# Toxicity of pentachlorophenol and chlorpyrifos in soil and in solution to a nematode and a plant species

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ABSTRACT

W.J.M. Aben, N.W.H. Houx & M. Leistra, 1992. *Toxicity of pentachlorophenol and chlorpyrifos in soil and in solution to a nematode and a plant species*. Wageningen (The Netherlands), DLO The Winand Staring Centre, Report 59, 39 pp.; 4 Figs; 8 Tables.

The toxicity of pentachlorophenol and chlorpyrifos to the soil nematode *Globodera rostochiensis*, exposed in three soils and in solution isolated from these soils, was studied. The exposure in isolated soil solution was only slightly effected by the adsorption of the chemicals to dissolved organic macromolecules. The toxicity of the chemicals to the nematodes in soil was less than one hundredth of their toxicity in solution. This was because of the decrease in concentration in the soil solution due to adsorption onto the solid phase. The toxicity of chlorpyrifos to the nematodes was more than a thousand times as high as that of pentachlorophenol, whereas the latter was more toxic to the plant *Arabidopsis thaliana*. The results of the study reveal the essential role of exposure dynamics on the effects of organic chemicals via the soil.

Keywords: soil contamination, organic chemicals, phytotoxicity, nematodes, bioavailability, adsorption, pesticides, exposure, effects.

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Project 7141 [025bw]

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# PREFACE

The three-year project: "Organic chemicals in soil pore water and their bioavailability, as criteria for soil contamination" commenced at the end of 1987 at the Institute for Pesticide Research. The project was sponsored by Shell Internationale Petroleum Maatschappij, Den Haag. The experimental part of the project was entrusted to W.J.M. Aben and N.W.H. Houx was the project leader. A reorganization of agricultural research at the end of 1988 led to the project being continued at The Winand Staring Centre. As the first author was unable to finish the report, this task was taken over by M. Leistra. This report is the second of the three reports on this project; the first report was prepared by J.J.T.I. Boesten and the third report by N.W.H. Houx.

SUMMARY

Organic chemicals are found in soil as a result of unintended contamination, for example by industrial waste, or as a result of application in agriculture. There is a lot of concern about the environmental consequences of the presence of synthetic chemicals in soil. A review is given of how the evaluation of the hazard of soil contaminants is currently approached. The scientific basis of such evaluations can be improved by considering the possible effects of the chemicals on organisms exposed via the soil, accounting for the bioavailability of the chemicals.

The effect of two organic chemicals, dissolved in isolated soil solution or mixed with soil, on two organisms was studied. The first test organism was *Globodera rostochiensis*, a potato cyst nematode. This nematode species can be bred on potato plants in the glasshouse and stored for a long time in the cyst stage. By means of a hatching agent, a rather uniform population of larvae can be obtained from the cysts. The second test organism was *Arabidopsis thaliana*, a hedge mustard plant. Under laboratory conditions, this plant species has a short life cycle, it produces a large number of seeds and its genetic variation is small.

Three soil materials were used in the experiments: a) a peaty sand soil from Schoonrewoerd, b) a humic sand soil from Wageningen and c) the artificial soil mixture proposed by the Organization for Economic Co-operation and Development (OECD) as an international standard soil mixture for toxicity studies with earthworms.

The two model compounds selected for this project were pentachlorophenol and chlorpyrifos. Pentachlorophenol is a wide-spectrum biocide with many potential applications, for example, in wood preservation. It has been found as a soil pollutant at many locations and it has been classified as a priority pollutant. Chlorpyrifos is an organophosphorus insecticide, with various applications in agriculture, animal husbandry and domestic insect control. It was selected because it had been used by colleagues as a model compound in aquatic toxicity studies. Basic data on physicochemical properties and on mammalian toxicity have been collected.

The contents and concentrations of pentachlorophenol were measured with a gas chromatograph, equipped with an electron-capture detector. Chlorpyrifos was measured with a liquid chromatograph, equipped with a diode-array detector. The compounds in pore water were separated into a fraction freely dissolved and a fraction adsorbed to dissolved macromolecules, by gel permeation chromatography with UV detection and radioactivity detection.

The rates of transformation of the two chemicals in the soils were measured. Half of the initial amount of pentachlorophenol in the two natural soils was transformed in about 1 month. The time taken for half of the initial amount of chlorpyrifos to be transformed ranged from 1 to 3.5 months.

A major part of the two chemicals in the isolated soil solutions was found to be in a freely dissolved state: the freely dissolved fractions were 93 to 97% for pentachlorophenol and 91 to 95% for chlorpyrifos. Under the present conditions, we can conclude that the adsorption to macromolecules dissolved in the soil solution produces only a minor decrease in the concentration of the freely dissolved chemical.

The toxicity of the chemicals to the nematodes in the isolated soil solution observed after two days was expressed in the solution-LC50 values: the concentration at which 50% of the nematodes died. The solution-LC50 value of pentachlorophenol ranged from 1 to 3.9 mg/l. Chlorpyrifos was much more toxic to the nematodes, expressed in solution-LC50 values ranging from 1.1 $10^{-3}$ to 1.6 $10^{-3}$ mg/l. The solution-LC50 values for the isolated soil solutions were similar to the values obtained for a dilute saline solution (without dissolved macromolecules).

The toxicity of the test chemicals to the nematodes in soil after two days exposure was expressed in the soil-LC50 value: the content in the soil (in mg/kg) at which 50% of the nematodes died. The soil-LC50 value of pentachlorophenol mixed into the three soils ranged from 130 to 640 mg/kg. The soil-LC50 values of chlorpyrifos ranged from 1.1 to 2.9 mg/kg. Therefore chlorpyrifos is much more toxic in soil than pentachlorophenol. The toxicity of both the compounds was comparatively high (lowest soil-LC50 value) in the Wageningen soil which contained the least organic matter.

The soil-LC50 values for the toxicity to nematodes in the soil were much higher than the solution-LC50 values for the toxicity in the isolated soil solution. This demonstrates that the adsorption to the solid organic matter has a tremendous effect on the toxicity of the chemicals in the soils.

The toxicity of the chemicals to the nematodes in the soil was related to their concentration in the soil solution in situ, which was collected and analyzed after the exposure time. The solution-LC50 values for exposure in soil were about as high as the solution-LC50 values for exposure in isolated soil solution. This means that at a certain level of soil contamination, expressed in mg per kg soil, the concentration in the soil solution should be measured or estimated first, so that the actual exposure of organisms such as nematodes in soil could be obtained. Then, a preliminary estimate on the effect of the contaminant in soil can be obtained using results from tests with exposure of nematodes in solution.

The results of the experiments with the soils varied a lot. Checks on the mixing of the chemicals in the soils showed that especially in large soil samples a homogeneous distribution, and thus exposure, is difficult to obtain. The recovery of the viable nematodes was very variable in the tests with the nematodes in the soils. To reduce the variations further development of the experimental procedures is needed.

The toxicity of the test chemicals to the plant *Arabidopsis thaliana* was expressed in soil-EC50 values: the contents in soil (in mg/kg) at which emergence is reduced

to 50% of the emergence from untreated soil, as observed after 10 days. The soil-EC50 values for pentachlorophenol in the three soils ranged from 3 to 9 mg/kg. Chlorpyrifos in soil demonstrated a distinctly lower toxicity, with soil-EC50 values ranging from 26 to 75 mg/kg.

Pentachlorophenol was most toxic to the plants (lowest soil-EC50) in the Wageningen soil; that with the lowest organic matter content. Contrary to expectation, there was no logical relationship between the plant toxicity of chlorpyrifos in the soils and their organic matter content.

Extensive testing of the toxicity of a chemical in soil to a particular organism may not be useful, as the high content of chlorpyrifos in soil required to induce a distinct effect on the plants illustrated. Information on the mode of action of a chemical should help determine the selection of suitable organisms for advanced toxicity testing.

# 1 INTRODUCTION

Toxic contaminants in soil as a potential cause of environmental problems now and in the future are a source of public concern. Even those soil contaminant levels that comply with established quality criteria are considered to present risks. Contaminated soil can lead to pollution of groundwater and surface water, even when no additional pollutant is spilled or dumped. The first establishment of soil quality criteria dates back to the early eighties and was set up according to the detection limits of the analytical methods at that time. The absence of scientifically-based soil quality criteria makes it difficult to assess the seriousness of the soil contamination problem and to decide on appropriate remediation actions. Better procedures for hazard evaluation are needed to assist the authorities in making decisions concerning problems of contaminated soil. The soil quality criteria should reflect available scientific knowledge for assessing the likelihood of undesirable environmental effects from contaminants in soil. A procedure to establish soil quality criteria and limit values should be applicable to a wide range of chemicals and to the range of soils encountered in practice. Such procedures are intended to protect the presence and functions of populations of soil organisms and to prevent irreversible ecological effects.

The Dutch government announced a major program for research on soil quality and its protection in 1987. Since then the extensive governmental First Priority Program for Soil Research and the sub-program on Soil Ecotoxicology have been carried out. The primary goal of the sub-program was the development of a comprehensive set of standardized toxicity tests with soil organisms. These tests should enable the design of an advanced risk assessment system for chemical substances in soil ecosystems. Subsequently, a system including a toxicological profile and a toxicological rating scheme could be developed and applied to each relevant chemical substance.

Recent standards developed for soil protection in The Netherlands were derived from research on heavy metal contamination of soil in post-industrial areas and in chemical waste sites. This has resulted in a scheme of limit values for taking decisions on soil sanitation. There are three levels of evaluation based on the presence of heavy metals in soil, the so-called A, B and C values. The A value is based on background contents in soil measured in nature conservation areas that are considered to be "normal". The contents below the A values are considered to be normal background levels not requiring action. Contents between the A and B values indicate that although the soil is contaminated, no further measurements are needed. Contents between the B and C values require further research to precisely determine the contents and spreading of the pollutant. Contents above the C value require that the soil is decontaminated. The government has extended the heavy metal list to include chlorinated hydrocarbons (e.g. pentachlorophenol) and polycyclic aromatic hydrocarbons (PAH's, e.g. phenanthrene). These extensions, however, have not been based on scientific research. Note, the behaviour of heavy metals in soil is completely different to the behaviour of organic compounds.

The soil reference value (A value) of a compound in the chlorinated hydrocarbons group is 10 µg/kg (dry mass basis). The A value for the total content of substances in this group is 20 µg/kg. The reference value of a compound in the PAH group is 25 µg/kg, while the reference value of the sum of the substances in this group is 50 µg/kg. These values are based on soils with an organic matter content of 10%.

The Provisional Soil Decontamination Act came into full operation in 1981 expecting the enactment of a Soil Protection Act. The former act deals with the removal or decontamination of polluted soil and of its damaging effects in cases where the soil is polluted to such an extent that there is serious hazard to public health and the environment. At this moment the Soil Protection Act is only a framework for judging the environmental risks of contaminated soil. In the future both the above-mentioned laws will be combined in an integrated Soil Protection Act. There are no scientifically-based criteria governing the soil protection and sanitation laws on which judgement could be based. Research projects should help to develop criteria and limit values needed. The limit values could have different functions: for the Soil Decontamination Act these values can be used to decide the extent to which the soil should be cleaned to achieve the acceptable concentration of the residue; for the Soil Protection Act these values can be used to set standards aimed at avoiding soil contamination.

In 1989, a committee (Committee Oele) installed by the government presented a report describing the planning and execution of a large scale operation called "Operation Soil Protection". This operation is based on a master plan in which an inventory (on a voluntary basis) is made of the extent to which present-day and former industrial sites are polluted. It should be completed before the beginning of 1994. Potential polluted sites will be evaluated according to the established scheme, together with the values guided by the current laws. Should new limit values emerge from scientifically-based criteria, the information available for the polluted soils could then be evaluated according to the updated scheme.

One of the major imperfections of the current limit values is that there is no differentiation between the chemicals or classes of chemicals, based on their toxicity towards soil organisms or plants. This may result in a substantial inventory of false positive "polluted" and false negative "not polluted" soils. In the case of a false positive evaluation (the C value for organic chemicals is exceeded) indicating that the soil has to be cleaned, high unnecessary costs could be incurred. Laboratory experiments with organic chemicals have proven that there are huge differences in toxicity of various chemicals to a particular test organism. In addition, huge differences in toxicity of an organic chemical to various soil organisms could also be expected. Therefore a system of soil quality criteria should be developed that includes toxicologically-based values. To avoid the errors being made by evaluating soil contamination with the old reference system, a new evaluation system for soil protection could be developed based on toxicity to organisms exposed via the soil.

To assess the potential toxicity of a chemical in soil, knowledge of the behaviour of the chemical and of how the soil characteristics influence this behaviour is of

primary importance. Organic chemicals in soil have a tendency to partition between the different soil phases and components (Karickhoff, 1984). Eadie et al. (1988) classified three states of partitioning of chemicals in sediments: chemical freely dissolved in the soil solution, chemical adsorbed to particulate organic carbon (POC) and chemical adsorbed to dissolved organic matter (DOM). In many instances there is only a small fraction of the chemicals in soil in solution. To determine the extent of exposure of organisms to organic chemicals in soil it is of vital importance to know the precise partitioning. Attempts should be made to correlate the partitioning with the characteristics of the soils. Earlier results from experiments with surface water (McCarthy & Jimenez, 1985; Landrum et al., 1987) and with soil (van Gestel & Ma, 1988) indicate that only the freely dissolved chemical is available for some organisms.

The aim of this project was to contribute to the development of scientifically-based criteria and limit values to enable risks of soil contamination by synthetic organic chemicals to be evaluated. The criteria for selection of the organic chemicals used as model compounds, the test organisms and the soils for the toxicity studies were described. The transformation rate of the chemicals in three soils and their partitioning between the phases in soil were measured. A nematode and a plant species were exposed to the chemicals via isolated soil solutions and via the soils. Measurements of the effects on the organisms were correlated to the bioavailability of the chemicals.

# 2 MATERIALS AND METHODS

## 2.1 Design of the experiments

### 2.1.1 Selection of the compounds

Two compounds with different physicochemical properties were selected for the experiments from the list of the Dutch Sub-program on Soil Ecotoxicology. Pentachlorophenol was selected because it is a moderately persistent compound on which a substantial amount of research has been done. Its toxicity to earthworms was studied by van Gestel & van Dis (1988) and by van Gestel & Ma (1990). It has been found in several places to be a soil pollutant. Pentachlorophenol uncouples the oxidative phosphorylation (Weinbach, 1957), therefore it would be suitable for use as a biocide for a wide spectrum of applications. Pentachlorophenol formulations have been used as herbicides and fungicides for many years. It can also be used as an insecticide and fungicide in wood preservation and as a molluscicide in the control of bilharzia (Crosby, 1981).

Because of its widespread use, pentachlorophenol has frequently been detected in soil, water courses and lakes. It has even been found in food and human urine. In soils, sediments and natural waters near wood preservation sites, contents as high as several g/kg have been found. Moreover, because of the extent and nature of its use, pentachlorophenol together with its transformation products has been designated a priority pollutant by the US Environmental Protection Agency (Keith & Telliard, 1979).

The environmental behaviour and fate of organic chemicals is largely dependent on their physicochemical characteristics. The physicochemical properties of pentachlorophenol, according to Schmidt-Bleek et al. (1982), are listed in Table 1. Several environmental factors play a key role in the transport and transformation of pentachlorophenol in soil. Volatilization of pentachlorophenol from soil depends on factors such as moisture, temperature and pH. Murthy et al. (1979) found that up to 0.5% of the pentachlorophenol added to the soil volatilized into the air. Baker & Mayfield (1980) were not able to detect volatilization of pentachlorophenol. In our experiments we took the volatilization of pentachlorophenol to be negligible.

***Table 1 Physicochemical properties of pentachlorophenol (Schmidt-Bleek et al., 1982)***

| | |
|---|---|
| **Log octanol-water partition coefficient** | **3.69** |
| **Water solubility at 30 °C** | **0.076 mol/m$^3$** |
| **Vapour pressure at 20 °C** | **0.0098 Pa** |
| **Molecular mass** | **266.4 g/mol** |
| **pKa** | **4.7** |
| **Boiling point** | **310 °C** |
| **Melting point (purity dependent)** | **188-191 °C** |

Sorption to soil particles retards transport in the soil. Pentachlorophenol shows substantial sorption to soil, especially to soil organic matter (Isaacson & Frink, 1984; Schellenberg et al., 1984; Lagas et al., 1986). Sorption of uncharged organic compounds to soil can be estimated on the basis of their octanol-water partition coefficient (Kow). The Kow value of pentachlorophenol is strongly influenced by the pH. The dissociation of pentachlorophenol as a function of the pH has been shown by Lagas et al. (1986). Both phenolic and anionic pentachlorophenol are present between pH 4 and 7. Pentachlorophenol is largely in the phenolic state at pH 4 or lower and it is almost completely ionized at pH 7 or higher. Verschueren (1983) reports a log Kow of 3.98 at pH 5 and 2.02 at pH 7.

Pentachlorophenol can be transformed in anaerobic as well as in aerobic soil environments. Most of the investigations demonstrated that the aerobic degradation processes were the quickest (Smith & Novak, 1987). Pentachlorophenol in soil is subjected to microbial transformation. Important microbial transformation processes are: methylation to pentachloroanisole (Murthy et al., 1979), acetylation to pentachlorophenylacetate (Rott et al., 1979), reductive dechlorination to tetrachlorophenols (Murthy et al, 1979) and hydroxylation to tetrachlorodihydroxybenzene (Suzuki, 1977). Photolysis, oxidation by metal ions ($Fe^{3+}$ and $Mn^{4+}$) and auto-oxidation may also play a role in the transformation of pentachlorophenol (Baker & Mayfield, 1980). Following the first transformation step, pentachlorophenol can be partly mineralized by micro-organisms to carbon dioxide, chloride and water.

The second compound selected for the experiments is the insecticide chlorpyrifos. Our institute uses chlorpyrifos as a model compound in aquatic ecotoxicological studies, both in the laboratory and in experimental ditches. Chlorpyrifos is an organophosphorus compound inhibiting the enzyme cholinesterase, which plays an essential role in neurotransmission. Chlorpyrifos forms colourless crystals with a mild mercaptan odour. Some physico-chemical properties of chlorpyrifos are shown in Table 2.

*Table 2 Physicochemical properties of chlorpyrifos (Worthing & Hance, 1991)*

| | |
|---|---|
| **Log octanol-water partition coefficient** | **4.6** |
| **Water solubility** | **0.0057 mol/m³** |
| **Vapour pressure at 25 °C** | **2.5 mPa** |
| **Molecular mass** | **350.5 g/mol** |
| **Melting point** | **42-43.5 °C** |

The predominant first step in the transformation of chlorpyrifos is the hydrolysis to 3,5,6-trichloro-2-pyridinol (Marshall & Roberts, 1978). The rate of hydrolysis in water and soil is dependent on the pH, temperature, presence of copper ions and of chelating agents (Marshall & Roberts, 1978). The time taken for 50% hydrolysis of chlorpyrifos ranges from 1.5 days (water, pH 7, 25 °C) to 100 days (phosphate buffer, pH 7, 15 °C). In soil the time taken for 50% transformation is between 60 to 120 days.

The acute oral toxicity of chlorpyrifos to mammals, expressed in the dose at which 50% of the test animals dies within 24 hours (24-h LD50) is: 135-163 mg/kg for rats, 500 mg/kg for guinea pigs and 1000-2000 mg/kg for rabbits. In chronic toxicity tests (two years feeding), the No Observed Effect Level (NOEL) based on blood plasma cholinesterase activity, was measured. The NOEL values, based on residue in food (mg/kg) were: 0.03 mg/kg for rats and 0.01 mg/kg for dogs. The Acceptable Daily Intake (ADI) for man was set at 0.01 mg/kg body mass.

Chlorpyrifos is used as a broad spectrum insecticide effective through contact action, ingestion and vapour action. Chlorpyrifos is not an effective systemic insecticide for plants. It is contained in several formulations, for example, Dursban (480 g/l). It is also used against household pests (cockroaches, houseflies, termites), mosquitos (larvae and adults) and for the control of ectoparasites on cattle or sheep. The volatility is sufficient to build up insecticidal deposits on nearby untreated areas, if applied indoors.

### 2.1.2 Selection of the soils

Three soils were selected for the experiments; some of their relevant properties are shown in Table 3. These data were measured by the Laboratory for Soil and Crop Testing in Oosterbeek, The Netherlands. The criteria for selecting the soils were the differences in pH and in organic matter content. The Schoonrewoerd soil contains a high percentage of organic matter and the Wageningen soil is a humic sandy soil, low in organic matter. The two soils were collected from the top 0.3 m layer of agricultural field plots. The artificial soil, as described in the OECD (1984) Guidelines and the EC (1985) Directive, was made up of a mixture of 10% peat (2 mm mesh sieved), 20% kaolin clay, 69.5% quartz sand and 0.5% $CaCO_3$. This mixture is used as a standard medium for testing the toxicity of chemicals to earthworms. The artificial soil is also proposed for tests involving nematodes, springtails and mites. It was included in our study for two reasons: to get experience with an artificial soil and to enable comparisons to be made with the results obtained with natural soils.

***Table 3 Some relevant chemical and physical properties of the test soils***

| | Schoonrewoerd topsoil | Wageningen topsoil | OECD artificial soil |
|---|---|---|---|
| **pH (1N KCl)** | **6.7** | **4.7** | **5.9** |
| **pH (water)** | **7.1** | **5.7** | **6.2** |
| **Organic matter (%)** | **8.7** | **2.4** | **8.1** |
| **Clay (%)** | **3.9** | **3.1** | **8.1** |
| **Silt (%)** | **5.2** | **4.7** | **7.4** |
| **Fine sand (%)** | **81.4** | **79.4** | **17.6** |
| **Coarse sand (%)** | **9.5** | **12.8** | **54.5** |

### 2.1.3 Selection of a plant species : *Arabidopsis thaliana*

*Arabidopsis thaliana* is a species of the mustard family with botanical information dating back to the 16th century. There are several advantages for its usefulness as a model plant for environmental toxicity testing. It has a short life cycle, a small size, a small genome size and many studies have been done on its classical genetics. There is little intraspecific variation and much of that variation has been described. Detailed descriptions have been published by Müller (1961) and Meinke & Sussex (1979).

*Arabidopsis thaliana* (a hedge mustard) is a small weed, approximately 0.3 m high at maturity. Under normal conditions it is a self-pollinating species. The length of the life cycle depends on the growing conditions and on eco-type. Short generation time can be induced by crowding, long day-length and high temperature. In the laboratory, at 25 °C and with additional light, the commonly used eco-type Landsberg usually starts to produce mature seeds in about six weeks. The plants continue to produce seeds for several months enabling 5 000 to 10 000 seeds to be harvested from each plant. The seeds can be stored for years in dry, cool conditions and still maintain their germinal force. Unlike most other plants, that require extensive greenhouse or field space, thousands of *A. thaliana* plants can be grown to maturity in the laboratory with minimal facilities. The US-EPA is also studying the use of *A. thaliana* as an alternative to the commonly used crop plants in toxicological tests.

### 2.1.4 Selection of a nematode : *Globodera rostochiensis*

Nematodes are small worms which are probably one of the most abundant groups of organisms on earth. They vary in length from 0.1 to 3 mm and in humid agricultural soils their density can range up to 10 000 nematodes per $dm^3$. The total population of nematodes in soil can be classified according to their feeding habits. Most abundant are the so-called bacteria grazing nematodes. There are also nematodes which are predatory to other nematodes. Other species are saprophytic or parasitic on plants. Nematodes move in the soil solution and they do not ingest soil (like earthworms do), so it may be expected that the exposure to chemicals in soil mainly occurs via the soil solution.

Some plant parasitic nematode species, have a specific stage in their life cycle, the cyst period, which is a period of latency to survive stressful times such as the winter when there is no food supply. For toxicological experiments, plant-parasitic cyst nematodes have advantages compared to other soil organisms.

*Globodera rostochiensis* is a cyst nematode that is parasitic on various potato cultivars. It has four larval instars, which can be described as the juvenile stages, after which it reaches the adult stage. The diameter of the cysts is about 1 mm and it consists of the former female body skins filled with 200 to 500 latent first instar larvae. When the cyst stage has been reached, the larvae can survive for several

years. The cysts can be stored in the refrigerator and at any time nematode larvae can be lured out of the cysts by adding attractant solution, which contains exudates of germinating potato plant roots. *Globodera rostochiensis* has a small genome size and its protein variation is well identified.

*Globodera rostochiensis* was chosen to test our working hypothesis that only the freely dissolved chemical can effect organisms. Cysts can be easily collected from infested soils planted with potatoes. Synchronization of the life stage is obtained when the nematode larvae are lured out of the cysts. Cysts and attractant solution were kindly supplied by Dr J. Bakker of the Department of Nematology, Agricultural University, Wageningen.

## 2.2 Experimental methods

### 2.2.1 Analysis of pentachlorophenol

Pentachlorophenol was extracted from the soil and the amount was measured by gas chromatography. A mass of soil corresponding to 30 g of air dry soil was mixed with 20 ml water containing HCl at 1 mol/l and with 20 ml of toluene. This mixture was vibrated in an ultrasonic bath for 10 min. After centrifugation the toluene phase was collected. The extraction with toluene was repeated twice and the organic layers were combined. A volume of 5 ml of the combined toluene phases was mixed with 10 ml hexane. A subsample of 50 µl of this solution was mixed with 10 ml n-hexane and the concentration of this solution was measured using a gas chromatograph. A stock solution was prepared by dissolving pentachlorophenol (phenolic) in n-hexane and the chromatographic standards were prepared from this solution.

The HP 5890A gas chromatograph (Hewlett Packard) was equipped with a HP 7673A autosampler and a HP 3393A integrator. The column was a WCOT (Wall Coated Open Tubular) widebore capillary column from Chrompack (type CP SIL 5B). The flow rate of the carrier gas was 55 ml/min. The injector temperature was 210 °C. The temperature of the column oven started at 200 °C and was programmed to increase linearly to 250 °C in 10 min. This temperature was maintained for another 10 min. Pentachlorophenol was detected at 280 °C with an electron-capture detector. The injection volume was 1 µl. Sample concentrations were estimated on the basis of the calibration curve made from the standard solutions (Fig. 1).

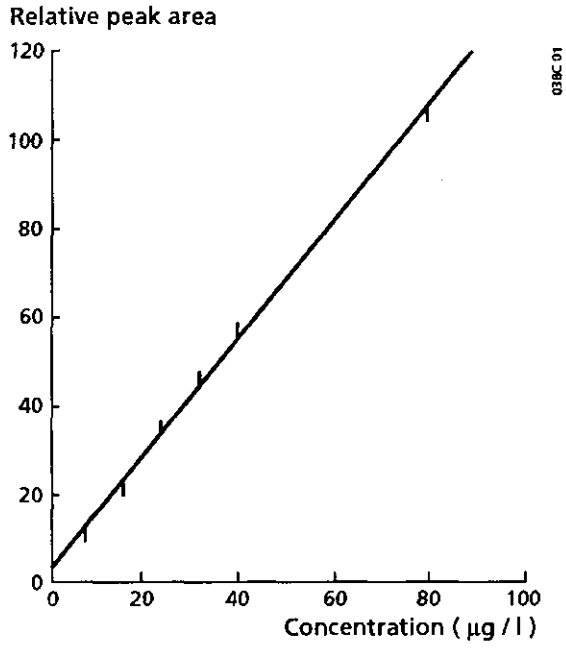


*Fig. 1 Calibration curve for the gas chromatographic analysis of pentachlorophenol*

### 2.2.2 Analysis of chlorpyrifos

The concentrations of chlorpyrifos were measured by a procedure using a liquid chromatograph (Perkin Elmer) equipped with a LC 235 diode-array detector. After adding 50 ml of acetone to 30 g of soil (dry mass basis), chlorpyrifos was extracted from the soil by vibration in an ultrasonic bath for 10 min. The acetone phase was collected and evaporated in a rotary evaporator. The residue was dissolved in 20 ml of a mixture of acetonitrile and water (20 : 80) and this solution was filtered through a 0.45 µm filter.

The chlorpyrifos in solution was preconcentrated in a C18 reversed-phase column (Brownlee RP-GU), to lower the limit of determination of the procedure. A 2 ml sample was pumped through this column by a Waters 590 HPLC pump which also controlled the valves to direct the solutions and solvents. The pre-concentration device was triggered by an ISS 100 autosampler (Perkin Elmer).

The separation column (Pecos C18, 150 mm) was mounted in a SSI 505 column oven, adjusted to 40 °C. The mobile phase for the separation column consisted of a mixture of acetonitrile and water (60 : 40). It was delivered by a LC 410 quaternary gradient pump (Perkin Elmer) from a controlled solvent cabinet.

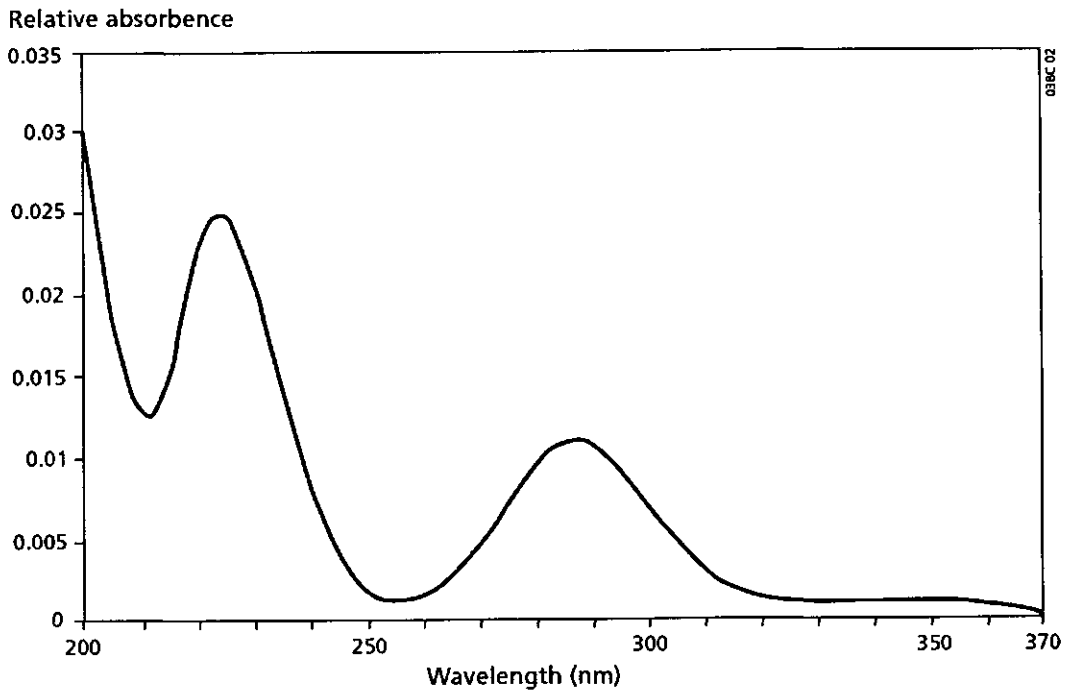


*Fig. 2 UV absorbence spectrum for chlorpyrifos*

The absorbence spectrum of chlorpyrifos was measured using an LC-235 diode-array detector (Perkin Elmer) to trace the most suitable wavelengths for its detection. The spectrum is given in Figure 2 and it shows two absorption maxima at 230 and 290 nm, respectively. Standard solutions could be measured very well at 230 nm. However, some soil extracts contained compounds also showing absorption at this wavelength. Therefore the samples were measured at 290 nm as well. The amounts of chlorpyrifos were quantified by integration of the peak areas with the data processing program Omega (Perkin Elmer) installed on an Epson PC AX2. The calibration curves for the analysis of chlorpyrifos at the two wavelengths are given in Figure 3.

The diode-array detector continuously scanned the absorbence spectrum from 200 to 370 nm as a further check for interfering substances. Three spectra were stored for each peak (front, top, rear) and at the end of the run the Peak Purity Index (PPI) was calculated by comparing the spectra at the three positions in the peak.

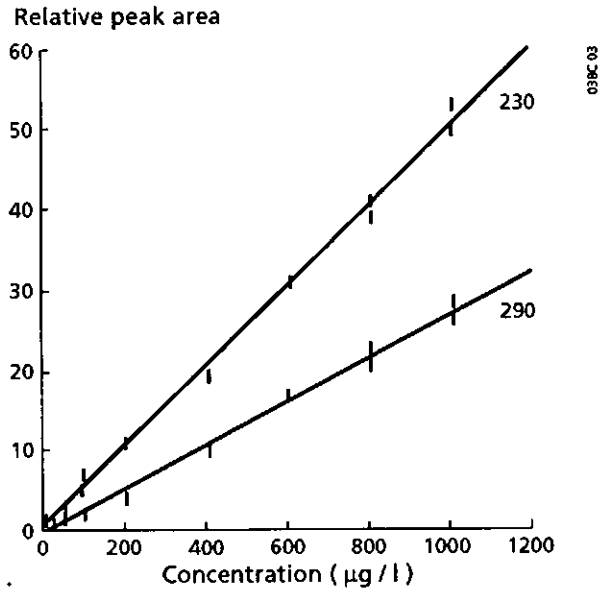


*Fig. 3 Two calibration curves of chlorpyrifos measured at wavelengths of 230 and 290 nm*

2.2.3 Incubation of the chemicals in the soils

The rates of transformation of pentachlorophenol and chlorpyrifos in the three soils were measured to find out the persistence of the compounds during the experiments with the test organisms. A portion of 300 g of each soil was weighed and 30 g water was added and mixed in. The soil was then divided into equal portions of about 30 g. Pentachlorophenol and chlorpyrifos were added to each portion at final contents of 50, 10, 5, 1, 0.5 and 0.1 mg/kg on dry soil basis. This was done by adding stock solutions of pentachlorophenol and chlorpyrifos to the soil and by gentle mixing in a mortar. At each sampling interval one portion of 30 g from each content series was analyzed. The sampling schedule was one sample each week in the first four weeks, and thereafter one sample at intervals of two weeks. The samples were analyzed as described in sections 2.2.1 and 2.2.2.

To determine the recovery and the variation in the recovery of the test chemicals from different soil samples, an experiment was carried out involving adding the chemicals to one large sample, which was subsequently divided into subsamples just before measuring the content of the chemicals. The test chemicals were also added to small individual samples before measuring the content of the chemicals. A mass of 3 g Schoonrewoerd soil was incubated with $^{14}$C-pentachlorophenol or $^{14}$C-chlorpyrifos, mixed thoroughly, and immediately divided into 10 subsamples of 0.3 g. These subsamples were analyzed in a D 306 Tri-Carb sample oxidizer (Canberra Packard).

### 2.2.4 Partitioning of chemical in soil solution

The partitioning of the chemicals between a molecularly dissolved fraction and a fraction bound to organic matter dissolved in pore water (soil solution) was assessed by size-exclusion chromatography. Pore water was isolated from the soil by centrifuge using the technique described by Boesten et al. (1983). The centrifuge tube consisted of an upper and a lower compartment, separated by a filter. Moist soil was placed in the upper compartment and the pore water was collected from the lower compartment after centrifugation. The centrifuge was a Heraeus Varifuge GL. The units were centrifuged at 2000 revolutions/min for 15 min (centrifugal force 200 g). The pore water in the lower compartment was filtered through a Millipore filter, pore size 0.45 μm.

The concentration of the organic matter dissolved in the pore water samples was measured. Before using the soil solutions for incubation, subsamples were made by dilution with tap water until a final dissolved organic matter concentration of 5 mg/l was reached. Artificial pore water composed according to Schouten & van der Brugge (1989) was used for comparison, containing: 0.1 mM KCl, 0.2 mM NaCl, 0.35 mM $Ca(NO_3)_2$, 0.3 mM $Mg(NO_3)_2$, 0.3 mM $NH_4NO_3$ and the pH was adjusted to 4.0 with 0.04 M $HNO_3$. Pore water samples were provided with $^{14}C$-pentachlorophenol and $^{14}C$-chlorpyrifos (both from Sigma Radiochemicals, St Louis, USA) to final concentrations of 1, 3, 5, 7 and 10 μg/l. The samples were equilibrated overnight at 10 °C in the dark.

Pore water samples were analyzed to determine the freely dissolved chemical and the chemical bound to dissolved organic material, using a liquid chromatograph (Spectra Physics SP8000) equipped with a size-exclusion column. The size-exclusion gel on silica basis (Serva Polyol Si100) is not likely to adsorb the organic chemicals. The mobile phase was distilled water. The detection unit consisted of an LC90 UV detector (Perkin Elmer) and an A-200 flow-through scintillation detector (Canberra Packard) equipped with a flow-through cell with a solid scintillator. Fractions were collected after the flow-through detector and counted separately in a 2200CA liquid scintillation analyzer (Canberra Packard).

All molecules above 20000 Dalton are shown in one peak at void volume. The molecules of chlorpyrifos and pentachlorophenol have masses well below 1000 Dalton. They are separated from the bound fractions which have masses far exceeding 1000 Dalton. To determine the analytical recovery, the total radioactivity measured in the combined fractions was compared with the total amount of radioactivity injected into the liquid chromatograph. It was found that the recovery of radioactivity was 99% or higher.

### 2.2.5 Exposure of *Globodera rostochiensis* in solution

Nematodes were exposed to the chemicals dissoved in pore water so that the toxicity of these chemicals in soil solution could be studied. A toxicity test with

nematodes in test solutions in Petri dishes was developed to determine the 48-hour solution-LC50. The soil solution was obtained from the different soils by centrifuge. Stock solutions of both chemicals were made in tap water in several concentrations and equal volumes were added to the pore water samples to avoid differences in dilution. Only pure chemicals were used in the solutions to avoid formulation effects.

Two weeks before starting the toxicity experiment, the nematode cysts were treated with attractant solution to lure the larvae out. About 0.05 g of cysts were added to 10 ml of attractant solution and placed in the dark at room temperature. After 12 days the hatched nematode larvae were removed and the freshly hatched nematodes were collected two days later. In this way age-synchronized nematodes were obtained for the toxicity tests. They were counted and, if necessary, concentrated by careful centrifugation (centrifugal force < 200 g). The nematode density in suspension is expressed as number per ml. The suspension containing the nematodes can be handled using pipettes and dispensers, provided that the suspension is gently stirred every two minutes to avoid segregation of the nematodes.

The nematodes were exposed to the test chemicals by adding 1 ml nematode suspension (1000 nematodes per ml) to 9 ml of the test solutions in the Petri dish. The test was done in the dark at room temperature. The nematode suspensions were inspected twice daily and motility and mortality counts were made. Motility scores were obtained by closely observing the movements of the nematodes and mortality scores by evaluating the appearance of the nematodes under the microscope. The skin of dead nematodes is grey and crumply. Accuracy in counting the dead nematodes requires some experience. Counting was done on a pattern of black squares placed on a piece of white paper under the Petri dish.

### 2.2.6 Exposure of *Globodera rostochiensis* in soil

The effect of the two test chemicals on nematodes in soil was also studied. The compounds were mixed at different contents into soil samples of 30 g (dry soil basis). Three series of soil samples were prepared with the same concentration range: the first for measuring the toxic effect, the second to collect pore water for chemical analysis and the third for chemical analysis of the bulk soil. A volume of 1 ml of nematode suspension (5000 nematodes per ml) was carefully sprinkled onto each sample of the first series and mixed in. All the samples were stored in the dark at 4 °C.

The nematodes were collected from the first series of soil samples according to the method used by the Nematology Department of the Agricultural University Wageningen. Briefly, the complete soil sample was stirred up with a mixture of water and glycerol (80 : 20) and the particles were allowed to settle for 1 min. The liquid column was gently decanted and centrifuged at 100 rpm for 5 min. The supernatant was discarded and the pellet with nematodes and soil debris on the bottom was

stirred up with the water-glycerol mixture and centrifuged again. After removal of the supernatant the pellet was resuspended in saline solution (0.9% NaCl) and the surviving nematodes were counted.

Pore water was collected from the second series of soil samples by centrifuge and the concentrations of pentachlorophenol and chlorpyrifos in this water were measured.

The soil samples of the third series were extracted, after which the concentrations of pentachlorophenol and chlorpyrifos in the extracts were measured (bulk soil analysis).

### 2.2.7 Exposure of *Arabidopsis thaliana* via the soil

The toxic effect of pentachlorophenol and chlorpyrifos on *Arabidopsis thaliana* was assessed by scoring the germination of the seeds and the growth of the emerging plants. Flower pots (8 cm diameter) filled with the three test soils were placed in holes in blocks of wet rockwool (100 x 15 x 17 cm) fitted in a tray. Approximately 20 to 30 seeds of *Arabidopsis thaliana* were sown on top of the soil in the pots in four replicates for the control and for each concentration level. In a preliminary experiment the concentration of the spray solution and the duration of the spraying were determined. Concentrations of chlorpyrifos (from Dursban 4E) and pentachlorophenol (from sodium salt) were prepared for application rates of 0.1, 0.5, 1.0, 5, 10, 50 and 100 mg/kg soil (dry mass basis); these solutions were carefully sprayed on the soil surface in the flowering pots.

The rockwool was watered regularly to keep the soil moist, the temperature was maintained at 21 °C and 14 hours light a day were given. Humidity in the air could not be controlled. Germination and plant growth (height) were scored daily. During the experiment small sub-samples were taken from the soil to measure the contents of pentachlorophenol and chlorpyrifos in the total soil and in soil solution.

### 2.2.8 Calculations

The persistence of a chemical in soil has been defined by the IUPAC as: "The residence time of a chemical species in a specifically defined compartment of the environment, which may be the parent compound or a derivative, but not both". The half-life or DT50 may be taken as a measure of the persistence of chemicals in soil. The DT50 is defined as the time required for a test substance in soil to decrease to 50% of the amount applied. To determine the DT50 values, samples were taken at various times and the content of the two chemicals was measured.

The toxicity of the two test chemicals to *Globodera rostochiensis* after 48 hours exposure was characterized by their solution-LC50, which is defined as the

concentration of test substance that kills 50% of the test animals within the test period. The soil-LC50 is defined as the content of the chemical in bulk soil that kills 50% of the test animals in the test period. The LC50 values were calculated using the trimmed Spearman-Kärber method (Hamilton et al., 1977), included in a computer program for an IBM personal computer. The program was downloaded from the Bulletin Board System at the Centre of Exposure Assessment Modeling (CEAM) of EPA (Athens, Georgia).

The effect of the chemicals on the plant species was expressed in EC50 values: the initial content in soil at which 50% reduction of germination and emergence occurred. The observations at 10 days after application were used for the calculations with the computer program mentioned above. The textbook by Sokal & Rohlf (1981) was used for the applied statistics.

# 3 RESULTS AND DISCUSSION

## 3.1 Persistence and distribution of the chemicals in soil

The results of the incubation of pentachlorophenol and chlorpyrifos in the three soils are shown in Table 4. The transformation rate of the substances is characterized by the time taken for the content to decrease to 50% of the initial content. This DT50 value for a chemical in soil depends on the physicochemical properties of the chemical as well as on the properties of the soil. Consequently, for different soils the DT50 value of a chemical may show substantial differences.

***Table 4 Times (days) for 50% transformation (DT50) of pentachlorophenol and chlorpyrifos in three soils. The standard deviation is given in parentheses***

| Soil | Pentachlorophenol | Chlorpyrifos |
|---|---|---|
| **Schoonrewoerd** | **34 (2)** | **64 (16)** |
| **Wageningen** | **28 (7)** | **111 (28)** |
| **OECD** | **-** | **34 (27)** |

The DT50 values of about a month, obtained for pentachlorophenol in the Schoonrewoerd and Wageningen soils (Table 4), correspond to those measured by Van Gestel & Ma (1990) for their soils. There was a marked difference in the DT50 values for chlorpyrifos obtained for the three soils (Table 4). As the values for the standard deviation were high, an analysis of variance did not reveal significant differences between the DT50 values for the three soils ($P > 0.9$). In the two-day experiments with nematodes, there was a minimal decrease in the contents of pentachlorophenol and chlorpyrifos in soil. Presumably, the decrease was somewhat more in the 10-day experiment with *Arabidopsis thaliana*. In more lengthy experiments the contents of both chemicals should be measured at given time intervals to assess the actual exposure.

The test incubations of the chemicals in large soil samples revealed a large variation in recovery from the soils. The recovery of pentachlorophenol was 83% (s.d. 28%) and the recovery of chlorpyrifos was 89% (s.d. 32%). These high standard deviations were attributed to inhomogeneous distribution of the chemicals in the samples. To check this, another incubation was carried out with $^{14}C$-labelled pentachlorophenol. The recovery from these subsamples ranged from 0.046 to 0.162 mg/kg pentachlorophenol, the average being 0.093 mg/kg. This confirmed that the distribution of the chemicals in the large soil samples was inhomogeneous. Therefore, only the results of the analyses for the small samples, which could be processed entirely, were used for the further presentation of results.

## 3.2 Partitioning of organic chemicals in soil

The adsorption of many organic chemicals on soil and sediment is mainly dependent on the organic matter fraction. Attempts are often made to correlate the extent of adsorption with the organic matter content. A linear relationship is not self-evident; Karickhoff & Morris (1985) suggested that higher organic matter contents promote the degree of aggregation, resulting in an increased fraction of the adsorption sites being blocked.

In earlier studies it was often assumed that the organic chemical is present in soil solution in the freely dissolved state. However, the question arose as to whether consideration of only two states in soil (freely dissolved and sorbed to solid organic matter) is enough to explain the partitioning of an organic chemical in soil and sediments. Benes & Majer (1980) and Gschwend & Wu (1985) found colloidal materials in solution after particle separation. Carter & Suffet (1983), Voice et al. (1983), Curl & Keolelan (1984) and Nelson et al. (1985) suggested dissolution of ligands or macromolecules by desorption from particles upon dilution. These large molecules would affect the partition of organic contaminants between the solid phase and solution. Eadie et al. (1988) distinguished three states for the partitioning of chemicals in sediments : freely dissolved chemical, chemical sorbed to dissolved organic matter (DOM) and chemical sorbed to particulate organic matter (POM). To assess the actual exposure of organisms via the soil system, it may be advisable to consider the distribution of organic chemicals over these three phases.

Distinction between freely dissolved chemical and chemical sorbed to dissolved organic matter requires a separation method. Landrum et al. (1987) used HPLC with a reversed-phase column. Such a method may have a discriminatory function based on the hydrophobicity of the components in a solution. However, during chromatography both the freely dissolved chemical and the chemical adsorbed to DOM can separate into the hydrophobic stationary phase. Therefore, it seemed preferable to use an approach to separate the components according to molecular mass, using size-exclusion chromatography, as was done in the present study.

The distribution of pentachlorophenol and chlorpyrifos in soil solution between freely dissolved state and adsorbed to dissolved organic matter, as measured in the present study, is illustrated in Figure 4. All the pore water samples were adjusted to the same concentration of dissolved organic matter to eliminate the effect of adsorbent (DOM) concentration as found by Di Toro & Horzempa (1983). Because all of the chemical was recovered at the end of the experiment, only the fraction freely dissolved chemical is shown. The supplementary fraction was adsorbed to dissolved organic matter. The freely dissolved fraction of both chemicals was lowest in the solution isolated from the Wageningen soil, indicating that the adsorption to dissolved organic matter from this soil was the most effective. Contrastingly, the dissolved organic matter in the solution isolated from the OECD soil was the least effective for both chemicals. In all three isolated solutions chlorpyrifos demonstrated a stronger adsorption to the dissolved organic matter than pentachlorophenol. As expected, no adsorption of the chemicals in the standard pore water, without dissolved organic matter, was detected.

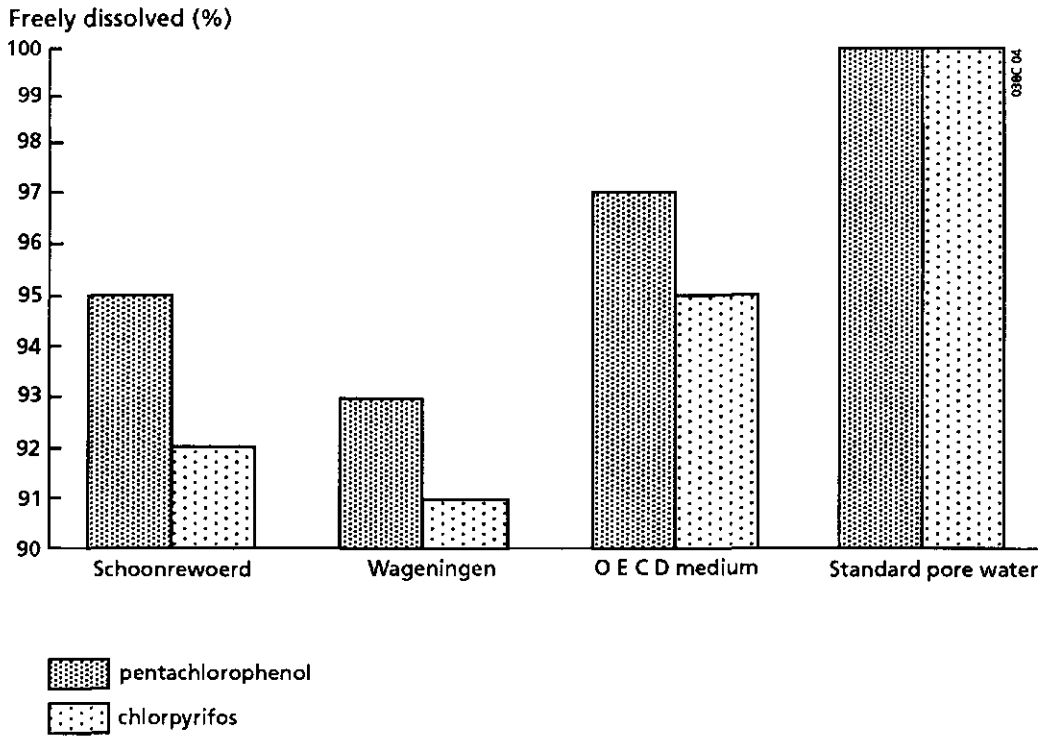


***Fig. 4 Percentage of pentachlorophenol and chlorpyrifos in the freely dissolved state in soil solutions isolated from three soils. The supplement is adsorbed to dissolved organic matter***

The difference in partitioning exhibited by the two chemicals supports the hypothesis that the partitioning to dissolved organic matter is greater as the octanol-water partition coefficient increases (Tables 1 and 2). Recent findings of R.C.M. Merkelbach (personal communication 1991, DLO The Winand Staring Centre), who used nine organic chemicals with marked differences in hydrophobicity, point out a linear relationship between the partitioning to dissolved organic matter in ditch water and the octanol-water partition coefficient. In future experiments with isolated soil solution and organic chemicals, a wider range of hydrophobicity could be included to check such a relationship.

The fraction of a chemical that is adsorbed to dissolved organic matter can be substantial if octanol-water partition coefficients are high. McCarthy & Jimenez (1985) using fish and Landrum et al. (1987) using an amphipod demonstrated that the uptake of PAH's by the organisms was significantly reduced if additional organic material was dissolved in the water. Such data indicate that chemicals sorbed to dissolved organic material are less bioavailable. However, the adsorption of pentachlorophenol and chlorpyrifos to dissolved organic material in the soil solutions, as we used them, can be considered to be of minor importance.

## 3.3 Toxicity to *Globodera rostochiensis*

Table 5 shows the toxicity data obtained for the nematodes exposed to pentachlorophenol and chlorpyrifos in the solutions isolated from the soils and to these chemicals in saline solution. The results are expressed in solution-LC50 values for 48 hours exposure. The 95% confidence intervals are given in parentheses. The toxicity of both pentachlorophenol and chlorpyrifos to *Globodera rostochiensis* in the soil solutions is not significantly different from that in the saline solution. This corresponds to the discovery that only a small fraction of the chemical is sorbed to dissolved organic matter. Our results do not support the findings of McCarthy & Jimenez (1985) and Landrum et al. (1987), who showed a decreased bioavailability of chemicals due to sorption to dissolved organic matter. Presumably, this was because of the comparatively low hydrophobicity of our test chemicals.

Results of the experiment in which *Globodera rostochiensis* was exposed to pentachlorophenol and chlorpyrifos mixed into the three soils are shown in Table 6. The results are expressed in the soil-LC50 value, which is defined as the content of chemical in soil (on dry mass basis) at which 50% of the nematodes die when exposed for two days. The soil-LC50 values of pentachlorophenol were higher than those of chlorpyrifos mainly because of the lower toxicity of the former compound in solution (see Table 5). The soil-LC50 values of the Wageningen soil were the lowest, which indicates that the bioavailability of the chemicals was highest in this soil. This corresponds to the fact that the organic matter content of the Wageningen soil was the lowest (Table 3). The soil-LC50 values in the Schoonrewoerd soil were higher (lower bioavailability) than those of the OECD artificial soil. This is remarkable because the organic matter contents of these soils differed only fractionally (Table 3). It seems that the peat fraction in the OECD mixture was a less effective adsorbent than the organic matter fraction in the Schoonrewoerd soil. Eadie et al. (1988) found similar differences in the adsorption of organic chemicals to natural organic matter of different origin.

***Table 5** Toxicity of pentachlorophenol and chlorpyrifos to Globodera rostochiensis in pore water previously isolated from three soils and in saline solution, expressed in solution-LC50 (mg/l) for 48 hours exposure.*
*The 95% confidence intervals are indicated in parentheses*

| Soil | Pentachlorophenol | Chlorpyrifos |
|---|---|---|
| **Schoonrewoerd** | **2.4 (2.0-2.9)** | **$1.4\ 10^{-3}$ ($1.3\text{-}1.6\ 10^{-3}$)** |
| **Wageningen** | **3.9 (2.8-5.1)** | **$1.6\ 10^{-3}$ ($1.4\text{-}1.6\ 10^{-3}$)** |
| **OECD** | **1.0 (0.2-3.0)** | **$1.1\ 10^{-3}$ ($1.0\text{-}1.2\ 10^{-3}$)** |
| **Saline solution** | **2.2 (1.2-3.2)** | **$0.9\ 10^{-3}$ ($0.8\text{-}1.0\ 10^{-3}$)** |

The variation in the soil-LC50 values in Table 6 is substantial, probably due to the isolation technique for the nematodes. Checks on the recovery of nematodes added to the untreated three soils also showed a considerable variation in the number of isolated nematodes. When 5000 nematodes were applied to untreated soils (three replicates per soil), only 68% (with a standard deviation of 15%) of the nematodes

was recovered after 48 hours. Extracting the nematodes from the OECD artificial soil was especially difficult because the organic material settled very slowly. To achieve accuracy, further development of the experimental procedures, to produce less variation in the distribution of the chemicals in soil and in the isolation of the nematodes, is necessary.

*Table 6 Toxicity of pentachlorophenol and chlorpyrifos, mixed into three soils, to Globodera rostochiensis, expressed in soil-LC50 (mg/kg) for 48 hours exposure. Contents (mg/kg) on dry soil basis. The 95% confidence intervals are given in parentheses*

| Soil | Pentachlorophenol | Chlorpyrifos | |
|---|---|---|---|
| Schoonrewoerd | 640 (470-820) | 2.9 | (2.0-3.8) |
| Wageningen | 130 (110-150) | 1.1 | (0.8-1.3) |
| OECD | 310 (very wide) | 1.7 | (1.2-2.2) |

The solution-LC50 values for the pore water in situ were derived from the mortality data for the nematodes exposed to the chemicals in the soils and the concentrations of the chemicals measured in pore water from these soils, isolated at the end of the exposure time. The results are given in Table 7. The solution-LC50 values for pentachlorophenol and chlorpyrifos in the soil solution in situ were about as high as the solution-LC50 values for the exposure in previously isolated pore water (Table 5). However, the variation of the solution-LC50 values for exposure in situ is higher, which is probably due to the variation in nematode isolation. The tentative conclusion is that toxicity testing for nematodes in isolated soil solution is a more accurate method of assessing the acute toxicity of chemicals than tests with nematodes added to soil. However, assessment of the effects of a chemical in contaminated soil with such a test requires measurement or estimation of its concentration in soil solution, in situ.

*Table 7 Toxicity of pentachlorophenol and chlorpyrifos, mixed into three soils, to Globodera rostochiensis, related to their concentration in soil solution isolated after the exposure of 48 hours and expressed in solution-LC50 (mg/l)*

| Soil | Pentachlorophenol | Chlorpyrifos | |
|---|---|---|---|
| Schoonrewoerd | 4.1 (2.2-6.1) | $5.2\ 10^{-3}$ | $(0.7\text{-}9.7\ 10^{-3})$ |
| Wageningen | 2.7 (1.2-4.2) | $4.9\ 10^{-3}$ | $(0.6\text{-}9.2\ 10^{-3})$ |
| OECD | 2.1 (very wide) | $1.9\ 10^{-3}$ | (very wide) |

The toxicity of some chlorophenols in four soils to two lumbricoid earthworms was studied by Van Gestel & Ma (1990). The difference in soil organic matter content was the most important parameter to determine the difference in toxicity of the chemicals to earthworms in these soils. These differences could be almost completely eliminated by calculating solution-LC50 values for the soils, using data on the adsorption of the chemicals to the soils. Thus the concentration of the chemicals in pore water was found to be of primary importance for their effect on earthworms in soil.

## 3.4 Toxicity to *Arabidopsis thaliana*

There is a large number of data available on the toxicity of herbicides to plants originating from dose-effect studies. However, the information on dose-effect relationships for the combination of non-herbicidal compounds and plants is scarce. It may be expected that most organic chemicals only effect plants at very high doses, far above the doses for selective use of herbicides in agriculture. However, even non-herbicidal pesticides may be phytotoxic to certain crops at comparatively low doses, as has been found repeatedly in the testing programmes for agricultural reliability and in practice. Comparatively high contents of organic chemicals may be expected after events such as disposal of manufacturing wastes or after accidental spillage during transport and handling.

In this preliminary study, the effect of soil-applied pentachlorophenol and chlorpyrifos on the plant *Arabidopsis thaliana* was studied. Only one experiment with each chemical was conducted because of limited time. In this experiment four pots were treated as replicates. The toxicity was expressed in the soil-EC50 value, which is defined as the content of the chemical in soil (in mg/kg, dry soil basis) at which plant germination and emergence is inhibited to 50%, observed at 10 days after sowing. The results given in Table 8 show that pentachlorophenol in soil is more toxic to *Arabidopsis thaliana* than chlorpyrifos.

Pentachlorophenol is most toxic to the plants via the Wageningen soil (Table 8), which has the lowest organic matter content. The toxicity of chlorpyrifos in the three soils was not clearly related to their organic matter content, which was not anticipated. The high variation in the soil-EC50 values which may be related to the non-uniform exposure of the plant roots to the chemicals; their contents were highest near the soil surface.

***Table 8** Toxicity of pentachlorophenol and chlorpyrifos in soil to Arabidopsis thaliana, expressed in soil-EC50 values (in mg/kg, on dry soil basis) after 10 days exposure. The 95% confidence intervals are given in parentheses*

| Soil | Pentachlorophenol | Chlorpyrifos |
|---|---|---|
| **Schoonrewoerd** | **7 (5.7- 8.3)** | **56 (42-66)** |
| **Wageningen** | **3 (2.4- 3.5)** | **75 (51-99)** |
| **OECD** | **9 (7.2-10.7)** | **26 (16-36)** |

Comparison of the toxicity data for pentachlorophenol in Tables 6 and 8 shows that this compound is much more toxic to plants (10 days exposure) than to nematodes (2 days exposure). The reverse holds for chlorpyrifos: this compound is much more toxic to nematodes (2 days) than to plants (10 days).

To assess the potential hazards of such xenobiotics in soil, more experiments need to be carried out with plants. More extensive testing of the toxicity to plants for compounds of low phytocidal activity, however, seems to be meaningless. This is illustrated by the extremely high contents of chlorpyrifos that had to be applied to soil in order to exert an effect on *Arabidopsis thaliana*.

# 4 CONCLUSIONS

There is a lot of concern about the environmental consequences of the presence of synthetic organic chemicals in the soil. The scientific basis of the evaluation of the hazards can be improved by considering the possible effect of the chemicals on organisms via the soil. This should include evaluation of the bioavailability of the chemicals in the soil.

The rate of transformation of an organic chemical in soil is important for the duration of the exposure and for the rate of recovery of the populations. Half of the initial amount of pentachlorophenol in the two natural soils was transformed in about 1 month. The time taken for half of the initial amount of chlorpyrifos to be transformed ranged from 1 to 3.5 months.

Most of the two chemicals in the isolated soil solutions was found to be in a freely dissolved state: the freely dissolved fractions were 93 to 97% for pentachlorophenol and 91 to 95% for chlorpyrifos. We can conclude that, under the present conditions, the adsorption to macromolecules dissolved in the soil solution produces only a minor decrease in the concentration of the freely dissolved chemical.

The solution-LC50 value (48 h) for the toxicity of pentachlorophenol to nematodes ranged between 1 and 3.9 mg/l. Chlorpyrifos was much more toxic to the nematodes, which was expressed in solution-LC50 values ranging from 1.1 $10^{-3}$ to 1.6 $10^{-3}$ mg/l. The solution-LC50 values for the isolated soil solutions were close to the values obtained for a dilute saline solution (without dissolved macromolecules).

The soil-LC50 value (48 h) for the toxicity of pentachlorophenol to the nematodes in the three soils ranged from 130 to 640 mg/kg. When chlorpyrifos was mixed into the soils, the soil-LC50 values ranged from 1.1 to 2.9 mg/kg, demonstrating that it is much more toxic in soil than pentachlorophenol. The toxicity of both compounds was comparatively high (lowest soil-LC50 value) in the Wageningen soil, which has the lowest organic matter content.

The soil-LC50 values for the toxicity of both chemicals to the nematodes in soil were much higher than the solution-LC50 values for their toxicity in isolated soil solution. This shows the enormous effect that the adsorption to the solid organic matter has on the toxicity of the chemicals in the soils.

The solution-LC50 values for exposure in soil solution in situ were about as high as the values for exposure in previously isolated soil solution. A tentative conclusion is that testing the toxicity in isolated soil solution provides a suitable preliminary estimate of the acute toxic effect in divergent soils. The content of the contaminant in soil should then be translated to its concentration in soil solution in situ, using data on the adsorption of the chemical to the soil.

There was a noticeable variation in the results of the experiments. Checks on the distribution of the chemicals in the soils by mixing revealed that it is difficult to obtain a homogeneous distribution, and thus exposure, especially in large soil samples. In the test with the nematodes in the soils, the recovery of viable nematodes was also very variable. Further development of the experimental procedures will be needed to reduce the variations.

The soil-EC50 values (10 d) for the reduction of germination and emergence of *Arabidopsis thaliana* from the three soils by pentachlorophenol ranged between 3 and 9 mg/kg. Chlorpyrifos in soil has a distinctly lower toxicity to these plants, with soil-EC50 values ranging from 26 to 75 mg/kg.

Pentachlorophenol was most toxic to the plants (lowest soil-EC50) in the Wageningen soil, with the lowest organic matter content. For the plant toxicity of chlorpyrifos in the soils no logical relationship with their organic matter content was found, which was not anticipated.

Extensive testing of the toxicity of a chemical in soil to a particular organism may not be useful, as the high content of chlorpyrifos needed to induce a clearly observable effect on the plants illustrated.

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