 3_I	3								Omitted
15	FB 3_I	4	26.8	0.1300	−0.0042	0.0052	19.2	0.2	0.22	B
45	FB 3_I	1	6.8	0.0877	−0.0127	0.0022	50.0	7.7	0.67	B
45	FB 3_I	2	13.6	0.0536	−0.0034	0.0003	86.4	0.4	0.14	B
45	FB 3_I	3	13.5	0.0598	−0.0039	0.0034	24.1	0.5	0.11	B
45	FB 3_I	4	9.2	0.0825	−0.0087	0.0013	70.8	3.7	0.91	B
75	FB 3_I	1	16.8	0.0265	−0.0016	0.0099	15.5	50.0	0.13	B
75	FB 3_I	2	15.5	0.0277	−0.0018	0.0095	16.6	100.0	0.14	B
75	FB 3_I	3	12.9	0.0278	−0.0022	0.0081	19.5	100.0	0.20	B
75	FB 3_I	4	14.2	0.0270	−0.0019	0.0093	15.7	50.0	0.14	B
15	FB 3_II	1	26.2	0.1791	−0.0056	0.0236	19.9	0.2	0.13	B
15	FB 3_II	2	6.3	0.3158	−0.0491	0.0399	32.7	6.3	0.11	B
15	FB 3_II	3	19.7	0.1386	−0.0050	0.0239	23.7	0.1	0.08	B
15	FB 3_II	4	27.7	0.1396	−0.0035	0.0153	15.4	0.1	0.16	B
45	FB 3_II	1	24.9	0.0278	−0.0008	0.0000	75.1	0.1	0.10	A
45	FB 3_II	2	22.8	0.0485	−0.0019	0.0000	77.2	0.4	0.33	A
45	FB 3_II	3	25.5	0.0415	−0.0014	0.0000	74.5	0.2	0.19	A
45	FB 3_II	4	11.5	0.0994	−0.0082	0.0020	88.5	1.7	0.08	A
75	FB 3_II	1								No fit
75	FB 3_II	2	4.5	0.0127	−0.0017	0.0005	95.5	0.4	0.28	A
75	FB 3_II	3								No fit
75	FB 3_II	4	21.8	0.0535	−0.0022	0.0003	78.2	0.5	0.54	A
15	FB 4_I	1	27.3	0.1403	−0.0013	0.0009	72.7	0.0	0.42	A
15	FB 4_I	2	11.8	0.5180	−0.0298	0.0356	28.2	0.2	0.35	B
15	FB 4_I	3	8.6	0.7467	−0.0700	0.0369	26.9	0.5	0.22	B
15	FB 4_I	4	24.4	0.2125	−0.0041	0.0215	21.1	0.0	0.03	B
45	FB 4_I	1	9.0	0.0784	0.0000	0.0003	91.0		0.05	A
45	FB 4_I	2	23.1	0.1674	−0.0043	0.0004	76.9	0.1	0.14	A
45	FB 4_I	3	29.4	0.0656	−0.0008	0.0010	70.6	0.0	0.07	A
45	FB 4_I	4	18.2	0.1018	−0.0021	0.0005	81.8	0.0	0.15	A
75	FB 4_I	1								No fit
75	FB 4_I	2	7.7	0.0669	−0.0083	0.0017	92.3	3.2	0.01	A
75	FB 4_I	3								No fit
75	FB 4_I	4	27.3	0.0379	−0.0009	0.0001	72.7	0.1	0.01	A

material; subsoil samples (45–75 cm) were kept undisturbed. The samples were stored at 5°C before incubation. To determine sorption and to incubate sterilised samples, the samples were irradiated with electron beam of 2×11 kGy. Former experience (Helweg, 1993) has shown that 2×11 kGy is sufficient to suppress biological activity.

2.2. Chemicals

The chemicals were obtained from Amersham. Ring ^{14}C-labelled mecoprop (2-(4-chloro-2-methylphenoxy)propanoic acid) had a specific activity of 24 $\mu Ci \times mg^{-1}$ and a radiochemical purity of 99%. ^{14}C-labelled Na-acetate had a specific activity of 667 $\mu Ci\ mg^{-1}$ and a radiochemical purity of 98.6%.

2.3. Pesticide degradation experiments

The incubation experiments were performed at the lowest possible concentration based on the specific activity (0.04 $\mu g \times g^{-1}$). The ^{14}C-labelled mecoprop was added to the plough layer soil samples by mixing in an Erlenmeyer flask, and to the subsoil samples by injecting it into the undisturbed soil column with a long needle to maintain incubation conditions as close to nature as possible. The pesticides were added in an aquatic solution to adjust the water content of the soil to approximately 50% of water holding capacity. The incubation temperature was 10°C to simulate Danish winter soil temperature. A gentle stream of atmospheric air was passed through 2 h/week. Evolved $^{14}CO_2$ was absorbed in traps of KOH according to Helweg (1993) and quantified by liquid scintillation counting to follow the mineralisation of the compound and a trap of glycerol was used to trap eventual volatile compounds. After the incubation period the soil samples were analysed for remaining ^{14}C-mecoprop. Extraction of ^{14}C-mecoprop was performed with 0.25 M $Ca(OH)_2$ by sonication and centrifugation. A total of 5 ml methanol/l extract

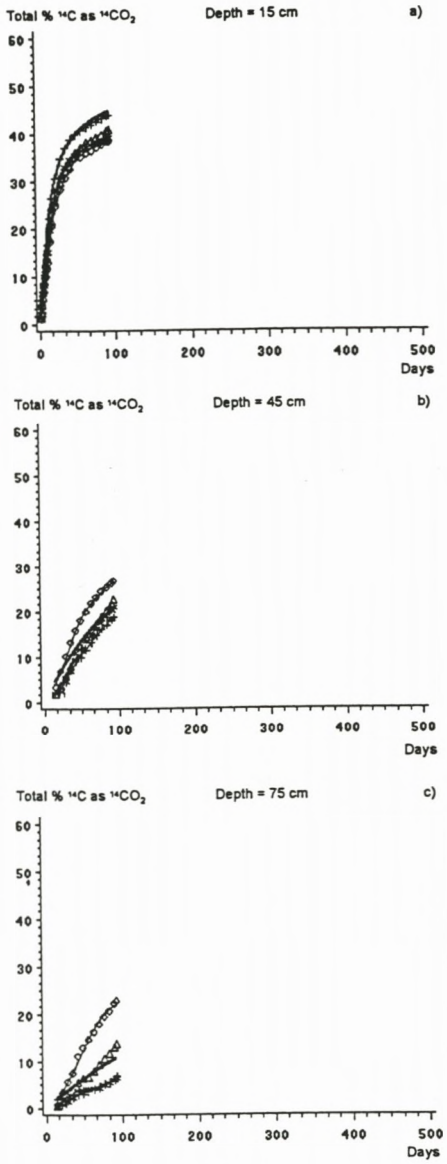

Fig. 4. Mineralisation of 0.04 $\mu g\ g^{-1}$ ^{14}C-mecoprop for each of four replicates in soil from 15-, 45- and 75-cm depths at site FB3_II. Symbols: data points. Broken lines: non-linear model fits. Bold solid line: composite model.

Fig. 4

was added and pH was adjusted to < 2 with HCl. Extraction was performed on a C-18 Empore disk and mecoprop was eluted with methanol. The methanolic extracts were evaporated to dryness with N_2 and dissolved in 1 ml mobile phase (methanol:tetrabutylammonia hydrogen sulphate 67:33) for HPLC separation in a Chrompack Spherisorb ODS2 column, 100 × 3 mm. The separated compounds were collected and residual mecoprop was quantified by liquid scintillation counting (Helweg, 1993). Recovery of mecoprop was > 85% in plough layer and > 93% in subsoil. Recovery experiments for the degradation product, 2-methyl-4-chlorphenol, were carried out, but no recovery was found, possibly because this compound is rapidly integrated into the organic soil components.

The remaining soil was combusted in a Packard Oxidizer to determine the amount of ^{14}C built into the organic matter of the soil.

2.4. Determination of sorption

Sorption (K_d) was determined according to OECD (1981). A total of three replicates of each 5 g of dried, sieved and sterilised soil was shaken for 16 h in 25 ml 0.01 M $CaCl_2$ with isotope-labelled pesticide (5 $\mu g \times g^{-1}$). The K_d-value was calculated as the ratio of the adsorbed amount to the concentration in water.

2.5. Degradation of ^{14}C-Na-acetate

Degradation of ^{14}C-Na-acetate was determined by adding ^{14}C-Na-acetate (5 mg kg^{-1}) to the soil in an Erlenmeyer flask, adjusting water content to approximately 50% of water holding capacity and incubating at 20°C according to the method of Dictor et al. (1992). Evolved $^{14}CO_2$ was absorbed in traps of KOH and quantified by liquid sointillation counting to follow the mineralisation of ^{14}C-Na-acetate. The mineralisation rate of ^{14}C-Na-

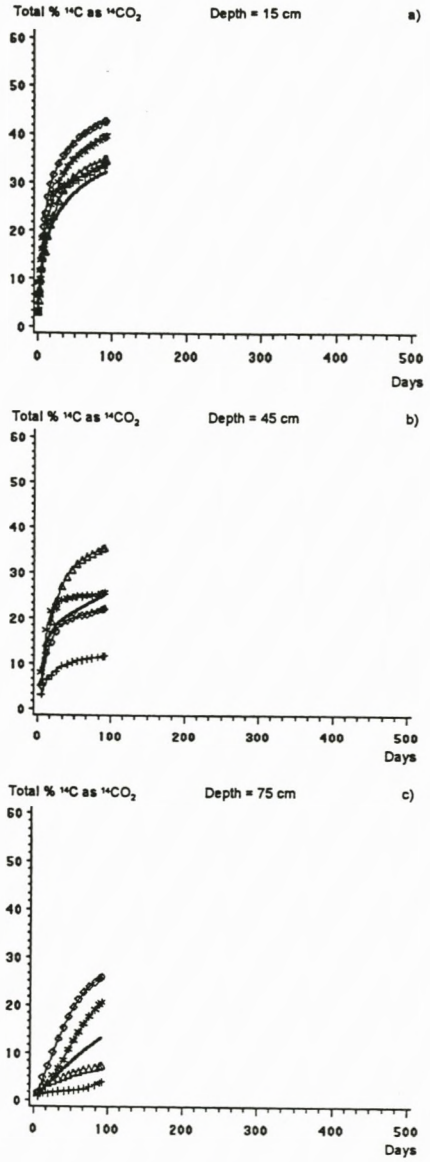

Fig. 5. Mineralisation of 0.04 $\mu g\ g^{-1}$ ^{14}C-mecoprop for each of four replicates in soil from 15-, 45- and 75-cm depths at site FB4_I. Symbols: data points. Broken lines: non-linear model fits. Bold solid line: composite model.

Fig. 5.

2.6. Determination of total organic carbon, NO_3-N and NH_4-N

Nitrate and ammonium were analysed according to the standardised methods of the Danish Agricultural Ministry (Landbrugsministeriet, 1994).

For the analysis of soluble organic carbon (SOC), 90 ml water was added to 20 g homogenised soil, shaken for 30 min and filtered. Then 2 ml H_3PO_4 was added, the sample was flushed with air to remove CO_2, and the sample was neutralised with 2 M NaOH. The volume of the extract was adjusted to 100 ml, and NVOC (non-volatile organic carbon) was measured in a Dohrman DX apparatus.

2.7. Determination of most probable number

Soil suspensions were made by mixing 10 g soil from each site and depth with 90 g Winogradski solution. A series of dilutions 1:10 was prepared and 1 ml of each dilution was added to test-tubes, to which 4.5 g sterilised soil from the same site and depth had already been added. ^{14}C-mecoprop was added in a concentration of 2 μg g^{-1} soil (Gardshodn and Fomsgaard, 1991). The test tubes were placed in scintillation vials with 2 ml 1 N KOH and incubated in the dark for 30 days. The evolved $^{14}CO_2$ was absorbed in KOH. The scintillation vials where changed, scintillation liquid was added to KOH in the scintillation vials, and the amount of $^{14}CO_2$ was counted. An amount of $^{14}CO_2$ above the detection limit was taken as a positive result. The MPN number was found from the number of positive test tubes according to Dansk Standard (1983) and American Public Health Association (1985) The incubation of the test-tubes in new scintillation vials with KOH continued for 30 days at a time until the MPN numbers at two successive measurements were equal.

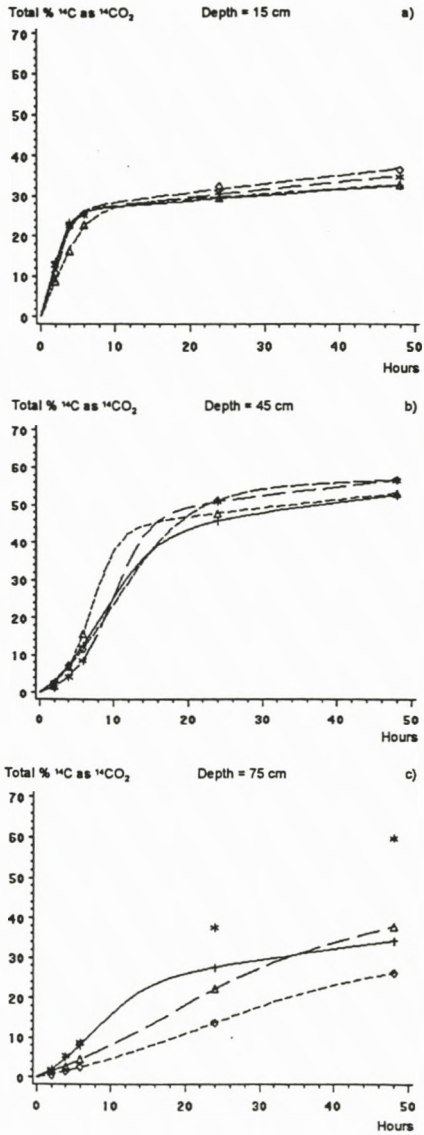

Fig. 6. Mineralisation of 5 μg g^{-1} ^{14}C-Na-acetate for each of four replicates in soil from 15-, 45- and 75-cm depths at site FB1_I. Symbols: data points. Broken lines: non-linear model fits.

Fig. 6.

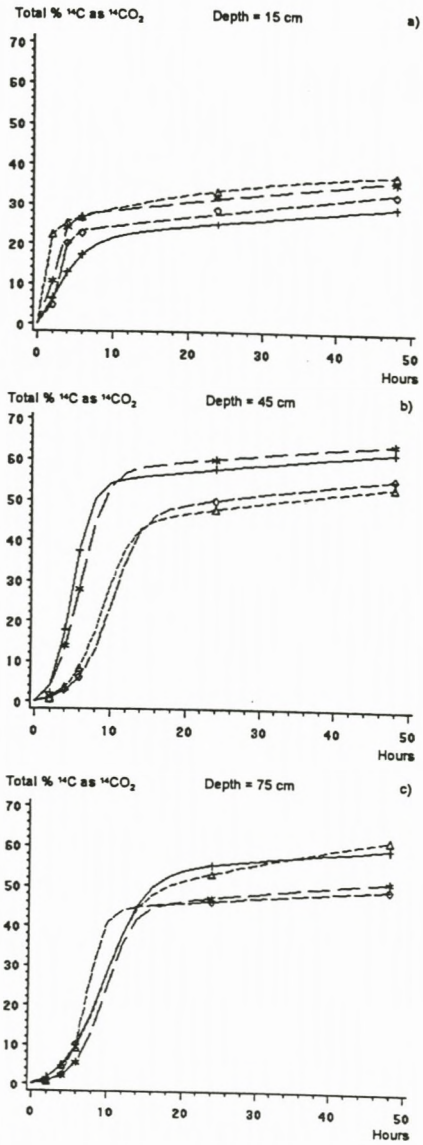

Fig. 7.

3. Models, results and discussion

3.1. Mineralisation model, part 1

In soil degradation studies, where undisturbed soil samples are used, it is not possible to remove aliquots of the sample to follow the disappearance of the parent compound. If pesticide mineralisation is to be followed in the same sample, $^{14}CO_2$, formed through the mineralisation of ^{14}C-labelled pesticide, must be quantified. For comparison of degradation rates and correlation with other parameters, the curves depicting the $^{14}CO_2$ formation must then be described by a mathematical model.

The data presented in the present study was formerly used by Fomsgaard (1997) to model the mineralisation kinetics; several mathematical models were fitted to the $^{14}CO_2$-formation data. The fitted models were very useful to elucidate the processes involved in the mineralisation, but comparison of degradation rates for different samples could not be performed when different models were used. Thus, a further model needed to be developed.

Liu and Zhang (1986) assumed that the degradation of pesticides in soil involves microbial utilisation of pesticide as an energy source (metabolicdegradation) and stated that their model, which described the decrease in pesticide concentration, was able to describe degradation curves whether they had an inflection point or not. Fomsgaard (1997) converted the Liu and Zhang model to express degradation of ^{14}C-labelled pesticides through the accumulated formation of $^{14}CO_2$ and found that only for cases where an inflection point was seen, generally in subsoil samples, did the model give useful fits. The converted Liu and Zhang model was used by Fomsgaard et al. (1998a) to model ^{14}C-maneb mineralisation in sediment, and by Fomsgaard et al. (1998b) to model ^{14}C-mecoprop degradation in

Fig. 7. Mineralisation of 5 μg g^{-1} ^{14}C-Na-acetate for each of four replicates in soil from 15-, 45- and 75-cm depths at site FB1_II. Symbols: data points. Broken lines: non-linear model fits.

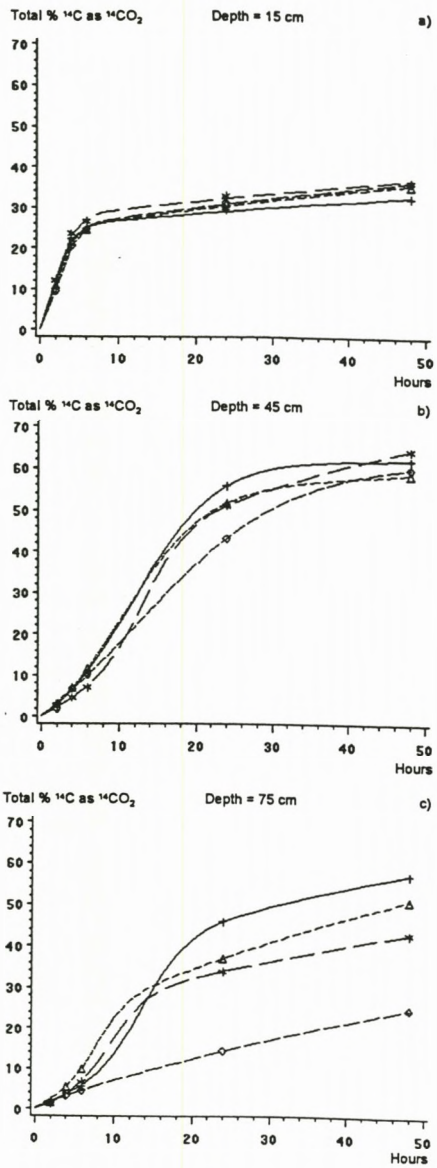

Fig. 8.

undisturbed subsoil. The converted Liu and Zhang model (Fomsgaard, 1997) was modified by adding a zero order term to describe mineralisation of 5 μg g^{-1} ^{14}C-mecoprop (Helweg et al., 1998). In the present study a further modification of the Liu and Zhang model (Fomsgaard and Kristensen, 1999), where a first order term is added, was used. The modified non-linear model had five parameters.

The mineralisation of ^{14}C-mecoprop was described according to the Fomsgaard and Kristensen (1999) modification of the Liu and Zhang (1986) model, assuming that the formation of $^{14}CO_2$ occurred as a result of at least two processes. One process was the immediate mineralisation of ^{14}C-pesticide and another process was the first order mineralisation of soil organic matter, into which ^{14}C from the pesticide had been built (or very strongly adsorbed). The same model was used to describe the mineralisation of ^{14}C-Na-acetate. The model used was thus:

$$C_t = C_m + C_h \tag{1}$$

$$C_m = c_n - \frac{k_1 c_n}{(k_1 + k_2 c_n)e^{k_1 t} - k_2 c_n} \tag{2}$$

$$C_h = c_b(1 - e^{-k_3 t}) \tag{3}$$

where

C_t = total concentration of mineralisation product ($^{14}CO_2$) formed at time t (measured as $\%^{14}$C evolved as $\%^{14}CO_2$)

$C_m = \%^{14}CO_2$ formed at time t according to Eq. (2)

$C_h = \%^{14}CO_2$ formed at time t according to a first order model (Eq. (3))

c_n = total % of ^{14}C-compound converted to $^{14}CO_2$ according to Eq. (2)

c_b = total % of ^{14}C-compound converted to $^{14}CO_2$ according to Eq. (3)

$$k_1 = k(m_0 + \lambda c_n) \tag{4}$$

$$k_2 = -k\lambda \tag{5}$$

where

Fig. 8. Mineralisation of 5 μg g^{-1} ^{14}C-Na-acetate for each of four replicates in soil from 15-, 45- and 75-cm depths at site FB3_I. Symbols: data points. Broken lines: non-linear model fits.

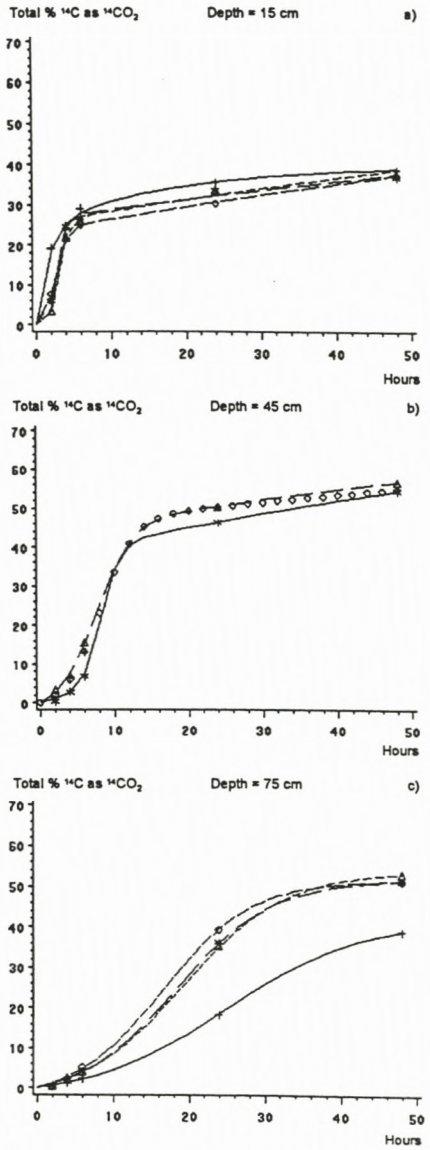

Fig. 9.

k_3 = degradation rate constant
k = degradation rate constant
m_0 = number of micro-organisms involved in the degradation of the compound at start time
λ = growth rate constant for micro-organisms
t = time in days.

The following two restrictions were put on the parameters because the total % of ^{14}C-compound converted to $^{14}CO_2$ cannot be larger than 100%, and because Eq. (2) does not describe a mineralisation curve if $c_n > -k_1/k_2$:

$$c_n + c_b \leq 100 \tag{6}$$

$$c_n \leq -\frac{k_1}{k_2} \tag{7}$$

Two different versions of this model were evaluated

Model version A: $c_n + c_b = 100\%$ and model version B: $c_n + c_b < 100\%$ according to Fomsgaard and Kristensen (1999).

Five coefficients (k_1, k_2, k_3, c_n and c_b) of the model were estimated using non-linear least squares method. As the model is non-linear, iterative methods were used. Different methods were used in parallel in order to ensure convergence. Two or more of the methods of steepest descent, the Gauss-Newton method, the intermediate method of Marquardt (Marquardt, 1963) and a derivative-free method (Ralston and Jennrich, 1978) were used for each sample. All statistical calculations were done using procedures from SAS Institute (1989) SAS Institute (1990) SAS Institute (1996). Parameters of simplified models were first estimated in order to obtain good initial estimates for the iterative process. The simplifications were that either k_2 or k_3 was assumed to be zero. It was possible to model all ^{14}C-mecoprop and ^{14}C-Na-acetate mineralisation curves with the described model (Eqs. (1)–(3)). The parameter estimates and the residual mean squares for the chosen model versions (A and B) are shown in

Fig. 9. Mineralisation of 5 μg g^{-1} ^{14}C-Na-acetate for each of four replicates in soil from 15-, 45- and 75-cm depths at site FB3_II. Symbols: data points. Broken lines: non-linear model fits.

Table 4
Estimates and mean square for model fits to Na-acetate mineralisation data and %^{14}C evolved as $^{14}CO_2$ after 2 h and after 4 h from ^{14}C-Na-acetate

Depth (cm)	Site	Replicate	c_{n_naac}	k_{1_naac}	k_{2_naac}	k_{3_naac}	c_{b_naac}	λ/m_{0_naac}	Mean square	Model version	%^{14}C as $^{14}CO_2$ (2 h)	%^{14}C as $^{14}CO_2$ (4 h)
15	FB1_I	1	25.7	0.872	−0.0262	0.0021	74.3	0.13	0.18	A	13.58	23.3
15	FB1_I	2	25.4	0.764	−0.0220	0.0029	74.6	0.11	0.48	A	12.88	22.6
15	FB1_I	3	26.4	0.461	−0.0121	0.0020	73.6	0.09	0.57	A	8.94	16.4
15	FB1_I	4	26.0	0.798	−0.0254	0.0033	74.0	0.18	1.17	A	10.89	22.2
45	FB1_I	1	39.0	0.272	−0.0063	0.0053	61.0	0.25	0.07	A	2.90	7.5
45	FB1_I	2	44.5	0.414	−0.0091	0.0052	55.5	1.31	0.04	A	1.35	4.2
45	FB1_I	3	41.7	0.565	−0.0133	0.0046	58.3	1.28	0.13	A	1.76	6.9
45	FB1_I	4	52.8	0.199	−0.0033	0.0018	47.2	0.14	0.44	A	2.55	7.4
75	FB1_I	1	20.8	0.281	−0.0121	0.0039	79.2	0.43	0.17	A	1.77	5.1
75	FB1_I	2								No fit	2.08	5.5
75	FB1_I	3	11.1	0.169	−0.0147	0.0075	88.9	2.31	0.00	A	1.53	3.0
75	FB1_I	4	30.9	0.074	−0.0020	0.0000	69.1	0.17	0.04	A	0.60	1.6
15	FB1_II	1	20.5	0.413	−0.0136	0.0024	79.5	0.10	0.01	A	6.38	12.7
15	FB1_II	2	26.0	1.187	−0.0426	0.0030	74.0	0.54	1.05	A	10.67	24.0
15	FB1_II	3	23.2	1.177	0.0000	0.0376	16.9		0.03	B	22.28	25.2
15	FB1_II	4	21.8	1.626	−0.0739	0.0032	78.2	4.65	1.43	A	4.73	19.9
45	FB1_II	1	52.7	0.816	−0.0152	0.0045	47.3	1.08	4.15	A	2.20	17.8
45	FB1_II	2	55.7	0.605	−0.0106	0.0043	44.3	0.68	6.07	A	1.51	13.7
45	FB1_II	3	41.2	0.544	−0.0131	0.0049	58.8	3.21	0.12	A	0.87	3.6
45	FB1_II	4	43.8	0.514	−0.0117	0.0048	56.2	4.27	0.32	A	0.45	2.9
75	FB1_II	1	51.1	0.383	−0.0073	0.0039	48.9	0.82	0.00	A	1.75	4.7
75	FB1_II	2	42.4	0.563	−0.0132	0.0035	57.6	5.72	0.00	A	0.74	2.2
75	FB1_II	3	42.6	0.462	−0.0107	0.0085	57.4	1.93	1.11	A	0.82	5.0
75	FB1_II	4	42.9	0.907	−0.0211	0.0025	57.1	19.16	0.05	A	0.77	2.3
15	FB3_I	1	24.9	0.817	−0.0255	0.0023	75.1	0.14	0.41	A	12.24	22.0
15	FB3_I	2	27.0	0.786	−0.0232	0.0030	73.0	0.14	1.34	A	12.12	23.4
15	FB3_I	3	24.2	0.870	−0.0301	0.0035	75.8	0.21	0.90	A	10.97	21.6
15	FB3_I	4	24.6	0.814	−0.0286	0.0035	75.4	0.26	0.37	A	9.29	20.3
45	FB3_I	1	62.1	0.185	−0.0027	0.0000	37.9	0.14	0.39	A	3.24	6.6
45	FB3_I	2	34.1	0.350	−0.0102	0.0129	65.9	2.76	0.36	A	2.56	4.6
45	FB3_I	3	53.1	0.189	−0.0031	0.0027	46.9	0.13	0.01	A	3.04	7.0
45	FB3_I	4	62.3	0.098	−0.0012	0.0145	37.7	0.05	0.72	A	1.92	6.7
75	FB3_I	1	32.9	0.348	−0.0105	0.0094	67.1	3.18	0.18	A	1.79	3.7
75	FB3_I	2	23.2	0.441	−0.0188	0.0062	76.8	2.75	0.26	A	1.01	3.8
75	FB3_I	3	18.7	0.559	−0.0295	0.0105	81.3	4.12	0.57	A	1.51	5.5
75	FB3_I	4	2.2	0.162	−0.0021	0.0055	97.8	0.01	0.01	A	1.61	3.2
15	FB3_II	1	24.7	0.584	0.0000	0.0495	15.3		1.82	B	19.10	23.6
15	FB3_II	2	26.1	1.740	−0.0661	0.0034	73.9	4.50	2.71	A	5.97	24.5
15	FB3_II	3	25.2	1.792	−0.0710	0.0041	74.8	11.29	2.99	A	3.31	21.9
15	FB3_II	4	23.0	1.380	−0.0583	0.0042	77.0	1.46	0.15	A	7.54	21.4
45	FB3_II	1	45.1	0.447	−0.0097	0.0041	54.9	0.83	0.85	A	1.31	6.6
45	FB3_II	2	36.7	0.839	−0.0228	0.0069	63.3	31.25	0.48	A	0.34	2.8
45	FB3_II	3	43.3	0.431	−0.0096	0.0057	56.7	0.60	0.54	A	3.31	7.1
45	FB3_II	4								No data		

Table 4 (*Continued*)

Depth (cm)	Site	Replicate	c_{n_naac}	k_{1_naac}	k_{2_naac}	k_{3_naac}	c_{b_naac}	λ/m_{0_naac}	Mean square	Model version	%^{14}C as $^{14}CO_2$ (2 h)	%^{14}C as $^{14}CO_2$ (4 h)
75	FB3_II	1	41.9	0.111	−0.0025	0.0000	58.1	0.39	0.16	A	0.26	1.2
75	FB3_II	2	51.6	0.157	−0.0029	0.0000	48.4	0.33	0.12	A	0.74	2.3
75	FB3_II	3	53.7	0.147	−0.0026	0.0000	46.3	0.31	0.25	A	0.61	2.2
75	FB3_II	4	51.5	0.168	−0.0031	0.0000	48.5	0.31	1.06	A	0.27	2.5
15	FB4_I	1	17.0	0.803	0.0000	0.0239	12.9		0.01	B	14.23	17.4
15	FB4_I	2	5.1	0.090	0.0000	0.0072	28.7		0.06	B	1.54	2.2
15	FB4_I	3	20.7	0.911	−0.0309	0.0031	79.3	0.11	0.31	A	13.03	20.1
15	FB4_I	4	18.4	0.613	0.0000	0.0223	18.4		0.01	B	13.68	18.4
45	FB4_I	1	27.0	0.673	−0.0245	0.0024	73.0	2.18	1.47	A	0.52	6.3
45	FB4_I	2	37.5	0.515	−0.0130	0.0029	62.5	0.49	0.12	A	3.27	10.7
45	FB4_I	3	33.2	0.692	−0.0202	0.0031	66.8	0.94	1.28	A	2.32	11.9
45	FB4_I	4	32.7	0.678	−0.0204	0.0028	67.3	1.77	1.01	A	1.03	76
75	FB4_I	1	68.9	0.283	−0.0041	0.0000	31.1	2.75	0.01	A	0.34	0.8
75	FB4_I	2	70.3	0.317	−0.0045	0.0000	29.7	3.55	0.00	A	0.21	0.8
75	FB4_I	3	68.2	0.300	−0.0044	0.0000	31.8	2.18	0.01	A	0.38	1.2
75	FB4_I	4	64.1	0.166	−0.0026	0.0000	35.9	0.97	0.02	A	0.49	1.1

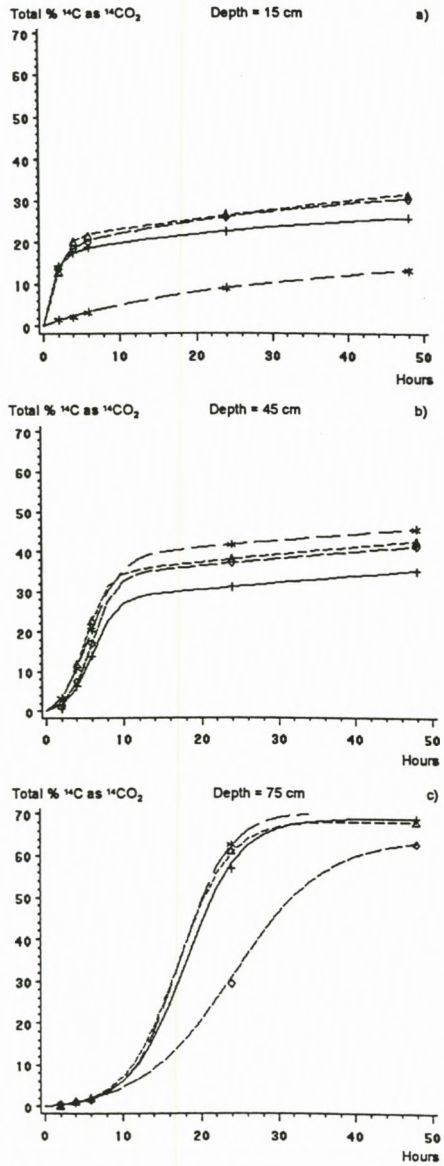

Fig. 10.

Table 3 for ^{14}C-mecoprop and in Table 4 for ^{14}C-Na-acetate. In some cases, only one of the versions converged. In many cases the mean squares for the two versions were significantly different, but the version yielding the smallest value were also chosen when there were no significant differences. The estimates for mecoprop are labelled with subscript '$_{-meco}$', and the estimates for Na-acetate are labelled with subscript '$_{-naac}$'. Table 4 also shows the amount of $^{14}CO_2$ developed from ^{14}C-Na-acetate after 2 h and the amount of $^{14}CO_2$ developed from ^{14}C-Na-acetate after 4 h. The accumulated amounts of $^{14}CO_2$ depicted as a function of time and the fitted non-linear model for ^{14}C-mecoprop are shown in Figs. 1–5 and for ^{14}C-Na-acetate in Figs. 6–10.

When the estimated value of k_{3_meco} was 0, the first order mineralisation (Eq. (3)) was insignificant. Such cases can be seen in Fig. 2b and c, where the development of $\%^{14}CO_2$ did not reach the flat part of the curve before it was stopped, while in Fig. 2a for example, the flat part of the curve where k_3 obtained a positive value can be seen. When the estimated value of k_{2_meco} was 0, no growth of micro-organisms occurred ($\lambda = 0$) and the model in Eq. (2) reduces to a first-order model

$$C_m = c_n - \frac{c_n}{e^{k_1 \cdot t}} = c_n \cdot (1 - e^{-k_1 \cdot t}) \tag{8}$$

Liu and Zhang (1986) stated that with $k_2 = 0$ only chemical degradation of the pesticide occurred. However, microbial degradation probably occurred in the present study even when no growth was seen. The degradation process was then a cometabolic process, where the micro-organisms degrade the pesticide without deriving energy from the degradation process. In cases where growth was seen, the curves have a sigmoidal form (show inflection point) before they bend over to the flat part. As stated by Foms-

Fig. 10. Mineralisation of 5 μg g^{-1} ^{14}C-Na-acetate for each of four replicates in soil from 15-, 45- and 75-cm depths at site FB4_I. Symbols: data points. Broken lines: non-linear model fits.

Table 5
Degradation of ^{14}C-mecoprop in soil[a]

Soil site, soil depth and days of incubation	% ^{14}C as $^{14}CO_2$	% ^{14}C in extract	% Mecoprop in extract	% ^{14}C in combusted soil	Total recovery of ^{14}C[b]
Field FB1_I ploughlayer, 500 days	43.5 ± 3.3	NA	NA	48.8 ± 6.2	92.3 ± 6.0
Field FB1_I 45 cm, 500 days	31.1 ± 6.4	NA	NA	44.7 ± 2.0	75.9 ± 8.1
Field FB1_I 75 cm, 500 days	29.3 ± 2.5	NA	NA	34.1 ± 4.2	63.4 ± 2.1
Field FB1_II ploughlayer, 93 days	51.0 ± 3.0	4.3 ± 0.2	ND	32.6 ± 1.5	87.8 ± 2.1
Field FB1_II 45 cm, 93 days	15.4 ± 6.2	26.1 ± 16.8	17.1 ± 16.1	25.0 ± 9.2	66.4 ± 2.0
Field FB1_II 75 cm, 93 days	11.1 ± 7.1	40.9 ± 4.7	24.6 ± 1.9	16.8 ± 1.2	68.8 ± 7.9
Field FB3_I ploughlayer, 500 days	37.7 ± 4.6	NA	NA	43.7 ± 2.9	81.5 ± 2.8
Field FB3_I 45 cm, 500 days	31.3 ± 6.2	NA	NA	37.3 ± 3.7	68.6 ± 5.4
Field FB3_I 75 cm, 500 days	31.2 ± 1.1	NA	NA	36.8 ± 4.2	67.9 ± 5.1
Field FB3_II ploughlayer, 93 days	40.8 ± 2.4	6.4 ± 0.8	ND	41.7 ± 4.3	88.8 ± 2.8
Field FB3_II 45 cm, 93 days	22.4 ± 3.4	11.5 ± 3.7	5.5 ± 3.1	26.7 ± 0.8	60.6 ± 3.9
Field FB3_II 75 cm, 93 days	12.5 ± 7.8	27.7 ± 12.9	18.5 ± 12.0	24.8 ± 7.7	65.0 ± 4.6
Garden FB4_I ploughlayer, 93 days	37.7 ± 4.2	5.7 ± 0.3	ND	42.7 ± 3.0	86.0 ± 4.1
Garden FB4_I 45 cm, 93 days	23.7 ± 9.8	3.5 ± 0.3	ND	41.2 ± 1.7	68.4 ± 0.5
Garden FB4_I 75 cm, 93 days	14.5 ± 10.5	25.7 ± 35.1	15.9 ± 27.3	30.9 ± 18.3	71.2 ± 12.1

[a] %^{14}C as $^{14}CO_2$, %^{14}C in combusted soil and total recovery of ^{14}C. Mean ± S.D. NA, not analysed; ND, not detected.
[b] ^{14}C in CO_2 + ^{14}C in extract + ^{14}C in combusted soil.

Table 6
Estimated mean parameters for mecoprop mineralisation at each site and depth according to composite model (i.e. Eqs. (11)–(14)) with the following estimated values for α_1: 0.982; β_1: 1.046; β_2: 0.427; α_2: −0.000254; β_3: −0.000633; α_3: 0.00404; β_4: 0.0145; α_n: −1.234; β_5: 0.930; α_b: −1.189; β_6: −0.0754; β_7 = −0.420

Site ID	Depth	k_1	k_2	c_n	k_3	c_b
FB1_I	15	0.1117	−0.00089	17.7	0.0186	20.3
FB1_I	45	0.0195	−0.00025	10.2	0.0040	28.3
FB1_I	75	0.0040	−0.00025	6.8	0.0040	28.6
FB1_II	15	0.1001	−0.00089	24.2	0.0186	20.4
FB1_II	45	0.0061	−0.00025	16.4	0.0040	28.6
FB1_II	75	0.0019	−0.00025	7.6	0.0040	28.9
FB3_I	15	0.0962	−0.00089	19.1	0.0186	20.5
FB3_I	45	0.0172	−0.00025	6.3	0.0040	28.8
FB3_I	75	0.0040	−0.00025	10.5	0.0040	29.6
FB3_II	15	0.1001	−0.00089	28.1	0.0186	20.3
FB3_II	45	0.0195	−0.00025	14.8	0.0040	28.1
FB3_II	75	0.0061	−0.00025	5.1	0.0040	28.3
FB4_I	15	0.1756	−0.00089	15.4	0.0186	20.1
FB4_I	45	0.1254	−0.00025	16.1	0.0040	28.1
FB4_I	75	0.0105	−0.00025	7.2	0.0040	28.9

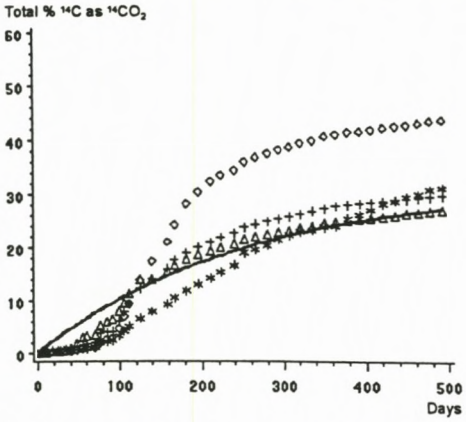

Fig. 11. Mineralisation of 0.04 μg g^{-1} ^{14}C-mecoprop for each of four replicates in soil from 75-cm depth at site TY1. Symbols: data points. Solid line: developed model based on %humus, %clay and rate constant k_{1_naac}.

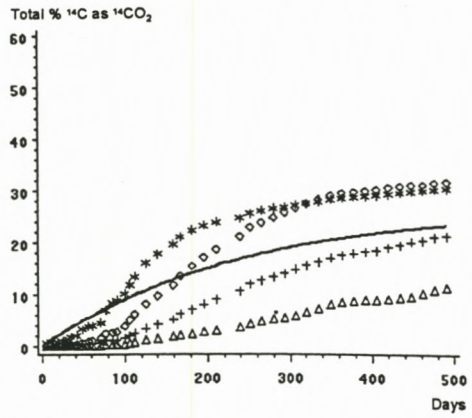

Fig. 12. Mineralisation of 0.04 μg g^{-1} ^{14}C-mecoprop for each of four replicates in soil from 75-cm depth at site TY2. Symbols: data points. Solid line: developed model based on %humus, %clay and rate constant k_{1_naac}.

gaard (1997), two sequential 1. order processes would cause a sigmoidal form of the curve, too. Such sequential processes could be a mineralisation of the parent compound followed by a mineralisation of a metabolite or a mineralisation of dissolved pesticide followed by desorption and subsequent mineralisation of sorbed pesticide. If such sequential processes occurred, they should also appear in ploughlayer, and not only in subsoil.

According to the farmers' cultivation and spraying program (Table 2) mecoprop had not been used for at least the last 5 years before the start of the present project, so no former adaptation of the microbial community to presence of mecoprop would be expected. Cross-enhancement with phenoxyacetic acids as historical herbicide and phenoxypropionic acids as challenge herbicide has been shown not to occur (Fryer and Kirkland, 1970). Previous field treatments with MCPA in the present study would thus not be expected to provoke any adaptation of the micro-organisms for degradation of mecoprop.

From the number of A's and B's in Table 3 it is seen that model version B generally was preferred (because of lowest mean square) for samples, which had been incubated for long time (mecoprop at site FB1_I and FB3_I was incubated for 500 days) or only for ploughlayer samples in cases of short time incubation (site FB1_II, FB3_II and FB4_I). When k_3 was equal to zero (FB1_II 45 and 75 cm, FB3_II 45 cm), the mineralisation of ^{14}C-mecoprop did not reach a level where the transformation to $^{14}CO_2$ of ^{14}C built into organic material had any importance, and the first order term (Eq. (3)) disappears.

The total recovery including ^{14}C evolved as $^{14}CO_2 + {}^{14}$C extracted + ^{14}C left in soil can be seen in Table 5. During the whole incubation period leakage checks were done frequently, so the low total recovery in subsoil is unlikely due to leakage of $^{14}CO_2$. Compounds could have been formed during the degradation of the pesticides, that evaporated but did not adsorb either in KOH or in glycerol (Helweg, 1993). For the samples from sites FB1_I and FB3_I , which were incubated for 500 days, no analysis for mecoprop was performed in the extract. Other studies (Helweg, 1993) showed that after long time incubation, when the flat part of the mineralisation curve had been reached, no mecoprop was left in the extract. For the samples from sites FB1_II, FB3_II and FB4_I which were incubated for 93 days, from site FB4-I. In all cases the Figs. 2 and 4, and Fig. 5 show that the flat part of the mineralisation curve was reached. When only small amounts of ^{14}C were evolved as $^{14}CO_2$, high

amounts of ^{14}C-mecoprop were present in the extract.

In a sterilised soil sample (radiated with 2 × 11 kGy), incubated with 0.04 μg g^{-1} mecoprop, less than 1.25% $^{14}CO_2$ was evolved after 125 days, which shows that the mineralisation of ^{14}C-mecoprop was microbial.

3.2. Mineralisation model, part 2

In addition to the model fitted as described in Section 3.1, which gave a tool for explaining the underlying processes in the mineralisation, the purpose of the project was to develop a model which related parameters of the ^{14}C-mecoprop mineralisation model to some other more easily obtainable parameters.

The chosen parameters were: (1) biological activity, measured as k_{1_naac} from the ^{14}C-Na-acetate mineralisation or as the amount of $^{14}CO_2$ developed from ^{14}C-Na-acetate after 2 or 4 h; (2) most probable number of mecoprop degraders (MPN); (3) humus content; (4) clay content; (5) sand content; (6) silt content; (7) soil pH; (8) soluble organic carbon content; (9) NO_3-N content; (10) NH_4-N content; (11) K_d-value; and (12) depth.

The number of mecoprop-degrading bacteria (MPN) had been expected to represent m_0 in Eq. (4) which would have made it possible to determine the growth rate λ and the rate constant k. Since no significant difference between MPN numbers at varying depths was seen, the values λ and k could not be determined.

Other published studies (Walker et al., 1983; Mueller et al., 1992) have reported linear correlation between pesticide degradation and one single parameter, so linear regressions were first of all made between mean values for the parameters k_{1_meco}, k_{2_meco}, k_{3_meco}, c_{n_meco}, and c_{b_meco}, obtained in the non-linear fit, part 1, and the other 12 soil parameters. Torstensson and Stenström (1986) showed good linear correlations between the basic respiration rate and degradationrate of linuron and glyphosate, respectively, but they stated that attempts to show linear correlation between degradation rates of the metabolically degraded 2,4-D and basic respiration rate had failed. A plot of residuals versus predicted values showed significant lack of homogeneity in variances for some variables and thus a lack in the assumptions for the regression analysis. In order to better fulfill the assumptions for the analysis, some variables were transformed.

Linear regressions between $\log_e k_{1\_meco}$, k_{2_meco}, k_{3_meco}, $R(c_{n\_meco})$, $R(c_{b\_meco})$ and the 12 soil parameters described above plus R(%humus), R(%clay), R(%sand), R(%silt), were also performed. Here

$$R(x) = \log_e \frac{x}{100 - x} \tag{9}$$

was used to make a linear relationship between the variables more likely, since parameters expressed in % will not have symmetric distribution for values close to 0 or 100%. Moreover variances, which generally are smaller close to 0 or 100 for %-values, were made more homogeneous, and predicted values under 0% or above 100% were avoided using the $R(x)$-function.

Based on the best linear regression models (Searle, 1971) between the individual (transformed) parameters of models 1–3 and the independent soil parameters, a starting point and initial estimates in a non-linear composite model were constructed. The estimates of the parameters α_1, α_2, α_3, α_n, α_b, β_1, β_2, β_3, β_4, β_5, β_6 and β_7 were improved by using a derivative-free algorithm (Ralston and Jennrich, 1978). The following composite model was then obtained:

$$\log_e k_1 = \alpha_1 + \beta_1 \cdot \log_e \frac{\%humus}{100\text{-}\%humus} + \beta_2 \cdot ploughlayer \tag{10}$$

$$k_2 = \alpha_2 + \beta_3 \cdot ploughlayer \tag{11}$$

$$k_3 = \alpha_3 + \beta_4 \cdot ploughlayer \tag{12}$$

$$\log_e \frac{c_n}{100 - c_n} = \alpha_n + \beta_5 \cdot \log_e k_{1\_naac} \tag{13}$$

$$\log_e \frac{c_b}{100 - c_b} = \alpha_b + \beta_6 \log_e \frac{\%clay}{100 - \%clay} + \beta_7 \cdot ploughlayer \tag{14}$$

where k_1, k_2, k_3, c_n and c_b are defined in Eqs. (1)–(3), *ploughlayer* was given the value 1 for ploughlayer samples (taken at 15-cm depth) and the value 0 for subsoil samples (taken at 45- and 75-cm depth, respectively).

Eqs. (10)–(14) determine the parameters of the model in Eqs. (1)–(3) for each combination of site and depth. Thus Eqs. (1)–(3) and Eqs. (10)–(14) in conjunction describe the model used to predict the production of $^{14}CO_2$.

All statistical calculations were done using procedures from SAS Institute (1989) SAS Institute (1990) SAS Institute (1996).

The parameter estimates of the composite model and the derived values of k_1, k_2, k_3, c_n and c_b are shown in Table 6. Figs. 1–5 show the data (%^{14}C as $^{14}CO_2$ versus time) and the non-linear model for each of four replicates, together with the predicted values (bold line) based on the composite model above.

3.3. Discussion of factors included in the model development

In the substrate-induced respiration method (Anderson and Domsch, 1978) glucose is added to the soil samples and CO_2 development is measured hour by hour. Anderson and Domsch (1978) indicated that 40 mg biomass C respires 1 ml CO_2 h^{-1} at the stage of maximum initial response. In the present study mineralisation of ^{14}C-Na-acetate was used as a measurement of microbial activity. Measurements of $^{14}CO_2$ were performed with scintillation counting, which was an already well-established method in our laboratory. The amount of $^{14}CO_2$ formed after 2 and 4h was used as a measurement of biological activity. Moreover, the mineralisation curves (Figs. 6–10) were analysed with the same non-linear model as the pesticide mineralisation curves and the mineralisation rate k_{1-naac}, determined with the non-linear modelling, was used as another variable related to microbial activity.

To assure that the flat part of the pesticide mineralisation curve was not caused by the death of degrading microorganisms, Na-acetate was added to a part of the soil after having stopped the incubation with ^{14}C-mecoprop for the samples at sites FB1_I and FB3_I. The biological activity was lower than at the beginning of the experiments, but a relatively high activity was still seen.

The amounts of $^{14}CO_2$ from ^{14}C-Na-acetate after 2 and 4 h were not among the most important variables for describing the parameters (k_1, k_2, k_3, c_n and c_b) of the model. However, the biological activity, measured as degradation rate for Na-acetate, k_{1-naac}, played an important role for the amount of ^{14}C-mecoprop mineralised according to Eq. (2), c_n.

The most probable number (MPN) of mecoprop degraders was determined and showed that microorganisms capable of degrading mecoprop were present. The number of mecoprop degraders did not vary significantly between layers, however, and could therefore not give any useable correlation to any of the determined parameters. This supports the hypothesis that other factors (texture, nutrients and organic material) influenced the degradation rate as well as the kinetics. Former modelling studies of the same data (Fomsgaard, 1997) showed that cometabolic degradation dominated in the plough layer and metabolic degradation dominated in subsoil.

An increasing amount of humus may increase degradation rates of pesticides because the organic material serves as energy for micro-organisms that degrade the pesticide cometabolically. In other cases, an increasing humus content may decrease degradation rates, because the pesticide is adsorbed to humus. In this study humus content was an important predictor of k_1, the rate constant in the ^{14}C-mecoprop mineralisation process. A positive relationship was found between log k_1 and R(%humus), so the mineralisation rate of ^{14}C-mecoprop increased with a higher content of humus. The parameter c_b, the amount of ^{14}C from pesticide built into organic material and then mineralised to $^{14}CO_2$, was negatively influenced by the amount of clay, probably because organic material can be sorbed to the surface of mineral particles and is then less available for degradation.

The adsorption of pesticide to soil generally depends upon the amount of humus, the chemical structure of the pesticide and pH. Mecoprop is a

Table 7
Table of values from mecoprop incubation experiments used for model validation*

Site	Sampling time	Depth (cm)	Humus (%)	Clay (%)	Silt (%)	Sand (%)	pH	Incubation time (days)	k_{1_nasc}	K_d mecoprop (l kg^{-1})	Soluble organic C (mg kg^{-1})	NO_3-N (mg kg^{-1})	NH_4-N (mg kg^{-1})
TY1	April, 93	75	0.2	9.7	5.7	84	6.6	500	0.130 ± 0.060	0.28	113.8	2.6	1.6
TY2	April, 93	75	0.1	6.9	1.9	91	7.1	500	0.436 ± 0.352	0.06	98.4	0.3	0.6

* Sampling site, sampling time, depth, texture, pH (H_2O), incubation temperature, humus, soluble organic carbon, NO_3-N, NH_4-N, identification of incubated mecoprop mineralisation experiments with replicate numbers and k_{1_nasc} values.

week acid at low pH ($pK_a = 3.78$) so in this soil with pH ~ 5.5–6.9, pH did not influence adsorption, or, as such, the degradation.

Pesticide degradation in soil mainly takes place in the soil water where contact between pesticide molecules and micro-organisms is possible. The organic material that can be used by micro-organisms is often considered to be the water soluble organic carbon. The correlation between degradation rates and mg kg^{-1} soluble organic carbon (SOC) was positive but not as strong as for the amount of humus.

NO_3-N is a nutrient and may be important as an alternative electron acceptor in the degradation process when the conditions are anoxic. The actual soil samples were incubated with a flow of atmospheric air, because the soil air in sandy soil at depths down to 75 cm normally is oxygen rich, so NO_3 as electron acceptor could not be expected to exert any influence. NO_3-N and in some cases NH_4-N could be used as N-nutrients by micro-organisms. In the present case, the influence of NO_3-N and NH_4-N on the degradation process was negligible.

K_d values give a measurement of sorption under standardised conditions (concentration of pesticide, amount of soil and water). K_d for mecoprop is very low, so it was no surprise that a correlation to K_d was not needed in the model.

Degradation rates of pesticides have been reported by many other authors to decrease with depth of soil as was the case in the present study, where mean k_{1_meco} values decreased with depth. Factors that often are reported as diminishing with decreasing depth are the amount of organic material and the number of micro-organisms/biological activity. The number of specific mecoprop degraders (MPN) was not significantly different between depths. In a parallel study carried out in soil from field FB3 (at the same time as FB3_II) and from FB4_I, (Vinter, 1998) direct counting with acridine orange staining was performed and a decrease in total number of cells g^{-1} from ploughlayer to 1 m depth from 10^9 to 10^7 was reported. Biological activity measured as the capacity of mineralising ^{14}C-Na-acetate decreased with depth. Comparing k_{1_meco} from all sites and depths, a steady decrease from ploughlayer to 75 cm is not clear, so a simple correlation between degradation rate k_{1_meco} and depth could not be shown. Humus content and K_d-value are other factors which decrease with increasing depth. Most of the parameters of the degradation model (Eqs. (1)–(3)) in the presented model were shown to depend on depth, but only between ploughlayer and subsoil was a clear shift seen. For this reason a factor was included to describe the change of the intersection in Eqs. (10)–(12)and Eq. (14).

Depth was the only factor that showed clear relationship to the parameters k_2 and k_3.

3.4. Validation of the model

All pesticide mineralisation studies used for the development of the model were made in Danish soil, sampled at different times at three different fields and at three depths. All samples were incubated with 0.04 μg g^{-1} ^{14}C-mecoprop at 10°C. It was shown very clearly, that factors other than temperature and initial concentration influenced the mineralisation rate of ^{14}C-mecoprop, factors which were humus content, clay content and biological activity.

Two set of mineralisation studies performed in German soil (TY1 and TY2) from 75-cm depth with the same concentration of mecoprop and at the same incubation temperature were used to validate the developed model. The characterisation of the German soil samples is shown in Table 7. Figs. 11 and 12 show the four replicates at each sampling site (symbols), and the model calculated on basis of the values in Table 7 for humus content, clay content and biological activity and the estimates of the parameters (α_1, α_2, α_3, α_n, α_b, β_1, β_2, β_3, β_4, β_5, β_6 and β_7), shown in the heading of Table 6. The first part of the mineralisation curve, where the increment in $^{14}CO_2$ formation is seen, was not modelled very closely, but the developed model can surely be used for prediction of the total mineralisation time as well as the amount of ^{14}C-pesticide developed as $^{14}CO_2$.

4. Conclusion

A model, which described the simultaneous effect of soil depth, biological activity, organic

content and soil texture on mecoprop mineralisation, was developed. In the future the model should be amplified to include effects of temperature and initial pesticide concentration. Moreover the model should be tested for other compounds. For compounds with higher sorption than mecoprop, sorption must be included as a part of the modelled process.

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