Norwegian Journal of Agricultural Sciences

Supplement No. 13 1994

Contamination of pesticides from agricultural and industrial areas to soil and water

Agricultural University of Norway – Advisory Service, Ås Norway

NORWEGIAN JOURNAL OF AGRICULTURAL SCIENCES

Norwegian Journal of Agricultural Sciences fills a need created by the folding of Scientific Reports of the Agricultural University of Norway and Research in Norwegian Agriculture for a forum for publishing Norwegian research with international interest within the following areas: Aquaculture, Animal Science, Soil Science, Agricultural Engineering and Technology, Natural Resources and Environment, Food Technology, Crop Science, Forestry, Economics and Society Planning.

PUBLISHER

The journal is published by the Agricultural University of Norway, Moerveien 12, N-1432 Ås, Norway. Norwegian Journal of Agricultural Sciences (ISSN 0801-5341) is published four times a year, each issue containing approximately 100 pages. Four issues comprise one volume. Annual subscription NOK 500,–. Subscribers receive supplement issues free of charge, but these can also be ordered separately through the publisher.

REFEREE

All papers submitted for publication will be sent to one or more competent referees.

CORRESPONDENCE

All Correspondence, editorial or otherwise, should be adressed to the Agricultural University of Norway.

The drawing on the cover is from Kjell Aukrust's *«Guttene på broen»*.

ISSN 0802-1600

Supplement No. 13 1994

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Major results from the project (1988–1992)

CONTAMINATION OF PESTICIDES FROM AGRICULTURAL AND INDUSTRIAL AREAS TO SOIL AND WATER

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Norwegian State Pollution Control Authority

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PREFACE

This volume of the *Norwegian Journal of Agricultural Sciences* addresses an important aspect of ecotoxicology - that of the fate of pesticides in the subarctic environment. Knowledge of the fate of herbicides, insecticides and fungicides is critical to environmental risk assessments and management decisions. To assess the exposure of pesticides to humans and the biota there is a need for understanding the fate and pathways for pesticides in soils and waters. These problems have been addressed in the project presented here: "Contamination of pesticides from agricultural and industrial areas to soil and water" (1988-92).

The various aspects of interactions of pesticides with soils, terrestrial insects, freshwater invertebrates, plankton and fish as well as improvements in analytical techniques constituted a challenge that required the interdisciplinary cooperation of scientists from a wide range of institutions. The following institutions have been involved in the project: the Norwegian Plant Protection Institute (SPV) including the Department of Herbology, the Department of Entomology and Nematology and the Department of Pesticide Analysis; the Agricultural University of Norway (NLH) including the Department of Soil and Water Sciences and the Laboratory for Analytical Chemistry (LAK), the Center for Soil and Environmental Research (JORDFORSK); the Norwegian Institute for Water Research (NIVA) and the Central Veterinary Laboratory (VI)/Norwegian College of Veterinary Medicine (NVH), Department of Toxicology and Chemistry. This volume gives a review of the different studies carried out during the project period. We hope that the results of the studies will be of value to the agricultural and environmental authorities as well as providing important additions to the state of basal knowledge in environmental science.

On behalf of the initiator of the project, The Plant Protection Institute, I express my deep appreciation to each contributing author and to the editorial board; Dag Berge, Eliann Egaas and Gunnhild Riise. Special thanks are due to the Agricultural Research Council of Norway (NLVF) and to the Norwegian State Pollution Control Authority (SFT) for financial support and to Agrolinz Agrarchemikalien Ges.m.b.h., BASF aktiengesellschaft, E.I.Du Pont de Nemours & Company, Ciba-Geigy Limited and the American Cyanamid Company for the supply of ^{14}C labelled pesticides.

Olav Lode

Content

Introduction

In the middle of the 1980s pesticides were detected in farmland rivers, streams and groundwater in many European countries, among them Sweden and Denmark. In 1986, as a direct result of these reports, the Norwegian State Pollution Control Authority (SFT) and the Norwegian Ministry of Agriculture launched a recipient surveillance on some ground- and surface waters in Norwegian farmland areas. This study was reported in 1987, and positive registrations of pesticides were made in six out of eight surface water bodies (SPV/GEFO 1987). The concentrations varied from the detection limit of 0.1 µg/l to as high as 15 µg/l for single pesticides. No positive registrations of pesticides were made in the groundwater sources included in this study. At the same time, however, pesticides were observed in another groundwater source, situated at Mysen, SE Norway (Lode *et al.* this volume).

Now it was confirmed that pesticides occurred in Norwegian water bodies, and there was a strong demand for information on how the pesticides were transported to ground- and surface waters, how this impacted on the freshwater ecosystems, the seasonal variation in pesticide contamination, the longevity of such contamination, etc. To provide this information, the Ministry of Agriculture and the SFT allocated funding for a five-year research project called the FPJV-project, where FPJV is a Norwegian abbreviation for "Contamination of pesticides from agricultural and industrial areas to soil and water". The project was organized through the Council for Agricultural Research of Norway (NLVF).

The following institutions were invited to participate:

1. *Norwegian Plant Protection Institute*
Dept. of Herbology
Dept. of Entomology and Nematology
Dept. of Pesticide Analysis

2. *Agricultural University of Norway*
Dept. of Soil and Water Science
Laboratory for Analytic Chemistry
(earlier the Isotope and Electron Microscopy Laboratories)

3. *Center for Soil and Environmental Research*

4. *Norwegian Institute for Water Research*

5. *Central Veterinary Laboratory/The Norwegian College of Veterinary Medicine*
Dept. of Toxicology and Chemistry

An agreed project outline was submitted to the NLVF by the turn of the year and was approved by the council in March 1988. The project, which started immediately, took the form of six part projects:

1. *Association of pesticides with different soil components*
The association of pesticides with different soil components was examined by sorption and desorption experiments using standard and model soils. Water suspensions containing ^{14}C-labelled pesticides were ultrafiltered by the use of hollow-fibres in order to study the size distribution pattern.

2. *Mobility of pesticides in laboratory scale lysimeters*
^{14}C-labelled pesticides were used to study the vertical transport of pesticides, whereas the movement of water was followed by ^{3}H-water.

3. *The mobility of pesticides in undisturbed soil profiles in different culture systems*
The influence of plant growth and the application of fertilizer on the mobility of pesticides in different soil-water systems was followed in large column- and field lysimeters. Barley, wheat and grass were cultivated. A controlled rainfall programme was applied.

4. *Pesticide runoff from agricultural fields under normal cultivation*
Field experiments were performed within well-defined catchment areas where pesticides were applied at normal rates. Surface runoff and drainage water were collected during the whole project period and analysed for pesticides.

5. *Induction of detoxification enzymes (biomarkers) in biota exposed to pesticides*
Biotest experiments using well-defined exposure solutions and terrestrial organisms (mainly Lepidoptera) were carried out. Biological uptake and the induction of detoxification enzymes were studied. The effects of some biological, environmental and chemical factors that may have an influence on the detoxification enzymes have also been studied.

6. *Effects of pesticide contaminated runoff on freshwater organisms*
Biotest experiments using well-defined exposure solutions at concentrations ranging from high to the levels found in Norwegian farmland water bodies, were carried out using aquatic organisms including algae, zooplankton, invertebrates and fish. The study included single species tests and community tests, acute toxicity tests and long-term sublethal tests, and induction of detoxification enzymes.

In Norwegian agriculture some 160 commercial pesticide products are currently in use, and obviously only a few could be included in the project. The selection of pesticides was based on the following criteria: All three groups of compounds, herbicides, insecticides, and fungicides should be represented. The compounds most commonly used should be included. In addition, some chemical properties, such as high solubility, high mobility, etc., should be given priority. The following compounds were selected as mandatory for all part projects: the herbicides MCPA, chlorsulfuron, atrazine, the insecticide dimethoate, and the fungicide propiconazole. In addition, several other compounds have been included in different studies: dichlorprop, tribenuron-methyl, simazine, difenzoquat, dazomet, endosulfan, glyphosate, lindane, DDT and DDE.

In order to coordinate the project, a steering committee consisting of members from the participating institutions/departments was appointed with Olav Lode as chairman and Ole Martin Eklo as secretary, both from the Norwegian Plant Protection Institute.

The results from the project were presented at three open seminars where sponsors and representatives from the environmental and agricultural authorities as well as the project co-workers were participants. Several publications and reports have already been printed from the project.

Olav Lode

Summary

PESTICIDES IN RUNOFF

Applied pesticides are supposed to disappear during the growth season. Under the climatic conditions that occur in Norway, however, some pesticides may persist in soil and water for a rather long period. In surface runoff from agricultural fields in conventional use at Ullensaker (SE Norway), dichlorprop was detected up to ten months after application (until May), propiconazole was detected from October to March and dimethoate from October to February. Chlorsulfuron was detected in drainage water from June to October during the year of application. Two percent of the total amount of chlorsulfuron applied was recovered in the drainage water.

The point in time and the amount of precipitation strongly influence the leaching of pesticides. Maximum concentrations of pesticides are generally found during the first rain event after application. The highest concentration measured for any pesticide during the field experiments at Ullensaker was 9.7 g/l propiconazole. In addition, a second peak was often found during snowmelt in the spring.

GROUND WATER POLLUTION

Simazine and atrazine, leached from an industrial area at Mysen (SE Norway), have contaminated a water source which supplied water for both human consumption and greenhouse crops. In 1986 greenhouse plants suffered considerable damage. Studies in the area have continued during the whole project period (1988-92).The water source, receiving water mainly from a groundwater aquifer, is in 1993 still contaminated with simazine and atrazine (seven years after the last application in 1986).

PESTICIDE MOVEMENT IN FIELD LYSIMETERS

Pesticides were frequently detected in leachate from field lysimeters receiving additional water. In field lysimeters covered by vegetation, the leaching of pesticides was delayed due to increased water uptake by plants. Furthermore, the application of fertilizers seemed to increase the leaching of pesticides. The vertical migration of pesticides through clay columns liable to cracking was more extensive compared to other soil types. Flow through macropores can also explain the relatively rapid appearance of pesticides in (a) the drainage water from agricultural fields at Ullensaker and in (b) the leachate samples from small-scale lysimeter experiments with silty clay loam from Ullensaker.

Of the total amount added, 0.1 % of dichlorprop, and up to 1 % of dimethoate and propiconazole were recovered in the leachate from the large-scale field lysimeters.

MOBILITY OF PESTICIDES IN SOILS

In small-scale lysimeter experiments, the vertical movement of ^{14}C labelled MCPA, dichlorprop, atrazine and tribenuron-methyl was higher in subsurface layers compared with that in surface layers with a higher content of organic carbon and a higher cation exchange capacity. The retention of tribenuron-methyl was particulary high in the silty clay loam from Ullensaker, where 25 % of the applied pesticides remained within the upper 2 cm after the columns had received an amount of water corresponding to 670 mm precipitation over a period of four weeks. Atrazine was especially mobile in fine sand columns, where the leachate curves for the [^{14}C] pesticide and the [^{3}H]water were almost overlapping.

Soil organic carbon is one of the single most important sorbents for pesticides in soils. The extent of sorption to soil organic carbon was dependent on the hydrophobicity of the pesticides (K_{ow}) and pH. For an organic-rich soil (pH 4.1) the sorption decreased in the following order: atrazine > dichlorprop, MCPA > tribenuron-methyl > dimethoate. All pesticides showed decreasing sorption to soil at increasing pH values. A direct relationship between K_{oc} and K_{ow} at given pH values was observed for phenoxy acids, suggesting a hydrophobic sorption mechanism. Similar relationships for the other pesticides due to other binding mechanisms than hydrophobic sorption (e.g. ionic binding), or pH-dependent changes in the properties of the sorbent were not observed. The sorption to inorganic components in soils (e.g. clays) was not dependent on the hydrophobicity of the pesticides. In a silty clay loam the sorption decreased in the following order: tribenuron-methyl > atrazine > dichlorprop, MCPA > dimethoate.

Although the desorption of dichlorprop increased with increasing pH values, a relatively large amount was still retained in organic-rich soils at high pH values (pH 7). Desorption of atrazine from soils low in organic matter was positively correlated to pH, while the desorption from organic rich soils was independent of pH. Thus, atrazine seems to be strongly associated with soil organic carbon.

Ultrafiltration of clay suspensions with hollow fibres (nominal molecular weight cut-off 3 kDa) showed that the association of tribenuron-methyl with illite and smectite was high, particulary at low pH values. Colloidal transport of tribenuron-methyl may therefore be of importance in clayey areas during tillage and soil erosion. It is suggested that transport in association with particles also occurs for propiconazole. For water souble pesticides showing high mobility in soil colloidal transport is probably of minor importance.

DETOXIFICATION ENZYMES (BIOMARKERS) IN BIOTA

The use of some biochemical characteristics of insects and fish (the so-called xenobiotic metabolizing enzymes (XME)) as biomarkers of contaminant exposure and environmental quality has been evaluated.

Our results support the use of the XME parameter P4501A1 as a biomarker for contamination of specific chemical structures (PAH-type compounds) in the aquatic environment. There are, however, important species differences to be considered. Furthermore, the specificity of the reaction has not been fully investigated. The pesticide dimethoate, for

example, which does not have a chemical structure resembling the PAH-type compounds, is an inhibitor of P4501A1 in fish. Thus, although most of the pesticides tested did not have any effect on the P4501A1 system in fish, we need more screening of the mechanisms involved in the induction process and of the chemical structures that may interfere with P4501A1 before this system can be fully used as a specific biomarker of PAH-type contamination.

The use of the XME parameter GST as a biomarker of contamination of pesticides and industrial chemicals in the aquatic environment needs more investigation than the P4501A1 system before it can be evaluated. Our results from experiments with pesticide-exposed limnic invertebrates indicate that insect GST activities may be changed by the presence of low levels of certain pesticides in the water. The specificity of this induction has, however, not been considered since in the herbivorous fish and insect larvae, plant allelochemicals also have a potential as inducers of XME.

In terrestrial organisms, XME parameters have a tradition as biomarkers for populations of insects that through selective breeding have become resistant to certain insecticides. The usefulness of insect XME as biomarkers of a pesticide contamination situation has not been studied earlier. We have documented that several factors (develomental stage, diet, sex) other than pesticide exposure may induce changes in the XME activities. Of these, dietary factors seem to be of particular importance in insects. However, our experiments also indicate that in selected species, a combination of information on several XME activity parameters and a study of specific isozymes in specific tissues may have a potential as a selective system for pesticide contamination in terrestrial as well as aquatic systems.

EFFECTS OF PESTICIDE RUNOFF ON FRESHWATER ORGANISMS.

The effects of low concentrations of the mandatory and some other pesticides on freshwater organisms are tested for acute and chronic toxicity, and with respect to induction of detoxification enzymes. The following test and research items are included in the studies:

- Phytoplankton algae, single species tests and community tests
- Benthic algae, community tests
- Zooplanton, single species and community tests
- Bentic invertebrates, single species and community tests
- Fish, single species tests and reproduction tests
- Accumulation of pesticides in lake sediments
- Bioaccumulation in fish muscle and liver
- Induction of detoxification enzymes - biomarkers

Algae

Studies of the effects of pesticide runoff on freshwater planktonic algae and cyanobacteria were carried out on three different scales, in microplates containing 1 ml, standard OECD bottles containing 100 ml, and in large enclosures of 20 000 litres lake water containing a natural lake water plankton community.

From the lab-scale tests it was demonstrated that there were great variations in impact depending on which algal species were used in the actual test, as well as that different pesticides

gave different results. The interspecies variation in EC50 -values ranged from a factor of 4.8 for atrazine to almost 104 for propiconazole. The sensitivity is not necessarily connected with phylogenetic position, which implies that one particular test alga should not be considered as representative of the phylum or genus to which it belongs, as far as sensitivity to chemicals is concerned.

For Hazard assessment purposes, a range of EC_{50}-values obtained from simple screening tests with a battery of test algae will provide more useful information than an EC50 -value determined with a high degree of precision for one test algae.

Among the pesticides tested, the phenoxy-herbicides MCPA and dichlorprop and the organophosphorus dimethoate showed low toxicity to algae, and effects of these pesticides on natural algal communities are unlikely to occur in streams and lakes situated in farmland areas.

The triazine herbicides atrazine and simazine affected the growth of algae at concentrations that have been recorded in natural waters, and the use of these pesticides may therefor cause environmental effects. Chlorsulfuron had a toxicity comparative with that of atrazine for the most sensitive species and should also be considered as a potential risk, although concentrations in the toxic range have not been recorded in Scandinavian waters.

The fungicide propiconazole has the potential to affect the species composition of algal communities at environmentally realistic concentrations because of the extremely high interspecies variation in sensitivity that was found for this pesticide. Propiconazole was also found to be generally toxic to benthic algae at 5 ug/l in running water, as a pre-established algal community on ceramic tiles totally disappeared in a five weeks test in an artificial stream. In the non-treated control stream nothing happened to the algal community.

The effects of four pesticides; chlorsulfuron, propiconazole, dimethoate and glyphosate on lake phytoplankton communities have been studied in a mesocosm experiment. The pesticides were added at initial concentrations of 1, 10 and 100 ug/l to 20 m^3 enclosures containing lake water. At the highest concentration, all pesticides affected the biomass development, measured as chlorophyll-a. With the exception of chlorsulfuron, this was also reflected by an initial slight depression of photosynthesis, and a lower rate of nitrate assimilation. Effects on the biomass development were also observed at 10 ug/l. The species diversity was reduced as compared to control enclosures at all pesticide treatments even at 1 ug/l. Chemical analysis revealed that approximately 100% of the dimethoate, 80% of the glyphosate and 60% of the propiconazole remained in the water after 16 days. These results indicate that structural changes on phytoplankton communities may occur at environmentally realistic concentrations of all tested pesticides (1-10 ug/l).

Zooplankton

With respect to zooplankton, the herbicide chlorsulfuron had low toxicity on the cladocerans *Daphnia magna* and *Daphnia pulex* in acute lab tests (LC50 > 100 mg/l), whereas glyphosate and propiconazole had a toxicity of 3.2 mg/l > LC50>10 mg/l. The insecticide dimethoate had a much higher toxicity for both species (LC50 = 0.02^{s} mg/l). This general toxicity pattern for *Daphnia* was confirmed by 12 days of enclosure experiments, whereas other species of cladocera gave a somewhat different response. While the crustacean zooplankton species were most susceptible to the insecticide, this was reversed for the planktonic rotifers which gave no negative response on dimethoate, but showed a negative population response in bags with added chlorsulfuron and glyphosate. No support was found for strong indirect effects of the

herbicides on zooplankton due to effects on algae (food depletion). In general, there was no correlation between total primary production, phytoplankton biomass, phytoplankton biomass and zooplankton. Community changes and species shifts in the phytoplankton community could, however, explain some of the negative effects on rotifers.

Benthic invertebrates

The toxicity of seven pesticides, atrazine, simazine, MCPA, dichlorprop, chlorsulfuron, propiconazole and dimethoate on the bottom-dwelling macro invertebrates of streams were tested in artificial laboratory streams. From the acute tests, dimethoate and propiconazole were found to be the most toxic, and these two compounds were investigated further in longterm tests with sublethal, environmentally realistic concentrations of the pesticides, 1 ug/l and 5 ug/l respectively. The other pesticides tested were judged unlikely to occur in concentrations harmful to benthic invertebrates in natural waters.

Relative to the untreated controls, the following observations were made in the dimethoate test: both drift of animals as well as the part of the population found in drift were larger in the dimethoate stream. The non-drifting movements away from the precolonized trays were higher in the dimethoate streams. Structural differences between the streams were small, but significant for some populations. In sum, a tendency to increased activity of the individuals and a reduction in the density in some populations were observed.

In the 5 ug/l propiconazole stream a somewhat opposite reaction was observed in the stream bed animal population. The drift rate was lower in the propiconazole stream compared with that in the untreated control stream. The proportion of the population found in the drift was lower in the propiconazole stream. The non-drifting animal movements away from the precolonized trays were lower in the propiconazole stream. Structural changes were observed, but they were generally small. There was a reduction of the filter feeding groups of Simulidae and *Hydropsyche* and an increase in the population of *Leuctra* and Chironomidae in the propiconazole-treated stream as compared to the untreated control. It is important to stress the considerable negative effect this low concentration of propiconazole seems to have on the communities of benthic algae. This will indirectly affect the benthic fauna in running water ecosystems because of the importance the benthic algae play as a food source.

Fish

Nine commonly used pesticides have been studied with respect to their acute effect (4-d LC50) on brown trout (*Salmo trutta*) and some other freshwater fishes. Early life stage tests were made with brown trout and zebra fish (*Brachydanio rerio*) in dimethoate and propiconazole. The 4-d LC_{50}-values (ppm) were as follows: MCPA (300), dichlorprop (78), simazine (70), chlorsulfuron (40), atrazine (27), glyphosate (4.5), propiconazole (1.2), dimethoate (0.13), and endosulfan (0.0009).

The lowest observed effect concentrations (LOEC) on newly hatched brown trout alevines in the early life stage tests were approximately 0.5 and 0.05 ppm for propiconazole and dimethoate, respectively.

Analyses of pesticides in fish muscle and liver were made in perch (*Perca fluviatilis*), pike perch (*Lucioperca lucioperca*), and pike (*Esox lucius*) and in sediments from two lakes, the one surrounded by intensively cultivated farmland. None of the above-mentioned substances were found, either in the fish nor in the sediments.

Only lindane and DDT derivates were found in detectable but low concentrations in fish. They were not found in the sediments in detectable amounts. The compounds were also detected in fish from the lake without agricultural fields in the catchment area, indicating that these pesticides may be of airborne origin. The concentrations of lindane were 10-20 ug/kg wet weight in the liver of perch and pike from the forest lake. The concentrations of the DDT derivates varied from 2 to 200 ug/kg with the highest concentration observed in the liver of a pike from the agriculturally influenced lake.

On the basis of these results it seems unlike that the tested pesticides could cause any direct harm to freshwater fish under normal use in Norway. For endosulfan, however, the toxicity was so high that heavy rain after spraying could cause direct effects on fish in farmland brooks and streams. For the other compounds, only indirect effects on fish via food organisms are likely to occur.There should be no risk in using the fillets of the fish species investigated for household purposes in Norwegian farmland areas.

Association of MCPA, dichlorprop, tribenuron-methyl, atrazine and dimethoate with different soil types - Laboratory experiments

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Riise, G., O. M. Eklo, M. Nandrup Pettersen & B. Salbu 1994. Association of MCPA, dichlorprop, tribenuron-methyl, atrazine and dimethoate with different soil types - Laboratory experiments. Norwegian Journal of Agricultural Sciences. Supplement No. 13: 17-29. ISSN 0802-1600.

Sorption and desorption experiments were conducted in order to study the mobility of [^{14}C]pesticides with different chemical properties in soils with different physical and chemical characteristics. The extent of sorption for dichlorprop, atrazine and dimethoate was linearly related to soil organic carbon and inversely related to pH. The sorption of tribenuron-methyl was also inversely related to pH, but the sorption behaviour was more dependent on clay content than the content of organic matter. Of the pesticides studied, dimethoate was the one most weakly sorbed to soil. With the exception of tribenuron-methyl, the extent of sorption was related to the hydrophobicity of the pesticides. For the phenoxy-acids the highest K_{oc} and K_{ow} coefficients were observed below a pH value of 4, while for atrazine the highest K_{ow} and K_{oc} coefficients were observed at pH values above 4. The K_{oc} coefficient of dichlorprop was correlated to its K_{ow} coefficient at given pH values, suggesting that hydrophobic sorption is a dominant sorption mechanism for the phenoxy acids. A direct relationship between K_{oc} and K_{ow} at given pH values was not observed for the other pesticides. This indicated that mechanisms other than hydrophobic sorption were involved and/or that pH-dependent changes in the properties of the sorbent have occurred (e.g. decreasing hydorophobicity of soil organic carbon at increasing values of pH).When pH increased, an increasing amount of dichlorprop was desorbed. Only a small amount of atrazine was desorbed from the organic rich soils. Desorption of atrazine from soils low in organic matter was positively correlated to pH, while the desorption from organic-rich soils was independent of pH. Ultrafiltration of illite suspensions spiked with [^{14}C]tribenuron-methyl indicated that the association of tribenuron-methyl with clay colloids was of major importance, especially at low pH values.

Key words: atrazine, desorption, dichlorprop, MCPA, organic carbon, pH, sorption, tribenuron-methyl

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Processes occurring at the soil-water interface are of major importance for the behaviour of organic micropollutants in the environment. Thus, factors influencing the sorption/desorption

processes at the surfaces of soil components will to a large extent control the mobility and transport of pesticides in the soil-water system. Furthermore, colloids present in the water phase may also interact with pesticides and thereby influence their transport behaviour.

Interactions between pesticides and soil surfaces are dependent on the physical and chemical properties of the soil and also on the pesticide. Sorption of *non-ionic* pesticides to soils is correlated to the soil organic carbon content, where the extent of sorption depends on the hydrophobicity of the pesticide (K_{ow}). Relationships between K_{ow} and K_{oc} (the soil-water distribution coefficient corrected for soil organic carbon content) for non-ionic pesticides are well established (Briggs 1973; Karickhoff *et al.* 1979). Chiou *et al.* (1979) explained this as a partitioning (dissolution) process between the organic matter and the water phase. Wershaw (1990) has later proposed that humic substances constitute a number of separate hydrophobic and hydrophilic phases (amphiphiles) where the hydrophobic interior of the humic aggregate represents a separate liquid phase able to interact with hydrophobic pesticides.

At given pH values, relationships between K_{ow} and K_{oc} for a weakly acidic herbicide have also been estimated (Riise & Salbu 1992). The neutral form predominating at low pH values is more hydrophobic and shows a higher degree of sorption to soils than the corresponding anionic form. Hydrophobic sorption seems therefore to be an important sorption mechanism for ionizable *anionic* pesticides as well.

There is usually a net negative charge on the surface of soil colloids. Electrostatic interactions (ionic binding, cation exchange) between permanent or ionizable *cationic* forms of pesticides and negatively charged soil colloids must therefore also be taken into consideration. In addition to those mentioned here, several other sorption/binding mechanisms for both non-ionic and ionizable pesticides are proposed, such as hydrogen bonding, charge transfer, van der Waals forces, ligand exchange and covalent bonding (e.g. Theng 1974; Senesi 1992).

In the present paper the associations of MCPA, dichlorprop, atrazine and dimethoate with different soil types are investigated. Special attention is given to the pH value of the soil-water system as it influences both the surface properties of the sorbent (soil) and the dissociation of weakly acidic/basic pesticides. The influence of pH on the hydrophobicity (K_{ow}) of the pesticides and also the relationships between K_{ow} coefficients and K_{oc} coefficients at given pH values are discussed. As easily extractable fractions of pesticides may be available for transport and biological uptake, the influence of pH on the desorption process is also studied.

MATERIALS AND METHODS

Samples

Five different soil samples, taken from the plough layer of cultivated soils, were air-dried and sieved through a 2 mm sieve. Soils A and B, with an organic carbon content of more than 14%, are described as organic-rich soils. According to texture analyses determined by the hydrometer method (Elonen 1971), soil C was loam, soil D silty clay loam and soil E sand. Organic carbon was determined in a LECO EC-12 (752-100) Carbon Determinator with an IR-detector. The organic carbon in soil samples ranged from 1.3 to 30.4%. Cation exchange capacity was determined by extraction with 1 M NH_4-acetate (pH 7.00). The concentrations of Ca, Mg, Na, K in solutions were determined by ICP (Phermo Jarrell Ash Cooperation ICP Poly Scan 61 E) and H^+ was determined by titration of the extract back to pH 7.0. The cation exchange capacity

varied from 7 to 99 mequiv/100g, while the base saturation was from 34 to 55%. pH was measured in a 1:1 liquid/solid slurry and was found to vary from 4.1 to 5.3 ($CaCl_2$) and from 4.3 to 5.7 (H_2O) (Table 1).

Table 1. Physical and chemical properties of soil types used in the experiments

Property	Soil A	Soil B	Soil C	Soil D	Soil E
Texture	Orc. rich	Org.rich	Loam	Silty clay loam	Sand
Size distribution (%)					
2-0.6 mm	8	18	7	1	1
0.6-0.2 mm	16	27	12	2	40
0.2-0.06 mm	29	27	21	2	57
0.006-0.02 mm	20	14	20	12	1
0.02-0.0006 mm	16	7	16	28	1
0.0006-0.002 mm	3	4	8	23	0
0.002 mm	8	3	16	33	0
Organic C (%)	30.4	14.6	3.3	1.3	1.4
CEC (mequiv/100 g)	99	55	18	20	7
pH (0.01 M $CaCl_2$)	4.4	4.1	5.0	5.1	5.3
(H_2O)	4.6	4.3	5.3	5.6	5.7
Base sat. (%)	49	34	49	54	55
Fe (%)	1.45	1.42	0.74	1.32	0.17
Mn (%)	0.062	0.003	0.013	0.081	0.027

Pesticides

The following pesticides were used in the experiments:
[1-^{14}C]MCPA ((4-chloro-o-tolyloxy)acetic acid)) with a specific activity of 297 µCi/mg (Amersham Int. UK), *[U-phenyl ring ^{14}C]dichlorprop* (2-(2,4-dichlorophenoxy) propionic acid) with a specific activity of 55.01 µCi/mg (Agrolinz, 1989), *[triazine-2-^{14}C]tribenuron-methyl*(2-(4-methoxy-6-methyl-1,3,5-triazin-2-yl (methyl) carbamoylsulphamoyl) benzoic acid) with a specific activity of 10.52 µCi/mg (Du Pont 1990),*[triazine^{14}C] atrazine* (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine) with a specific activity of 53.5 µCi/mg (CIBA-Geigy AS) and*[^{14}C)dimethoate* (O,O-dimethyl S-methylcarbamoylmethyl phosphorodithioate) with a specific activity of 74 µCi/mg (BASF 1989).

Sorption/desorption experiments

Unless otherwise stated, sorption was studied by equilibrating 0.5 g soil with 5 ml 0.01 M $CaCl_2$ spiked with ^{14}C-labelled pesticides. The suspension was shaken for 24 h in a chamber under temperature control (T 17°C). Corresponding tubes without soil were also equilibrated to control sorption of pesticides to the interior walls. Prior to separation, the tubes were centrifuged for 30 min. at 11.000 x g. Sorption was estimated from the decrease in concentration of ^{14}C in the liquid phase after equilibration.

Linear and Freundlich sorption isotherms were calculated from four to seven different concentrations of ^{14}C-labelled pesticide in accordance with the formulae:

$C_s = a + K_d C_w$ (Linear)

$\log C_s = \log K_f + (1/n) \log C_w$ (Freundlich)

where C_s is is the amount sorbed (µg/g) and C_w is the final concentration in the solution (µg/ml). K_d and K_f are the Linear and the Freundlich distribution coefficients respectively, while 1/n is the intensity factor (slope of the regression line).

The influence of pH on the sorption process was studied by adjusting the pH of the spiked $CaCl_2$ solutions (dilute HCl/NaOH). Single distribution coefficient points (K_d) were determined in accordance with the formula:

$$K_d = \frac{\mu g \text{ dissolved} \cdot g \text{ solute}^{-1}}{\mu g \text{ sorbed} \cdot g \text{ soil}^{-1}}$$

The influence of pH on the desorption process was studied by extracting equilibrated soil residues with 0.01 M $CaCl_2$ adjusted to different pH values by dilute NaOH/HCl.

The suspension was shaken for 1 h at room temperature prior to separation.

Octanol/water extractions

Water samples (0.2 mM KCl or humic water) spiked with ^{14}C-labelled pesticides were adjusted to different pH values (dilute NaOH/HCl). The water samples (50 ml) were transferred to 100-ml flasks with screw caps and 5 ml of 1-octanol (PS 97% purity, Kebo Lab) was added. The flasks were shaken for 16 h and stored for 1-2 h before separating the two phases.

Ultrafiltration

Water suspensions spiked with ^{14}C-labelled pesticides were ultrafiltered using Amicon hollow fibres (nominal molecular cut-off value 3kDa). Sorption was minimized by conditioning the system with a sample aliquot prior to collection of the ultrafiltrate.

^{14}C measurements

^{14}C activity in the extracts was measured with a Packard Tri-Carb 5430 liquid scintillation counter. The ratio between sample and the scintillation cocktail was 1:10.

RESULTS AND DISCUSSIONS

Sorption isotherms

Soil type as well as type of pesticide influenced the Linear (K_d) and the Freundlich (K_f) soil-water distribution coefficients (Table 2). The intensity factor 1/n (determined by the slope of the regression line in the Freundlich isotherms) is in most cases rather close to 1, which indicates only minor deviation from linear behaviour. With the exception of results from some of the sorption experiments with silty clay loam (soil D) and fine sand (soil E), the agreements between the K_d and K_f coefficients were in general rather good (mostly within 10% variation). As soil organic carbon content is one of the most important factors contributing to the retention of pesticides in soils, the K_d coefficients are often normalized to K_{oc} ($K_{oc} = K_d$/fraction of organic carbon). The range in K_{oc} coefficients for dichlorprop, atrazine and dimethoate varied by a

factor of 2-3, which is considerably less than the observed range in K_d coefficients, which varied by a factor of 41-65. This indicates that much of the variation in the capacity of different soil types to sorb dichlorprop, atrazine and dimethoate can be attributed to the content of organic matter. Wang *et al.* (1993) have proposed that sorption of organic compounds is a combination of two different processes: adsorption and partitioning. The authors claim that the adsorption process can be described either by a Langmuir or a Freundlich equation ,while the partitioning

Table 2. Linear and Freundlich sorption isotherms determined from batch experiments with ^{14}C pesticides and soils A (organic rich), B (organic rich), C (loam), D (silty clay loam) and E (sand)

	A	B	C	D	E
MCPA					
K_d		16.6*		0.82*	
K_f					
K_{oc}		114*		63*	
Dichlorprop					
K_d	34.7	17.4	1.62	0.84	0.72
K_f	31.5	18.3	1.61	0.84	0.59
1/n	0.96	0.94	0.91	0.90	0.87
K_{oc}	113	118	50	62	54
Tribenuron-methyl					
K_d	17.9	12.5	55.5	28.3	1.21
K_f	16.5	11.3	59.3	19.9	1.16
1/n	0.94	0.91	0.99	0.81	0.87
K_{oc}	59	86	1673	2173	86
Atrazine					
K_d	89.1	38.6	5.8	1.37	2.16
K_f	85.1	33.9	6.27	1.67	2.43
1/n	0.98	0.96	1.02	1.06	1.01
Koc	293	265	175	106	154
Dimethoate					
K_d	7.42	3.55	0.74	0.31	0.18
K_f	8.40	3.55	0.74	0.31	0.18
1/n	1.08	1.08	1.00	1.01	0.75
K_{oc}	24.4	24.3	22.4	23.8	12.9

*Distilled water

process can be described by a linear equation. In general, the distribution of pesticides between soil and water in this study fitted well with the linear equation ($r^2 > 0.96$), indicating that the sorption behaviour can be explained by a partitioning mechanism. However, at low pesticide concentrations, where the amount of pesticide sorbed is considerably less than the total amount of pesticide capable of being sorbed ($C_s << V_m$), the Langmuir equation reduces to a linear equation (Riise & Salbu 1992). Thus, at low pesticide concentrations, where the pesticides distribute linearly between soil and water, several interactions are possible. Hence, in order to identify the binding mechanisms involved additional information is needed.

For an organic-rich soil (B), the K_{oc} coefficients of the different pesticides decreased in the following order: atrazine > dichlorprop, MCPA > tribenuron-methyl > dimethoate (Fig. 1).

With the exeption of tribenuron-methyl, this corresponds to the water solubility of the pesticides; i.e. atrazine being least soluble while dimethoate is the most soluble of the five pesticides compared. Accordingly, atrazine will show higher retention in an organic-rich soil than the more mobile phenoxy-acids and dimethoate. Sorption of atrazine to humic substances is well documented in the literature (e.g. Weber et al. 1969; Hayes 1970).

Dimethoate was the pesticide most weakly sorbed in the silty clay loam (soil D) as well as in the organic-rich soil (soil B), compared with MCPA, dichlorprop, atrazine and tribenuron-methyl (Fig.1).

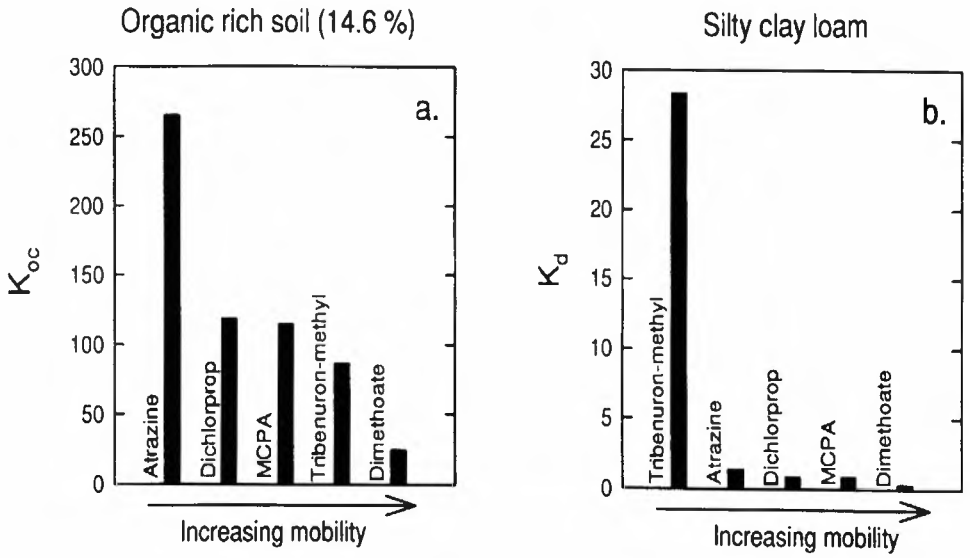

Fig. 1 a) Soil-water distribution coefficients corrected for content of organic carbon in soils - K_{oc} for different pesticides. The K_{oc} coefficients are determined on an organic-rich soil and organized in descending order.
b) Soil-water distribution coefficients for different pesticides - K_d for different pesticides. The K_d coefficients are determined on a silty loam soil and organized in descending order. (The K_d coefficients are not corrected for content of soil organic carbon, as sorption of tribenuron-methyl is not very dependent on soil organic carbon.)

The silty clay loam showed high sorption of tribenuron-methyl compared to the other pesticides, indicating low mobility of tribenuron-methyl in clayey and silty soils. According to Rahman & James (1989) the mobility of tribenuron-methyl is low compared with that of other sulphonyl-urea herbicides.

K_{ow}, K_{oc} vs pH

MCPA and dichlorprop are weak organic acids with pKa values close to 3 (Kearney & Kaufmann 1975). Depending on pH, weakly acidic pesticides dissociate in aqueous solutions in accordance with the equation: $HA \rightleftarrows H^+ + A^-$. The neutral form of the phenoxy-acids predominating at low pH is more hydrophobic than the anionic form. This explains the steady

decrease in the octanol-water partitioning coefficient, as an increasing amount of the acids are dissociated at increasing pH (Fig. 2a). Dichlorprop is somewhat more hydrophobic than MCPA at low pH values. Otherwise, only small differences in K_{ow} were observed.

There is a good agreement between K_{ow} and K_{oc} for dichlorprop at given pH values, and the following relationship has been calculated (Riise & Salbu 1992):

$$\log K_{oc} = 0.5 \log K_{ow} + 0.2 \ (r^2 = 0.986).$$

Hydrophobic sorption is therefore considered to be of major importance for the sorption of dichlorprop in the soil types studied. In addition, positively charged metal oxides may be important sorbents for anionic pesticides in soils rich in oxides (e.g. Borggaard & Streibig 1988).

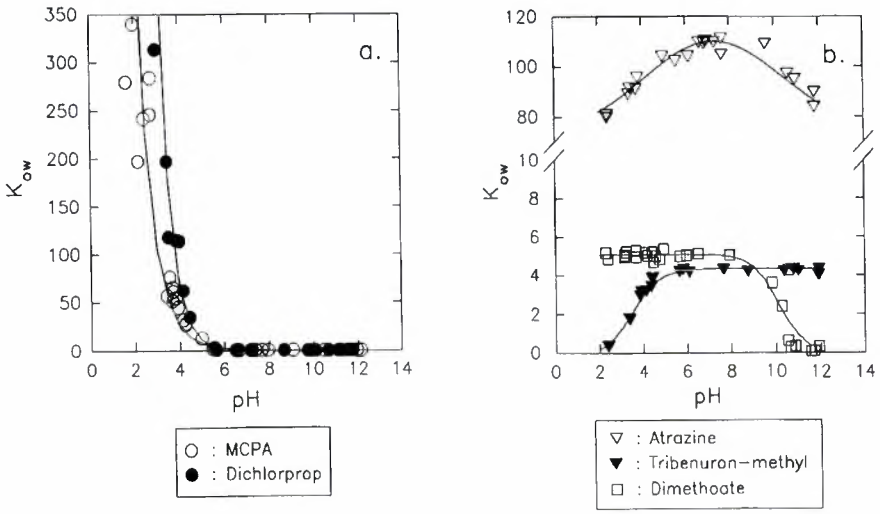

Fig.2. (a) Relationships between K_{ow} and pH for MCPA and dichlorprop, (b) Relationships between K_{ow} and pH for atrazine, tribenuron-methyl and dimethoate

Protonization of the weakly basic atrazine is also pH dependent, as can be seen from the following equation: $B + H^+ \rightleftarrows HB^+$. Atrazine showed a small decrease in hydrophobicity at decreasing pH values, indicating an increase in the protonated form of atrazine (Fig. 2b). However, as the pKa value of atrazine is quite low (pKa 1.7), the neutral form is supposed to predominate within a normal pH range in soils. A small decrease in hydrophobicity is also seen at high pH values (pH > 10), which indicates an ionization or formation of an atrazine derivate (e.g. hydroxylation) with lower hydrophobicity than the parent compound. Because of the relatively high K_{ow} coefficient of atrazine, hydrophobic sorption of atrazine to soil organic matter should be an important sorption mechanism. Other mechanisms suggested to be involved in the sorption of atrazine are H-bonding, ligand exchange, charge transfer reactions and covalent bonding (Senesi 1992). Atrazine showed very low sorption in clayey soils (Table 2). Heteroaromatic compounds, such as atrazine and simazine, contain nitrogen atoms bearing

partially localized electron density. These partially negative charge sites on the pesticide are thought to repel negative sites on the clay surfaces (Cohen et al. 1984) and may explain the low sorption observed in clayey soil.

Dimethoate is classified as a non ionic insecticide and has a low K_{ow} coefficient which is relativly stable at pH-values of less than 9. At high pH values (pH > 9) ionization seems to occur as the K_{ow} coefficient decreases. Dimethoate has a high water solubility, a low K_{ow} coefficient and the sorption to soil is low (Table 2). Decreasing sorption at increasing pH values is perhaps attributable to the dissociation of functional acidic groups of humic substances; e.g. carboxylic groups. Thus, pH influences the charge properties of the *sorbent*, making it less hydrophobic and reducing its capacity to interact with hydrophobic components. Changes in the sorbent's properties with pH may also contribute to the decreased sorption of atrazine within the pH range 6-8 where the K_{oc} coefficient was relatively stable.

Being a sulphonyl-urea herbicide, tribenuron-methyl is classified as a weak acid. The hydrophobicity is low and seems to decrease even further at low pH values (Fig 2b). This indicates ionization at low pH-values and does not correspond to the pattern reported for other sulphonyl-urea herbicides, for which the neutral form is supposed to predominate at low pH values (Beyer et al. 1987).

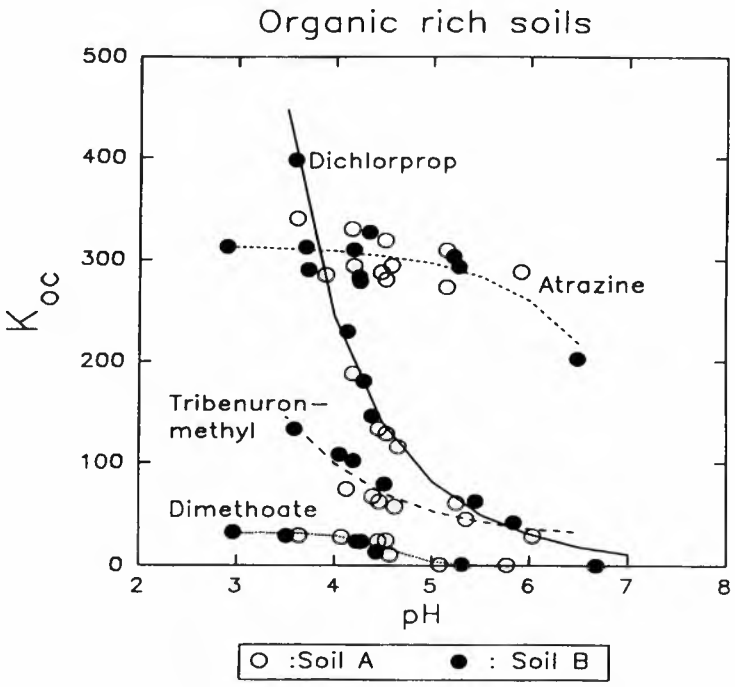

Fig. 3. Relationships between K_{oc} and pH for different pesticides. The K_{oc} coefficients are determined on two organic rich soils (A and B)

Within the pH range where the K_{ow} coefficient of tribenuron-methyl decreases (Fig. 2), the sorption increases (Fig. 3), suggesting that ionic binding or cation exchange reactions are involved. Soil persistence of tribenuron-methyl is shown to be low compared with that of other sulphonyl-urea herbicides (Ferguson et al. 1985). If chemical hydrolysis of the sulphonyl-urea bridge has occurred, the ^{14}C measurements in this study may represent the behaviour of the amino heterocyclic part containing the ^{14}C-labelled triazine ring instead of the parent compound.

The soil's sorption capacity depends on the pesticide in question and on soil pH (Fig. 3). Pesticides with a K_{ow} coefficient of less than 10 are generally considered to be weakly sorbed in soil, and thereby rather mobile. MCPA and dichlorprop at pH values above 5.5, and dimethoate and tribenuron-methyl within the whole pH range studied fall into this category. However, as can be seen from Table 2 and Fig. 1b, the retention of tribenuron-methyl in soils rich in clay and silt is supposed to be high, even though its K_{ow} coefficients are low. Thus, the K_{ow} coefficients of pesticides do not reflect the interaction of pesticides with inorganic components in soil.

Comparing K_{ow} coeffcients for the different pesticides within certain pH-values, MCPA and dichlorprop are found to have the highest hydrophobicity at pH values of less than 4, while atrazine has the highest hydrophobicity at pH values above 4 (Fig. 2a, b). This is in agreement with the results presented in Fig. 3 for organic-rich soils, where dichlorprop showed highest sorption compared to the other pesticides at pH values of less than 4, while atrazine showed highest sorption at pH values above 4. At pH values of less than 6, dichlorprop was more hydrophobic and showed higher sorption to organic-rich soils compared to tribenuron-methyl, while at pH values above 6, tribenuron-methyl was more hydrophobic and seemed to show higher sorption to organic-rich soil than dichlorprop. Accordingly, for pesticides whose major sorbent is soil organic carbon, the sorption seems to a certain degree to be related to their K_{ow} coefficients at given pH values.

However, at high pH values and within a pH range where the K_{ow} coefficients were stable, the sorption of atrazine and dimethoate decreased. This perhaps indicates that changes in the surface properties of the sorbent (soil) influence the sorption behaviour, i.e. decreasing sorption of pesticides is due to the decreasing hydrophobicity of the sorbent.

Desorption vs pH

The pH in the soil solution had a major influence on the amount of dichlorprop desorbed from organic-rich soils (Fig. 4). For soils with a low content of organic carbon the effect was considerably less. However, even though approximately half of the amount initially sorbed was desorbed at high pH values (pH 7-8), a relatively large amount was still retained in the organic-rich soils. This indicates that the mobility of phenoxy-acids in organic-rich soils is low within a relatively large pH range ($pH < 7$). In acidic soils high in organic matter the mobility of phenoxy-acids is assumed to be low.

Within the chosen pH range, the desorption of atrazine from the organic-rich soils was low and not dependent on pH (Fig. 4). For soils with less organic carbon there was a small increase in the amount desorbed with increasing pH. Atrazine shows a high degree of sorption to organic- rich soils and the leachability is low. Irreversible sorption of atrazine to soil has been reported earlier, where the amount desorbed was inversely related to soil organic carbon (Raman *et al.* 1988)

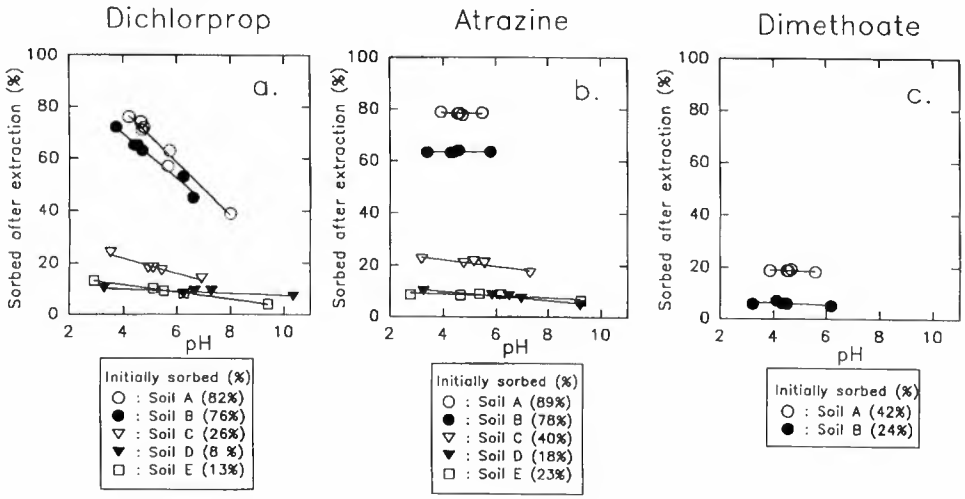

Fig. 4. a) Desorption of dichlorprop from different soil types at different pH values
b) Desorption of atrazine from different soil types at different pH values
c) Desorption of dimethoate from different soil types at different pH values

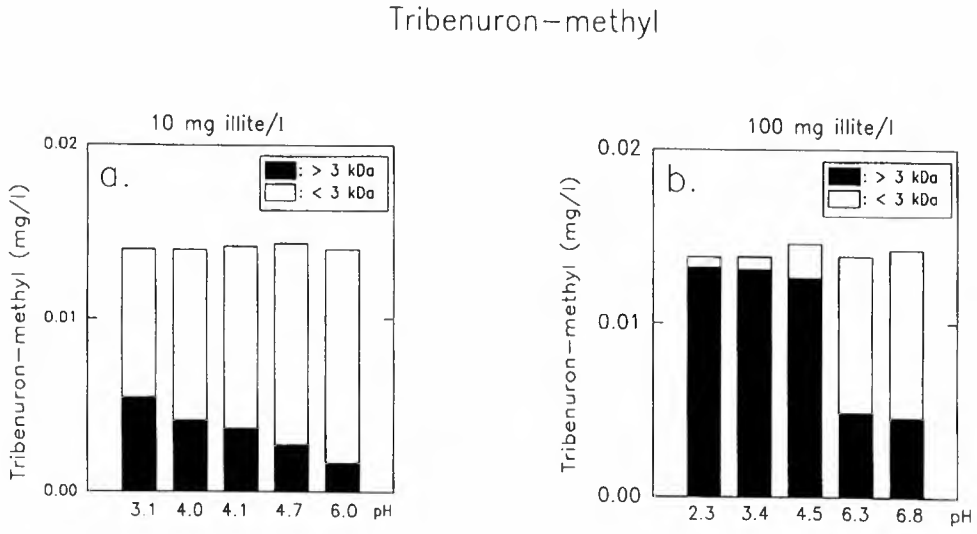

Fig. 5. Association of tribenuron-methyl with clay colloids > 3 kDa at different pH values. The clay suspensions (a) 10 mg illite/l and (b) 100 mg illite/l were ultrafiltered by the use of hollow fibres

As dimethoate was only slightly sorbed to soils poor in organic matter, the results presented refer to soils high in organic carbon (Fig. 4). Although the sorption of dimethoate was related to pH (Fig. 3), the amount desorbed was only to a minor degree dependent on the pH of the soil water. Lower pH values close to the microlayer of colloidal surfaces compared to the bulk solution or irreversible binding of a small amount of dimethoate are possible explanations for this behaviour.

Association of tribenuron-methyl with clay colloids

As tribenuron-methyl exhibited high sorption to soils rich in clays, the importance of clay colloids in suspensions was further examined. Illite suspensions spiked with [^{14}C]tribenuron-methyl were ultrafiltered by means of hollow fibres. The result indicated that the sorption was inversely related to pH, and that a large amount of tribenuron-methyl associates with clay colloids (>3kDa) at low pH values (Fig. 5). In low pH suspensions containing 100 mg illite/l, more than 90% tribenuron-methyl was associated with colloids. Thus, in areas rich in clays and susceptible to surface erosion, colloidal transport of tribenuron-methyl may be of importance.

SUMMARY

The extent of sorption to soil organic carbon was dependent on the hydrophobicity of the pesticides (K_{ow}). For an organic-rich soil (pH 4.1) the sorption decreased in the following order: atrazine > dichlorprop, MCPA > tribenuron-methyl > dimethoate. All pesticides exhibited decreasing sorption to organic-rich soils at increasing pH values.

A direct relationship between K_{oc} and K_{ow} at given pH values was only observed for phenoxy-acids. Similar relationships for the other pesticides were not observed, because of binding mechanisms other than hydrophobic sorption (e.g. ionic binding), or pH dependent changes in the properties of the sorbent.

The sorption to inorganic components in soils (e.g. clays) was not dependent on the hydrophobicity of the pesticides. In a silty clay loam the sorption decreased in the following order: tribenuron-methyl > atrazine > dichlorprop, MCPA > dimethoate.

Although the desorption of dichlorprop was strongly related to pH, a relatively large amount was retained in organic-rich soil at high pH values (pH 7-8). The desorption of atrazine from soils low in organic carbon increased with pH, while the desorption from the organic- rich soils was low and not dependent on pH. The desorption of dimethoate from organic-rich soils was to a minor degree dependent on pH.

Ultrafiltration of clay suspensions showed that the association of tribenuron-methyl with illite was high, especially at low pH values. Colloidal transport of tribenuron-methyl may therefore be of importance in clayey areas.

ACKNOWLEDGEMENT

The authors express their thanks to the personnel at the Isotope Laboratory, Agricultural University of Norway for assistance with ^{14}C measurements and their gratitude to the following companies for providing [^{14}C]pesticides: Agrolinz, BASF Aktiengesellscaft, CIBA-GEIGY

and Du Pont. G.R. thanks the Norwegian Research Council for Science and Humanities for a Research Fellowship.

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Mobility of atrazine and tribenuron-methyl in the soil-water system – Lysimeter experiments

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Riise, G., O. M. Eklo, O.Lode & M. Nandrup Pettersen 1994. Mobility of atrazine and tribenuron-methyl in the soil-water system – Lysimeter experiments. Norwegian Journal of Agricultural Sciences. Supplement No. 13: 31-41. ISSN 0802-1600.

Lysimeter experiments were performed in order to study the vertical movement of [^{14}C]atrazine and [^{14}C]tribenuron-methyl through different soil layers. [^{3}H]water was used as a tracer for water transport. The leaching of atrazine increased with decreasing amounts of soil organic carbon; i.e. the retention of atrazine was higher in silt loam surface layers (1.4% organic carbon) compared with that in subsurface layers poor in organic carbon (<0.1 % organic carbon). [^{14}C]atrazine was especially mobile in fine sand columns (70-91 cm), where the [^{3}H]water and [^{14}C]atrazine leaching curves were almost overlapping. The transport of [^{14}C]tribenuron-methyl through the soil columns was less than that for [^{14}C]atrazine in all soil columns studied. The retention of tribenuron-methyl was particularly high in silty clay columns where 25% of the applied pesticides stayed within the upper 2 cm, after having received 670 mm precipitation. Soil-water distribution coefficients (K_f) estimated from batch experiments were in fair agreement with the leaching data. However, in soils where most of the flow drained through macropores, the batch experiments seemed to overestimate the soil's sorption capacity for atrazine. Furthermore, for tribenuron-methyl, whose sorption behaviour is rather pH dependent, the batch experiments seemed to underestimate the sorption capacity of the soil. This can be attributed to differences in soil surface pH in the batch and lysimeter experiments.

Key words: Atrazine, lysimeter experiment, mobility, tribenuron-methyl.

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In order to assess the possibility of pesticides reaching groundwater, factors influencing the vertical migration of water and mobile species have to be considered. In this respect the persistence and water solubility of the pesticides as well as soil type, texture and climate in the actual area are of major importance.

Atrazine degrades slowly with a half-life of months or years, depending on soil conditions. In aerated subsoils the half-life of atrazine is expected to be longer than five years, while in anaerobic subsoils the half-life may be less than one year (Boesten & van der Pas 1993). Widespread occurrence of atrazine in surface waters and groundwaters is reported; for example, in the United States (Pereira & Rostad 1990; Kolpin & Kalkhoff 1993) in Germany (Lang & Hurle 1993; Pestemer *et al.* 1993) in Poland (Sadowski *et al.* 1993) and in Denmark (Kristiansen 1993). In Norway, leaching of atrazine and simazine through a groundwater aquifer occurred for several years (6-7 years) after the application of these pesticides in the area ceased (Lode *et al.* this volume).

Tribenuron-methyl belongs to a relatively new class of herbicides, the sulphonyl-urea herbicides, which are highly active against a wide spectrum of weeds. The degradation rate of tribenuron-methyl is rapid (Ferguson *et al.* 1985; West 1989) and its mobility in soil is low (Rahman & James 1989) compared with that for other sulphonyl-urea herbicides (e.g. chlorsulfuron). The behaviour of tribenuron-methyl in the soil-water system is pH dependent as the water solubility is related to pH while degradation rate is inversely related to pH.

The aim of the present work was to study the vertical transport of atrazine and tribenuron-methyl in different Norwegian soil types. Transport through upper soil horizons with higher content of organic carbon and higher cation exchange capacity was compared with transport through underlying horizons with a more sandy texture poor in organic carbon. Silty clay loam columns susceptible to cracking were also included in the study. In order to simulate conditions occurring in field, intact soil columns were used in the lysimeter experiments. The leaching of [^{14}C]pesticide through the columns was compared with information on Freundlich sorption coefficients determined from batch experiments.

METHODS

Samples

Intact soil columns were obtained from agricultural fields at Haslemoen and Ullensaker by pressing PCV tubes (10 cm i.d.) into the soil. The soil samples were taken from three different layers at Haslemoen and from the upper layer at Ullensaker. The different soil layers from Haslemoen were classified as silt loam (0-22 cm), sandy loam (35-57 cm)and fine sand (70-91 cm) and the upper layer from Ullensaker as silty clay loam (0-23 cm) (Table 1). Soil properties were analysed following the methods by Riise *et al.* (this volume). The pore volumes of the soils were determined at the Department of Agricultural Engineering, Agricultural University of Norway. While there were only minor differences in total pore volume among the soils (42-51%), there were major differences in the distribution of pore sizes containing water accessible for plants. In the upper silt loam layer and the underlying fine sand layer from Haslemoen, the pore size volumes containing water accessible for plants were 38% and 5% respectively. Prevailing clay types in the upper soil layers from Haslemoen were illite and mixed illite-vermiculite (Sperstad 1991).

Pesticides

The following two pesticides were used in the experiments: [triazine^{14}C]atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine) with a specific activity of 53.5 μCi/mg (CIBA-Geigy AS) and[triazine-2-^{14}C]tribenuron-methyl (2-(4-methoxy-6-methyl-1,3,5-triazin-2-yl(methyl)carbamoyl)benzoic acid) with a specific activity of 10.52 μCi/mg (Du Pont 1990).

Lysimeter experiments

A 50 ml solution, containing 8.19 μCi [^{3}H]water, 2.58 μCi [^{14}C]atrazine and 5.89 mg inactive atrazine (primatol, 470 g a.i./l) was applied to the top of each atrazine column. In the tribenuron-methyl experiment a 30 ml solution containing 8.39 μCi [^{3}H]water, 3.05 μCi [^{14}C] tribenuron-methyl and 0.60 mg inactive tribenuron-methyl (Gran Star, 750g a.i./1000g) was applied to each column. The experiments were carried out on water-saturated columns in triplicate, i.e. three columns for each soil type. Accurate amounts of water with a similar ionic composition as precipitation at Narbuvollen (Overrein *et al.* 1980) were added to the columns during the

experiment. The columns received 1300 ml artificial rainwater per week, equivalent to approx. 24 mm precipitation/day. Water leaching through the columns was sampled once a day. ^{14}C and ^{3}H were measured in 1 ml x 3 aliquotes of the leachate.

^{14}C recovery from soil

After the end of the leaching experiment, the columns were divided into 2-5 cm sections. Representative samples (1-2 g) from each section were oxidized by means of a Biological oxidizer OX 200 (R.J. Harvey Instr. Corp.), whereupon $^{14}CO_2$ was directly transferred into scintillation vials.

Table 1. Physical and chemical properties of soil types used in lysimeter experiments

	Columns treated with atrazin				**Columns trated with tribenuron-methyl**			
Property	Haslemoen			Ullensaker	Haslemoen			Ullensaker
	0-21 cm	35-55 cm	70-91 cm	0-22 cm	0-24 cm	35-59 cm	70-91 cm	0-24 cm
Texture	Silt loam	Sandy loam	Fine sans	Silty clay loam	Silt loam	Sandy loam	Fined sand	Silty clay loam
Sand (%)	15	55	97	7	15	55	97	7
Silt (%)	77	42	37	1	77	42	3	71
Clay (%)	7	2	0	29	7	2	0	29
Pore volume (%)	51	42	45	44	51	42	45	44
Water* acc. to plants vol.(%)	38(8)	32(4)	5(2)	17(3)	38(8)	32(4)	5(2)	17(3)
CEC (mequiv/100g)	7	1	0.1	18	7	5	6	12
Base saturation (%)	100	100	100	58	74	16	2	74
pH(H_2O)	6.8	6.5	6.5	6.5	6.3	6.1	5.9	6.4
pH($CaCl_2$)	6.1	5.7	5.6	5.7	5.6	5.3	4.8	5.6
Org. C (%)	1.4	0.1	<0.05	1.0	1.2	0.2	0.05	0.9
Fe (%)	0.60	0.45	0.16	1.31	0.59	0.25	0.08	1.33
Al (%)	0.18	0.12	0.06	0.15	0.15	0.11	0.03	0.13

*The numbers in parentheses give the amount (vol. %) easily accessible to plants

Batch experiment

Freundlich sorption isoterms were determined following the methods presented in Riise *et al.* (this volume). The same soil and pesticide solutions as those in the lysimeter experiment were used.

Liquid scintillation

^{14}C and ^{3}H were measured with a Packard Tri-Carb 4530 liquid scintillation counter. The ratio between sample and scintillation cocktail was 1:10.

RESULTS AND DISCUSSIONS

Haslemoen - vertical transport with depth

Both atrazine and tribenuron-methyl, in different soil layers from Haslemoen showed considerable variations with respect to mobility (Fig. 1). While only small amounts of herbicides were found in the leachate from the silt loam surface layer (0-22 cm), more than 75% of tribenuron-methyl and 87-95% of atrazine were leached through the sandy loam (35-57 cm) and fine sand (70-91 cm) columns during the experiments (Tables 2 and 3). The transport of atrazine through the silt loam surface layer was faster than that for tribenuron-methyl, as the migration of tribenuron-methyl was very slow through the upper soil layer from Haslemoen and no distinct peak was observed for the leachate curve.

Table 2. Results from columns with added [^{14}C]atrazine and [^{3}H]water

Soil type	**Haslemoen**			**Ullensaker**
	0-21 cm	35-59 cm	70-91 cm	0-22 cm
Volume (ml)				
^{3}H-peak	571±171	809±123	625±246	
^{14}C-peak	1933±672	870±174	720±89	
Ratio:				
^{3}H-peak/^{14}C-peak	0.3	0.9	0.9	
Volume (ml)				
^{3}H Σ(C/CT)/2	653±137	723±36	513±133	70±29
^{14}C Σ(C/CT)/2	2012±495	867±63	553±123	41±2
Leaching (%)				
^{3}H Σ(C/CT)100	91±3	91±3	91±2	87±0.4
^{14}C Σ(C/CT)100	18±16	87±2	95±1	71±4

To a large extent [^{14}C]atrazine followed the [^{3}H]water leaching curves for the two deeper horizons, and only a small delay in the ^{14}C peak compared with the ^{3}H peak was observed (Table 2). In the fine sand columns the leaching curves of ^{3}H and ^{14}C were rather close, indicating a very low retention of atrazine in fine sand soils. According to the ^{14}C-atrazine analyses on the soil columns, more than 70% of atrazine was retained by the silt loam, while less than 10% and 5 % of atrazine were retained by the sandy loam and fine sand columns respectively (Table 4).

The transport of tribenuron-methyl through the deeper soil layers was slower for tribenuron-methyl than for atrazine, as the ratio between the ^{3}H-peak and ^{14}C-peak was 0.6 and 0.9 for tribenuron-methyl and atrazine respectively (Tables 2 and 3). In addition, the [^{14}C]tribenuron-methyl curve showed a marked tailing in both the sandy loam and fine sand layers (Fig. 1 and Table 3). The recovery of [^{14}C]tribenuron-methyl from soils was in agreement with the leaching data, as the largest amount was found in the upper horizon (71 ± 4%) and considerably less in the sandy loam (16 ± 17%) and fine sand (12 ± 9%) layers (Table 5). There was a small loss of pesticides which can be attributed to evaporation. However, total recovery of ^{14}C was generally above 90 percent, which is considered as acceptable. As only ^{14}C analyses were carried out, information concerning the formation of possible degradation products is not available.

Table 3. Results from columns with added [^{14}C]tribenuron-methyl and [^{3}H]water

Soil type	**Haslemoen**			**Ullensaker**
	0-18 cm	35-60 cm	70-95 cm	0-18 cm
Volume (ml)				
^{3}H-peak	930±87	808±40	751±88	338±112
^{14}C-peak		1466±521	1210±173	235±165
Ratio:				
^{3}H-peak/^{14}C-peak		0.6	0.6	1.4
Volume (ml)				
^{3}H Σ(C/CT)/2	886±64	773±40	695±13	498±50
^{14}C Σ(C/CT)/2	3663±437	1978±964	1568±455	770±114
Leaching (%)				
^{3}H Σ(C/CT)100	92±0.3	93±1	90±3	85±5
^{14}C Σ(C/CT)100	8±5	76±19	78±11	7±4

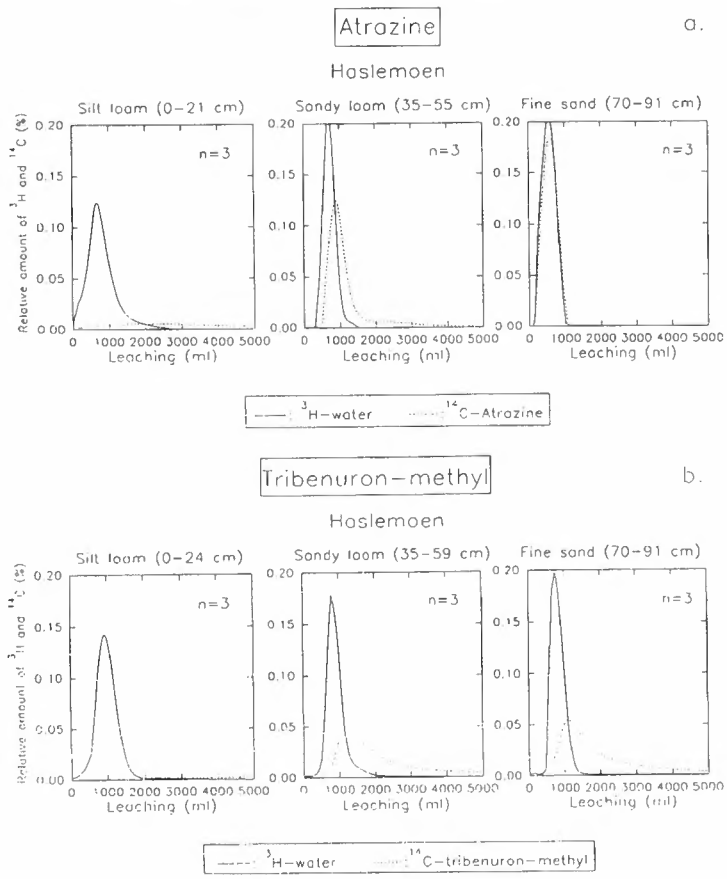

Fig. 1. a) Leaching of [^{3}H] and [^{14}C] atrazine through silt loam, sandy loam and fine sand colums. b) Leaching of [^{3}H] water and [^{14}C] tribenuron-menthyl through silt loam, sandy loam and fine sand columns

Table 4. Mass balance of [^{14}C]atrazine in lysimeter experiments and Freundlich sorption isotherms determined from batch experiments

	Lysimeter experiment			Batch experiment		
	^{14}C leachate (%)	^{14}C recovered from soil (%)	Total recovery (%)	Kf	1/n	r^2
Haslemoen						
0-21 cm	18±16	73±19	91±3	3.10	0.90	0.992
35-55 cm	87±2	9±1	95±1	0.723	0.93	0.990
70-91 cm	95±1	3±0.2	97±1	0.284	0.79	0.790
Ullensaker						
0-22 cm	71±4	23±4	94±0.03	1.95	1.00	0.982

Earlier lysimeter experiments with similar soil types have shown that the leaching of dichlorprop also increases once the herbicides have passed the surface layer (Riise *et al.* 1992). The microbial degradation is often less and the transport of pesticides faster in subsurface compared to surface layers. It is therefore important to keep the surface layers intact in order to prevent pesticides from reaching groundwater.

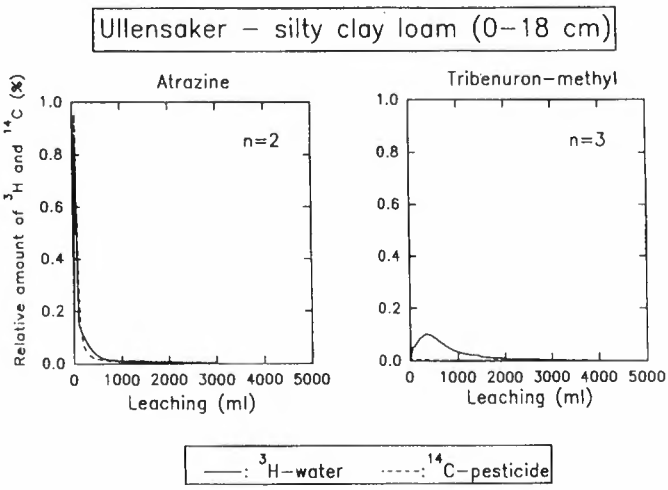

Fig. 2.a) Leaching of [^{3}H] water and [^{14}C] atrazine through silty clay loam columns.
b)Leaching of [^{3}H] water and [^{14}C] tribenuron-methyl through silty clay loam columns

Ullensaker - flow through macropores

The silty clay loam soil from Ullensaker is susceptible to cracking, and much of the vertical water transport is probably being drained through these preferential flow paths, or so-called macropores. During wet periods large amounts of water may rapidly drain through these macropores, representing only a small fraction of the total pore volume.

Field experiments in the Ullensaker area have shown that pesticides quickly appear in

subsurface drainage water after periods with heavy rain (Eklo *et al.* 1993). In silty, crack-free, clay soil columns from Ullensaker, the vertical migration of water was too slow to allow measurements of leachate during the observation period. As the macropores are not homogeneously distributed, large variations among the different columns should be expected. The differences in the [^{3}H]water leaching curve between the atrazine and tribenuron-methyl columns are also attributed to heterogeneous distribution of macropores (Fig. 2). In the atrazine columns the maximum amount of [^{3}H]water and [^{14}C]atrazine was found in the first leachate. While a large amount of atrazine (71 ± 4%) had leached through the columns within a short period of time, only a small amount of tribenuron-methyl (7% ± 4) leached through the columns during the whole experiment. Distribution data for ^{14}C recovery from the soil revealed that a major fraction of tribenuron-methyl was retained in the upper 2 cm (approx. 25%) indicating very low mobility of tribenuron-methyl in the silty clay loam (Fig. 3).

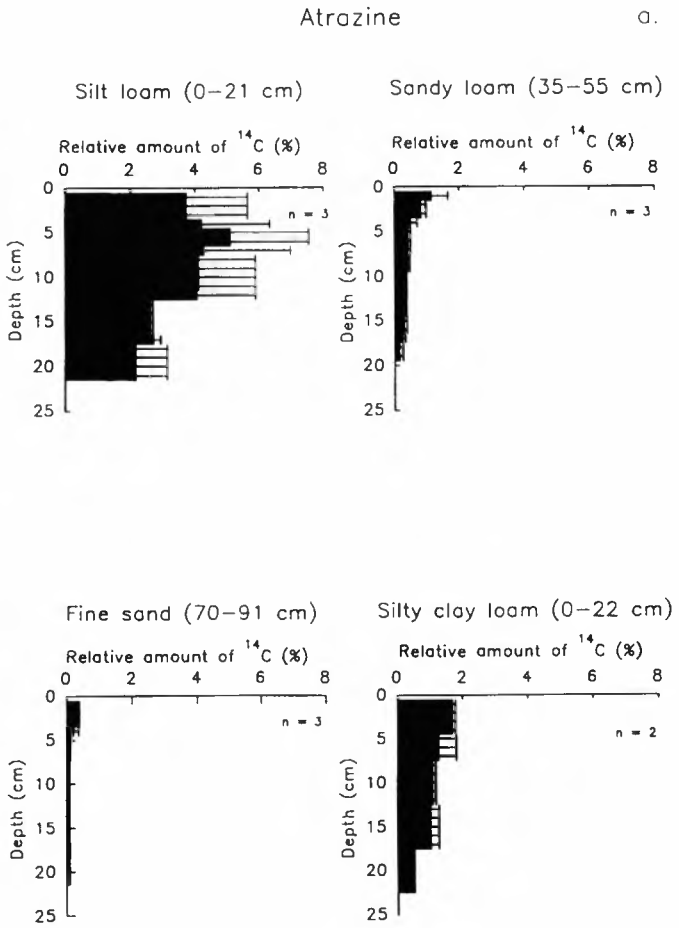

Fig. 3 a) Recovery of [^{14}C] atrazine in soil columns after the end of the leaching experiment

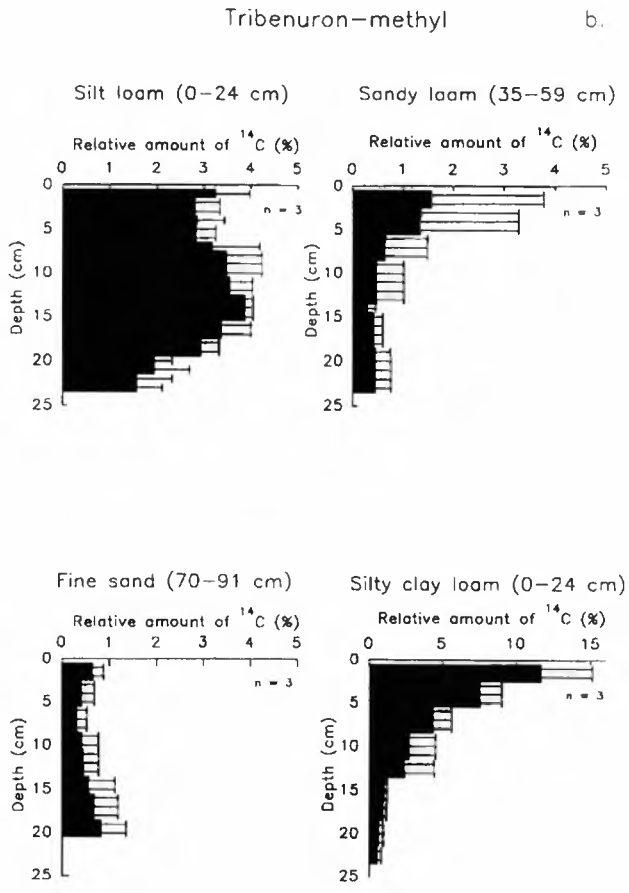

Fig. 3 b) Recovery of [^{14}C] tribenuron-menthyl in soil columns after the end of the leaching experiment

Batch experiments

Results from the batch experiments confirmed that the sorption capacity of the surface layer at Haslemoen is higher compared with that of the underlying layers (Tables 4 and 5). The variation in the sorption behaviour of atrazine could to a large extent be explained by differences in the concentrations of organic carbon in the soil, as the leaching decreases and the K_f coefficients increase with increasing levels of soil organic carbon. Similar K_{oc} values are also obtained for the Haslemoen silt loam surface layer and the Ullensaker silty clay loam surface layer (221 and 195, respectively). Differences in the flow pattern probably influence the sorption behaviour also, as the leaching of [^{14}C]atrazine through the silty clay loam columns from Ullensaker was large compared to the K_f coefficients for the same soil type (Fig. 4). Rapid movement through macropores in the soil from Ullensaker may have increased the leaching of atrazine through the columns. Thus, when water rapidly flows through macropores, batch experiments seem to overestimate the soils sorption capacity.

Table 5. Mass balance of [14C] tribenuron-methyl in lysimeter experiment and Freundlich sorption isotherms determined from batch experiments

	Lysimeter experiment			Batch experiment		
	14C leachate (%)	14C recovered from soil (%)	Total recovery (%)	Kf	1/n	r^2
Haslemoen						
0-24 cm	8±5	71±4	79±3	2.23	0.85	0.998
35-59 cm	76±19	16±17	91±28	0.83	0.79	0.996
70-91 cm	78±11	12±9	90±2	0.46	0.66	0.999
Ullensaker						
0-24cm	7±4	80±3	87±0.3	2.25	0.80	0.996

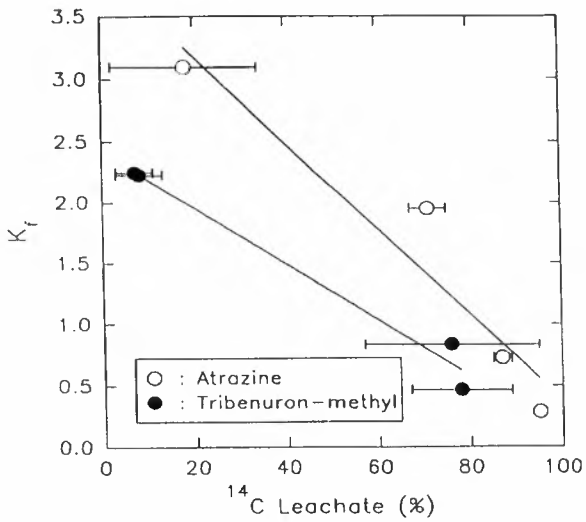

Fig. 4. Relationship between Freundlich sorption coefficients K_f and the percentage amount og [^{14}C] pesticide transported through the columns

The high retention of tribenuron-methyl in the soil columns implies that the K_f values should be higher than those obtained from the batch experiments. The $CaCl_2$ solutions added to the soil in the batch experiments may have an influence on the pH of the soil surface by taking part in ion exchange processes. Consequently, since the sorption of tribenuron-methyl is very pH dependent (Riise *et al.* this volume), an increase in soil surface pH will decrease the sorption of tribenuron-methyl. Thus, deviations in the results obtained from the lysimeter experiments compared with the results from the batch experiments can be attributed to differences in soil surface pH.

SUMMARY

The vertical movement of tribenuron-methyl was slower than that for atrazine in all soil types studied. The mobility of tribenuron-methyl was especially low in the silty clay loam.

The vertical movement of both atrazine and tribenuron-methyl was higher in subsurface layers compared with that in surface layers with higher content of organic carbon and higher cation exchange capacity. Atrazine was particularly mobile in the fine sand subsurface layer, where the leaching curves for water and atrazine were almost overlapping.

Results from batch and lysimeter experiments were in fair agreement. However, in silty clay loam columns, where a major part of the flow drained through macropores, the batch experiment seemed to overestimate the sorption capacity of the soil to some extent. Furthermore, for pesticides whose sorption behaviour is very pH dependent, deviation in results among batch and lysimeter experiments can be attributed to differences in pH at the soil surface.

ACKNOWLEDGEMENTS

The authors express their thanks to the personnel at the Isotope Laboratory, Agricultural University of Norway for assistance with ^{14}C and ^{3}H measurements and their gratitude to the following companies for providing [^{14}C] pesticides: CIBA GEIGY and Du Pont. G.R. thanks the Norwegian Research Council for Science and Humanities for a Research Fellowship.

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Leaching and surface transportation of pesticides in lysimeter experiments and runoff plots

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Uhlen, G., O. M. Eklo & O. Lode 1994. Leaching and surface transportation of pesticides in lysimeter experiments and runoff plots. Norwegian Journal of Agricultural Sciences. Supplement No. 13:43-51. ISSN 0802-1600

The pesticides MCPA, dichlorprop, dimethoate and propiconazole were applied at normal rates and time for spring grain. Four soil types were used in 550-1 lysimeter cylinders and given different treatments. Surface runoff was investigated in 20 m long plots on 4% slope. In the first years 1988-90, MCPA, dichlorprop and dimethoate were detected in only a few cases and in very small quantities in drainage samples, but it was found that there were somewhat higher concentrations of propiconazole, particularly in surface runoffs. A rainy period following the spraying in June 1991 resulted in some drainage and leakage of pesticides. Dichlorprop and less MCPA were found in drainage samples from sandy soils. In clay soils, however, the leaching of dichlorprop was delayed to a second runoff episode in September-October. Leaching of dimethoate and propiconazole occurred in the first period for the clay soils. Dichlorprop and propiconazole were found in all samples of surface runoff in February 1992 after spraying in 1991 and earlier. Despite a dry period during the first three weeks after spraying in June 1992, the results from the lysimeters in autumn 1992 were almost identical to those after the 1991 spraying. Dichlorprop was detected in most of the lysimeter water samples taken from a silty clay soil. The proportion of added dichlorprop leached in the lysimeter was in both years no more than 0.1%, and the proportion of dimethoate and propiconazole was in some cases about 1%.

Key words: Dichlorprop, dimethoate, MCPA, propiconazole, sandy and clay soils, water regimes.

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Investigations on the transport of pesticides were carried out in lysimeters and runoff plots at the Department of Soil Sciences, Agricultural University of Norway. The application of pesticides was performed by the Norwegian Plant Protection Institute and the pesticide analysis at the Department of Pesticide Analysis.

MATERIAL AND METHODS

Lysimeter experiments

Seventy-two weighing cylinders, 110 cm deep and 80 cm in diameter, were placed outdoors above a under-ground cellar (Uhlen *et al*. 1992). The soils were either removed as undisturbed soil monoliths or filled layer by layer in the cylinders. Four soil types from agricultural areas

in Norway were used:

A. Sandy loam morainic soil: 14 % clay at a depth of 0-20 cm and less than 10% in the deeper layers. 34-40% silt.

B. Silty clay: 20% clay and 55% silt in the upper layers, increasing to 23% clay and 67 % silt at a 50-100 cm depth.

L. Sandy soil: Only 3-4% clay and decreasing silt (26-4%) and increasing sand (70-94%) content in the deeper layers.

Ø. Clay soil: 32% clay and 44 % silt at 0-20 cm depth, and 50% clay at 50-100 cm.

Organic matter content was high (5-7 %) at a depth of 0-20 cm for all four soils. Ca saturation was high in the topsoil of A and L, and Ca and Mg were especially high in the subsoil of B and Ø. Physical available water (pF2-4,2) at 0-100 cm was 140 mm for the sandy soil (L) and 180-200 for the A, B and Ø soils.

Two types of water regimes were investigated:

Water regime 1: The cylinders were protected from precipitation from January to March. No irrigation.

Water regime 2: No protection in the winter months and, in addition, 100 mm water was applied in five doses in June to July.

The four fertilizer treatments were: three levels (a, b, c) of fertilizer, 30-95-160 kg N per hectare (+ P and K), and *d*, farm manure treatment, 40 t per year, applied in either autumn or spring.

The cylinders were sown with barley in 1990, 1991 and 1992. Four cylinders of soil B were kept without plants and fertilizers, and two of the four were kept indoors in the cellar. The remainder were exposed to normal freezing in winter.

Spraying treatments in 1990, 1991 and 1992 were:

MCPA, herbicide	425	g/hectare	of	active ingredient
Dichlorprop, herbicide	1250	"	"	"
Dimethoate, insecticide	300	"	"	"
Propiconazole, fungicide	125	"	"	"

In 1989, only herbicides were applied. The spraying was carried out in the first or second week of June each year, and four sylinders were left as controls without spraying treatments.

Field lysimeter for surface runoff measurements:

The field lysimeter was built in 1973 for measuring surface runoff after different crops, fertilizers and soil cultivations (Uhlen 1989). The plots measured 4 m x 20 m and with 4% slope. In autumn 1988 propiconazole was applied to winter wheat.The same treatment + dimethoate was given in June or July in 1989. In the subsequent years, 1990, 1991 and 1992,

the spraying took place in June and with the same mixture of the four pesticides on spring grain as that given above for the lysimeter cell experiment.

Sampling
Samples of leachates or surface water runoffs were taken at first runoff after spraying in 1-1 dark glass bottles and brought to the laboratory for pesticide analysis. Composite samples for autumn and for winter runoffs, which were collected in large plastic cans in the cellar, were also analysed.

Chemical analysis
The analytical procedures are described in the paper of Holen & Svensen (This volume). Detection limits were 0,05 ppb for herbicides in water samples, and 0,2 for dimethoate and propiconazole. From 1991 detection limits are 0,05 ppb also for dimethoate and propiconazole.

RESULTS

Pesticides in drainage and surface water after spraying in 1988-90

Because MCPA, dichlorprop and dimethoate were detected in only a few cases, and in rather small quantities, a summary of the results for the first two to three year only will be given in Table 1. Propiconazole was found in higher concentrations in surface waters compared to the other pesticides.

Table 1. Pesticides in lysimeter and surface runoff water 1988-91. Concentration in ppb

Pesticide	No. of samples analysed	No. above detection limits	Max. concentration
Lysimeter water:			
MCPA	78	3	0.29
Dichlorprop	78	0	
Dimethoate	41	2	0.5
Propiconazole	41	7	0.4
Surface runoff water:			
MCPA 1990	7	2	0.23
Dichlorprop 1990	7	1	0.29
Dimethoate 1989-91	26	0	
Propiconazole 1988/89	35	35	5.7
" 1989/90	22	11	1.4
" 1990/91	19	14	4.7

The high concentrations of propiconazole in the first runoffs in autumn 1988 and in 1990 represent only small quantities of surface water, and might be influenced by direct contamination in the form of wind drift to the water collecting gutter. However, a relatively high concentration of propiconazole was found in some surface water samples containing visible content of clay particles, indicating the presence of pesticides associated with particles.

Leaching in lysimeters after the spraying in 1991

After spraying on 12.June 1991, a period with a rainfall totalling 60 mm resulted in some drainage water and apparently also a downward transport of pesticides. Drainage runoff occurred especially from cylinders with reduced plant growth, fertilizer treatment *a*, from monoliths with additional irrigation and from cylinders without plants.

For the sandy soil, L, MCPA was detected in three out of six samples and dichlorprop in all six samples, whereas the herbicides were not found in leachate from the clay soils B and Ø (Table 2).

Table 2. Pesticides in lysimeter water after spraying on 12 June 1991

1. runoff 12 June to 1 July

		MCPA		Dichlorprop		Dimethoate		Propiconazole	
	mm	n	x	n	x	n	x	n	x
L. Sandy soil	17	3/6	0.10	6/6 c	0.29	4/6 a	0.33	0/6 a	-
A. Sandy loam	21	0/6	-	3/6 b	0.10	4/6 a	2.3	3/6 a	0.11
B. Silty clay	18	0/7	-	0/7 a	-	7/9 b	7.8	7/9 b	0.75
Ø. Clay soil	27	0/5	-	0/5 a	-	5/7 a	2.5	1/7 a	0.30

2. runoff 20 September to12 October *

		MCPA		Dichlorprop		Dimethoate		Propiconazole	
	mm	n	x	n	x	n	x	n	x
L.Sandy soil	62	0/7	-	3/9 a	0.08	0/9 a	-	0/9 a	
A.Sandy loam	37	0/7	-	3/7 a	0.14	0/7 a	-	0.7 a	-
B.Silty clay	37	0/11	0,07	9/11b	0.42	5/11b	0.21	5/11b	0.55
Ø.Clay soil	40	0/10	-	9/10b	0.16	1/10a	0.13	2/10a	0.12

n = Proportion of samples with higher content than detection limits (0.05 ppb)
x = Mean content in ppb (for samples > detection limits only) a, b and c: Different letters indicate significant differences between soil types

In the next runoff events, after heavy rain in September to October, a significantly higher concentration of dichlorprop was found in the runoff from the clay soils than that from the sandy soil (L) and the sandy loam soil (A).

For both period the concentrations of dimethoate and propiconazole were highest in leachates from the silty clay soil B, and were practically not present at all in the sandy soil (L) leachate. The results of a simple t-test between soil types, in accordance with Snedecor & Cochran (1956), are indicated by the small letters a, b and c in Table 2. It should be noted that in the mean content in ppb, only samples with values above the detection limits are included. In testing differences between soil types, however, all samples were used, and the samples below detection limit was set to 1/2 of 0.05 ppb. The test of significance ($P < 0.05$), therefore, does not refer directly to the mean (x values) for the soils in the tables.

The highest concentrations of dichlorprop were 0,17, 0.96 and 0.55 ppb for soils A, B and Ø, respectively, in the second runoff period, whereas the concentration reached 0.67 ppb for soil L in the first period.

The concentrations of dimethoate reached 4,6 ppb in the first runoff period in leachate from a cylinder of soil A, 6.5 in soil Ø and 15.5 ppb in soil B, and the highest level of propiconazole occurred in a sample from soil B without plants (2.2 ppb).

In composite samples from the entire period, 12. June to 31. December 1991, analyses of pesticides in water were carried out for 30 samples. The results are shown below:

Dichlorprop 10 out of 30 samples. Mean (x) 0.10 ppb
Dimethoate 9 out of 28 samples. Mean (x) 0.38 ppb

None of the composite samples had concentrations of MCPA or propiconazole above the detection limits. The amount of leaching water in the whole period varied from 210 to 460 mm (influenced by irrigation and water use). The pesticides from the early part of the period were diluted considerably. Good agreement was found between total amounts in leachates in the first two periods and the content in the composite samples for the whole period. Nevertheless, breakdown of pesticides during the long storage in the cellar cannot be completely ruled out.

Some of the samples from a leaching period in March to April in 1992 were also examined. Again, dichlorprop, but not the other pesticides, was detected in leachates from cylinders exposed to frost and thawing, (see Table 3).

For samples represented in the period September to October 1991 and March to April 1992 a significant correlation (r= +0.67***) existed between the concentration of dichlorprop in the two seasons. The amount of leachate was 24-86 mm in September to October compared with 140-200 mm in March to April.

In relation to the amounts of dichlorprop applied in June 1991, the amounts in the leachates for the whole year from June 1991 to April 1992, are rather small, and only in three to four cases did they exceed 0.1%.

For some cylinders with the silty clay soil the total leakages of dimethoate from June through October 1991 reached 1% of the added amount.

Table 3. Dichlorprop (ppb) in lysimeter water March-April 1992

Soil	No. of samples = cylinders	No. above detection limits	Concentrations
L. Sandy soil	7	3	0.08, 0.19, 0.52
A. Sandy loam	4	2	0.07, 0.49
B. Silty clay	8	7	0.10-0.96, mean 0.44
Ø. Clay soil	3	2	0.07, 0.5

Leaching in lysimeters after the spraying in 1992

In the first three weeks after spraying, only a few millimeters of rain fell in June 1992. High rainfall in July to August, totalling 235 mm, might have caused downward transportation of residual pesticides in the soil.

The leaching behaviour of the pesticides in 1992 (Table 4) was rather similar to the results in autumn 1991 (Table 2). Dichlorprop was found in more samples than was the case for MCPA, and similar to the previous year, the silty clay soil, B, displayed higher leaching figures than the other soils. For both years, dimethoate and propiconazole were more frequently in

leachates from the clay soil than those from sandy soils.

The amounts added pesticides leached of in the 340 mm runoff in 1992 were for the herbicides less than 0.1% and for propiconazole in some samples about 1%.

Four cylinders of soil B, silty clay, were kept without plants and two of these were placed in the cellar. They were given rather rough watering and sometimes some odd leaching figures were obtained. The propiconazole figures for the indoor cylinders were omitted from the test of significance in Table 4. A comparison between all cylinders with plants of soil B and the four cylinders without plants for the whole autumn period in 1992 showed a higher mean concentration without plants for propiconazole and MCPA, and the opposite result for dichlorprop and dimethoate.

Table 4. Pesticides in lysimeter water after spraying on 12 June 1992

First runoffs, 15 August to 20 October

	mm runoff	MCPA n	MCPA x	Dichlorprop n	Dichlorprop x	Dimethoate n	Dimethoate x	Propiconazole n	Propiconazole x
L. Sandy soil	41	1/9 a	0.06	7/9 a	0.12	1/9a	0.48	3/9 a	0.11
A. Sandy loam	42	1/4	0.13	4/4	0.12	1/4a	0.07	1/4	0.14
B. Silty-clay	51	7/9b	0.11	9/9c	0.54	8/9a	0.26	4/8b	0.40*
								5/9	0.64
Ø. Clay soil	48	0/8a	-	8/8b	0.22	6/8a	0.21	5/8b	0.18

Average runoff samples, 15 August to 22 October 1992

	mm runoff	MCPA n	MCPA x	Dichlorprop n	Dichlorprop x	Dimethoate n	Dimethoate x	Propiconazole n	Propiconazole x
L.Sandy soil	283	1/10a	0.06	2/10 a	0.08	0/10 a	-	0/10 a	-
A. Sandy loam	290	3/4	0.15	1/4	0.07	0/4	-	0/4	
B. Silty clay	321	4/10	0.16	9/10b	0.20	6/10b	0,29	4,8b	0.23*
								6/10	0.85
Ø. Clay soil	285	2/8a	0.06	3/8a	0.10	1/8a	0,15	1/8ab	0.30

n = Proportion of samples with higher content than detection limits (0,05 ppb)
x = Mean content in ppb (for samples > detection limits only)
* Results from indoor cylinders omitted from the test of significance

Surface runoffs 1991-1992

No surface runoff, overland flow, occurred in summer or the autumn of 1991. The only surface runoff period in the 1991/92 season took place in February 1992. The amount of surface runoff was relatively small (Table 5). Thus, on average over a 10-year period, 1972-81, Uhlen (1989) measured about 150 mm surface runoffs per year for these 13 plots.

The concentrations of MCPA, dichlorprop and propiconazole in the surface water in February 1992 are shown in Table 5. Dimethoate was not detected. The concentration of MCPA was only slightly higher than the detection limit, whereas dichlorprop was found in

samples from all sprayed plots. In earlier years, dichlorprop was found only in a single sample in 1990, and it is somewhat surprising that the dichlorprop added in June 1991 to spring grain was not completely broken down during late summer and autumn. Rapid infiltration in the rainy period and preservation in the soil layer is not a plausible explanation for herbicides in the overland flow samples. For some reason breakdown also of surface applied dichlorprop has been markedly delayed.

Ploughing in late autumn reduced the volume of surface water and with the exception of plot no. 2, the concentrations of propiconazole were reduced as well. It should be born in mind that the runoff losses of dichlorprop are very small in relation to the amount added, less than 0.01%.

Despite the fact that plot nos. 1 and 10 were not sprayed in 1991, some propiconazol was detected in the surface runoff from these two plots in February 1992 (Table 5). The plots were, sprayed in 1990 and in earlier years however.

In these plots, surface runoff of propiconazole has occurred in the earlier years as well. Thus, after spraying in the autumn of 1988, up to 0.8 % of the added propiconazole was found in surface runoff .

Table 5. Pesticides in surface water February 1992 Concentration in ppb

Plot no.	mm	Propic-onazole	MCPA	Dichl-orprop	Spraying
13	29	nd	nd	nd	No spraying
1	24	0.24	nd	nd	Sprayed 1990
10	12 fpl	0.23	nd	nd	Sprayed 1990
2	5 "	1.18	0,09	1.02	Sprayed 1991 and 1990
3	9 "	0.71	nd	0.11	Sprayed 1991 and 1990
4	24	0.52	0.08	0.32	Sprayed 1991 and 1990
6	25	1.44	0.07	0.36	Sprayed 1991 and 1990
8	12 fpl	0.91	nd	0.21	Sprayed 1991 and 1990
9	27	1.49	nd	0.24	Sprayed 1991 and 1990
11	7	1.68	0.05	0.47	Sprayed 1991 and 1990
12	3 fpl	0.93	nd	0.11	Sprayed 1991 and 1990

fpl = Ploughed in autumn 1991. The rest spring ploughed
nd = Pesticides not detected (< 0.05 ppb)

DISCUSSION AND CONCLUSION

In the first two years of this lysimeter study only trace amounts of pesticides were detected in leaching water. In surface water from runoff field plots the fungicide propiconazole was found.

Conditions favouring rapid downward transport and leaching occurred in 1991 where the spraying on 12. June was followed by a rainy period. In 1992 a rainy period occurred three weeks after the addition of pesticides. Transportation below topsoil layers may have protected the pesticides from a rapid and complete breakdown. This seems to have been the case, especially for the herbicide dichlorprop in the clay soils. Soil B, a silty clay, appeared to give more pesticide leakages than the other soils. This soil has a dense structure, without cracks and larger pores, and possibly also reduced aeration in the deeper layers.

Dichlorprop was found in leachate from sandy soils and not from the clay soils in the first runoff in 1991, whereas more dichlorprop was detected in leachate from the clay soil than from

sandy soils after the growth season (September to October 1991). Even in April to March the next year, dichlorprop was detected in 14 out of 22 leachate samples from the four soils.

The herbicide MCPA was detected in only a few samples in 1991 and in one-third of the samples in 1992. MCPA was applied at a rate of about one-third of the quantity used for dichlorprop. However, this fact cannot explain the different behaviour of the two pesticides. Lode *et al*. (1991) found a more rapid leaching of MCPA than that of dichlorprop in a clay soil, whereas in a Swedish investigation (Kreuger 1985) dichlorprop, but not MCPA, was found in drainage water from a clay soil and MCPA and dichlorprop were both found in sandy soil drainage.

The breakdown of the herbicides in laboratory incubation is rapid. Ghorayshi & Bergstrøm (1991) refer a half-life of dichlorprop of only 10 days. In an investigation of the adsorption of dichlorprop in Swedish soils they found no significant correlation between adsorption coefficients and organic matter or clay content.However, a much higher adsorption coefficient was found in a subsoil horizon of sandy loam with extremely low pH (3,7). Of the four soils used in the lysimeter study the pH values were in the range 5,6-7 in the different layers. The topsoil of the two clay soils, was limed at the start of experiment.

The somewhat delayed leaching of dichlorprop can partly be explained as a delayed solution transport in clay soil compared to the sandy soil. The sandy soil (L) has a lower water content, available for capillary transports, wheras the sandy loam (A) did not have volume percentage of water in the pF range 2 to 4.2 any lower than that of the clay soil. Furthermore, it should be noted that the leaching of dimethoate and propiconazole was not delayed and was highest in the silty clay loam in both years. In the sandy soil (L), and the sandy loam (A) breakdown of these two pesticides was more rapid and, execpt for in the early periods, no leaching was apparent.

The transport of water in the soil is also a key factor in leakages of pesticides, even if modified by soil chemical and biological processes. In the lysimeter experiments pesticides were more frequently detected in drainage from cylinders with additional watering, low fertilizer rate and also more frequent from monoliths of clay soils than from filled cylinders. The clay soils are more liable to shrinkage, making it possible for the passage of water through cracks, as well as larger pores. It is possible, but has not been proved in this investigation, that propiconazole might be transported in association with particles.

In a large proportion of the water samples from the surface runoff investigation, propiconazole was found and, in the period 1991-1992, also dichlorprop. Propiconazole seems to be more persistent in topsoil than the other three pesticides. It should be borne in mind that the amounts found in leachates, as well as in overland flow, are very tiny in relation to the amount added by spraying - for dichlorprop, less than 0.01 % in surface runoff and 0.1 % in drainage. Therefore, transfer processes other than through the aqueous phase of the soil media and movement over the soil surface could be involved. According to Weber (1991) such processes may be exudation by crop residues and also vapour-phase diffusion through soil pores and the atmosphere.

In this study it was found that the effect of plants was not consistent. Pesticide leakages from soil without plants are found to be higher than those from soil with plant cover (Lode *et al.* 1991). A proportion of the sprayed pesticide will be withheld and absorbed by the leaves, and without plants less water usage will result in more leachate and sometimes earlier runoff as well.

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Runoff and leaching experiments of dichlorprop, MCPA, propiconazole, dimethoate and chlorsulfuron in outdoor lysimeters and field catchment areas

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Eklo, O.M., R. Aspmo & O. Lode 1994. Runoff and leaching experiments of dichlorprop, MCPA, propiconazole, dimethoate and chlorsulfuron in outdoor lysimeters and field catchment areas. Norwegian Journal of Agricultural Sciences. Supplement No. 13: 53-78. ISSN 0802-1600.

The mobility of the pesticides MCPA and dichlorprop, propiconazole, dimethoate and chlorsulfuron[1)] was studied in two types of field experiment: (1) leaching from a field lysimeter at Syverud, and (2) runoff and leaching from parts of areas in conventional use at Ullensaker. The runoff pattern of chlorsulfuron is described after one year of monitoring. Studies carried out over several years on leaching and runoff give an account of the fate of the phenoxy-acids, dimethoate and propiconazole after application. Results from the field lysimeter experiments revealed that leaching was at its most extensive from plots without vegetation receiving the highest amounts of fertilizer. From plots with permanent grass, the leaching of pesticides was generally delayed. The typical runoff pattern from the fields at Ullensaker was found to be one peak during autumn with highest concentrations at the runoff events close to the application, and one peak in spring during snowmelt. The highest concentrations were measured for propiconazole at 9.7 µg/l, while the most persistent compound was dichlorprop, which occurred in the runoff in May, ten months after application. In relation to the low soil-water distribution coefficients and the usually rapid degradation rate for the phenoxy-acids, these late occurrences were unexpected. Extensive flow through macropores was confirmed for the silty clay loam from Ullensaker.

[1)]only in catchment areas.

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The aim of this study was to measure the leaching from small field lysimeter plots and leaching and runoff from areas in conventional use. These monitoring data were expected to confirm the reliability and the usefulness of the parameters and results obtained in the laboratory experiment with the same soil (Riise *et al.*, this volume).

The monitoring data were also to provide information about the effect of different soil management practices, the effects of different crops and ground cover on pesticide leaching, and indicate the concentration levels to be used in biological experiments to ensure that they were seing carried out with realistic concentrations appearing in the field.

Pesticides from each of the three main groups - herbicides (3), fungicide (1) and insecticide (1) - were used. The criteria for the selection are described by Lode (this volume).

MATERIALS AND METHODS

The equipment for the field studies was implemented at two different locations. The field lysimeter experiment was carried out at Syverud, Ås, 30 km south of Oslo, and the runoff studies from three small catchment areas in conventional use at Ullensaker, 30 km north of Oslo.

Field lysimeter studies at Syverud

Site description

The experimental area covered 30.2 m x 38.2 m = 1153.64 m^2 and was established by the Agricultural University of Norway in 1977. The experimental area comprised 36 different plots, each covering 6 m^2. Along the border of each plot the upper 1.30 m soil was removed and the bottom was packed with clay and the walls were covered with PVC foil (Fig. 1). A drainage system from each plot was established. The water was chanelled through separate drainpipes from each plot into PVC vessels (175 dm^3) in an excavated cellar. The texture of the soil is classified as silty clay and clay.

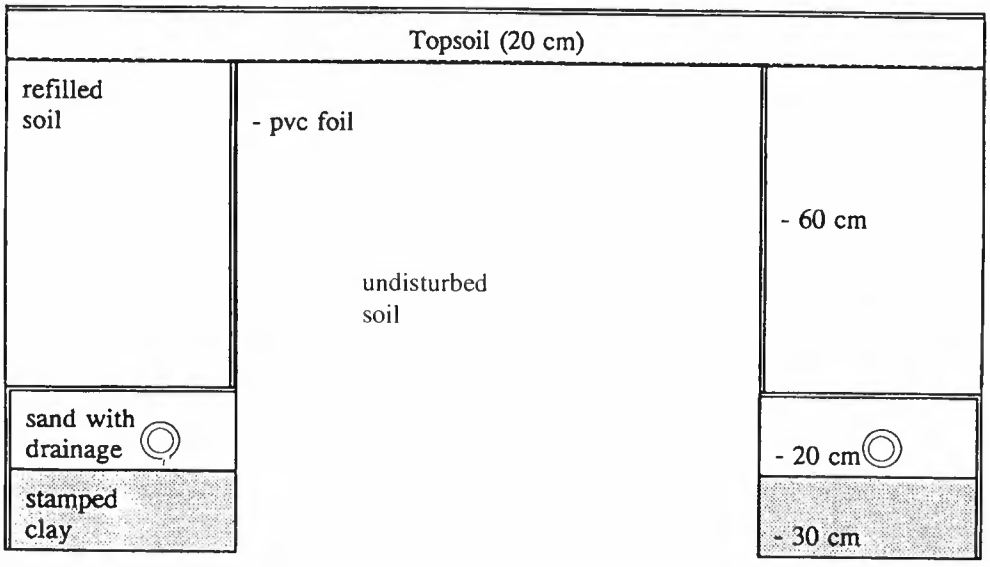

Fig. 1. Sectional drawing of a plot from Syverud (Bjerve 1981)

Climate

Average temperature, precipitation, and measurements for the two years are described in Table 1.

Table 1. Precipitation and temperature at Ås for the years 1990 and 1991

	Precipitation, mm			Temperature, °C		
Month	1990	1991	Normal	1990	1991	Normal
January	94	74	55	1.3	-3.7	-5.2
February	138	23	34	3.6	-4.7	-4.6
March	20	70	27	4.6	1.5	1.2
April	72	21	48	6.4	5.0	4.3
May	11	0	49	11.8	10.3	10.2
June	94	93	70	14.6	11.3	14.4
July	62	55	79	16.0	17.1	16.8
August	96	30	96	15.7	16.2	15.6
September	53	79	86	9.9	11.2	10.9
Oktober	99	105	86	6.0	6.1	5.7
Nowember	43	162	83	-1.0	2.6	0.9
December	102	29	72	-1.3	0.3	-2.3

Treatment

Two concentration levels of fertilizer were used, 300 and 600 kg/ha NPK fertilizer, 21-4-10 respectively. The pesticides dimethoate, propiconazole, MCPA and dichlorprop were applied at the following concentrations: 0.300, 0.125, 0.425 and 1.250 kg/ha (Table 2). One-third of the plots was seeded with barley (Pernilla), one-third was covered with permanent grass and one-third was left free from vegetation. The whole area was conventionally tilled and harrowed in the spring. The experiment was run in triplicate.

Table 2. Pesticides used in lysimeter and field experiment during 1988-1991

Pesticide	Type	Commercial name	Dose (g/ha)	Active ingredient
chlorsulfuron	herbicide	Glean		750 mg/g
MCPA	herbicide	FK-kombi	425	167 g/l
dichlorprop	herbicide	FK-kombi	1250	500 g/l
propiconazole	fungicide	Tilt	125	250 g/l
dimethoate	insecticide	Rogor	300	200 g/l

Sampling and measurements

The amount of drainage water from the plots was continuously measured and representative one litre samples were collected and analysed for pesticides. Samples from 1990 were analysed following the methods described by Eklo *et al.* (1991a), and analyses from 1991 were conducted in accordance with the methods described in Holen *et al.* (this volume).

Field studies at Ullensaker

Site description

The field experiments were carried out at three locations at Ullensaker. These sites were originally established by GEFO (Institute for Georesources and Pollution Research) in 1985 in order to study the nutrient runoff and effect of different land-use practices (Øygarden 1989). From 1988 to 1992 pesticides in runoff and drainage water were monitored for the selected pesticides used in the areas. The areas consisted of depositions of marine sediment with ravines. To increase the area available for agricultural practice, the steep slopes of the ravines were levelled by bulldozer in the 1970s. Since the topsoil was removed, the upper clay layer has been exposed to surface erosion.

Field 1 (Fig. 2)

This was a long and extended area measuring 0.86 ha representing a smaller part of a field. Clay, clay loam and silty clay loam were the predominant soil textures, classified as Orthic Gleysol. The physical and chemical properities of this soil are described in Table 3. The maximum slope length was 155 m with a 4% inclination. From the short sides across the main slope length the inclination was 7.7-8.5%.

Table 3. Physical and chemical properties of soil from Ullensaker, Norway

Experimental site	Size distribution (%)						
	Sand	Silt	Clay	pH	Organic C (%)	CEC MEQ/ 100g	Base sat. (%)
Field 1	5	63	33	5.1	1.3	20	54

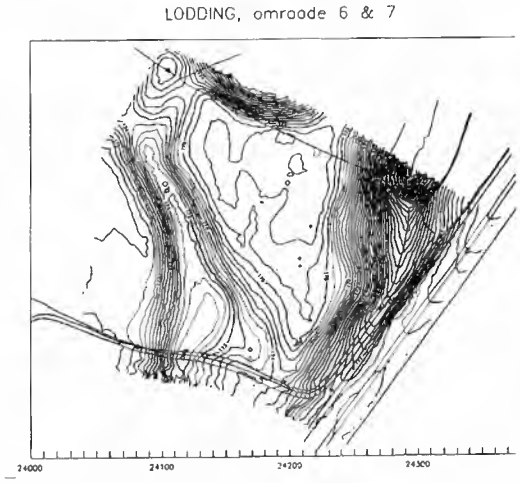

Fig. 2. Map showing the catchment area of field 1 (omraade 6 & 7), Ullensaker

Field 2 (Fig. 3)

The size of this area was 3.2 ha, also representing a larger field. Clay, clay loam and loam were the predominant soil textures and the soil was classified as Orthic Gleysol. The topography of the area comprised a mixture of ridges and small slopes. At the end (northern) and through the central part of the area the inclination was 3-6%, but at the southern end the inclination was 14%. The maximum length of the slope was 175 m and the maximum height difference was 12 m.

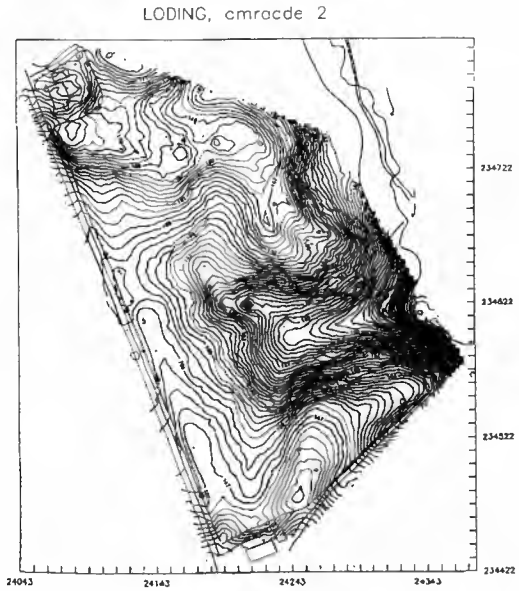

Fig. 3. Map showing the catchment area of field 2 (omraade 2), Ullensaker

Field 3 (Fig. 4)
The area was 0.44 ha in size. Silty clay loam was the prevailing texture and the soil mixture was classified as Orthic Gleysol and Gleyed, Sombric Brunisol. The area had an elongated shape with steep inclinations down to a slope running along the area. The maximum length along the main slope was 113 m and the inclination was 6%, while from the short sides the inclination was 16% with a maximum length of 25 m.

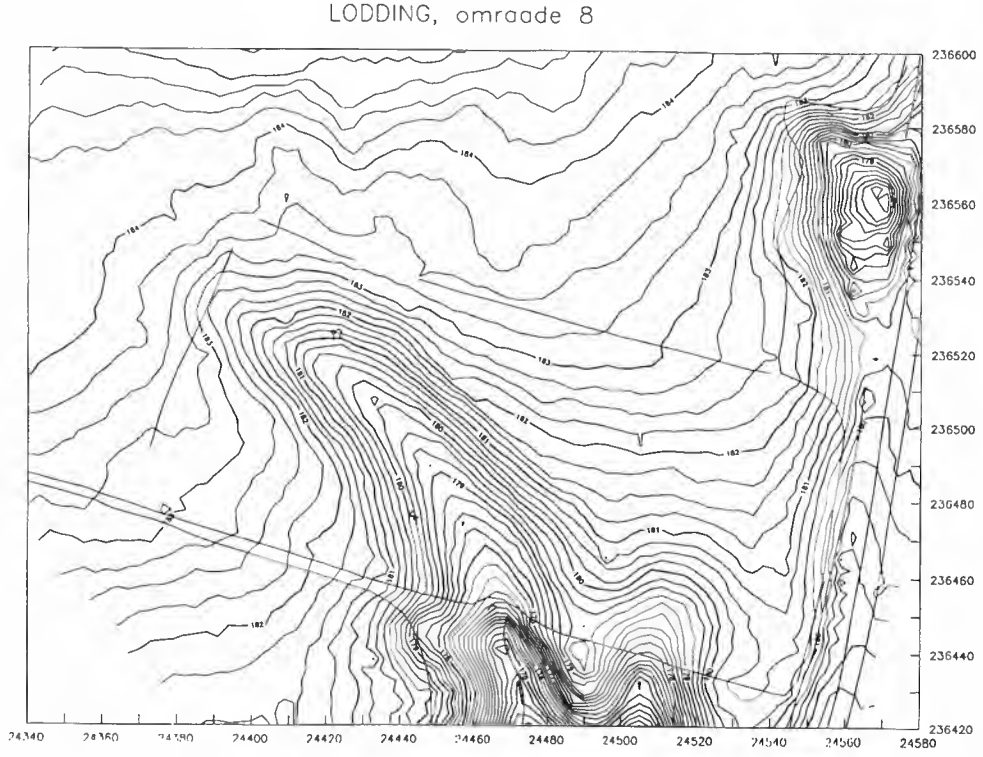

Fig. 4. Map showing the catchment area of field 3 (omraade 8), Ullensaker

Climate
Tables 4 and 5 give the normal precipitation and temperature at Gardermoen for the period 1931-60 and the years 1988-92. During 1988-92, the temperature was high in winter with heavy rainfall in the spring compared to the normal.

Table 4. Precipitation at Gardemoen for the period 1931 - 1960 (normal period) and the years 1988 - 1992

	Precipitation, mm					
Month	1988	1989	1990	1991	1992	Normal
January	135	14	111	77	58	58
February	115	84	113	27	24	41
March	123	81	20	63	69	30
April	53	83	81	25	70	50
May	31	35	9	7	54	53
June	60	70	81	137	10	79
July	141	54	57	43	106	93
August	150	158	87	75	106	95
September	13	158	87	64	72	87
October	79	51	67	102	50	85
Nowember	31	73	41	124	176	82
December	37	52	86	32	66	73

Table 5. Temperature at Gardemoen for the period 1931 - 1960 (normal period) and the years 1988 - 1992

	Temperature, °C					
Month	1988	1989	1990	1991	1992	Normal
January	-0.7	0.7	-0.9	-5.6	-2.3	-6.9
February	-3.1	1.5	2.0	-6.7	-2.0	-6.3
March	-3.4	2.1	3.4	0.3	1.4	-2.3
April	1.8	3.8	4.9	4.2	2.8	3.2
May	10.7	10.5	11.6	9.5	12.6	9.4
June	17.7	14.2	14.2	10.0	16.8	13.6
July	15.5	15.5	15.5	17.3	15.4	16.0
August	13.9	15.1	15.1	15.3	12.9	14.7
September	11.1	10.8	8.9	9.7	9.4	10.0
October	3.6	3.9	4.8	4.6	1.3	4.5
Nowember	-3.7	1.0	-2.1	0.6	-1.0	-0.6
December	-5.7	-5.5	-3.2	-1.6	-2.4	-3.9

Treatments and management

All three fields have been cultivated for cereals (small grain). The cereals and soil management practices varied from one year to another. An overview of soil management, crops cultivated and pesticide treatment during the project period is presented in Table 6.

Sampling and measurements

A monitoring station was built in order to collect and register the amount of surface runoff from each of the fields. In addition to surface runoff, field 1 had a separate station for collecting drainage water. From all the stations, representative samples proportional to the waterflow for each runoff period were collected. A principle outline of the sampling station can be seen in Fig. 5. The monitoring station was located on a slope at the edge of the area. The water was led into an overflow pond via an inlet pipe. The water level of the pond was registered by a limnigraph to measure the waterflow. From the overflow pond the water flowed out through two V-notches, a small one and a larger one. Ten percent of all the water passed through the

small outlet into a sampler, collecting a mixed sample representing the precipitation event. After each event, the sampler was emptied.

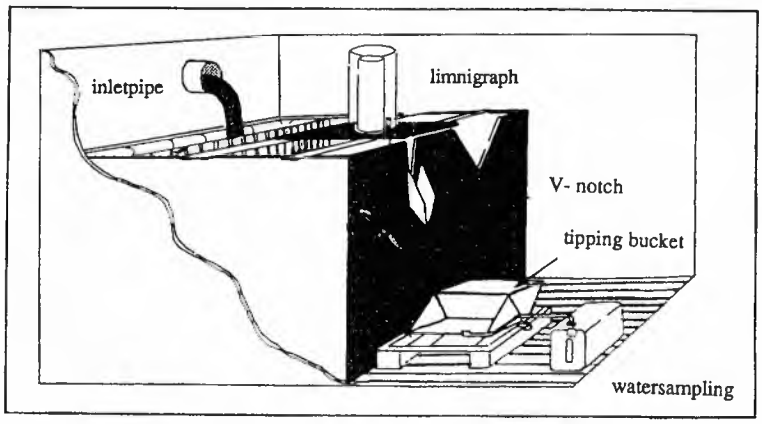

Fig. 5. Principle outline of the monitoring stations at Ullensaker

Table 6. Soil management, crops and pesticide treatment of the different fields of Ullensaker

Field, year	Soil management, spring	Crop	Pesticide treatment	Soil management, autumn
1988				
1	dragged? harrowed	barley	chlorculfuron 31/5 propiconazole 24/6,4/7	tilled 13/10
2	dragged harrowed	wheat	MCPA, dichlorprop 7/6	tilled - not in the slopes
3	harrowed - not in the slopes	barley	MCPA, dichlorprop 10/6	tilled - not in the slopes
1989				
1	harrowed	barley	propiconazole 7/7	tilled oct.
2	harrowed	cereals	MCPA, dichlorprop 9/6	stubble harrowing
3	harrowed - not in the slopes	cereals	dimethoate 15/7	stubble harrowing
1990				
1	harrowed	oats	chlorsulfuron 30/5 propiconazole 1/7	tilled oct. lime
2	harrowed	barley	MCPA, dichlorprop 28/5	harrowed
3		winter wheat	MCPA, dichlorprop 7/5 propiconazole 20/5 11/6	tilled lime
1991				
1	harrowed 24/4	barley	MCPA, dichlorprop 28/6 propiconazole 10/7	tilled 20 oct
2		oats	MCPA, dichlorprop 17/6 1/7 dimetoat, propiconazole 1/7	no
3	harrowed 26/4	oats	MCPA, dichlorprop 2/7 propiconazole 12/7	tilled 8/10

The soil samples were analysed at the Department of Soil Science at the Agricultural University of Norway, while the pesticide analyses were conducted at the Pesticide Laboratory and at the Department of Herbology, both at the Norwegian Plant Protection Institute. Chlorsulfuron was analysed at National Laboratory for Agricultural Chemistry, Sweden.

RESULTS AND DISCUSSIONS

Syverud

In the first year, 1990, only dichlorprop and MCPA were found in the leachate from the plots, wheras dimethoate and propiconazole were not detected. The vegetation had a clear-cut effect on the pesticide leaching. Areas without vegetation showed the highest amount of leaching,

while areas with grass had almost no leaching (Figs. 6 & 7). This was evidently a secondary effect as the leaching of the two phenoxy-acids was closely associated with the amounts of water leached from the different vegetation types. Five months after application no more pesticides were detected. The amount of fertilizer applied did not influence the leaching of pesticides that year.

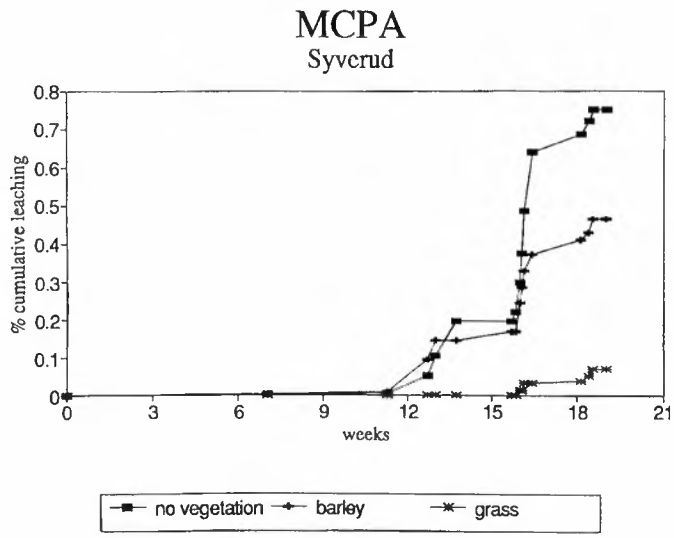

Fig. 6. Cumulative leaching of MCPA from plots with no vegetation, barley and grass, 1990/91 Syverud, Ås

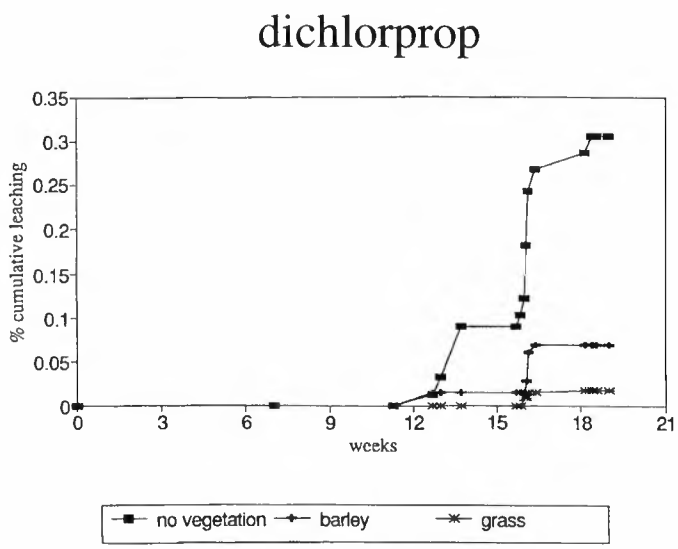

Fig. 7. Cumulative leaching of dichlorprop from plots wlith no vegetation, barley and grass, 1990/91 Syverud, Ås

During the year 1991/92 the same leaching pattern was observed in line with the differences between vegetation types as that for 1990, but because of the variability only the highest level of fertilizer without vegetation showed significantly more leaching than the others (Figs. 8, 9, 10 & 11). Two to three months after application, no more pesticides were detected in the leachate. In contrast to 1990, dichlorprop had the highest concentration of the two phenoxy-acids. This corresponds to the result from the field experiment at Ullensaker, where dichlorprop displayed the highest concentrations during the last year. The high concentration for dichlorprop coincided with a change in methodology (with respect to the analyses of the phenoxy-acids). It is uncertain whether this had an influence on the result.

For the year 1991/92 both dimethoate and propiconazole were found in the leachate. Dimethoate, which was thought to be the most mobile of the pesticides studied, showed the same leaching tendency according to the effects of the ground cover as the other pesticides studied. The frequencies of findings seem, however, to reflect the ratio between the different amounts of the pesticides applied. The amount of dimethoate applied was a quarter of that of dichlorprop, so, in order to get a good comparison the detection limit for dimethoate might be four times lower than that for dichlorprop. Propiconazole was thought to be the least mobile pesticide, and the delay in the leaching curve of that compound is in accordance with this assumption. Neither dimethoate nor propiconazole was found in the leachate from plots with grass. In most of the plots with grass, the leachate appeared at least two months later than for the other plots. This slow migration of the water is due to the uptake of water by the plants in plots with well-established grass cover. A vegetation ground cover may also have the opposite effect. Plants might make root channels, thereby causing fast preferential flow through macropores.

The total amount of dichlorprop leached is ten times higher than that for MCPA. This result is in accordance with the field experiment.

Plots without vegetation and with the highest amount of fertilizer had significantly more pesticide leaching than the other plots. Higher concentrations and more frequent findings were documented, although the amount of water from these plots was not significantly different.

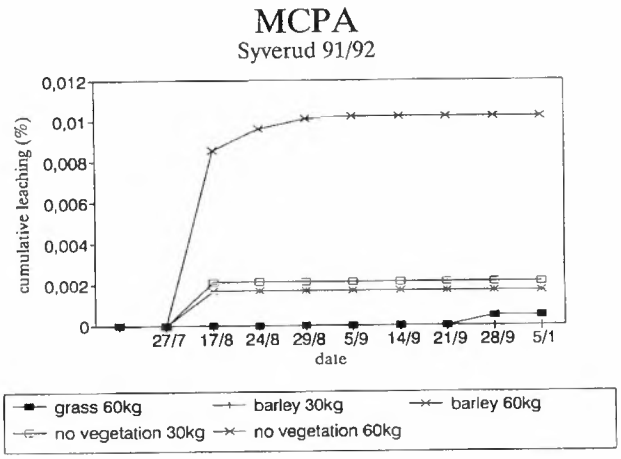

Fig. 8. Cumulative leaching of MCPA from plots with no vegetation, barley and grass, 1991/92 Syverud, Ås (n=3)

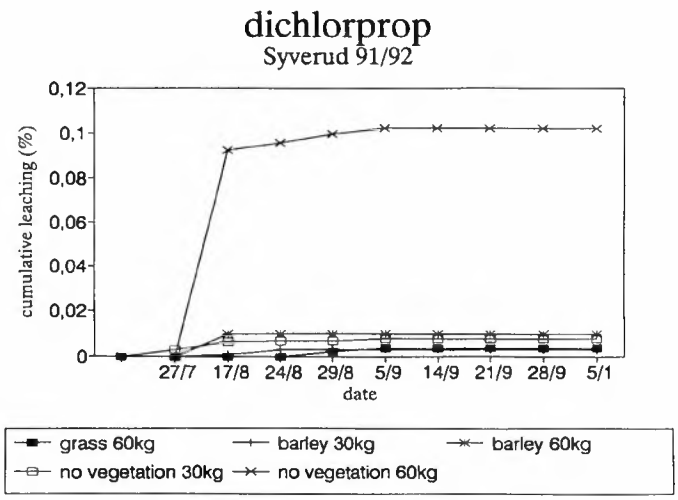

Fig. 9. Cumulative leaching of dichlorprop from plots with no vegetation, barley and grass, 1991/92 Syverud, Ås (n=3)

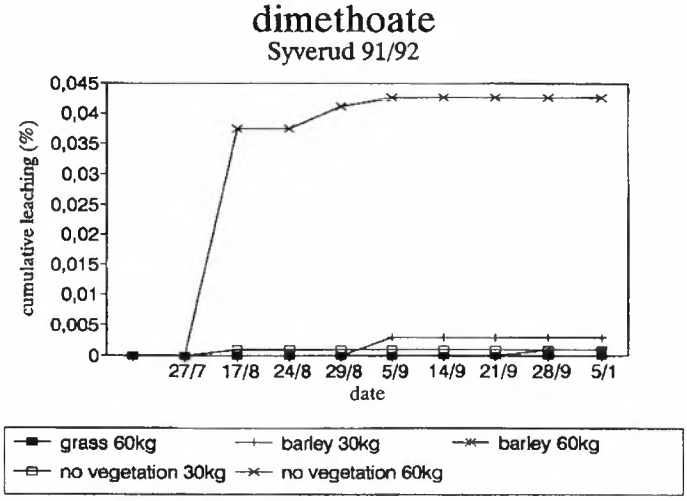

Fig. 10. Cumulative leaching of dimethoate from plots with no vegetation, barley and grass, 1991/92 Syverud, Ås (n=3)

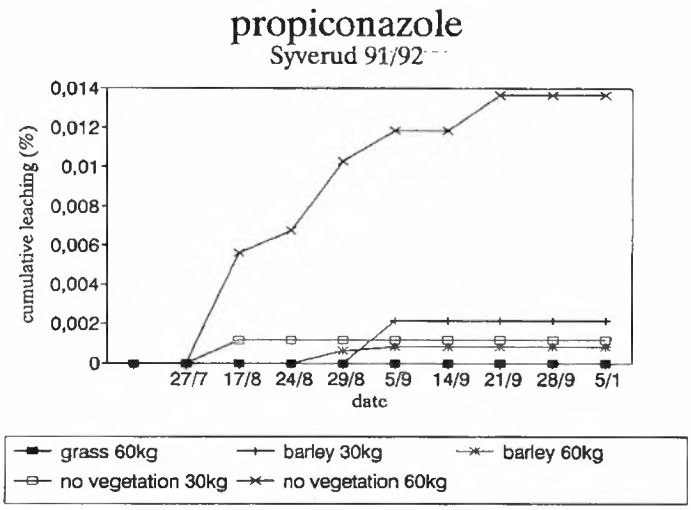

Fig. 11. Cumulative leaching of propiconazole from plots with no vegetation, barley and grass, 1991/92 Syverud, Ås (n=3)

Ullensaker

Chlorsulfuron

Chlorsulfuron was found in the first samples in June in both the surface and the drainage runoff, with the highest concentrations in drainage water. Decreasing amounts were found up until the middle of October. After ploughing, between 12 and 13 October, the concentration increased again. These samples showed higher concentrations of pesticides in the surface water than in the drainage water just after ploughing (Fig. 12).

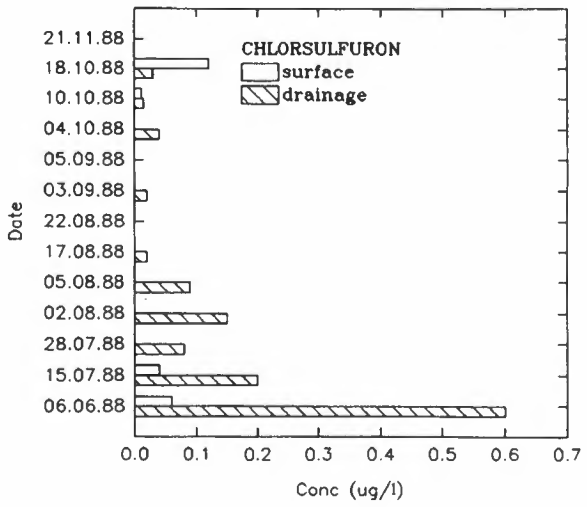

Fig. 12. Concentration of chlorsulfuron in surface and drainage water from field 2, Ullensaker 1988/89

Despite low application rates (more than 100 times lower concentration of active ingredient per ha than for the phenoxy-acids) relatively high concentrations were found in the runoff. Chlorsulfuron is a weak acid with anionic properties and with a generally low estimated soil-water distribution coefficient (K_d). Thus, the presence of chlorsulfuron in the first runoff in both the surface and the drainage water confirms the predicted high mobility of this compound. The rapid movement of chlorsulfuron is not explained by the properties of the pesticide alone, but also because of preferential flow through cracks and macropores. This type of flow is stated for other pesticides used in the project for this soil type in both field and lysimeter experiments (Riise *et al.* this volume). Because of the high biological activity of this compound, these concentration levels found may result in phytotoxic effects on some organisms.

In total, the reported findings represent 2% of the amount applied. According to the classification of the potential of leaching from different soils types, for chlorsulfuron this corresponds to soil representing the highest sensitivity to leaching described by Strada *et al.* (1993) based on a system worked out by Boesten & van der Linden (1991).

Dimethoate

Dimethoate was used for two years in fields 2 and 3. This compound was found only in runoff from field 3 during the season 89/90 (Fig. 13). Since dimethoate is generally not often reported in runoff (Kreuger 1992), the results from 89/90 are rather exceptional. According to the low K_d - value (Riise *et al.* this volume), it might be expected that dimethoate would be found more frequently, especially immediately after spraying.

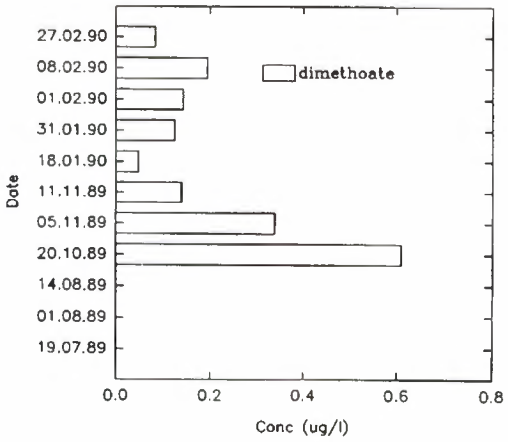

Fig. 13. Concentration of dimethoate in surface runoff from field 3, Ullensaker 1989/90

According to the biological experiments in the project, these concentrations have effects on the activity of the xenobiotic metabolizing enzymes of fish and organisms in soil (Skåre & Egaas, this volume). Rather large negative changes in the benthic fauna at sublethal concentrations of 1 µg/l dimethoate have been proved. Of all the sustances tested in our project this was the compound that had the most serious effect on the benthic fauna (Aanes 1992).

Propiconazole

During the project period propiconazole represented the pesticide most frequently found at Ullensaker, in a total of 62 samples. However, propiconazole was the most extensively used pesticide during the project. One-third of the samples collected from areas treated with propiconazole contained the compound. Since the detection limit of the pesticide analyses has improved during the project period, the data from the different periods are not directly comparable. Nevertheless, the quite frequent occurrence of propiconazole was surprising considering the relatively high K_d - values in the soil (6 - 60).

Propiconazole was found in the first runoff after treatment from all areas. This was the situation even for the drainage water, which should confirm that transport through macropores also exists for this substance. The highest concentration found for any of the pesticides used was for propiconazole at 9.7 µg/l. As there is a paucity of data on waterflow for some periods, the total load of the pesticide applied has not been calculated for all the fields. For field 1 the total load of propiconazole varied from 0.04 to 0.3% of the amount applied. According to the sensitivity scale of Strada *et al.* (1993), this soil represents medium to high sensivitity to leaching of propiconazole. When calculating the load of pesticide, the removal of pesticide absorbed to the particles transported from the area was not taken into account. Calculations by means of distribution coefficients and amount of dry matter transported from the area were performed, but the contribution from the amount of propiconazole absorbed to the particles was found to be insignificant.

Looking at the general pattern of the runoff (Figs. 14 - 21) it would appear that when runoff occurs in July the concentration of propiconazole is often 1 µg/l or more. Thereafter the concentration decreases and the compound is not detected after October/November. This was the situation during the first three years, 1988-89-90, for the fields treated with propiconazole. During the last year, 91/92, there was no runoff before October. The concentrations were often not more than 0.2 mg/l, but propiconazole remained in the runoff during the winter. In fact, in one field, traces were still detected at the end of April after the field had been harrowed. The concentration in the first runoff will be a function of time and degradation, but also a function of reaction time with soil colloids. As a function of time, the pesticides will be transferred to a hardier desorbable pesticide in the soil (Barriuso *et al.* 1992), called aged residues (Schribner *et al.* 1992).

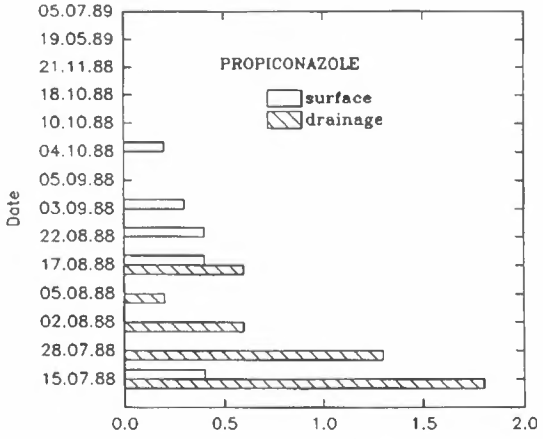

Fig. 14. Concentration of propiconazole in surface and drainage water from field 1, Ullensaker 1988/89

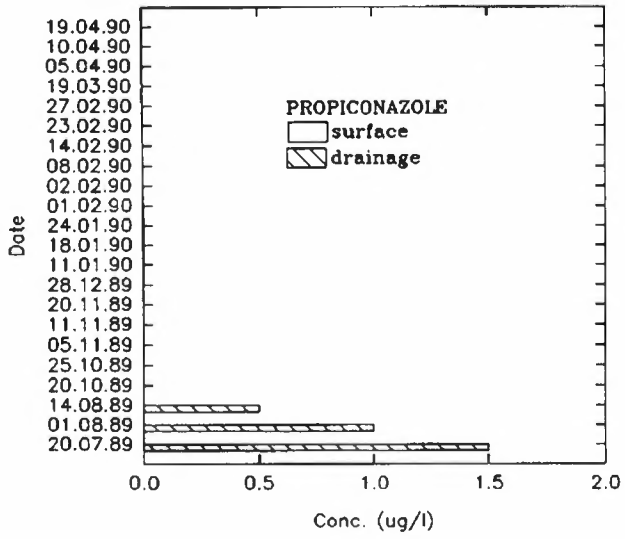

Fig. 15. Concentration of propiconazole in surface and drainage water from field 1, Ullensaker, 89/90

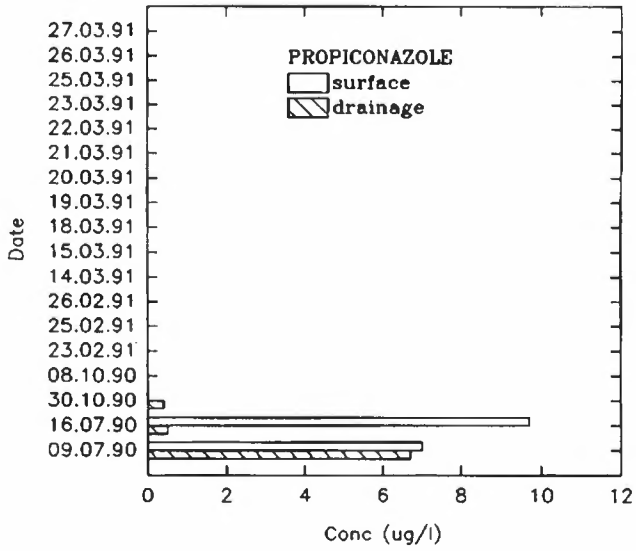

Fig. 16. Concentration of propiconazole in surface and drainage water from field 1, Ullensaker 1990/91

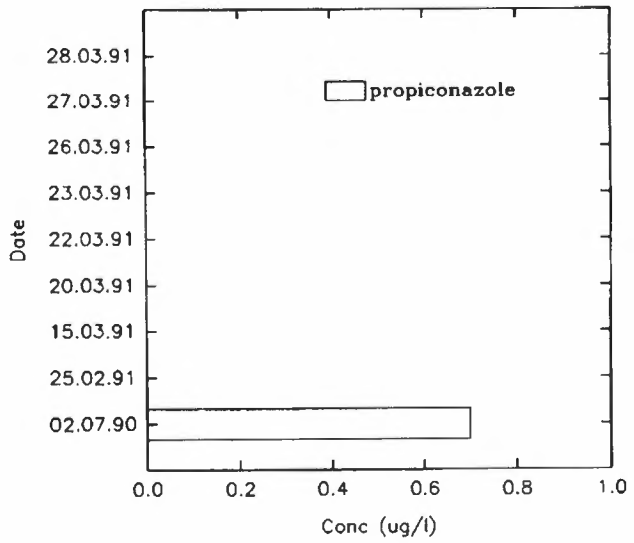

Fig. 17. Concentration of propiconazole in surface runoff water from field 3, Ullensaker 1990/91

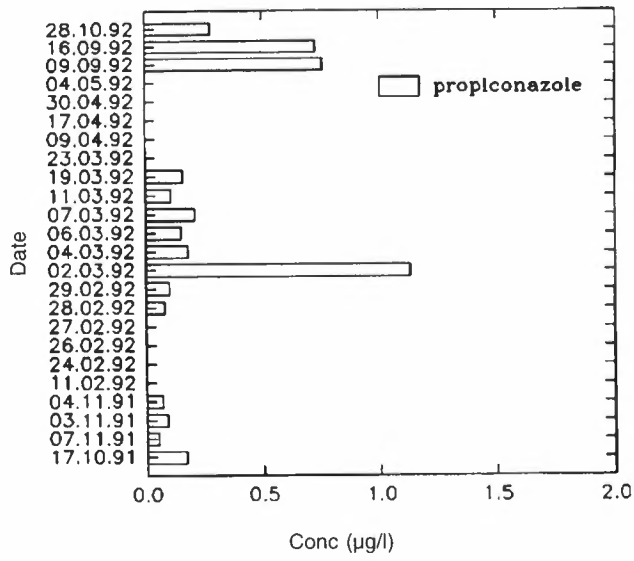

Fig. 18. Concentration of propiconazole in drainage water from field 1, Ullensaker 1991/92

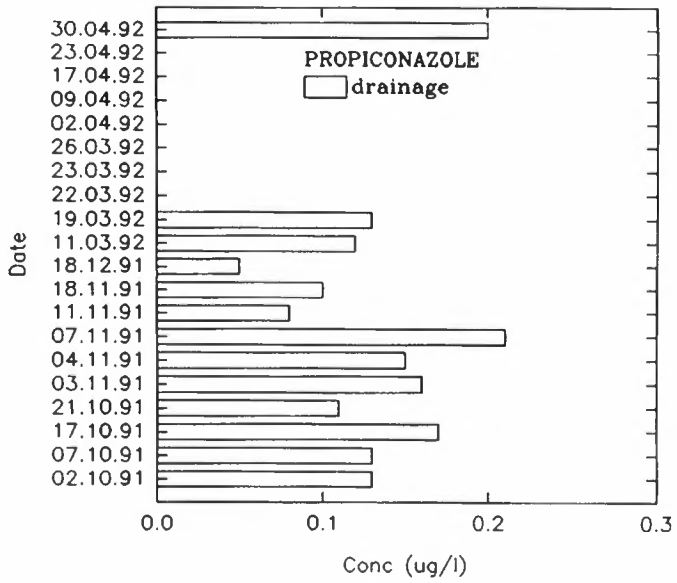

Fig. 19. Concentration of propiconazole in drainage water from field 1, Ullensaker 1991/92

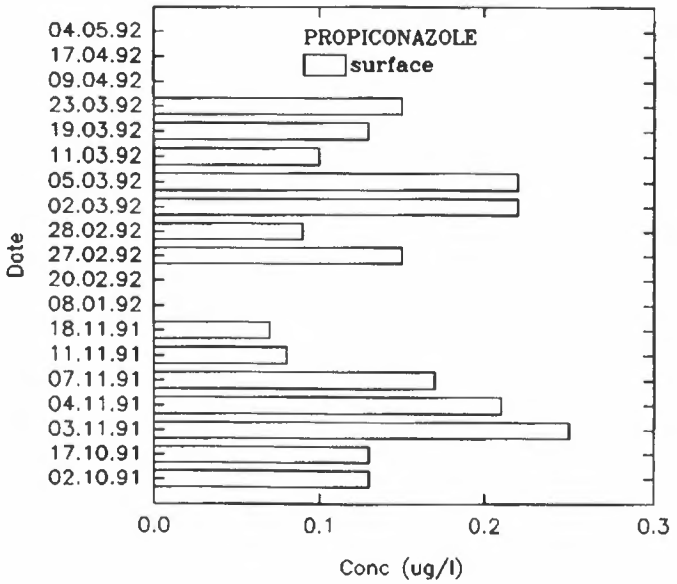

Fig. 20. Concentration of propiconazole in surface runoff water from field 2, Ullensaker 1991/92

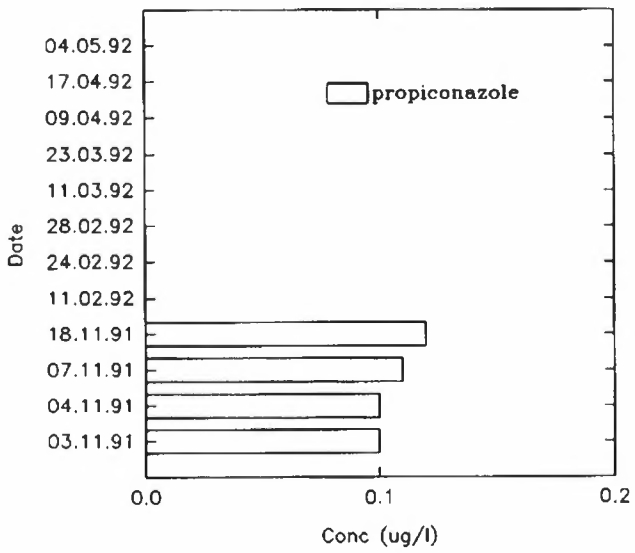

Fig. 21. Concentration of propiconazole in surface runoff water from field 3, Ullensaker 1991/92

The only year in which all fields were treated with propiconazole was 1991 and this makes it possible to compare the different fields under similar weather conditions. The differences between the runoff from the fields might be caused either by topography or by treatment. Field 3 seems to have the shortest period of propiconazole in the runoff. This is the steepest area and available pesticides will be eroded or transported away during the first runnoff event. There is an increase in the concentration of propiconazole in surface runoff from fields 1 and 2 and this is probably due to the snowmelt in the spring.

Comparing pesticide concentration with the amount of suspended dry matter in water and the waterflow for field 1, there is a positive correlation between suspended dry matter and concentration of propiconazole (Fig. 22). There is no correlation between concentration of the pesticide and the waterflow, however. To some extent one might expect some increase in pesticide with increasing waterflow, but the pesticide soon becomes undetectable because of dilution.

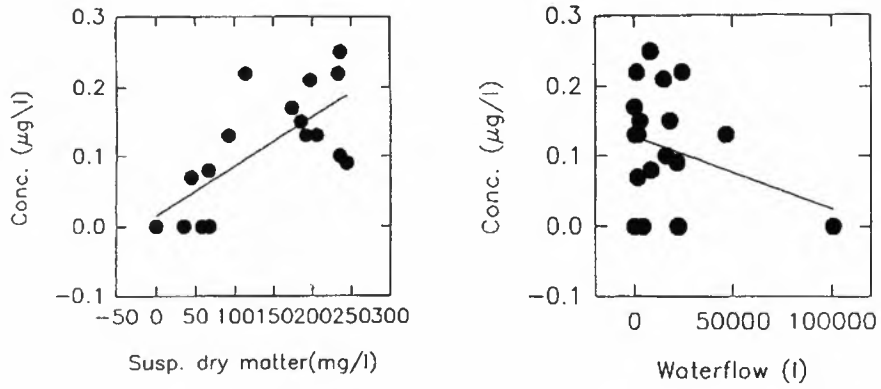

Fig. 22. Correlation between suspended dry matter, waterflow and concentration in runoff from field 1, Ullensaker 1991/92

According to the biological experiments in this project, propiconazole was evidently the most phytotoxic compound of all those used. This means that the concentrations found of this substance will probably have biological effects in the streams. In particular, strains of the blue green algae *Chlamydomonas* were very sensitive and propiconazole affected the growth at concentrations below 0.2 µg/l (Källqvist & Romstad 1991). In tests in an artificial running-water system, dosed with 5 µg/l propiconazole, the whole algal community was wiped out and replaced by fungus. However, there were no clear-out effects on the invertebrates at these concentrations (Aanes 1992).

Dichlorprop and MCPA

Dichlorprop is the most frequently found pesticide, calculated as a percentage of the samples collected from the area treated with these compounds. More than 50% of the samples from areas treated with the phenoxy-acids contained dichlorprop. In field 3, dichlorprop was found in 11 of 12 samples from November 1991 to May 1992. The behaviour of MCPA is similar to that of dichlorprop, but generally the concentration of dichlorprop is up to or more than three times greater than that of MCPA. This can be explained by the fact that the formulation used contains a mixture of these two pesticides. The difference reflects the relation between the concentrations of the two pesticides in the formulation. Often the ratio between dichlorprop and MCPA was more in the runoff (3.4 - 8.6) than for the formulation (3.0).

The concentration and distribution of dichlorprop and MCPA in the surface runoff and drainage water from the three field sites are shown in Figs. 23-29. Except for in 1988, both MCPA and dichlorprop were found in runoff from all the treated fields. This is probably due to different detection limits during the project.

Typically, the phenoxy-acids in the runoff showed two annual peaks, one in October/ November connected with one of the first runoff events, and the other in February/March during snowmelt. In 1990/91 the runoff during the autumn was restricted to one event and nothing was found until the spring snowmelt.

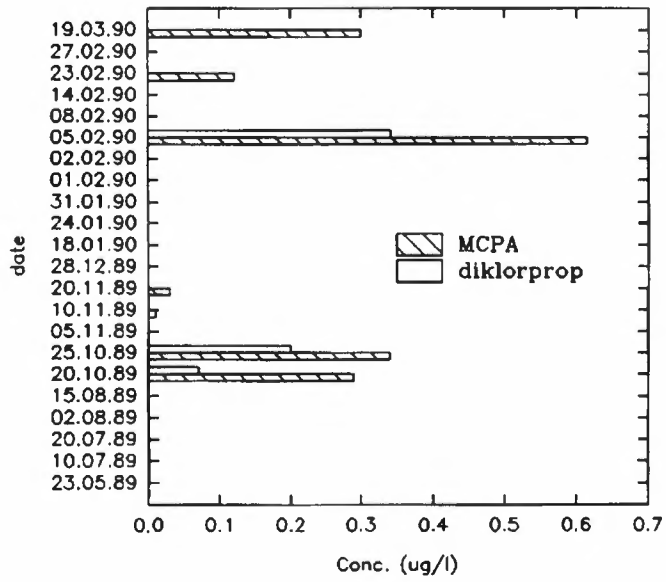

Fig. 23. Concentration of MCPA and dichlorprop in surface runoff water from field 2, Ullensaker 1989/99

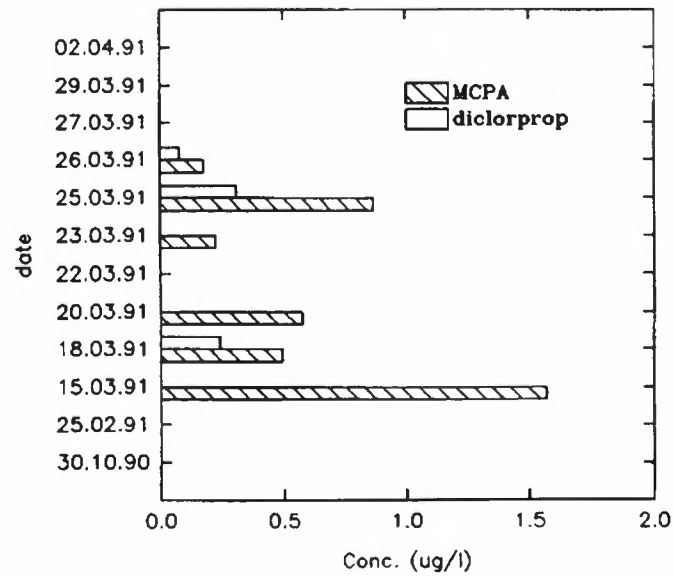

Fig. 24. Concentration of MCPA and dichlorprop in surface runoff water from field 2, Ullensaker 1990/91

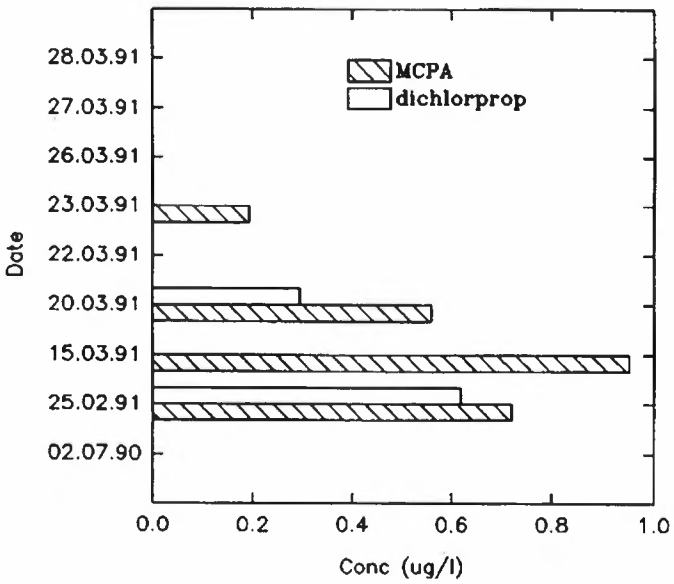

Fig. 25. Concentration of MCPA and dichlorprop in surface runoff water from field 3, Ullensaker 1990/91

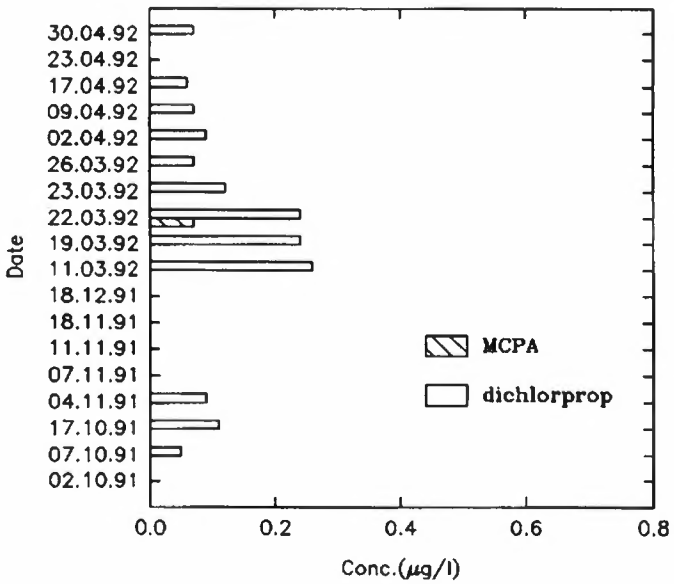

Fig. 26. Concentration of MCPA and dichlorprop in drainage water from field 1, Ullensaker 1991/92

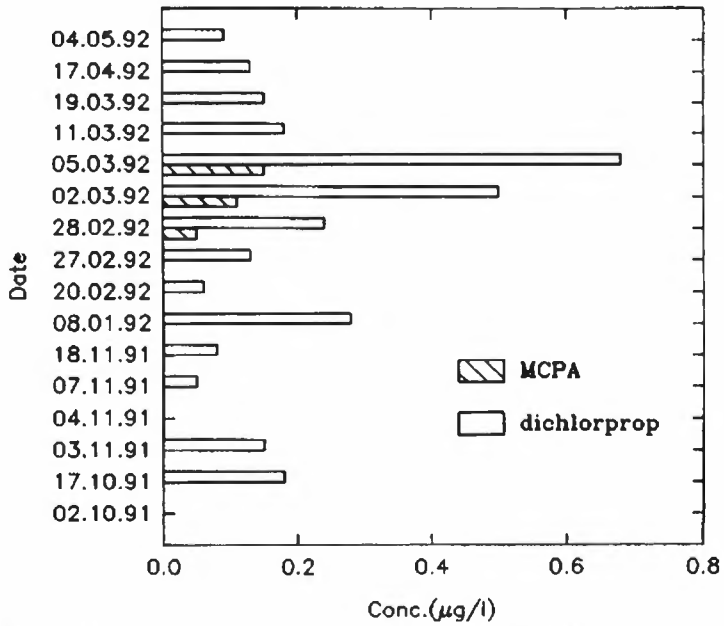

Fig. 27. Concentration of MCPA and dichlorprop in surface runoff water from field 1, Ullensaker 1991/92

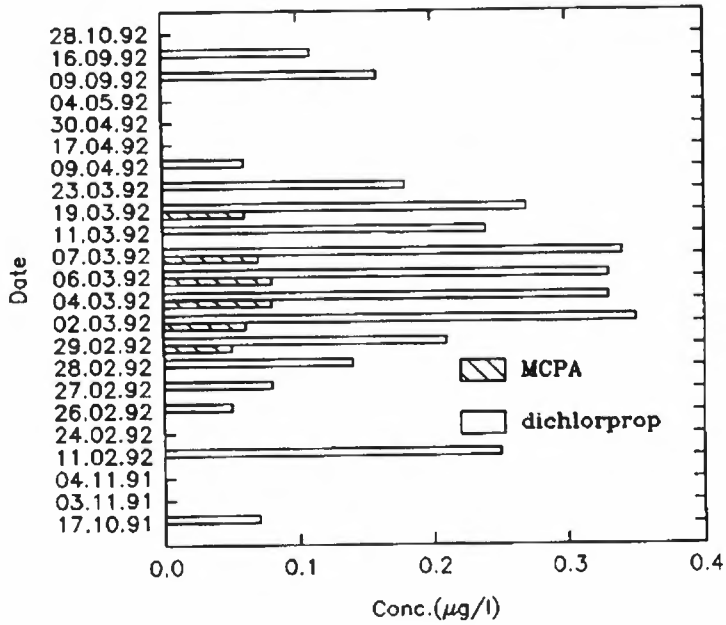

Fig. 28. Concentration of MCPA and dichlorprop in surface runoff water from field 2, Ullensaker 1991/92

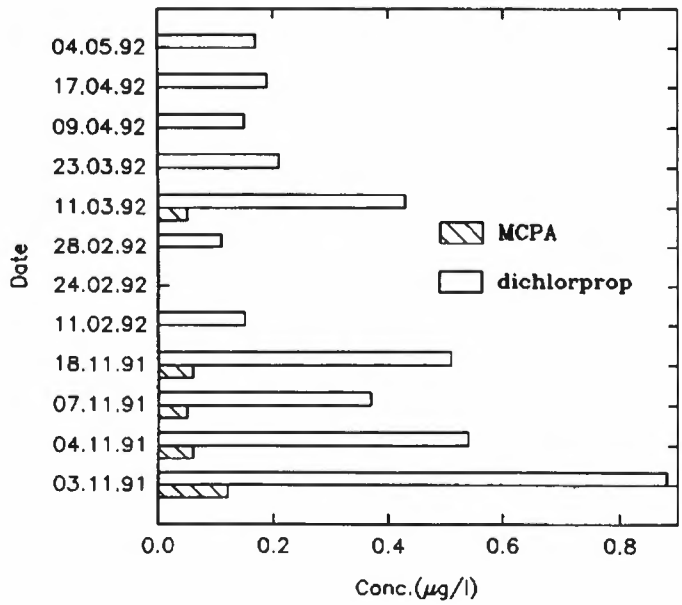

Fig. 29. Concentration of MCPA and dichlorprop in surface runoff water from field 3, Ullensaker 1991/92

The low soil-water distribution coefficients of these pesticides ($K_d = 0.8$) suggest that pesticide mobility should be high. In accordance with the results from the sorption and lysimeter experiments, most of the phenoxy-acids from the field areas were thought to be washed out or degraded during the growth season. After repeated treatments with MCPA in field experiments, Torstensson (1980) found that 90% of the applied dose was degraded within 12-42 days. Degradation of 90% of the applied dichlorprop has been reported to occur within three months (The Agrochemicals Handbook 1986). The frequent findings of phenoxy-acids 10 months after application is quite surprising, but Lang & Hurle (1993) reported phenoxy-acids from May until December in the river Rems in Germany.

Low temperatures during the winter and some protection from degradation during sorption by soil particles could also explain why some phenoxy-acids remained in the water late in the spring. Unusually warm wheater conditions and heavy rainfall in March 1990 and 1991 caused serious surface erosion and leaching that year. Freezing and thawing can be important factors in releasing the pesticides in the spring, causing peak concentrations in the runoff. Studying the night and day mean temperatures in February and March, great variations could be documented. This resulted in continous thawing of the topsoil, while the frozen soil underneath prevented downward movement and caused surface erosion.

Another obvious difference between the runoff curves from field 3 compared to those from the other fields was the maximum peak concentration of the pesticides in the first water sample in the autumn. This could be explained by field a 3's steep slopes causing immediate pesticide runoff after the first precipitation. In the other fields the downward migration through the soil layers was so rapid that the pesticide, to some extent, was protected from surface runoff.

Lysimeter experiments demonstrate that MCPA and dichlorprop move rapidly through

macropores (Eklo & Lode, 1991b), Riise *et al.* 1992 in this volume). According to the runoff monitoring, this also seems to happen in the field at Ullensaker with dichlorprop, MCPA and propiconazole.

As MCPA and dichlorprop are ranked among the least toxic compounds used in the project (Källqvist *et al.* 1991), the concentrations observed should not cause serious toxic effects on the tested species and communities. However, the presence of pesticides in the runoff water in late spring indicates that there is a risk of toxic accumulation in the soil. Different tillage treatment in areas late in the autumn would probably increase these risks, especially in countries with cold winters.

SUMMARY

During two years of monitoring, all pesticides used were found in the leachate from the outdoor lysimeter field. The phenoxy-acids were the most frequently found compounds and at the highest concentrations. Propiconazole was the least mobile pesticide. The typical leaching pattern of the pesticides was only one peak during the autumn. Plots with bare soil and the highest level of fertilizer had the highest leaching. From the plots with permanent grass there was a delay of three to four months before the water reached the drainpipes. Leaching varied considerably between plots and between years, but the total amount of leaching was in good accordance with the amounts collected from the areas in conventional use at Ullensaker.
From the catchment areas at Ullensaker, dichlorprop was found at the highest frequency. Dichlorporp was detected in 50% of the samples from areas treated with that pesticide. Propiconazole was found in 62 samples representing the largest numer of any single pesticide detected during the project period. This compound also represents the highest concentration with 9,7 µg/l measured in the runoff quite near to the time of application. Expressed as a percentage of the amount applied, 2% of chlorsulfuron was washed out. The typical pattern of leaching was one peak in the autumn and another in the spring during snowmelt. The phenoxy-acids were still found in the runoff just before the spring farming. Soil management with subsequent runoff increased the risk of pesticide leaching and runoff. The silty clay loam of Ullensaker was frequently subjected to cracking during dry periods and preferential flow of pesticides through macropores was observed.

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Leaching of simazine and atrazine from an industrial area to a water source. A long term case study

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Lode, O., O.M. Eklo, P. Kraft & G. Riise 1994. Leaching of simazine and atrazine from an industrial area to a water source. A long term case study. Norwegian Journal of Agricultural Sciences. Supplement No. 13: 79-88. ISSN 0802-1600.

For 20 years (up until 1986), the herbicides simazine and atrazine were used for vegetation control on an industrial area at Mysen, Norway. Because of leaching, a water source which received water mainly from a groundwater aquifer within the same catchment became contaminated with simazine and atrazine. This water source supplied water both for human consumption and for greenhouse crops. The plants that received water contaminated with simazine and atrazine sustained considerable damages. The aim of the present case study is to investigate the longevity of these herbicides in the catchment in order to assess the risk for residues in the water source. Until now both herbicides have been permanently present in amounts varying from about 0.1 µg/l to 0.9 µg/l. High concentrations of both simazine and atrazine (8.6µg/l and 4.5 µg/l, respectively) were measured in a manhole (drain) receiving surface runoff from the treated area. Results from lysimeter experiments with the same soil (sandpit) indicated that the vertical migration of simazine was relatively fast, but very dependent on the amount of water added. An estimate of the longevity of simazine and atrazine based on percolation time in the unsaturated zone indicates that the herbicides need 10-15 years to reach the groundwater table. In addition factors such as degradation rate and residence time in the saturated zone will have an influence on the duration of simazine and atrazine in the water source.

Key words: Atrazine, drinking water, flower crops, leaching, sand and simazine.

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Pesticides applied to agricultural and industrial areas may find their way into aquatic environments depending on hydrogeology, properties of the pesticides and their mode of application.

Hydrogeology deals with the interactions between soil, geology and water. In this context it is important to include information about the groundwater level, recharging of the aquifer, i.e. the relation between groundwater formation and precipitation, topography (e.g. slope) and the influence of porosity and geology on processes in the unsaturated and saturated zone.

The solubility of the pesticides, their sorption to soil colloids and their degradation in both abiotic and biotic environments are important factors influencing the behaviour of pesticides in the soil-water system. Provided they are rapidly degraded in soils, water soluble pesticides weakly sorbed to soils may not reach groundwaters. This may also be the case for persistent pesticides if they migrate very slowly in soil due to strong sorption to soil colloids.

The application mode also plays a key role with regard to pollution of groundwaters. Frequency, method and time of application and applied dose are factors influencing the leaching behaviour of the pesticides.

At The Nordic Plant Protection Conference held in Denmark in 1989, Lode et al. presented results from a case study at Mysen, Norway. For 20 years (up until 1986) the triazines simazine and atrazine have been used on an industrial area in order to keep five acres free from vegetation. The treated area lay within the catchment of a water source supplying water to flower crops in a glasshouse. Seven species of young annual and biennial flower crops *(Cineraria maritima, Dianthus carthusianorum, Dianthus chiensis, Fuchsia hybrida, Petunia hybrida, Sinningia speciosa* and *Saintpaulia ionantha)* which received water contaminated with simazine and atrazine were severely damaged. Simazine and atrazine have a high affinity for soil organic carbon. But, even though the plants were grown in humus-rich soil, the herbicides were apparently available for uptake from the drip watering system.

In order to assess the risk of simazine and atrazine in the water source, this case study has investigated the longevity of the applied triazines in the Mysen area.

MATERIAL AND METHODS

The catchment area

The catchment of the water source is shown in Fig. 1. The geology within the area is dominated by relatively coarse mass (sand/gravel) upon ground rock. The dominating particle size fraction of the soil material is sand (Fig. 2). Most of the precipitation within the catchment infiltrates the groundwater and drains towards the water source along a funnel-shaped fall gradient. Masses of sand have earlier been taken out from this sandpit area and the remaining holes act as infiltration wells which contribute significantly to the recharging of the groundwater.

The catchment area is about 0.35 km^2. The yearly mean precipitation is approximately 700 mm, with about 30% of the precipitation reaching the groundwater. According to this data the groundwater input is calculated to be roughly 8.4 m^3/t.

The water sources

The water source has delivered about 900 l/hr. for water consumption. In addition, an unspecified amount of water has been taken from well B (Fig. 1) to supply the factory. Thus, a considerable amount of water is exported from the area. Furthermore, the supply of water from both these sources has been stable throughout the year. The size of the groundwater aquifer must therefore be relatively large. The recharging of the groundwater takes place mainly during the rainy spring and autumn seasons.

Using drilling equipment, we collected a soil profile at AA' (Fig. 1). A schematic survey of the profile is presented in Fig. 3. A dot-and-dash line indicates the groundwater level. The results from the three sites S1, S2 and S3 revealed that the groundwater magazine has an increasing and relatively vast dimension (>10 m) south of the magazine. Sand dominates the masses with a minor but varying content of silt and gravel. The depth of the soil layer varies to a great extent. Furthermore, rock ridges create thresholds which change the water flow, which is normally perpendicular to the profile. The site where the particle-size distribution was determined was located at U1, a 1-2 m deep well. (Fig. 2).

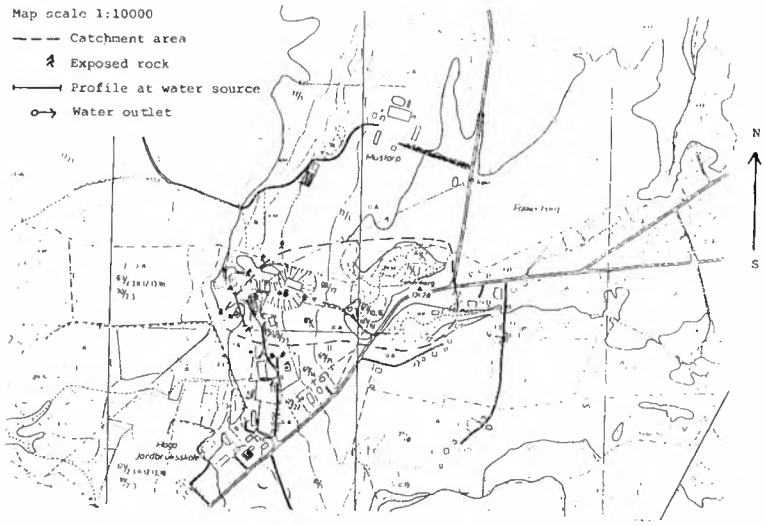

Fig. 1. The catchment of the studied area. Scale 1:1000

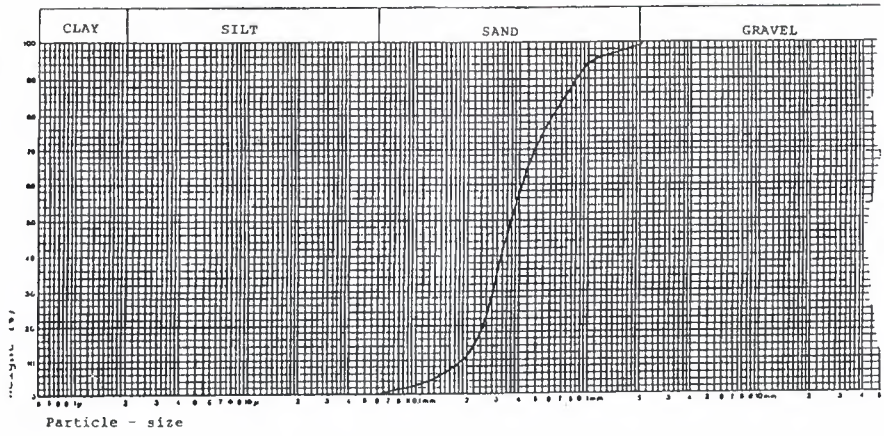

Fig. 2. Particle size distribution of the soil in the area at W1

Surface runoff

In addition to water samples from the open source, runoff samples from the sprayed area were also taken from a manhole (drain), depth = 1 m.

Size fractionation

In situ size fractionating of the water source was performed with an Amicon CH2 concentrator equipped with hollow fibre cartridges with a nominal molecular weight cut-off of 0.1 µm, 10 kDa and 3 kDa.

Lysimeter

Surface soil cores from the *sprayed* area, without any additional herbicides, were included in lysimeter experiments. The total amount of water added to the cores was 1000 ml. Both simazine and atrazine were analysed in the leachate. In addition, a lysimeter experiment with simazine was performed with *unsprayed* surface soil cores (d = 10 cm, h = 35 cm). Two doses were used, 1.62 mg and 8.10 mg. The water regimes are presented in Table 1 and the experiment was run for 13 days. Water transported through the columns was sampled once a day and analysed for simazine.

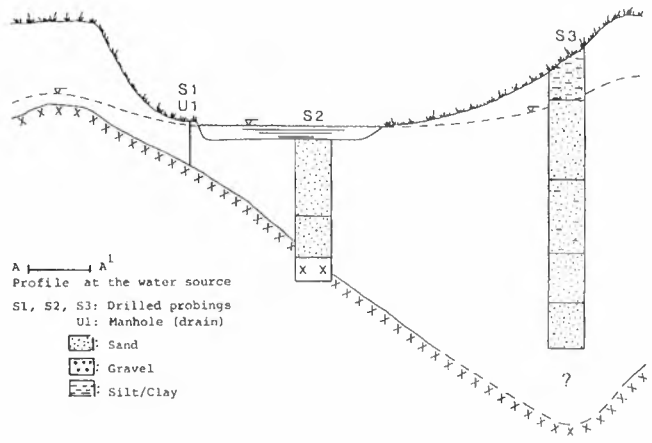

Fig. 3. Profile of the water source

Chemical analyses

The chemical analyses of simazine and atrazine were performed by gas liquid chromathography. The detection level varied from 0.1 µg/l in the first years to 0.01 µg/l at the end of the period (Appendix).

Table 1. The ratio between precipitation and percentage of leached simazine

Dosage	Fig.	Water added ml	mm	Leached simazine %	Leach. (mm)/ 164 mm	Simazine leach. (%)/ 26.4%
8.1 mg	6	1285	164	26.4	1	1
8.1 mg	7	2615	333	82.7	2	3.1
1.62 mg	8	2815	359	96.9	2.2	3.7
1.62 mg	9	2615	333	82.1	2	3.1

RESULTS AND DISCUSSION

The water source

Although eight years have passed since simazine and atrazine were applied for the last time in the industrial area, these herbicides are still present on the water source in 1993 (Fig. 4). With the exception of the first three years, both simazine and atrazine were measured in all samples collected within the time period 1986-1993. Atrazine was not measured in the first years of the study, as only information concerning the use of simazine was given. After having confirmed the presence of this chemical in the water samples in 1989, it was admitted that atrazine had been used together with simazine.

As the water source was used for drinking water purposes, the EEC norm (EEC directive 80/778) for the concentration of pesticides is of interest. The limits are set at 0.5 µg/l for the total amount of pesticides and at 0.1 µg/l for any individual pesticide. Both simazine and atrazine exceeded the 0.1 µg/l norm in more than 90% of the samples measured. As for the 0.5 µg/l norm, the concentrations of simazine and atrazine were higher in 44% and 33% of the samples respectively. The 0.5 µg/l norm for the total amount of pesticides (simazine + atrazine) was exceeded in 61% of the samples. The highest concentrations measured for simazine and atrazine were 0.7 µg/l and 0.9 µg/l, while the lowest concentrations were 0.04 µg/l and 0.09 µg/l, respectively.

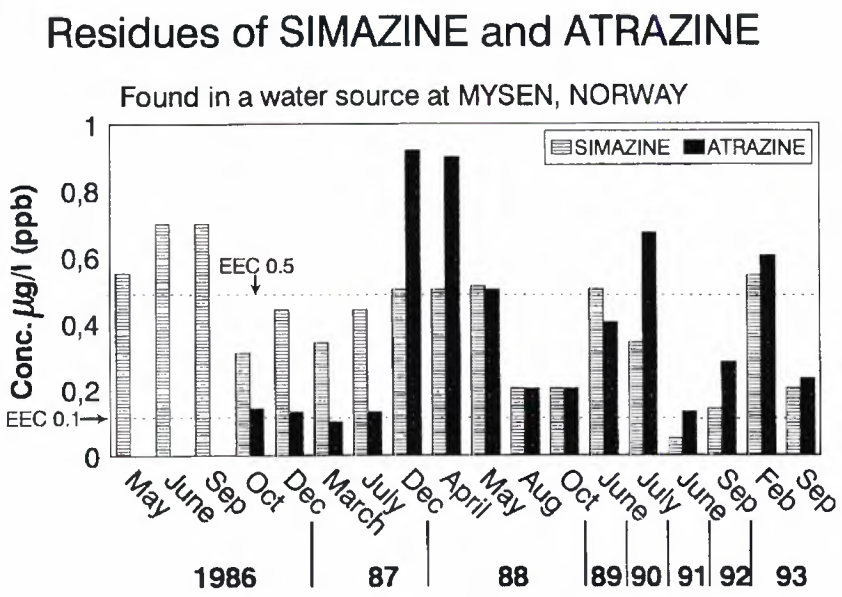

Fig. 4. Simazine and atrazine (µg/l) measured in a water source at Mysen in the period 1986-1993. The water source is fed from a groundwater aquifer in the area

No tendency toward to seasonal variations was observed. On the other hand, the relative amounts of simazine compared with atrazine have varied during the study. While simazine was dominant at the beginning of the study, the relative importance of atrazine has increased during recent years. The retention of pesticides in soil depends to a great extent on two main factors, solubility in water and the sorption to soil particles. Although both herbicides have low water

solubility, atrazine (33 mg/l) is almost seven times more soluble than simazine (5 mg/l). On the other hand, the sorption of atrazine to soil is generally higher compared to simazine. In a review concerning environmental transport and persistence of pesticides, Cohen et al. (1984) have given values of K_d and K_{oc} for simazine and atrazine. For atrazine the K_d and K_{oc} values were 0.4-8 and 163±80 and for simazine 0.26-5 and 138. Riise *et al.* (1993a, 1993b) have studied the mobility of atrazine in both batch and lysimeter experiments. A comparison between the vertical migration of atrazine in silt loam (1.4% organic carbon) and soil poor in organic carbon (<0.1%) confirmed that leaching of atrazine increased with decreasing amounts of organic carbon. The authors, results are in agreement with Bailey & Rothberg (1968) who found that the leaching of seven triazines in sandy loam corresponds rather poorly with solubility, i.e. the vertical migration of atrazine in soils poor in organic carbon is high concerning its low water solubility. However, if we assume that simazine and atrazine have been used in equal amounts, the following leaching event might have occurred. At the beginning of our study the extent of sorption was higher for atrazine compared to simazine, explaining why the residues of simazine exceeded those of the atrazine. Later in the study, the degradation pattern of the herbicides probably gained increasing importance for the leaching eveent. Low temperature and shortage of nutrition are not conducive to the growth of micro-organisms in the subsoil. Thus, a rapid biotic decomposition of the triazines cannot be expected. On the other hand, abiotic hydrolysis may be more pronounced. Hydrolysis half-lives (weeks) have been found to lie between 10 and 106 for atrazine and 8 and 30 for simazine (Cohen *et al.* 1984). The slow rate of hydrolysis of atrazine vs. simazine can explain the higher residue levels of atrazine during recent years. According to Boesten & Van der Pas (1993) the half-life of atrazine is expected to be more than five years in aerobic subsoils with pH values above 6. The pH of the water source at Mysen was 7.5. Thus, atrazine can persist in the unsaturated subsoil for a rather long period.

Surface runoff

The residue levels of atrazine and simazine in the manhole (drain) can be seen in Fig. 5. The values in the manhole were always higher than those in the water source. Maximum concentrations measured were 8.6 µg/l and 4.5 µg/l for simazine and atrazine respectively. No residues were observed in 1989. From 1990, no samples were available, as the runoff was prevented from reaching the manhole. Compared to the EEC norms, each measurement for each individual herbicide exceeded the limit for the total amount (0.5 µg/l). At corresponding times of sampling these results show the same elapse as for the water source - residues of simazine were at higher concentrations than those of atrazine at the beginning of the study.

Size fractionation

The ultrafiltration studies with hollow fibres indicated that both simazine and atrazine were in a low molecular form (<3 kDa) in the water source.

Lysimeter

After adding 1000 ml water to surface soil cores from the sprayed area, both simazine and atrazine were found in the leachates (1986). The results from different parallel columns varied. However, by comparing the results from the two most similar columns, 2.4 µg/l and 4.2 µg/l simazine and atrazine had leached through the columns respectively. The results from the lysimeter experiments where simazine was added to unsprayed soil columns are shown in Figs. 6-9. The vertical migration of simazine (percolation) through the columns was fast, but to a large extent dependent on the amount of water added to the top of the columns.

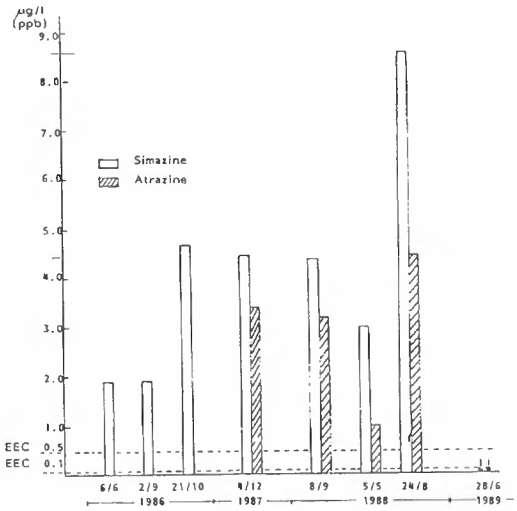

Fig. 5. Simazine and atrazine (µg/l) measured in a manhole (drain) in the period 1986-1989. The manhole was receiving surface runoff from the area treated with herbicides

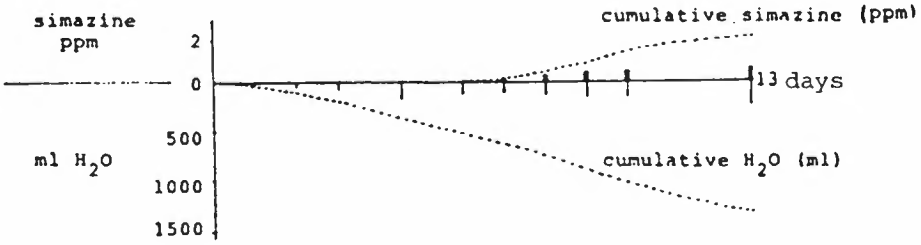

Fig. 6. Leaching of simazine through a soil column, where 8.1 mg simazine was applied to the top of the column. Of the applied simazine, 26% had leached through the column within 1285 ml leachate (13 days)

The ratios between the amount of water added and percentage amount of leached simazine are presented in Table 1. The result from the column receiving the smallest amount of water (Fig. 6) is used as a base level and is set to 1. By adding 20 to 25 mm water daily to the water-saturated monoliths, simazine appeared in the leachate after three days. If we assume linearity between precipitation (mm) and leaching distance, this gives a yearly downward movement of simazine of about 12 cm, provided that 30% of the annual precipitation percolates through the soil. As the average depth of the subsoil in the area is assumed to be between 1 and 2 m, it will take from 10 to 15 years before simazine reaches the groundwater table.

Thus, the herbicides may still be present in the water source for at least seven years, depending on the degradation rate of the herbicides, the amount of herbicides still sorbed in the unsaturated zone, and the residence time of the groundwater aquifer.

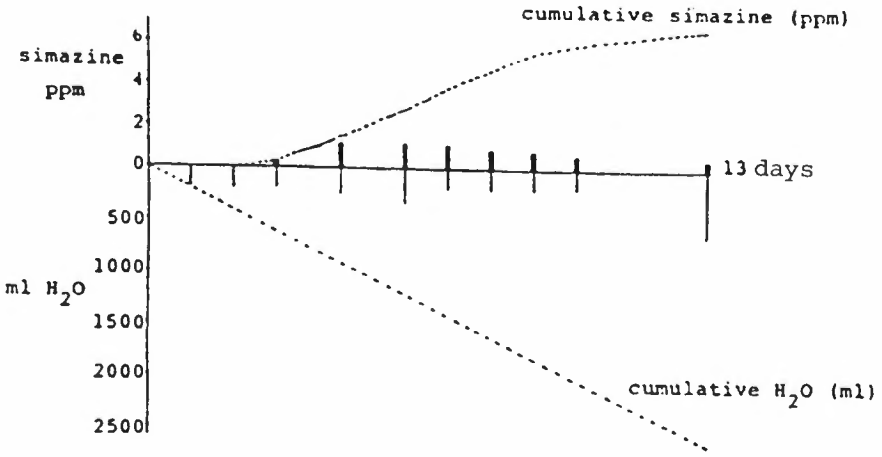

Fig. 7. Leaching of simazine through a soil column, where 8.08 mg simazine was applied to the top of the column. Of the applied simazine 83%, had leached through the column within 2615 ml (333 mm) leachate (13 days)

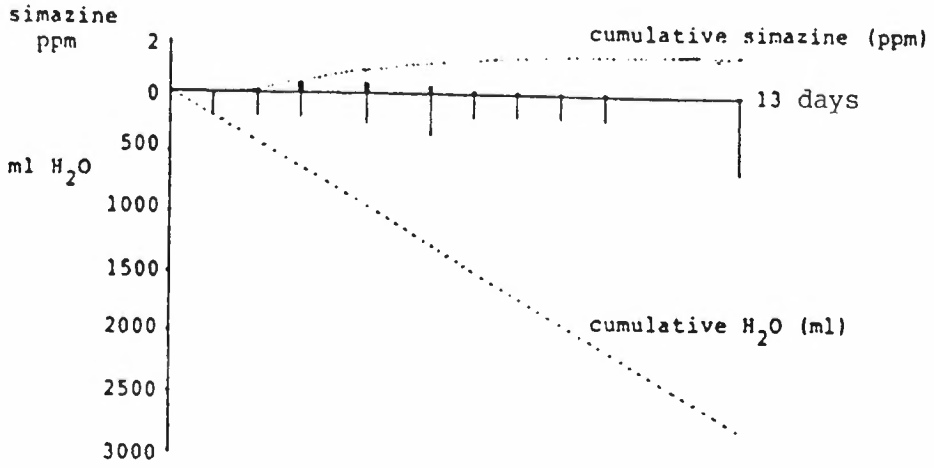

Fig. 8. Leaching of simazine through a soil column, where 1.62 mg simazine was applied to the top of the column. Of the applied simazine 97%, had leached through the column within 2815 ml (359 mm) leachate (13 days)

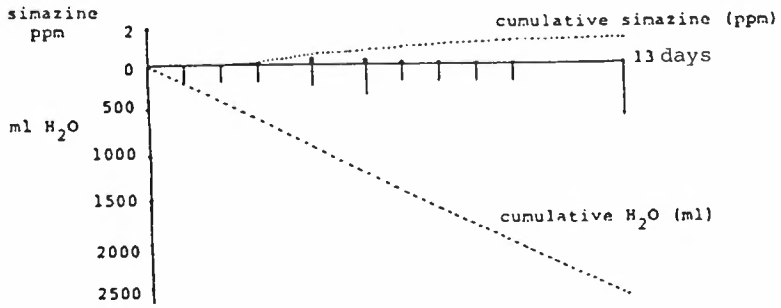

Fig. 9. Leaching of simazine through a soil column, where 1.62 mg simazine was applied to the top of the column. Of the applied simazine 82%, had leached through the column within 2615 ml (333 mm) leachate (13 days)

CONCLUSIONS

- The presence of small amounts of simazine and atrazine in a drip watering system caused considerable damage to flower crops in a greenhouse.

- Simazine and atrazine are shown to persist in the subsoil below an industrial area for a long period of time. The herbicides were still leaching to a water source in 1993, eight years after the last application of simazine and atrazine in the area.

- Based on the percolation time of the water in the unsaturated zone, it was estimated that the water needs approximately 10-15 years to reach the groundwater table. Thus, atrazine and simazine may continue to exist in the water source for at least seven years, depending on degradation rate and the retention time of the herbicides in the saturated zone.

- Despite the lower water solubility of simazine compared to atrazine, the sorption of atrazine seems to be higher than that for simazine in this sandy area.

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Pesticide induced biochemical changes in terrestrial insects, benthos and fish as markers of contamination of soils and waters

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Egaas E., J.U. Skaare, N.O. Svendsen & M. Sandvik 1994. Pesticide induced biochemical changes in terrestrial insects, benthos and fish as markers of contamination of soils and waters.(Biomarkers for pesticide contamination). Norwegian journal of Agricultural Sciences. Supplement No13: 89-116. ISSN 0802-1600

The major results from a five year research project on the biochemical effects of selected pesticides and some industrial chemicals on terrestrial and aquatic species are summarized and discussed. The appropriate analytical methods that have been used to detect induction/inhibition events in XME activities are described. Furthermore, some biological (tissue distribution, developmental stage, sex and strain), environmental (temperature) or chemical (plant allelochemicals, pesticides, polychlorinated biphenyls and 2,3,7,8-tetrachlorodibenzo-p-dioxin) factors that may have an influence on XME in the species investigated are discussed.

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During the past decade, considerable attention has been given to the concept that biochemical and physiological characteristics can serve as sensitive biomarkers of contaminant exposure and enviromental quality. The effects of chemical contaminants can be viewed as occurring at different levels of biological organization, extending from the molecular or biochemical level to the physiology of the individual, and ultimately to the levels of population and ecosystems. Changes at the biochemical level offer distinct advantages as markers of exposure to specific environmental contamination for two major reasons:

First, biochemical alterations are usually the first detectable, quantifiable responses to environmental change, and as such are often more sensitive indicators than those at higher levels of biological organization. Thus, changes at the molecular level will underlie the effects at higher levels of organization. Depending on the function of the systems affected and the nature of the response, biochemical perturbations can indicate whether additional effects at higher levels of organization are likely to occur.

Second, depending on type of marker and species of interest, biochemical alterations can serve as markers of both exposure and effect. A major group of enzymes, the so-called xenobiotic metabolizing enzymes (XME), have a central role in detoxification of foreign compounds (xenobiotica). This would seemingly make them better indicators of exposure to

damage. In 1987, Payne *et al.* concluded that a variety of organic pollution conditions can result in XME induction in fish, and that the extreme sensitivity of this response might permit discrimination of water quality over broad geographic regions. The changes in XME usually occur within hours or a few days after exposure, and may disappear when the contaminant is removed from the organism. Thus, XME induction has been suggested as a set of parameters to be used as sensitive biomarkers of contaminant exposure and environmental quality.

XMEs include several families and superfamilies. The cytochrome P450 superfamily (P450) is one important group of XMEs investigated as a potential biomarker of exposure to xenobiotics in fish (McCarthy & Shugart 1990; Goksøyr & Förlin 1992; Skaare *et al.* 1991b). The P450s are involved in the first phase (phase I) of the biotransformation of organic chemicals, resulting in molecular changes and either their activation to toxic metabolites or their inactivation. P450 induction by specific classes of hydrocarbon compounds has been well documented experimentally, and has been linked to such chemicals in the environment. Less is known of the utility of P450 as biomarkers of exposure in terrestrial wildlife and invertebrates. Field studies in avian embryos and hatchlings suggest that P450 induction has utility for documenting contaminant exposure; however, findings in adult birds and mammalsare inconclusive (Rattner *et al.* 1989).

Conjugating enzymes aid in the second phase (phase II) of the detoxification and excretion of foreign compounds, including reactive metabolites formed in the first phase, by linking them to water-soluble endogenous compounds. One superfamily of phase II enzymes, the glutathione S-transferases (GST), has been given attention as a marker of development of some cancer forms and as a component playing a role in the acquired resistance of some tumours to chemotherapeutic agents (Mannervik & Danielson 1988). The contribution of GST in insecticide resistance has long been acknowledged (Oppenoorth 1985). Furthermore, atrazine resistance in corn is explained by an increased GST-linked atrazine metabolism (Dauterman 1990, Tu*et al.* 1990). Our preliminary studies have demonstrated significant differences in the GST-linked atrazine metabolism between the male and female mouse *(Mus musculus)*. This is of interest since a mixture of atrazine and simazine induces a variety of tumours in Swiss albino mice (Donna *et al.*, 1981), and rats (*Rattus Norvegicus*, Sprague-Dawley) develop mammary tumours upon treatment with atrazine (Seiler*et al.* 1992). Collier*et al.* (1992) found substantial differences in the GST activities of starry flounder (*Platichtys stellatus*) and English sole (*Pleuronectes vetulus*), which are two closely related benthic fish with substantial differences in prevalence of developing contaminant-associated hepatic cancers. Recently, Pascual *et al.* (1991) have reported different GST activities in the same species of marine fish and molluscs sampled from both polluted zones and non-contaminatedareas, indicating GST as a potential biomarker of pollution.

In both aquatic and terrestrial ecosystems, age, sex, nutrition and season are sources of variation that require consideration when undertaking field trials. Furthermore, little is known about the long-term effects of pesticide-induced changes in insect XME on later developmental stages or generations. A chronic exposure may select the survival of insecticide-resistant strains with inherent higher XME activities, thus disfavouring XME as parameters in situations with incidental exposure. In these cases, however, the XME parameters may have validity as indicators of insecticide resistance (this is discussed by Clark 1990).

In a previous NLVF-supported project, "Comparative aspects of pesticide detoxifying enzymes in the phylogenesis" (1984-88), the cooperate work of the scientists involved at the Norwegian College of Veterinary Medicine/Central Veterinary Laboratory and the Norwegian Plant Protection Institute led to the description of XME activities in more than 60 animal

species (Stenersen *et. al.* 1987). Furthermore, new evidence of an insecticide-mediated change in XME activities (induction/inhibition) in fish and insects has been presented (Egaas et al. 1988 a,b, Jensen *et al.* 1991). These studies were continued in the NLVF and SFT supported project, "Contamination of soils and waters from agricultural and industrial use of pesticides" (1988-92). Some of the results have already been published, documenting that a range of pesticides, including herbicides, fungicides, insecticides, some important polychlorinated biphenyl (PCB) congeners and 2,3,7,8-tetrachloroodibenzo-p-dioxin (TCDD), are potential inducers of XME (Egaas *et al.* 1988a, b, 1989a, b, c, d, 1990, 1991a, b, c, d, 1992a, b, 1993a, b, c, d; Skaare *et al.* 1991a, b, c; Sandvik *et al.* 1991; Jensen *et al.* 1991; Bernhoft *et al.* 1993; Hektoen *et al.* 1993.

The aim of presentation is to summarize and discuss the major results from the last five-year research project on biochemical effects of selected pesticides and some industrial chemicals on terrestrial and aquatic species. The appropriate analytical methods that have been used to detect induction/inhibition events in XME activities will be described. Thereafter, some biological (tissue distribution, developmental stage, sex and strain), environmental (temperature) or chemical (plant allelochemicals, pesticides, PCB and TCDD) factors that may have an influence on XME in the species investigated will be discussed.

MATERIALS AND METHODS

Experimental procedures

The pesticides used throughout this project are described by Lode (this volume). Other materials and equipment have been described in detail by Egaas *et al.* 1988a, b, 1991d, 1992a, b, 1993a; Skaare *et al.* 1991 b; Bernhoft *et al.* 1993 and Hektoen *et al.* 1993.

Females of the Hebrew character moth (*Orthosia gothica L.*), the *Orthosia stabilis L.*, the red sword grass moth (*Xylena vetusta* Hb.) and the small winter moth (*Operophtera brumata* L.) were collected in light traps in southern Norway in the early spring and left in mesh-covered plastic buckets. After 2-4 days, clusters of eggs were collected from the mesh and randomly distributed on leaves of apple (*Malus domestica* cult.), willow (*Salix caprea* L.) or birch (*Betula pubescens* Ehrh.). After hatching, the larvae kept at 23°C and a 16/8 h light/dark cycle were given a fresh supply of leaves every other day or a semi-synthetic diet (Fisher & Otto 1976). One group of *O. gothica* was kept in an out-door shield on fresh leaves and exposed to the temperature and light variations of the spring of 1990. Local meterological data for this period were registered by the Norwegian Plant Protection Institute. During the experimental period, the lowest and highest day-time temperatures were respectively 2.7 and 16.2°C, with a median of 10°C. Two days after the last moulting, the final instar larvae at 23°C on a diet of leaves were given fresh leaves that had been pretreated with solutions for concentrations (see Table 6) of the selected pesticides and then left to dry in the air.

Larvae of the cabbage moth (*Mamestra brassicae* L.) were collected in August in a field of cabbage and allowed to pupate. The pupae were stored at 3°C for at least 10 weeks and then used for establishing a laboratory culture of larvae. After hatching, the larvae grew either on a semi-synthetic diet (Fisher & Otto 1976) or on cabbage. Most of the larvae were kept at 24°C and at a light cycle of 16 h light and 8 h darkness. Two days after the last moulting (20-25 days after hatching), five groups containing 10 final instar larvae of cabbage moth on a semi-synthetic diet were given pesticide in the feed (details in Tables 6, 7 and 8). After 48 h, the larvae were weighed and decapitated. In the long-term exposure experiments, the larvae were given

pesticides in the semi-synthetic diet from the day of hatching. Four days after the last moulting, the larvae within each treatment group were pooled in 5 groups of 10 larvae, counted, weighed and decapitated. In all experiments, after decapitation, the midgut and the soft tissue excluding the digestive tract were separated, and the tissue from pools of 10 larvae was homogized in 0.1 M K-phosphate, pH 7.8, added 1 mM EDTA and 2 mM GSH. Microsomes and post-microsomal supernatant were prepared as described earlier (Egaas *et al.* 1988a).

The peach-potato aphid (*Myzus persicae* S.) originated from a stock kept by parthenogenetic reproduction on second-year swedes (*Brassica napus rapifera* M.), and were a gift from Dr. Eline Hågvar, Agricultural University of Norway. Paprika (*Capsicum annuum* L.) and swedes were grown and infested by aphids as described by Egaas *et al.* 1991d.

Yellow mealworm (*Tenebrio molitor* L.) was initially bought in a local pet shop in Oslo, Norway, and reared and treated with pesticides as described by Egaas *et al.* 1988a.

Final instars of European rhinoceros beetle (*Oryctes nasicornis* L.), garden chafer (*Phyllopertha horticola* L.) and summer chafer (*Amphimallon solstitiale* L.) were collected from natural habitats near Ås, Norway, and treated with pesticides as described by Egaas *et al.* 1992b.

The benthic organisms, *Gammarus lacustris, Baetis rhodani, Heptagenia sulphurea, Hydropsyke siltalai, Physa fontinalis, Isoperla,* Tipulidae sp., Corixa sp. and Leuctra sp. were collected in Maridalen, Oslo, kept in a flow-through system and treated with pesticides as described by Aanes, this volume.

Rainbow trout of both sexes, gonadally immature, weighing 150-270 g, were purchased from a local fish farm (OFA, Sørkedalen, Oslo). The fish were acclimatized at a constant temperature for four weeks prior to the start of the experiments. In the short-term experiments, the fish were fasted and kept in 200 l glass tanks with recirculated (biological filters) and aerated water (specification of conditions in Skaare *et al.* 1991b). The pesticides and the environmental toxicants were administered as described in Table 5. The fish were sacrificed by cervical dissection.The livers were carefully separated from the gall bladder, washed in ice-cold 0.15 M KCl, weighed, and prepared for microsomes and microsomal supernatant as described by Skaare *et al.* 1991b. In the two weeks` experiments, administration of the pesticides through water was carried out at the Norwegian Institute of Water Research in cooperation with Dr. Magne Grande. The fish were kept fasting in runnning water in which the pesticide was continually added. After two weeks, the fish were sacrificed and treated as in the short-term experiment.

Cod (*Gadus morhua*) of cultivated origin were obtained from the Sea Farm A.S., Bergen, Norway. The fish were of both sexes and gonadally immature. The doses of environmental chemicals are given in Table 6, and the experiments are described in detail in Bernhoft *et al.* 1993 and Hektoen *et al.* 1993.

English sole and starry flounder were caught by otter trawl in the Puget Sound, Washington, in an area considered to be relatively non-contaminated. Male and female rats (*Rattus norvegicus*, Sprague-Dawley and Fisher) and mice (*Mus musculus* CD-1, C-57 BL/6, Swiss Webster, DBA/2) were obtained from Charles-Rivers, Raleigh, North Carolina. The phase II metabolism of atrazine in the 100,000 g supernatant of liver from starry flounder and English sole was studied with the 100,000 g supernatant prepared in 0.1 M Na-phosphate, pH 8, 1 mM EDTA and 2mM GSH. The 100,000 g supernatant from 44 day old rat and mouse was obtained in accordance with standard methods (Levi & Hodgson 1983), and for young leaves of common groundsel in accordance with Tu *et al.* 1990.

The chemical assays included ethoxyresorufin O-deethylase (EROD) (Klotz *et al.* 1984

as modified by Skaare *et. al.* 1991b), aldrin epoxidase (AE) (Moldenke & Terriere 1981, modified as described by Egaas *et al.* 1988a, NADPH cytochrome c reductase (NCCR) (modified as described by Egaas*et al.* 1988a), 1-chloro-2,4-dinitrobenzene (CDNB), ethacrynic acid and 1,2-dichloro-4-nitrobenzene (DCNB) (Habig *et al.* 1974), atrazine (Guddevar & Dauterman, 1979) and P4501A1 as measured by the indirect P4501A1 ELISA method of Goksøyr *et al.* 1992. The GST purification method is described in Egaas*et al.* 1991d. Statistical methods were carried out with the Mann-Whitney test for the difference between two population means (Ben-Horim & Levy 1984), using a significance level of $p< 0.05$.

RESULTS AND DISCUSSION

Choice of *in vitro* substrates for activity assays

The *in vitro* studies of XME often involve choice of substrates such that the products formed can be made the basis of convenient spectrometric or chromatographic assays (Table 1). Although the biological significance of some of the substrates is sometimes doubtful, these data can supply two types of valuable information on the status of an XME family. First, the catalytic activities of the 100,000 g supernatant towards specific substrates can be used as a screening device to reveal xenobiotica-mediated changes in XME. Second, certain substrates are only metabolized by specific subfamilies or isozymes of an XME group, making these substrates suitable as biomarkers for inducers of the specific isozymes. Thus, a major feature of the P450 systems in fish as well as in mammals is the discovery of a specific induction of P4501A1 by polyaromatic hydrocarbon (PAH) type compounds. The increased amount of P4501A1 can be evaluated by measuring increases in catalytic activity, using ethoxyresorufin or benzo[a]pyrene as substrates (Table 1). These activities are largely specific in their response to P4501A1. More recently, specific antibodies have also been produced to identify and quantify specific members of the P450 superfamily (Goksøyr 1985). In cooperation with Dr. A. Goksøyr, University of Bergen, we have been able to verify induction/inhibition of P4501A1 in rainbow trout and cod mediated by pesticides and industrial chemicals by using antibodies to cod liver P4501A1.

Although the different XME superfamilies have been well conserved through the phylogenesis, great variations within the subfamilies are found between the species. For example, in contrast to mammals, birds and insects, the evidence for phenobarbital (PB)-type induction, which is mediated by P4502B (Table 1), is ambiguous in fish (Kleinow*et al.* 1987). As for P4501A1, the induction of P4502B can be evaluated by specific substrates. In our studies, we have been using the insecticide aldrin. This is not the most traditional substrate for P4502B, but was chosen because of our studies of aldrin as an inducer of P450 in insects. To our surprise, we found that the P450-mediated conversion of aldrin to dieldrin was inducible in fish (see section headed "Effects of pesticides on *in vitro* XME activities". Unfortunately, no antibodies against P4502B were available at the time when these studies were carried out. In cooperation with Dr J. Stegeman, who is now in possession of these antibodies, we will attempt to verify our results in the near futurue.

Table 1. Examples of model substrates used to identify specific forms of an XME superfamily.

Substrate	Super family	Sub family	Isoenzyme class	Ref.
ethoxyresorufin	P450	1A	1A1	Payne *et al.* 1987
benzo(a)pyrene		1A	1A1	
aldrin		2B		Moldenke & Terriere 1981
1-Chloro-2,4-dinitrobenzene	GST[1]	all	all except 5-5 and 9-9	Habig *et al.* 1974
1,2-Dichloro-4-nitrobenzene		mu	3-3, 3-4	Habig *et al.* 1974
Ethacrynic acid		pi	7-7	Ketterer 1988
1,2-Epoxy-3-(p-nitrophenoxy)propane		theta	5-5	
Coumene hydroperoxide		alpha, theta	2-2, 5-5	

[1]) Rat GSH transferases

The 1-chloro-2,4-dinitrobenzene (CDNB) has traditionally been used as a model substrate for GST activity, since most major subfamilies, or classes, of GST have been considered able to conjugate this molecule to glutathione (Table 1). Thus, compared to some other substrates (Table 1 and the following paragraph), the CDNB assay is considered to be less suitable with regard to the detection of specific isozymes of the GST.

Based on the properties of the purified GST isozymes, three distinct and species-independent classes of the cytosolic GSTs have been suggested: alpha, my and pi. Recently a class theta was also added. In an initial screening of GST in a species, substrate specificity may help to distinguish between GST classes. Ethacrynic acid (ETHA), for example, is considered a fairly specific substrate for class pi in all the mammalian species investigated (Table 1). For some substrates, however, the pattern of specificity may vary between species. Thus, 1,2-dichloro-4-nitrobenzene (DCNB) is a good substrate for class mu in the rat and the mouse, and for classes alpha and pi in the human. In mammals, specific antibodies have been produced for all known classes of GST.

Although the complete primary structures are known for only a few isozymes, it would seem that the primary structure of GST is well conserved throughout evolution. The isolation and purification of non-mammalian GST are, however, often complicated because of the higher instability of these molecules compared to the corresponding mammalian GST. Thus, little attempt has been made to establish whether the classification of alpha, my and pi holds in species other than mammalian. However, Dominey *et al.* 1991 reported that the major GST in the salmonoid fish liver is homologous to class pi, while flatfish liver GST is more related to other classes of GST (George 1989).

We have compared the *in vitro* GST activity towards CDNB, DCNB, ETHA and atrazine in fish, insects, mammalian and one plant species. Atrazine was included since plant GST metabolizes this herbicide with an isozyme that cannot conjugate CDNB to glutathione (Tu *et al.* 1990). We found that the GST activity towards CDNB was evenly distributed between fish, insects and mammals, while the activity towards DCNB was high in terrestrial animals compared to a very low activity in all the fish species studied. The GST activities towards atrazine and ETHA were equally distributed between the species. The results indicate that atrazine, like ETHA, is a specific substrate for class pi GST. Mammalian class pi is normally

not present in the rat and human livers. In the mouse liver, only traces of the pi enzyme are found in the females, whereas it is the quantitatively major GST in the male (McLellan & Hayes 1987). We found that the GST activity towards atrazine was highest in the liver from the male mouse and the trout, and very low in the female mouse and the rat (Egaas *et al.* 1993b). Our observations of the distribution of GST activity towards ETHA and atrazine in the different species support the assumption of pi as a major hepatic GST in the male mouse and the salmonoids. This is interesting, since properties of class pi are used as markers of preneoplastic lesions (Ketterer *et al.* 1988).

XME activity and isozyme composition in different tissues

It appears almost invariably that there is some kind of XME activity in whatever tissue studied. Using fractions obtained from the 100,000 g centrifugations and the most common substrates (see Table 1), the activities are, however, generally highest in preparations from organs with functions analogous to the vertebrate liver (Sipes & Gandolfi 1991; Clark 1989).

In insects, the fatbody has an important liver function in the organism; it serves as a store for food reserves and, in some insects, for the storage of excretory materials (Chapman 1982). It may contribute substantially to the total body weight of the larva. In the full-grown larva of the honey-bee, for example, the fatbody makes up 65% of the total body weight (Wigglesworth 1972). The fatbody is, however, made up of cells aggregated to form a rather irregular and diffuse tissue and may be difficult to separate from other tissues.

When dissecting a tissue for the purpose of XME activity studies, important requirements to be met are low temperature, a rapid speed of handling and obtaining a satisfactory yield of protein. Although studies on XME activity in insect fatbody have been performed for many species, the midgut often fulfils these requirements better and thus has often been the preferred tissue. In studies with the midgut, however, care must be taken to remove all gut contents. Furthermore, the presence of endogenous proteases may destroy XME activities, in particular phase I activities (discussed in Egaas *et al.* 1988a).

We have been studying two types of insect tissue; the midgut (when the size has permitted a proper cleaning for the gut contents) and the mixture of tissue that remains when the cuticle and the whole alimentary canal tract has been removed (named "soft tissue" in this manuscript). A major portion of this tissue consists of fatbody.

In the insect species covered by our investigation, the XME activities were generally higher in the soft tissue compared with the corresponding activities in the midgut. In the two types of chafer that were studied (*P. horticola* and *A. solstitiale*), soft tissue GST activities towards CDNB were indeed extremely high compared to the values normally observed in mammals and fish (Egaas *et al.* 1992b). This may be related to their feeding habits (grass root), or to some important endogenous function of the fatbody. Thus, chafer soft tissue GST may in fact suit as an excellent model for the purification and study of invertebrate GST.

As seen from the ratio between the XME values in soft tissue and midgut, the phase I and II activities were also generally highest in soft tissue in *O. gothica, O. stabilis, X. vetusta* and *M. brassicae* (Table 2). As in the chafers, the GST values for *O. gothica* (Egaas *et al.* 1992 a) were higher than the corresponding activities in mammalian tissues. The XME values observed in the remaining species, were not extreme compared with those of other animals. Several of the XME activities seemed to be influenced by the diet (details on the observations of GST in *X. vetusta* and *O. gothica* are described in Egaas *et al.* 1992a). However, the XME activities in the midgut were generally more easily affected by the diet than the corresponding activities in the soft tissue. Thus, with the phase I activities in *O. gothica* on apple leaves as

the only exception, the diet-related changes in the precentage activity within a species (Table 2) were mainly due to increased activities in the midgut. The general impression was that the midgut XME activities in these insects were lower but more easily inducible compared to the corresponding activities in the soft tissue. We did, however, observe exceptions to this pattern. Thus, in the larva of the *O. nasicornis,* the midgut phase I activities (AE and NCCR) were higher and less inducible by pesticides than the corresponding activities in the soft tissue (Egaas *et al.* 1992b). The GST activities were however equal (CDNB) or higher (DCNB) in soft tissue compared with those in midgut.

Table 2. Activities of some xenobiotic metabolizing enzymes (AE = aldrin epoxidase, NCCR = NADPH cytochrome c reductase, CDNB = 1-chloro-2,4-dinitrobenzene, DCNB = 1,2-dichloro-4-nitrobenzene) in soft tissue as a percentage of the corresponding value in midgut of *Orthosia gothica* L., *Orthosia stabilis* L., *Xylena vetusta* Hb. and *Mamestra brassicae* L. on apple leaves (AL), willow leaves (WL), cabbage (C) or a semi-synthetic diet (SS). The larvae were sacrificed on day 3 of the last instar. The data on CDNB and DCNB in *O. gothica* and *X. vetusta* have been published earlier (Egaas *et al.* 1992a). All values that are significantly different ($p<0.05$) from the corresponding value in the midgut have been marked with an *, and are the means of 5-25 replications from experiments performed over a two year period (except for *O. stabilis*, which was studied in one season only). nc = not calculated because of no detectable activity in the midgut.

Species	Diet	XME activities in soft tissue (% of midgut)			
		AE	NCCR	CDNB	DCNB
O. gothica	AL	3500*	550*	34000*	750*
	WL	110	150*	1900*	56*
	SS	nc	156*	610*	129
O. stabilis	SS	nc	nc	881*	50*
	WL	nc	nc	1164*	24*
X. vetusta	AL	69	135	318	225*
	WL	62	124	145	37*
M. brassicae	C	1100*	300*	500*	50*
	SS	230*	280*	240*	49*

Studies of isozyme composition of different XME superfamilies in mammalian species have revealed substantial tissue differences in isozyme composition. Such tissue-specific differences in the expression of certain isozymes have been related to function and to the state of health (cancer tissues) of the organ in question. The subject has been discussed in numerous papers (e.g. Proceedings of the 6th International Conference on Biochemistry and Biophysics of Cytochrome P450, Vienna, 1988, and the Proceedings of the 3rd International GST Conference, Edinburgh, 1989).

We have not found any papers describing tissue-specific distribution of GST isozymes in insects. Our results from studies on partly purified GST from *X. vetusta*, however, indicate a difference in composition of GST isozymes between soft tissue and midgut in the last instar of this larva (Egaas *et al.* 1992a).

Effects of developmental stage on in vitro XME activities

Several studies in fish have demonstrated different effects of inducing agents depending upon developmental stage (Förlin 1980). The P4501A1 content in the liver of rainbow trout increased following administration of 3-methylcholanthrene and Clophen A50 (a technical PCB mixture), and the observed induction was greater in older fish. Hormonal factors were thought to be involved. We have been using juvenile fish in all our exposure studies. Thus, the observed effects of pesticides and industrial chemicals on hepatic XME activities should be related to this age group only.

In four strains of mouse, we found significant differences in specific GST activities and isozyme composition between immature and mature males but the difference was more pronounced in some strains than in others (details in Egaas *et al.* 1993b). This may be related to genetic influences on the timing of puberty (Nelson *et al.* 1990).

The changes in insect midgut phase I activities with development have been reviewed by Brattsten (1979), who pointed out that high levels of activity in the midgut generally coincide with active periods of life. In the Madagascar cockroach (*Gromphadorhina portenttosa*), active periods with high levels of midgut phase I activity alternate with moulting with very low levels of activities. Furthermore, the level of activity in one active period is always higher than that in the preceding instar (Brattsten 1979).

The study of *G. portenttosa* was made on the midgut; an organ which has to be very active during the feeding periods of the insect. From an energy conservation point of view, it stands to reason that the insect XME activities in the midgut are low during moulting periods without food intake.

Increases in insect phase II activities with increasing instars have been reported for economically important Lepidopterans such as the fall armyworm (*Spodoptera frugiperda*) (Yu 1983) and the *Helicoverpa (Heliothis) zea* (Chien & Dauterman 1991).

In our studies of the effects of developmental stage on phase I and II activities in insects, we have found that not all XME activities necessarily increase with increasing instars. Factors like insect order, which tissue is studied and which XME activity is measured all have to be taken into account. Thus, in the Coleopteran *T. molitor*, all the soft tissue phase I activities studied (AE, NCCR, content of P450 and cytochrome b-5) were inversely related to the weight of the larva in the range 0.10-0.21 g body weight. Furthermore, no changes in the phase II activity (GST activity towards CDNB and DCNB) were observed (Egaas *et al.* 1992b). This is different from *O. portenttosa* and the two Lepidopterans referred to above, but may be explained by the developmental pattern of *T. molitor,* which may pass 9 to 20 instars before pupation (Mehl 1989). The quality of feed and care is probably involved in the triggering of the pupation process (Mehl, personal commmunication). Thus, body weight may not be a good parameter when the purpose is to select *T. molitor* at the same stage of development.

The Lepidopterans seem to have a fixed number of instars before reaching the pupal stage. Furthermore, the different instars are easily distinguishable by the size of the head capsule. Thus, the Lepidopteran larvae included in our exposure studies have a synchronized development. We observed a similar increase in XME activities with increasing instars in these Lepidopterans as was reported for *S. frugiperda* and *H. zea.* Thus, in *O. gothica, X. vetusta* and *M. brassicae*, the GST activities towards CDNB and DCNB generally increased with increasing instars. The only exception was the GST activity in whole homogenates of *O. brumata* which were unchanged between the second and the fifth (last) instar (Egaas *et al.* 1989d).

During the last instar, the soft tissue GST activities changed in all the Lepidopteran larvae to such an extent that this must be considered when evaluating these species as potential

biomarker organisms. Thus, in soft tissues of the last instar of *M. brassicae*, the GST activity with CDNB as a substrate was at an optimum at days 2 and 3 after the moult (Table 3). With DCNB as a substrate, the GST activity was highest at day 5. This indicates a change in the GST isozyme composition during the last instar. A similar pattern of variations in the GST activities was observed for *O. gothica* soft tissue (Table 4), only that in this type of larva, the optimas for CDNB and DCNB coincided. In midguts of both *M. brassicae* and *O. gothica* reared under laboratory conditions, only the GST activity towards DCNB changed during the last instar (Tables 3 and 4). Furthermore, in both types of larva, the DCNB activity increased significantly just before pupation. Thus, in both soft tissue and midgut, it would seem that the GST isozymes with specific DCNB activity become more important towards the end of the larval stage.

Table 3. Body weight (mg) and GST activities (nmol/min, mgp) towards CDNB and DCNB in soft tissue and midgut on each day of the last instar of the *Mamestra brassicae* L. on a semi-synthetic diet. Each number is the mean of four replicated experiments, each containing tissue from 10 larvae.

	Weight	Soft tissue		Midgut	
Day	(mg)	CDNB	DCNB	CDNB	DCNB
1	314±28	2007±318	27± 7	910±100	25± 6
2	500±17	3280±275[a]	40±11	1094±84	45±38
3	586±26	3260±560[a]	39±12	955±200	45±23
4	682±30	2330±280	28± 8	605± 50	32±12
5	684±21	2704±210	51±12[a]	805± 67	57±16[a]

[a] = Significantly different ($p<0.05$) from the corresponding value at day 1

In the two days exposure studies, the larvae are usually killed on day 3 of the last instar. We have not found any indication in the literature that sublethal doses of pesticides can provoke an earlier start of pupation and, as a secondary effect, a change in the GST isozyme composition in specific tissues. This is, however, a possibility that should be considered in later studies of effects of low levels of pesticides on insect XME.

Effects of sex on *in vitro* XME activities

Sexual differences in the P450 mediated metabolism of drugs have been rather extensively studied in mammals (Sipes & Gandolfi 1991) and fish (Kleinow *et al.* 1987). Thus, in all our studies, care was taken to use only-juvenile fish. Much less information is available on such differences in phase II reactions (reviewed by Mulder 1986). However, sex-related differences in hepatic GST isozyme composition have been observed in the rat (reviewed by Ketterer et al.1988) and in the mouse (McLellan & Hayes 1987).

Table 4. GST activities (nmol/min/mgp) in soft tissue and midgut of *Orthosia gothica* L. on willow leaves, on days 1, 2, 3 and 5 of the last instar. The larvae were kept at 23°C or in an outdoor shield where they were exposed to the diurnal temperature fluctuation of June 1991. The results are means of four replicated experiments, each containing tissues from 10 larvae.

Day	Weight	Temp	Soft tissuses		Midgut	
	(mg)	°C	CDNB	DCNB	CDNB	DCNB
1	157 ± 22	23	9750 ± 1500	49 ± 10	1380 ± 380	170 ± 30
	204 ± 8	D*	8700 ± 1000	90 ± 11[c]	1030 ± 270	100 ± 30
2	243 ± 12	23	11700 ± 1120	40 ± 5	1625 ± 580	170 ± 60
	260 ± 18	D	13600 ± 800	55 ± 22[a]	800 ± 120	120 ± 8[c]
3	364 ± 14	23	19900 ± 3600[a]	84 ± 11[a,b]	1780 ± 640	200 ± 20
5	421 ± 7	23	15600 ± 1400[a,b]	74 ± 8[a,b]	1860 ± 430	200 ± 30
	434 ± 6	D	47000 ± 1000[a,b,c]	150 ± 50[a,b,c]	3000 ± 320[a,b,c]	280 ± 45[a,b,c]

* = Day and night fluctuations
[a] = Significantly different ($p<0.05$) from the corresponding value at day 1
[b] = " " " " " " " " 2
[c] = " " " " " " larvae kept at 23°C

Using the hepatic mouse GST activities towards CDNB, DCNB, atrazine and ETHA, we observed significant differences between the sexes. These differences were present in assumed immature as well as mature animals; however, no such differences were observed in the rat (Egaas *et al.* 1993b). These findings can be explained as a difference in the distribution of the GST class pi in the mouse (discussed under 'choice of in vitro substrates for activity assays'), since class pi is normally not present in rat liver cells.

Effects of strain on *in vitro* XME activities

Although significant interstrain differences in the amount of GST class pi were observed between four strains of the mouse, class pi was present in all the strains investigated (Egaas *et al.* 1993b). This is in accordance with McLellan & Hayes (1987), who concluded that in spite of some differences in GST activities in three strains of mouse, no interstrain differences in the properties of the individual GSTs were observed.

Effects of ambient temperature on *in vitro* XME activities

The liver microsomal P450 dependent system in fish responds to acclimation temperature in a compensatory manner. Fish acclimatized to colder temperatures exhibit higher P450 enzymatic activity than those acclimatized to warmer temperatures (Stegeman 1979). Low temperature also influences the process of P450 induction by increasing the time necessary to reach the maximal enzyme activity in the rainbow trout (Egaas & Varanasi 1982; Andersson & Koivusarri 1985). There is a limited amount of information regarding effects of temperature

& Koivusarri 1985). There is a limited amount of information regarding effects of temperature on phase II activities. Cytosolic GST displayed a marked seasonal variation in plaice, with the highest activity in March to April (George & Buchanan 1990). In the present fish studies, care was taken to avoid temperature effects. Thus, the fish were acclimatized for several weeks and the temperature kept constant throughout the experiments.

GST activities in *O. gothica* larvae kept in outdoor conditions (the highest day- and night fluctuations observed were between 2.7 and 16.2°C) or at a constant room temperature (23°C) were roughly similar until the last day before pupation, when the activities in the larvae kept under outdoor conditions were 2-3 times higher (Table 4).

It remains to be investigated whether changes in the isozyme composition are also involved. Thus, growing up in outdoor conditions did seem to exert an influence on the GST activities, but only at the end of the last instar. The day/night light period was very similar in the field and the laboratory set up. Even so, factors other than the temperature may still be responsible for the differences observed in the GST activities between the laboratory and the field conditions.

Effects of diet on *in vitro* XME activities

Two P450 isoenzymes that are believed to have evolved in response to dietary allelochemicals in mammals, P4502B and 3A, also seem to be higher in fish that feed on a diet rich in allelochemicals. One observation of changes in GST activity in response to certain allelochemicals has also been presented as a paper at the PRIMO Conference in Gothenburg (Vrolijk *et al.* 1993). In addition, antioxidants in the diet induce GST activitiy in fish (George & Buchanan 1990). In the present fish studies, the fish were not fed for two days before each experiment.

Molecular aspects of insect-plant interaction have recently been extensively reviewed (edited by Brattsten & Ahmad 1986). Strong evidence indicates that the XME activities of herbivorous insects are related to feeding strategies. Furthermore, this relationship may affect the ability of insects to metabolize and tolerate synthetic insecticides.

We found that the activities of AE, NCCR and GST towards CDNB and DCNB in whole homogenates of peach-potato aphids on swedes were respectively 0.4, 1.6, 2.0 and 4.6 times the corresponding activities in aphids on paprika. Further purification of GST revealed the presence of different GST isozymes in aphids on swedes and paprika (Egaas *et al.* 1991d).

In the final instars of *X. vetusta* (midgut and soft tissue) and *O. gothica* (midgut) reared on leaves from apple, GST activities were significantly higher than in the corresponding tissues in larvae on willow (Egaas *et al.* 1992a). Similarily, the GST activities in whole body of the last instar of the small winter moth were significantly higher by respectively 150 and 200% (CDNB) and 200 and 300% (DCNB) in the larvae fed leaves of willow or birch compared with the larvae fed on apple leaves (Egaas *et al.* 1989d). Furthermore, on a diet of cabbage, the cabbage moth GST activity towards CDNB was significantly increased by 260 and 120 % in soft tissues and midgut respectively compared with the corresponding value in cabbage moth on a semi-synthetic diet (see section "Effects of pesticides on *in vitro* XME activities in insects on a natural diet").

Effects of PCB and 2,3,7,8 TCDD on *in vitro* XME activities

The industrial chemicals, polychlorinated biphenyls (PCBs), are complex mixtures of isomers and congeners which have been detected in air, water, wildlife and human beings throughout the world. The individual PCBs differ in persistence in the environment and in toxicological mechanism and potency depending on the chlorine number and substitution pattern of the

biphenyl rings. The mono-ortho chlorine analogues of the most toxic PCBs the coplanar 3,3',4,4'-tetrachlorobiphenyl (PCB-77), 2,3',4,4,5'-pentachlorobiphenyl (PCB-118) and 2,3,3', 4,4'-pentachlorobiphenyl (PCB-105) are of the major PCB congeners found in fish and marine animals along the coast of Norway (De Boer 1988; Jensen & Sundstrøm 1974; Muir*et al.* 1988; Kannan *et al.* 1989). The biological effects of the mono-ortho PCBs contribute considerably to the total PCB toxicity (Ahlborg *et al.* 1992). Thus, mono-ortho PCBs are known to induce both the P4501A and P4502B subfamilies in mammmals (Parkinson *et al.* 1980; Safe1984; Safe *et al.* 1985; Clarke 1986).

In fish very little is known about the structure-induction relationship of PCBs. Our studies have included i.p. and oral administration (for details of PCB isomers, doses and species, see Table 5) to gonadally immature cod and rainbow trout. The results have already been published (Skaare*et al.* 1991b; Bernhoft*et al.* 1993) but are summarized in Table 5. Both PCB-118 and PCB-105 were moderate inducers of the subfamily cytochrome P4501A1 in rainbow trout liver. P4501A1 is considered an early indicator of environmental exposure to organic contaminants, as discussed earlier in this paper. Cod exposed to PCB-105 did not show significantly different enzyme and ELISA values from the controls. The distribution pattern of the highly lipophilic PCB-105 differed between fish species. Thus, the potential for induction of XME seemed to be lower in cod as compared with rainbow trout.

The industrial byproduct 2,3,7,8-tetrachlorodibenzo-p-dioksin (TCDD) is known as one of the most potent inducers of the hepatic P450 system in mammals and fish (Huff*et al.* 1980; Lech & Bend 1980; Goksøyr & Förlin 1992). In our studies, TCDD was administered orally twice at 4-day intervals to juvenile cod and rainbow trout (for details, see Table 5). A very strong induction of EROD activity, supported by induction of P4501A was found in both fish species. Furthermore, the relative induction of EROD activity in rainbow trout was 3-5 times higher than that in cod. The results have already been published (Hektoen *et al.* 1993). The difference between rainbow trout and cod may reflect interspecies differences in sensitivity to TCDD which also was supported by clinical symptoms. No induction was found of AE or GST in any of the species. In contrast, rainbow trout exposed to PCB-105 and 2,3,7,8-TCDD had significantly reduced GST activity towards CDNB. It was suggested that this depression could be due to an analytical assay effect, however, the depression could also be a toxic effect. Furthermore, exposure time may play a role in the response of the GST system since longer exposure time is required for GST induction compared to induction of P450 activites (Andersson *et al.* 1985; Boon *et al.* 1992).

Effects of pesticides on *in vitro* XME activities

The pesticides used in this project have been described by Lode (this volume), and only properties important for effects on the XME will be discussed in this text. The effects of the various pesticides on XME in rainbow trout liver and midgut, soft tissue or whole homogenate of various insects are summarized in Tables 5, 6, 7 and 8, respectively. Since some of these results have already been published in detail earlier, the purpose of the present review is to

focus on comparative aspects of species and pesticides.

Table 5. Effects of different pesticides and some environmental toxicants on activities of some xenobiotic metabolizing enzymes (EROD = ethoxyresorufin O-deethylase, AE = aldrin epoxidase, CDNB = 1-chloro-2,4-dinitrobenzene, DCNB = 1,2-dichloro-4-nitrobenzene, P4501A1 as measured by the indirect P4501A1 ELISA method of Goksøyr *et al.* 1991. Arrows indicate significant different from corresponding control values. Empty circles indicate that no effect was found.

1: Short term experiments. Endosulfan was given as i.v. injections (75 ug/kg) to rainbow trout (*Oncorhynchus mykiss*), and the fish were sacrificed after day 1 (for details, see Jensen *et al.*1991b). Atrazine, propiconazole, dimethoate, MCPA, dichlorprop and chlorsulfurone were given to rainbow trout i.p. on 5 (atrazin), 4 (dimethoate) or 3 (the remaining pesticides) consecutive days (the respective doses were 20, 25, 20, 200, 200, 200 ug/kg) and the fish were sacrificed one day after the last injection (details on atrazine in Egaas *et. al.* 1993). PCB-118 was injected i.p. (30 mg/kg) and the fish were sacrificed after four days (Skaare *et al.* 1991b). PCB-105 and TCDD were administered orally twice with two day interval to cod (*Gadus morhua*) and rainbow trout (PCB-105; 10 mg/kg (Bernhoft *et al.* 1993), TCDD 5ug/kg (Hektoen *et al.* 1993). The fish were sacrificed on days 5 and 13 after the last administration.

2: Two-week experiment. The pesticides were administered to rainbow trout (*Oncorhynchus mykiss*) or brook trout through water for 14 days. The doses were; endosulfan (8.3 ppb) (Jensen et al. 1991); atrazine 10 ppb (Egaas *et al.* 1993a), dimethoate (1 ppb) and propiconazole (20 ppb).

	EROD		AE		GST				ELISA	
					CDNB		DCNB			
	1	2	1	2	1	2	1	2	1	2
ENDOSULFAN [1]	↑	↑	↑	↑		○		○	↑	
ATRAZINE[1]	○	○	○	○	○	○		○		
DIMETHOATE [1]	↓		↑						↓	
DIMETHOATE [3]	○	○	○	↑		↑		↑		
PROPICONAZOLE [1]	○	○	○	↓	↑	○		○		
MCPA [1]	○		○		○					
DICHLORPROP [1]	○		○		○					
CHLORSULFURONE [1]	○		○							
PCB-118 [1]	↑		○						↑	
PCB-105 [1]	↑		○		↓				↑	
PCB-105 [2]	○		○		○					
2,3,7,8-TCDD [1]	↑		○		↓				↑	
2,3,7,8-TCDD [2]	↑		○		○				↑	

[1] rainbow trout
[2] cod
[3] brook trout

Endosulfan is an organochlorine insecticide and acaricide which is considered to have the same mode of action on the insect central nervous system as lindane and the cyclodienes. In spite of a low solubility in water, endosulfan is more rapidly metabolized by hydrolysis than most other organochlorine insecticides. Thus, the use of endosulfan has not been restricted in the same way as other members of this particular group of insecticides. It is, however, extremely toxic to fish. Furthermore, like DDT, endosulfan is an effective inducer of hepatic XME in the mouse (Fabacher *et al.* 198O, Kulkarni *et al.* 1980).

In trout, as little as 8.3 ppb of endosulfan administered through the water sufficed to increase phase I activities (Table 5). Furthermore, the induction was verified by specific antibodies towards cod P4501A1 (Jensen *et al.* 1991). The effects of endosulfan on XME in different insect species are reviewed in Tables 6 and 7. With the exception of cabbage moth, these experiments and results have been presented in detail in earlier publications (Egaas *et al.* 1988a, b, 1992a, b). Endosulfan induced phase I activities in most of the species, while GST activites were induced only in the three Lepidopterans, not in the Coleopterans and the trout. Thus, GSTs in the Coleopterans studied seem to have both different activity development during the larval instars (discussed earlier in this manuscript) and different ability to be induced by endosulfan compared to the Lepidopterans investigated.

Atrazine is a persistent s-triazine herbicide of widespread use on essential crops (Seiler *et al.* 1992; DeMarco 1992). At certain periods each year, most major rivers in Western Europe exceed the EEC drinking water directives for atrazine (Van Hoof *et al.* 1992; Foster *et al.* 1992; Reme 1992). Despite its remarkably low acute toxicity to mammals (Seiler *et al.* 1992), information on the metabolic fate of atrazine is necessary to establish the safety of atrazine. Most studies have been performed from the residue point of view, demonstrating that essentially all agricultural products are free of the parent compound after exposure to the herbicide through the feed (Knuesli *et al.* 1969). Some investigations have, however, dealt with the general detoxification and excretion of atrazine. The dominant phase I metabolic reaction in plants and mammals seems to be a P450-mediated N-dealkylation (Dauterman & Muecke 1974; Adams *et al.* 1990), while the primary phase II reaction is the GST catalysed conjugation with glutathione (Guddewar & Dauterman 1979; Adams *et al.* 1990). In plants (reviewed by Ketterer 1988, and Dauterman 1990) the ability of GST to conjugate atrazine to GSH is important in conferring plant resistance to this herbicide. Furthermore, apparent induction of GST can be produced by treating seeds with "safeners", thus creating crops more tolerant to atrazine treatment. A sex-related induction of P450 and GST has been reported in rats and offspring after chronical exposure to atrazine in the diet (Ugazio *et al.* 1991). Those results also indicate that atrazine and/or metabolites of atrazine are transferred to the offspring via the mammary and/or placental route. Furthermore, an increased incidence of mammary gland tumours was recorded in females of the Sprague-Dawley strain (Donna *et al.* 1981). A causal link between the tumour formation and a triazine-mediated hormonal imbalance has been suggested (Seiler *et al.* 1992).

In the trout and *M. brassicae,* only the highest dose of atrazine increased AE (Tables 5 and 7). Insect GST activity, however, was induced by lower doses of atrazine than were needed for the AE induction (Egaas *et al.* 1993a). Furthermore, after a life-long exposure to atrazine in the diet, the GST activity towards DCNB in the last instar of *M. brassicae* was significantly inhibited compared to the controls. This indicates that during the growth, a selection of larvae (or the induction of specific isozymes in the larvae) with a different GST isozyme composition in the exposed animals has occurred.

A 14-day exposure to a realistic dose of atrazine (20 ppb) in the water did not significantly affect GST activities in the limnic invertebrates *G. lacustris* and a species of *Corixa* (Egaas *et al.* 1991b).

Dimethoate is considered a moderately water-soluble organophosphorus systemic insecticide of moderate persistence (Hassal 1990). An inhibitory effect on haem biosynthesis in the mouse has been reported (El-Toukhy *et al.* 1989). Thus, dimethoate may indirectly disturb the synthesis of specific subfamilies of the haem-protein P450. This may explain the, at first sight, conflicting results of dimethoate on phase I activities in the trout, which indicated

that P4501A1 was inhibited at the highest dose whereas the aldrin epoxidase activity was induced (Skaare *et al.* 1991c). Dimethoate treatment affected the GST activities as well. In the trout, activities towards both CDNB and DCNB were induced after two weeks of realistic concentrations (1 ppb) of dimethoate in the water (Table 5). However, the possibility of dimethoate mediating such an effect in the marine environment may be rather small. In limnic invertebrates, a 14-day exposure to a realistic dose of dimethoate (1 ppb) induced the GST activity towards CDNB in *Leuctra.* Furthermore, in 2-day exposure studies, dimethoate also induced GST activities in the *O.gothica* and *M.brassicae* (Table 7, and Egaas et al. 1991a). However, in life-long exposure studies with *M. brassicae*, higher doses of dimethoate in the diet were inhibitory on the activity towards DCNB (Table 7).

Table 6. The effects of technical grade endosulfan (Thiodan 35) on xenobiotic metabolizing enzymes (NCCR = NADPH cytochrome c reductase, AE = aldrin epoxidase, CDNB = 1-chloro-2,4-dinitrobenzene, DCNB = 1,2-dichloro-4-nitrobenzene) on some insect species. Arrows indicate significant difference from corresponding control values in either soft tissue or/and midgut. Empty circles indicate that no effect was found. For 24 h, last instars of *O. gothica* and *X. vetusta* were given leaves of willow (*Salix Caprea* L.) that had been dipped in a solution of 7 ppm endosulfan and then air-dried (Egaas *et al.* 1992a). Last instar of *M. brassicae* were given endosulfan for two days in the semi-synthetic diet (50 ppm) or cabbage that had been dipped in a 1 ppm solution and then air-dried, both experiments giving similar effects on the parameters. *T. molitor*, *O. nasicornis* and *P. horticola* were given doses of endosulfan ranging from 1 to 1000 ppm in a basal flour mix, manure and garden soil respectively (Egaas *et. al. al.*1988a, b and 1992b)

Species	NCCR	AE	GST	
			CDNB	DCNB
O. gothica	↑	○	↑	↑
X. vetusta	○	○	↑	↑
M. brassica	↑	↑	↑	↑
T. molitor	↑	↑	○	○
O. nasicornis	↑	↑	○	○
P. horticola	↑	↑	○	○

Propiconazole belongs to a group of triazole agricultural fungicides which is known to act through an inhibition of an endogenous form of P450 catalysing the 14-alpha demethylation of lanosterol, an important intermediary in the synthesis of cholesterol. The binding to haem iron is rather unspecific and several of these compounds are potent inhibitors of many P450 dependent activities. On the other hand, propiconazole, like several of these C-14 demethylation inhibitors, is an effective inducer of total P450 (reviewed by Hassal 1990). Prochloraz, which belongs to the same group of fungicides, has been reported to be a mixed inducer of P450 in the rat (Laignelet *et al.* 1989). Propiconazole in low concentrations through the water

decreased AE activity in the trout, whereas higher i.p. doses did not have any effect on AE (Table 5). These, at first sight, conflicting results at different levels of propiconazole exposure may be the superimposed result of several parallel events in the endoplasmic reticulum. At low doses of propiconazole, the inhibition of AE may be a result of competitive inhibition, which at higher concentrations may be masked by a specific P450 induction. Furthermore, a low concentration of propiconazole induced the GST activity towards CDNB in the trout. In a 14-day exposure to a low dose of propiconazole (5 ppb) in the water, the *G. lacustris* GST towards CDNB was induced by 40% compared to the control. Furthermore, evidence indicated that GST activity towards CDNB in some mayfly and *Corixa* were also induced by the treatment (Egaas et al. 1991c). Propiconazole also induced GST activities in *O. gothica* (Egaas *et al.* 1991c) and cabbage moth (Table 7).

Table 7. Effects of some pesticides mixed in a semi-synthetic diet for two days or from hatching on xenobiotic metabolizing enzymes (AE = aldrin epoxidase, NCCR = NADPH cytochrome c reductase, CDNB = 1-chloro-2,4-dinitrobenzene, DCNB = 1,2-dichloro-4-nitrobenzene) in the last instar of *M. brassicae*. Arrows indicate significant difference from corresponding control values in either soft tissue or/and midgut. Empty circles indicate that no effect was found. For details, see Egaas *et al.* 1991d and 1993a. The doses were; atrazine 1:500 ppm for two days, 2:5 000 ppm for two days, 3:500 ppm from hatching, propiconazole 1:100 ppm for two days, 2:100 ppm from hatching, 3:500 ppm from hatching, dimethoate 1:100 ppm for two days, 2:1 ppm from hatching, 3:10 ppm from hatching, MCPA 1:10000 ppm for two days, dichlorprop 1:5000 ppm for two days.

PESTICIDE	NCCR	AE	GST	
			CDNB	DCNB
	1 2 3	1 2 3	1 2 3	1 2 3
ATRAZINE	○ ↑ ○	○ ↑ ↑	↑ ↑ ↑	↑ ↑ ↑
PROPICONAZOLE	↑ ○ ○		↑ ○ ↑	↑ ○ ↑
DIMETHOATE	↑ ○ ○	○ ↑ ○	↑ ○ ○	○ ○ ↑
MCPA	↑	↑	↑	↑
DICHLORPROP	○	○	↑	↑

We have not found any information on effects of the herbicide chlorsulfuron on XME. The phenoxyacids, however, are known to have potent effects on the metabolism of xenobiotica in mammals. Thus, MCPA increased P450 activities and inhibited GST activities in rat liver (Hietanen *et al.* 1983). None of the herbicides MCPA, dichlorprop or chlorsulfuron induced XME in the trout liver (for doses and administration routes, see Table 5). In *M. brassicae*, however, MCPA and dichlorprop induced the GST activities towards CDNB and DCNB (Table 7).

Effects of pesticides on *in vitro* XME activities in insects on a natural diet

As has been demonstrated and discussed in the preceding sections, both the natural diet and pesticides may have a considerable influence on the *in vitro* XME activities in animals. How

specific, then, could the XME system be as a biomarker of pesticide contamination in the environment? In other words, will we ever be able to distinguish between a dietary, and a pesticide-induced change in *in vitro* XME activities?

In our project, we have studied the specificity of XME induction in allelochemical- or pesticide-induced insects by measuring changes in *in vitro* activities and by purifying different XME and searching for the production of isozymes specific for the chemical(s) used as inducer.

In *M. brassicae,* the plant allelochemicals present in the cabbage increased the GST activity level compared to the larvae on a semi-synthetic diet, but only when measured with CDNB as the substrate (Table 8). Furthermore, the phase I activities AE and NCCR were not significantly different in the larvae on the two diets. In contrast, the GST activity towards DCNB, the AE and the NCCR activities were induced when the cabbage moth larva was exposed to endosulfan both in cabbage and in the semi-synthetic diet, while the CDNB activity was unchanged (it is worth noting that the dose of endosulfan used on cabbage in this experiment was only 1/50 of the recommended dose for agricultural purposes). Thus, a screening of several *in vitro* XME activities in this case gave specific patterns for the two types of treatment.

In *X. vetusta* reared on leaves from apple and willow, the GST activities (CDNB and DCNB) were different for the two types of food. However, the same isozymes were present in midgut and soft tissues of the larvae on both feeds. Endosulfan treatment of the willow and apple leaves, in contrast, led to the appearance on SDS-PAGE of a new band, indicating the presence of an endosulfan-induced GST isozyme (Egaas *et al.* 1992a). It remains to study whether this GST isozyme is inducible by still other chemicals. However, in this case, the chemical effected a specific isozyme of one XME family. Thus, a study of to detect which of the XME activities affected and whic of the isozymes specifically induced may have a potential as specific biomarkers of pesticide contamination. However, the methods for the detection will have to be specially designed for each species and type of chemical.

Table 8. Activities of aldrin epoxidase (AE), NCCR (NADPH cytochrome c reductase), 1-chloro-2,4-dinitrobenzene (CDNB) and 1,2-dichloro-4-nitrobenzene (DCNB) as a percentage of the corresponding activity in the respective controls in soft tissue (S) and midgut (M) of last instar of *Mamestra brassicae* L. on a semi-synthetic diet (24 replicated experiments) containing 50 ppm of endosulfan or cabbage leaves (12 replicated experiments) that had been dipped in a 1 ppm solution of endosulfan corresponding to 1/50 of the recommended dose and then air-dried. Each replication contained tissues from 10 larvae. The values are given as a percentage of the corresponding value in the respective controls.

Enzyme activity	Tissue	Synthetic diet	Cabbage leaves
AE	S	373*	427*
	M	nc	nc
NCCR	S	135	100
	M	220*	137*
CDNB	S	108	104
	M	138	107
DCNB	S	200*	183*
	M	246*	205*

a = significantly different ($p<0.05$) from the control. nc = values not calculated because of a very low control value.

CONCLUSIONS

For five years, we have been studying biochemical effects of selected pesticides, industrial chemicals and one of their by-products on terrestrial and aquatic species. One major goal has been to contribute to the on-going discussion on whether the XME parameters can be used as biomarkers for pesticide and industrial chemical contamination. The results of our efforts can be concluded as follows:

1. Our results support the use of the phase I reaction (P4501A1) as a biomarker for contamination of specific chemical structures (PAH-type compounds) in the aquatic environment. There are, however, important species differences to be considered. Furthermore, the specificity of the reaction has not been fully investigated. Thus, the pesticide dimethoate, which does not have a chemical structure resembling the PAH-type compounds, is an inhibitor of P4501A1 in fish. Thus, although most of the pesticides tested did not have any effect on the P450 system in fish, we need more screening of the mechanisms involved in the induction process and of the chemical structures that may interfere with P4501A1 before this system can be fully used as a specific biomarker of PAH-type contamination.

2. The use of the phase II reaction with GST as a biomarker of contamination of pesticides and industrial chemicals in the aquatic environment needs even more investigation than the P450 system before it can be seriously discussed. Our results from experiments with pesticide-exposed limnic invertebrates indicate that insect GST activities may be changed by the presence of low levels of certain pesticides in the water. The specificity of this induction has, however, not been considered since in the herbivorous fish and insect larvae, plant allelochemicals also have a potential as inducers of XME.

3. In terrestrial organisms, XME activities have a tradition as biomarkers for populations of insects that through selective breeding have become resistant to certain insecticides. The usefulness of insect XME activities as biomarkers of a pesticide contamination situation has not been studied earlier. We have documented that several factors (develomental stage, diet, sex) other than pesticide exposure may induce changes in the XME activities. Of these, dietary factors seem to be particularly important in insects. However, our experiments also indicate that in selected species, a combination of information on several XME activity parameters and a study of specific isozymes in specific tissues may have a potential as a selective system for pesticide contamination in terrestrial as well as aquatic systems.

SUMMARY

1. Induction/inhibition of P4501A1 in rainbow trout and/or cod due to exposure to endosulfan, dimethoate, PCB-118 and PCB-105 has been documented using enzyme assays and/or antibodies towards cod liver P4501A1.

2. Induction of P4502B in rainbow trout due to exposure to endosulfan, atrazine, dimethoate and propiconazole has been indicated by using an enzyme assay with the insecticide aldrin as substrate.

3. Induction of GST in rainbow trout due to exposure to dimethoate and propiconazole exposure has been indicated by using enzyme assays.

4. Exposure to low concentrations of the pesticides in the water sufficed to induce/inhibit fish XME parameters. Thus, unless further evaluated as XME inducers, pesticide contamination may complicate the use of XME activites as biomarkers for contamination of PAH-similar compounds.

5. In the cabbage moth, lower concentrations of the pesticides (endosulfan, atrazine, propiconazole, dimethoate, MCPA and dichlorprop) were needed to induce the GST activities compared to the P450 system. However, in the Coleopterans studied, endosulfan was a better inducer of the P450 activity than of the GST.

6. The *in vitro* GST activities towards CDNB, DCNB, ETHA and atrazine in fish, insects, mammalian and one plant species were distributed so that the GST activity towards CDNB was evenly found in fish, insects and mammals, while the activity towards DCNB was high in terrestrial animals compared to a very low activity in all the fish species studied. The GST activities towards atrazine and ETHA were equally distributed between the species. These results indicate that atrazine is a very specific substrate for class pi GST. Furthermore, class pi seems to be a major GST in the male mouse and the salmonoids.

7. When initiating the study of XME activities and induction processes in an insect, care must be taken to control the sampling of specific tissue from synchronous cultures with a similar genetic and feeding background. Our results indicate, however, that pesticide exposure may give characteristic changes in a set of XME parameters, so that it eventually may be distinguished from induction processes due to diet or developmental processes.

ACKNOWLEDGEMENTS

This project was funded by the Agricultural Research Council of Norway and the State Pollution Control Agency. The pesticide exposure studies of benthic organisms were performed by Torleif Baekken and Karl Jan Aanes, the Norwegian Institute for Water Research, as a part of this project. We thank the Ciba-Geigy Co. for their generous gift of ^{14}C atrazine, atrazine and atrazine-GSH conjugate. One of the authors spent a year at North Carolina State University, Dept. of Toxicology, and the work on rat and mouse was performed in cooperation with J.G. Falls and Dr. W.C. Dauterman at this institution. Dr. U. Varanasi and Dr. T. Collier have cooperated on the flounders, and Dr. A. Goksøyr, University of Bergen, has kindly verified P450 induction in the trout by using polyclonal cod antibodies.

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Effects of agricultural pesticides on planktonic algae and cyanobacteria – examples of interspecies sensitivity variations

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Källqvist, T. & R.Romstad 1994. Effects of agricultural pesticides on planktonic algae and cyanobacteria - examples of interspecies sensitivity variations. Norwegian Journal of Agricultural Sciences. Supplement No. 13: 117-131. ISSN 0802-1600

The effects of seven pesticides (atrazine, simazine, MCPA, dichloroprop, chlorsulfuron, propiconazole and dimethoate) on the growth rate of six species of freshwater phytoplankton have been investigated using a microplate technique. The interspecies variation in EC_{50}-values ranged from a factor of 4.8 for atrazine to almost 10^4 for propiconazole. Results from the microplate tests with *Selenastrum capricornutum* were in reasonably good agreement with those obtained using the standard OECD bottle test. The results indicate that the herbicides atrazine, simazine and chlorsulfuron and the fungicide propiconazole may affect natural populations of algae in streams draining arable land. The phenoxy-herbicides MCPA and dichloroprop and the insecticide dimethoate are not likely to reach concentrations toxic to algae in natural waters. It was concluded that simple multispecies tests may provide useful information for hazard assessment of chemicals.

Key words: Algae, pesticides, phytoplankton, toxicity

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A wide range of pesticides is used to protect agricultural crops. Although the instructions for use of these chemicals aim at minimizing the risk of contamination of aquatic environments, residuals of pesticides can be detected in water courses draining agricultural areas (Nilsen 1989; Torstensson 1989). The concentrations reported are usually below those known to cause acute toxic effects on aquatic organisms, but for many pesticides, toxic effects have been investigated only for a rather restricted number of species, usually fish and invertebrates.

Algae and cyanobacteria are known to be comparatively sensitive to many chemicals, and the inclusion of these organisms in test batteries has been shown to improve the capacity of the battery to predict the most sensitive ecosystem responses (Slooff *et al.* 1983). Furthermore, the importance of these organisms as dominating primary producers in most aquatic ecosystems speaks for their use in test batteries for environmental hazard assessment.

Phytoplankton is an extremely diverse group of organisms, physiologically as well as phylogenetically, comprising 12 different classes of eucaryotes in addition to the procaryotic cyanobacteria. This implies that the sensitivity to toxic chemicals may be quite different for different phytoplankton species. Blanck *et al.* (1984) found that the interspecies variation in

sensitivity among 13 freshwater algal species could be more than three orders of magnitude, and that no species was universally the most sensitive when a range of chemicals was tested.

It is generally believed that the interspecies variation in sensitivity is less for chemicals with a nonspecific mode of action than for those which act specifically. This has been demonstrated by LeBlanc (1984), who found less variation in susceptibilities among different organisms to non-pesticides than to pesticides. Pesticides are purposely specific in their mode of action and, consequently, a large variation in sensitivity among species may be expected.

In order to investigate whether agricultural pesticides can affect the growth of algae and cyanobacteria in the concentration range that has been observed in water courses draining arable land, six high-volume pesticides were selected for toxicity testing with phytoplankton. The pesticides included five herbicides, one fungicide and one insecticide. These are listed in Table 1.

Table 1. Pesticides used in the toxicity tests with phytoplankton

Pesticide	Trade name	Type	Category
Atrazine	Primatol A	triazine	herbicide
Simazine	Gesatop	triazine	herbicide
MCPA	San-75	phenoxy	herbicide
Dichloroprop	Hedonal DP	phenoxy	herbicide
Chlorsulfuron	Glean	urea, triazine	herbicide
Propiconazole	Tilt 250 EC	triazole	fungicide
Dimethoate	Rogor L 20	organophosphorus	insecticide

MATERIALS AND METHODS

The pesticides were tested as composite commercial products. The amount of active ingredients was found to be within 100-116% of the nominal concentrations specified by the producers (specific analysis performed at the Agricultural University, Ås, Norway. Eklo pers. com.).

Six species of phytoplankton including cyanobacteria, green algae, a diatom and a cryptophycean were selected for the toxicity tests (see Table 2).

Table 2. Phytoplankton species used in the microplate toxicity tests

Species	NIVA-strain	abbreviation
Selenastrum capricornutum	CHL 1	Sel.
Chlamydomonas noctigama	CHL 25	Chlam.
Cyclotella sp.	BAC 8	Cycl.
Cryptomonas pyrinoidifera	2/81	Crypt.
Microcystis aeruginosa	CYA 43	Micr.
Synechococcus leopoliensis	CYA 20	Synec.

Toxicity tests of propiconazole were also carried out with three additional strains of *Chlamydomonas, C. reinhardtii* (CHL 13 and CHL 21) and C. *cf. obesa* (CHL 24).

All species are held in pure (non-axenic) cultures at the culture collection of the Norwegian Institute for Water Research (Skulberg & Skulberg 1990). Inoculum cultures were incubated in an inorganic nutrient medium Z8 (Staub 1961), diluted to 20% concentration in glass-distilled water at the same light and temperature conditions as during the tests. The toxicity tests were started while the inoculum cultures were in the exponential growth phase.

The toxicity tests were carried out on polystyrene microplates with 6 x 4 flat bottom wells of 3 ml capacity. The inoculum cultures were diluted with growth medium (20% Z8) to give a cell density of 10^7 cells/ml. Each well on the plates was filled with 1.8 ml of the dilute cultures.

Solutions containing 3.2 and 1.0 g/l of active ingredient were prepared from each pesticide. Of the 3.2 g/l solution, 0.2 ml was added to the first well on the plates. Similarly, 0.2 ml of the 1.0 g/l solution was added to the second cell. The cell contents were mixed and 0.2 ml transferred from well no. 1 to no. 3 and from no. 2 to no. 4. The serial dilutions were repeated down the row of wells on two plates to give 11 concentrations in the range 320 mg/l to 0.0032 mg/l. Some tests were repeated with a closer spacing of concentrations to obtain a better defined response curve.

The microplates were incubated on a reciprocal shaker continuously illuminated by cool-white fluorescent tubes. The photon flux was approx. 70 $\mu E\ m^{-2} s^{-1}$ in tests with green algae and approx. 30 $\mu E\ m^{-2}\ s^{-1}$ for the other species.

The incubation period was restricted to the exponential growth phase of the control cultures. Since the maximum growth rates differed among the species, the test duration was adjusted accordingly to 3 days for the green algae, 5 days for *Synechococcus leopolensis* and 6 days for the remaining species.

At the end of the test, the cell density in each well was measured by counting using a Coulter Multisizer. The average growth rate during the test was calculated as suggested by Nyholm & Källqvist (1989), from the initial and final cell density.

The growth rate at each pesticide concentration was calculated as a percentage of the control growth rates and plotted against the logarithm of concentration. A curve was fitted to the points by third level polynomial, and the EC_{50}-value derived from the curve.

In addition to the microplate technique described above, all pesticides were also tested on *Selenastrum capricornutum*, using a conventional bottle test according to the OECD Guideline 201 (OECD 1984). Bottle tests were also used to study the effect of propiconazole on *Chlamydomonas noctigama*. The growth medium and incubation conditions were the same as those for the microplate tests. The growth rate in each culture was calculated as for the microplate tests (average growth rate for 72 h) and normalized against the growth rate in control cultures (mean value of six controls). The normalized responses were transformed to probit values (Finney 1952). The response curve with confidence limits was determined by least squares linear regression (Guttman et al. 1971) of probit values against the logarithm of concentrations. The EC_{50} and EC_{10}-values were calculated from the regression equation.

RESULTS

Response curves showing the effect of each pesticide on the six species of phytoplankton are shown in Figs. 1-8. The EC_{50} values are listed in Table 3.

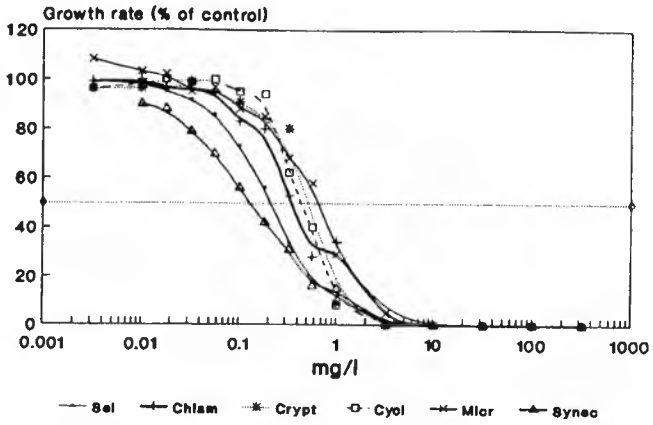

Fig 1. Effect of atrazine on the growth rate of 6 species of phytoplankton. (Abbreviations, se table 2).

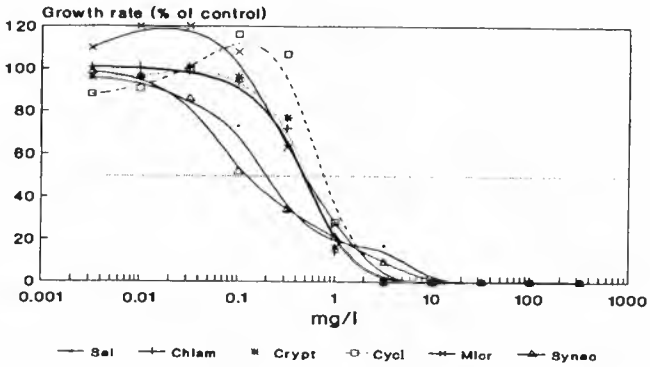

Fig. 2. Effect of simazine on the growth rate of 6 species of phytoplankton. (Abbreviations, see table 2)

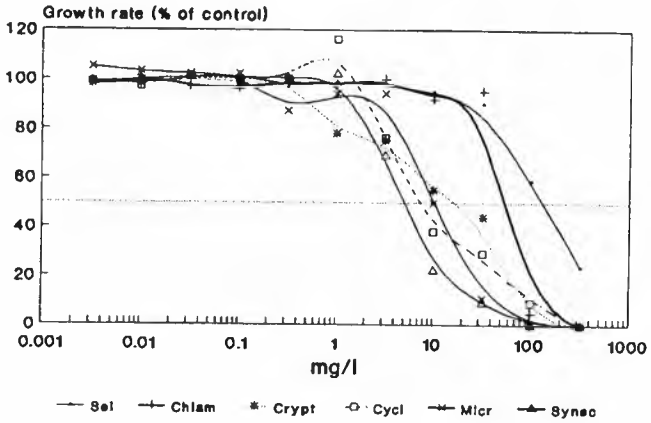

Fig. 3. Effect of MCPA on 6 species of phytoplanktorn (Abbreviations, see table 2)

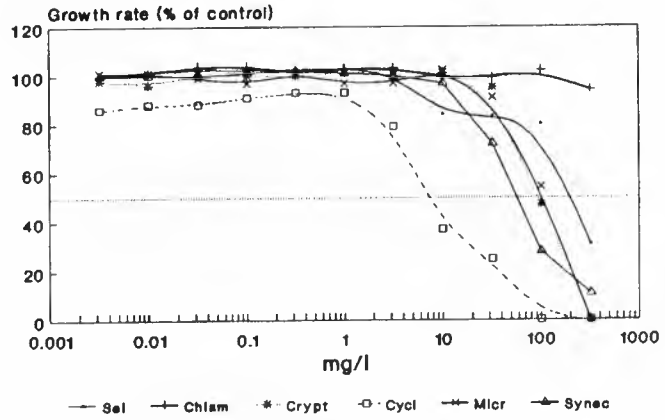

Fig. 4. Effect of dichloroprop on 6 species of phytoplankton. (Abbreviations, se table 2)

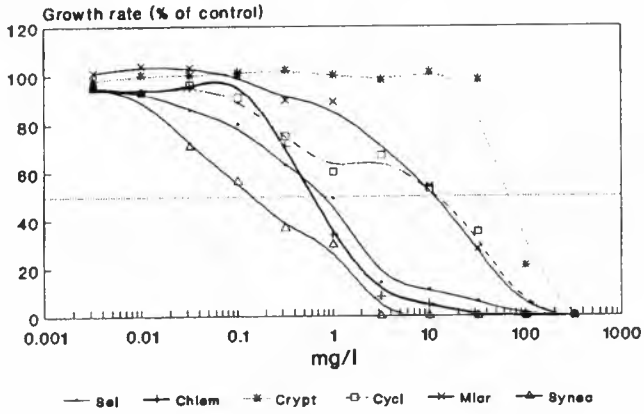

Fig. 5. Effect of chlosulfuron on 6 species of phytoplankton. (Abbreviations, see table 2)

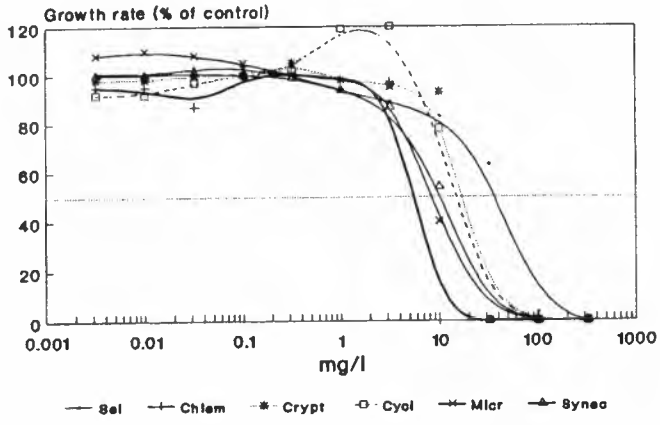

Fig. 6. Effect of dimethoate on 6 species of phytoplankton. (Abbreviations, see table 2)

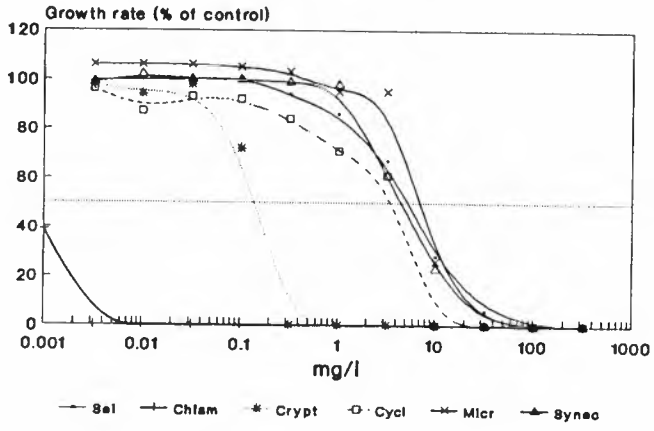

Fig. 7. Effect of propiconazole on 6 species of phytoplankton. (Abbreviations, see table 2)

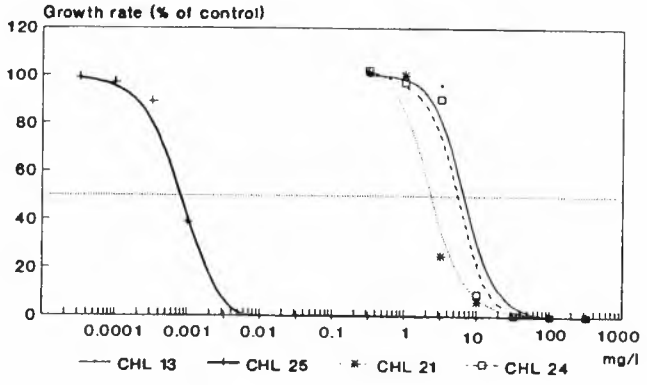

Fig. 8. Effect of propiconazole on 4 strains of Chlamydomonas spp. (CHL 13 and CHL 21 = C. reinhrdtii, CHL = C. cf. obesa, CHL 25 = Chlamydomonas noctigama

The response of the phytoplankton species to the two triazines, atrazine and simazine, was rather similar. The growth rate of the most sensitive species, *Synechococcus leopoliensis*, was reduced at concentrations above 0.01mg/l, and the EC_{50}-values were 0.13 and 0.12 mg/l for atrazine and simazine respectively. The total range of EC_{50}-values was only a factor of 4.8 for atrazine and 6.7 for simazine. Low concentrations of simazine stimulated the growth of *Microcystis aeruginosa* and *Cyclotella sp.* No growth was observed for any of the algae at concentrations of the triazines above 10 mg/l.

Table 3. EC_{50}-values (mg/l active ingredient) for the effect of pesticides on the growth rate of the tested phytoplankton species. (Abbreviations, see table 2).

Pesticide	Sel.	Chlam.	Cycl.	Crypt	.Micr.	Synec.
Atrazine	0.20	0.33	0.43	0.50	0.63	0.13
Simazine	0.20	0.45	0.80	0.50	0.50	0.12
MCPA	120	50	7	15	10	5
Dichloroprop	200	>320	7	90	7	100
Chlorsulfuron	0.8	0.6	10	60	10	0.15
Propiconazole	5.0	0.0008	3.3	0.13	10	4.5
Dimethoate	35	5.5	14	16	8.5	10

MCPA did not affect any of the species at concentrations below 1 mg/l. At higher concentrations first *Cryptomonas pyrenoidifera* and then *M. aeruginosa* were inhibited. The two green algae were the least sensitive to MCPA. The EC_{50}-values ranged from 5 mg/l for *S. leopoliensis* to 120 mg/l for *S. capricornutum*, i.e. a range factor of 24.

Dichloroprop was, for most of the phytoplankton, the least toxic herbicide. The exception was the diatom *Cyclotella sp.*, which was affected at concentrations above 3 mg/l and the EC_{50} value was 7 mg/l. The least sensitive species, *C. noctigama* was not significantly affected even at the highest concentration tested (320 mg/l) and the total range factor for the EC_{50} values is thus >46 for dichloroprop.

Toxic effects of chlorsulfuron were observed above 0.01 mg/l on *S. leopoliensis*, while *C. pyrenoidifera* was unaffected up to 32 mg/l. The slope of the concentration/response curves for this herbicide was comparatively gentle, indicating a wide concentration range between no effect and total growth inhibition. The EC_{50}- values ranged from 0.15 mg/l (*S. leopoliensis*) to 60 mg/l (*C. pyrenoidosa)*, i.e. a total range factor of 400.

The insecticide dimethoate reduced the growth of the tested species only at concentrations above 1 mg/l. *C. noctigama* was the most sensitive, and *S. capricornutum* the least sensitive, but the range of EC_{50} values was only 5.5-35 mg/l (range factor 6.4).

The widest range of sensitivity was found for the fungicide propiconazole. Four of the tested species were significantly affected only at concentrations above 1 mg/l, with EC_{50}-values between 3 and 7 mg/l. *C. pyrenoidifera* was much more sensitive, with EC_{50} at 0.13 mg/l. For *C. noctigama*, the concentration range had to be extended below 1 µg/l to obtain a complete response curve. The growth rate was reduced above 0.3 µg/l and the EC_{50}-value was 0.8 µg/l. The total range of EC_{50}-values thus spanned almost four orders of magnitude.

Because of the exceptionally high sensitivity to propiconazole shown by *C. noctigama*, the effect of this pesticide was tested also on three other *Chlamydomonas* strains to investigate whether high sensitivity to propiconazole was a general property of the genus or unique to the species *C. noctigama.*

The response curves for the four *Chlamydomonas* strains (see Fig. 8.) show that the three additional strains tested were not particularly sensitive to propiconazole. The EC_{50}-values were in the same range as for the majority of phytoplankton species, i.e. 2.2 mg/l (*C. reinhardtii* strain CHL 21), 5.4 mg/l (*C.* cf. *obesa*) and 6.5 mg/l (*C. reinhardtii* strain CHL 13) . Thus, it appears that the extreme sensitivity of *C. noctigama* to propiconazole is not a characteristic of *Chlamydomonas* species in general.

In order to further establish the effect of propiconazole on *C. noctigama*, toxicity tests were carried out with this species according to the OECD guideline 201 (OECD 1984), using

the same formulation (Tilt 250 EC) and the pure chemical obtained as analytical master standard (CIBA-GEIGY CGA 64250). This was done to verify that the toxicity was caused by the active ingredient in the formulation. The test with the formulation showed an EC_{50} value of 0.9 µg/l with 95% confidence limits 0.75-1.1 µg/l), which is in agreement with the results from the microplate tests (see Fig. 9).

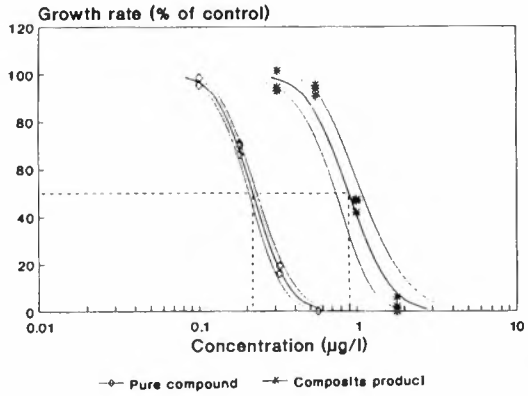

Fig. 9. Effect of propiconazole as pure compound and composite formulation (tilt EC 250 on the growth rate of Chlamydomonas noctigame (bottle tests)

In the test with the pure compound, the growth rate of *C. noctigama* was reduced already at 0.18 µg/l, and the EC_{50}-value was calculated at 0.22 µg/l with confidence limits 0.21-0.23 µg/l. The significantly higher toxicity of propiconazole as pure compound as compared to the formulation shows that the toxicity is caused by the active ingredient, and that the carrier in the pesticide formulation reduces the toxic effect on *C. noctigama.*

Bottle tests were also carried out with *S. capricornutum* on the six pesticides to check whether the microplate tests give similar results to those with the standard OECD procedure. The EC_{50} and EC_{10}-values from the bottle tests are presented in Table 4.

Table 4. EC_{50} and EC_{10} values (mg/l active ingredient) obtained from OECD bottle tests with *Selenastrum capricornutum*

Pesticide	EC_{50}	95% conf. limits	EC_{10}	95% conf. limits
Atrazine	0.11	0.099-0.111	0.027	0.024-0.030
Simazine	0.35	0.33-0.38	0.091	0.086-0.096
MCPA	297	228-385	54	42-70
Dichloroprop	200	175-225	74	61-90
Chlorsulfuron	0.31	0.29-0.33	0.036	0.030-0.043
Propiconazole	1.87	1.75-2.00	0.61	0.54-0.67
Dimethoate	14	13-15	3.4	2.8-4.1

DISCUSSION

The screening tests performed with six phytoplankton species show that, for certain chemicals, sensitivity may vary over a wide range (factor 10^4). Furthermore, no species was consistently the most sensitive to all pesticides tested. These findings are in agreement with previous reports (Blanck *et al.* 1984).

Some pesticides, e.g. dimethoate and simazine, caused an increase in the growth rate for certain species at low concentrations. This phenomenon, known as hormesis, is well known from toxicity tests where sublethal, continuous responses such as growth are studied (Stebbing 1982).

The significance of the large interspecies variation in sensitivity among phytoplankton species is not obvious. Exposure of a natural phytoplankton community to a toxic chemical will tend to extinguish the most sensitive species. Because of the generally high reproduction potential of phytoplankton, other species will rapidly replace those that disappear, and the standing crop and level of primary production may remain merely unchanged. The new community will have a higher tolerance to the specific pollutant, as has been shown experimentally by Blanck *et al.* (1988), who introduced the term "pollution induced community tolerance" (PICT) for this phenomenon.

The loss of a few, particularly sensitive, phytoplankton species from a community containing hundreds of species may not be considered significant, as long as the function of the community remains unchanged. However, this may be one of the most sensitive reactions of an ecosystem to a pollution stress, and thus be an important indication of the potential of ecologically harmful effects. Qualitative changes in the phytoplankton community may also have consequences for higher trophic levels, if edible species are replaced by less edible species.

If structural changes in phytoplankton communities are considered as ecologically significant, toxicity screening tests to find the range of response among a battery of species should be a more fruitful approach than to determine the EC_{50}-value of one single species of green algae with a high level of precision. Similar views have been expressed as conclusions of some recent reviews on algal toxicity (Goulding & Adams 1985; Lewis 1990a and b.

Table 5. EC_{50}-values for effect of atrazine on algae (mg/l)

Alga	Effect parameter	EC_{50}	Reference
Cyclotella meneghiniana	photosynthesis	0.099-0.105	Millie & Hersh 1987
8 species	photosynthesis	0.037-0.308	Larsen *et al.* 1986
Scenedesmus subspicatus	area under growth curve	0.11	Geyer *et al.* 1985
5 species	growth rate	0.1-5	Stratton 1984
5 species	photosynthesis	0.1-0.5	Stratton 1984
Skeletonema costatum	growth	0.265	Walsh 1983
Selenastrum capricornutum	produced biomass	0.059	Turbak *et al.* 1986
6 species	growth rate	0.13-0.6	This study
Selenastrum capricornutum	growth rate	0.11	This study

The microplate technique used in this study allows a sufficiently precise determination of EC_{50}-values for an assessment of the range of sensitivity among the species used. With this approach it is possible to produce toxicity data for six species with a degree of effort comparable to that

of a traditional single species bottle test.

An important consideration when using the microplate technique in toxicity tests is the possible consequences of miniaturization and use of plastic materials for the outcome of the test compared to a traditional bottle test. The use of polystyrene instead of glass and the higher surface/volume ratio in the microplate wells may decrease the actual concentration to which the algae are exposed, especially for less soluble chemicals. Blaise et al. (1986), who used 96-well microplates with only 0.2 ml culture volume, found slightly less toxic responses to heavy metals with algae in microplate tests than in bottle tests. Generally, however, there was good agreement between the two methods in describing toxicity of metals and waste waters. The capacity of the microplate wells used in this study was 10 times higher than that used by Blanck et al. (1984) and Blaise et al. (1986), which means that the surface/volume ratio was much lower.

The difference in EC_{50}-values obtained from the microplate and bottle tests was on an average a factor of 1.98, and 2.67 at the most. The bottle tests with *S. capricornutum* gave lower EC_{50}-values for four pesticides, higher for two, and identical values for the last pesticide (dichloroprop). This divergence between the two methods must be regarded as acceptable, in view of the variation that is usually experienced in data generated from toxicity tests. A ring test organized by ISO showed, for example, that bottle tests performed according to a standardized method with *S. capricornutum* at 10 laboratories yielded range factors for EC_{50}-values of 1.75 to 3.3 for four chemicals, after deletion of some extreme results that were believed to have been caused by insufficient pH control. (Hanstveit & Oldersma 1981).

For several of the pesticides tested in this study, no data on toxicity on algae appear to have been published. Hence, no comparison with previously reported toxicity can be made. For atrazine, which is probably the most studied pesticide as far as effects in the aquatic environment are concerned, several records on algal toxicity can, however, be found. Some of these have been compiled in Table 5. Most of the EC_{50}-values reported are in the range 0.1-1 mg/l, which is a comparatively narrow range, considering that several different species as well as test procedures and effect parameters are included. Reviews of toxicity to algae of other pesticides and surfactants have shown much wider ranges (Goulding et al. 1985; Lewis 1990). The results of the microplate tests are thus in agreement with previously published toxicity ranges for atrazine. EC_{50}-values that have been published for dimethoate (4.5-13 mg/l for effect on biomass production of five marine and freshwater algae) (Ibrahim 1983, 1984) are also in agreement with the level of toxicity found in this study.

The present study demonstrated that the toxicity of simazine was comparable to the related atrazine. This is in agreement with studies of effects on photosynthethis in isolated chloroplasts, which have shown only slightly lower EC_{50}-values for atrazine than for simazine (Cesareo et al. 1987). In contrast, Turbak et al. (1986) reported an EC_{50}-value as low as 0.61 µg/l for the effect of simazine on biomass production of *S. capricornutum*, while the corresponding value for atrazine was 100 times higher.

The results of the algal toxicity tests show that the phenoxy-herbicides MCPA and dichloroprop have a low toxicity to algae, and that effects on natural populations of algae in streams draining arable land are unlikely to result from proper use of these pesticides. Analyses of water from streams in the Scandinavian countries have not shown concentrations of these herbicides in the range that is toxic to algae. Traces of MCPA and dichloroprop were found in several streams in Norway, but the highest concentrations recorded were 2.6 µg/l MCPA and 6.2 µg/l dichloroprop (Nilsen 1989). Similar levels (0.1-8 µg MCPA/l and 0.1-16 µg dichloroprop/l) have been reported from Swedish rivers (Brink 1985;Torstensson 1989).

For the insecticide dimethoate, effects on algae in natural waters are unlikely to result from normal application on arable land, but toxic effects on invertebrates have been observed at concentrations in the ppb range (Bækken & Aanes 1991).

The toxic effects of triazines, which have also been documented in numerous earlier studies, indicates that these pesticides may cause ecological effects at environmentally realistic concentrations. deNoyelles et al. report that atrazine has been detected in many natural waters in concentrations of 0.1-30 µg/l. The highest concentration found in a survey of Swedish rivers was 6 µg/l Torstensson 1989). The lowest concentrations that affected the growth of algae were only slightly higher than this level.

The relevance of toxicity tests with algae for predicting ecological effects of atrazine has been demonstrated through model ecosystems and ecosystem tests, which have shown effects at the community level in the same concentration range as that in the single species tests (deNoyelles et al. 1982; Larsen et al. 1986; Moorhead & Kosinski 1986; Krieger et al. 1988; Pratt et al. 1988; Stay et al. 1989).

The effect of chlorsulfuron is very species dependent, and the most sensitive algae (the green algae) were almost as sensitive to chlorsulfuron as to atrazine. The OECD bottle test with *S. capricornutum* showed 10% reduction of the growth rate (EC_{10}) at 36 µg/l, which is only slightly higher than for atrazine. The analyses of stream waters in Scandinavia have, however, not revealed concentrations in this range. Bechmann et al. (1990) measured the concentration of chlorsulfuron from a treated field and found up to 0.6 µg/l.

The extremely high interspecies variation in sensitivity to propiconazole indicates that this pesticide may have effects on the species composition in algal communities at environmentally realistic concentrations. Eklo this volume measured up to 7 µg/l in ditch water from a field after spraying and estimated that 0.33% of the applied pesticide was washed out over a period of approx. four months. *C. noctigama*, which was particularly sensitive to this fungicide, was significantly affected at concentrations down to 0.018 µg/l of the pure substance.

SUMMARY

The study of the toxic effects of pesticides on several phytoplankton species have shown that the interspecies variation in sensitivity may be considerable (up to a factor of 10^4) for certain chemicals. The sensitivity is not necessarily connected with phylogenetic position, which implies that a certain test alga should not be considered as representative for the phylum or genus to which it belongs, as far as sensitivity to chemicals is concerned.

For hazard assessment purposes, a range of EC_{50}-values obtained from simple screening tests with a battery of test algae will provide more useful information than an EC_{50}-value determined with a high degree of precision for one test alga. However, it is also important that the screening tests are performed according to sound principles so as to avoid, e.g., high inoculum density and inadequate pH -control.

The microplate technique used for the multispecies screening tests showed acceptable agreement with the standard OECD bottle test.

Among the pesticides tested, the phenoxy-herbicides MCPA and dichloroprop and the organophosphorous insecticide dimethoate showed low toxicity to algae, and effects of these pesticides on natural algal communities are unlikely to occur in streams draining arable land.

The triazine herbicides atrazine and simazine affected the growth of algae at concentrations

that have been recorded in natural waters, and the use of these pesticides may therefore cause environmental effects. Chlorsulfuron had a toxicity comparative with that of atrazine for the most sensitive species and should also be considered as a potential environmental risk, although concentrations in the toxic range have not been recorded in Scandinavian waters.

The fungicide propiconazole has the potential to affect the species composition of algal communities at environmentally realistic concentrations because of the extremely high interspecies variation in sensitivity that was found for this pesticide.

ACKNOWLEDGEMENTS

The study was funded by the Agricultural Research Council of Norway and the Norwegian State Pollution Control Agency .

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Effects of agricultural pesticides on freshwater plankton communities in enclosures

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Källqvist T., M. I. Abdel-Hamid & D. Berge 1994. Effects of agricultural pesticides on fresh water plankton communities in enclosures. Norwegian Journal of Agricultural Sciences. Supplement No. 13: 133-152. ISSN 0802-1600

The effects of four pesticides; chlorsulfuron, propiconazole, dimethoate and glyphosate, on lake phytoplankton communities have been studied in a mesocosm experiment. The pesticides were added at initial concentrations of 1, 10 and 100 µg/l to 20 m^3 enclosures containing lake water. At the highest concentration, all pesticides affected the biomass development, measured as chlorophyll-a. With the exception of chlorsulfuron, this was also reflected by an initial slight depression of photosynthesis and a lower rate of nitrate assimilation. Effects on the biomass development were also observed at 10 µg/l. The species diversity was reduced as compared to control enclosures at all pesticide treatments. Chemical analyses showed that approx. 100% of the dimethoate, 80% of the glyphosate and 60% of the propiconazole remained in the water column after 16 days. The results indicate that structural changes on phytoplankton communities can occur at environmentally realistic concentrations of all tested pesticides (1-10 µg/l). Short-term functional effects can be induced at concentrations in the range 100 µg/l.

Key words: Mesocosms, pesticides, photosynthesis, phytoplankton, species diversity, toxicity

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The use of agricultural pesticides can present a risk of environmental effects in natural waters if loss of pesticides occurs in runoff from arable land after spraying. Several studies have revealed that pesticide residues occur regularly in surface waters in agricultural areas (Nilsen 1989; Torstensson 1989; Kreuger 1992). During the summer of 1987, eight Norwegian waterbodies were monitored for a selection of commonly used pesticides in agriculture (GEFO and SPV 1987). In six of these water bodies a variety of pesticides were found in concentrations ranging from the detection limit (0.1 µg/l) up to about 15 µg/l (Lode, this volume).

In 1989, the Norwegian State Pollution Control Authority and the Agricultural Research Council of Norway launched a research programme on contamination of soils and water from agricultural and industrial use of pesticides. Experimental studies have been performed in the laboratory and in the field to assess the effects of several extensively used pesticides on aquatic life (Bækken & Aanes 1990, Källqvist & Romstad, this volume). These studies have indicated that toxic effects may occur at environmentally realistic concentrations of some of the pesticides investigated. The variation in sensitivity, even among related organisms, to pesticides has been shown to be substantial. This may have consequences for the community and ecosystem levels, where both the structure and function of the systems may be altered by pesticide stress.

One effect of exposure to low concentrations of pesticides may be an increased tolerance to the pesticides due to selection of more tolerant species and individuals as well as physiological adaptation. This phenomenon, which has been termed "pollution-induced community tolerance" (PICT), is described by Blanck *et al.* (1988).

While single species laboratory tests can provide useful information on concentration/response relationships and the potential for a chemical to cause ecological effects, the actual, direct and indirect effects of exposure of natural ecosystems can never be accurately predicted. Experiments in mesocosms, which are confined compartments of natural ecosystems, may give useful information on the mechanisms which are not included in the single species tests, but which are decisive for the effect of chemicals in the natural ecosystem. Mesocosm experiments with plankton communities are usually carried out in enclosures suspended in the water (limnocorrals).

The present paper describes an experimental study that was carried out in July-August 1990 on the effects of four pesticides on natural phytoplankton communities in limnocorrals.

MATERIAL AND METHODS

The study was performed in enclosures (limnocorrals) of 4 m depth and 2.5 m in diameter, making a total volume of 20 m^3. The enclosures were situated in Lake Omdalsvatn, 50 km north of Oslo. The lake is oligotrophic (total P = 7 mg/l, total N = 200 mg/l, Secchi depth = 6 m), medium rich in calcium (19 mg Ca/l) and slightly alkaline (pH between 7.5 and 8.3). The maximum depth is 35 m, and the depth at the experimental site is 12 m. The thermocline was located at about 6 m depth during the experiment.

The enclosures were filled by means of an electric outboard engine pumping water from about 0.5 to 1 m depth into the enclosure through a narrow V-notch formed by pressing down the enclosure rim between two suspensions. It was assumed that the phytoplankton introduced by this filling technique was representative of the lake epilimnion phytoplankton. As it was observed that the zooplankton migrated downwards from the surface layer during daytime, the enclosures were stocked with a vertical zooplankton net haul (45 µm mesh size) from 10 m to the surface.

Based on experience from earlier enclosure experiments in the lake, nutrient depletion occurs rapidly in enclosures filled with the oligotrophic lake water. (Berge & Källqvist 1990). To avoid this, the water in the enclosures was enriched by the addition of 5 µg P/l as K_2HPO_4 and 50 µg N/l as $NaNO_3$.

Fourteen enclosures were used in the experiment. Two served as controls. To the remaining 12 enclosures four pesticides were added from stock solutions to obtain the concentrations 1 µg/l, 10 µg/l and 100 µg/l. The pesticides included two herbicides, glyphosate and chlorsulfuron, a fungicide, propiconazole and an insecticide, dimethoate. The experimental setup is presented in Fig. 1. Both nutrient and pesticide additions were followed by 3 min. of thorough mixing of the water using the electric outboard engine.

Integrated samples from 0-3.5 m depth were taken with a flexible tubesampler. Sample volume varied from 15 to 16 l. The samples were poured into a 20 l plastic can from which subsamples for water chemistry, chlorophyll, phytoplankton and measurement of photosynthetic activity were taken after mixing.

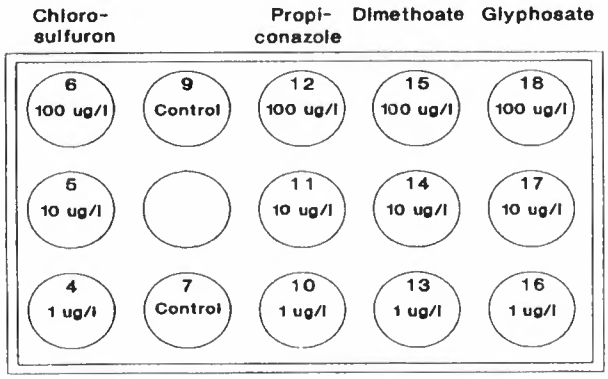

Fig. 1. Experimental setup for enclosusre experiment in Lake Omdalsvatn

The integrated water samples from each enclosure were analysed for plant nutrients (total P, PO_4, total N and NO_3) as well as chlorophyll-a after 2, 6, 9 and 13 days. The initial concentrations were found by analysis of samples from the two control enclosures after addition of nutrients (5µg P and 50 µg N). Water samples for pesticide analysis were taken at the start and at the end of the experiment (16 days).

The photosynthetic activity of the phytoplankton was measured after 2, 6, 9 and 13 days. The incubations were started immediately after sampling in a field laboratory. Integrated samples from each enclosure were transferred to 25 ml glass bottles with stoppers (two light bottles and one dark bottle from each enclosure) and 0.2 ml of a ^{14}C-labelled $NaHCO_3$ solution (activity 4 µCi/ml) was added to each bottle. The bottles were incubated for 2 h on a rotating wheel in a water bath at 20°C under constant illumination from fluorescent tubes. (Gargas 1976). The flux of PAR was approx. 14×10^{-2} cal cm^{-2} min^{-1}. After incubation the bottles were transferred to an insulated dark box, and the contents filtered through 25 mm membrane filters with 0.45 µm pore size. The filters were transferred to scintillation vials which were left open for the filters to dry.

Five ml of scintillation fluid (Opti-fluor) was added to each vial before measuring ^{14}C activity in a liquid scintillation counter (Packard Instruments Tri Carb.).

The degree of adaptation of the phytoplankton community to the pesticides was investigated after 13 days', incubation. Aliquots of the integrated samples from the enclosures with the highest added concentration (100 µg/l) of the four pesticides, and from one control enclosure (no. 9) were transferred to 25 ml bottles with glass stoppers, to which the pesticides were added in 0.00, 0.10, 0.32, 1.0, 3.2 and 10 mg/l concentrations. The phytoplankton from the control enclosure was tested with all four pesticides, while those that had been exposed to 100 µg/l of the different pesticides were tested with the same pesticide only. This was done to discover whether the tolerance of the phytoplankton community in enclosures exposed to pesticides differed from that in the control cultures. The photosynthetic activity of the phytoplankton in each bottle was measured as described above.

The phytoplankton samples were settled and counted using an inverted microscope. Specific volumes were measured and used together with algal density for calculating the biovolumes (mm^3 /l). Algal taxa (species and varieties) were identified according to Prescott (1962), Desikachary (1959), Hustedt (1930), Skuja (1948) Javronicky & Popovsky (1971), Ettl

(1983), Komàrek & Fott (1983), and Starmach (1985).

The Shannon-Wiener index (Shannon & Weaver 1963) was used to calculate the species diversity. The index is usually expressed as :

$$d = - \sum_{i=1}^{s} p_i + \log_2 p_i$$

where"s" is the number of species and "p_i"is the proportion of the total sample belonging to the "ith" species.

RESULTS

Pesticide analysis

The results of analysis of pesticide concentrations in the enclosures are presented in table 1. The initial concentrations were close to the nominal concentrations in all enclosures. After 16 days, the propiconazole concentrations had dropped to approximately 60% of the initial values. (The sample from the 100 µg/l enclosure was lost). For dimethoate, only a marginal reduction in the concentrations was recorded. Obviously no significant degradation of this pesticide occurred during the experiment. For glyphosate, the detection limit was inadequate for analysis of the two lowest concentrations. At 100 µg/l of glyphosate, the concentration dropped to 80% of the initial concentration after 16 days. No method was available for analysis of chlorsulfuron.

Table 1. Pesticide concentrations in enclosures at the start and end (16 days) of the experiment. All concentrations are given as µg/l

Encl. no.	Pesticide	Nominal conc	Conc. day 0	Conc. day 16
10	Propiconazole	1	1.12	0.67
11	Propiconazole	10	9.41	5.3
12	Propiconazole	100	92.5	-
13	Dimethoate	1	1.0	0.9
14	Dimethoate	10	12	11
15	Dimethoate	100	105	101
16	Glyphosate	1	n.a.	<20
17	Glyphosate	10	<20	n.a.
18	Glyphosate	100	98	81

Plant nutrients

The initial concentrations of phosphorus and nitrogen compounds in the control bags (nos. 7 and 9) indicate that the content of bag 7 was not completely homogenized before the sample was taken. The average initial concentrations of total P and N in the control bags were 10 and 245 µg/l respectively. The concentration of total P remained almost constant in the control enclosures throughout the experiment, with average levels of approx. 11µg/l. (see Fig. 2). Total N decreased slightly towards the end of the experiment from the initial 245µg/l to 214 µg/l after 13 days (See Fig. 3).

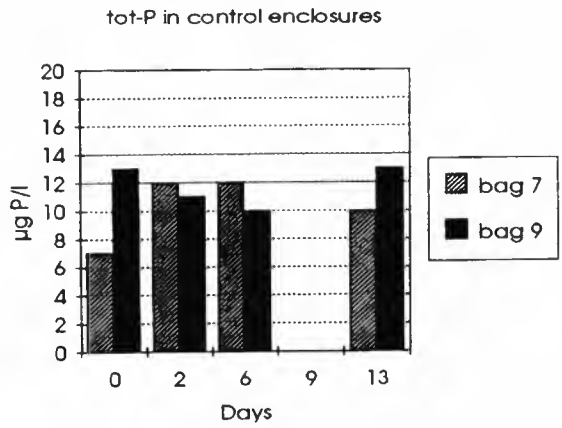

Fig. 2. Tot.-P in the two control enclosures

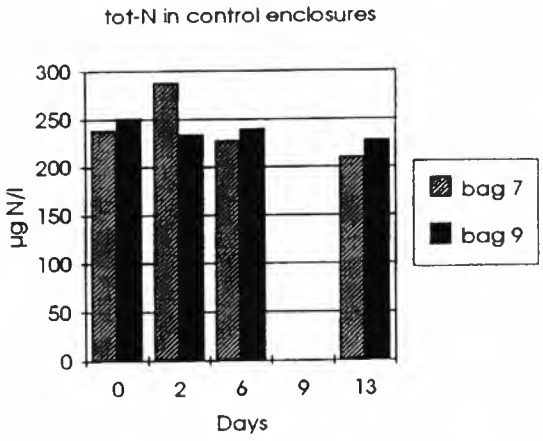

Fig 3. Tot.-N in the two control enclosures

Both of the bags showed lower concentrations of phosphate than the nominal concentrations (5 µg P) that were added shortly before sampling. Obviously the concentration of this nutrient was very low in the lake at the start of the experiment, and very rapid uptake of the added phosphate by the plankton may account for the low initial phosphate concentrations. The phosphate concentrations dropped rapidly to below the detection limit (1 µg/l) and remained at this low level throughout the experiment. Similarly, nitrate concentrations declined from the initial 50 µg N/l to 32 µg/l after 2 days, and only 1µg/l after 6 days (See Fig. 4). This means that the nutrients added at the start of the experiments were completely assimilated after 6 days.

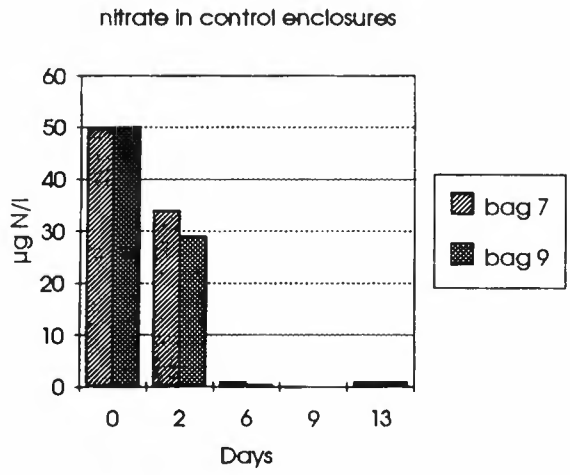

Fig. 4. Nitrate in the two control enclosures

The nutrient variations in the bags with pesticides generally followed the same pattern as those in the control bags. One exception to this was the concentration of total N after two days, which was lower than the mean value for the two controls in all bags to which pesticides had been added. The reason for this, however was, probably a too high value in control bag no. 7 on the second day (see Fig. 3).

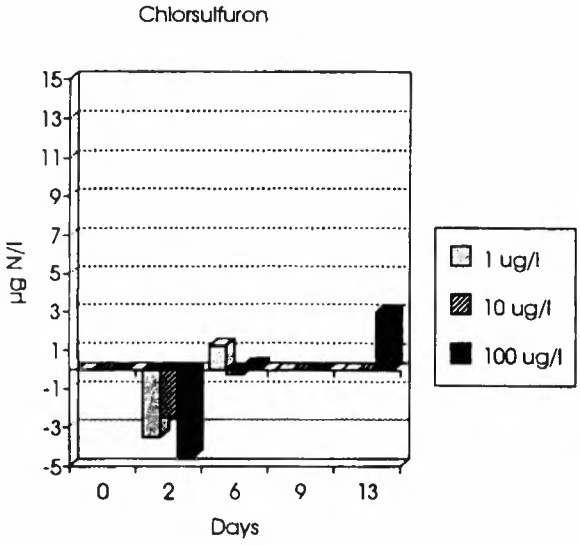

Fig. 5. Deviation of nitrate concentrations relative to control enclosures - Propiconazole

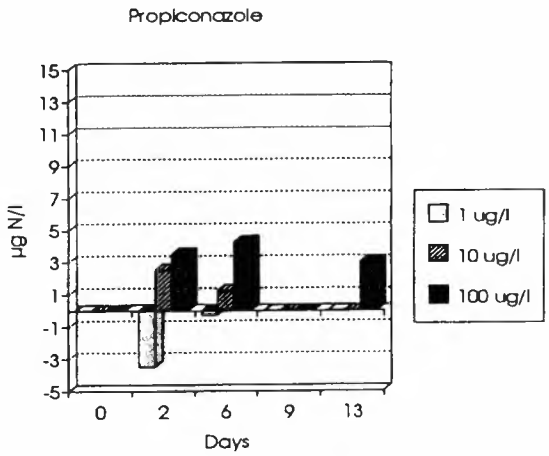

Fig. 6. Deviation of nitrate concentrations relatiave to control enclosures - Propiconazole

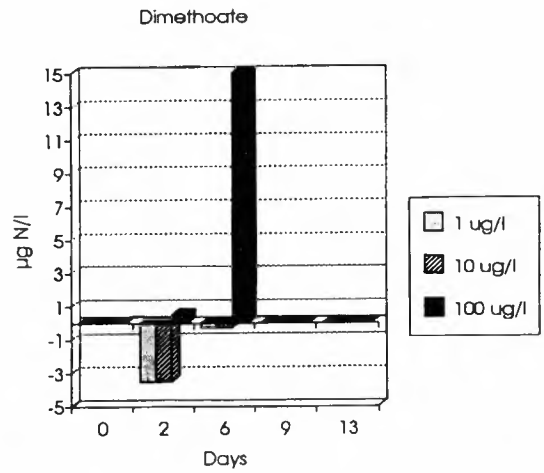

Fig. 7. Deviation of nitrate concentrations relative to control enclosures- Dimethoate

The only deviations of nutrients compared to the control bags that can be ascribed to the pesticide effects were observed for nitrate. However, the deviations were usually less than 5 µg/l. The deviation of the nitrate concentration in enclosures with pesticides as compared to the control enclosures are shown in Figs. 5-8. In the bag with the highest concentration of glyphosate, nitrate concentrations significantly higher than those in the control bags were observed after 2 and 6 days (see Fig. 8). The reason for this was perhaps reduced assimilation of nitrate by the phytoplankton. Release of nitrate during degradation of the pesticide cannot account for the observed deviation in nitrate concentration, since the chemical analysis of glyphosate at the end of the experiment showed that not more than 20% was lost after 16 days,

which would yield only 3µg N as nitrate. A high nitrate concentration (15 µg/l higher than that in the control) was also recorded at the highest concentration of dimethoate after 6 days (see Fig. 8).

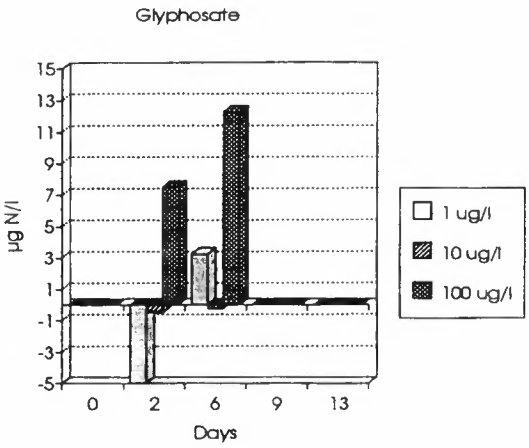

Fig. 8. Deviation of nitrate concentrations relative to control enclosures - Glyphosate

Chlorophyll-a

Chlorophyll-a can be used as an indicator of biomass variations of the integrated phytoplankton community. Since the zooplankton density and thus the grazing activity in the enclosures were fairly low (Hessen *et al* . this volume), the variations in chlorophyll concentrations at the beginning of the experiment can be interpreted as direct effects of the pesticides on the development of phytoplankton. In the last part of the experiment indirect effects mediated by differences in zooplankton density may also have occurred.

The initial chlorophyll-a concentration was only 2 µg/l. As a result of the nutrient addition, the chlorophyll levels in the control bags increased during the first 6 days of the experiment to 4 µg/l. After that, the chlorophyll concentrations declined again to only approx. 1.5 µg/l after 16 days. The chlorophyll levels in the two control bags were very similar (see Fig. 9).

Figures 10 to 13 show the deviation in chlorophyll levels in the pesticide bags as compared to the controls. The results show that the highest concentration (100 µg/l) of all pesticides reduced the development of algae during the initial phase of the experiment. For chlorsulfuron, this effect persisted throughout the 16 days. At 10 µg/l chlorsulfuron, the chlorophyll concentration was also lower than that of the controls after 2 days, but not later (Fig. 10).

At 100 µg propiconazole/l, the deviation in chlorophyll concentration increased to 1.2 µg/l less than that in the controls after 6 days, but after 16 days the levels were again close those of to the controls (see Fig. 11). In addition, at 10 µg/l propiconazole an initial drop in chlorophyll was observed. Glyphosate caused a considerable drop in chlorophyll compared to the controls at 100 µg/l concentration after 2 and 6 days (Fig. 13). However, the phytoplankton appeared to recover from the initial inhibition. The data also indicate a small initial inhibition at 10 µg/l glyphosate.

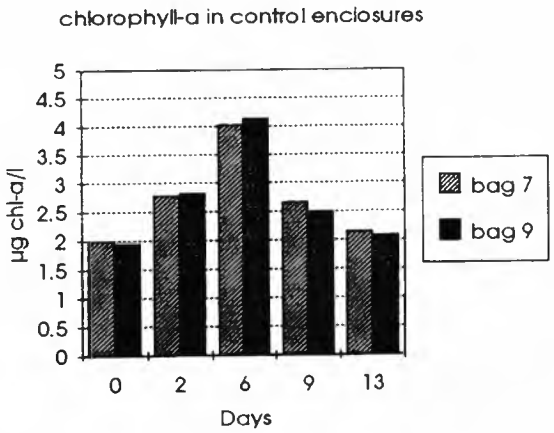

Fig. 9. Chlorophyll-a on the two control enclosures

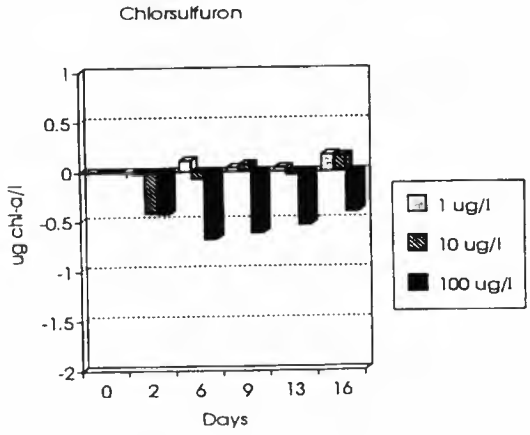

Fig. 10. Deviation of chlorophyll-a concentrations relatiave to control enclosures -Chlorsulfuron

The highest concentration of dimethoate (100 µg/l) also appeared to inhibit the initial phytoplankton increase, but after 6 days the chlorophyll concentrations were close to those of the control (Fig. 12). At the intermediate concentration of dimethoate (10 µg/l), the chlorophyll levels were lower than those of the controls after 13 and 16 days.

Photosynthetic activity

The photosynthetic activity (PA), measured as ^{14}C-uptake in an incubator, was highest after two

days in all the enclosures. Later, the photosynthesis in the control enclosures dropped by approx. 50% and remained at that level throughout the experiment. Thus, the variation in photosynthesis was different from chlorophyll-a, which was highest after 6 days. Obviously, the nutrient addition at the start of the experiment stimulated the photosynthetic production which resulted in the biomass (chlorophyll) increase. On the sixth day, when the chlorophyll concentration peaked, the nutrients were exhausted and the photosynthetic activity had already declined.

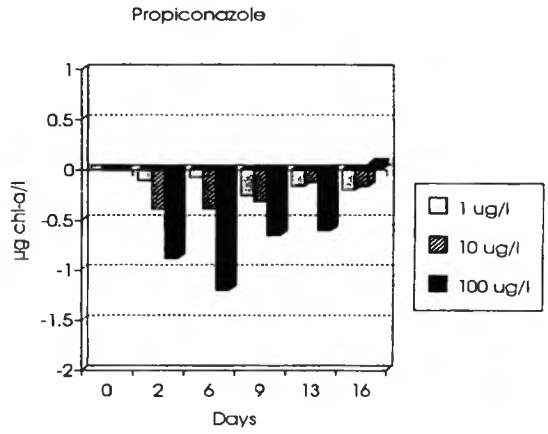

Fig. 11. Deviations in chlorophyll-a concentrations relative to control enclosures - Propiconazole

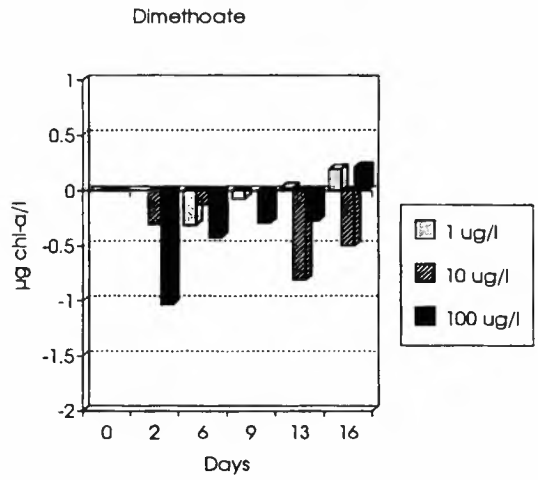

Fig. 12. Deviations of chlorophyll-a concentrations relative to control enclosures - Dimethoate

Figures 14-17 show the photosynthetic activity (PA, measured as ^{14}C-activity) of the plankton for each of the enclosures after 2, 6 and 9 days. The results from the two control bags (mean values) are shown as bars in the figures.

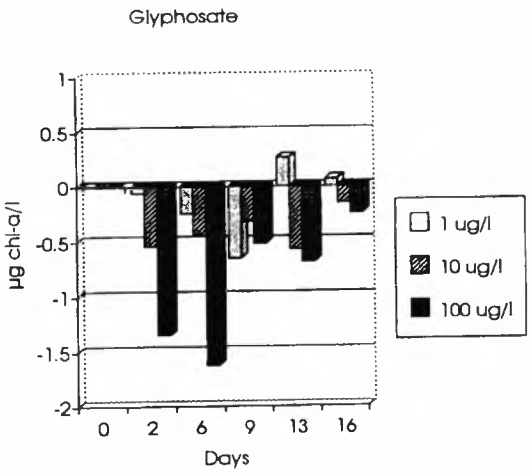

Fig. 13. Deviations in chlorophyll-a concentrations relative to control enclosures - Glyphosate

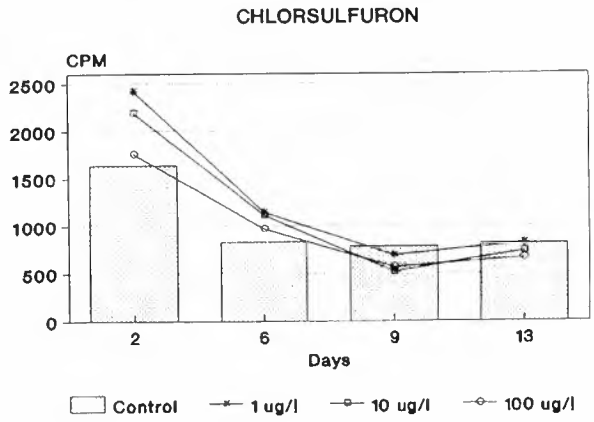

Fig. 14. Photosynthetic activity in enclosures with chlorsulfuron and in controls (bars)

At the two lowest concentrations of chlorsulfuron, 1 and 10 µg/l, the PA was higher than that in the controls after 2 and 4 days, but later, the deviations from the control were small. At 100 µg chlorsulfuron/l, the PA was not much different from that of the controls, but much lower than at 1 and 10 µg/l after two days.

It was found that propiconazole also seemed to stimulate the PA at the lowest concentration. At 1 µg/l, PA was higher than that in the controls after 2 and 6 days. At 10 µg/l, PA was higher than that in the controls only after 6 days. As for chlorsulfuron, 100 µg/l of propiconazole, seemed to decrease the PA initially, but after 6 days it was higher than that in the controls.

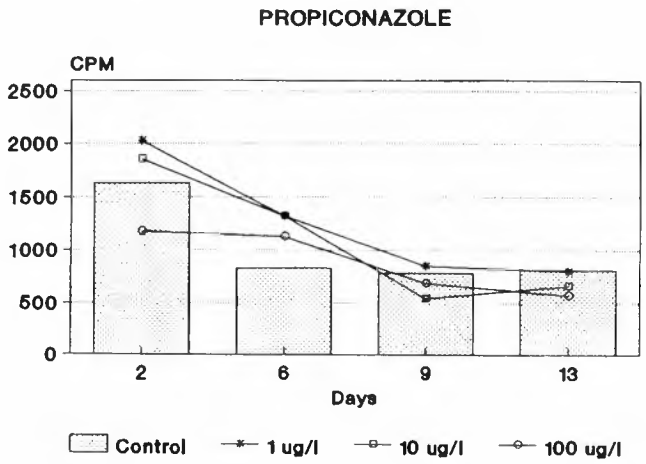

Fig. 15. Photosynthetic activity in enclosures with propiconazole and in controls (bars)

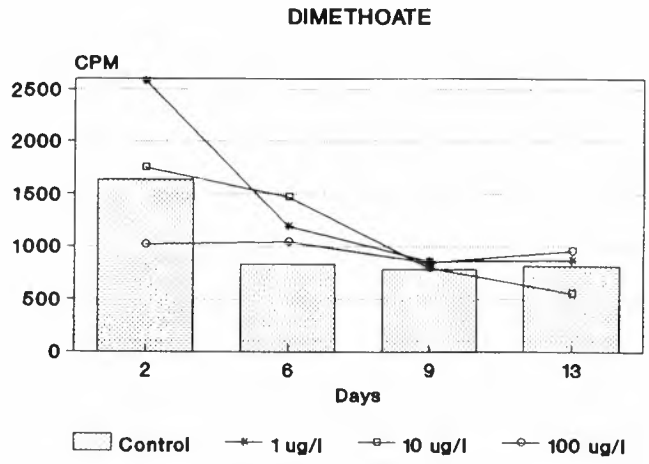

Fig. 16. Photosynthetic activity in enclosures with dimethoate and in controls (bars)

The lowest concentration of dimethoate appeared to stimulate PA after two days. At 100 µg/l, there was a significant initial decrease in PA. After 6 days PA was higher than that in the controls at all concentrations of dimethoate, but later no deviations from the controls were observed.

The two highest concentrations of glyphosate reduced the PA slightly after two days, but after 6 days PA was higher than that in the controls. By the end of the experiment, PA was again lower than that found the controls, particularly at 100 µg/l. At 1 µg/l of glyphosate, no deviations from the controls were observed.

A general picture arises for the effect of all the pesticides on PA. The highest concentrations of pesticides cause a delay of the peak in PA by a few days, which tends to give lower values

than those found in the controls after 2 days and higher values after 6 days. This conforms with the nitrate values, which tend to be higher than those in the controls after 2 and 6 days as a result of the lower PA in those bags where the photosynthesis peak is delayed.

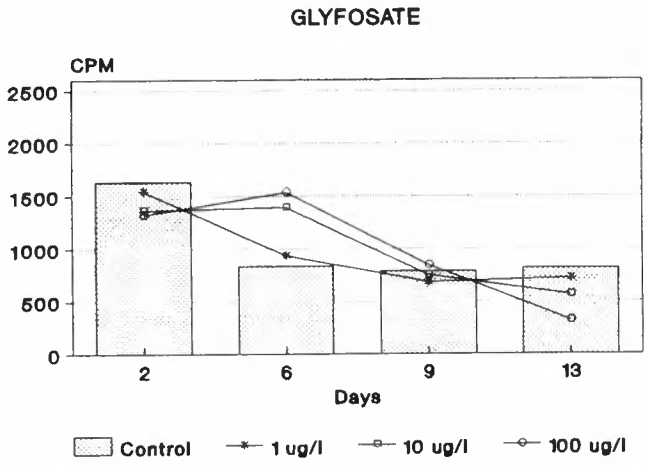

Fig. 17. Photosynthetic activity in enclosures with glyphosate and in controls (bars)

Pesticide tolerance of the phytoplankton community

To investigate whether the plankton communities exposed to the highest concentration of each pesticide had developed higher pesticide tolerance as compared to the control communities, toxicity tests were carried out after 13 days' exposure in the enclosures.

No effect of chlorsulfuron on the carbon assimilation was observed at concentrations up to 10 mg/l, and consequently it is not possible to assess whether there was any difference in sensitivity to this pesticide between exposed and non-exposed plankton.

With propiconazole, there was no detectable effect on the carbon assimilation below 1 mg/l. A 50% reduction of the carbon assimilation (EC_{50}) occurred at 3 mg/l in the control and at 6.5 mg/l in the enclosure pre-exposed to 100µg propiconazole/l. Thus, in this case the plankton that had been exposed to propiconazole was less sensitive to this pesticide than plankton from the control enclosure.

Dimethoate stimulated the carbon assimilation at low concentrations, especially in the sample from the control enclosure. The assimilation was reduced at 10 mg/l and higher concentrations. The response curves indicate that the plankton previously exposed to dimethoate was more sensitive than plankton from the control enclosure. The EC_{50}-values were approx. 20 and 30 mg/l respectively.

With glyphosate too, the pre-exposed plankton was more sensitive to this pesticide than the plankton from the control enclosure. The EC_{50}-values were approx. 7 mg/l and >10 mg/l respectively.

The results from the toxicity tests with the plankton communities show that short-term carbon assimilation measurements are not a sensitive response parameter for toxic effects on

algae for all types of toxicants. This is particularly the case for chlorsulfuron which did not affect the short-term carbon assimilation at 10 mg/l, while the growth of several algae tested in the laboratory was affected below 1 mg/l (Källqvist & Romstad this volume). This has to do with the mode of action of the pesticides. For those that affect the photosynthetic system directly (such as atrazine), short term carbon assimilation measurements may be a more adequate response parameter for assessing toxic effects on algae.

Phytoplankton species composition

A total of 63 taxa belonging to 35 genera were identified. The species composition was mainly represented by Chlorophyceae (29 taxa), Chrysophyceae (19 taxa) , Cryptophyceae (8 taxa) and, to a small extent, Bacillariophyceae (3 taxa), Cyanophyceae (2 taxa) and Dinophyceae (2 taxa). The smallest planktonic forms with diameters between 0.5 and 1.0 µm were grouped under µ-algae.

In terms of biomass (total volume), the most dominant species were the Bacillarophyceae with 41-54% of the total biomass in the control enclosures followed by Dinophyceae (14-32%), Cryptophyceae (7-18%), Chlorophyceae (4.5-11%), Chrysophyceae (2.0-11%) and µ-algae (1.2-4.6%).

The Shannon-Wiener diversity index summarizes in a single value two important aspects of the community under study; the species richness and the evenness in distribution of the individuals amongst the species. The diversity index of control bags was remarkably stable and attained values between 2.69 and 2.76. The diversity index in enclosures with pesticides and in the controls is shown in Figs. 18-21 Significantly ($p < 0.05$) lower values were found at all pesticide treatments with the lowest values in most cases recorded after two days and at the end of the incubation period. Compared to other pesticides, chlorsulfuron (Fig. 18) and propiconazole (Fig. 19) exerted the strongest impacts on species diversity, especially at the highest concentration level (100 µg/l).

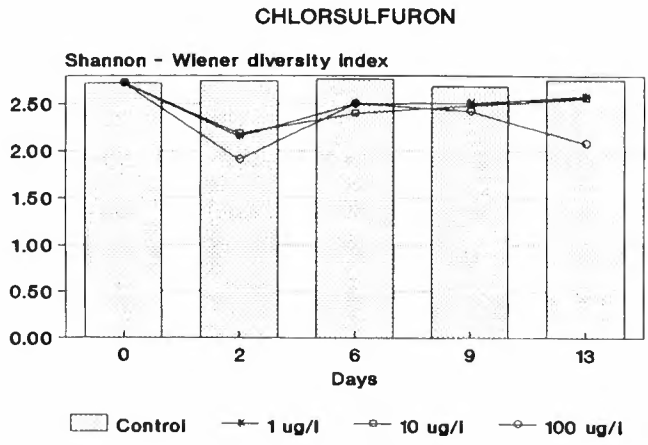

Fig. 18. Shannon-Wiener diversity index for phytoplankton in enclosures with chlorsulsfuron and in controls (bars)

Distinctly different responses to the various pesticides were observed among the most frequent phytoplankton species. The cyanobacterium *Anabaena flos aqua* dissappeared completely

from the enclosure with 100 µg chlorsulfuron/l. At 1 and 10 µg/l of this pesticide, an initial depression of *A. flos aqua* was observed, but the organism recovered and attained a higher biomass than that in the control bags after 13 days. The occurrence of this species in enclosures with propiconazole, dimethoate and glyphosate was irregular and did not indicate particular toxic effects. The green alga *Oocystis submarina* appeared to be affected by all pesticide treatments, with lower biomass concentrations than those in the controls, particularly on day 2. For propiconazole and dimethoate a dose/response relationship for the initial depression of *O. submarina* was observed, but with the other two pesticides all three concentrations caused the same level of response.

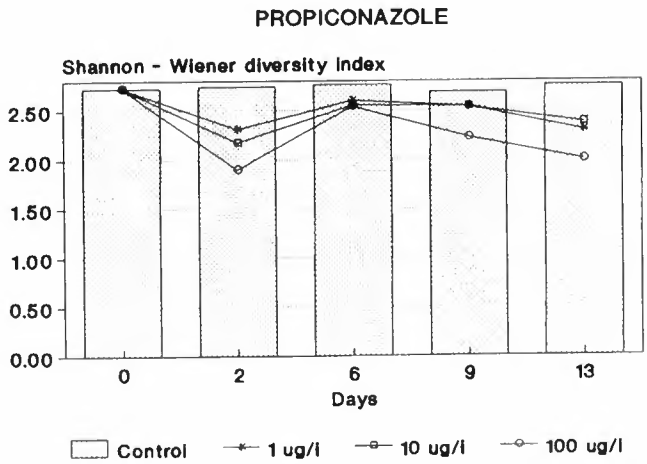

Fig. 19. Shannon-Wiener diversity index for phytoplankton in enclosures with propiconazole and in controls (bars)

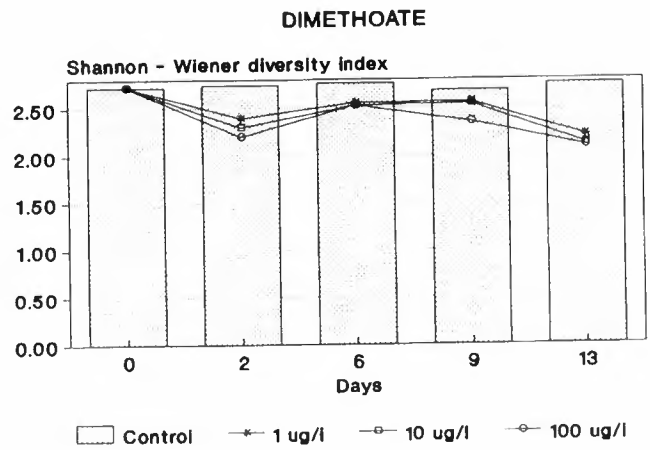

Fig. 20. Shannon-Wiener diversity index for phytoplankton in enclosures with dimethoate and in controls (bars)

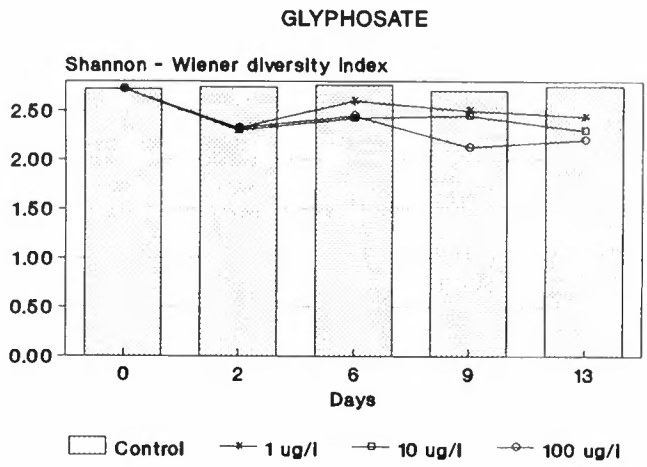

Fig. 21. Shannon-Wiener diversity index for phytoplankton in enclosures with glyphosate and in controls (bars)

The cryptophycean *Rhodomonas lacustris var. nannoplanctica*, was affected by propiconazole, dimethoate and glyphosate in a dose-related fashion. At 100 µg/l of these pesticides, a significant biomass reduction was observed after only 2 days. Later, a reduction was also observed at 10 µg/l of propiconazole and dimethoate. With glyphosate, no significant effect of the lower concentrations could be detected, and at 100 µg/l this alga recovered after the initial depression and attained a much higher biomass than that in the control after 9 days. Chlorsulfuron caused no apparent effect on the development of *R. lacustris var. nannoplanctica*.

The development of the diatom *Cyclotella comta* was similar in all enclosures including the controls, indicating that this species was not significantly affected by any of the pesticide treatments.

DISCUSSION

The development of all parameters that were studied, i.e. plant nutrients, chlorophyll-a, primary production and phytoplankton species composition, was very similar in both the control enclosures. This fact indicates that any deviations from the development in the controls, observed in enclosures treated with pesticides, can be interpreted as an effect of the specific treatment. However, the lack of replicates of pesticide treatments calls for caution in drawing conclusions from single observations unless they are supported by other parameters or a dose/response relationship can be demonstrated when comparing enclosures with different concentrations of the same pesticide.

Effects observed on the structure and function of the phytoplankton in mesocosm toxicity experiments may be either direct effects of the toxic agents on the individual phytoplankton or indirect effects, e.g. by reduced nutrient cycling or grazing by herbivores. It is not possible to distinguish clearly between such direct and indirect effects unless all relevant prosesses and interactions are monitored. The low density of zooplankton that was present in the enclosures throughout the experiments implies, however, that grazing was an insignificant factor regula-

ting the phytoplankton, and, thus, that indirect effects induced by toxicity to grazers were limited.

The enclosure experiment has shown that all four pesticides affected the function of the plankton ecosystem by reducing the rate at which the phytoplankton responded to the initial nutrient additions. This was most significant in the enclosures to which the highest concentration of pesticides (100 µg/l) had been added, where chlorophyll concentrations were consistently lower than those in the control enclosures. The slower phytoplankton development in these enclosures was frequently accompanied by a higher concentration of nitrate as compared to the controls, which may be an indication of reduced nutrient assimilation.

Also at the intermediate concentration (10 µg/l) of the pesticides, chlorophyll concentrations showed that the phytoplankton biomass was reduced as compared to the control enclosures. With chlorsulfuron this was observed only after two days, but with the other pesticides the deviation persisted throughout the experiments.

At the 1 µg/l level, little effects on the plant nutrient and chlorophyll concentrations was observed. At 1µg/l of glyphosate, however, chlorophyll levels were lower than those in the controls after 6 and 9 days' exposure.

The measurements of photosynthetic activity confirm that the 100 µg/l propiconazole, dimethoate and glyphosate reduced the phytoplankton production initially, and delayed the peak in photosynthesis that occurred as a result of the nutrient addition. Three of the pesticides, chlorsulfuron, dimethoate and propiconazole, appeared to stimulate photosynthesis slightly at low concentrations. This may be explained by the phenomenon known as hormesis (Stebbing 1982).

The phytoplankton in enclosures with initial concentrations of 100 µg pesticide/l did not develop increased tolerance to dimethoate and glyphosate. In fact these plankton communities were slightly more sensitive to the pesticides to which they had been exposed than the plankton from a control enclosure. Only for propiconazole an increased tolerance was observed.

According to these results, increased tolerance to pesticides as a result of exposure does not seem to be a general phenomenon as it was only observed for propiconazole. This pesticide was also the one for which the variation in sensitivity among different algal species was highest when tested in the laboratory. It seems reasonable that pollution-induced community tolerance (PICT, Blanck *et al.* 1988) will be most pronounced for toxicants for which variation in sensitivity is high. The opposite effect, i.e. decreased tolerance, which was indicated by the tests with propiconazole and glyphosate is difficult to account for. However, the high concentrations of pesticides required to cause effects on the photosynthesis in short-term experiments indicate that the mechanism for toxicity for these pesticides does not primarily involve the photosynthetic reactions and, hence, an increased tolerance to the pesticides is perhaps not reflected in the photosynthetic response.

Species composition and diversity of the plankton community deviated from the controls at all pesticide treatments. This indicates that structural changes were induced already at a concentration of no more than 1µg/l of the four pesticides. It is difficult to explain why four pesticides with different mode of action and levels of toxicity to single species of algae would all cause similar effects on the species diversity. However, since the species diversity in both control enclosures was stable and similar throughout the experiment, we have interpreted the observed reduction in diversity in the pesticide-treated enclosures as an effect of the pesticides. Furthermore, a more or less obvious concentration/response relationship was demonstrated, with increasing concentration causing increasing effect on the species diversity.

The enclosure experiment has revealed ecological effects on plankton communities that could not have been predicted from previous laboratory tests (Källqvist & Romstad this

volume). The most surprising observation was the reduction in species diversity at all treatment levels of all the pesticides. The insecticide dimethoate, which would not be expected to cause a direct effect on phytoplankton in the concentration range tested, appeared to affect species diversity even at 1 µg/l and hampered the initial increase of phytoplankton biomass at 100 µg/l. The laboratory tests with six species of plankton algae did not show any effect on the growth at concentrations below 1 mg/l dimethoate (Källqvist & Romstad this volume). On the other hand, the laboratory tests showed dimethoate to be very toxic to invertebrates (Bækken & Aanes 1990, Hessen *et al.*, this volume). For propiconazole and chlorsulfuron, the observed effects on phytoplankton at the 100 µg/l concentration could be expected from the results obtained with laboratory toxicity tests.

The experiment indicates that low concentrations of pesticides can affect species diversity of the phytoplankton community without reducing the community production. This is an effect of the wide variation in sensitivity among phytoplankton species to many toxic chemicals, leading to a replacement of sensitive species with less sensitive species. The ecological implications of such changes are not obvious. As long as the level of primary production is maintained, effects on higher trophical levels in the food chain may be insignificant. However, changes in the species composition may also involve changes in the nutrition quality of the produced algal biomass with consequences for the phytoplankton grazers.

The analyses of pesticides in the enclosures show that agricultural pesticides may be fairly persistent in surface water. For dimethoate, practically no degradation occurred during the experiment, and for glyphosate and propiconazole approx. 80% and 60% respectively was recovered in the water column after 16 days.

ACKNOWLEDGEMENTS

The study was funded by the Agricultural Research Council of Norway and the Norwegian State Pollution Control Agency .

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Effects of pesticides on different zooplankton taxa in mesocosm experiments

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Hessen, D.O., T. Källqvist, I. A.-H. Mohammad & D. Berge 1994. Norwegian Journal of Agricultural Sciences. Supplement No. 13: 153-161. ISSN 0802-1600.

In a series of acute laboratory tests it was found that the herbicide chlorsulfuron had low toxicity on the cladocerans *Daphnia magna* and *D. pulex* (LC_{50} > 100 mg/l), whereas glyphosate and propiconazole had a 3.2 mg/l < LC_{50} < 10 mg/l. The insecticide dimethoate had much higher toxicity for both species (LC_{50} ~ 0.02 mg/l). This general toxicity pattern for *Daphnia* was confirmed after 12 days' enclosure experiments, whereas other species of cladocera gave a somewhat different response. While the crustacean zooplankton species were the most susceptible to the insecticide, this was reversed for the planktonic rotifers, which gave no negative response on dimethoate, but showed negative population response in bags added chlorsulfuron and glyphosate. No support was found for a strong indirect effect of herbicides on zooplankton due to food reductions (algal effects). In general there was no correlation between total primary production, phytoplankton biomass and zooplankton. Community changes and species shifts in the phytoplankton community could, however, explain some of the negative effects on rotifers.

Key words: Freshwater, mesocosm, pesticides, toxicity, zooplankton

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The use of *Daphnia* spp. in short-term assays is a common tool for studying acute toxic effects. Since *Daphnia* is a parthenogenic species, the use of clones with (more or less) identical genotypes in standardized tests is permissible. Such tests have their obvious shortcomings, however. Various clones of *Daphnia* can show quite pronounced genetic differences which may strongly affect toxin susceptibility, and between-test variability with regard to food regime may also strongly affect survival (Baird *et al.* 1989). Moreover, *Daphnia magna*, most frequently used in tests, is a pond form of the genus *Ctenodaphnia*, and may not represent well the other *Daphnia* species (genus *Daphnia*), most of them lake forms. More obviously, effects and toxicity levels on *D. magna* may not represent well the effects on other cladocerans, particularily the calanoid and cyclopoid copepods, although they all represent the class Crustacea (order Arthropoda). For other important groups within the zooplankton community, such as the rotifers (phylum Rotifera), which are taxonomically distant from the crustaceans, as well as other sectors of the aquatic biota, one could expect quite a different response for several toxins (Slooff *et al.* 1983; Mayer & Ellesieck 1986; Hickey 1989). The principal purpose of most toxicity studies is to gain knowledge on expected effects in natural environments, meaning that indirect effects through food chains could be more important than the direct effects (cf. Lampert et al. 1989). While the non-target organism (in this case zooplankton) could

be rather insensitive to herbicides in short-term assays, excluding the effects of food availability during the experimental period, a decreased phytoplankton production or altered community composition could cause adverse effects in natural systems. With this list of precautions, it is advisable that such laboratory tests should be used merely as a first screening, followed up by more comprehensive life-table studies or *in situ* experiments including communities and various trophic levels.

To compare results from short-term LC_{50} laboratory bioassays with "natural" conditions, we conducted a series of screening laboratory assays with *D. magna* and *D. pulex*, and a total of 14 enclosures including all natural planktonic compartments and multitaxa zooplankton communities. This approach, as well as testing both herbicides and an insecticide, enabled us to separate some of the direct and indirect effects.

MATERIALS AND METHODS

The species *Daphnia pulex* was exposed to a toxicity screening in a geometric dilution series of 10 concentrations (320, 100, 32, 10...0.01 mg l^{-1}) of the toxins glyphosate, propiconazole, chlorsulfuron and dimethoate. For all concentrations of toxins as well as the control, five individuals (< 48 h juveniles) were added to a 50 ml bottles. The number of immobilized individuals was inspected after 24 and 48 h. In general, these two time periods showed good conformity, and in the following discussion, we mainly refer to the results after 24 h. As a control, an additional test was performed for a cultured clone of *D. magna*. We here selected fewer concentrations, but more replicates. This test was performed strictly after the standardized procedure (ISO-standard, 1989), with five replicates, each of 5 > 48 h juveniles in 50 ml bottles for each concentration. The number of immobilized animals was counted after 24 h.

A field study was performed in 14 pelagic enclosures situated in Lake Omdalsvann, 60°16'N 10° 37'E, SE. Norway. The lake is oligotrophic (7µg total P and 200 µg total N l^{-1}), relatively rich in Ca (19 mg l^{-1}) and slightly alkaline (pH 7.5 - 8.3). The bags were sealed below, but open to the air and had a volume of approx. 22 m^3. Inspection after filling with surface water revealed an almost complete absence of crustacean zooplankton, and a net tow (45 um mesh, 27 cm diameter) from 6 m depth (thermocline) – O m was thus added to each bag to ensure an equal inoculum of macrozooplankton to each bag. This gives a lower concentration when compared to the lake, but a low concentration was preferred to avoid strong effects of grazing which would mask the direct effects of toxins on algae. In addition, by using low initial concentrations of macrozooplankton, effects of food depletion and community changes caused by grazing would not affect the overall judgement of indirect toxic effects on zooplannkton.

Quantitative zooplankton and phytoplankton samples were taken every three days with a 10-l rubber hose, penetrating the whole depth of each bag. Because of low concentrations of macrozooplankton, the effects on the crustacean zooplankton community could not be judged from the daily quantitative samples. Effects on this community were thus based on the cumulative yield of quantitative samples and net hauls at the end of the experiment (after 12 days). The rotifers were persistently high in number in all bags throughout the experimental period, allowing a day-to-day evaluation of responses. Photosynthetic activity was determined from 14C assimilation in 25 ml bottles (4 µCi/ml) after 2, 6, 9 and 13 days. At the experimental initiation, each bag received PO_4 and NO_3 equalling 5 and 50 µg $^{-1}$ respectively, to prevent severe nutrient limitation. Each of the toxins was added to final concentrations of 1, 10 and 100 µg l^{-1}, i.e. three bags for each toxin. Two bags were kept as controls. Interactions between phyto- and zooplankton were tested by Spearman rank correlation coefficient.

RESULTS

Initial and final concentrations of propiconazole, glyphosate and dimethoate were analysed (Table 1). For all substances the measured concentrations were in general accordance with the nominal concentrations. While the concentrations of glyphosate and propiconazole declined to respectively 83 and 58% of initial concentration after 15 days, the concentrations of dimethoate had a negligible decrease over the experimental period.

Table 1. Initial (25. July) and final (10. August) conc. mg L^{-1}) of pesticides

	Nominal	Initial	Final	% of initial
Dimetoate	1	1	0,9	90
	10	12	11	92
	100	105	101	96
Propoconazole	1	1,1	0,67	62
	10	9,4	5,3	58
	100	92,5	-	-
Glyphosate	1	-	-	-
	10	-	-	-
	100	98	81	83

The four substances had markedly different effects in the screening assay, while no clear-cut differences between the two *Daphnia* species were detected (Fig. 1). For chlorsulfuron, immobilization was induced first at concentrations close to 100 mg l^{-1}. Glyphosate gave no immobilization at levels of 3.2 mg or below, but almost complete immobilization at 10 mg l^{-1}. Propiconazole resembled closely the pattern of glyphosate, while the insecticide dimethoate was by far the most toxic, with a complete immobilization of *D. pulex* at 0.032 mg l^{-1}, suggesting a LC_{50} near 0.02 mg l^{-1}.

In the field experiment, the rotifer communities in both lake and bags were almost exclusively composed of the three herbivorous species *Conochilus unicornis, Kelicottia longiseta, Polyarthra sp.* and the carnivorous *Asplanchna priodonta.* The same species dominated the lake community. No clear-cut effects were revealed from a simple comparison of numbers in the various bags, mainly due to density oscillations within all bags. In particular, the colony-building species *C. unicornis* had pronounced density variations in the samples as even rather small variations in number of colonies strongly affected the number of individuals. The effects were better visualized by comparing total number of individuals (with the exception of *C. unicornis* for the reasons given above) in each treatment as the percentage deviation from controls (Fig. 2). All concentrations of chlorsulfuron and the highest concentration of glyphosate gave negative deviations compared with controls, but the effect of both decreased during the first week and for chlorsulfuron there was a final positive net effect at the end of the experiment. For propiconazole, the effects seem more accidental. Medium and high concentrations (10 and 100 µg l^{-1}) yielded a negative net effect, while the second sampling gave a positive response at all concentrations. Dimethoate had apparently little effect, even at the highest concentrations. For *Conochilus*, not included in these figures, no clear-cut effects could

be traced. This species declined in all bags, including the controls. The two dominant species, *Polyarthra* and *Kellicottia*, had a more or less similar response to all treatments. The large carnivorous species *Asplanchna* was negatively affected by the highest concentrations of propiconazole, glyphosate and dimethoate, while chlorsulfuron had no visible effects.

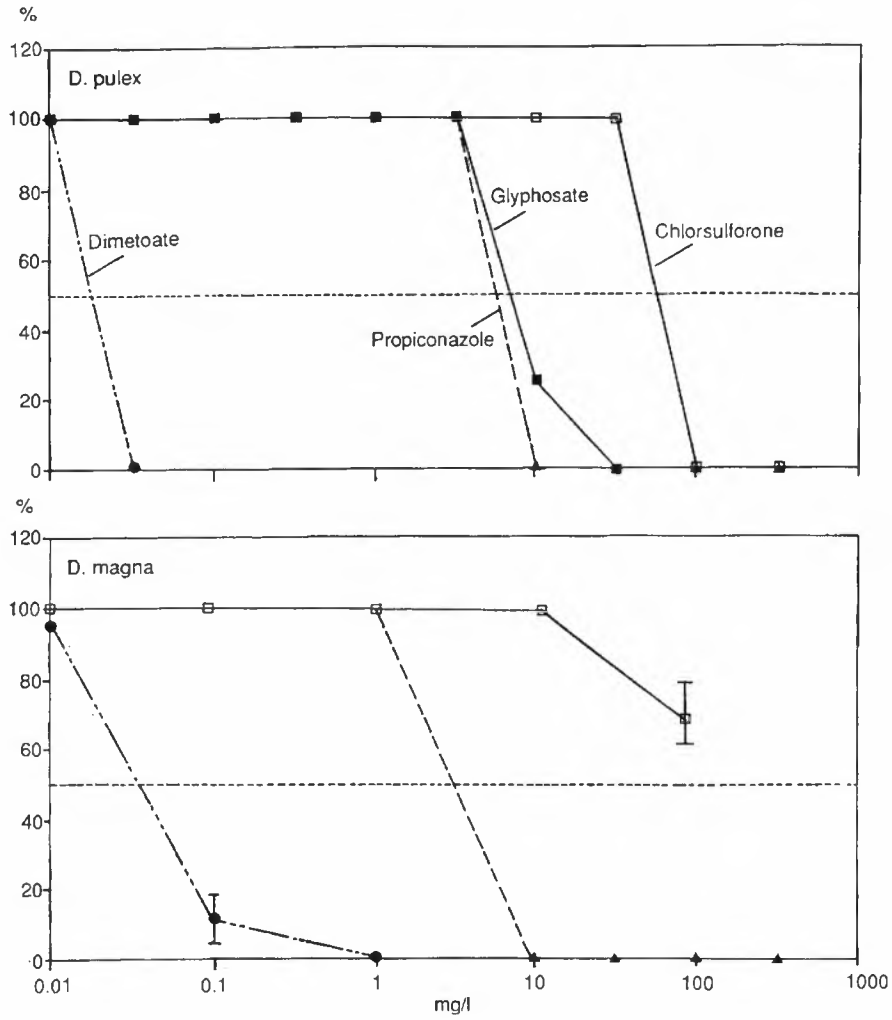

Figure 1. Acute laboratory tox-tests with *Daphnia* pulex (upper) and *D. magna*

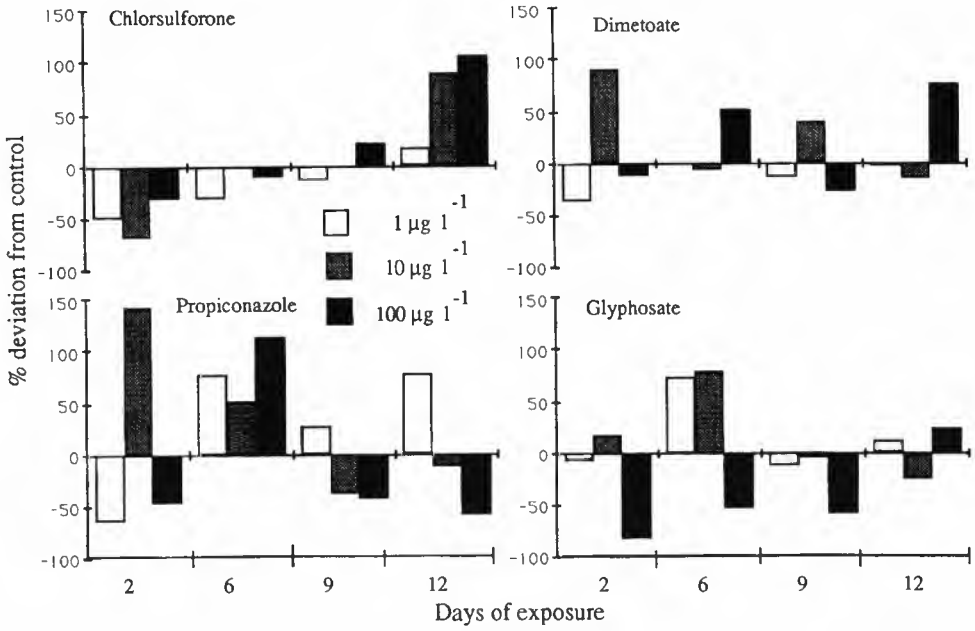

Figure 2. Total numbers of rotifers given as deviation from controls

The crustacean community was composed of the copepod *Acanthodiaptomus gracilis* and the cladocerans *Holopedium gibberum*, *Bosmina longispina, Daphnia longispina, Ceriodaphnia quadrangula* and the semibenthic *Sida crystallina*. The last-mentioned species mainly colonized the bag walls, but also occurred free-living. All species were initially added to each bag from nethauls, but, as previously mentioned, a subsequent recording of the crustacean community was not possible because of low numbers of all species. The final nethauls from the bags revealed highly variable effects on the various species (Table 2). The most pronounced effect was found at the highest concentration of dimethoate, where all cladocera disappeared. *Daphnia* seemed also susceptible to the medium concentration (10 µg l^{-1}), while the other species were not. In general, *Ceriodaphnia* was the most susceptible species, and disappeared at 100 µg for all tested substances. The highest conccentration of propiconazole wiped out all species except *Daphnia*. This supports the laboratory test for *Daphnia*, but indicates that other species of cladocera might be more sensitive.

Table 2. Final net-haul numbers of crustacean zooplankton in the bags at the experimental termination

Bag	Cal.cop.III-ad.	Sida	Holopedium	Bosmina	Ceriodaphnia	Daphnia
Control	12	2	1	2	5	2
Control	27	2	2	1	1	
Chl., 1 µg/L	75	4	5	9	5	11
Ckl., 10 µg/L	29	16	-	5	-	5
Chl., 100 µg/L	77	36	4	-	-	14
Prk., 1 µg/l	24	19	1	11	8	2
Prk., 10 µg/L	435	14	1	3	10	9
Prk., 100 µg/L	188	-	-	-	-	14
Dim., 1 µg/L	104	11	6	16	9	7
Dim., 10 µg/L	105	140	2	9	2	-
Dim., 100 mg/L	42	-	-	-	-	-
Gly., 1 µg/L	40	2	4	6	4	5
Gly., 10 µg/L	26	1	4	6	2	4
Gly., 100 µg/L	23	3	-	28	-	9

In general primary production showed a positive, initial response at low toxicity levels, whereas biomass decreased in all bags relative to controls. More detailed data on phytoplankton biomass and production will be given elsewhere (Källqvist et al., this volume). The indirect effects of food availability were tested by Spearman rank correlation (not bivariate distribution of parameters) of phytoplankton biomass and production versus zooplankton biomass. Since there is a time-lag in zooplankton response to changes in phytoplankton biomass and production, averaged data from the four dates of sampling rather than a day-to-day comparison were applied for all parameters. All species of rotifers, as well as total numbers were tested. For the crustaceans, final numbers in net hauls were tested against mean phytoplankton biomass and production. No positive correlations were detected (Table 3), suggesting that indirect effects due to changes in total phytoplankton biomass and production were not a major cause of the observed effects in the zooplankton community. For the carnivore rotifer *Asplanchna*, correlation analysis indicated that indirect effects caused by oscillations in prey density could not explain its negative response to the highest concentrations of propiconazole, glyphosate and dimethoate.

Table 3. Spearman rank correlation coefficients between mean number of rotifers and phytoplankton, production (as 14C incorporation) and the predator *Asplanchna*. Positive or negative directions indicated. No correlations were significant at a 10% level

	Conochilus	Polyarthra	Kelicottia	Asplanchna	Total
Algal biomas	0.04 (-)	0.08 (+)	0.07 (+)	0.25 (+)	0.03 (-)
14C-incorp.	0.07 (-)	0.40 (-)	0.13 (-)	0.14 (-)	0.31 (-)
Asplanchna	0.19 (+)	0.51 (+)	0.20 (+)		

DISCUSSION

A comparison between laboratory and field responses is only relevant for *Daphnia* spp. In the lake and bags, *D. longispina* occurred, while *D. magna* and *D. pulex* were tested in the laboratory. Chlorsulfuron, propiconazole and glyphosate had no detectable negative impact on D. longispina even at a concentration of 100 μg l^{-1}. For dimethoate, there was, however, a complete mortality at 10 μg l^{-1} (initial concentration), which is in good accordance with the laboratory data. In this experiment, a previous test thus seemingly gave a good indication of the "real" field effects. Lampert et al. (1989), by testing the effects of the herbicide atrazine on zooplankton, recorded profound differences in laboratory and field tests. Response levels for *Daphnia* in acute toxicity tests, feeding, growth and reproduction tests, laboratory food-chain tests and enclosure tests were 10, ~ 2, 0.05-0.1 and 0.001 mg atrazine l^{-1}, respectively. They concluded that indirect effeects could be more important than direct effects, that laboratory tests would give a poor estimate of the real effects, and that non-target organisms (zooplankton) could be a more sensitive tool than target organisms (phytoplankton) in such tests. The reason why such strong indirect effects were not observed in our tests was simply that total biomass of edible algae gave no strong response to additions of toxins, and that *Daphnia*, feeding on a broad spectrum of algae and bacteria, would not suffer from the observed phytoplankton community changes.

Because of low concentrations and only one sample from each bag, strict statements on tolerance limits are premature, but, nevertheless, these data support previous findings (Baird *et al.* 1989; Hickey 1989) pointing towards consistent differences in tolerance between various species of cladocera. The calanoid copepod *Eudiaptomus gracilis* occurred in relatively high densities in all bags, indicating higher tolerance to all toxins than the cladocera. It is, however, not unreasonable that copepods, which in temperate waters have slow growth rate and most often a univoltine life cycle (i.e., no eggproduction during the experimental period), are less susceptible than the fast-growing, multivoltine and more short-lived cladocera. This is also because the moult process itself increases the susceptibility to toxins (Lee & Buikema 1979).

The rotifers represent a phylum of their own. These species also show a totally different response to the various toxins compared with the crustaceans. While chlorsulfuron and the highest concentrations of propiconazole and glyphosate had a strong initial effect, no effect of dimethoate could be traced. This is not surprising, as the crustacean zooplankton, like insects, belong to the arthropods, and thus act as a target organism on dimethoate additions. Rotifers, on the other hand, are taxonomically distant from the crustaceans, and thus have a lower susceptibility to this arthropod toxin. The physiological effects of dimethoate are mainly nervous, and probably rather specific. A highly variable susceptibility has, however, also been reported in acute tests with Daphnia (Kenaga 1979; Slooff & Canton 1983).

Phytoplankton biomass declined in all bags, relative to the controls, while at low concentrations of chlorsulforon, propiconazole and dimethoate, primary production was initially elevated. Thus the initial effect in most bags at low and modest concentrations of toxins (1 and 10 μg l^{-1}) was a strong increase in production/biomass ratio. These fluctuations could not be traced in the zooplankton community, suggesting that either the phytoplankton biomass was not directly limiting for the production of rotifers or the direct effects were superimposed on the indirect. The fact that higher concentrations of both rotifers and crustaceans were recorded in some of the treated bags relative to controls, in spite of lowered algal biomass, might have been a consequence of the initial increase in primary production, and a subsequent increase in bacterial biomass utilizing dissolved organic matter from the decaying algae. Microscopic

examination revealed a high number of bacterial even in high conccentrations of toxins, but no attempt was made to quantify bacterial biomass.

The zooplankton community would also be affected by changes in species composition in the phytoplankton community which could not be traced by changes in production or total biomass. All treated bags, and in particular those with high concentrations of toxins, showed reduced phytoplankton diversity. The various species of phytoplankton, even within the same class, responded very differently to the various toxins. Among the phytoplankton, Chlorophyceans, Chrysophyceans and Cryptophyceans were most susceptible to medium and high concentrations of chlorsulfuron and glyphosate, probably causing the decline in rotifers in these bags. In conclusion, various species and taxa of zooplankton respond differently to the various toxins, clearly showing that general conclusions based on single-species tests are premature.The strikingly low degradability of dimethoate in water should call for special attention to this pesticide with regard to effects on crustacean zooplankton and other aquatic arthropods.

Compared with the detected concentrations in Norwegian surface waters, the tested herbicides should not represent a major problem for zooplankton in lakes. The results of Lampert *et al.* (1989) still suggest that various toxins may be active in very low concentrations, and persist in surface waters for weeks. In particular, insecticides like dimethoate have a strong toxic effect on all arthropodes, including crustacean zooplankton, and may cause effects in the natural environment in very low concentrations. What is more, the strikingly low degradability of dimethoate in water should call for special attention to this pesticide with regard to effects on crustacean zooplankton and other aquatic arthropods. Various species and taxa of zooplankton, however, respond highly differently on the various toxins, clearly confirming that general conclusions based on single-species tests are premature.

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Sublethal effects of the insecticide dimethoate on invertebrates in experimental streams

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Bækken, T. & K.J. Aanes 1994. Sublethal effects of the insecticide dimethoate on invertebrates in experimental streams. Norwegian Journal of Agricultural Sciences. Supplement No.13: 163-177. ISSN 0802-1600.

Sublethal concentrations (1 ppb) of the insecticide dimethoate were tested on (a) an autumn and (b) a spring benthic macroinvertebrate community in indoor experimental streams. The communities were sampled using small substrate-filled trays colonized by natural stream biota for five weeks. Drift and other animal movements away from the trays during the four weeks tests as well as structural changes in the communities were measured. Some responses were common for the two tests: (1) Drift rate was higher in the dimethoate streams compared to the control as well as the proportion of the populations found in drift: (2) the non-drifting movements away from the trays were higher in the dimethoate streams; (3) structural differences between the streams were small, but significant for some populations. Thus, in both autumn and spring systems, dimethoate mediated an increased activity of the individuals and a reduction in the density in some populations.

Key words: Abundance, community, dimethoate, drift, experimental, insecticide, macroinvertebrates, movements, population, stream

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During recent years, increasing attention has been paid to the environmental effects resulting from the use of pesticides in agriculture and forestry. Pesticides from treated fields eventually enter watercourses and the ecosystems of brooks, rivers, ponds and lakes may be affected (Muirhead-Thomson 1987). In the FPJV-Project (Contamination of Soils and Waters from Agricultural and Industrial Use of Pesticides) the Norwegian Institute for Water Research (NIVA) has been responsible for water pollution related issues. The studies have included acute and chronic testing of several pesticides on various aquatic plant and animal species and communities as well as accumulation studies of pesticides in lake sediments and fish flesh (Bækken & Aanes 199; Aanes 1992, Aanes & Bækken, this volume; Hessen*et al.*, this volume; Grande *et al.*, this volume; Källquist & Romstad, this volume, Källqvist *et al.*, this volume).

One of the groups of organisms tested was the bottom-dwelling macro-invertebrates of streams, an important component of this ecosystem. These communities are generally dominated by insect larvae and nymphs but snails, worms and crustaceans are also common.

Acute toxicity tests of pesticides commonly used in Norwegian agriculture were performed as well as long-term tests with low pesticide concentration on benthic communities. The two most toxic pesticides to macro-invertebrates, as observed from the acute tests, were further tested in the long-term tests at sublethal concentrations. These two were the fungicide

propiconazole and the insecticide dimethoate (Bækken & Aanes 1991). The sublethal effects of the fungicide propiconazole on benthic communities are presented by Aanes & Bækken in this volume.

The present paper presents results from two tests using experimentally set up benthic invertebrate communities in indoor streams exposed to sublethal doses of the insecticide dimethoate. As drift is a characteristic feature of several populations of stream invertebrates, drift rate was one parameter to be measured. Other parameters on moving activity and structural changes in the community were also included.

MATERIALS AND METHODS

For each of the spring and autumn tests a total of 30 trays (15·116 cm^3) filled with a defined combination of sand, gravel and pebbles were colonized by a natural stream biota for five weeks. Ten trays, randomly selected, were placed in each of two indoor experimental streams. The remaining 10 trays represented the invertebrate communities at the start of the experiment. Each stream was 5 m long (Figs. 1 and 2). Most of the water was recirculated, but there was a continuous renewal of 105 l/h. One stream was continuously treated with a low dose of dimethoate (1 ppb), the other was left untreated. A peristaltic pump was used for administering the pesticide. The water current ranged between 2 and 10 cm/s. One part of the stream water was channelled into a net (250 mm) and pumped back again, and the number of drifting animals counted. The water used in the tests was natural surface water pumped directly from a drinking-water source. The water quality was representative of Norwegian not polluted lowland water (Table 1). The water temperature was 15°C.

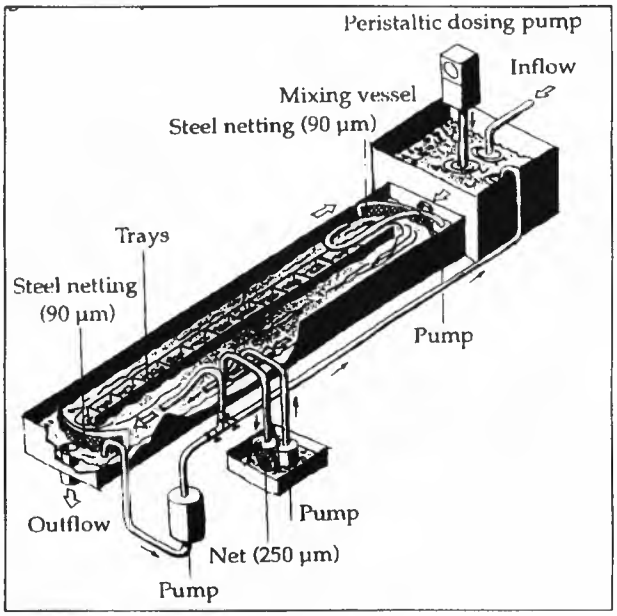

Figure 1. The experimental stream

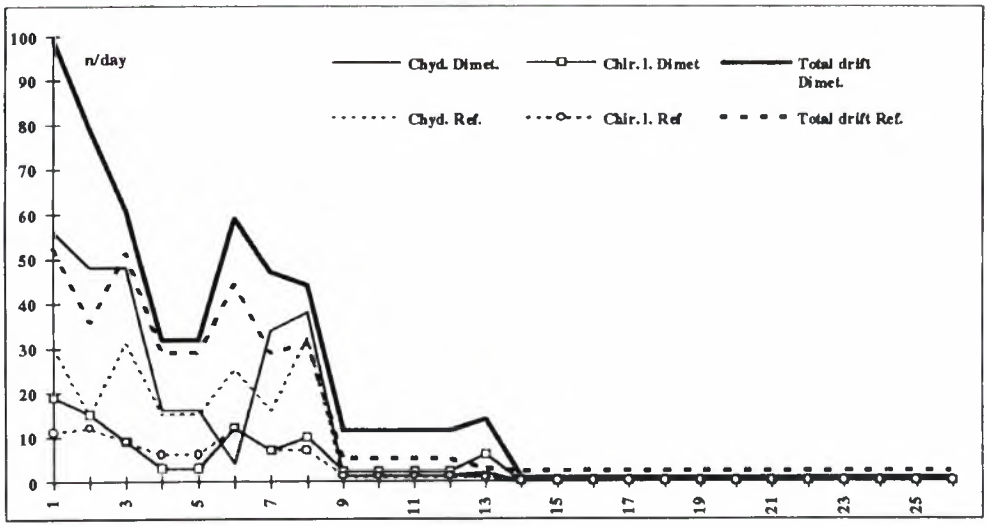

Figure 2. The drift rate of macroinvertebrates during the experimental period in test A

Table 1. Chemical composition of the test water

Parameters	Unit	Values
pH		6,6
Conductivity	mS/m	3,4
Alkalinity	mmol/l	0,09
TOC	mg C/l	2,4
Tot N	µg N/l	300
Tot P	µg P/l	4,5
Cl	mg/l	2,7
Ca	mg/l	2,9
Na	mg/l	1,6
K	mg/l	0,38

In order to study the sublethal responses of the benthic community to the insecticide dimethoate the following parameters had to be registered:

1. Behavioural changes in populations

(a) The drift as (i) the average number of animals drifting every 24 h and (ii) the total number of individuals of a particular taxa found in the drift during the experimental period relative to the size of its population at the end of the experiment. The population size in this context means the number of animals found in the experimental streams at the end of the experiment together with the total number of drifting individuals found during the experimental period.

(b) Other animal movements away from the trays. The total number of individuals found outside the trays relative to the total number of individuals in the stream. The animals caught in drift were not included.

2. Structural changes in the communities

The population sizes at the start, as represented by the 10 start trays, and at the end of the experiment.

The experiments were first conducted on the invertebrate community in Aug./Sept. (autumn period, Test A), then repeated on a community from the same locality in May/June the following year (spring period, Test B).

In order to increase the population density of the mayfly, *Baetis rhodani*, 40 medium-sized individuals sampled in the same stream, as used for colonization of the trays, were added to each of the experimental streams at the start of test B.

RESULTS

Drift

Test A: the autumn community

The total number of drifting animals was higher in the dimethoate stream than in the reference stream with totals of 518 and 353 individuals, respectively (Table 2). Except for the last two weeks having almost no drift at all, the average number of animals found drifting each day was always equal or higher in the dimethoate stream relative to the reference stream (Fig. 2). During the first days the chydorids were the most frequently encountered animal taxa in drift. But chironomids were also common. At the end of the experiment the number of drifting individuals of each taxon was very low, and the chydorids were no longer observed.

Table 2. The total number of individuals of different taxa caught in the drift fauna in test A and test B

	test A : autumn		test B: spring	
	Dimethoate	Reference	Dimethoate	Reference
Oligochaeta	0	0	7	7
Gastropoda	0	0	0	0
Lamellibranchiata	0	0	0	0
Hydracarina	57	30	48	32
Copepoda	0	0	20	26
Chydoridae	267	184	132	15
Ostracoda	0	0	2	1
Ephemeroptera	3	8	91	125
Plecoptera	23	19	495	333
Coleoptera, larvae	0	0	1	4
Coleoptera, imago	0	0	1	3
Trichoptera	6	14	9	10
Simuliidae, larvae	0	0	18	5
Simuliidae, pupae		0	0	0
Chironomidae l.	132	96	399	409
Chironomidae p.	0	0	6	15
Other diptera	0	0	7	1
Total drift	525	355	1236	981

When considering the total drift fauna in relation to the total population at the end of the experiment (potential drift fauna), this relative drift was also higher in the dimethoate stream.

The tendency for drifting was, however, very different among the different taxa (Fig. 3). For example, almost 100% of the total population of chydorids was found in the drift fauna, whereas drifting beetle larvae were not observed. A total of 10.4% and 6.8% of individuals of the benthic macro-invertebrate communities were caught in the drift fauna of the treated and untreated stream respectively.

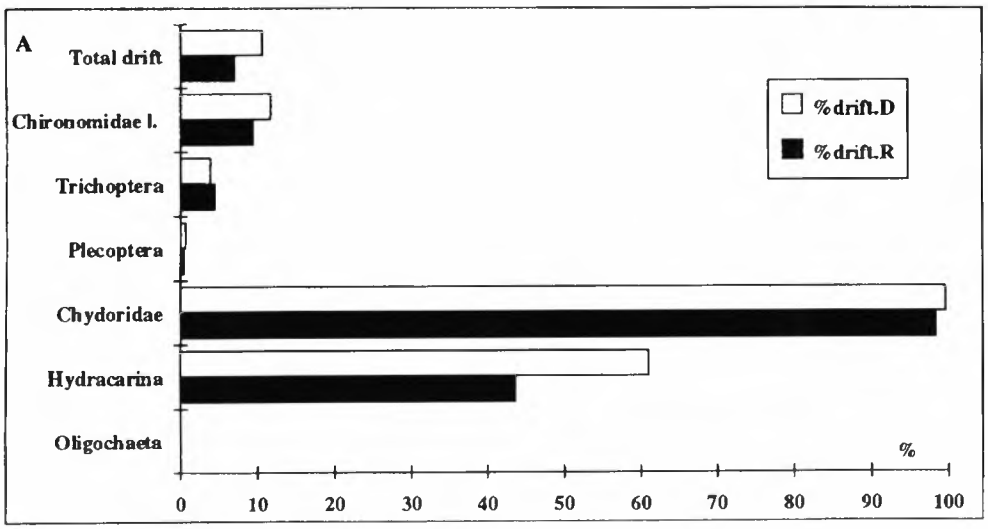

Figure 3. The proportion of the populations found drifting in test A

Test B: the spring community

In the spring community the total numbers of drifting animals was more than twice that in test A. The total numbers of individuals were 1239 and 982 in the dimethoate-treated and the reference streams respectively (Table 2). Most of the time the average daily drift rate for the total drift fauna was higher in the dimethoate stream than in the reference stream (Fig. 4). The drift rates varied between 24 and 68individulas each day in the dimethoate-treated stream and between 13 and 82 each day in the reference stream whereas the average daily drift rates for the total experimental period were 42 and 34 each day.

Chironomids and stoneflies made up most of the drifting animals (Table 2). For chironomids there were only small differences between the treated and the untreated streams (Fig. 4). A large proportion of the stoneflies consisted of small nymphs of the stonefly *Leuctra sp.* Most of the time stoneflies were caught in considerably higher numbers in the treated stream than in the untreated one. A total of 463 and 302 *Leuctra sp.* were found in the dimethoate and the reference streams respectively. *Baetis sp.* made up most of the mayfly drift fauna. A total of 84 and 118 individuals were found in the treated and untreated streams respectively. Chydorids also were common in the drift fauna and were much more frequently encountered in the dimethoate-treated stream than in the reference stream with a total of 128 and 15 individuals in the two respective streams.

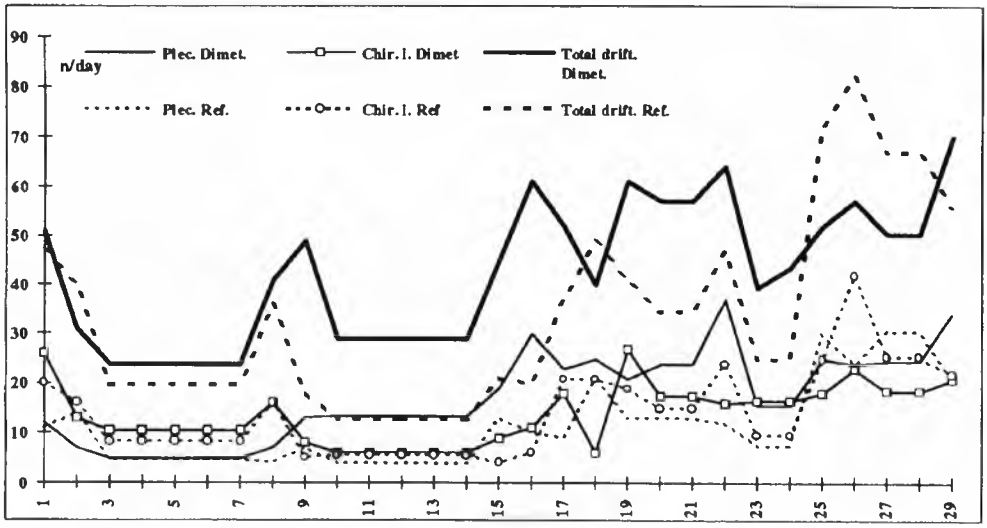

Figure 4. The drift rate of macroinvertebrates during the experimental period in test A

For most species/groups the proportion of the population caught in the drift was higher in the dimethoate stream (Fig. 5). The differences between different taxa were, however, considerable. About 1% of the oligochaets were caught in the drift fauna whereas 68% of the mayfly fauna was found there. Among the taxa found in sufficient numbers the chironomid pupae constituted the only group with considerably lower drift activity in the dimethoate stream than in the reference stream. A total of 28% and 24% of the individuals of the invertebrate communities were caught in the drift fauna during this experiment.

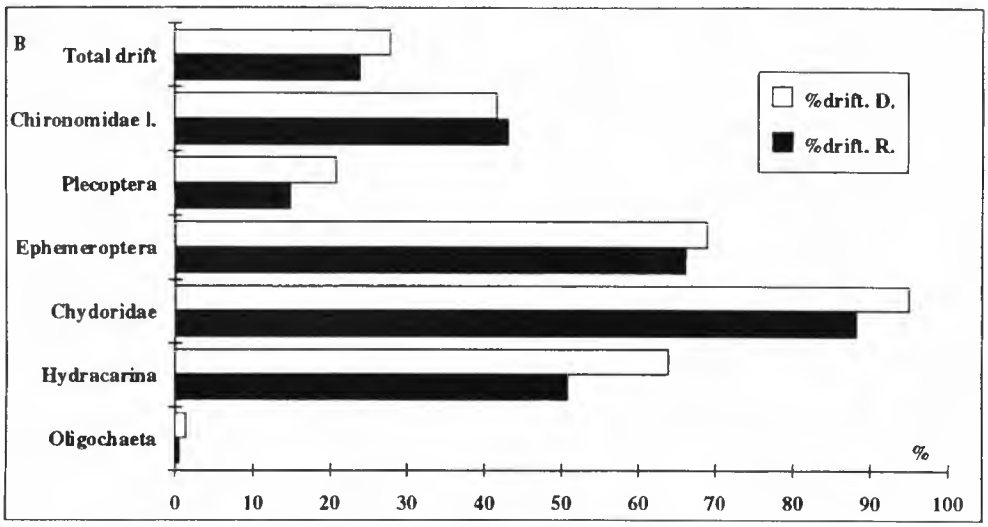

Figure 5. The proportion of the populations found drifting in test B

Other movements

Test A

The tendency of moving out of the trays varied between the different populations (Fig. 6). In both dimethoate and reference streams large and/or mobile species such as mayflies and last instars of stoneflies were more dispersed than smaller or less mobile species. As much as 70% of the mayfly fauna was found outside the trays in the dimethoate-treated stream and 54% in the untreated one. The total moving activity was higher in the dimethoate stream than in the reference stream with a total of 22% and 19% found outside the trays.

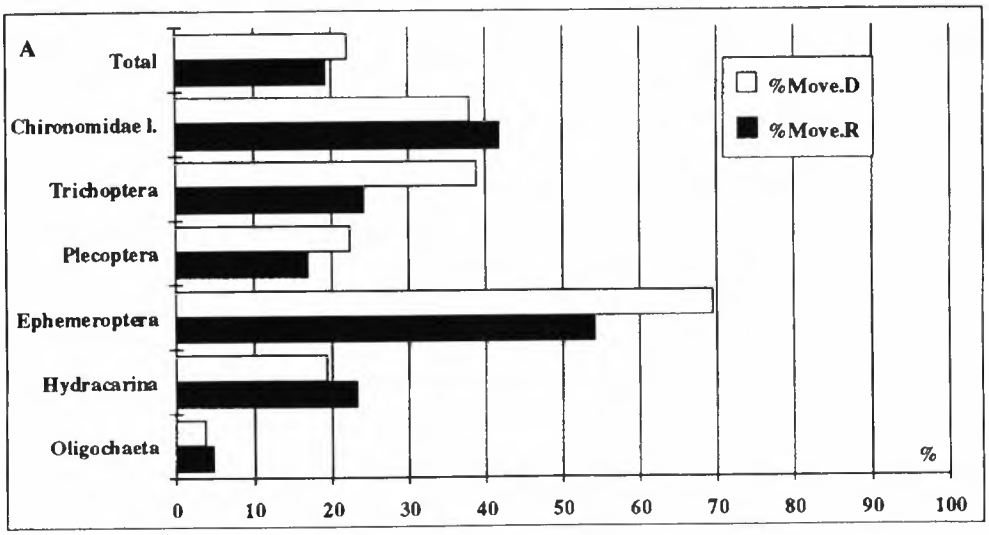

Figure 6. The proportion of the populations moving out of the substrate filled trays in test A

Test B

The tendency of moving away from the trays was for most populations higher in the dimethoate stream. It was found that only for stoneflies and the simulids the proportion of animals moving out of the trays was almost equal in the two streams (Fig. 7). For the mayflies, a large proportion of the community was found outside the trays in both streams. However, the proportion was higher in the dimethoate-treated stream than in the untreated streams with 68% and 55% respectively. The 40 extra *Baetis rhodani* added to the mayfly community in each stream at the start of the experiment were assumed to be equally distributed in the two streams. A total of 36% and 32% of the fauna were found outside the trays.

Structural changes

Test A

Even though only small differences in total abundance between the two streams were observed, 9 out of 13 populations had a lower number of individuals in the dimethoate stream than in the reference stream at the end of the experiment. For example, the beetle larvae and the caddisflies were reduced by 70% and 50% respectively in the dimethoate stream relative to the reference stream. However, the oligochaets were 70% more abundant in the dimethoate stream (Fig. 8).

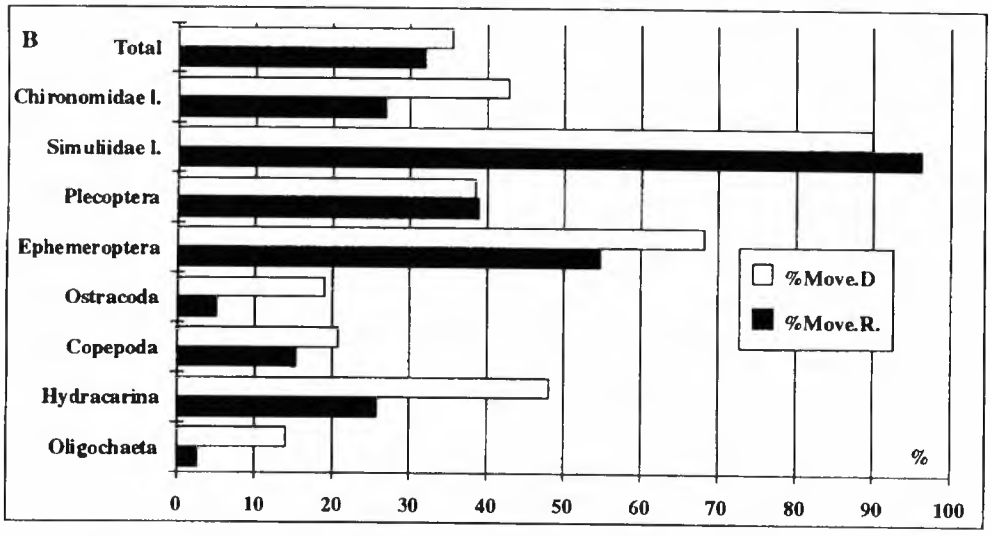

Figure 7. The proportion of the populations moving out of the substrate filled trays in test B

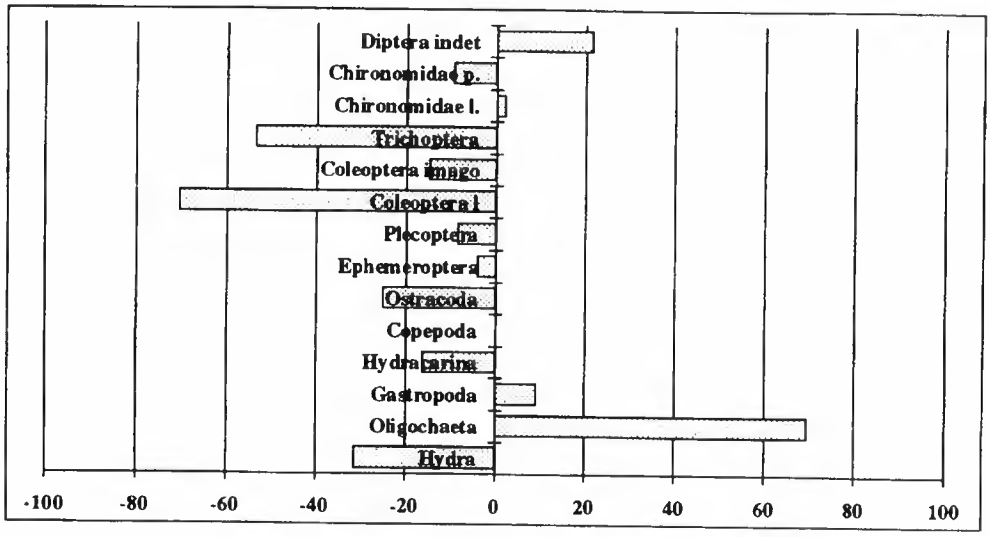

Figure 8. The percentage deviation in abundance at the end of the experiment in the dimethoate stream relative to the reference stream in test A

In both streams in test A the total number of animals was almost doubled during the experimental period (Table 3). This was to a great extent caused by an increase of newly hatched individuals of stonefly species in the two streams. The most extensive increase was observed for the species *Capnopsis schilleri* (Table 4). In contrast, the stonefly species *Leuctra digitata* reduced its abundance by two-thirds from the start to the end of the experiment. With the exception of *Capnopsis schilleri,* which had about the same abundance in both streams, the abundance of stoneflies in general was about 20% lower in the treated stream than in the untreated one. The abundance of mayflies was low both at the start and at the end of the experiment. The most common species was *Paraleptophlebia sp.*, having an equal abundance in both streams. A newly hatched but unidentified caddisfly species was absent in the start trays, abundant in the reference stream, but almost absent in the dimethoate stream.

Table 3. The abundance of different taxa at the start and at the end (reference and dimethoate) of the experiments of test A and test B

	test A: autumn			test B: spring		
	Start	Ref.	Dimet	Start	Ref.	Dimet
Hydra	29	54	37	0	1	0
Nematoda	0	2	5	4	3	2
Oligochaeta	465	375	636	110	342	490
Hirudinea	0	0	2	0	0	0
Gastropoda	14	98	107	5	5	4
Lamellibranchiata	3	2	0	0	0	0
Hydracarina	20	43	36	23	31	27
Copepoda	0	0	0	33	39	24
Chydoridae	0	3	1	2	2	7
Ostracoda	15	232	174	30	98	47
Ephemeroptera	3	24	23	54	64	41
Plecoptera	1236	2563	2343	4139	1902	1876
Coleoptera l	25	125	37	120	9	19
C. imago	24	27	23	13	18	12
Sialidae	0	0	2	0	0	0
Trichoptera	98	321	149	24	16	18
Simuliidae l	0	3	6	4	26	30
S.pupa	0	4	6	0	7	3
Chironomidae l.	565	695	709	1008	540	560
Chironomidae p.	31	21	19	23	13	25
Other diptera	74	107	130	86	17	22
Total	2602	4699	4441	5678	3132	3207

Table 4. The abundance og different species of mayflies, stoneflies and caddisflies at the start and at the end (reference and dimethoate) of the experiments of test A and test B

	test A: autumn			test B: spring		
	Start	Ref.	Dimet.	Start	Ref.	Dimet.
Ephemeroptera						
Baetis sp.	0	2	5	4	4	2
B.rhodani	1	0	0	46	40	28
Paraleptophlebia sp.	1	19	20	0	11	15
Ephemerella sp.	1	1	0	4	6	6
Plecoptera						
Isoperla sp.	0	24	6	3	0	0
Siphonoperla burmeisteri	0	176	153	1	0	1
Amphinemura sp.	5	726	558	0	23	20
Amphinemura borealis	0	0	0	35	0	1
A.sulcicollis	0	0	0	14	0	0
Nemoura sp.	3	4	4	0	1	6
Leuctra sp.	0	121	95	4086	1878	1848
Leuctra digitata	1228	480	380	0	0	0
Capnopsis schilleri	0	1012	1083	0	0	0
Trichoptera						
Rhyacophila nubila	5	4	2	0	0	1
Polycentropus flavomaculatus	84	129	133	16	13	14
Hydropsyche siltalai	5	1	1	0	2	2
Trich. spA	0	182	1	0	0	0
Trich. spB	0	14	11	0	0	0

Test B

At the end of this experiment there was approximately the same number of animals in the dimethoate and reference streams. However, there were differences between the taxa. Oligochaets, unidentified dipterans and chironomid pupae were more abundant in the dimethoate stream, whereas mayflies, ostracods were and copepods less abundant. Only for mayflies and ostracods the differences were statistically significant. For the other taxa there were only minor differences (Fig. 9).

A considerable reduction in the number of animals was observed during the experiment, mainly due to the reduction of young stonefly nymphs of the genus *Leuctra* and chironomids (Tables 3 and 4). The reductions were of the same magnitude in the two streams. Mayflies (mostly *Baetis rhodani*) were, however, significantly more reduced in the dimethoate stream.

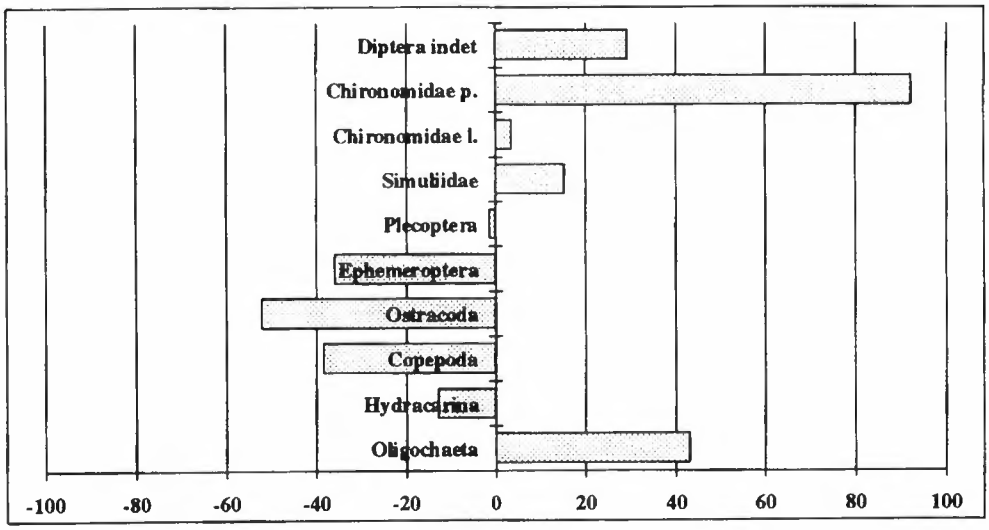

Figure 9. The percentage deviation in abundance at the end of the experiment in the dimethoate stream relative to the reference stream in test B

DISCUSSION

Although the communities were from the same place in the same river, the autumn and spring experiments were conducted on two different communities. The autumn community included last instars of the summer populations as well as the hatching of nymphs and larvae of winter populations. The spring community included late instars as well as young nymphs and larvae of the summer populations. This must be born in mind when comparing the results of the two tests.

In the autumn community the drift rates of both streams were high at the start. During the experiment, however, there was a steady decline in the number of drifting animals. One explanation for this may be that the animals are stressed by this new environment and struggle to get away. This would lead to an accumulation of drift in the streams during the days before the registration starts. The most active animals will, as time goes by, be caught in the drift and taken out of the system. For example no chydorids were found in the community at the end of the autumn test, but this taxon was abundant in the drift fauna at the start of the experiment.

Except for a decline in the drift rates during the two first days, the autumn pattern of drift rates was not seen in the spring community. On the contrary, the total number of individuals increased during the last two weeks of the test. This happened despite the fact that the abundance of the dominating taxa, the chironomids and the stoneflies, was reduced by about 50% during the test. This increased drift therefore does not seem to be a function of abundance, but of other factors of the systems such as, e.g., age, food availability, and so on. In addition to stress factors common to the communities of the two streams, there seems to be a stress factor that increases the drift rate of the stoneflies of the dimethoate stream over a long period. This is probably an effect of the added dimethoate. Only for the active mayfly *Baetis spp.* there were lower drift rates in the dimethoate-treated stream during the last week, perhaps a result of

reduced abundance of the potential drift fauna.

The drift activity as measured in relation to the abundance of populations in the streams in general confirmed the tendency observed for the actual drift rates. The general mobility of the species was the basis for the proportion found in the drift fauna, as was clearly observed for in mayflies both test A and test B. However, the added stress in the form of an insecticide seemed to increase the proportion of the community found in the drift fauna. The opposite result was found for chironomid pupae in the spring community (B). Probably the dimethoate creates problems for the sensitive metamorphosis from larvae to pupae, reducing its activity.

The changes in general mobility of the benthic invertebrates, excluding the fauna caught in the drift net, showed a similar pattern to that for the drift rates; the transfer of the community from a natural stream to an experimental one causes the basic stress for the community in both streams. Dimethoate adds stress to one of the communities and is assumed to be the reason for the increased general moving activity in this community compared to the community of the reference stream.

The changes in abundance from the start to the end of the autumn and spring experiments were surprisingly similar in the reference and treated streams in both tests. However, differences were observed. During the autumn test the stonefly species *Capnopsis schilleri* hatched in great numbers from eggs to nymphs in quite similar quantities in both streams. Thus, dimethoate did not seem to have any negative effect on the egg hatching process. The same seemed to apply to the mayfly *Paraleptophlebia* and some caddisflies. In contrast, the hatching of some other stonefly species was 20% lower in the dimethoate stream relative to the reference. Furthermore, the hatching of an unidentified caddisfly species in high numbers was observed in the reference stream. The species, however, was almost absent in the dimethoate-treated stream. Thus, for the caddisfly it is quite probable that dimethoate had a negative effect on eggs, the hatching process or on the early larvae.

During the spring test the number of invertebrates was reduced mainly due to reduced abundance of chironomids and stoneflies. The reductions were, however, similar in both streams indicating no effect of the dimethoate treatment. Rather, the reduction, at least for the dominant stonefly *Leuctra* shows the natural decline in young insect populations. It should be noted that this species was already found in large numbers in the start trays. This was quite a different situation from that of the dominating stonefly of the autumn test. Mayflies (mainly *Baetis rhodani*) were, however, significantly more reduced in the dimethoate stream, probably an effect of dimethoate.

Studies on effects of insecticides on macroinvertebrate communities in streams frequently report on increased drift and reduced abundance as a typical response (Wallace & Hynes 1975; Muirhead-Thomsen 1987; Sebastien & Brust 1989; Wallace*et al.* 1989; Kreutzweiser & Sibley 1991). The magnitude of the responses depends on the concentrations of the pesticides relative to their toxicity, as well as the water quality, but also on the kinds of macroinvertebrate communities included in the studies (Dermott & Spence 1984). Most of the above cited studies deal with rather high concentrations. In laboratory streams, Hansen & Garton (1982) assessed the effects of diflubenzuron on the total biological community for several months. The insect fauna suffered direct toxic effects at concentrations above 1 µg/l. Differences in sensitivity were observed; mayflies and stoneflies were affected at 1 µg/l, whereas dipterans were affected at 10 µg/l. This insecticide is, however, asssumed to be 3-5 times more toxic than dimethoate, so in relation to our study the concentrations used were rather high and more serious effects are expected. Different taxa also respond in different ways, it has often been observed that amphipods and mayflies are sensitive species. Arthur *et al.* (1983) reported that effects were

noted at the lowest concentrations (diazinon; 0,3 µg/l) for the amphipods and insects, lower number of mayflies and damselflies emerged from the treated channels. Acute tests on dimethoate showed that the mayfly species *Baetis rhodani* was by far the most sensitive species (Bækken & Aanes 1991).

Behavioural effects have been studied that support our results on increased activity. Both methoxychlor and fenitrothion exposure at sublethal concentrations on the stonefly species *Acroneuria lycorias* caused abandonment of microhabitats, associated with increased locomotor activity and drift (Scherer & McNicol 1986). It has been observed that the caddisfly *Brachycentrus* leaves its case when exposed to pesticides (Symons & Metcalfe 1978; Anderson & DeFoe 1980) and chironomids have a tendency to leave their burrows when exposed to insecticides (Sebastien & Lockhart 1981).

Although not clearly stated in our experiments, the insecticide treated streams may result in mortality of exposed macroinvertebrates. However, the number of dead chironomids counted in the drift during the autumn test was higher in the dimethoate than that in the reference stream; 46 and 26 respectively (Baekken & Aanes 1991). Lugthart *et al.* (1990) observed that insecticide application resulted in significantly reduced density and biomass. In a study of the insecticide temephos on benthic community of a model stream, the majority of the benthic animals were killed. This resulted however in a bloom of epilithic algae because of the reduced grazing (Yasuno *et al.* 1985). Morever, the use of herbicides may seriously affect the benthic invertebrate community. Dewy (1986) found herbivores to be greatly reduced with the addition of atrazine. Both these examples demonstrate that changes forced on some part of the ecosystem will eventually have effects on other parts.

SUMMARY

Ecotoxicological testing on benthic invertebrate communities in experimental streams may be useful in long-term testing of sublethal concentrations of pesticides and other pollutants. The present tests of the insecticide dimethoate (1 ppb) on communities at different stages of development revaled similar effects that may be a general response to this pesticide:

- Drift rate was higher in the dimethoate streams.
- The proportion of the populations found in the drift was higher in the dimethoate streams.
- The non-drifting movements away from the trays were higher in the dimethoate streams.
- Structural changes were observed during the experiments. Structural changes between the streams mostly were small, but with significant differences for some populations.

In sum, a tendency toward increased activity of the individuals and a reduction in the population density of some species.

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Acute and long-term effects of propiconazole on freshwater invertebrate communities and periphyton in experimental streams

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Aanes, K.J. & T. Bækken 1994. Acute and long-term effects of propiconazole on freshwater invertebrate communities and periphyton in experimental streams. Norwegian Journal of Agricultural Sciences. Supplement No. 13: 179-193. ISSN 0802-1600.

Results from screening test on the toxicity of pesticides commonly used in Norwegian agriculture revealed that the fungicide propiconazole was toxic to macroinvertebrates in the stream-bed community, with 96 h LC_{-50} -values ranging from 0.9 ppm to 1.3 ppm. A five week test was set up using indoor experimental streams to study possible sublethal effects generated by propiconazole (5 ppb) on natural benthic communities from running water ecosystems. At the end of the test period the results indicated a greater increase in the number of young individuals hatching from eggs during the experiment in the propiconazole stream compared with that in the control stream. However, for the two groups Coleoptera and Simuliidae the abundance in the contaminated stream was reduced compared with that in the control stream. The only two taxa with a marked increase in the drift rate were the larvae of the filter feeders *Hydropsyche* and the Simuliidae. To provide information about effects generated by propiconazole on natural communities of periphyton, unglazed ceramic tiles colonised in a natural stream were implanted at the beginning of the five week test. At the end of the test period no algae could be detected on the tiles in the propiconazole-treated stream. This may have an indirect affect on the benthic fauna in recipients draining arable land sprayed with propiconazole by reducing the growth of periphyton, an important food source for the benthic fauna.

Key words: Benthic algae, experimental streams, freshwater organisms, fungicide, macro invertebrates, pesticides, propiconazole, sub lethal concentrations, toxicity.

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The FPJV - Project: Contamination of Soils and Waters from Agricultural and Industrial Use of Pesticides (1988-1992) was established in order to obtain more information about the environmental effects of some commonly used pesticides in Norway (Lodhe 1993). One important part of the project was to study possible effects in the aquatic environment generated by our use of pesticides. Studies elsewhere have shown on several occasions that lentic and lotic ecosystems have been affected by pesticides from treated fields (Morrison & Wells 1981; Deway 1986; sMuirhead-Thomson 1987). In Scandinavia several studies have shown residues of pesticides in surface water (Torstensson 1992; Lode *et al.* 1992; Kreuger 1992; Mogensen 1992). During the summer period in 1987, eight Norwegian water bodies where monitored for a selection of commonly used pesticides (GEFO and SPV 1987). A variety of pesticides were

found in concentrations ranging from the detection limit up to about 20 µg/l.

In the joint cooperative research project, the Norwegian Institute for Water Research (NIVA) has been responsible for the study of issues relating to water pollution. The effect studies have included acute and sublethal toxicity tests on algae, zooplankton, macroinvertebrates and fish as well as accumulation studies of pesticides in lake sediments and fish flesh. Both single species and natural communities have been tested (Aanes 1992; Aanes & Bækken 1989; Abdel-Hamid *et al.* 1991; Bækken & Aanes 1991; 1993; Hessen *et al.* 1991; Grande *et al.* 1993; Källqvist *et al.* 1991).

In toxicity tests benthic macroinvertebrates from lotic environments were exposed to seven pesticides commonly used in agriculture in Norway. The acute tests revealed that two of the tested pesticides listed in Table 1, dimethoate and propiconazole, were more toxic than the others (Bækken & Aanes 1991). It was decided to go further with sublethal concentrations in order to search for possible effects over a five week period for these two pesticides. Long-term effects have previously been shown both for herbicides and insecticides (Streith & Peter 1978; Arthur *et al.* 1983; Seuge & Bluzat 1983; Dewey 1986). A study of the sublethal effects of dimethoate are presented by Bækken & Aanes (1993).

Table 1. Pesticides studied in the FPJV - Project: Contamination of Soils and Waters from Agricultural and Industrial Use of Pesticides (1988-1992)

Pesticide	Trade name	Type	Category
Atraxine	Pramitol A	triazine	herbicide
Simazine	Gesatop	triazine	herbicide
MCPA	San-75	phenoxy	herbicide
Dichlorprop	Hedonal DP	phenoxy	herbicide
Chlorsulfuron	Glean	urea - triazine	herbicide
Propiconazole	Tilt 250 EC	triazole	fungicide
Dimethoate	Rogor L 20	organophosphorus	insecticide

In the present paper we focus on the fungicide propiconazole, its effect on some important organisms in the benthic communities of macroinvertebrates and algae under the following test conditions:

(1) short periods with high concentrations of the fungicide, in order develop 96h LC_{50} -values for the invertebrate species tested.

(2) A five-week period of sublethal concentrations of 5 ppb propiconazole/l. During the test period behavioural changes and drift activities of invertebrates were registered, as well as any structural changes observed in the communities of benthic macroinvertebrate fauna in the two experimental streams at the end of the test period.

(3) Algae. In this test with concentrations of 5ppb propiconazole we investigated effect of the fungicide on natural communities of benthic algae from a running water biotope. Growth and structural changes in the communities of benthic algae in the two experimental streams were registered at the end of the test period. During the test period colonisation of algae on implanted substrates were studied.

MATERIALS AND METHODS

Acute effects

The biotest laboratory with through-flow aquaria were used in the acute tests (Fig. 1). Each concentrations of the fungicide was continuously dosed to the aquaria using peristaltic pumps to give a constant concentration throughout the test period. Propiconazole was tested at concentrations of 10, 5, 1, 0.5 and 0.25 ppm. To each of the circular aquaria there was an input of 30 l test water/hour (Fig. 1). The test water was natural lake water from Lake Maridalsvann and the water quality was representative for Norwegian unpolluted lowland waters (Table 2). To prevent particles from entering the test system, the input water was filtered through a series of 10, 5, and 1 µm column filters. In the aquaria an artificial substrate was made up of differently sized glass spheres with the exception of the two ephemeropteran species the tested organisms were put into separate boxes in the lower row of aquaria to minimize interspecific disturbances. All experiments were run for 96 h using five test solutions and one control. The water temperature was 15±1°C. Dead animals were removed, and severaly affected animals as well as exuviae were registered twice times a day. LC_{50} -values were calculated according to an Logit model.

Table 2. Physico-chemical data of the test water used in the experiments with propiconazole. Mean values

Parameter		Parameter	
pH	6.6	Ca, m/l	2.9
Conductivity, mS/m at 25°C	3.4	Mg, m/l	0.41
Colour, mg Pt/l	21	Na, mg/l	1.6
COD, mg 0/l TOC = 2.4 mg C/l	4.0	K, mg/l	0.38
Total nitrogen, µg N/l	300	Cd, µg/l	0.30
NO_3, µg N/l	180	Cu, µg/l	2.0
Total phosphorous, µg P/l	4.5	Zn, µg/l	< 10
Cl, mg/l	2.7	Pb, µg/l	<1.0
Hardness, mg $CaC0_3$/l	11	Al, µg/l	110

Five common species of the macro fauna were used in the tests: The snail *Physa fontinalis*; the crustacean *Gammarus lacustris* (an amphipod); three insects: The mayflies *Baetis rhodani* and *Heptagenia sulphurea*, and the net-spinning caddisfly *Hydrophsyche siltalai*. The first two are primarily lake species, but are also found in lotic environments. The last three species are confined to running waters. The animals were in the last instar and were sampled a few days before the tests.

All concentrations referred to in the tests are calculated values of the active pesticide chemical in the commercial product: Tilt 250 EC from Ciba-Geigy AG, Basel.

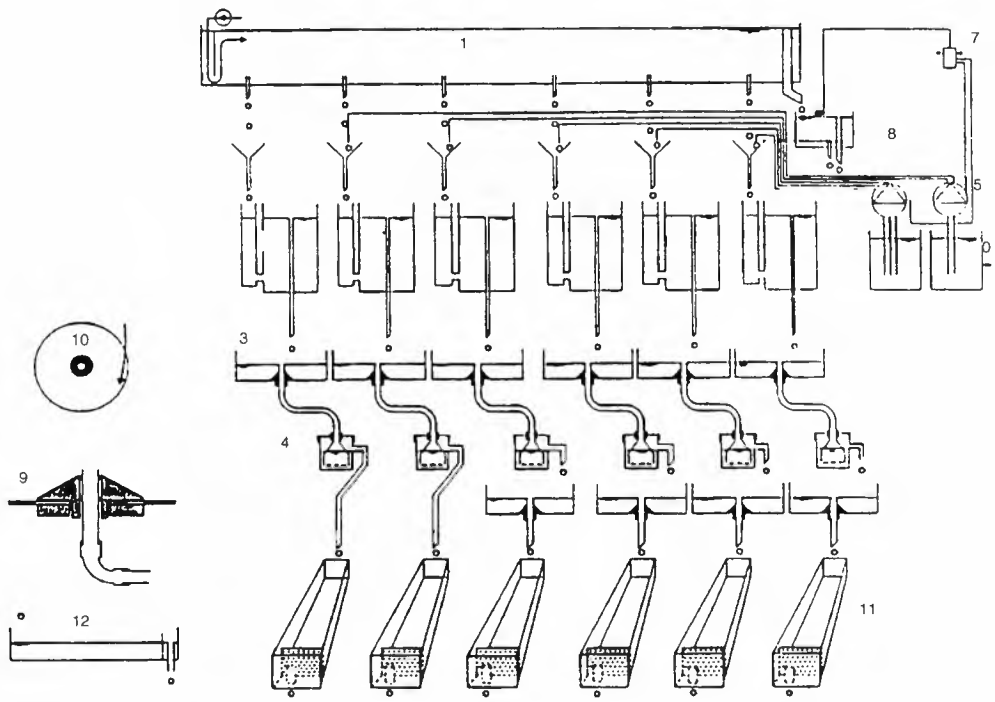

Fig. 1. The throug-flow test system used for the 96 h-LC_{50} test

1. Reservoir for test water
2. Mixing vessel for test solutions
3. Test aquaria
4. Sieve and test aquaria
5. Peristalic pumps with separate channels to each mixing vessel
6. Tank for chemicals to be tested
7. An automatic swith to stop the peristalic pumps if the water flow changes
8. Thank to control the flow of test water
9. Detail of the outlet from the circular aquaria
10. Test aquaria for lotic macroinvertebrates
11. Test vessel for fish and bigger evertebrates as crayfish and molluscs
12. Details of the test vessel no 11

Sublethal effects

Sublethal effects on the benthic fauna in running water ecosystems were studied in two indoor experimental streams, each one 5 m long (Fig. 2). Most test water was recirculated, but there was a continuos renewal of 105 l/h. Propiconazole was continuously dosed to one of the streams to give a constant concentration of 5ppb. The other stream was left untreated.

Thirty trays filled with a defined combination of sand, gravel and pebbles (990 cm^3) were placed in a nearby unpolluted stream for a period of five week for colonization. Trays colonised by the stream biota and with a composition reflecting a natural benthic community were used to test possible sublethal effects of propiconazole. Ten trays, randomly selected, were placed in each of two indoor experimental streams. The remaining 10 trays represented the animal community at the start. During the test some of the stream water was piped out into a net and

pumped back again (Fig. 2), and the number of drifting animals counted. The mesh size of the net was 250 µm.

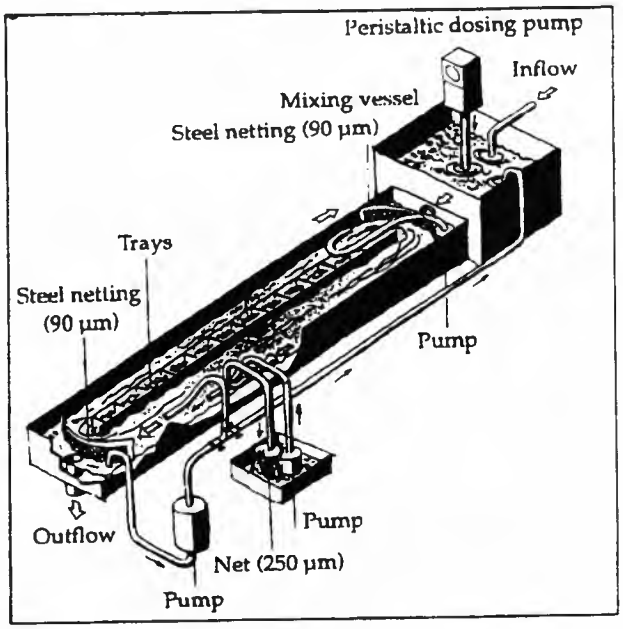

Fig. 2. One of the indoor experimental streams

In this experiment we also studied how benthic algae was affected. A community of algae was established on unglazed ceramic tiles in the same streams and for the same time period as that for the trays used to collect communities of benthic invertebrates. Together with these tiles we also placed sterilised tiles in the experimental streams to investigate how the colonization of algae on new tiles was affected. The tiles were illuminated and the day length was 16 h. After five week the tiles were analysed. The chlorophyll a was measured and a semi-quantitative study of the algae community on the tiles was carried out.

RESULTS

Macro invertebrates - acute effects

All the test animals died within few hours in the 10 ppm solution (Fig. 3). In the 5 ppm solution the animals survived for a longer time, but all died within a period of 96 h. At the lower concentrations between 50% and 100% survived. The toxicity level of propiconazole measured as 96 h-LC_{50} was quite similar for all species, with values ranging from 0.87 ppm for *Baetis rhodani* to 1.3 ppm for *Gammarus lacustris* (Table 3).

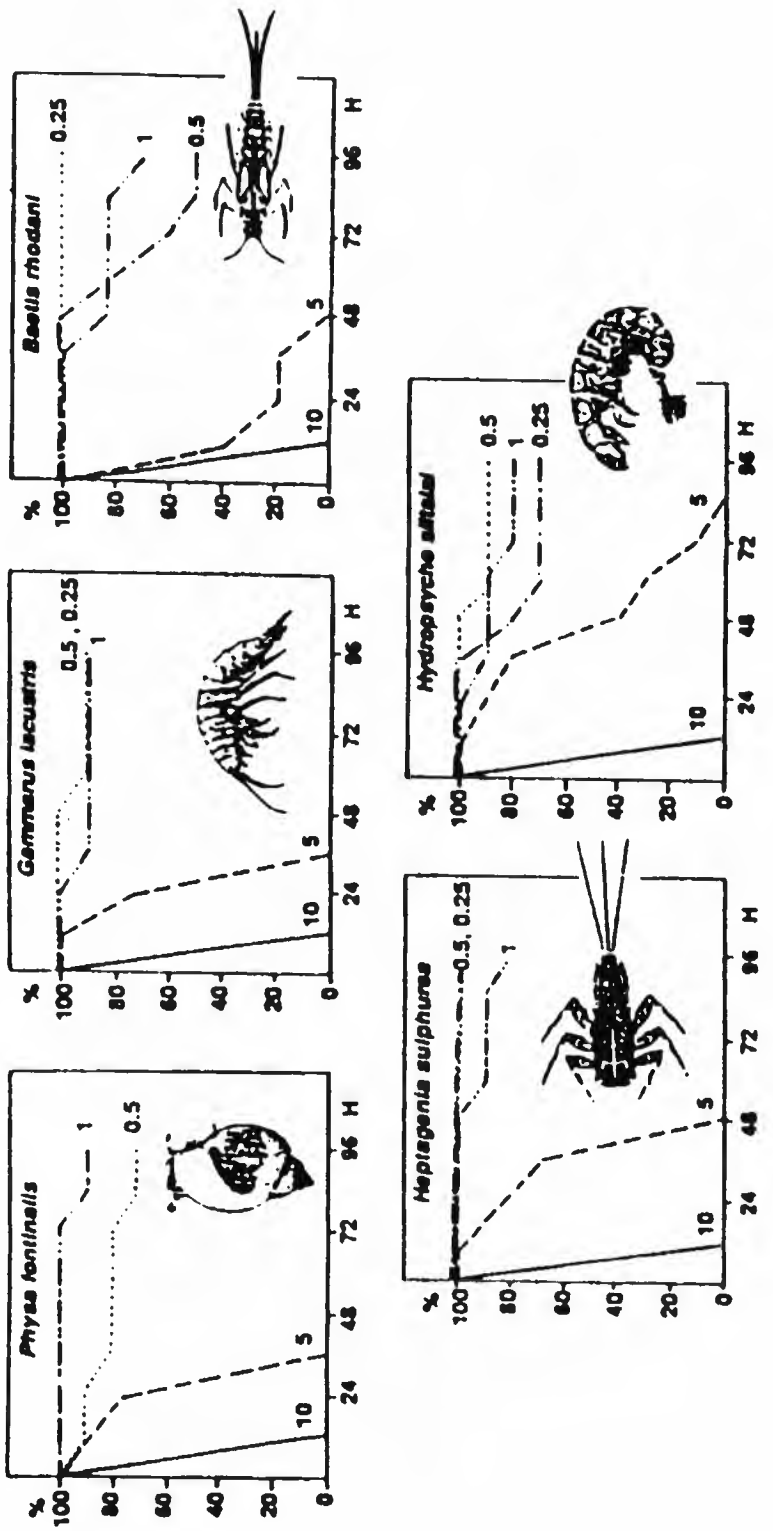

Fig. 3. Survival curves for five macroinvertbrate species exposed to propiconazole

Table 3. 96 h-LC_{20}, LC_{50} and LC_{80} -valies for five invertebrates in relation to the fungicide propiconazole. The units are ppm. (From Bækken & Aanes 1989)

Species	Propiconazole		
	LC_{20}	LC_{50}	LC_{80}
Gastropoda			
Physa fontinalis	0.7	1.3	2.6
Crustacea			
Gammarus lacustris	0.7	1.3	2.5
Ephemeroptera			
Baetis rhodani	0.4	0.9	2.0
Heptagenia sulfurea	-	1.0	-
Trichoptera			
Hydropsyche siltalai	0.5	1.2	2.8

Macro invertebrates - sublethal effects

In the material from the drift analysis which was sampled daily during the five week test period, there seemed to be a tendency for more animals to drift in the untreated stream (Fig. 4). During the test period 712 individuals were caught in the drift net in the reference stream compared to 542 in the propiconazole-treated stream. This came to 6.9% and 11.0% of the total number of macroinvertebrates found in the treated and untreated stream, respectively, at the end of the test period (Fig. 5). Chironomids dominated the drift fauna. For most taxa the drift rate was higher in the reference stream. The only two groups with a higher drift rate in the propiconazole stream were the larvae of the caddisflies: *Hydrophsyche* and the black flies: Simuliidae (Fig. 5). Both are filter feeders living on the top of the substrate. Registration of the dispersion of the different populations showed a higher proportion of animals outside the trays in the reference stream than in the propiconazole-treated stream at the end of the test period.

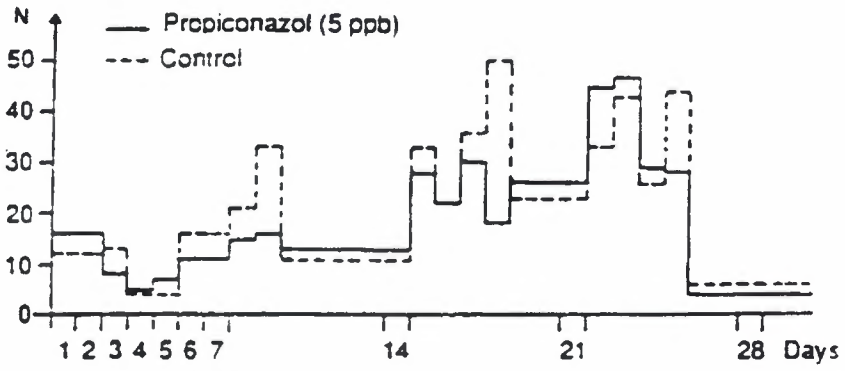

Fig. 4. Total number of animals found in the drift/day

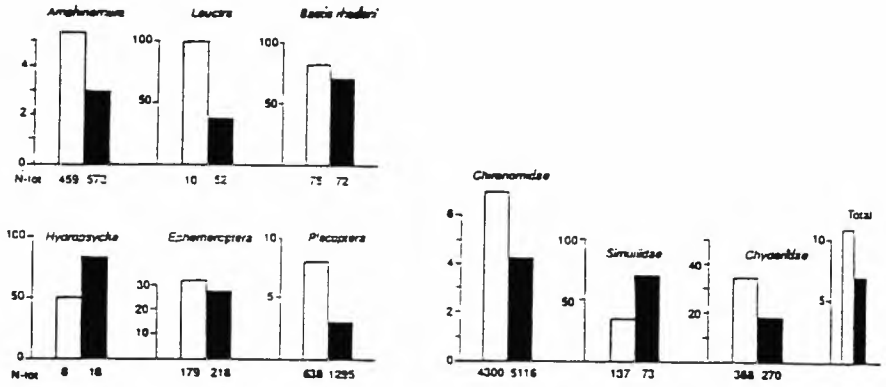

Fig. 5. The number of drifting animals in relation to their frequency in bottum fauna

Changes in the structure of the benthic fauna were observed from the start to the end of the test period in both streams. The total number of invertebrates increased in both streams due to young individuals hatching from eggs during the experimental period (Table 4 and 5). This increase was greater in the stream treated with propiconazole. Small and unidentified dipterans, nymphs of plecoptera and Ostracods doubled their density in the treated stream (Fig.5). However, the simuliidae population, in the propiconazole-treated stream was reduced by 75% compared with that in the control stream.

Table 4. The abundance of different taxa at the start and at the end of the five-week experiment with propiconazole

Group		Start	Reference	Propiconazole
Hydra		0	0	0
Nematoda		11	7	8
Oligochaeta		115	84	92
Hirudinea		0	0	0
Gastropoda		2	0	0
Bivalvia		0	0	1
Hydracarina		48	51	53
Chydoridae		13	243	222
Ostracoda		102	53	110
Copepoda			268	222
Odonata		1	3	3
Ephemeroptera		167	122	157
Plecoptera		919	587	1256
Coleoptera	larvae	22	16	9
	imago	3	2	0
Sialidae		0	0	0
Trichoptera		128	83	84
Simuliidae	larvae	188	89	22
	pupae	5	4	5
Chironomidae	larvae	1250	4005	4899
	pupae	25	135	167
Andre diptera		50	21	40
Total		3049	5773	7350

Table 5. The abundance of different species of mayflies, stoneflies and caddisflies at the start and at the end of the five week experiment with propiconazole

	Start	Reference	Propiconazole
Ephemeroptera			
Ameletus inopinatus	0	19	35
Baetis rhodani	87	22	13
Baetis niger	18	21	42
Heptagenia sp.	62	53	67
Plecoptera			
Protonemura meyeri	7	1	0
Amphinemura sp.	565	434	556
Nemoura sp.	1	0	0
Leuctra sp.	282	101	588
Capnopsis schilleri	2	7	30
Isoperla sp.	18	13	10
Siphonoperla burmeist.	26	30	32
Trichoptera			
Rhyacophila nubila	7	4	8
Wormaldia sp.	1	0	0
Polysentropidae	19	16	16
Hydropsyche sp.	5	4	3
Hydroptilidae	4	4	3
Trich. indet	88	54	54

Algae

During the test period the algae community on the tiles from the natural stream was gradually reduced in the propiconazole stream . At the end of the experiment it was not possible to detect any remaining algae by eye examination. A microscopic examination showed small numbers of only four algae species while at least nine were present at start in the propiconazole-treated stream (Table 6). Surprisingly as propiconazole being a fungicide, we found a lot of fungus on these tiles. In the control stream only small changes were observed.

For uncolonised tiles placed in the streams at the beginning of the experiment there had been an active colonisation in the control stream, but only a few single cells were found in the treated stream. An examination of the chlorophyll concentration on these tiles showed 5,3 mg chlorophyll a per tile (10,9 cm^2) in the control stream. In the propiconazole-terated stream chlorophyll a was not detected.

Table 6. Results after five week in an experiment with 5 ppb propiconazole on benthic algae, given as semi-quantitative analyses of the natural algal community on "old" ceramic tiles, (colonized in a natural stream and placed in the experimental stream). When the test was started "new" sterilized tiles were placed in the streams to study the colonization of algae (from Aanes & Bækken 1989)

At start		Control		Propiconazol	
		old	new	old	new
*	Anabaena flos-aqua				
	Binuclearia tectorum	*			
**	Bulbochaete sp.	**		*	
	Cosmarium spp.	*	*		
	Euastrum elegans	*			
***	Muogeotia sp. (5 µm)	***	*		+
***	Muogeotia sp. (11-14 µm)	***	**		+
**	Muogeotia sp. (20-23 µm)	**	**		
**	Oedogonium sp. (5-e µm)	**	*		+
	Oedogonium sp. (20 µm)			*	
	Staurastrum sp.	*			
	Teilingia granulata	*	*		
***	Zygnema sp.	**	*	*	
***	Achnantes sp.	***	**		
	Eucocconeis lapponica	*			
***	Tabellaria flocculosa	***	*	*	+
*	Vorticalia sp.				
	Fungus			**	

*** dominating species
** common
* rare
+ single cells

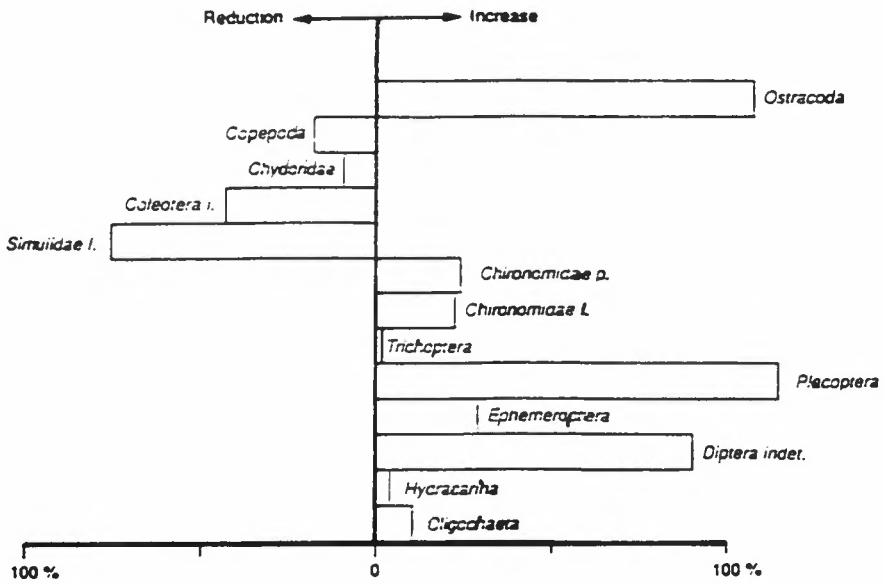

Fig. 6. Changes in the community structure at the end of the experiment

DISCUSSION

When testing stream organisms a continuos through-flow system is highly recommended (Muirhead-Thomson 1987). This ensures a constant level of pesticides and minimizes additional stress caused by stagnant water and variable water quality. The animal species used in this study are not common in standard toxicity tests, but as common inhabitants of affected ecosystems they should be used more frequently. Because of this, information about their reactions to the present pesticide is scarce in the literature. Data on the acute toxicity of propiconazol to *Daphnia magna* given as 48h LC_{50} -values are 4.8 ppm (information supported by Ciba-Geigy AG, Basel). Results from our study on acute toxicity gave 96h LC_{50} -values for propiconazole ranging from 0.87 to 1.3 ppm.

The low pesticide concentrations found in Norwegian surface waters are not thought to produce any acute effects on the macroinvertebrate fauna, with perhaps the exception of behavioural responses such as, for instance the response seen as increased drift among certain groups of organisms in the benthic fauna. But there may be long-term effects. This has been shown for both herbicides and insecticides (Streit & Peter 1978; Arthur *et al.* 1983; Seuge and Bluzat 1983; Dewey 1986).

A proportion of the invertebrate fauna of streams may respond to an impairment of the environmental conditions with escape behaviour resulting in increased drift. In the bioassay with propiconazole a clear-cut drift reaction was not given by the concentration of 5 ppb . One explanation for this might be that propiconazole slows down the mobility of the animals. Only for two groups, the *Hydrophsyche* and Simuliidae, we found an increased drift rate in the treated stream. We had an unexpected increase in the abundance for most groups in the propiconazole stream indicating that this fungicide did not negatively affect the hatching of eggs from these species. The total number of drifting animals/day in the propiconazole treated stream did not reveal any pattern indicating effects like those we found in the bioassay with dimethoate, which significantly increased the drift (Bækken & Aanes 1993). That propiconazole seems to have the effect to reduce the activity in the benthic fauna was also seen when the test was ended. In the affected stream the proportion of the organisms found outside the trays was much less than in the reference stream.

The data generated from the tests carried out with benthic algae were very interesting. Here the results from the bioassay with propiconazole were very clear-cut. The total algae community was wiped out and replaced by fungus, which is surprising since propiconazole is a fungicide. The potential of propiconazole to affect the growth and species composition of the algae communities at environmentally realistic concentrations was also shown by Källqvist *et al.* (1993). At very low concentrations propiconazole may thereby affect the benthic fauna in running water because of the negative effect it seems to have on the benthic algae, which constitute important food organisms for the benthic fauna.

In tests like those presented in this paper the relevance of the data collected in laboratory situations compared to the natural situations is always a crucial point. With respect to propiconazole this fungicide has been measured in Scandinavian surface water in concentrations above the 5 ppb level used in this five week test (Torstenson 1988; Aspmo 1991). We can therefore assume that the effects observed in this test are also likely to be found in running water ecosystems with an increased level of propiconazole. But at the same time the bioavailability of the active components in the pesticide and thereby its effect on the biota may be to some extent different in the recipient (Fisher 1991). This is due, for instance, to a higher levels of organic material and inorganic particles in the water of recipients draining arable land than

those found in our test water. For this reason accurate predictions about this pesticide, its fate and toxicity in aqueous environments, are hindered to some extent by lack of information about how site-spescific water quality parameters affect the biological activity of propoconazole. Therefore further investigation should be undertaken on the bentic fauna in contaminated localities with an increased content of propiconazole in order to verify our results from the laboratory tests.

CONCLUSION

With respect to toxic effects on benthic fauna in lotic environments the 96 h LC_{50}-values (0.87 - 1.3ppm) ranged propiconazole after dimethoate as the second most serious pesticide of the seven tested in the FPJV - Project.

The results from the present test with the sublethal concentration of 5 ppb propiconazole using experimental streams with natural communities of benthic invertebrates revealed the following effects:

- Drift rate in the propiconazole-treated stream was lower than that in the untreated stream.
- The proportion of the populations found in the drift were lower in the propiconazole-treated stream.
- The non-drifting movements away from the trays were lower in the propiconazole stream. This may indicate that the animals in the bentic fauna are less active when stressed by low concentrations of propiconazole.
- Structural changes were observed, but were generally small. There was a reduction in the filter feeding groups of Similiidae and *Hydropsyche* and an increase of the population of *Leuctra* and Chironomidae in the propiconazole-treated stream.

In order to get the correct picture of the water-related environmental effects of this fungicide it is important to pin-point the considerable negative effect these low concentrations of propiconazole seem to have on the communities of benthic algae. This will indirectly affect the benthic fauna in running water ecosystems because of the importance of benthic algae as a food source.

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Effects of pesticides on fish

Experimental and field studies

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Grande, M., S. Andersen & D. Berge 1994. Effects of pesticides on fish. Experimental and field studies. Norwegian Journal of Agricultural Sciences. Supplement No. 13: 195-209. ISSN 0802-1600.

Nine commonly used pesticides were studied with respect to their acute effect (4-d LC_{50}) on brown trout (*Salmo trutta*) and some other freshwater fish. Early life stage tests were carried out with brown trout and zebrafish (*Brachydanio rerio*) in dimethoate and propiconazole.The 4-d LC_{50} -values (ppm) for brown trout were as follows: MCPA (300), dichlorprop (78), simazine (70), chlorsulphurone (40), atrazine (27), glyphosate (4.5), propiconazole (1.2), dimethoate (0.13) and endosulfan (0.0009). The lowest observed effect concentrations (LOEC) on newly hatched brown trout alevins in the early life stage tests were approximately 0.5 and 0.05 ppm for propiconazole and dimethoate respectively. Analyses of the effects of pesticides were carried out of perch (*Perca fluviatilis*), pike perch (*Lucioperca lucioperca*), pike (*Esox lucius*) and sediments from two lakes, the one surrounded by intensively cultivated farmland. None of the above-mentioned substances were found and only lindane and the DDT derivates were found in detectable but low concentrations in fish.

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A great deal of field and laboratory work has been done in order to study the effect of pesticides on fish. Murty (1986) has critically reviewed much of this in his book "Toxicity of Pesticides to Fish". Here summaries of the lethal and sublethal effects of different pesticides and their derivates are presented. The effect of different biotic and abiotic factors, the joint effects of different pesticides, biochemical and physiological features are described and discussed. Of other reviews concerning effects of pecticides the work of Alabaster (1969), Johnson (1973), Holden (1973) and Johnson &Finley (1980) can be mentioned.

However, although much has been done here there are still questions to be asked about the effects of the different compounds on different species of fish and water quality which are of relevance in Norway. For sensitive salmonid fish species, the effects of soft and acid waters and the cold and wet climate typical for Norway may be different from those observed under other circumstances. One of the main purposes of this investigation was to test some commonly used pesticides in Norwegian agriculture on Norwegian fish species under conditions typical for Norway. It was also deemed of interest to measure concentrations of pesticides in fish from localities influenced by agriculture.

MATERIALS AND METHODS

Experimental

The pesticides tested can be seen in table 1.

Table 1. Pesticides used for toxicity testing

Pesticide	Trade name	Type	Category
Atrazine	Primitol A	Triazine	Herbicide
Dichlorprop	Hedonal DP	Phenoxy	Herbicide
Dimethoate	Rogor L 20	Organophosporus	Insecticide
Endosulfan	Thiodan 35	Chlorinated hydrocarbon	Insecticide
Glyfosate	Roundup	Organophosporus	Herbicide
Chlorsulfuron	Glean	Urea-triazine	Herbicide
MCPA	San 75	Phenoxy	Herbicide
Propiconazole	Tilt 250Ec	Triazole	Fungicide
Simazine	Gesatop	Triazine	Herbicide

The substances were delivered from the Norwegian Plant Protection Institute as solutions. They were stored in narrow dark glass bottles at 10°C. All substances were tested for acute toxicity to trout. (4-d LC_{50} = the concentration of a poison lethal to one-half of a test population of fish at four days' exposure). One of the substances - propiconazole - was also tested for acute effects on roach (*Rutilus rutilus)* and minnow (*Phoxinus phoxinus*) and for effects on the early life stage of brown trout. In addition, dimethoate was tested for acute effects on Atlantic salmon (*Salmo salar*), arctic charr (*Salvelinus alpinus*), lake trout (*Salvelinus namaycush)* and on early life stage of zebrafish (*Brachydanio rerio)* and brown trout. Some of the data on the test fish used in acute tests are listed in Table 2.

Table 2. Fish used in acute tests

Species	Weight (mean)	Origin (Strain)
Brown trout *(Salmo trutta)*	1.9	OFA-Grenland strain
Atlantic salmon *(Salmo salar)*	1.1	DOFA-Lierelva strain
Arctic charr *(Salvelinus alpinus)*	2.1	SNERTA-Korssjøen, Røros
Lake charr *(Salvelinus namaycush)*	1.8	NIVA-Lake Superior strain
Minnow *(Phoxinus phoxinus)*	0.74	Akerselva river
Roach *(Rutilus rutilus)*	0.42	Nitelva river

The tests were mainly performed in semistatic systems with a change of solution once a day. A preliminary test was carried out in a through-flow test (Aanes 1992). In all tests laboratory water pumped from a depth of 3 m in a nearby lake was used. Chemical data of the water used are presented in Table 3.

Table 3. Chemical data of laboratory water. Mean values

Parameter		Parameter	
pH	6.3	Ca, mg/l	3.7
Conductivity, mS/m at 25°C	3.2	Mg, mg/l	0.41
Colour, mg Pt/l	21	Na, mg/l	1.1
COD, (perm.no.), mg O/l	4.0	K, mg/l	0.35
Total nitrogen, µg N/l	300	Cd, µg/l	0.30
NO_3, µg N/l	180	Cu, µg/l	2.0
Total phosphorous, µg P/l	4.5	Zn, µg/l	<10
Cl, mg/l	1.3	Pb, µg/l	1.0
Hardness, mg $CaCO_3$/l	11	Al, µg/l	110

The short-term tests were run for four days and the experiments were performed in glass aquaria of 10 l with seven fish in each and different concentrations of the pesticides to be tested. Solutions were renewed every day. The experiments were performed in a termostatically controlled room at a temperature of 10 ± 1°C. There was a slight aeration through glass sinters. The fishes were observed several times every day and their reactions and time of death recorded. The 4-d LC_{50} -values were calculated graphically.

The reproduction tests with brown trout were run from the eyed egg stage, through the hatching process and yolk-sac period and for a few days during the swim-up stage. Fifty eggs were placed on a nylon net kept 2 cm above the bottom on a plastic frame in a 3 l glass aquarium. Two litres of the solution was renewed every day without moving or disturbing the eggs.

There was a slight aeration of the solutions with an air pump and the temperature was maintained at 9.5 ± 1°C during the experiments which had a duration of up to 45 days. The eggs and fry were inspected daily and mortality recorded. Eggs were considered as dead when they became white (opaque) and the yolk-sac fry when they showed no reaction to disturbance by a glass tube and no heart beats were visible. It was found that the exact point of death was difficult to determine for both eggs and yolk-sac fry.

The early life stage test was conducted with zebrafish because this fish is a cyprinid. The results may therefore be comparable with those for the Norwegian cyprinids such as roach and minnow ,which spawn in the spring and have a short egg and yolk-sac period. The tests were mainly carried out in accordance with a standardized method described by Dave et al. (1987). In short, the newly fertilized eggs were placed in Petri dishes with series of concentrations of the substance to be tested. The solutions were changed every day (semistatic system). The tests were completed when the larvae had absorbed the yolk sac after a period of approximately 12 days.

Field investigations

Two lakes in Vestfold county in SE Norway were selected for analysis of pesticides in sediments and fish. Some of the morphometric and hydrological data of the two lakes are presented in Table 4.

The drainage area of Akersvatnet consists of 43% intensively cultivated land and 20% forest. In addition some urban areas (0.7 km^2) are situated in the drainage area. The Foksetjern lake is surrounded only by forests which mainly consist of spruce and fir.

Table 4. Akersvatnet and Foksetjern. Morphometric and hydrological data

		Akersvatn	Foksetjern
Drainage area	km^2	14	0.73
Altitude	m	14	64
Area of lake	km^2	2.3	0.13
Volume	m^3	$14.5 \cdot 10^6$	-
Maximum depth	m	13	-

Akersvatnet can be characterized as a eutrophic lake after Norwegian conditions, while Foksetjern is an oligotrophic lake (Table 5).

Table 5. Physical/chemical data from Akersvatnet (1984) and Foksetjern (1993) (Mean values)

	Akersvatn	Foksetjern
pH	7.2	6.5
Conductivity mS/m	23	5.9
Turbidity, FTU	4.7	0.6
COD, mg O/l	4.5	
Ca, mg Ca/l	15	5.5
Tot.N mg N/l	1.1	
Tot. P µg P/l	36	4

The sediments were sampled by means of a plastic core sampler on 18 February from depths of 8 m and 5 m in Akersvatn and Foksetjern respectively. Only the upper 2 cm of the cores was analysed. The sediment samples were analysed at the Norwegian Plant Protection Institute with GC-MS (MCPA and dichlorprop) and GC (atrazine, simazine, permetrine, propiconazole, dimethoate, DDT, DDE, DDD and lindane). The detection limits were 0.5 µg/kg for lindane and the sum of DDT, DDD and DDE and 1.0 µg/kg for the other pesticides.

The fish were caught using gillnets on 10-11 September 1992. Three species were examined two of which were present in both lakes, i.e. perch (*Perca fluviatilis*) and pike (*Esox lucius*). The pike perch (*Lucioperca lucioperca*) was only found in Akersvatnet. All three species are carnivorous and as the upper link in the food chain, are likely to accumulate persistent substances to a higher degree than organisms lower in the food chain (see table 6 for some data on the fish).

Table 6. Weight and number of fish analyzed

Species	Akersvatn		Foksetjern	
	No. anal.	Mean weight, kg	No. anal.	Mean weight, kg
Pike	2	2.91	6	966
Perch	16	0.361	9	513
Pike perch	15	0.953	-	-

The analyses were carried out on muscle and liver. Samples of muscle of approximately the same weight (16-249 g - somewhat dependent on the size of the fish and their numbers) from each fish were mixed together and homogenized. The same procedures were used for whole liver from each fish. The analyses were conducted at the National Laboratory for Agricultural Chemistry, Uppsala, Sweden.

RESULTS

Experimental

Fig. 1 presents the survival of brown trout exposed to pesticides for four days, calculated 4-d LC_{50} - values for the same tests are presented in Table 7.

Fig. 1.

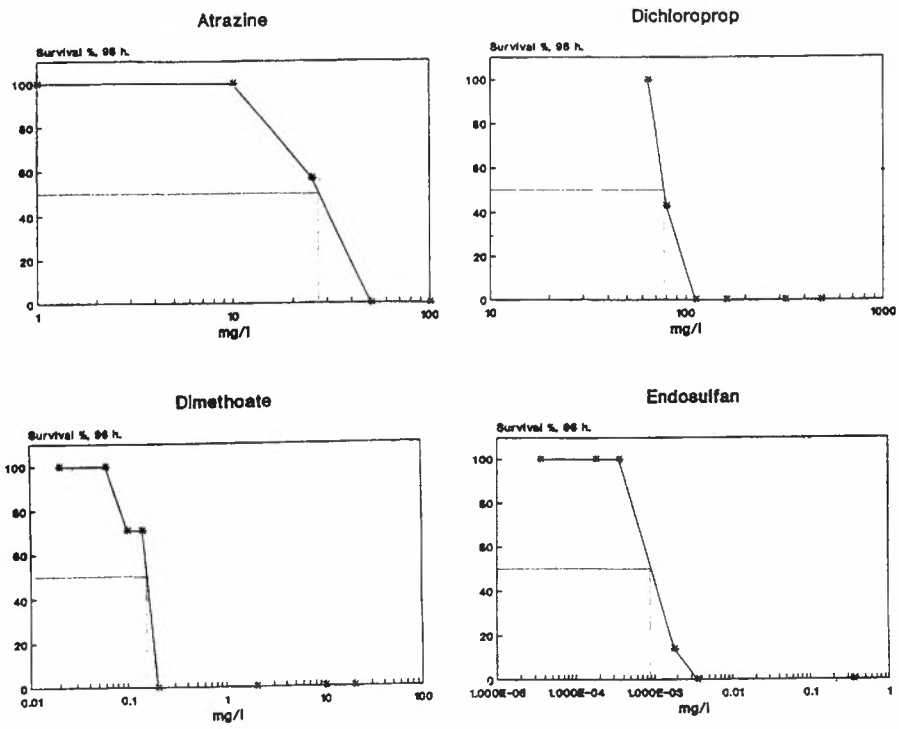

Fig.1a

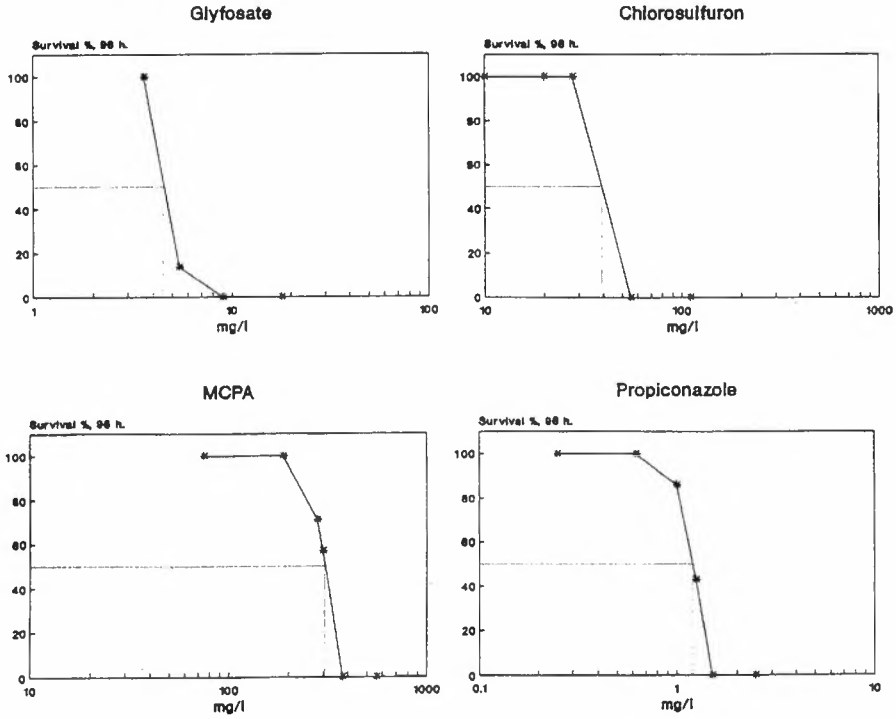

Fig. 1b

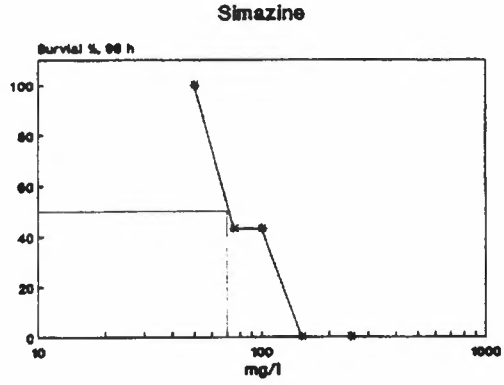

Fig. 1c

Fig. 1a, b and c. Survival of trout exposed to pesticides

Table 7. 4-d LC_{50} - values for trout exposed to pesticides

Substance		4-d LC_{50}, mg/l
MCPA	(H)	300
Dichlorprop	(H)	78
Simazine	(H)	70
Clorsulfuron	(H)	40
Atrazine	(H)	27
Glyfosate	(H)	4.5
Propiconazole	(F)	1.2
Dimethoate	(I)	0.13
Endosulfan	(I)	0.0009

[1]) H = Herbicide
[2]) I = Insecticide
[3]) F = Fungicide

The results show that the six herbicides had a low acute toxicity to brown trout, the 4-d LC_{50} -values ranging from 4.5 to 300 mg/l. The fungicide propiconazole and the insecticide dimethoate were somewhat more toxic with 4- d LC_{50} -values ranging from 1.2 to 0.13 mg/l. Endosulfan was the most toxic with a 4-d LC_{50} -value of 0.0009 mg/l.

The results of tests with minnow and roach in solutions of propiconazole and dimethoate are presented in Fig. 2. For comparison, the results from a parallel test with brown trout, arctic char and lake trout are also shown. There were only slight differences between the two cyprinids and the three salmonids. The cyprinids, however, were more tolerant than the salmonids. The 4-d LC_{50} -values for roach and minnows were 0.5 and 1.8 mg/l in dimethoate and propiconazole respectively. The corresponding values for the salmonids were 0.13 and 1.2 mg/l.

In Fig. 3 and table 8 the results of tests with eggs and alevins of brown trout are presented. The results of tests with zebrafish are given in Fig. 4 and Table 8.

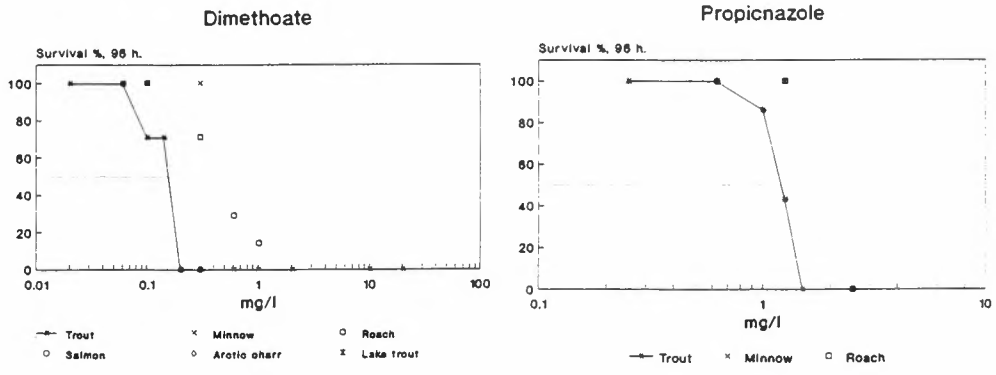

Fig. 2. Survival of different fish species in dimethoate and propiconazole

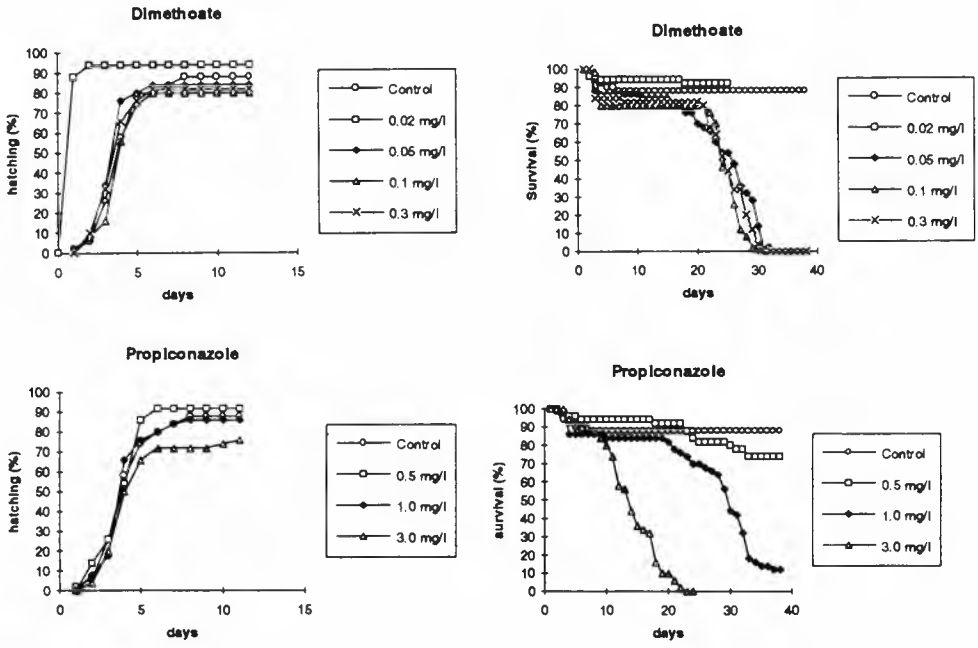

Fig. 3. Hatching and survival of eggs and fry of trout in dimethoate and propiconazole

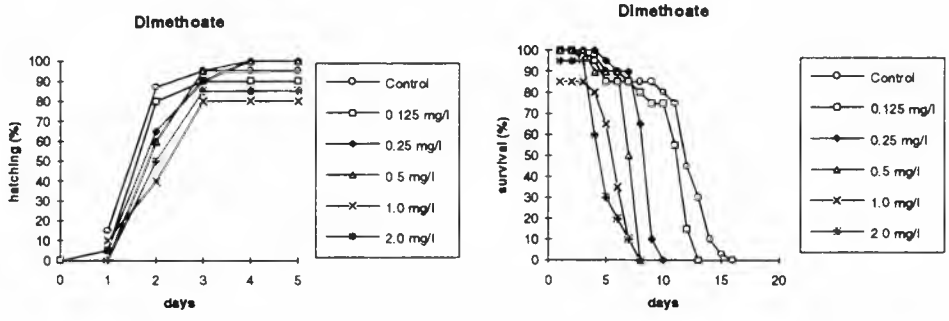

Fig. 4. Hatching and survival of eggs and fry of zebrafish in dimethoate

The hatching process was evidently not greatly affected in the solutions with dimethoate and propiconazole. It was found that only in 3.0 mg/l propiconazole there was a somewhat more reduced hatching than in the lower concentrations (LOEC). However, this is such a high concentration that it would produce acute lethality to older fish.

The mortality among alevins took place, however, in concentrations lower than those that were acutely toxic to older fish. In dimethoate there was an effect at 50 µg/l on brown trout and 25 µg/l on zebrafish.

Table 8. Hatching of eggs and survival of yolk sac fry of trout and zebrafish in dimethoate and propiconazole mg/l
NOEC = no observed effect concentration
LOEC = lowest observed effect concentration

Substance/fishspecies	Hatching		Survival	
	NOEC	LOEC	NOEC	LOEC
Propiconazole				
Trout	1.0	3.0	≈ 0.5	1.0
Dimethoate				
Trout	0.3		0.02	0.05
Zebrafish	0.2		≈ 0.0125	0.025

Field investigation

Pesticides were not found in the sediments beyond the detection limits, which were 0.5 µg/kg for lindane and the same for DDT, DDD and DDE and 1.0 µg/kg for dimethoate, simazine, atrazine, permetrine, propiconazole, MCPA and dichlorprop.

In fish no semipolar pesticides were detected. Their detection limits were as follows:

Dimethoate	0.005 (0.03-0.2 in liver) mg/kg
Atrazine	0.02 mg/kg
Simazine	0.001 (0.003-0.01 in liver) mg/kg
Propiconazole	0.01-0.03 mg/kg

The results of the fish analyses of lindane and the DDT derivates are listed in Table 9.

As can be seen from the table, p,p'-DDE was found in nearly all samples in concentrations ranging from 0.002 to 0.2 mg/kg. The other derivates of DDT were found only in the liver of the pike from Akersvatnet. Lindane was found only in the liver of perch and pike from Foksetjern (see Table 9).

Table 9. Content of lindane and DDT-derivates in fish. Mg/kg (ppm) wet weight - means not detected

Locality/Species		Lindane	p,p'-DDE	p,p'-DDD	o,p'-DDT	p,p'-DDT
Akersvatn						
Pike	M	-	0.009	-	-	-
	L	-	0.2	0.04	0.01	0.02
Perch	M	-	0.02	-	-	-
	L	-	0.02	-	-	-
Pike perch	M	-	0.002	-	-	-
	L	-	0.02	-	-	-
Foksetjern						
Pike	M	-	0.002	-	-	-
	L	0.002	0.1	0.002	-	-
Perch	M	-	-	-	-	-
	L	0.001	0.01	-	-	-
Detection limits		0.001	0.001	0.001-0.005	0.002-0.005	0.002-0.005

DISCUSSION

Experimental

A great many experiments have been performed to test the acute toxicity of those pesticides examined in this investigation. For some of the substances the results are in good agreement with other findings while there are considerable deviations for others . The disparites can be explained by differences in experimental conditions such as water quality, temperature, species and life stage of the fish, etc.s. Hartley & Kidd (1987) report of 4-d LC_{50} -values for rainbow trout *(Oncorhynchus mykiss)* at 232 mg/l for MCPA, 8.8 mg/l for atrazine and > 250 mg/l for chlorsulfuron. For bluegill sunfish *(Lepomis macrochirus)* exposed to dichlorprop, the 2-d LC_{50} -values were 1.1-165 mg/l dependent on the composition of the substance. For dimethoate and mosquitofish *(Gambusia affinis)* a 4-d LC_{50} -value of 60 mg/l was given. The 4-d LC_{50} -value for propiconazole and brown trout was 20 mg/l. Murty (1986) reports of 4-d LC_{50} -values for endosulfan and rainbow trout of 0.3-0.8 µg/l depending on temperature. For glyphosate and rainbow trout Hildebrand et al. (1982) found a 4-d LC_{50}-value of 55 mg/l.

The greatest difference between the results of this investigation and others cited in the literature was found for dimethoate with an LC_{50-} value for mosquitofish more than 300 times higher than that found for trout which were tested in our experiments. As for the rest, there were deviations with a factor of up to 15 times (propiconazole).

The concentrations of the tested pesticides leading to acute toxicity are so high that they can only likely to occur by accident in small natural water bodies in Norway. Many attempts have been made to determine long-term safe concentrations by multiplying the short-term LC_{50} -value by an application factor. Alabaster & Lloyd (1982) suggest that if the maximum concentration of a chemical in the water is much smaller (say by a factor of 10^{-4}) than the acutely lethal level for a particular species of fish, then it might be safe to assume that such a chemical poses no pollutional problems for fisheries. This applies to pesticides that are not found to bioaccumulate.

The maximum concentrations found in Nordic surface water (Kreuger 1992) are presented in Table 10. These are divided by the 4-d $LC_{50} \cdot 10^{-4}$ in the column to the right in the table. If these values are above 1.0, problems may occur. MCPA, dichlorprop, simazine, chlorsulfuron and atrazine all have values below 1.0, but propiconazole, dimethoate and endosulfan are above 1.0. For these chemicals it cannot be guaranteed that damage will not occur to aquatic life, according to Alabaster & Lloyd (1982). Such substances should therefore be tested further in different long-term tests to establish water quality criteria (Alabaster & Lloyd 1982).

Because endosulfan is used in small quantities, only dimethoate and propiconazole were tested further. According to investigations by McKim (1977) the early life stage tests give a reliable basis for establishing maximum acceptable toxic concentrations (MATC). This is based on comparisons of results from tests which have been run for several years and with up to three generations of fish with results from relatively short-term tests with eggs and alevins. In our investigation it was decided to test eggs and alevins of brown trout and zebrafish.

The results of the early life stage tests showed that the difference between these and the acute tests was slight for propiconazole. Here all that was found was a small, non-significant effect in 0.5 mg/l i.e. 4-d $LC_{50} \cdot 0.4$. For dimethoate, however,effects was found down to 10-25 µg/l, which is 1/10 of the 4-d LC_{50} -value for young brown trout. If the MATC value was set to 10 µg/l for dimethoate, this would nevertheless be above the highest measured value in the Nordic countries (4.6 µg/l in Finland, cf. Kreuger 1992).

Table 10.The proportion between maximum measured concentrations in Nordic surface water (B) and "safe" concentrations (4 dLC_{50} 10^{-4}). See also text

Stoff	A $4dlC_{50}$ mg/l	B Max.conc.in water mg/l	C $(B/A)10^{-4}$
MCPA	300	0.0028	0.09
Dichlorprop	78	0.0062	0.79
Simazin	70	0.0045	0.64
Chlorsulfuron	40	0.00012	0.03
Atrazine	27	0.0018	0.67
Glyfosate	4.5	-	-
Propiconazole	1.2	0.0004	3.3
Dimethoate	0.13	0.0046 [1)]	324
Endosulfan	0.0009	0.00007[2)]	778

[1)] Finland - not found in Norway

[2)] Sweden - not analyzed in Norway

It can be concluded from these experiments that the tested chemicals, with the exception of endosulfan, are only by accident likely to represent any direct risk to fish in Norwegian water bodies of any size.

The experiments have shown that dimethoate and propiconazole in concentrations that are likely to occur in natural waters induce detoxification enzymes in rainbow trout and brook trout (Skaare *et al.* 1991). The biological significance of these findings is still questionable, however.

Another aspect which may be of importance is the indirect effect on fish through damage to plants and animals of importance as food organisms. Parallel to this study on fish some investigations were also carried out on the effect of pesticides on fish food organisms (Aanes & Bækken, 1989, Bækken & Aanes, 1990, Bækken & Aanes, 1991a, Bækken & Aanes, 1991b and Aanes, 1992). Aanes (1992) has summarized the results and concludes that MCPA, simazine and dichlorprop showed no acute toxicity at concentrations below 10 mg/l (4 d LC_{50} > 10 mg/l) to different species of the macroinvertebrates tested. Atrazine and chlorsulfuron were somewhat more toxic. The fungicide propiconazole was found to have a relatively high acute toxicity to invertebrates and possibly also to have an effects on algae which might be important food organisms for the benthic fauna. For dimethoate negative changes in benthic communities occurred down to 1 µg/l. According to this findings both dimethoate and propiconazole may have an indirect effect on fish production through their effect on the food chain. In Table 11 some 4 dLC_{50}-values are listed for propiconazole and dimethoate in relation to some important fish food organisms.

Table 11.Table 11. 4-dLC_{50}-values for some fish food organisms for propiconazole and dimethoate (mg/l). From Aanes og Bækken, 1989

Species	Propiconazole	Dimethoate
Snail		
Physa fontinalis	1.3	
Crustacean		
Gammarus lacustris	1.3	0.18
Mayflies		
Bactis rhodani	0.9	0.007
Heptagenia sulphurea	1.0	0.081
Caddisfly		
Hydropsyche siltai	1.2	0.023

Hessen et al. (1991) found that the 4-d LC_{50} -values for *Daphnia pulex* were approximately 1.6 and 0.02 mg/l for propiconazole and dimethoate, respectively.

The conclusions to be drawn from our investigations are that probably none of the tested substances are toxic to fish under normal use in water systems of any size in Norway. In small creeks and by accident, however, toxic action may occur for dimethoate, propicanazole and endosulfan.

Propiconazole and dimethoate may have an effect on the algae and/or the invertebrates which serve as food for the fish and thus, indirectly, an effect on the fish fauna.

Field investigations

Two freshwater localities were selected in order to determine the content of pesticides in sediments and fish. One of the localities, Akersvatn, a relatively shallow and eutrophic lake, was surrounded by intensively used farm-land while the other, Foksetjern, was situated in a forest. No pesticides were found in the sediments of either lake and in the fish there were generally only small amounts of the DDT metabolites. The DDE-residues in fish are probably equivalent to or less than residues found earlier in fish from Lake Tyrifjorden, Lake Mjøsa and some other lakes in Norway (Brevik 1981, 1993; Carlberg *et al.* 1993).

Only a few fish have been examined - at least 10 specimens of each species have to be examined to be sure of having a representative sample (Holden 1970). This was only possible for the perch and pike perch in Akersvatn. The results must therefore only be looked upon as indicative.

Pike, perch and pike perch are fishes at the top of the food pyramid and should therefore have the highest amounts of residues among fish. However, according to Murty (1986) the trophic level of the fish seems to have little influence on body residue level. Other factors such as lipid content, size and condition of the body and reproductive stage seem to be more important. The fishes examined have a low lipid content in flesh compared with fish such as, for example, eel and salmonids. However, the relationship between lipid content and residue concentration in fish is not fully understood. The correlation may be both positive and negative (Murty 1986).

The fact that pp'-DDE was the main component of the DDT derivates indicates that the animals have not been exposed to these contaminants in recent times (Murty 1986). There was little difference between the values in Akersvatn and Foksetjern which indicates that the compounds may have originally been airborne.

It is possible that the shallow and eutrophic character of Akersvatn will cause a more rapid

breakdown of pesticides than a deeper, colder and more oligotrophic lake. On the other hand, oligotrophic and deep lakes are – at least in Norway – seldom surrounded by farm-land to a degree that would result in a great transport of pesticides into the water system. Therefore it does not seem probable that fish and sediments from other lakes or rivers of any size in Norway will have significantly higher concentrations of pesticides from agriculture than those from Akersvatn.

SUMMARY

Nine commonly used pesticides have been studied with respect to their acute and long-term effect on some freshwater fishes. 4-d LC_{50}-values have been determinded for all substances and early life stage tests have been carried for dimethoate and propiconazole. Mainly the brown trout (*Salmo trutta*) was used for testing but the arctic char (*Salvelinus alpinus*), roach (*Rutilus rutilus*), minnow (*Phoxinus phoxinus*) and zebrafish (*Brachydanio rerio*) were also tested for dimethoate and/or propiconazole. As a test medium, untreated water from a nearby oligotrophic lake (pH 6.3, hardness 11 ppm as $CaCO_3$) was used. The 4-dLC_{50}-values (ppm) for brown trout were as follows: MCPA (300), dichlorprop (78), simazine (70), chlorsulphuron (40) atrazine (27), glyphosate (4.5), propiconazole (1.2), dimethoate (0.13) and endosulfan (0.0009). Only small differences in tolerance were found between thee salmonid species tested, but roach and minnows were somewhat more tolerant. Tests in the early life stage of brown trout in propiconazole and brown trout and zebrafish in dimethoate showed that the hatching process was not affected in concentrations below the 4-d LC_{50} -values. In propiconazole there was a slightly increased mortality of newly hatched alevins in 0.5 ppm while in dimethoate an LOEC (lowest observed effect concentration) at 0.05 ppm was found for brown trout and 0.025 ppm for zebrafish. The NOEC (upper no observed effect concentration) was 0.0125 ppm for dimethoate and zebrafish.

Field studies were carried out in two lakes – one of them surrounded by intensively cultivated farm-land – the other by forests. Analyses of MCPA, dichlorprop, 2.4D, atrazine, permetrine, propiconazole, dimethoate, DDT, DDE, DDD and lindans were made in sediments and liver and musele of three species of fish; perch (*Perca fluviatilis*), pike perch (*Lucioperca lucioperca*) and pike (*Esox lucius*). Only lindane and the DDT derivates were found in detectable but low concentrations in fish – none were found in sediments. The concentrations of lindane were 10-20 µg/kg wet weight in the liver of perch and pike from the forest lake. The concentrations of the DDT derivates varied from 2 to 200 µg/kg with the highest concentration in the liver of a pike from the agriculture influenced lake. On the basis of these results it does not seem to be probable that the tested pesticides could cause any direct harm to freshwater fish under normal use in Norway. Neither should there be any risk in domestic use of the flesh of the fish species investigated. However, indirect effects on fish via food organisms may occur.

For endosulfan, the toxicity was so high that heavy rain after spraying, could cause direct effects on fish in farmland brooks and streams.

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Appendix

Analysis of pesticides in water by gas chromatography

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Determination of pesticide residues in water is mainly performed by gas chromatography with selective detectors after extraction with organic solvents. (Åkerblom et al. 1990; Brauch *et al.* 1991.) In addition, some of the more polar herbicides like the phenoxy- acids require a derivatization step before the chromatographic analysis . (Åkerblom *et al.* 1990; Olson *et al.* 1978.) Two methods have been used; a multiresidue method (no. 03) for analysis of atrazine, simazine, dimethoate and propiconazole and another method (no. 08) for the analysis of the phenoxy acids MCPA and dichlorprop.

EXPERIMENTAL

Samples were taken in one litre glass bottles and stored for not more than one week at + 4°C before analysis.

Method no. 03

Sample preparation

250 ml water with an additional 5 g NaCl was extracted with dichloromethane; 1 x 75 ml and 2 x 50 ml. The extracts were combined and dried over anhydrous Na_2SO_4, concentrated on a Kuderna-Danish apparatus to near dryness and then dissolved in 1 ml iso-octane:acetone 95:5.

Gas chromatography

A Hewlett Packard 5890 GC equipped with an autosampler, split/splitless injector, electron capture and nitrogen/phoshorus detector and two capillary columns HP Ultra-2, 25 m x 0.2 mm i.d., 0.33 µm film thickness was used. The oven was programmed from 90°C (1 min) at a rate of 40°C/min to 160°C (0 min) and then at a rate of 3.5°C/min to 270°C (19 min). Injections (2 µl) were made with the injector (230°C) in splitless mode. The temperatures of the NP- and the EC-detectors were 250°C and 300°C, respectively. The NP-detector was used for analysis of atrazine and simazine, while dimethoate and propiconale were determined on both detectors. The detection limit was 0.10 µg/l (ppb) for the pesticides.

Method no. 08

Sample preparation:

200 ml water was acidified to pH 1.0 with an addition of HCl and then extracted with 1 x 50 ml and 1 x 25 ml dichloromethane. The combined extracts were dried over anhydrous Na_2SO_4, evaporated on a Kuderna-Danish apparatus to near dryness and the residue dissolved in 2 mL dichloromethane. The phenoxy-acids were methylated with an additional of 2 ml BF_3/MeOH (14% BF_3) ata temperature of 30-35°C for 30 min and washed with 1 x 5 ml of water. The aqueous layer was discarded and the organic solution dried over anhydrous Na_2SO_4 before injection.

Gas chromatography - mass spectrometry

A Hewlett Packard 5890 GC with an autosampler, a mass selective Hewlett Packard 5971 detector, a split/splitless injector and a capillary column HP-1, 25m x 0.2mm i.d., 0.50 µm was used. The oven was programmed from 80°C (1 min) at a rate of 20°C/min to 160°C (0 min), then 4°C/min to 196°C (19 min) and finally 25°C/min to 270°C (5 min). Injections (2 µl) were made with the injector (240°C) in splitless mode. The temperature of the MS-detector was 200 °C. Selected ion monitoring (SIM) was used for the derivatives. The ions at m/z 155, 214 and 216 were monitored for the methyl ester of MCPA while ions at m/z 162, 189 and 248 were used for the ester of dichlorprop. The detection limit is 0.05 µg/l (ppb) for both acids but a limit of 0.10 µg/l has been used for analyses of water giving high background on the detector.

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