LABORATORY-SCALE APPARATUS FOR THE STUDY OF PESTICIDE VOLATILITY FROM SOIL AND PLANT SURFACES

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ABSTRACT

Under the regulatory requirements of the BBA in Germany, certain physical and chemical properties of pesticides trigger the need to determine the volatility of pesticides from plant and soil surfaces. This paper describes apparatus developed to assess the volatility of a fungicide under the prescribed conditions within small chambers. The advantage of this approach facilitates the use of radio-labelled test material and the ability to produce a quantitative recovery of the applied pesticide.

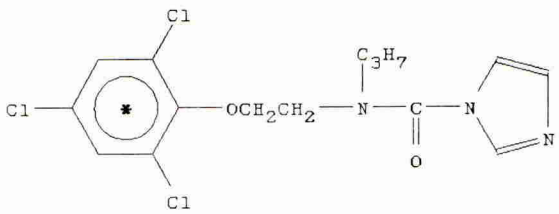
INTRODUCTION

Prochloraz (N-propyl-N-[2-(2,4,6-trichlorophenoxy)ethyl]imidazole-1-carboxamide) is a protectant and eradicant fungicide effective against a wide range of stem base and foliar diseases affecting field crops, fruit, turf and vegetables. Its hydrolytic and photolytic half-lives are greater than 4 days. Therefore, the extent of volatilisation of prochloraz as SPORTAK® 40EC.HF from the surfaces of soil (Speyer 2.1) and french bean (Phaseolus vulgaris var. Pros-Gitana) leaf has been investigated in accordance with the requirements of the relevant BBA Guideline, Part IV, 6-1 (July 1990).

MATERIALS AND METHODS

Test materials

^{14}C-Prochloraz



* Position of [^{14}C] radiolabel

Specific Activity:	97.8μCi/mg
Molecular Weight:	376.7
Water Solubility:	34mg/litre at 25°C
Vapour Pressure:	1.5 x 10^{-4}Pa
Henry's Constant:	1.7 x 10^{-3}Pa/mM

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Soil

The Speyer 2.1 soil was obtained from the Landwirtschaftliche Untersuchungs und Forschungsanstalt, Speyer in Germany. It was passed through a 2mm sieve and stored in a loosely sealed plastic container at 20 ± 2°C until use. The moisture content was determined by drying to constant weight at 110°C and the moisture holding capacity determined by the Hilgard cup technique.

Plant

French beans (Phaseolus vulgaris var. Pros-Gitana) were grown in John Innes compost at a density of one seed/pot under glass. Plants were selected at the required growth stage i.e. flowering.

Description of apparatus for soil studies

The soil volatility apparatus (see Figure 1) is comprised of two perspex blocks. The lower block (B) contains a well (25 x 2 x 2cm) for the test soil. The upper block (A) has a uniformly machined groove of 1mm depth. The two parts are joined by a series of bolts and separated by a 1mm thick PTFE gasket thereby providing a 2mm headspace above the soil surface. In Figure 1 the bolts are not shown to aid clarity. Air (at ca. 35% relative humidity) is passed through the system over the 25 x 2cm soil surface at a rate of 2.4 l/min which corresponds to a velocity of 1m/sec. The baffle chambers at the entrance and exit from the apparatus serve to minimise pressure fluctations and to ensure laminar flow over the soil surface. The air flow is supplied by a Model A85.DEP pump (Charles Austen Pumps Ltd.) which is then passed through a Dreschler bottle containing saturated calcium chloride solution. The condition of the incoming airstream to the apparatus is monitored continuously using a Grant combined temperature/ humidity probe Model VH-L-ZI connected to a Grant "Squirrel" data logger. To offset the drying of the soil by the air flow, provision exists for the addition of water during the course of an experiment. In this study a Walters M-6000A hplc pump (0.1ml/min) was attached to a time switch (Model 1311, Superswitch, Stockport, England) which facilitated the slow, intermittent addition of water over the study period. The outflowing gas from the apparatus then passed through a series of ethanediol traps to remove ^{14}C-labelled volatiles and finally to a Platon flowmeter (0-5l/min).

Description of apparatus for plant studies

The design concept of the apparatus for the study of volatility of pesticide from the surface of French bean leaf (Figure 2) is very similar to that of the soil apparatus. The flow path length is 10cm and, with a headspace of 3mm, a total flow of 5.4l/min of air through the apparatus is equivalent to a velocity of 1m/sec at the leaf surface. The temperature/ humidity are recorded using the same type of probe and data logger located in a separate perspex block placed in-line between the outlet from the Dreschler bottle(s) and the volatility apparatus. Because a higher flow-rate is required with the greater headspace, a flowmeter of 0-10l/min capability is required. As a further consequence the air outflow from the chamber was split into two and each passed through two ethanediol traps in series. The outflow from these was then recombined to provide a single stream for the measurement of flow through the apparatus.

Formulation of ^{14}C-prochloraz

Radio-labelled prochloraz was formulated as an emulsifiable concentrate equivalent to the commercial product. An aliquot (0.7922mg) of ^{14}C-prochloraz and 14.2028mg of unlabelled prochloraz were dissolved in the blank formulation (22.48mg) and transferred quantitatively with tap water to a measuring cylinder to give a final volume of 10ml. The solution was checked for purity by tlc in two solvent systems and stored at -20°C in the dark prior to use. The radiochemical purity was determined as 97%.

Soil volatility

The lower block (B) of the soil volatility apparatus was prepared by fitting a base pad of Whatman No.1 filter paper to the bottom of the 25 x 2cm trough and priming the water ducting to remove any residual pockets of air. The unit was weighed and soil added and compacted to achieve a bulk density of ca. 1.5g/cm^3. Any required adjustment of the water content to achieve 60% MHC (moisture holding capacity) was made by addition of distilled water using a Pasteur pipette. The flat surface of the soil in the lower block was sprayed with 200μl of the formulated ^{14}C-prochloraz at field rate (600g AI/400l/ha ≡ 6μg/4μl/cm^2) using a modified Camag Linomat III tlc applicator. To determine the amount applied to the soil, two equivalent areas of graph paper were each sprayed with the same volume of formulated material, one prior to and one subsequent to the soil treatment. The average of these two values was taken as the dose at time zero.The upper and lower units were combined and the apparatus connected to the air supply. A flow rate of 2.5l/min was applied to the system for 24 hours. The ethanediol traps were not removed until the conclusion of the 24 hour period of the study at which time the respective volumes were recorded and sub-samples (3 x 1ml) were taken for analysis by LSC (liquid scintillation counting). The soil was likewise removed and extracted with acetonitrile (2 x 250ml); the combined extracts were analysed by LSC. The residual, extracted soil was subsequently air-dried, weighed, sub-sampled, combusted and the resultant $^{14}CO_2$ determined by LSC.

The experiment was carried out twice.

Plant volatility

The boundaries of a 2.5 x 2cm area on a leaf of a glass-house grown french bean plant (at the growth stage where both flowers and beans were present) were marked using non-phytotoxic permanent ink. This area was sprayed with the same apparatus as for the soil system with the same formulated ^{14}C-prochloraz solution at the rate of 20μl/5cm^2. Prior to and subsequent to the spraying of the leaf equivalent areas of graph paper were sprayed with the same volume of solution. The average of these two results provided the time zero value for the application to the leaf. The sprayed leaf was allowed to dry prior to winding PTFE tape around the stem to afford a gas-tight seal when introduced to the upper part of the leaf chamber (Figure 2). The upper and lower portions of the chamber were sealed together with adhesive tape and connected to the air supply with a flow rate of 6 l/min for 24 hours. The ethanediol traps were replaced at 1h, 3h, 6h and, with the concluding samples at 24h, were sampled for LSC. The leaf was excised from the plant and washed with acetone (3 x 25ml). The combined washings were sampled for LSC and the residual, washed leaf material combusted and analysed by LSC.

The experiment was carried out twice.

RESULTS AND DISCUSSION

Deposition of radioactivity

Analysis of the graph papers used to assess the level of deposition of prochloraz on the soil targets showed that the amount applied was reproducible across the four paper targets (mean = 299.9μg / 50cm^2; coefficient of variation 3.9%). Furthermore, as a consequence of dividing the target areas into 2 x 1cm samples for combustion it was demonstrated that the material was uniformly distributed along the 25cm length of the target in each case.

As observed with the soil experiments above, the deposition of prochloraz onto the graph paper targets for the plant study showed a high level of reproducibility (50.6μg / 5cm^2; coefficient of variation 1.7%).

Soil volatility (Table 1)

A quantitative recovery of material within the system was achieved; 97.8 and 100.0% of applied radioactivity for the two respective experiments.

The extent of volatilisation was <1.5% in both experiments. The bulk of the radioactivity (>84%) was recovered through acetonitrile extraction of the soil. The remaining ca. 10% was determined through combustion of the extracted soil residues.

The temperature of the air stream to the soil chamber was maintained at ca. 20°C and 47% relative humidity during the course of the two replicate experiments.

Plant volatility (Table 2)

As above, recoveries of radioactivity were quantitative in each experiment.

The ^{14}C-volatiles trapped in ethanediol accounted for <19% of the applied material. The bulk (≥77%) of the applied radioactivity was retained on the leaf surface and was readily removed in the acetone wash. A further ca. 5% was recovered during combustion of the extracted leaf material.

The temperature of the air stream varied as a result of fluctuations in the glasshouse temperature during the course of these experiments whereas the relative humidity was more stable at ca. 45%.

CONCLUSIONS

From data derived from use of the apparatus described above, it was found that prochloraz showed, as would be predicted from its physicochemical properties, low volatility especially from soil surfaces. This may be attributed to adsorption to soil organic matter reducing the amount of pesticide available for volatilisation.

It has been demonstrated that, using the apparatus described, quantitative recovery of applied radio-labelled pesticide from soil and leaf surfaces is possible.

It is evident that the system lends itself to the use of different types of trap (eg acid, base, activated carbon, Tenax, cold trap) to facilitate recovery of different classes of volatiles derived from target pesticides.

The apparatus will be used to assess the characteristics of compounds of higher vapour pressures.

TABLE 1. Distribution of radioactivity following application of formulated ^{14}C-prochloraz to the surface of Speyer soil 2.1 after 24 hours exposure to an air flow of 1m/s.

	Sample	% of applied radioactivity Replicate 1	Replicate 2
VOLATILES			
	Ethanediol		
	Trap 1	0.3	0.8
	Trap 2	ND	0.5
	Total volatiles	0.3	1.3
SOIL			
	Acetonitrile		
	Extract 1	62.5	64.6
	Extract 2	26.5	19.8
	Non-extracted residue	8.5	14.3
	Total recovery	97.8*	100.0*

* = the sum of non-rounded values
ND= Not detected as below detection limit. Detection limit defined as 2 x background cpm from reference vials.

TABLE 2. Distribution of radioactivity following application of formulated ^{14}C-prochloraz to the surface of french bean leaf after 24 hours exposure to an air flow of >1m/s.

	Sample	% of applied radioactivity Experiment 1	Experiment 2
VOLATILES			
	Ethanediol traps		
	0-1h	4.7	3.8
	1-3h	4.8	ND
	3-6h	3.7	1.2
	6-24h	5.7	4.8
	Total Volatiles	18.9*	9.8*
LEAF			
	Acetone extract	77.0	91.7
	Non-extracted	6.5	4.7
	Total Recovery	102.3*	106.1*

* = the sum of non-rounded values
ND= Not detected as below detection limit. Detection limit defined as 2 x background cpm from reference vials.

FIGURE 1. Soil Apparatus

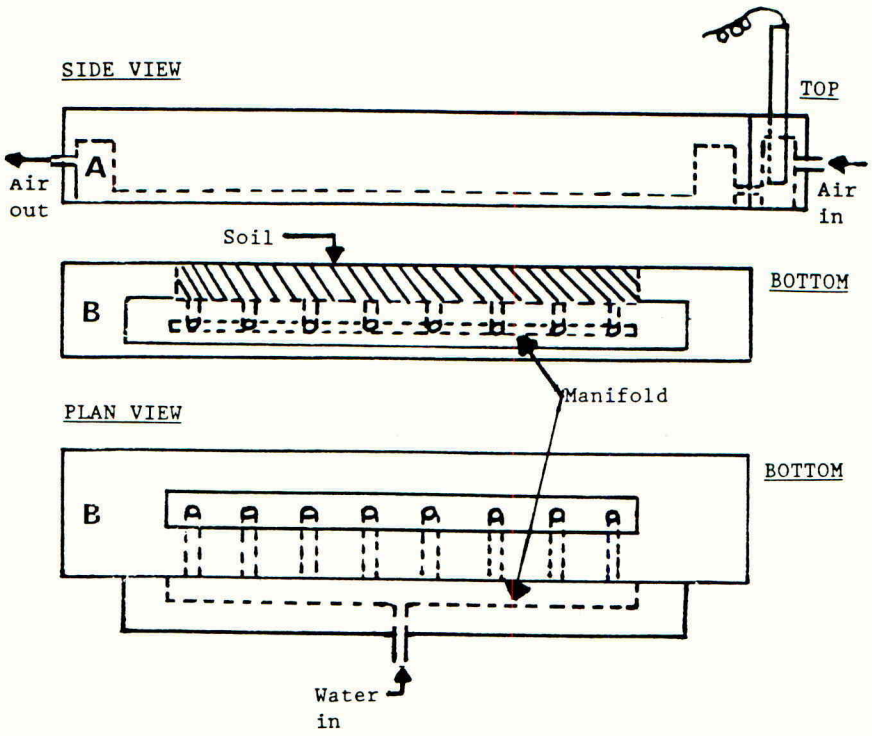
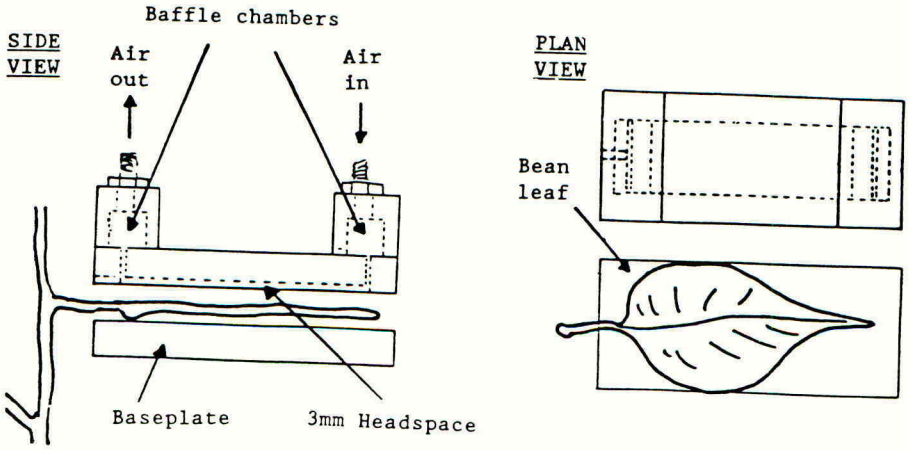


FIGURE 2. Plant Apparatus



A FIELD STUDY TO MEET UNITED STATES ENVIRONMENTAL PROTECTION AGENCY REGULATORY REQUIREMENTS FOR MEASUREMENT OF MOVEMENT OF PESTICIDES TO GROUND WATER

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ABSTRACT

The influence of a dynamic field environment on the fate and transport of pesticides through the soil profile can best be measured in the field. Laboratory testing alone cannot determine the actual field leaching potential of a compound. The small-scale prospective ground-water study described in this paper is designed to measure pesticide fate and transport by collecting soil, soil water, and ground water in an instrumented field. The test plots are established in an existing field, under typical farming practices, which represent a "reasonable worst-case" situation under which to evaluate leaching potential.

INTRODUCTION

The potential for pesticides applied under normal agricultural practices to leach through the soil and reach ground water was considered to be remote until the late 1970s. The conventional wisdom was that pesticides would fully degrade within the first few meters of soil and would not have the opportunity to percolate through the unsaturated zone, ultimately reaching ground water. However, detections in ground water of the nematicide DBCP in California and the insecticide/nematicide aldicarb on Long Island, New York, in 1979 forever changed how the scientific community and regulators viewed the environmental fate of pesticides (Holden, 1986).

Responding to concerns raised about the potential of pesticides to leach to ground water, the USEPA screened over 800 pesticide active ingredients during the early 1980s to assess their "leaching potential" based on environmental fate characteristics. Persistence and mobility of the active ingredients were evaluated using standard studies required for pesticide registration such as product chemistry, hydrolysis, photolysis, column leaching, aerobic and anaerobic soil metabolism, and field dissipation. Guidelines or so-called triggers indicating that the pesticide may leach were established as part of this review and included the following: Kd <5; K_{oc} <300; solubility >30 ppm; soil half-life >14-21 days; and hydrolysis half-life >175 days. If this evaluation concluded that a pesticide was sufficiently persistent and mobile to leach to ground water, then it was likely that a requirement to perform a ground-water study would be placed on the registrant.

The goals of the USEPA in requiring ground-water studies are numerous. The principal goal of this study requirement is to assess the leaching potential of a pesticide under actual field conditions and, by doing so, not to rely too heavily on laboratory studies performed under overidealized conditions. Other goals of this study type are to identify sources of pesticides reaching ground water (i.e., are detections that have been observed associated with normal labelled uses or are they more likely the result of spills or poor handling at mixer/loader sites?); and to evaluate the environmental impact associated with a range of uses (e.g., are there specific regions of the country or uses on certain crops that are causing problems?). Ultimately, the overriding goal of USEPA in requiring this study is to obtain the data needed to regulate a specific compound in order to protect the environment and human health.

The USEPA usually follows a "tiered approach" when evaluating the potential for a pesticide to leach to ground water. If a pesticide (parent and/or metabolites), based on its persistence and mobility, is believed to have a high leaching potential and has been demonstrated to move below a 0.9-metre depth in a field dissipation study, the next step from the regulator's perspective is to require a ground-water study (Behl & Eiden, 1990). The study design currently preferred by the USEPA is the small-scale prospective ground-water study (FIFRA Guideline 166-1). A study to meet criteria laid down by this FIFRA guideline is described in this paper.

GENERAL DESIGN OF FIELD STUDY TO ADDRESS GROUND-WATER CONCERNS ASSOCIATED WITH PESTICIDES

Sensitivity analysis

The objective of the sensitivity analysis phase is to identify a hydrogeologically vulnerable test site for monitoring the test substance. This test site should represent a "reasonable worst case." Data bases such as Database Analyzer and Parameter Estimator (DBAPE) and a superposition of a relative ranking system (DRASTIC) are utilized to identify regional areas having the greatest potential hydrogeologic vulnerability. Suitable soil series within these vulnerable regions are selected as the most favourable for further characterisation based on DRASTIC indexes, soil acreage, average rainfall and irrigation for crop production, and permeability. Once identified, these areas are visited by a geologist to identify and select candidate sites.

The geologist characterises the test sites for the following minimum criteria: 1) no past history of any test compound use; 2) slope of topography of less than 2 percent; 3) water table less than 9.1 metres below land surface; 4) agricultural land that has been cropped for several years; 5) permeable soils with low organic matter (<2%) and free of restrictive layers (i.e., sandy clay or clay loam layers); 6) irrigation system in place; and 7) easily accessible test site of 0.81 to 2.02 hectares. Additionally, the following information should be documented: 1) history of agricultural chemical use; 2) history of irrigation; 3) history of land use; 4) locations of point-source mixing areas and their relative distance from the candidate site; and 5) documentation regarding any nearby sources of the chemical which could inadvertently affect the proposed study.

Protocol development

The study protocol which is unique for the particular agrochemical and/or its proposed use is developed to describe in detail the activities involved in the study. The protocol follows GLP which is required by EPA. It therefore acts as a "road map" for the study, and all persons involved utilize the protocol as a guide in its conduct.

Site selection and characterisation

The second phase of site screening consists of hand-auguring a series of soil borings at each candidate test site to a depth of 1.5 to 3.0 metres below land surface to characterise the general texture of the soils and to evaluate the unsaturated zone conditions for restrictive layers. An attempt is made to advance at least one soil boring downward to the water table to collect deeper soil samples and verify reported depths to ground water. Based upon these visual field observations, the most eligible test sites are selected. Soil samples are taken and analyzed for background chemical levels and identification of interfering compounds to further characterise the site for suitability. The soil samples are analyzed for the following: 1) grain size distribution; 2) water holding capacity; 3) wilting point (percent of moisture at 15 bar); 4) organic matter content; 5) soil pH; 6) cation exchange capacity; and 7) bulk density. This phase of the site selection process results in the identification of at least one candidate test site for each product usage scenario. The selected site is then characterised in further detail in the third phase.

In the third phase of the site selection and characterisation process, a truck-mounted, hollow-stem auger drilling rig collects at least two sets of soil samples down to the water table to verify the suitability of the soils and the depth to ground water. The deeper soil samples are analyzed for the same parameters evaluated for the shallow soil samples and the soils series classification is verified for the selected test sites. The test sites are selected to have permeable soils underlain by unsaturated zone sediments that are low in organic matter and percent clay.

Four piezometers are positioned in the field to roughly delineate its four corners. The water level in each piezometer is measured prior to chemical application. Water level data are plotted to determine the general direction of ground-water flow beneath the study area. This facilitates the orientation and placement of a smaller 0.81 hectare treatment area which is aligned with the primary direction of the ground-water flow and the subsequent placement of monitoring wells and suction lysimeters.

The site design will represent reasonable worst-case conditions for vulnerability in potential product usage areas.

Site instrumentation

The site area of 2.0 hectares is bound by four piezometers installed at each one of the study areas. The treated area is approximately 0.81 hectare and divided into three equal size subplots. Markers are placed at the intersecting points of each treated subplot and labelled with the plot identification and study number. An approximate 30.5 x 60.9 metre control

area is established upgradient from the treated test plot. This control area provides treatment-free samples of soil, soil water, and ground water at different intervals during the study. These control samples are analyzed along with the authentic samples to identify background interferences.

A total of eight monitoring wells are installed in four well clusters within or immediately adjacent to the treated areas before the compound is applied. The four clusters are arranged so that two are positioned upgradient and two are downgradient with respect to ground-water flow under the test site. One additional well cluster is installed in the control area to collect ground-water samples for residue analysis. Suction lysimeters are installed to extract soil-water samples from the unsaturated zone. The number of lysimeters and their depth are a function of soil type and depth to ground water. Typically, three to six lysimeters are placed in each subplot with depth ranging from 1-5 metres. One group of lysimeters is placed in the control area. The appropriate crop is established following normal cultural practices for the study location.

Crop establishment

The treatment zone is agricultural land that has been cropped for the last 4 to 5 years. An appropriate field crop is established on the test plot following typical planting schedules for the region in which the plots are located. All agronomic practice (tillage, fertilization, planting, irrigation, etc.) is typical for the crop. The buffer zone around the crop is planted in a cover crop to protect the soil from erosion and facilitate movement around the test plot. Field notes record equipment used, soil moisture status, weather conditions, direction and depth of tillage, and the number of passes over the treatment area. All recorded field notes become part of the study raw data.

Compound and tracer application

The test compound is applied in the typical manner and at the maximum rate as defined by the product label. All equipment is calibrated to ensure the proper amount of compound is applied to the treated area. An aliquot from the tank mix is taken before and after application to determine the actual concentration of active ingredient applied. Spray deposition cards are placed within the treatment area to determine application efficiency. One application of ionic tracer is applied to the test plot. The tracer is applied at levels which clearly indicate breakthrough of the wetting front through the soil into ground water.

Crop maintenance

Post-treatment maintenance of the crop consists of normal agronomic practices. The test site is irrigated to maintain a typical irrigation schedule to assure that the combination of irrigation and rainfall exceeds 150 percent of the normal monthly volumes. Normal monthly volumes are determined from a 30 year period of recorded rainfall averages from the nearest National Weather Service Station. Completed records are maintained regarding irrigation including dates of application and amount applied.

Sampling of soil and water

Scheduled samples are collected from monitoring wells, suction lysimeters, and soil cores at various depths from the treated and control areas.

Sampling typically occurs approximately 1 day prior to treatment; day of treatment; 1, 3, 7,and 14 days after treatment; and then monthly thereafter for the first year after application.

Sample analysis

All samples are analyzed by analytical residue methods that are validated prior to study initiation. The analytical results of the water, soil water, and soil are tabulated and reviewed to determine the fate and transport of the parent and degradation compounds. Sample storage and transportation stability studies may be established to allow for determination of any degradation of or changes to the chemical that might occur prior to analysis. The transportation samples are spiked in the field and transported in the same manner as the authentic samples. If samples need to be stored prior to analysis then a storage stability study will be required.

Quality assurance and good laboratory practices

Critical events in each phase of the field work and the analytical laboratory are audited by a quality assurance unit (QAU) to ensure full compliance with the study protocor and to meet GLP requirements. The study report in the format specified by the EPA combining the field and analytical phases and which includes all raw data is also fully audited by a QAU. The GLP component of this type of study is of great importance and should not be overlooked if the work is to be of regulatory value.

CONCLUSION

The prospective ground-water field study allows for measurement of the fate and transport of a compound applied at a known rate. Laboratory leaching studies develop data in a controlled environment and cannot duplicate the dynamic field conditions to which compounds are subjected. By establishing test plots in existing agricultural fields and utilizing typical farming practices in the actual field, leaching can be determined. This information is critical to the USEPA in determining the leaching potential of pesticides under field conditions.

Eight ground-water studies have been conducted to date on a range of pesticides following the design specified in this paper. The results from these field studies indicate that the regulatory triggers are a valuable guideline for predicting leaching potential. However, the triggers were established eight years ago based upon a subjective review of environmental fate data. Current ground-water studies offer the USEPA the opportunity to re-examine the triggers and make changes, if required, based on actual field data.

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EARTHWORM POPULATIONS UNDER CONSERVATION TILLAGE AND THEIR EFFECTS ON TRANSPORT OF PESTICIDES INTO GROUNDWATER

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ABSTRACT

Greatly increased earthworm populations occurred in seven direct-drilled fields compared with seven ploughed fields, and in 20 direct-drilled experimental sites compared with 20 ploughed experimental sites. The numbers and area of earthworm burrows/m^2 in seven direct-drilled fields were calculated and their potential for pesticide transport assessed.

In laboratory experiments, earthworms were inoculated into intact soil profiles for one month, then killed by fumigation. Large doses of aldicarb, carbofuran and diazinon were applied and 17 l of water passed down each profile. Residues in the leachate were analyzed by glc. In the field alachlor and atrazine solutions were poured into individual earthworm burrows, and the leachate collected into mini-lysimeters, pumped to the surface and analyzed for residues by glc. Between 3% and 18% of the pesticides applied to earthworm burrows was recovered in leachates. Probably, the highly organic and microbial lining of the burrows adsorbed the pesticides and minimized groundwater pollution.

INTRODUCTION

Earthworms are important in improving soil structure, in burying organic matter, releasing plant nutrients, turning over soil and improving its aeration, water-holding capacity and drainage (Edwards & Lofty, 1977; Lee, 1985). Their activities are most important under decreased cultivations, particularly with direct drilling, since earthworms are then the main agents in moving organic matter through soil profiles and increasing soil porosity.

Studies of the effects of cultivation on earthworm populations have reported increased earthworm numbers in

direct-drilled soils compared with ploughed soils (Ehlers, 1975; Barnes & Ellis, 1979; Edwards & Lofty, 1982).

The residue-covered surface of the direct-drilled fields affects infiltration and creates a favourable environment for earthworms. Increases in infiltration of between 2 and 15 fold due to Lumbricus terrestris L. burrows have been reported (Lee, 1985; Klavidko et al 1986). Hence, there is a potential for insecticides and herbicides used in direct drilling to percolate through earthworm burrows to groundwater.

MATERIALS AND METHODS

Earthworm populations were measured in 14 neighbouring fields in Sussex, U.K., seven of which had been direct-drilled and seven which had been ploughed. Earthworm populations were assessed by pouring nine litres of dilute formalin (containing 50 ml of 40% formaldehyde) onto sixteen quadrats, (0.25 m^2), randomly distributed in each field, collecting the earthworms that came to the surface, and storing them in 5% formalin until they could be fully identified. Populations of earthworms were also assessed in the same way at 20 sites in South East England, U.K., which compared crop yields in replicated direct-drilled plots with those in ploughed ones.

Average numbers of earthworm burrows/m^2 were assessed in seven long-term direct-drilled sites by taking off the surface soil carefully to a depth of 10.0 cm and counting the numbers and size of distinct earthworm burrows using a large graduated, low-power hand lens.

The infiltration of the insecticides aldicarb, carbofuran and diazinon, through L. terrestris burrows in twelve intact soil profiles (15 cm x 15 cm x 30 cm deep), was measured in the laboratory. Intact soil profiles were dug out from a field, with a silty clay loam soil, that had been direct-drilled for five years. They were enclosed in tightly-fitting wooden sides lined with polythene, with open tops and bottoms. The top of the sides of each profile was sealed to the polythene lining with a band of hot wax. At the bottom, a 7.5 cm dia. glass funnel was fitted into a circular aperture in a metal square attached to the wooden sides. Each profile was inoculated with ten adult L. terrestris and a layer of finely-chopped straw added to promote burrowing activity. After one month at 20°C, the soil profiles were fumigated for 24 hours with methyl bromide to kill all worms; and left for 7 days for the worms to decompose.

Each insecticide was applied to the surfaces of four soil profiles in amounts equivalent to 3 kg/ha AI aldicarb, 4 kg/ha AI carbofuran and 4 kg/ha AI diazinon. Seventeen litres of water (the equivalent of one year's rainfall) was

added in small successive applications to the surface of each soil profile over a period of 24 hours and collected into flasks. Insecticide residues were extracted with hexane and then analyzed with a capillary glc using a nitrogen-phosphorus detector.

The infiltration of atrazine and alachlor through L. terrestris burrows was investigated in a direct-drilled field with a silt loam soil. Soil pits were dug to a depth of 45 cm and then excavated laterally to intercept earthworm burrows at this depth. A 125 ml plastic bottle was placed on a soil shelf beneath each earthworm burrow with a short section of polyethylene tubing leading from the burrow directly into the bottle. A wider tube which led from the bottle to the soil surface, served as an access port to the buried sampler. After installing such samplers under several burrows, each pit was refilled with soil. After rainfall, the contents of the bottles could be collected by inserting a slender tube through the above-ground access port. The contents of the bottle was pumped into individual containers, for measurement of volume and transport to the laboratory for analysis.

Seven 50 ml aliquots of solutions containing 223 mg/l alachlor or 29.4 mg/l atrazine (the limits of solubility of these chemicals) were poured directly into the surface openings of newly-abandoned earthworm burrows and adjacent man-made channels and collected from the 45 cm depth within 1 minute. Alachlor and atrazine residues were passed through a solid phase column and eluted with ethyl acetate. Residues were analyzed with a Varian model 3500 capillary glc with a nitrogen-phosphorous detector.

RESULTS

The populations of earthworms in seven direct-drilled and seven ploughed fields are summarized in Table 1 and those in the 20 direct-drilled and ploughed experimental sites in Table 2. The estimated numbers of earthworm burrows/m^2, the mean burrow diameter, mean burrow surface area/m^2 and total burrow wall area/m^2 are given in Table 3. The amount of aldicarb in the leachate through the soil profiles was 0.0740 mg/l, that of carbofuran 0.0156 mg/l, and that of diazinon 0.0035 mg/l. The concentration of alachlor poured into the earthworm burrows was 223 mg/l, that in the eluent from these burrows 39.8 ($\pm$6.4) mg/l and that in the eluent from the artificial burrows 154 ($\pm$30.3) mg/l. The concentration of atrazine poured into the earthworm burrows was 29.4 mg/l, that in the eluent from these burrows 3.3 ($\pm$0.51) mg/l and that in the eluent from the artificial burrows 13.4 ($\pm$2.7) mg/l.

TABLE 1. Earthworm populations/m^2 in seven ploughed and seven neighbouring direct-drilled fields. (Mean ± S.E.)

Earthworm Species	Direct drilled	Ploughed
Lumbricus terrestris	31.3 ± 11.7	6.2 ± 2.1
Apporrectodea longa	10.2 ± 4.1	3.1 ± 1.6
Allolobophora chlorotica	27.9 ± 8.3	10.9 ± 4.2
Apporrectodea caliginosa	9.5 ± 4.2	9.0 ± 3.6
Other species	6.3 ± 2.8	4.1 ± 2.1
TOTAL	85.2	33.3

TABLE 2. Earthworm populations/m^2 in twenty experimental sites with direct drilled and ploughed plots. (Mean ± S.E.)

Species	Direct drilling	Ploughed
L. terrestris	42.4 ± 14.3	19.7 ± 7.2
A. longa	11.2 ± 5.3	6.1 ± 2.8
A. chlorotica	28.6 ± 8.1	17.7 ± 7.9
A. caliginosa	46.7 ± 10.2	28.4 ± 9.8
Other species	7.8 ± 3.1	5.3 ± 2.1
TOTAL	136.7	77.2

TABLE 3. Mean earthworm burrow numbers and dimensions in seven fields.

Mean number of earthworm burrows/m^2 at depth of 10 cm	Mean diameter of burrows (cm)	Mean total surface area of burrows/m^2	Mean total burrow wall area/m^2
123.0 ± 48.0	4.4 ± 1.9	18.71 cm^2	691 cm^2

DISCUSSION

In all of the 27 field comparisons, there were significantly more earthworms in direct-drilled soils than in ploughed soils (Tables 1 & 2). In particular, populations of Lumbricus terrestris, which occurred at all of the sites, and has relatively deep permanent burrows, were much greater

under direct-drilling than under ploughing. About 15% of the L. terrestris populations were adult.

The mean population of L. terrestris in seven direct-drilled sites was 31.3/m^2 and this could be equated to 123 burrows/m^2 10 cm below the soil surface. This compares with data from Ehlers (1975) who reported 42 burrows/m^2 20 cm below the soil surface and 180 burrows/m^2 at 180 cm depth. The larger number of burrows than earthworms was probably due to the existence of old burrows from earthworms that had died. The mean surface area of the burrows of 18.71 cm^2/m^2, which although a conservative estimate that probably increased considerably with depth, was sufficient to allow considerable infiltration to occur through them (Edwards et al., 1988). For instance, Ehlers (1975) estimated that 6.15 l/m^2 could pass down earthworm burrows (0.12 mm water/minute). It seems probable that the numbers of earthworm burrows, their diameter and depth, are such that a large proportion of the water falling on direct-drilled soils can percolate through earthworm burrows thereby minimizing surface runoff (Edwards et al., 1989).

The maximum potential for leaching of the insecticides through the earthworm burrows and the surrounding soil, based on the amounts of insecticides applied and their solubility, was 0.397 mg/l of aldicarb and 0.529 mg/l of carbofuran and of diazinon in the leachates. Such concentrations would mean that none of the insecticides were adsorbed on the soil or the lining of the earthworm burrows. Since the equivalent of one year's rainfall was applied to the profiles, over a period of 24 hours, the potential for adsorption of the insecticides onto the lining of the earthworm burrows was stressed to the maximum, so probably the amounts appearing would be greater than with normal rainfall. The concentrations of insecticides actually appearing in the leachate was about 18% of the aldicarb, 3% of the carbofuran and 0.7% of the diazinon, i.e. much less than those in the water entering the burrows, even though the leaching was greatly accelerated. In similar experiments with no earthworm burrows 39-74% of aldicarb applied appeared in the leachates down soil columns with a range of soil types (Awad et al., 1984).

The concentrations of dissolved atrazine and alachlor in the samples from the earthworm burrows was 11.2% and that of alachlor 17.8%. These compared with 45.5% of atrazine and 69.0% of alachlor in the eluent from the artificial burrows. This suggests that the material lining the earthworm burrows had a high affinity and/or capacity for adsorbing atrazine and alachlor, because of the greater content of finely divided organic matter that is typically associated with the drilosphere surrounding the walls of earthworm burrows. Edwards (1966) demonstrated the capacity of organic matter to adsorb pesticides strongly. The amounts of herbicide in the eluent from the L. terrestris burrows tended to remain relatively constant, or even decrease, during successive

leachings, suggesting that the amounts of finely-divided organic matter in the wall materials of the earthworm burrows did not limit the adsorption. The estimated 691 cm^2/m^2 of burrow wall area which could adsorb the pesticides is conservative, since there is good evidence that burrows become more convoluted and numerous with depth. By contrast, the leachings through the man-made channels showed progressive increases in amounts of dissolved herbicide present with each successive leaching, suggesting at least a partial saturation of available sorption sites for atrazine and alachlor.

All of the evidence from these investigations suggest that there is little potential for groundwater pollution by pesticides passing through earthworm burrows, due to the adsorption of chemicals on the organic matter lining the large surface area of the burrows.

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SOURCE-SEDIMENT CONTROLS ON THE RIVERINE TRANSPORT OF PESTICIDES

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ABSTRACT

The results of a pilot study of the distribution of pesticides at three locations in the R. Windrush catchment in Oxfordshire are presented. The concentration of selected pesticides in the river water, suspended sediments and bed-sediments were determined and compared with chemical and mineralogical information on representative samples of source and river sediments. Simazine and atrazine were found in the water at all the sites with prometryn and lindane at two sites. DDE, dieldrin, DDT, heptachlor and parathion were detected in the bed and suspended sediments with concentrations in the suspended solids appreciably higher than in the bed-sediments. The sorption of atrazine, simazine, prometryn, malathion, and parathion on the bed and source sediments indicated a mineralogical control on sorption: the composition of source sediments may therefore be an important guide to the transport of pesticides in river catchments.

INTRODUCTION

The main objectives of this work were to measure the distribution of selected pesticides between water, bed sediment and suspended sediment at three locations in the river Windrush catchment and to assess the significance of the nature of the source sediments in controlling the pesticides sorption behaviour of riverine particulates.

The sites were chosen to include samples from the catchment (Figure 1) which were dominated by carbonate - Great oolitic White Limestone, WLS (site 1), clay - Lower Lias, LLC (site 2) and also a downstream location, site 3. Site 1 was at Harford bridge in the upper reaches of the R. Windrush, Site 2 was on the R. Dikler, a tributary to the R. Windrush. Site 3 was below the confluence with the R. Dikler. A water sample was also taken from the confluence with the R. Thames.

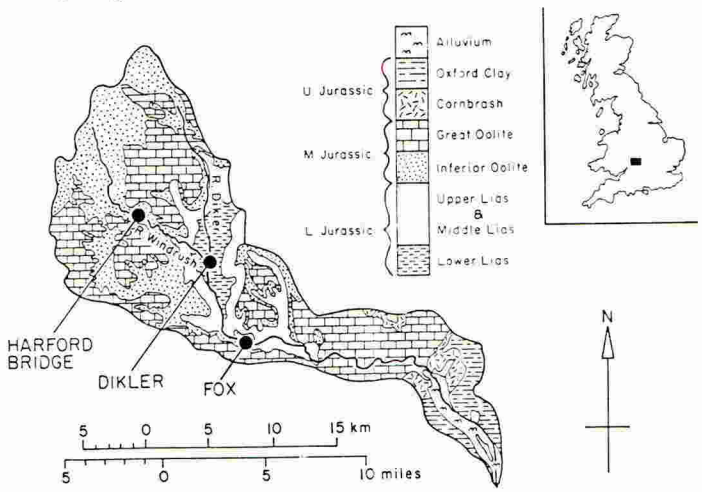
METHODS

Sampling

All samples were collected on 02.04.92 starting at site 1 and moving down-stream. Standard precautions were observed in the preparation of the sampling equipment and the inclusion of control blanks from the sampling through to the final analysis. Water samples were collected for pesticide

analysis in pyrex bottles fitted with PTFE screw caps. Further samples were collected for major-ion analysis; pH, temperature, conductivity and oxygen concentration were measured *in-situ*. Approximately 32 litres of water were collected in glass demijohns for the separation and analysis of suspended sediment. Surface bed-sediments to a depth of *ca.* < 5 cm, were collected using either a stainless steel scoop or a pond net (1 mm mesh). The sediments were immediately transferred through a 1 mm screen into glass jars with tops lined with aluminium foil. Very fine particulate material associated with the bed-sediment that remained in suspension was stored separately in glass demijohns. This was later centrifuged and combined with the rest of the bed sediment. The water samples were immediately extracted with solvent, the bed sediments were freeze-dried until the weight loss was < 0.1 % in 24 h; the samples of suspended solids were stored in the dark at *ca.* 5 °C and the solids separated over the following 5 days using an MSE 18 centrifuge with a continuous-flow-rotor operated at 110-120 ml min^{-1}. Samples of source sediments (WLS and LLC) were collected from appropriate sites within the catchment.

FIGURE 1 Study area showing the geology of the catchment and the location of sampling sites.



Analytical methods

The water samples were extracted with dichloromethane (DCM) with Kuderna-Danish (KD) concentration and a solvent change to 5% acetone in hexane. The freeze-dried sediments (10g) were soxhlet extracted for 5 h with 60 ml of DCM. The extracts were then concentrated using KD apparatus and the solvent changed to 5% acetone in hexane. The extracts were analysed using a Perkin-Elmer 8700 instrument with split-splitless/PTV injectors, an electron-capture-detector (ECD) and nitrogen-phosphorus thermionic detector (NPD). The pesticides were confirmed by GC/MS in selective-ion mode. The samples were analysed for the following compounds: α-HCH, γ-HCH(lindane), *p,p'*-DDE, *p,p'*-TDE, *p,p'*-DDT, heptachlor, dieldrin, endrin, *cis/trans*-permethrin, cypermethrin, fenvalerate, deltamethrin, atrazine, simazine, prometryn, malathion, parathion, fenitrothion and dichlorvos. The limits of determination were *ca.* 1.0 µg/kg and *ca.* 10 ng/l for the synthetic pyrethroids and *ca.* 0.1 µg/kg and 0.5 ng/l for the other compounds for the sediment and water analysis respectively. The organic carbon (OC) content of the source (WLS and LLC), bed and suspended sediments was determined by

combustion and corrected for inorganic carbon. X-ray diffraction was carried out on the source, bed and suspended sediments using a Philips PW 1380 horizontal goniometer with PW 1710 diffractrometer control. The specific surface areas of the freeze-dried sediments were determined by nitrogen gas adsorption using a flow-technique with overnight outgassing at 110°C and analysis by the single-point BET method. The sediments were examined using scanning electron microscopy (SEM) and EDAX.

Adsorption measurements

The adsorption of parathion, malathion, fenitrothion, atrazine, simazine and prometryn at 10±0.2°C was determined simultaneously using a batch procedure; the adsorption of parathion was studied separately using a flow-cell. The batch method (BM) involved the addition of freeze-dried sediments to 220 ml of 0.01 M $CaCl_2$ containing 3-30 µg dm^{-3} of each pesticide (adsorbent concentration of 0.03-0.14 kg/l depending on the adsorption affinity of the sediment).

RESULTS AND DISCUSSION

Pesticide analysis of the field samples (Table 1) showed that the triazines were found in all the waters but not in the sediments. Parathion was in all the sediments with concentrations in the suspended sediments much higher than in bed-sediments. The results of the sediment characterization are collected in Table 2. The field pH values were 8.4, 7.7 and 8.2 at sites 1 to 3 with oxygen concentrations between 79-105% saturation and water temperatures between 6.8 and 7.3°C. The major-ion analysis showed the waters to have similar compositions with calcium concentrations of 2.4-2.7 mM. The suspended sediment concentrations were 5.1, 26.4 and 13.7 mg/l for sites 1-3 respectively.

TABLE 1. Concentration of pesticides in samples from R. Windrush.

Compd	Water / ng/l				Sediments/ µg/kg					
					Bed			Suspended		
	1	2	3	4	1	2	3	1	2	3
lind'	-	T	0.7C	5.9	-	-	-	-	-	-
DDT	2.8	T	-	-	0.2C	-	-	T	1.1	4.7
DDE	-	-	T	-	T	0.2	0.2	T	1.6C	3.7
hept'	-	-	-	-	-	0.1	0.2	13C	5.1C	4.7
diel'	-	-	-	-	-	0.2C	0.2C	17C	8C	12
sim'	4.9	1.5C	22C	26C	-	-	-	-	-	-
atr'	15C	7C	17C	32C	-	-	-	-	-	-
prom'	-	-	1.2C	4.4	-	-	-	-	-	-
para'	-	-	-	-	0.3C	0.6C	1.0C	13C	3.3C	8.8C

Cypermethrin was detected in the water at site 2 at a concentration of 0.07 µg/l. Key:C; confirmed by GC/MS. T; trace detected. -; not detected

TABLE 2. Specific surface area, Σ, and percentage composition by percentage mass of organic carbon, OC, and crystalline components of the sediments.

Sediment	OC	Σ m^2/g	Q	C	K	I	E	F
LLC	3.1	15.5	77	13	47	-	53	-
WLS	0.09	1.8	-	99	16	40	64	-
			bed-sediments					
1	0.1	3.6	53	42	27	27	46	3
2	3.5	29.1	69	2	42	24	34	8
3	4.7	10.0	45	45	39	24	37	3
			suspended sediments					
1	12.9	20.5	58	34	30	37	33	7
2	7.1	13.5	69	3	42	42	36	6
3	7.4	18.3	45	29	42	36	22	3

Quartz(Q), calcite(C), feldspars(F) determined by whole rock analysis; kaolinite(K), illite(I) and expandable clays(E) (totalled to 100 %) from the clay analysis. Key: -: not detected. LLC: Lower Lias Clay. WLS: White Limestone.

The results of the batch adsorption experiments are shown in Table 3 expressed as the distribution coefficients normalized with respect to organic carbon, K_{oc}, and specific surface area, K_d/Σ. The results from the flow-cell obtained after 1 h contact between parathion and the bed-sediments gave K_d values of 98,19,53 and 99 l/kg for LLC and sediments from sites 1-3 respectively. The pH during the batch adsorption experiments was *ca.* 8.5 for WLS and between 7.6 and 7.9 for the other sediments with an oxygen saturation > 79%. The final solution compositions of the supernatants were also determined. Both nitrate and phosphate leached from the sediments to produce concentrations of 2-4 mM and 0.1-4 μM of nitrate and phosphate respectively with the highest concentrations from sediment 3 ; the final concentrations of calcium differed < 10% from their original values with traces of Mg, Na, K also present.

The mineral composition was estimated from the percentage clay determined by the whole rock analysis and the detailed composition of the clay fraction. On this basis, the most noticeable differences between the sediments were the general increase in % by mass of the OC, illite and Σ of the suspended compared with the bed-sediments. An exception to this trend was at site 2 where the suspended sediments appear aggregated in comparison with the bed material leading to a lower external surface of the suspended solids. It was also interesting that the ratio of the % quartz to % kaolinite shown in Table 2, decreases from *ca.* 1.95 at site 1, 1.64 at site 2 to 1.11 at site 3 for both the bed and suspended sediments. The relative proportions of the major mineral components were similar for both the bed and suspended sediments at all sites with important differences between the sites.

TABLE 3. Results of the batch adsorption experiments at 10 °C.

	simazine			atrazine			prometryn		
	c	K_{oc}	K_d/Σ	c	K_{oc}	K_d/Σ	c	K_{oc}	K_d/Σ
LLC	10.4	128	0.26	11.1	96	0.19	4.6	320	0.65
WLS	10.8	-	-	10.9	-	-	10.9	-	-
1	9.3	7000	1.9	9.4	7000	1.9	9.9	1000	0.28
2	9.1	171	0.21	9.6	171	0.21	6.2	200	0.24
3	4.2	702	3.3	4.2	872	4.1	3.4	808	3.8
	fenitrothion			malathion			parathion		
	c	K_{oc}	K_d/Σ	c	K_{oc}	K_d/Σ	c	K_{oc}	K_d/Σ
LLC	0.4	5256	10.6	0.3	3782	7.6	0.4	5160	10.4
WLS	6.2	-	-	3.7	-	-	6.7	-	-
1	1.8	37000	10.2	1.1	38000	10.5	2.1	38000	10.5
2	0.8	2943	3.5	0.2	5943	7.2	0.8	3086	3.7
3	0.5	4298	20.2	0.7	1723	8.1	0.4	6404	30.1

c (µg/l) denotes the concentrations of pesticide in solution after 24 h mixing with the sediments. K_{oc} is in l/kg and K_d/Σ is in units of µm.

The relationships between the composition of the source and bed/suspended sediments and their application for the prediction of OC and K_d's was elucidated further using the equation:

Fraction of LLC in sediment = [P(sed) - P(WLS)]/[P(LLC) - P(WLS)]

where P(sed), P(WLS) and P(LLC) are the percentage by mass of the mineral components in the sediment, WLS and LLC respectively. This was attempted with quartz, calcite and kaolinite as the predictive minerals, P(sed), and the linear combinations necessary to produce the correct sediment compositions were determined. In general illite was always underestimated with more in the sediments than can be explained by a linear combination or multiple regression using WLS and LLC as the predictors. For site 1, kaolinite gave the best prediction for OC and the K_d's for fenitrothion, malathion and parathion with similar combinations of source sediments (*ca.* < 0.1 LLC and > 0.9 WLS). It was the only site for which the prediction of OC in the bed-sediment was greater than the observed value. At site 2, only quartz was able to predict the composition of the bed and suspended sediments (*ca.* > 0.8 LLC and < 0.2 WLS) but underestimated all the clay fractions. In contrast quartz, calcite and kaolinite were all reasonable predictors for the bed sediment from site 3 (*ca.* 0.6 LLC and 0.4 WLS). The OC was underestimated but the K_d for malathion was in agreement with the experimental value (Table 3). The influence of the source sediments at sites 1 and 2 was evident: site 1 was dominated by WLS, site 2 by LLC, with more integrated sediments downstream at site 3.

The K_{oc}'s for LLC and the sediments from sites 2 and 3 (Table 3) were within the range reported by Karickhoff (1981) for soils. The correlation between the K_d's and the composition of the bed-sediments revealed that the adsorption affinity of parathion correlates well with the triazines and fenitrothion but not malathion. There was also a good correlation between the flow-cell results for parathion and the other pesticides except malathion. This suggested fundamental differences in the adsorption mechanism for malathion which has a distinctly different molecular structure from fenitrothion and parathion. The lower values of K_d from the flow-cell indicated significant kinetic effects probably associated with diffusion of the pesticides into the bulk matrix. An excellent correlation between the K_d for malathion, clay content and Σ (K_d = 2.85 + 7.20(±0.50)Σ; R=0.993: the standard deviation of the slope is shown in brackets) was evident. In contrast, the correlation with OC was poor (R=0.678) for malathion but reasonable for both parathion and fenitrothion (K_d = 5.0 + 50.9(±13.6)OC; R = 0.91 and K_d = 15.4 +37.4(±8.4)OC; R = 0.93 respectively). A reasonable correlation between the concentration of parathion in the sediments and the OC was also found (R = 0.902) which suggested that the concentration of parathion in the water was similar at all the sites but below the limits of determination of our method ,ie at a lower concentration than was expected from the laboratory adsorption studies. The specific surface areas correlated best with the individual clay fractions particularly kaolinite, R = 0.99, and the expandable clays, R = 0.97, but very poorly with OC, R = 0.65.

The high K_{oc}'s for the bed-sediments from site 1 indicated that the partition of the pesticides with this OC was not consistent with the partition behaviour with the OC in the other sediments. In contrast, the values of K_d/Σ for this sediment were in the range found for the other sediments suggesting that the exposed surface of this sediment, was a key influence on the pesticide interaction. Similarly, the sorption of malathion with K_d/Σ = 8.4 ± 1.5 μm for all the sediments, appeared closely related to the exposed surface area of the sediments rather than to the OC content. The only sediment showing the traditional linear relationship *viz* (standard deviation of coefficients shown in brackets):

$$\log K_{oc} = 1.34(0.26) + 0.66(0.09) \log K_{ow};\ R = 0.975,$$

was that from the downstream site 3. However, a good correlation:

$$\log K_d/\Sigma = -1.00(0.27) + 0.66(0.09) \log K_{ow};\ R = 0.973,$$

for site 3 sediment was also found which suggests that the interaction of the pesticides was with a surface film rather than partition with discreet organic particles.

This preliminary study shows there are important source-sediment controls on the composition of both bed and suspended sediments which have implications for the transport of pesticides in catchments. Further research is necessary to examine these effects in detail particularly for the more persistent pesticides in current usage.

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THE ROLE OF OUTDOOR LYSIMETERS TO EVALUATE LONG-TERM SOIL DEGRADATION AND LEACHING POTENTIAL OF PESTICIDES

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ABSTRACT

Under certain circumstances chemicals used as protection agents in the cultivation of agricultural crops may have detrimental effects on man, animals and the environment.Thus a catalogue of tests including the degradation, percolation, volatilisation and accumulation behaviour of all active substances and their main metabolites are required prior to registration. In practice laboratory, lysimeter and field studies are conducted to fulfil these requirements. However, it raises the question, to what extent do these totally different kinds of study give complementary results and provide realistic information on the behaviour of the active substances in agricultural ecosystems. In order to answer this question results from laboratory, field and outdoor lysimeter studies with the insecticide carbosulfan and its metabolite carbofuran are discussed.

INTRODUCTION

Both theory and practical experience have shown that pesticides may have detrimental effects on man, animals and the environment under certain circumstances. Therefore, in addition to other parameters the degradation, percolation, volatilisation and accumulation behaviour of all active substances and their main metabolites in agriculture, forestry and horticulture are systematically investigated prior to registration. Laboratory, lysimeter and field studies are conducted to fulfil these requirements. Immediately following application, pesticides are present in high concentrations near the edges of the compartments of the agricultural ecosystem - namely in the soil or crop layers immediately adjacent to the air. Since the inner regions of the compartments themselves are free of pesticide immediately after application, high concentration gradients of the pesticides are present at the compartment edges, which set diffusion processes in motion - either into the deeper soil layers or plant compartments or into the air compartment. In view of the complex distribution (migration, volatilisation, up-take, and accumulation) and degradation (hydrolysis, photolysis, biotransformation and mineralisation) processes involved the degree to which the results of the laboratory, lysimeter and field studies present a realistic picture, should be constantly reviewed, with the aim of establishing:

- To what extent is there agreement between the predictions obtained from laboratory experiments and the corresponding results from outdoor lysimeter or field studies?
- What relevance have lysimeter experiments in the evaluation of the degradation and distribution behaviour of pesticides in agricultural ecosystems?

In an attempt to answer these questions, results for the active substance carbosulfan and its primary degradation product carbofuran obtained in laboratory, lysimeter and field studies are discussed.

DEGRADATION, VOLATILISATION AND LEACHING POTENTIAL OF CARBOSULFAN AND CARBOFURAN

Degradation/metabolism in soils

Carbosulfan (2,3-dihydro-2,2-dimethyl-7-benzofuranyl(di-n-butylaminosulfenyl)-methylcarbamate) is rapidly degraded in soils under both aerobic and anaerobic conditions. The estimated DT_{50} and DT_{90} values range from 2.5 to 5.0 and from < 3 to 38 days, respectively. The initial step of the degradation of carbosulfan (thiolysis) is a first order reaction, leading, mainly, to the equally biologically active carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl-methylcarbamate). The few metabolites formed in parallel appear in the soil in insignificant quantities. Carbofuran is unstable in alkaline aqueous media. Under these conditions, hydrolysis of the carbamate ester occurs, yielding carbofuran 7-phenol and methyl carbamic acid, the latter decomposing spontaneously to CO_2 and CH_3NH_2. This hydrolysis does not occur as rapidly in acidic aqueous media and therefore carbofuran is relatively stable under acidic conditions. However, it appears that pH is not the controlling factor in determining the rate of degradation of carbofuran in soil. For example, the shortest half-life of carbofuran (11 days) was observed in a soil with a pH value 5.7. Rather, the rates of degradation are primarily determined by the presence of soil microflora capable of metabolising carbofuran.

The dissipation of carbofuran under laboratory conditions is also affected by soil type with most DT_{50} values in the range from < 30 to 100 days and DT_{90} values mainly ranging from 40 to 200 days. The half-life of carbofuran in the field is similarly affected by soil type, but is nevertheless generally shorter than in the laboratory. The corresponding DT_{50} and DT_{90} values are typically below 50 and 150 days, respectively. The longest DT_{90} value (ca. 200 days) was found in a silt loam soil treated with carbofuran granules.

Volatilisation

Under indoor model environment conditions (25 ± 2°C; flow rate 30 l air/h; 24 h), the volatility of carbofuran, produced in soil from carbosulfan, was examined together with three other active ingredients. The volatilisation rates of the

pesticides from water were in the order trifluralin > lindane > methyl parathion > carbofuran and from soil surfaces in the order carbofuran > methyl parathion > lindane > trifluralin (Table 1).

TABLE 1. Volatilisation rates of carbofuran and three other pesticides from water and soil surfaces

Pesticides	Physicochemical properties			Volatilisation rates [%]	
	Molecular weight	Water solubility [ppm]	Vapour pressure [x 10^{-6} mmHg]	Water	Soil surfaces
Carbofuran	221	500.0	20.0	3.6	15.6
Methyl parathion	263	60.0	9.7	15.8	14.4
Lindane	290	10.0	9.4	89.4	8.6
Trifluralin	335	0.3	65.0	92.6	6.0

The experiments demonstrate that the volatilisation rates of the pesticides from water were related not only to their vapour pressures but were also primarily affected by their water solubilities. The volatilisation rates decreased as water solubility increased. The volatilisation rates of the pesticides from soils were determined primarily by their soil adsorption constants. In a further study, also performed under indoor model environment conditions (25°C and 40°C; flow rate 1002 l air/h; 39 h) using a ^{14}C-carbofuran containing formulation, a maximum value for the volatility of the active ingredient from soil of 0.81 % was found.

Mobility and leaching

Carbosulfan has a relatively low water solubility (0.03 mg/l at 25°C) and an extremely short half-life in soils. Therefore, it is not regarded as having a potential to leach. Carbofuran is significantly more soluble in water (700 mg/l; 25 °C) and has a much longer half-life. It exhibited rather low K_{OC} values ranging from 9.6 to 36.0 in 5 soil types studied, which encompassed a wide range of textural characteristics.

Therefore, numerous laboratory and field studies were performed in order to obtain as reliable information as possible on the distribution behaviour of carbofuran in agriculturally utilised soils. The majority of the results from these studies indicated that carbosulfan and carbofuran (or their associated metabolites) applied to the surface were retained in the top layers of soils (i.e. 0 to 25 cm) and were transported into lower soil layers only to a very small extent, if at all. However, some of the experimental results were not in complete agreement with this prediction. Furthermore, doubts concerning the immobility of carbofuran in soil were strengthened by its "soil ubiquity score", an index based on the soil/water partition coefficient (K_{OC}) and the soil half-

life of the chemical. Carbofuran was classified by this index as being in the transition zone between leaching and non-leaching chemicals. Therefore, a 2 year outdoor lysimeter study was performed, using [benzofuranyl-^{14}C]carbosulfan and intact soil cores, in order to clarify this question.

The undisturbed soil cores used in the lysimeter study had diameters of 113 cm and depths of 110 to 120 cm. They were taken from a representative field specified by the appropriate authority. The soil was classified as a light, loamy sand, with a low humus content, down to a depth of 50 cm. Below this, the layers consisted of virtually pure sand. The soil cores were collected using a proven, standardized procedure. Investigations have demonstrated that the procedure does not damage the soil cores and soil characteristics typical of the location are preserved. Once, placed in the lysimeter units, the cores were cultivated manually following the principles of "Good Agricultural Practice". Carbosulfan treatment of the soil cores was based on the maximum application rate of 1.5 kg AI/hectare plant culture. However, since carbosulfan is applied to soils with plant growth, in practice only about 35 % of the spraying solution would land on the soil surface. Consequently, 2 applications per year would result in 1.05 kg carbosulfan being distributed over 1 ha of soil surface. Accordingly, quantities of 104.6 and 111.3 mg ^{14}C-carbosulfan (35.26 and 37.53 MBq) were applied to the surfaces of the soil cores in lysimeters 1 and 2 at time t_o.

The rainfall during the study equalled 1642 mm precipitation (1642 l/m^2) in 740 days (including the additional irrigation). During the same period, 994 and 1008 litres of leachate were collected from lysimeter 1 and 2, respectively (Figure 1). The percolated water samples obtained from both soil cores contained only low quantities of radioactivity, relative to the quantities applied at time t_o (equivalent to 0.85 and 0.82 mg carbosulfan or 0.49 and 0.48 mg carbofuran, respectively). More than 20 % of the radioactivity present in the leachate was $^{14}CO_2$. Radiochromatographic analysis showed that the remaining radioactivity was not carbosulfan or carbofuran. This indicated that all samples of percolated water, obtained during the two years study, contained little or no carbosulfan and its major metabolite carbofuran.

At the end of the study, soil samples to a depth of 28 cm were taken from the topsoil of the cores before they were completely sectioned into layers 10 cm thick. The radioactivity in all of the soil samples was determined. The values obtained were used to calculate the radioactivity profiles in the topsoils and soil cores (Figures 2 and 3). It can clearly be seen that after completion of 2 years of the study, the remaining radioactivity was present mainly in the upper 20 cm layer of the soil core. The radioactivity found at the end of the study in the soils and leachates from lysimeters 1 and 2 were 20.3 and 19.5 % of applied, respectively. This means that during the 2 year period of the study, approximately 80 % of the radio activity applied as ^{14}C-labelled carbosulfan passed into the atmosphere, i.e. mineralisation of the main metabolite

FIGURE 1. The cumulative quantities of precipitation, percolated water and leached radioactivity for the duplicate lysimeter study with ^{14}C-labelled carbosulfan

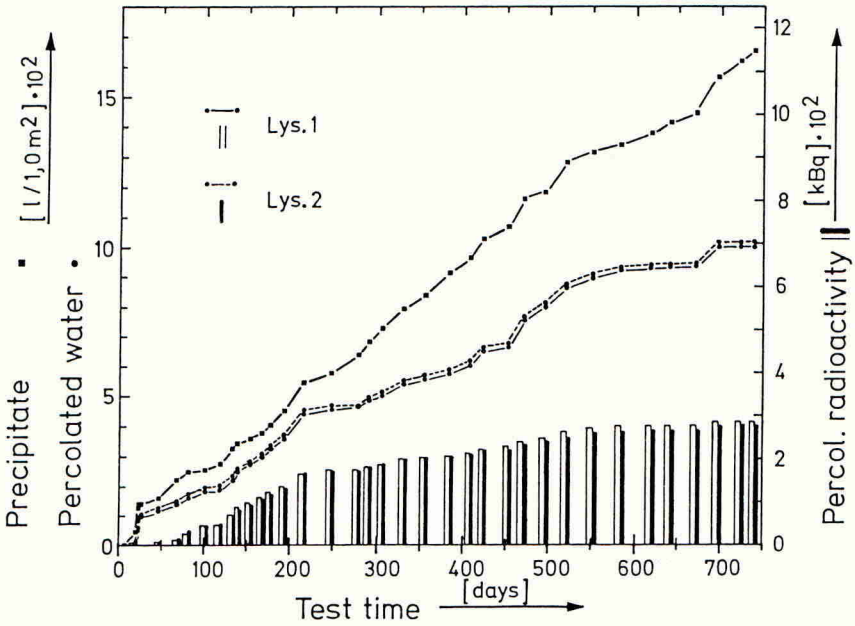


FIGURE 2. Distribution of the radioactivity, applied as ^{14}C-labelled carbosulfan, in the topsoils of duplicate soil cores at time t_n = 740 days

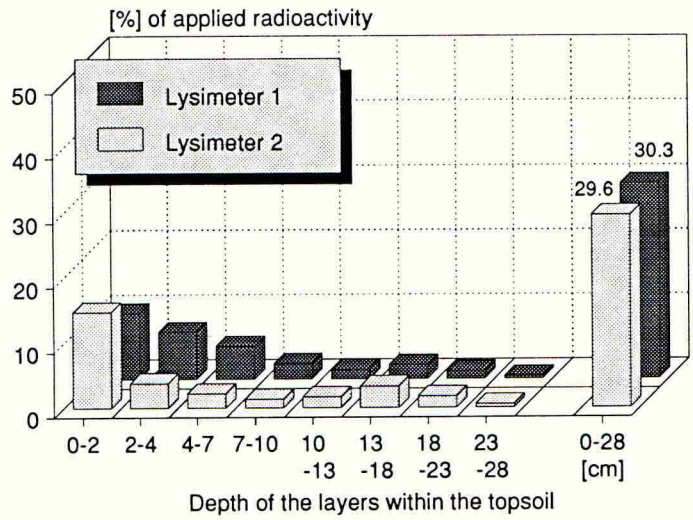
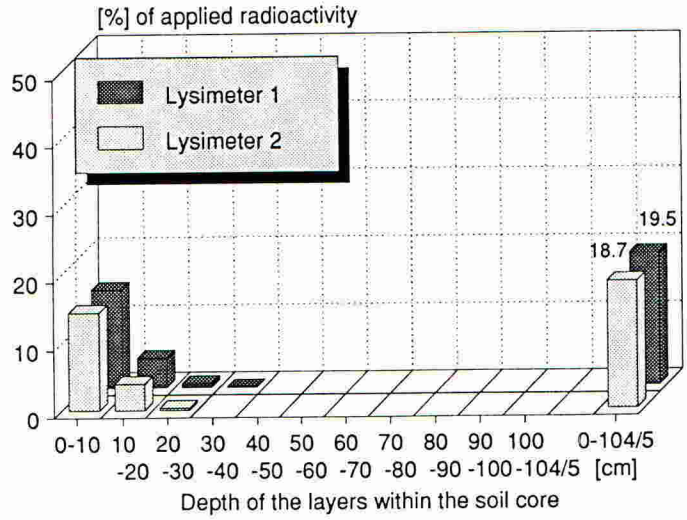


FIGURE 3. Decrease of radioactivity, applied as ^{14}C-labelled carbosulfan, in duplicate soil cores as a function of depth at time t_n = 811 days



carbofuran to CO_2 was a dominant influence on the fate of the chemical. It can additionally be concluded from the shape of the radioactivity profiles shown in Figures 2 and 3 that the carbosulfan residues still present would not have been transported into the lower soil layers and into the groundwater, if the study had been continued.

CONCLUSION

The results of the laboratory studies performed with the insecticide carbosulfan and its major metabolite carbofuran are insufficient to construct a reliable picture of the actual behaviour of the active substances in agricultural ecosystems. This is often the case in practice, because only limited information on the behaviour of chemicals in individual, isolated processes selected from the complex array in agricultural ecosystems can be obtained from the standardised laboratory tests.

However, the laboratory studies have an important role within active substance testing. These studies, generally easy to perform according to guidelines, produce the database upon which decisions can be taken on the type and extent of further field and/or outdoor lysimeter studies, even though they rarely produce unequivocal results which can be extrapolated to the real behaviour in agricultural ecosystems. It should also be realized that field studies involve significant technical and analytical difficulties which can compromise the quality of the results. In contrast, outdoor lysimeter studies generally give defined, valid and interpretable data.

UPTAKE AND ELIMINATION OF FENAZAQUIN BY RAINBOW TROUT IN RELATION TO PREDICTED ENVIRONMENTAL CONCENTRATIONS

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ABSTRACT

The bioaccumulation of radiolabelled fenazaquin by rainbow trout was studied under dynamic flow conditions. A rapid increase in radioactivity was observed initially followed by temporary plateauing between 7 and 14 days. Concentrations at cessation of exposure were approaching Day 1 values. Mean bioconcentration factors after exposure for 21-28 days were ca. 500. After transfer to clean water, approximately 80% of the radioactivity was eliminated within the first 24 hours and less than 10% remained after seven days.

Predicted aquatic concentrations based on a "worst case" scenario are similar to those used in the bioaccumulation study. Physico-chemical data indicate that fenazaquin is rapidly lost from water. A microcosm study confirmed a half-life of 1-2 days. Accumulations of radioactivity in fish were rapidly excreted once the source of exposure was removed. Thus, bioaccumulation of fenazaquin at maximum predicted environmental concentrations will not represent a hazard.

INTRODUCTION

Fenazaquin is a new quinazoline-based acaricide with a spectrum of control that includes Panonychus, Tetranychus and Eotetranychus on apples and citrus fruit. The chemistry and mode of action have been reviewed by Dreikorn et al., (1991) and Hollingworth et al., (1992). As part of the regulatory requirements, a bioaccumulation study in rainbow trout was undertaken in accordance with OECD Guideline No. 305E using fenazaquin which was uniformly ^{14}C-labelled in the quinazoline portion of the molecule.

METHODS AND MATERIALS

Preliminary study

The suitability of a semi-static renewal system was investigated. Tanks containing nominally 0.2 and 1.0 µg/l fenazaquin were allowed to equilibrate for 24 hours prior to addition of fish. The concentration in the water was monitored at intervals and the chemical composition of the radioactivity from the higher dose determined. The analytical conditions were identical to those reported for the main study. The results are described in detail in the results section. Nominal concentrations could not be maintained using a semi-static renewal system and, therefore, the main study was conducted using continuous flow conditions.

Main study

Test tank system

Rainbow trout (Oncorhyncus mykiss), approximately one year old (length 6.9 ± 0.9 cm), were acclimatised in fibreglass tanks for one month prior to commencement of the study. Three 150-litre glass aquaria (two test and one control), fitted with overflow to maintain a volume of 100 litres at a loading of <3 g of fish/litre at initiation of exposure, were used for the study.

Radiolabelled fenazaquin stock solutions were prepared in acetone (6 and 27 µg/ml) and injected into the diluent stream via a mixing unit by means of a Braun syringe infusion pump. The system was calibrated to achieve nominal concentrations of 0.2 and 1.0 µg/l, respectively, with the control tank receiving only acetone.

Sampling regime

Water samples (500 ml) and fish (3) were taken from each test tank at Days 1, 3, 7, 14, 21 and 28 during the exposure. During depuration, water was sampled at Days 1 and 14 and fish at Days 1, 3, 7 and 14.

Analytical methods

Total radioactivity

Fish tissues were homogenised. A subsample was mixed with dry cellulose powder and oxidised using a Packard Instruments Co. Model 306 MK2 Tricarb. The oxidation products were collected in Optisorb I and mixed with a scintillation cocktail (Optisorb S) prior to counting.

Water and stock solutions were mixed with M 31 Special scintillator before measurement. The radioactivity in these samples was determined by scintillation counting. Quench correction was by external standard method and radioactivity with less than twice background gross counts was considered to be below the limit of accurate quantitation.

Radiolabel purity

Water samples (Days 0, 7, 14 and 28) of tank water (ca. 300 ml) from the 1.0 µg/l exposure were extracted three times with chloroform (3 x 60 ml) and the pooled extracts evaporated to dryness. The residuum was redissolved in acetone for examination by thin-layer chromatography (tlc).

Separation was carried out on commercially available silica gel (0.25 mm thickness) or reverse phase C18 (0.2 mm thickness) plates using one of the following solvent systems:

System 1 hexane: ethyl acetate (70:30 V/V), silica
System 2 chloroform: ethyl acetate (80:20 V/V), silica
System 3 acetonitrile: water (80:20 V/V), reverse phase

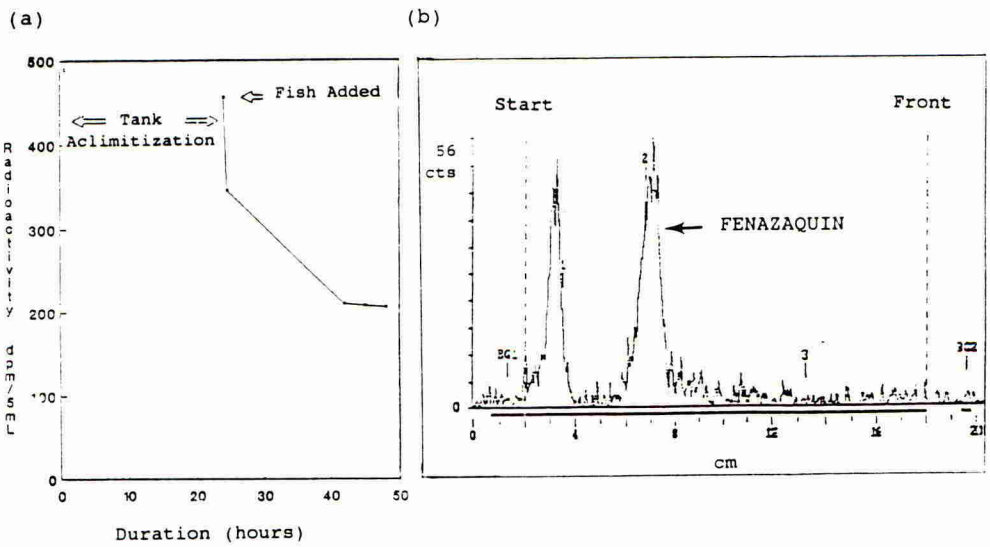
All three tlc systems were used to check the stock solutions for 0.2 and 1.0 µg/l treatments. Tlc systems 2 and 3 were used to analyse water samples. Quantitation was obtained using a Berthold Linear Analyser. The retention of ^{14}C-fenazaquin was determined by co-chromatography with unlabelled fenazaquin and located by quenching of a fluorescent indicator under u.v. light at 254 nm.

RESULTS

Preliminary study

Analysis of the water samples from the semi-static tanks showed that a small amount of fenazaquin was lost from solution during a 24-hour equilibration period prior to addition of fish. However, rapid loss (ca. 50%) of fenazaquin from water was observed during the first 18 hours after addition of fish. The concentration of radioactivity then stabilized during the next six hours (Figure 1a). Analysis by tlc of the water residue 24 hours after commencement of the exposure showed that the fenazaquin had been rapidly degraded (ca. 35%) to an uncharacterised metabolite (Figure 1b).

FIGURE 1. Reduction of fenazaquin concentration in water from a semi-static system and accompanying production of an uncharacterised metabolite.



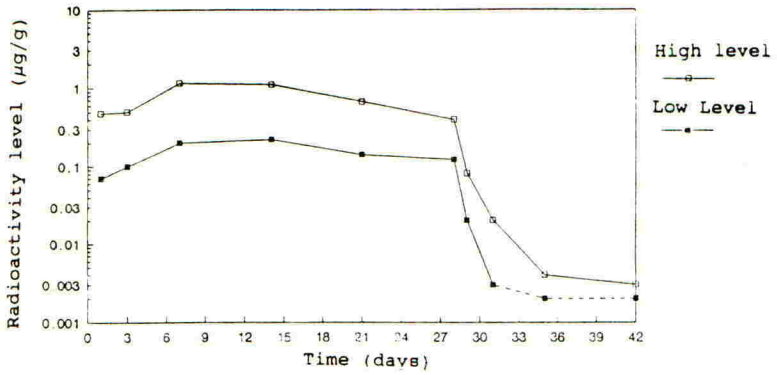
Main study

The chemical composition of the radioactivity in water samples from the higher dose only was measured due to limitation in detection. Samples were taken at selected times (Days 0, 7, 14 and 28) during the study and were found to contain, generally, >90% fenazaquin.

Nominal concentrations of fenazaquin of 0.2 and 1.0 µg/l were maintained over the duration of the 28-day exposure period with mean values of 0.22 ± 0.03 and 0.97 ± 0.07 µg/l, respectively.

The radioactivity profiles for the fish from each test concentration are shown in Figure 2. The concentration in fish rose rapidly over the first three days for both test concentrations before temporarily plateauing between Days 7 and 14 and then declining to values comparable with the start of the study by Day 28.

FIGURE 2. Concentration of radioactivity in fish during the study period.



Broken line indicates the limit of reliable measurement.

The bioconcentration factors (BCF) were calculated as a ratio of the concentrations in fish compared to those in the tank water. Although levels of radioactivity were generally three to five times greater in fish from the higher test concentration compared to the lower, the BCF were similar for both exposure groups with values ca. 400-500 on Days 1-3 and 400-600 on Days 21-28. The temporary nature of the raised BCF throughout Days 7-14 may be attributable to the induction of enzymes during this period which subsequently increased the rate of elimination of the compound from the fish and resulted in the return of the BCF to lower levels. Minor variations in concentration of fenazaquin in the tank over this period could not account for any variation in the residue level in the fish. Further support for the enzyme induction hypothesis was obtained in 90-day toxicity studies with rats and hamsters where increased hepatic enzyme activity was observed in animals which had been fed fenazaquin. The mean BCF, based on the equilibria which had established at Days 21-28, were 520 and 500 for the 0.2 and 1.0 µg/l concentrations, respectively.

At the end of the exposure period, fish from the two test tanks were transferred to fresh tanks containing control water. Water at the beginning and end of the depuration period (Days 1 and 14) contained no radioactivity above the detection limit of 0.05 µg/l. Residues in the fish were observed to fall rapidly with approximately 80% of the radioactivity eliminated within the first 24 hours and less than 10% remaining after seven days (Figure 2). Half-lives were calculated to be 0.7 days (0.2 µg/l) over depuration Days 1-3 and 1.4 days (1.0 µg/l) over Days 1-7.

No fish mortalities or other adverse reactions were observed throughout either of the exposure or depuration periods.

DISCUSSION

Fenazaquin is intended for use in orchards and is not expected to reach bodies of water in concentrations significant to cause any environmental impact.

Fenazaquin has a solubility in water of ca. 0.1 mg/l at 20°C. It is rapidly adsorbed onto soil particles; ca. 88% and 96% of the adsorption from a calcium chloride solution onto a sandy loam soil and a clay loam soil, respectively, occurred within two hours. It is also strongly bound to soil particles; the organic carbon adsorption coefficient, K_{OC} (18700 - 42100), generally increases with the organic content (0.5% - 2.0%) and clay content (5% - 32%) of the soil. Little movement of fenazaquin down the soil column has been observed with residues remaining in the top 15 cm. No fenazaquin has been observed in the leachate of column leaching studies. These data indicate that movement of fenazaquin to bodies of water would be unlikely to be in the form of free fenazaquin but more likely to be closely associated with soil particles.

If fenazaquin reached bodies of water in the free form, some loss would occur, mainly attributable to photolysis (half-life ca. 15 days). However, the main parameters governing stability in the aqueous environment are likely to be the hydrophobic and adsorptive nature of fenazaquin. As described above, adsorption onto soil from solution occurs rapidly (mainly within two hours). In an adsorption/desorption experiment, the slopes of the desorption isotherms were nearly horizontal, indicating that little of the soil-adsorbed fenazaquin was released to re-establish equilibrium with the aqueous phase. Therefore, loss from water onto particulate material would be expected to be rapid whilst only slow release back into the aqueous environment would occur.

This has been confirmed in an aquatic microcosm study with a simulated "worst case" scenario of 5% drift and 1% runoff. Fenazaquin application rates equivalent to 225, 450 and 1125 g AI/ha (nominally ca. once, twice and five times the maximum European field rates) were studied. Applications were made to microcosms (1.8 m diameter and 0.6 m depth, containing 1100 l of water and 2 to 3 cm of natural pond sediment), three per treatment level, to simulate 5% drift reaching receiving waters of a model pond (0.4 ha area and 1.83 m average depth, equivalent to ca. 7.4 million litres of water). The concentration of fenazaquin in the model pond water was calculated for each application rate, assuming that 5% was applied directly to its surface. Fenazaquin was sprayed on the surface of each microcosm to achieve the theoretical concentration appropriate for the treatment level. After two hours, respective fenazaquin concentrations of 0.58, 1.07 and 2.68 µg/l were measured in the water, corresponding to 89-97% of the theoretical values. Over the next 22 hours, concentrations dropped by ca. 60-70%.

A second application was made to each microcosm to simulate 1% runoff from a treated crop area of 4 ha. The amount of fenazaquin to be added to the model pond was calculated for each of the three application rates, assuming that 1% of the amount applied to 4 ha would reach the pond either in solution or adsorbed to soil particles. A typical suspended sediment level in water following a 1% runoff event would be 200 mg/l. In order to simulate this situation, the theoretical amount of fenazaquin was added to soil and aged for 24 hours. This soil was slurried (220 g of soil in 10 l) of water) and was added to each microcosm to simulate runoff. Two hours after the addition, fenazaquin concentrations in the water were 0.54, 1.00 and 2.87 µg/l, respectively, for the three treatment levels. These values represented only 23-29% of the theoretical concentrations in the water,

assuming that all the fenazaquin could desorb into the aqueous phase and subtracting the background contribution from the drift application. The half-life of fenazaquin in the water following the runoff treatment was 1.7 days (with 95% confidence limits of 1.4 to 2.0 days) for the first seven days and 2.2 days (with 95% confidence limits of 2.0 to 2.4 days) for the 21-day period. These data fitted a first order decay model with a degree of fit of R^2 = 0.99 and 0.94, respectively. No fenazaquin was detected (detection limit 0.03 μg/l) in the water after 21 days.

The aqueous concentrations of radiolabelled fenazaquin used in the bioaccumulation study (0.2 and 1.0 μg/l) were in the range which might be expected from a "worst case" scenario of 5% drift and 1% surface runoff at rates higher than the maximum anticipated field rate of 200 g AI/ha. The microcosm study has indicated that fenazaquin would be rapidly removed from the water unlike the continuous flow conditions of the bioaccumulation study where the concentration of fenazaquin was maintained over the 28-day study period. Furthermore, in the preliminary bioaccumulation study fenazaquin was shown to degrade in the presence of fish. Therefore, under natural conditions, continuous exposure to these levels is unlikely to occur.

Although the mean BCF at equilibrium in the bioaccumulation study were ca. 500, cessation of exposure brought rapid elimination of the radioactivity from the fish with ca. 80% loss in the first 24 hours and subsequent half-lives of between 0.7 and 1.4 days.

Thus, it is concluded that the bioaccumulation of fenazaquin in fish at maximum predicted environmental concentrations will not represent a hazard.

ACKNOWLEDGEMENT

The authors wish to thank the staff of Huntingdon Research Centre for conducting the bioaccumulation study and in particular Dr. B. Mayo as Senior Study Director. We also thank Dr. P. Francis and Dr. P. Cocke of Lilly Research Laboratories for the data on the microcosm study.

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INFLUENCE OF GROUNDCOVER ON THE DEGRADATION OF ^{14}C-IMIDACLOPRID IN SOIL

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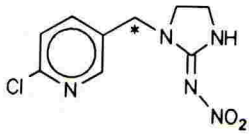
ABSTRACT

The influence of vegetation on the degradation of imidacloprid was investigated. A second experiment carried out in the dark and without vegetation, but otherwise in identical conditions, served as a control. The rate of degradation of the active ingredient was considerably influenced by ground cover. Under simulated practical conditions (with vegetation) the half-life was 48 days while in the parallel guideline experiment without vegetation a half-life of 190 days was determined. The imidazolidine ring was degraded stepwise to the key metabolite 6-chloronicotinic acid which ultimately led to the formation of considerable amounts of CO_2.

INTRODUCTION

Imidacloprid, 1-[(6-chloro-3-pyridinyl)methyl]-4,5-dihydro-N-nitro-1H-imidazole-2-amine, is a new systemic insecticide with a nitroguandine structure. It can be applied as a soil, foliar, or soil-drench insecticide (Moriya *et al.*, 1992), and will be used to control sucking insects (for example leaf hoppers and aphids) in a wide variety of fruits, field crops and ornamentals. It acts as a stomach and contact poison.



* ^{14}C-label

In standardized laboratory tests imidacloprid metabolized relatively slowly in soil under aerobic conditions. In contrast to these tests, results from soil surface photolysis studies indicated a relatively rapid initial degradation of the active ingredient. The metabolism of imidacloprid in maize also confirmed the rapid uptake of the active ingredient by plants after seed treatment. Therefore the influence of vegetation on the rate of degradation and metabolism of the active ingredient in soil was investigated in an extended laboratory experiment under simulated field conditions to assess the behaviour of imidacloprid.

MATERIAL AND METHODS

Radiochemical

The investigations were conducted with [pyridinyl-^{14}C-methylene] imidacloprid. The radiolabelled compound had a specific radioactivity of 5.18 MBq/mg (140 µCi/mg), and a radiochemical purity of >99 % determined by tlc and hplc.

Soil used

The experiments were carried out with freshly collected BBA Speyer soil 2.2 (75.9% sand, 16.5% silt, 7.6% clay, 2.2% C org, and a microbial biomass of 1047 mg microbial carbon/kg). The soil was screened to < 2mm in moist conditions and fertilized with liquid manure and straw to maintain the nutrient supplies for the plants during the course of the experiment.

Soil treatment

An application rate of 0.23 mg AI/kg soil (dry weight) was taken as a basis for all experiments. The active ingredient for the entire experiment was added as a soil subsample (100 g) in order to avoid possible differences between the individual samples of the experiment and in order to preclude influences of the solvent (1ml acetonitrile). The treated subsample was thoroughly mixed with the total soil sample (4.5 kg) in a 6 l drum resulting in a radioactive concentration of 1.2 MBq/kg soil (dry weight). For the experiments with ground cover, two kg (dry weight) of the soil batch was applied as a layer on to a quartz sand bed (1 kg) in a 3 l glass beaker (Figure 1). The glass beaker was darkened by covering the outside with black cardboard. The soil was kept moist (about 50% of the maximum water capacity) by means of an automatic plant irrigator (Blumat Co.). Grass ("Berliner Tiergarten") was sown as vegetation. The containers were incubated in a greenhouse at a temperature of about 20°C during the day (15 hours) and about 17 °C during the night (9 hours). The climatic data were recorded. The light intensity (12 hours) was ≥ 35 kLUX and the humidity of the air about 50%. The air above the glass beaker was extracted every 6.25 hours for 1 hour and passed through a tube (inner diameter 2.2 cm) filled with soda lime (15 g) to collect evolved $^{14}CO_2$.

Samples 100 g of the soil batch spiked with 0.23 mg/kg imidacloprid were incubated in 300 ml Erlenmeyer flasks without ground cover (Figure 2). The incubation vessels were darkened and kept in the greenhouse beside the glass beakers. The soil moisture (50% of the maximum water capacity) was maintained during the incubation period by addition of an amount of water which corresponded to the quantity evaporated.

FIGURE 1: Metabolism in soil with ground cover

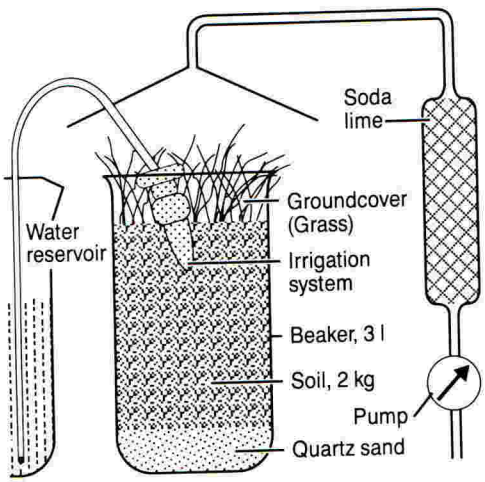
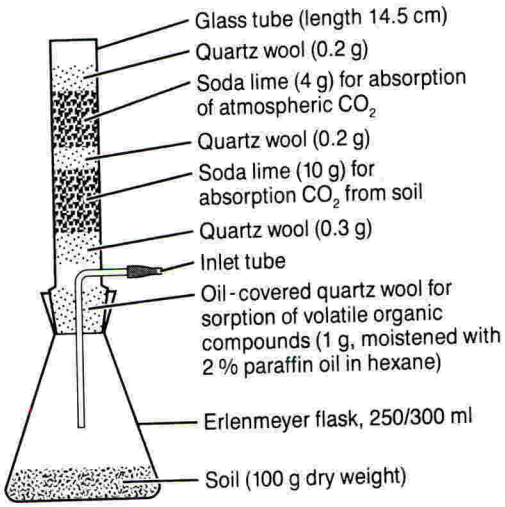


FIGURE 2: Incubation vessel for aerobic soil metabolism



Sample processing

Experiments with ground cover

At the time of sampling the soil, the grass was cut immediately above the ground. Maceration of the grass in liquid nitrogen using an Ultraturrax was followed by extraction with acetone. Soil samples were taken 0, 56, 77, 113, 149, 201 and 274 days post treatments and subsequently extracted on a mechanical shaker with acetonitrile/water (1:1), acetonitrile and dichloromethane.

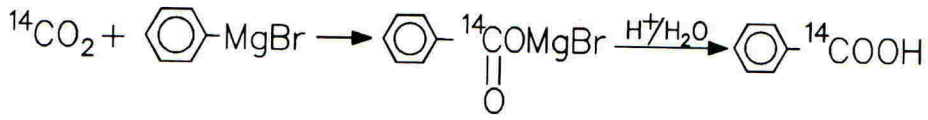
Experiments without ground cover

At day 149, 201 and 274 the entire contents of the Erlenmeyer flasks were processed as described above.

Radioactivity measurments

Liquid samples were measured in a liquid scintillation counter (PW 4700, Philips Co. or Rackbeta 1219, LKB Co.) using scintillation gel (®Instant Scint. Gel, Packard Co.). Soil samples which had been extracted with solvents were dried at approximately 20°C and then ground in a mortar. The radioactivity in the soil was determined by combustion of three samples (each of approximately 1g) in an oxidizer (OX-300, Harvey Instrument Corporation). The liberated $^{14}CO_2$ was collected in a suitable scintillation cocktail (®Carbosorb and ®Permafluor V, Packard Co.).

For determination of $^{14}CO_2$, the soda lime was dissolved with 18% hydrochloric acid (10 g soda lime with 60 ml hydrochloric acid) and the liberated $^{14}CO_2$ was passed into a ®Carbosorb-Permafluor V cocktail. Furthermore the amount of radioactivity being assigned as $^{14}CO_2$ was determined qualitatively and quantitatively by conversion to ^{14}C-benzoic acid (Grignard reaction):



The ^{14}C-benzoic acid was determined qualitatively by means of the inverse isotope dilution technique.

Chromatography

The hplc conditions were as follows:

Instrument:
-Liquid chromatograph HP 1090 with DAD (Hewlett Packard)
-Radioactivity flow-through detector Ramona 5 with evaluation programme IM 2300 (Raytest).

Stationary phase:
Precolumn: ®Li Chrospher, 60 RP-select B, 5µm, 4x4 mm
Separation column: ®Li Chrospher, 60 RP-select B, 5µm, 250x4mm
Flow rate: 1ml/min
Oven temperature: 40°C
Injection volume: 10-250µm

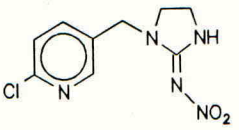
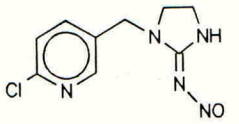
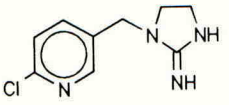
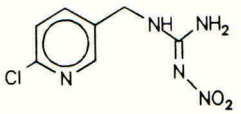
Mobile phase: Water (600 µl orthophosphoric acid/l)-acetonitrile (87:13)
Detection: u.v. 254 nm, analogized radioactivity signal

Thin-layer chromatgraphic separations (tlc) were carried out on precoated tlc-plates, silica gel 60 F254 0.25mm (Merck Co.) and the following solvent systems were used (volume ratios):

A: Ethyl acetate-toluene-methanol-acetic acid (80:20:20:1)
B: Ethyl acetate-2-propanol-water (65:23:12)

Chemical structure of the reference substances and their R_f-values are given in Table 1.

TABLE 1: Identification, chemical structures and R_f-values of reference substances.

structure	R_f-values solvent-systems A	B
	0.69	0.75
	0.41	0.55
	0.02	0.10
	0.69	0.91

Quantitation was derived from the radio thin-layer chromatograms.

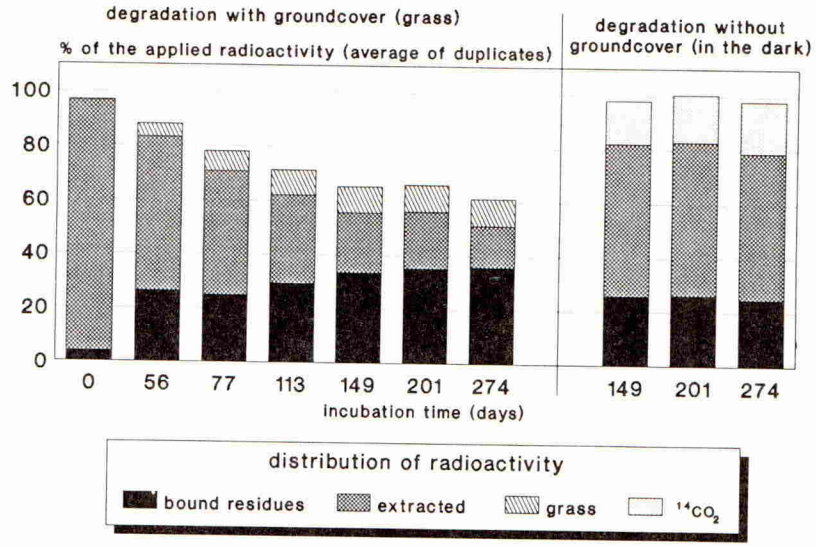
RESULTS AND DISCUSSION

Distribution of radioactivity

The basis of the calculation in this study was the radioactivity recovered at day 0 (1.2 MBq/kg dry weight soil = 0.23 mg/kg). Due to the test conditions a complete balance could not be established in the degradation experiment with vegetation (Figure 3). The percentages of the extracted radioactivity decreased from 93.1% to 13.7% in the experiment with vegetation whereas the amount of bound residues increased continously from 3.7% to 35.8% of the applied radioactivity. At the end of the experiment a maximum of 10% of the applied radioactivity was recovered in the grass. The loss of radioactivity in the material balance was mainly attributed to evolved $^{14}CO_2$. Since this was not measured continuously, total $^{14}CO_2$ could not be quantified.

These results are in good agreement with those results from experiments reported in the literature. For example, an increased degree of mineralization under vegetation was determined in a study of the degradation of two organo phosphate insecticides (Hsu & Bartha, 1979).

FIGURE 3: Material balance of imidacloprid in soil

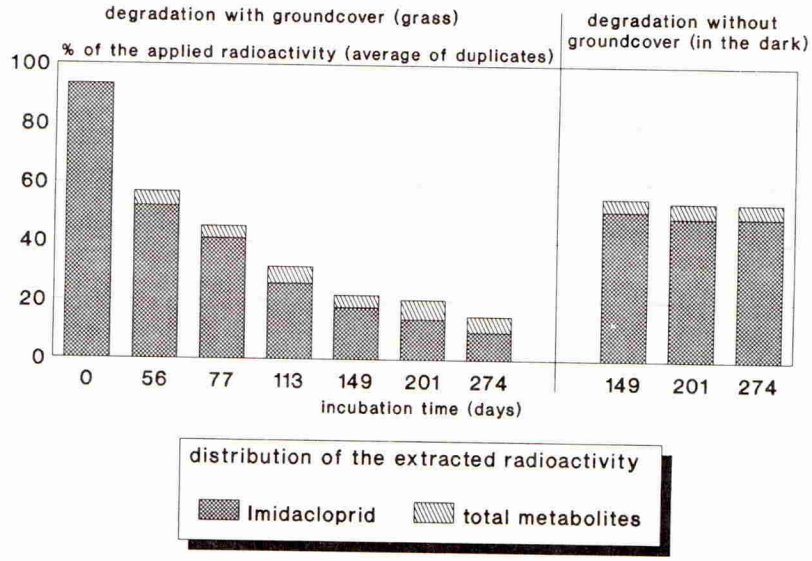


The recovered radioactivity ranged from 96.8 to 100.5% during the test period of 274 days in the control experiment without vegetation. Radioactivity levels for extracted and bound residues remained relatively constant over the incubation period. The $^{14}CO_2$ formed unlike the experiment with vegetation could be collected quantitatively and amounted to 19% after 274 days.

Degradation of imidacloprid and formation of metabolites

The decline of imidacloprid in soil with and without vegetation is shown in Figure 4.

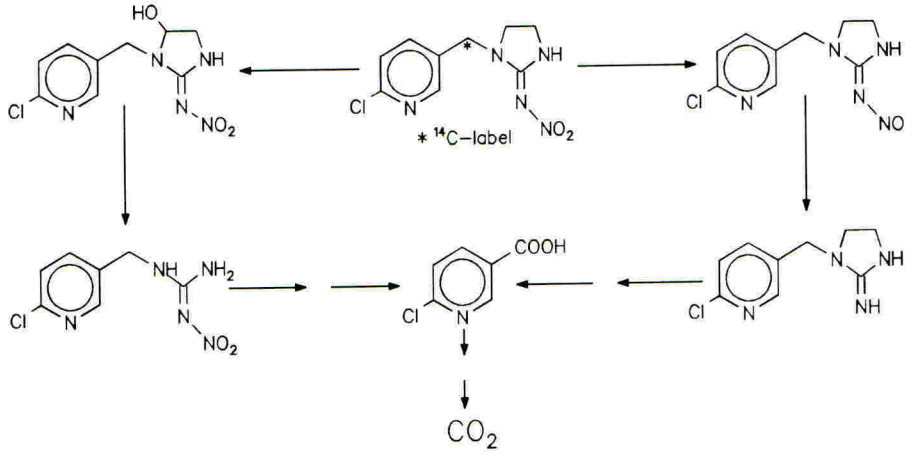
FIGURE 4: Degradation of imidacloprid in soil



Imidacloprid was degraded much more rapidly under vegetation. The half-life was 48 days for the experiment with groundcover whereas a half-life of 190 days resulted from the experiment similiar to the BBA-guideline (no vegetation, dark: Schinkel *et al.*, 1986). The large differences in the degradation behaviour are primarily due to the influence of vegetation, although variations in the soil moisture may be a factor. In the experiments with vegetation the soil moisture was controlled via plant irrigators and was consequently subjected to certain variations. Light had certainly no, or only a negligible influence on the degradation behaviour since the active ingredient was incorporated into the soil and consequently subjected to photodegradation only at the surface.

The metabolites shown in Figure 5 occurred in only small amounts (≤ 4%) irrespective of the presence of vegetation. It is concluded that the rate determining step in the degradation of imidacloprid is the primary attack of the parent molecule. The imidazolidine ring is stepwise degraded to the key metabolite 6-chloronicotinic acid which leads to the final product carbon dioxide (Figure 5).

FIGURE 5: Proposed degradation pathway of imidacloprid



CONCLUSIONS

The experiments demonstrated that use of vegetation, in the determination of imidacloprid degradation in soil, was essential to provide data relevant to the intended field use. The approach presented here is expected to be of benefit for those compounds which show an apparent persistence under current standard laboratory conditions used for the registration of pesticides.

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Schinkel, K.; Nolting, H.-G.; Lundehn, J.-R. (1986) BBA, Richtlinien für die amtliche Prüfung von Pflanzenschutzmitteln, Teil IV, 4-1 Verbleib von Pflanzenschtzmitteln im Bodenabbau, Umwandlung und Metabolismus.

A PHOTOGRAPHIC FLUORESCENT TRACER TECHNIQUE FOR ASSESSING OPERATOR EXPOSURE TO PESTICIDES

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ABSTRACT

Most current methods of assessing dermal exposure of sprayer operators to pesticides are essentially volumetric. They rely on recovery of chemical or dye from protective suits or absorptive pads and analysis of the washings. An area-based technique is under preliminary investigation as a quick and cost-effective alternative. Using data and photographic prints from field trials in Pakistan, a comparison was made between the volume of pesticide deposited on operators' suits and the area it covered. Volumes were determined fluorimetrically and areas determined by a combination of a fluorescent photographic technique and computer-based image analysis. Although the relationship was statistically significant, correlation was poor. This is partly due to experimental error, partly to the number of variables in the sample but also due to factors intrinsic to the technique. A reliable universal relationship between area and volume exposure is unlikely, but a relationship for specific cases may be possible.

INTRODUCTION

Application of pesticides always presents some degree of exposure hazard to operators. This applies particularly to operators of portable sprayers in less developed countries where proper protective clothing may be impractical or not available (FAO, 1990). The health risk to the operator depends on a combination of the inherent toxicity of the formulation and the duration and extent of exposure (Plestina, 1989). In order to assess this risk to the operator, information is required on these three factors. Mammalian toxicity data are freely available and the duration of exposure can be taken as the time spent spraying (assuming the operator washes and changes after spraying), but measuring the extent of exposure is more difficult.

Strictly speaking, dermal exposure occurs when pesticide falls on the skin. Since most operators wear some sort of clothing during spraying, for a given amount falling on the clothing, dermal exposure will depend on its resistance to penetration. For this reason, the amount falling on a protective suit represents a potential dermal exposure, giving an indication of relative rather than absolute risk. However, potential exposure will be referred to here as exposure.

Various procedures for measuring operator exposure to pesticides have been developed and published. They are generally based on analysis of absorptive pads attached to the sprayer operator's clothing (Durham & Wolfe, 1962; WHO, 1975). Other methods have included the quantitative analysis of protective suits worn during spraying, when the amounts of

pesticide residue or dye tracer on designated body part sections have been determined (Ambridge *et al.*, 1990). Unfortunately, for any given sprayer and mode of use, the extent of exposure will always be highly variable because of chance occurrences such as spills or gusts of wind, and large numbers of samples are required to make statistically significant comparisons of exposure from different spraying options. As a result, thorough studies are expensive at present; the development of more rapid exposure assessment techniques is desirable.

METHODS AND MATERIALS

Preliminary work has been carried out to test a method of exposure assessment based on image analysis of photographic records of suit deposits. Exposure area data were compared with volume data from fluorimetric analysis of the suits to determine whether one could be estimated from the other.

Details of the field trials which provided the spray suits are described by Sutherland *et al.*, (1990). In brief, a number of spray applications were made on cotton crops by spray operators wearing polyolefine fabric protective suits (Tyvek, Du Pont Nemours) and using a range of portable sprayers. Sufficient fluorescent tracer was included in the spray mixtures to give a nominal concentration of 1%, which was confirmed by analysis of tank samples taken before and after spraying. Visible dyes were not used because operators tend to spray more carefully when they have visual feedback of exposure. Uvitex OB and Tinopal CBS-X were used for oil and water-based sprays respectively. All relevant application parameters such as sprayer emission rate, operator walking speed, track spacing, total volume emitted, duration of spraying and meteorological conditions such as wind speed, wind direction and temperature were recorded. After spraying, the suits were allowed to dry, turned inside out, and shipped to England in labelled plastic bags.

The suits were mounted on a tailor's dummy and photographed front and back, illuminated by flash units filtered to give solely ultra-violet output. The areas of exposure were visible on the photographic prints as bright white areas against the much darker background of the rest of the suit. The suits were then cut into fifteen sections corresponding to different parts of the body and subjected to fluorimetric analysis, details of which are also given by Sutherland *et al.*, (1990).

The photographs were originally only intended to indicate broadly which areas of the body were most exposed to pesticides and to distinguish between spray deposition and leaks/spills. Indeed they provided valuable qualitative information on exposure processes. However, after the purchase and installation of a Cambridge Instruments Q520 Image Analyser (Leica Cambridge Ltd, Clifton Rd, Cambridge) it was realised that further quantitative information could be obtained from the photographs. Ten examples from the fifty suits analysed were selected, representing a range of different equipment and levels of exposure. The procedure adopted for the image analysis of the photographs consisted of the following steps:

(a) on each photograph, a marker pen was used to delimit the suit body part sections;
(b) a photograph was then placed into position under the Q520 video camera;

(c) a dimensional calibration was carried out based on the known height of the suit;
(d) the total apparent area of a suit section was measured;
(e) after setting an appropriate grey level threshold, the apparent exposure area was measured;
(f) the procedure was repeated for all sections on all photographs;
(g) for hood, arms and legs, data from front and back photographs were summed.

Because of creases in the suits and depth effects as a result of presenting them on a three dimensional dummy, the projected area of each suit section and its exposure area were less than the real values. To allow for this, the data were analysed as apparent exposure area per apparent unit area of suit for each section, in other words, proportion of area with deposits. Provided exposure is distributed uniformly across a section, this gives the same result as if the suit had been photographed perfectly flat.

Data for both variables (area and volume) were transformed to natural logarithms to allow use of the regression technique. In addition, 0.1 was added to the area proportions to prevent the logarithm of zero appearing in the data.

RESULTS

Table 1 shows details of the field trials from which the spray suits derived. Different sprayers, volume application rates, durations and tracer dyes were involved.

TABLE 1. Details of sprayers and modes of use for each suit

Suit no	Sprayer type	Date -1987	Vol appln rate (l/ha)	Emitted Volume (l)	Fluore-scent tracer
1	Micron Micro-Ulva spinning disc	28-Jul	1.63	0.44	Uvitex
2	Micron Micro-Ulva spinning disc	1-Oct	2.50	0.99	Uvitex
3	Chinese made LOK *	19-Sep	30.00	13.00	Tinopal
4	Cooper Pegler LOK *	28-Jul	28.90	7.80	Tinopal
5	Cooper Pegler LOK *	30-Jun	13.40	3.60	Tinopal
6	Cooper Pegler LOK *	1-Oct	12.00	4.00	Tinopal
7	Solo motorised mistblower	15-Oct	30.00	27.50	Tinopal
8	Solo motorised mistblower	19-Sep	6.00	3.40	Tinopal
9	Solo motorised mistblower	14-Jul	33.30	9.00	Tinopal
10	ICI Electrodyne (electrodynamic)	13-Oct	0.90	0.17	Uvitex

* Lever-Operated Knapsack sprayer

Initially, all data were included in the statistical analysis. After consideration of the results, further analysis was carried out on increasingly selective elements of the data, down to a level of part of one of the suits. Table 2 shows this progression to more specific examination.

TABLE 2. A summary of the statistics for different treatments of the data

Sprayers	Sections	R2 (%)	n	P
all	all	10.21	149	<0.0001
all	all except legs	17.84	109	<0.0001
LOKs	all except legs	19.61	43	<0.01
all except LOKs	all except legs	29.97	65	<0.0001
Micro-Ulva	all except legs	52.56	21	<0.001
Electrodyne	all except legs	54.60	10	<0.01

R2 (correlation coefficient squared) is a measure of the variability accounted for by the proposed relationship between volume and area

n is the number of data points

P is the probability that there is no relationship

DISCUSSION

Results for all sections of all suits (see table 2) show that the correlation between the exposure area and the exposure volume is poor with only 10% of the variation being attributable to the relationship. There are many possible reasons for this:

(a) the exposure area produced by heavy deposits, especially those caused by spills, may be less per unit volume than that produced by light spray deposits. The lower molecules of the layer will be obscured and quenched by those above, meaning that a deep layer fluoresces no more brightly than a shallow one;
(b) distribution may not be uniform across the suit sections, meaning that the creasing and depth effects are not affecting apparent exposure area uniformly. For example, the area of a deposit on the side of the suit would be under-estimated by the image analysis;
(c) lighting was not uniform over the suit; deposits are less bright or missed altogether on some sections, particularly the legs;
(d) where fields had been recently irrigated, mud obscured some of the deposits on the lower parts of the suits; staining with green plant pigments, caused by pushing through the denser plots, had the same effect in some cases;
(e) different sprayers have different drop spectra which spread differently on the suit;
(f) oil-based formulations, such as those used in the Micro-Ulva and Electrodyne, spread and penetrate differently from the water-based formulations used in the other sprayers. Even within the water-based formulations, higher pesticide concentrations associated with lower volume application rates mean higher concentrations of wetting agents and other additives which would affect spread after deposition;

(g) the two dyes used may have different fluorescence characteristics resulting in a different intensity from similar volume deposits.

Investigating these possibilities by focusing on selected data produced improvements in the correlation (see table 2). Omission of leg data, for the reason given in (c) above, improved the variability accounted for to 18%. The area/volume relationship for lever-operated knapsack sprayers (LOKs) seems to differ from that for other sprayers and omission of LOK data ((a) and (e) above) increased the variability accounted for to 30%.

Modification to the methods could be expected to improve the situation further. Recently, techniques of suit presentation and analysis have been refined; the suit is stretched on a flat surface to eliminate creases and depth effects ((b) above) and flash lighting is arranged to illuminate the suit uniformly ((c) above). The problem of mud and plant pigments ((d) above) will always arise in dense crops and wet fields.

The type of sprayer, formulation used and volume application rate, may also affect the way exposure area relates to volume ((e) and (f) above). The Electrodyne and Micro-Ulva exhibit a much more reliable area/volume exposure relationship than the LOKs (see table 2). This is to be expected since they are ULV sprayers producing a narrow spectrum of small drops (typical Volume Median Diameter of 50 μm). They are also held away from the body which reduces the chance of spills onto the operator. The LOKs produce larger drops (typical Volume Median Diameter of 250 μm) in a wider spectrum, are carried on the back and are usually used at higher volume application rates. They are more likely to give a poor area/volume correlation for the reason given in (a) above.

CONCLUSIONS

With all data represented in this preliminary investigation, there is a statistically significant relationship, but a poor correlation, between area and volume of exposure. Experimental error is thought to be partly responsible and the correlation is improved when some of the suspect data is excluded. However, a good estimate of volume from area would still not be possible.

This is a preliminary investigation using photographs in a quantitative way for which they were not originally intended. Modifications to the technique may reduce error and improve the correlation, but aspects of the spray system such as sprayer type, formulation and volume application rate can still alter the relationship between volume and area and make a universal relationship unlikely. However, useful estimates of volume from area, for any particular combination of circumstances, may be possible providing an initial calibration of the relationship is carried out.

The two main processes causing exposure, often occurring together, are spills and spray deposition. They have different volume/area relationships and discrimination between them may be the most serious obstacle to overcome before exposure volume can be reliably estimated from exposure area.

ACKNOWLEDGEMENTS

Grateful thanks are due to Mr Stephen Causley for image analysis of the photographs and to Miss Sheila Green for statistical analysis of the data.

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THE APPLICATION OF GAS CHROMATOGRAPHY-SELECTED ION MONITORING TO THE DETERMINATION OF RESIDUES OF THE NOVEL ACARICIDE FENAZAQUIN IN ENVIRONMENTAL AND CROP SAMPLES.

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ABSTRACT

The criteria used to develop residue methodology for the determination of fenazaquin, a quinazoline based acaricide are reviewed. A method based on capillary gas chromatography utilizing automated cold on-column injection with selected ion monitoring (gc-sim) has been successfully employed over an 18 month period. Fenazaquin has been analysed in a wide range of matrices: apple, apple process fractions, citrus peel and pulp, grape and soil. A validated determination limit of 0.01 mg/kg has been achieved for these substrates and 0.05 μg/l for drinking water.

INTRODUCTION

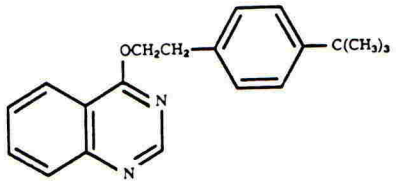
Fenazaquin represents a novel class of compounds that show good selectivity to a range of mites (Dreikorn et al., 1991, Hollingworth et al., 1992).

The main criteria used in the development of residue methodology encompasses sensitivity, selectivity and robustness of the method over a range of substrates. The analytical approach is determined by the range of substrates requiring analysis and the physicochemical characteristics of the compound (Table 1).

The applicability of high performance liquid chromatography with u.v. detection is dependent on the variability of the background profile of the substrates and the intrinsic extinction coefficient of the compound. Extensive clean-up is often required for hplc with fine tuning to cope with a range of crop samples. Small coeluting peaks in the region of the test substance or apparent residues near the determination limit of 0.01 mg/kg can often require confirmatory tests, for example mass spectrometry. These problems were encountered with fenazaquin and therefore an alternative approach was evaluated.

The use of gas chromatography with selected ion monitoring can allow the unequivocal identification and quantification of low residues. This technique has been used extensively with heated split/splitless injection and a wide range of thermally stable compounds can be detected (Liao, et al., 1991). Fewer authors have reported the use of automated cold on-column injection and often these refer to direct injection into the analytical column. Injection into a 530 micron retention gap with a 200 micron analytical column has been reported by Hewlett Packard (Foror, 1990).

TABLE 1. Physicochemical properties of fenazaquin.



4-tert-butylphenethyl quinazolin-4-yl ether

Molecular Weight: 306.4amu Melting Point: 77.5-80.0°C

Vapour Pressure: 1.60×10^{-4} Pa

Solubility (g/ml) Hexane: 0.033-0.05, Dichloromethane: 70.6, Acetone:0.4-0.5, Acetonitrile: 0.33-0.50, Water 0.0001

Molar extinction coefficient at 262.2nm 1.24×10^{4} at pH 7.83

Fenazaquin will chromatograph on non-polar capillary columns. In order to achieve the required sensitivity and to avoid any discrimination or degradation in the injection port, cold on-column injection was selected. Initial studies with splitless injection indicated that long term reproducibility of the standard response was poor.

True automated cold on-column injection into an analytical capillary column linked directly into the ion source was not possible, due to limitations in injector design or the flow rate capacity of the mass selective detector. Instead injection into a 530 micron (2.5 m) retention gap which was connected with a deactivated glass press fit connector to a 200 micron (12 m) analytical column was used. This system has the added advantage of allowing an increased injection volume with minimal loss in peak shape while protecting the analytical column from the deposition of extraneous material which could shorten its effective lifetime.

Quantification using a surrogate internal standard to compensate for the enhancement of the mass selective detector response by the presence of coeluting material in the ion source has been reported by Carmichael and Perkins 1989. No suitable surrogate standard was available for fenazaquin. In order to compensate for any enhancement effect, calibration standards were prepared in control sample extract.

This poster reviews the implementation of capillary gas chromatography with automated cold on-column injection and selected ion monitoring to routine residue analysis.

EXPERIMENTAL

Methods for the following substrates have been validated, apples, apple pomace and puree, orange peel and flesh, lemon peel and flesh, grapes, soil and water. All substates except water were extracted with hot acetonitrile/water. After centrifugation and the addition of aqueous sodium bicarbonate the extracts were partitioned with hexane. The organic phase was cleaned up using a Florisil Sep Pak cartridge before gc-ms analysis. Soil, orange and lemon peel, orange flesh and grape samples received an additional clean-up on an aminopropyl Bond Elut cartridge prior to analysis. Fenazaquin residues in water were determined by partitioning sodium bicarbonate treated water with hexane which was then purified using an aminopropyl Bond Elut cartridge before gc-ms analysis.

The gc and ms conditions used are summarised below, an example of the chromatography and spectrum is given in Figure 1.

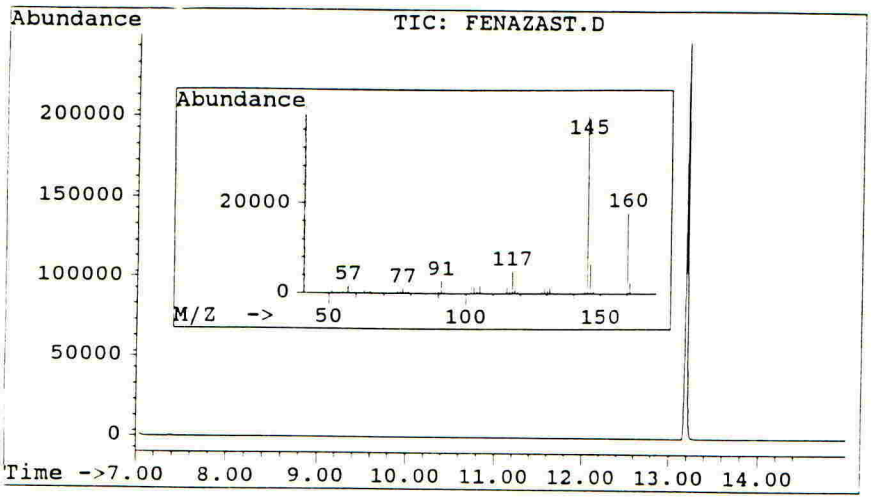
Gas Chromatograph: Hewlett Packard 5890 with 7673A autosampler

Column: Retention gap 2.5m x 0.53mm id (Chrompak) with glass connector (Jones).
: Analytical column 12m x 0.2mm id Ultra 2 (Hewlett Packard).
: Injection volume 5μl

Injection Port Temp. : 40°C Transfer Line Temp.: 280°C
Oven Initial Temp/Time : 40°C for 1 min Oven Ramp Rate: 20°C/min
Final Temp/Time : 300°C for 12 mins

Mass Spectrometer: Hewlett Packard 5970/5971 Mass Selective Detector
Masses Monitored (amu) : Fenazaquin 145.1 and 160.1,
: Internal Standard 285.9
Multiplier Voltage: ca 2200 eV Dwell Time: 100 microseconds

FIGURE 1. Typical chromatography and mass spectrum of fenazaquin.



A batch standard was prepared with each analytical batch from a suitable control sample (i.e. control of similar growth stage or variety). This standard was used to bracket samples and to quantify the residue. The linearity of the system was checked with each analytical run (typical coefficient of correlation >0.999).

To compensate for any variation in injection volume an internal standard was incorporated in the trimethyl pentane used to reconstitute the final residuum. 1,4-Dibromonapthalene was chosen as it was well separated from fenazaquin and with a high mass ion was unlikely to suffer interference from the chromatographic background from the various substrates examined.

RESULTS

A summary of mean recovery data for a range of substrates is given in Table 2. These methods were applied to samples from field trials where crops were treated at rates up to either 150g AI/ha or 5.0g AI/hl.

TABLE 2. Mean % recovery data for fenazaquin.

	Fortification Rate (mg/kg)			
SUBSTRATE	0.01	0.05	0.10	0.25
Apple	90 (11)	89 (6)	94 (2)	85 (6)
Apple Puree	85 (6)	107 (2)	104 (2)	-
Orange Flesh	99 (12)	105 (2)	98(10)	93 (4)
Orange Peel	89 (12)	104 (1)	93 (5)	85 (4)
Lemon Flesh	86 (6)	98 (3)	92 (3)	-
Lemon Peel	88 (3)	86 (2)	89 (2)	77 (1)
Grape	98 (6)	89 (6)	94 (2)	-
Soil	83 (6)	88 (4)	84 (4)	-
		Fortification Rate (µg/L)		
		0.05	0.50	5.0
Water		78 (6)	91 (2)	88 (2)

( ) denotes number of analyses

In routine use the mean overall recoveries and standard deviation are shown for representitive crops in Table 3 together with typical residues at harvest.

TABLE 3. Typical recovery data and residues from selected substrates.

Crop	Mean Recovery(%) (SD)	Range of residues (mg/kg)
Apple	90 (9.8)	<0.01 - 0.06
Orange Flesh	98 (9.5)	not detected

DISCUSSION

Routine use of these methods over an 18 month period on a range of substrates has resulted in over 1000 analyses. In all cases the monitoring of two masses has allowed unequivocal assignment of residues. The concentrations derived from each mass were within 10% of the mean value based on the average of the two results. No residues have been detected in untreated samples.

The main challenges encountered in introducing automated cold on-column capillary gc-sim into routine residue analysis related to the use of the 530 micron retention gap.

The following conclusions have been drawn:

1. The quality of the deactivation of the fused silica retention gap is extremely variable and several manufacturers should be evaluated.

2. The design of the press fit glass connectors allowing joining of the 530 micron and 200 micron columns is crucial. Columns can be subjected to considerable stress during the oven cooling cycle. With high sample throughput the seal between the column coating and the glass connector can fail giving a small leak. Poor quality control in terms of the internal diameters of these connectors has been observed from some manufacturers, adding to this problem.

   Ultimately a universal designed connector suitable for a wide range of column sizes was chosen. This had a longer glass stem giving better support and hence giving more physical strength to the connection.

   Washing the connectors with acetone and heating to ca. 100°C prior to connection were found to be beneficial. To ensure a prolonged gas tight fit, the addition of polyamide resin a few millimetres from the tip of the column combined with a slow baking of the the freshly connected columns at 10°C/min to ca. 250°C has proved to be successful.

3. Loss of chromatograhic resolution or peak shape is usually attributable to a build up of coextracted material in the retention gap. Shortening of the retention gap or replacement of

the push fit connector alone, are not usually successful. Both items should be treated as disposable and both replaced at the same time.

The frequency of replacement can be reduced by the addition of the aminopropyl cleanup. The soil and grape methods have been shown to produce cleaner extracts which do not exhibit signal enhancement following standard addition. This has the added benefit of eliminating the necessity for using surrogate standards or standards in matrix.

Maintenance of the gc-ms system only required the replacement of the retention gap and glass connector and routine cleaning of the ion source. During this period the analytical column was only replaced on two occasions whereas the retention gap was replaced after 100-400 injections.

In conclusion automated on-column capillary chromatography with mass selective detection operating in the selective ion mode has been successfully implemented as a routine residue procedure ensuring unequivocal assignment of residues. Use of the retention gap and glass connector as disposable items helped to ensure low instrument downtime and high sample throughput.

ACKNOWLEDGEMENTS

The authors wish to thank the contribution of their colleagues, R. Teasdale, G. Betts, R. Draper and T. Long for their assistance in the production of data for this poster.

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COMPARATIVE METABOLISM OF [PYRIDINYL-^{14}C-METHYL]IMIDACLOPRID IN PLANT CELL SUSPENSION CULTURES

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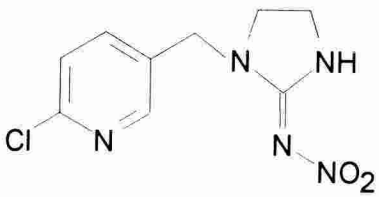
ABSTRACT

The metabolism of [pyridinyl-^{14}C-methyl] imidacloprid was investigated in heterotrophic and photomixotrophic plant cell suspension cultures of different plant species. It was shown, that the degradation of this new insecticide was qualitatively and quantitatively different in the tested cell cultures. Imidacloprid was degraded by all the cell cultures, but the extent of degradation varied amoung the different species. For an apple cell culture, only 39 % of the recovered radioactivity was attributed to the parent compound. The degradation in other cell cultures ranged from 1.5 % (maize) to 23 % (soybean and wheat). The major metabolites were the monohydroxy and the olefinic compound, which were found in all cultures. Some minor metabolites, which were not detected in all cell cultures, were 6-chloronicotinic acid, 6-chloropicolyl alcohol and 6-chloropyridinyl glucopyranoside.

INTRODUCTION

Imidacloprid (1-[(6-chloro-3-pyridinyl)methyl]-4,5-dihydro-N-nitro-1H-imidazol-2-amine) is a new systemic insecticide with a nitroguandine structure , which can be applied as a soil, foliar, or soil-drench insecticide (Moriya et al., 1992). It will be used to control sucking insects (for example leaf hoppers and aphids) in a wide variety of fruits, fields crops and ornamentals. It acts as a stomach and contact poison.



Imidacloprid (NTN 33893)

The aim of this study was to continue the investigation of the metabolism of imidacloprid in cell suspension cultures of different plant species. In an earlier study (Koester, 1989) the metabolism of imidacloprid was investigated in potato, wheat and maize cell suspension cultures. It was shown, that imidacloprid was degraded by three metabolic pathways: 1. oxidation of the ethylene bridge of the imidazolidine ring to form the mono- and bis-hydroxy-, the keto- and the olefin metabolites, 2. oxidation of the pyridinyl-methylene group followed by cleavage and subsequent conjugation with glucose to form the 6-chloropyridinyl gluco-pyranoside, and 3. reduction of the nitro- to the nitroso-group.

MATERIAL AND METHODS

Radiochemical

The investigations were conducted with [pyridinyl-^{14}C-methyl] imidacloprid. The radiolabelled compound had a specific radioactivity of 140 µCi/mg (5.18 MBq/mg), and a radiochemical purity of > 99 % determined by tlc and hplc. By diluting with non-radiolabelled compound a final concentration of 50 µM (511 µg/40 ml cell suspension; specific radioactivity 3.91 µCi/mg (9.79 µCi/mg: apple induction experiment)) was used for the experiments.

Plant cell cultures, application and sampling

The experiments were carried out with the following heterotrophic plant cell suspension cultures: citrus var. "Keyline", apple var. "Boskop", potato var. "HH 258", tomato, soybean var. "Mandarin", vine var. "Kerner", tobacco, peanut, parsley, wheat var. "Heines Koga II", cotton var. "Coker 310" and maize var. "Black Mexican sweet". In one additional experiment a mixotrophic green cell culture of tobacco was used and in another an apple cell culture was used, which was treated with 10 µM of the non-radiolabelled compound over 5 weeks (induction experiment) before application of the radiolabelled compound. The experiments were performed with cells from the beginning of the stationary growth phase (day 7 - 14 after transfer of cells into fresh nutrient medium depending on the cell culture species). A sterile acetonitrile solution of the active ingredient was applied directly to the cells. The amount of solvent added did not exceed 120 µl/40 ml cell suspension (= 0.3 %).

The cell cultures were grown in 200 ml Erlenmeyer flasks containing 40 ml nutrient medium. They were kept in climate cabinets at +25°C. The heterotrophic cell cultures were cultivated under dark conditions, and the mixotrophic cell cultures under light conditions with cool white fluorescent lamps. They were shaken on a rotatory shaker operating at 130 cycles per minute. Different media were used for the various cell cultures. The composition of some basic nutrient media is described by Gamborg (1984).

Sample processing

At harvest time (day 7 after application), nutrient media and cells were separated by filtration. The nutrient media were concentrated by lyophilization, redissolved with water and separated on an Extrelut - gel column (10 g Extrelut gel/20 ml extract) by consecutive elution with dichloromethane, ethyl acetate and methanol. The plant cells were extracted with acetonitrile / water (8 : 2 (V/V)). The cell extract was further partitioned with ethyl acetate to yield an organic and an aqueous phase. The aqueous phases with sufficient radioactivity were additionally separated on a XAD 4 column by successive elution with water, water / methanol (9 : 1 (V/V)), methanol and methanol / conc. HCl (99 : 1 (V/V)). The solid residues from the extracted cells were dried by lyophilization before determination of the radioactivity.

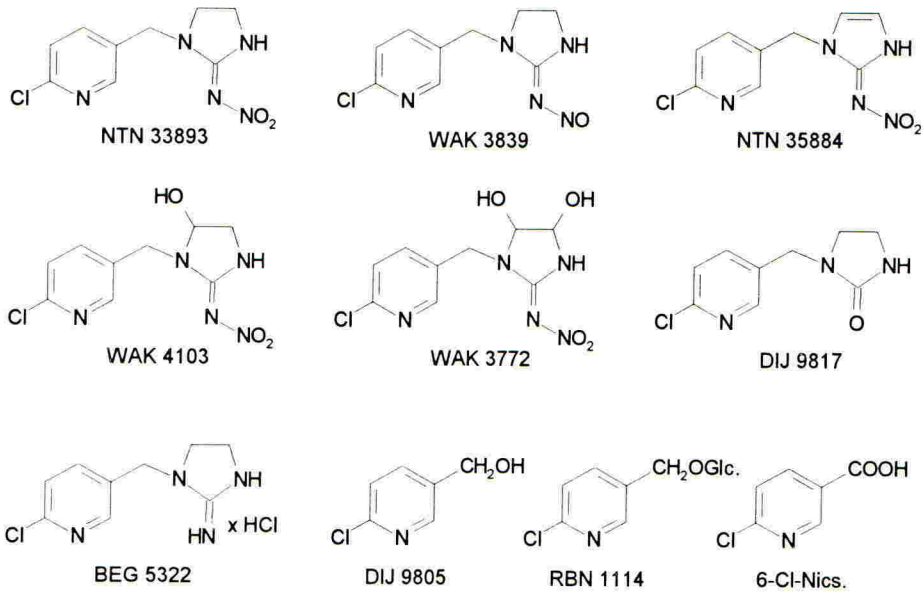
Thin layer chromatography

The analysis of the samples was carried out on silica gel plates (Merck, Germany 60 F_{254}, 0.25 mm, 20 x 20 cm) by two dimensional thin layer chromatography with the following solvents:

Direction 1: ethyl acetate / isopropanol / water = 65 : 23 : 12 (V/V) and
Direction 2: chloroform / methanol / acetic acid / water = 65 : 25 : 3.5 : 3.5 (V/V).

The tlc-spots were detected by quenching of the u.v.-induced fluorescence at 254 nm and by autoradiography using Curix Clear Base X-ray films (AGFA, Germany). For identification of the metabolites and the parent compound, the concentrated samples were mixed with the reference compounds (Figure 1) before application to the tlc-plate. When the reference compound co-chromatographed with the radioactive metabolite, it was assumed that the chemicals were identical. For quantitation of the radioactive substances, the silica gel was scraped off and the radioactivity was measured by liquid scintillation counting.

FIGURE 1. Chemical structures of imidacloprid (NTN 33893) and reference substances [6-Cl-Nics.: 6-chloronicotinic acid; RBN 1114: 6-chloropyridinyl glucopyranoside].



RESULTS AND DISCUSSION

Distribution of radioactivity

The radioactivity which was found in the insoluble residues and the wash solution of the Erlenmeyer flasks was generally low (0.0 % to 1.9 %). With the exception of soybean, vine and the heterotrophic (hcc) tobacco cell culture, most of the radioactivity was found in the nutrient media (42.1 % to 94.9 %). The cell extracts contained 3.2 % to 56.3 % of the recovered radioactivity. The majority of this radioactivity could be transferred into the organic phase using ethyl acetate. The largest amount of radioactivity in the aqueous phase of the cell extract was detected in the soybean cell culture (14.3 %).

Identification and quantitation of metabolites

All fractions were analysed using two dimensional tlc. The results are summarized in table 1, which show the distribution of imidacloprid and metabolites in the tested plant cell cultures.

TABLE 1. Distribution of metabolites and imidacloprid in different plant cell cultures (values are expressed as percent of radioacitivty recovered).

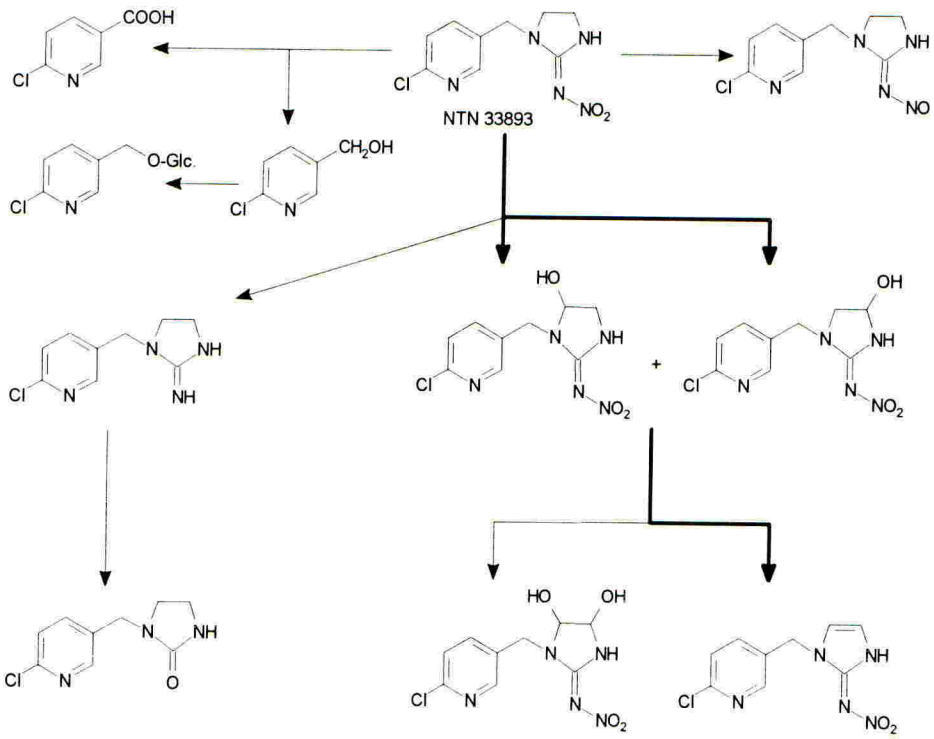
cell culture	NTN 33893	WAK 4103	NTN 35884	WAK 3772	WAK 3839	DIJ 9817	BEG 5322	DIJ 9805	6-Cl Nics	RBN 1114
Citrus	95.79	1.13	0.28	----	0.06	0.12	0.12	----	----	----
Apple	39.41	20.01	28.48	0.21	0.10	0.11	----	----	3.12	----
Apple (induc.)	82.76	4.10	5.30	0.02	0.36	0.11	----	----	1.65	----
Potato	83.42	8.07	4.33	0.15	0.02	0.20	----	0.23	0.45	----
Tomato	97.62	0.40	0.08	----	----	----	----	----	----	----
Soybean	77.38	2.25	0.97	----	0.11	0.20	0.03	0.27	8.65	1.27
Vine	85.02	2.05	0.98	----	----	0.38	0.10	0.14	----	4.15
Tobacco (hcc)	96.75	0.77	0.17	----	----	0.06	----	----	----	----
Tobacco (mcc)	92.21	1.23	0.42	----	0.14	0.09	0.03	----	0.44	0.07
Peanut	99.10	0.14	0.06	----	----	----	----	----	----	----
Wheat	77.12	1.40	16.09	----	0.09	0.14	0.04	----	----	----
Parsley	80.24	0.34	11.66	----	----	----	----	----	----	----
Cotton	86.71	3.70	1.10	----	0.02	0.13	0.08	----	0.69	3.10
Maize	98.48	0.10	0.10	----	0.09	0.25	0.04	----	----	----

(6-Cl-Nics: 6-chloronicotinic acid; induc.: induction experiment; hcc: heterotrophic cell cultures; mcc: mixotrophic cell culture)

With the exception of the apple cell culture, the parent compound was the major radioactive substance, showing that little degradation of imidacloprid had occured in most of the tested plant cell cultures. The main metabolites were the monohydroxylated (WAK 4103) and the olefinic compound (NTN 35884), which were detected in all cell cultures. The amount of all other metabolites was generally below 10 %, including the nitrosimine compound (WAK 3839). 6-chloronicotinic acid was detected in the apple, potato, soybean, tobacco (mcc) and cotton cell cultures. 6-chloropyridinyl glucopyranoside (RBN 1114) was found in soybean, vine, tobacco (mcc) and cotton cell cultures. 6-chloropicolyl alcohol (DIJ 9805), the precursor of 6-chloropyridinyl glucopyranoside, could be detected only in potato, soybean and vine cell cultures. The guanidine metabolite (BEG 5322) was a minor metabolite of citrus, soybean, vine, tobacco (mcc), wheat, cotton and maize cell cultures and the dihydroxy metabolite (WAK 3772) was only observed in apple and potato cell cultures. The urea metabolite (DIJ 9817) was found in nearly all cell cultures with exception of parsley, tomato and peanut.

From these results, the following degradation scheme is proposed (Figure 2), essentially confirming the results of the earlier study (Koester, 1989).

FIGURE 2. Degradation pathway of imidacloprid in plant cell cultures



The quantitative differences between the two apple cell cultures were significant. Imidacloprid was degraded more by the cell culture grown under standard conditions compared to the preincubated cell culture (39.4 % AI vs. 82.2 %). Nearly all of the metabolites (with the exception of the guanidine compound (BEG 5322), 6-chloropicolyl alcohol and the corresponding glucoside), were detected in both cell cultures. Obviously the degradation of imidacloprid was hindered by the pretreatment with the non-radiolabeled active ingredient. Additionally, significant quantitative differences for the nitrosimine metabolite (WAK 3839) in both cell cultures were detected . A higher amount was found in the preincubated cell culture (0.36 % vs. 0.1 %), indicating a slight preference for this alternate route of the degradation of imidacloprid.

A similar difference in the formation of the nitrosimine metabolite (WAK 3839) was observed for the heterotrophic (hcc) and green mixotrophic (mcc) tobacco cell culture. This metabolite was only detected in extracts of the green mixotrophic cell culture (0.14 %). In this case, the formation of NADPH via photosynthesis might be one reason for the presence of the metabolite.

Some of the identified metabolites in this study were found in the cell extracts and in the nutrient medium. It seems, that these compounds were partly released into the nutrient medium, the biggest "lytic" compartment of the plant cell culture system. Similar effects have been noticed for several secondary products, which are normally stored in the vacuole (Wink, 1984; Hallard et al., 1992), and also in other metabolism studies with pesticides (Edwards & Jones, 1986; Cole & Loughman, 1984; Cole & Owen, 1987).

The quantitative and qualitative differences of the metabolites of imidacloprid in the various plant cell cultures are not surprising (Owen, 1989). Normal variations in the expression of degradating enzymes, the age of the cell cultures and differences in the sensitivity of the plant cells to this pesticide might be the reasons for these results. Other metabolism studies showing similar effects are those of chlortoluron in cell suspensions of Lactuca sativa (Cole & Owen, 1988) and wheat (Canivenc et al., 1989) as well as bentazon in suspension cultures of alfalfa, carrot, maize, potato, rice, tobacco, wheat and soybean (Sterling & Balke, 1989). This study has clearly shown, that due to little degradation of the applied compound in most cases, a single specific plant cell culture is not always sufficient, to determine the range of pesticide degradation products formed in the plant cell. Nevertheless, it was demonstrated by the results of these in vitro experiments, that studies with different plant cell cultures were useful to understand the biodegradation of a pesticide by plant cells and can complement investigations on metabolism of pesticides with whole plants (Mumma, 1987).

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THE ENVIRONMENTAL DISTRIBUTION OF HEXAFLURMURON

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ABSTRACT

Hexaflumuron (DE-473) is a new insecticide, and may be used in orchard fruits, vegetables and cotton.

In plant metabolism studies hexaflumuron appears to be stable and does not penetrate into the plant tissue, or translocate.

The degradation and leaching behaviour of hexaflumuron in soil will control its environmental behaviour. In aerobic soil, hexaflumuron degraded with half-lives in the range of 100-160 days. In soil leaching column and desorption studies hexaflumuron was essentially immobile (Kd in the range 147 -1326) as were the major metabolites. Allied with a very low water solubility, it will not leach into surface or ground waters. Any hexaflumuron entering surface waters will be removed by rapid adsorption to suspended soil particles plus aqueous photolytic degradation. When these properties are considered together with its lack of toxicity to non-target organisms, it can be concluded that, under normal agricultural practises hexaflumuron is unlikely to have an adverse effect on the environment.

INTRODUCTION

Hexaflumuron is the accepted common name for the new benzoylphenylurea insecticide, 1-[3,5-dichloro -4- (1,1,2,2-tetrafluoroethoxy) -phenyl] -3-(2,6-difluorobenzyl) urea.

CONSULT, SONET, CONSUL and CARION are registered trademarks of DowElanco for formulations containing hexaflumuron.

Hexaflumuron acts by inhibition of chitin synthesis and it affects insects at all stages of development including, dependent on insect species, ovicide activity. Larvicide activity is by ingestion; the primary and most visible mode of action is at ecdysis, when treated larvae fail to moult to the next instar resulting in death. The fertility of treated adults may also be reduced. Hexaflumuron is active on a broad range of insect pests especially caterpillars, beetles, flies and a few sucking pests including psylla and whiteflies. Products containing hexaflumuron are used on apples, pears, potatoes, vegetables, cotton and in forestry. Combined with this excellent activity against target pests, hexaflumuron has been shown to be safe to a wide range of important beneficial arthropods. Results of a recent large scale apple orchard study (K. Brown et. al., 1992) showed that hexaflumuron, formulated as CONSULT (100g A.I./litre) at its recommended field rate, was safe to predatory bugs, ladybirds, spiders, earwigs, parasitic wasps and to predatory hoverfly larvae.

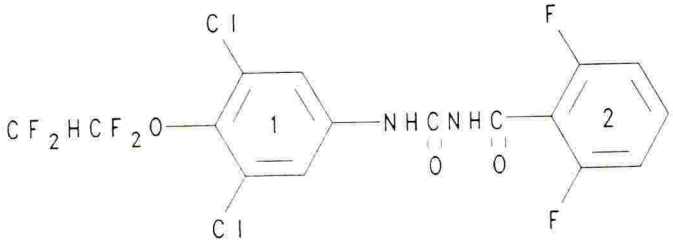
Hexaflumuron is of low mammalian, avian and fish toxicity (DowElanco internal reports). Mammalian toxicity tests on rats, mice and dogs showed the lowest NAOEL (no adverse observed effect level) of 2mg/kg body weight/day. Hexaflumuron toxicity to bird species was tested in acute tests where the NOEL (no observed effect level) was >2g/Kg and in dietary tests with the obtained LC50 of >5g/Kg of food. Fish toxicity testing showed no effect upto and exceeding the maximum solubility in water (30μg/litre). The effects on aquatic crustacea, however, can be significant, but since it is rapidly metabolised and eliminated the resultant risks are minimal. At 10 times application rate hexaflumuron had no effect on soil microorganisms active in soil respiration or N-mineralisation. Hexaflumuron has a very low v.p. of 5.87×10^{-9} Pa @ 298K and will thus not readily volatilise, the potential for exposure by this route is therefore minimal.

Results of the plant metabolism and environmental fate investigations are described in this paper. The environmental fate studies were conducted in accordance with U.S. EPA guidelines (Hitch, 1982).

MATERIALS AND METHODS

These studies were carried out using hexaflumuron uniformly labelled with carbon-14 (Figure 1) in either (1) the aniline ring (three batches with specific activities in the range 10.02 - 16.75uCi/mmole, purities > 99%); or (2) the benzoyl ring (three batches, specific activities 10.78 - 14.28uCi/mmole, purities >99%). Non-labelled reference samples of hexaflumuron plus the 3,5-dichloro-4-(1,1,2,2-tetrafluoroethoxy)aniline, 3,5-dichloro-4-(1,12,2-tetrafluoroethoxy)phenyl urea, 2,6-difluorobenzoic acid, 2,6-difluorobenzamide and 3,5-dichloro-4-hydroxyaniline (degradation products) were also used.

FIGURE 1. Structure of hexaflumuron showing position of ^{14}C-label



Plant metabolism

To investigate the metabolism of hexaflumuron in plants, apples, cabbage and cotton were chosen to represent each major target crop. Both aniline and benzoyl forms of ^{14}C-labelled hexaflumuron were applied to the crops according to the details given in Table 1. Samples were taken for analysis at the time of application and at harvest (crop maturity). Plant material was also collected at intermediate timepoints if treated leaves or fruit fell from the plants.

Parts of the plants were left untreated so that these areas or new growth could be analysed to give an indication of the ability of hexaflumuron to translocate within the plant. In the apple study, treated apples collected at harvest were subjected to a solvent wash with dichloromethane (DCM) prior to homogenisation so that an estimate of the degree of penetration of hexaflumuron into the apple tissue could be made.

TABLE 1. Application conditions for plant metabolism experiments

Crop	Application stage	Application rate (gAI/ha)
Apples	1. 3 weeks post blossom	100
	2. 6 weeks post blossom	100
Cabbage	7 - 8 leaf	60
Cotton	10 - 12 leaf	50

Soil degradation and mobility studies

Castle Rising sandy clay loam was weighed (50g oven dry equivalent) into one side of a biometer flask. The moisture content of the soil was adjusted to that required (50, 75, or 100% of MHC at 0.33bar) with the addition of distilled water. [^{14}C-aniline]Hexaflumuron was added to the soil at a rate of 1mg/kg and well mixed. The other side of the biometer flask was charged with 0.1M NaOH solution (100ml) which served as a CO_2 trap. The biometer flasks were then incubated in the dark at 25°C for the soils adjusted to 50, 75, and 100% 0.33bar; and at 10°C, and 35°C for further samples adjusted to 75% 0.33bar. The CO_2 traps were changed at regular intervals and monitored using liquid scintillation (LS) counting. Biometer flasks were taken for analysis 0, 14, 28, 56 and 84 days after application. In a further experiment, four soils (see Table 2) treated at the same rate and incubated at 75% 0.33bar and 25°C for 30 days were subsequently flooded with distilled water, amended with powdered straw and flushed with N_2 to promote anaerobic conditions. These flasks were then sealed and incubated for a further 30 or 60 days. The aerobic soil samples were extracted three times with acetonitrile/water (1:1) which was subsequently partitioned with DCM. The anaerobic samples were extracted three times with acetonitrile/acetone (1:1). The radioactivity in the extracts was determined by LS counting and those extracts with greater than 10% of the applied radioactivity were analysed by thin layer chromatography (tlc) with authentic reference standards.

Leaching and desorption experiments were conducted in 4 soils (Table 2) with both labelled forms of hexaflumuron at rates equivalent to 150g AI/ha. The leaching behaviour was investigated in soil columns (5cm i.d. x 30 cm long), saturated with 0.1M $CaCl_2$, treated with labelled hexaflumuron and eluted with 1 litre of 0.1M $CaCl_2$ (equivalent to 20 inches of rainfall). Leachate was collected and analysed by LS counting. Radioactivity in the soils were determined by combustion. The top 2 segments of each soil column were then extracted with acetone, portions of which were concentrated and analysed by tlc. The desorption behaviour of soil residues of hexaflumuron was investigated after ageing for 30 days

under aerobic conditions in the dark in biometer flasks. Portions of soil (50g, dry weight equivalent) were extracted with 0.1M $CaCl_2$ followed by acetone and 0.5M NaOH. Portions of the $CaCl_2$, acetone and NaOH extracts were partitioned with DCM (after acidification). The DCM solutions were analysed by tlc.

TABLE 2. Soils used for degradation and mobility experiments

Soil	Type	%o.m.	pH	cec
Catlin (USA)	silty loam	2.7	6.6	17.8
Hanford (USA)	sandy loam	1.1	7.5	4.9
Castle Rising (UK)	sandy loam	14.9	7.9	39.1
Alconbury (UK)	clay loam	3.2	8.1	21.6

cation exchange capacity (cec) is in units of mEq/100g soil

Aqueous hydrolysis and photolysis studies.

The hydrolysis of hexaflumuron was investigated in sterile buffered distilled water (pH 5.0, 7.0 and 9.0) and in a natural water sample (nominally pH 8) at 25°C over 33 days in the dark. Flasks were treated with labelled hexaflumuron at a nominal concentration of 15-20μg/l and were removed for analysis approximately 0,1,3,7,14,21, and 33 days after treatment. Samples were analysed by direct LS counting and then partitioning with DCM, concentration of the residue and tlc analysis. The photolysis was conducted in sterile aqueous pH 5.0 buffer with samples irradiated for the equivalent of 33 days of natural sunlight using a xenon-arc light source filtered to remove wavelengths less than 290 nm.

RESULTS AND DISUSSION

Analysis of harvest samples from apples, cabbage and cotton experiments showed average recoveries from 80.4 to 103.4%. Greater than 99% of the radioactivity recovered was identified as unchanged hexaflumuron. No degradation products were observed and <5% of the applied radioactivity was found to be associated with plant tissue after extraction.

All untreated plant tissue analysed showed only trace amounts of radioactivity (<1% of applied). Thus it can be concluded that hexaflumuron does not translocate within the plant.

In the apple metabolism study the majority of the radioactivity recovered was found in the dichloromethane wash indicating that hexaflumuron does not penetrate into the pith.

Levels of radioactivity in the $^{14}CO_2$ traps from the aerobic soil degradation experiment were very low, with a total of only 0.7 - 2.1% of the applied activity observed 84 days after treatment with [^{14}C-aniline]hexaflumuron. However, previous investigations using benzoyl - ^{14}C-labelled hexaflumuron have shown greater amounts of $^{14}CO_2$

suggesting that this part of the molecule is more readily degraded to carbon dioxide. Tlc of the extracts of the soil samples incubated under aerobic conditions showed the presence of two metabolites, 3,5-dichloro-4-(1,1,2,2 - tetrafluoroethoxy)aniline (M1) and 3,5 - dichloro -4- (1,1,2,2 - tetrafluoroethoxy)phenylurea (M2) in addition to parent. Levels of both metabolites generally increased with incubation time, and the greatest levels were observed at the higher temperatures and moisture contents. The half-lives of [^{14}C-aniline]hexaflumuron under the different temperature and moisture regimes are presented in Table 3. Temperature has a marked effect on half-life. For samples with a moisture content of 75% of 0.33bar the half-life was 190 days at 10°C compared to 56 days at 35°C. Moisture content also influenced the half-life of hexaflumuron with an increase from 64 days to 159 days observed in samples incubated at 25°C as the moisture content decreased from 100% 0.33bar to 50% 0.33bar respectively.

TABLE 3. Half-life of [^{14}C-aniline]hexaflumuron in Castle Rising soil under different temperature and moisture regimes.

Temperature (°C)	Moisture (% of 0.33bar)	Half-life (days)
25	50	159
25	75	90
25	100	64
10	75	190
25	75	90
35	75	56

Analysis of the anaerobic soils showed the presence of M1 as the major metabolite (8 - 18% of applied activity), with an unknown metabolite of greater polarity than any of the reference standards observed at levels up to 5% of applied activity. Half-lives of [^{14}C-aniline]hexaflumuron at 25°C under anaerobic conditions were in the range 40 - 64 days (Catlin 64d, Hanford 54d, Castle Rising 54d and Alconbury 40d).

The results of the leaching experiment showed that hexaflumuron did not leach in any of the soils despite the extreme "rainfall" conditions indicating that it is very strongly sorbed to soil. In the desorption experiment, hexaflumuron was the major component seen in all samples but small amounts of M1 and M2 metabolites were observed along with traces of the 2,6-difluorobenzoic acid (M3). Desorption constants (Kd, units ml/g) were calculated for the 4 components as follows: hexaflumuron Kd = 147 - 1326, M1 Kd = 35 - 392, M2 Kd = 32 - 142 and M3 Kd = 3 - 27. These results confirm that hexaflumuron is strongly sorbed and also indicate that the 2 major metabolites, M1 and M2, will have only a limited tendancy to move in soil solution. The component M3 will be more likely to move but was only detected in trace amounts so it will not be of significance.

The hydrolysis experiment showed that hexaflumuron is hydrolytically stable in sterile buffers at pH 5.0, is slowly degraded at pH 7.0 (6% breakdown in 33 d) and is more rapidly degraded at pH 9.0 (ca. 60% breakdown in 33 days). Hydrolysis at pH 9.0 proceeds by 2 separate routes, with the more facile producing M2 and M3 and the slower producing M1 and the 2,6-difluorobenzamide (M4). The overall dissappearance of hexaflumuron was a first order process with a half-life of 22 days. The hydrolysis of hexaflumuron in natural water was also a first order process with a mean half-life of 42.3 days and resulted in the formation of M3 and two unknown products, one of which was tentatively identified as M2. The aqueous photolysis of hexaflumuron resulted in M4, 3,5 - dichloro -4- hydroxyaniline (M5) and CO_2 as the major degradation products. The photodegradation was a first order process with a half-life of approximately 6.3 days.

CONCLUSIONS

Plant metabolism studies on apples, cabbage and cotton, showed that hexaflumuron is stable and does not translocate within the plant or penetrate into the fruit tissue. Under normal use conditions plant residues of hexaflumuron had no adverse effect on beneficial insects such as hoverflies, ladybirds and bees.

The degradation and leaching behaviour of hexaflumuron in soil will control its environmental behaviour. Despite the slow degradation rate of hexaflumuron in soil, soil residues have been shown not to effect beneficial soil organisms. Allied with low mobility and very low water solubility, it will not leach into surface or ground waters under normal use conditions so will have no effect on aquatic organisms. Any hexaflumuron entering surface waters (overspray or run-off) will be removed by rapid adsorption to suspended soil particles plus aqueous photolytic degradation. When these properties are considered together with its lack of toxicity on non-target organisms, it can be concluded that, under normal agricultural practises hexaflumuron is unlikely to have an adverse effect on the environment.

ACKNOWLEDGEMENTS

The authors would like to thank Drs. Hawkins, Mayo and McEwan at the Huntingdon Research Centre who conducted the aqueous photolysis and anaerobic soil studies described in this paper. Thanks are also due to Dr. Beiber of the Natec Institute, Germany for conducting the cabbage metabolism study.

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THE METABOLISM OF CGA 173506 IN THE RAT

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ABSTRACT

Independent of sex and dose the experimental phenylpyrrole fungicide CGA 173506 was well and rapidly absorbed from the gastrointestinal tract into the general circulation and rapidly and almost completely eliminated, mostly in the faeces. As a consequence the residual radioactivity in the tissues was low.

The dominating metabolic pathway of CGA 173506 was oxidation of the pyrrole ring at two positions. A minor pathway was hydroxylation of the phenyl ring. All the metabolites were excreted as conjugates, mainly as glucuronides.

Blue-violet colouration of urine was observed after long term administration of CGA 173506. It was detectable only at the 1000 and 3000 mg/kg diet feeding levels during a chronic toxicity/ oncogenicity study. The colouration was due to a dimeric compound formed by oxidation of CGA 173506. At the higher feeding level (3000 mg/kg) and at steady state it accounted for about 1% of the daily intake of CGA 173506 in male rats.

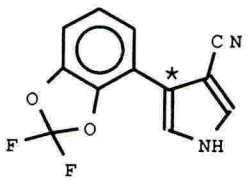
INTRODUCTION

CGA 173506 is a new broad spectrum fungicide being developed for both foliar and seed treatment. It is foreseen as having potential in a broad range of crops such as cereals, rice, fruits, and vegetables.

During toxicology studies it had been noticed that this substance caused blue-violet colouration of urine and tissues. The purpose of the studies described here was to investigate the fate of CGA 173506 including the extent and nature of urine colouration in order to aid in the evaluation of test results from toxicology studies.

FIGURE 1: Chemical name, structure and site of labeling of CGA 173506

4-(2,2-difluoro-1,3-benzdioxol-4-yl)-1H-pyrrole-3-carbonitrile



* = [^{14}C]

MATERIAL AND METHODS

Male and female rats were dosed at 0.5 and 100 mg/kg body weight with single oral doses of [4-^{14}C]pyrrole CGA 173506. Radioactivity was determined by liquid scintillation counting. Metabolites from bile, and urine were isolated by chromatographic means (tlc and hplc) and their structures elucidated by ^{1}H-nmr spectrometry and ms. Unchanged CGA 173506 present in faeces was identified by co-chromatography.

The time dependent extent of urine colouration was determined by measuring the absorbance at 595 nm in the urine of male and female rats from a chronic toxicity/oncogenicity study. The coloured metabolite was isolated from the urine of male rats of a satellite group (3000 mg/kg diet feeding level) after oral dosing with radiolabeled test substance.

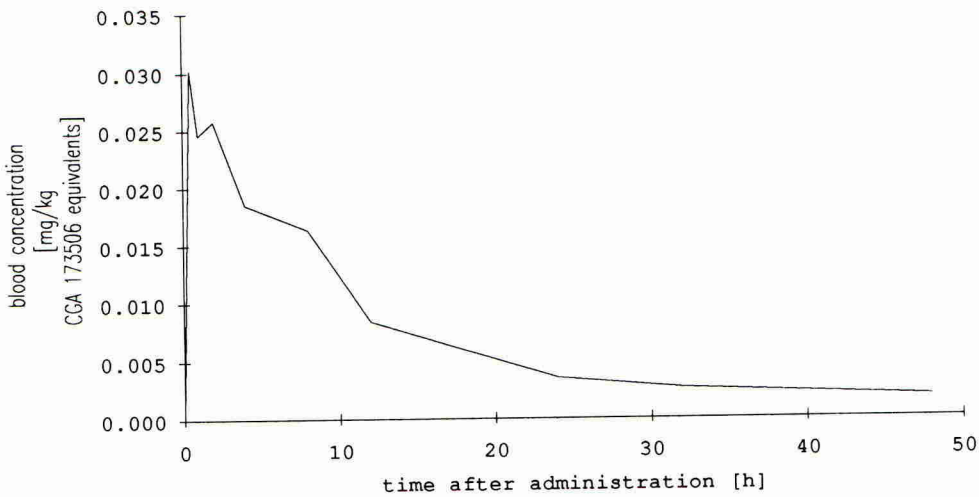
RESULTS AND DISCUSSION

Toxicokinetics

Blood kinetics

The results showed a fast absorption rate of the orally administered test substance. The maximum blood level was reached half an hour after administration. Rapid clearance of radioactivity from blood was observed.

FIGURE 2: Blood kinetics after single oral administration of approximately 0.5 mg/kg body weight to female rats



Excretion

At least 75% of an orally administered dose was absorbed from the gastrointestinal tract into the general circulation by estimation from the amount of radioactivity biliary and renally excreted. Rapid elimination was indicated by the fact that more than 90% of the dose was excreted within 48 hours, mostly in the faeces. Less than 0.01% of the dose was detected in the expired carbon dioxide.

TABLE 1: Excretion of radioactivity from rats after a single oral administration of CGA 173506 (% of dose)

Dose	[mg/kg]	0.5	100	100[1]
Urine	0 - 24 h	15.8	16.7	4.9
	24 - 48 h	0.6	0.9	5.2
	48 -168 h	0.2	0.6	---
	Subtotal	***16.6***	***18.2***	***10.0***
Bile	0 - 24 h	---	---	42.3
	24 - 48 h	---	---	25.2
	Subtotal	---	---	***67.5***
Faeces	0 - 24 h	69.7	63.9	6.0
	24 - 48 h	9.6	12.5	8.3
	48 -168 h	0.9	1.2	---
	Subtotal	***80.1***	***77.6***	***14.3***
Total Excretion	0 - 48 h	95.6	94.0	91.8
	0 -168 h	96.7	95.8	---

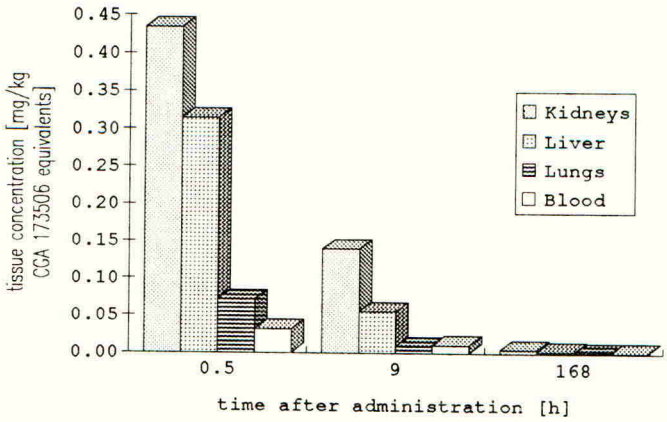
[1] administration to bile-duct cannulated rats

Within 7 days after administration the test substance was almost completely excreted, resulting in low tissue residues.

Tissue residues

After single oral administration of approximately 0.5 mg/kg body weight to female rats, tissue residues 0.5 hours after administration (time of maximum blood concentration) were below 0.45 mg/kg CGA 173506 equivalents and declined very fast. After 7 days all tissue residues were below 0.005 mg/kg.

FIGURE 3: Depletion of radioactivity from selected tissues after single oral administration of approximately 0.5 mg/kg body weight to female rats



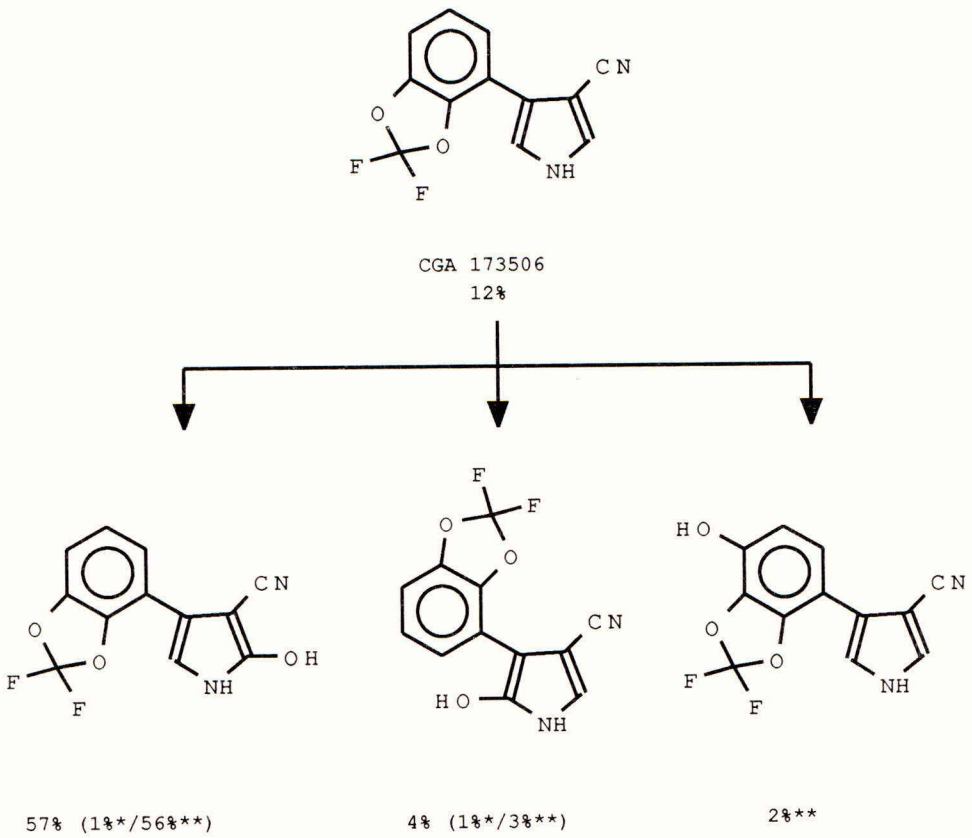
Metabolic pathways

Based on the structures of the metabolites isolated from bile and urine, and unchanged CGA 173506 present in faeces the following metabolic pathways were derived:

- oxidation of the pyrrole ring
- hydroxylation of the phenyl ring

These phase I metabolites were excreted as glucuronide and, to a lesser extent, sulphate conjugates.

FIGURE 4: Proposed metabolic pathways of CGA 173506 in the rat (% of dose after administration of approximately 100 mg/kg body weight to bile-cannulated female rats)



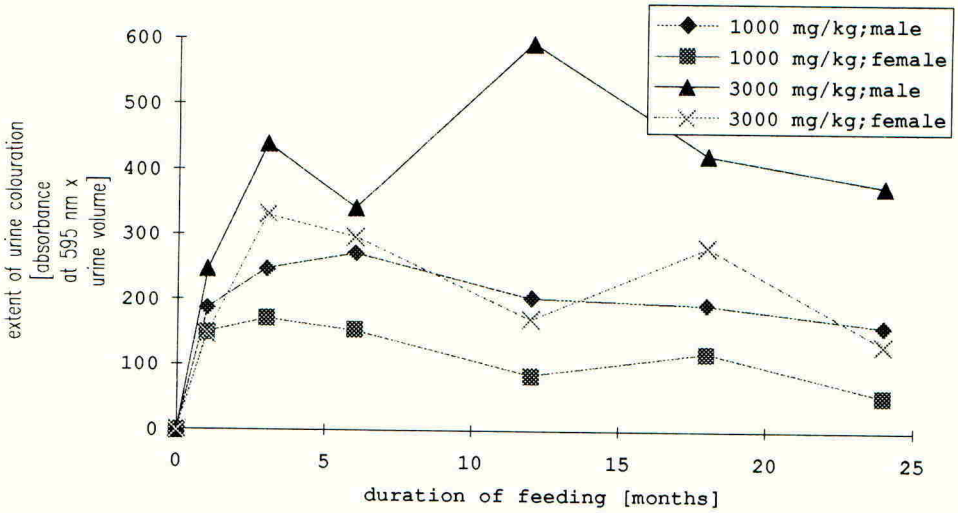
57% (1%*/56%**) 4% (1%*/3%**) 2%**

* excreted as sulphate conjugate
** excreted as glucuronide conjugate

Extent and nature of urine colouration

Urine colouration was detectable at the 1000 and 3000 mg/kg diet feeding level in a chronic toxicity/oncogenicity study. The extent was clearly dose dependent and possibly sex dependent, with lower amounts found in female rats. A plateau was reached after about 3 months. At steady state the coloured urine metabolite accounted for approximately 1% of a daily dose at the 3000 mg/kg diet feeding level in male rats.

FIGURE 5: Extent of colouration of urine from male and female rats at the 1000 and 3000 mg/kg diet feeding level

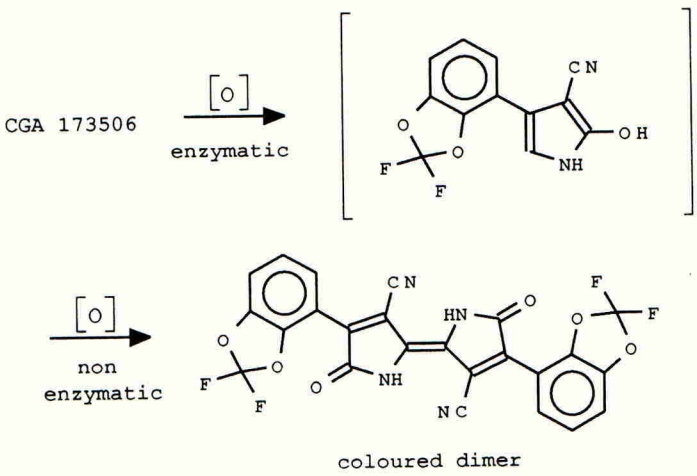


The coloured metabolite showed a maximum absorption at about 595 nm. It was isolated and the structure investigated by ms, ^{1}H-nmr, and i.r. spectrometry.

The nmr spectra showed a group of aromatic protons indicating an intact phenyl ring. The i.r. spectrum showed the presence of cyano and carbonyl groups. Accurate mass determination by high resolution ms was in agreement with a dimeric compound (Figure 6) formed by oxidation of CGA 173506 [note: the product was identified as an artifact (m/z = M + 14) which is assumed to be due to the addition of methanol followed by dehydration or due to the methylation of the pyrrole nitrogen].

The same compound could be generated from the major metabolite by treatment with β-glucuronidase.

FIGURE 6: Generation and proposed structure of the coloured urine metabolite



coloured dimer

CONCLUSION

CGA 173506 was rapidly and well absorbed (at least 75% of the administered dose) from the gastrointestinal tract into the general circulation and rapidly and almost completely eliminated. The main route of excretion was the faeces. The residual radioactivity in the tissues was low, even at the time point of maximum concentration in the whole blood. No sex or dose dependent changes could be observed.

The dominating metabolic pathway of CGA 173506 was oxidation at position 2 of the pyrrole ring. Two minor pathways were oxidation of the pyrrole ring at position 5 and hydroxylation of the phenyl ring. All of these metabolites were excreted as conjugates, mainly as glucuronides.

Urine colouration was detectable only at the 1000 and 3000 mg/kg diet feeding levels during a chronic toxicity/oncogenicity study, which was far beyond expected exposure. The colouration was due to a dimeric compound formed by oxidation of CGA 173506. At the higher feeding level (3000 mg/kg) and at steady state it accounted for about 1% of the daily intake of CGA 173506 in male rats.

ACKNOWLEDGMENTS

The skilled technical assistance of Mrs. M. Imhof and Mr. J.-L. Bohn and the cooperation of Mr. J.C. Ritter, Dr. G.D. Fisher, and Dr. J.C.F. Chang from *CIBA-GEIGY Ltd.*, Farmington are gratefully acknowledged.