5.
Effects on Aquatic Ecosystems

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RECENT ATTEMPTS TO MEASURE THE BEHAVIOUR AND IMPACT OF PESTICIDES IN AQUATIC ENVIRONMENTS

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ABSTRACT

Recent surveys have shown that U.K. freshwaters are widely contaminated with triazine herbicides, and more locally with pyrethroids and organotin compounds. Furthermore, the organotins and other pesticides used in timber preservatives have caused large fish kills following spills. These examples highlight two central questions; 1) What exposure to a new pesticide will aquatic organisms receive?, 2) How harmful will the product be to aquatic populations? This paper describes attempts to solve these problems at the Fisheries Laboratory.

For the approval of agricultural pesticides, the environmental exposure is initially estimated from 'worst-case' scenarios, for example accidental overspray of a shallow waterbody. Although tempered by physicochemical and degradation rate data, this 'predicted environmental concentration' (PEC) is usually a gross over-estimate. To make the PEC more realistic, a model developed by the Building Research Establishment is being field validated. It uses physicochemical data and degradation rates to predict residue concentrations to be expected in fields and associated water bodies. The validation includes both monitoring pesticide inputs to a small water-catchment and measuring subsequent residues in various compartments, including the outflowing stream.

It is widely believed that ecosystem-level effects cannot be predicted from single-species laboratory data on, for example, life-cycle and growth rate responses. Recent work in the USA has shown that single test-species are not reliable surrogates for all related taxa, although their sensitivity to some pollutants can be used to predict certain ecological effects. A more specific approach is to study toxicant effects on multi-species systems such as laboratory 'microcosms' or pond-scale 'mesocosms'. We are studying the effects of dissolved tributyltin (TBT) on fauna in small freshwater mesocosms, and of particulate TBT on faunal recolonisation rates in marine sediments. The observed effects are being related to the results of sensitive laboratory tests to assess whether these adequately predict ecosystem-level effects.

Early results suggest that the mesocosm method is probably no more sensitive than laboratory tests, while the recolonisation work has shown that at least two groups (polychaetes and crustacea) appear more susceptible than expected from laboratory exposures to TBT in the absence of sediment. This and other studies raise the question of the potential toxicity to benthic in-fauna of pesticides adsorbed onto sediments, an area which is now receiving greater attention.

INTRODUCTION

The EC 'Drinking Water' Directive (EEC, 1975) enforced in 1986 limits the total pesticide concentration permitted in category A1 waters to 1 µg l^{-1} in order to protect human consumers. Irrespective of the scientific justifications for this limit, its promulgation by the EC provoked studies of pesticide concentrations in European surface waters. In the UK, for example, the Anglian Water Authority (Croll, 1986) found widespread surface-water contamination with phenoxyalkanoic acid herbicides (up to 2.7 µg l^{-1}) and triazine herbicides (up to 1.4 µg l^{-1}) in their region. A more recent study (Department of the Environment, 1987) of approximately 70 rivers and 10 estuaries throughout the UK found low, localised concentrations of synthetic pyrethroid insecticides (maximum pyrethroid total = 110 ng l^{-1} for 10 compounds), whereas triazines were found in ca 70% of the samples (maximum triazine total = 2.0 µg l^{-1} for 5 compounds). Similar widespread contamination by water-soluble pesticides has been found in the USA (Wauchope, 1978; Frank et al., 1982; Spencer et al., 1985). However, the common water-soluble herbicides are not particularly toxic to aquatic fauna (96h LC50 values >1 mg l^{-1}), although algal growth may be reduced at lower concentrations, and degradation in natural waters may be slow. The distribution and effects of pesticides in freshwater sediments are less well-known, although some highly adsorbed insecticides such as the pyrethroids may persist in sediment and affect burrowing invertebrates (Friesen et al., 1983). Pesticides in our surface waters (and ground waters) are, therefore, a major problem for Water Authorities in the context of the 'Drinking Water' Directive. Whether aquatic life is at risk from pesticides at these concentrations is less clear but is a question that needs to be addressed.

Pesticides are mainly used by farmers, although some (e.g. triazine herbicides) are used in non-agricultural areas; others (e.g. synthetic pyrethroids and some organophosphates) can occur in effluents from textile mills and pesticide manufacturing plants, and some (e.g. pentachlorophenol, dieldrin, tributyl tin) may be spilled into waterways from timber-treatment plants. Marine ecosystems may be contaminated from antifouling paint usage (e.g. tributyl tin) and from riverine inputs. Farm water-pollution incidents, although numerous (3510 in 1985 in England and Wales - Anon, 1986) comprise only 17% of pollution incidents recorded by Water Authorities. Only about 2% of these (ca 70 per annum) are attributed to pesticides and very few appear to result from approved agricultural practice. Certainly, the national River Quality Survey (Department of the Environment, 1986) did not ascribe the measured declines in water quality to pesticides. Most pesticide-related fish kills are undoubtedly caused by accidental spills, but the possibility remains that some subtle effects (such as gradually declining fisheries) may be related to low-level pesticide contamination. There is, thus, a continuing need for improved techniques to study and predict the behaviour of pesticides in agricultural ecosystems, and for measuring their impact on aquatic organisms under field conditions.

As Fisheries Advisers to the UK pesticide registration scheme, this laboratory has a commitment to develop, inter alia, improved field techniques and this paper describes some methods with which we are currently involved. Section 1 deals with measurement of pesticide distribution in fields and an adjacent stream, and development of a model which predicts environmental concentrations using physicochemical and degradation rate data. Section 2 describes an investigation of the utility of small pond mesocosms for hazard assessment in contrast to the more commonly used

laboratory-based tests. The final section concerns a sediment bioassay technique which can be used for studying the impact of pesticide-contaminated sediments on benthic fauna.

1. PESTICIDE BEHAVIOUR IN AGRICULTURAL ECOSYSTEMS

Assessment of pesticide hazards for aquatic life requires two items of information; a predicted environmental concentration (PEC) and an estimate of the 'safe' concentration for fish, crustacea and algae. At this laboratory, we currently derive the PEC by calculating the concentration in a 1m deep waterbody resulting from an accidental overspray at the maximum recommended application rate. If leaching from soils is a possibility, we also calculate the maximum possible concentration in drainage water derived from a heavy rainstorm. Although the PEC is tempered by knowledge of degradation rates, it is obviously a worst case estimate, and we cannot yet make rigorous use of the notified physico-chemical data (aqueous solubility, octanol-water partition coefficient (Kow), vapour pressure, soil organic-carbon partition coefficient (Koc), and degradation rates in soil and water) in order to refine the calculation of the PEC.

Many models for predicting water pollution from non-point sources have been developed (Haith, 1980; Donigan *et al.*, 1977; Burns *et al.*, 1982; Knisel, 1982). They require complex data inputs, were constructed primarily for use in large American agro-ecosystems, and have not been validated for use under our considerably different UK conditions. Another widely-used model is based on the concept of fugacity, or the 'escaping tendency' of a chemical from one environmental compartment to another (Mackay *et al.*, 1985). This uses a minimum of input data based on physico-chemical coefficients and degradation rates of the relevant pesticide or other chemical. A further advantage is that this model has been modified (D. Brooke, UK Building Research Establishment (BRE), pers. comm.) to operate *inter alia* using the dimensions of a 'typical' English arable field adjacent to a small stream (the FIELD model). It can calculate the chemical's distribution in this system soon after application, and its concentrations in soil, soil biota, soil water, air, stream water, stream sediment and stream biota.

In collaboration with BRE, we are validating the FIELD model by measuring the behaviour of a range of pesticides applied to two fields on the MAFF Rosemaund Experimental Husbandry Farm (EHF) in Herefordshire. Rosemaund EHF is possibly unique in that the farm boundary almost completely encloses a small water catchment area where run-off is being monitored by a joint Institute of Hydrology/Welsh Water Authority (IH/WWA) study (Williams & Bird, 1987). The direct chemical inputs to the whole area are therefore well-known, and we have access to continuously-recorded hydrological and meteorological data.

The 17.5 ha area at Rosemaund used for our experiments (Fig. 1) is drained by plastic pipes at 1m depth which empty into the stream at sites D and C. The site is at an altitude of 90-100m, and consists of gently sloping silty clay-loam of the Bromyard series. The crops in 1987/88 consisted of 4.5 ha of winter wheat, 5.5 ha of winter barley and 6.0 ha of rye grass. A small stream originating as surface soil drainage flows in a southwesterly direction, joining the River Lugg after about 10 km.

The preliminary experiment reported here involves the herbicide mecoprop (2-[4-chloro-2-methyl phenoxy] propionic acid) which is prone to leaching from many soils and could be expected to appear in the stream in measurable amounts. Over the next three years, it is intended to study approximately 10 further pesticide groups in a similar manner.

Following a mecoprop application (2.05 kg a.i. ha^{-1}) to 5.5 ha of winter barley on 17/11/87 (see Fig. 1), mecoprop residues were measured 6 times in the following 4 weeks in surface soil (4 sites), soil drainage water (Sites C and D), stream sediment (Sites A-D), stream water (Sites A and B) and caged fish (Gymnocephalus cernua) (Site A). In addition, 22 hourly water samples were taken automatically by a rainfall-actuated vacuum sampler at site B during and after a 25mm rainstorm on 19/11/87.

The mean mecoprop level in the surface 1m of soil dropped by 47% between 18 and 20/11/87, and was below the detection limit (0.5 µg kg^{-1} wet wt.) by the end of the experiment (D. Brooke, pers. comm.). Mecoprop levels in the stream on 19/11/87 peaked within 10 h of rain onset (Fig. 2), and declined below the detection limit (0.1 µg l^{-1}) within 24 h. Simultaneous pH and conductivity measurements showed that sampling commenced at the time when floodwater first reached site B. Samples taken on 20/11/87 revealed

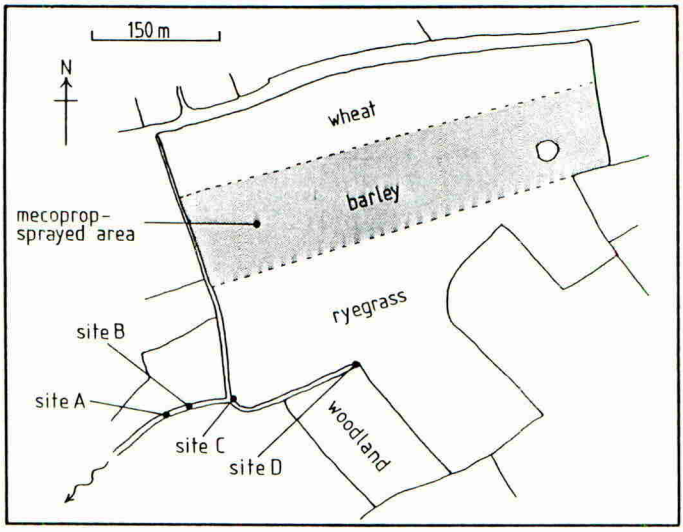

Fig. 1 Field site at Rosemaund Experimental Husbandry Farm

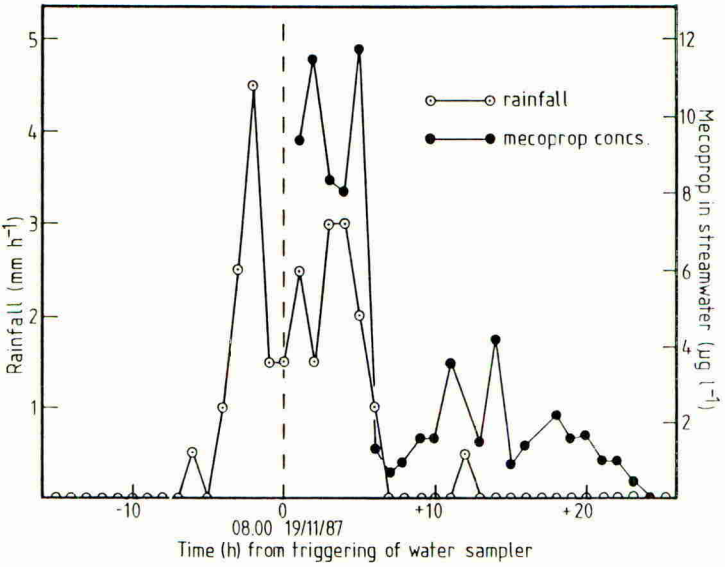

Fig. 2 Mecoprop concentrations in stream water at Site B, and rainfall on 19 Nov. 1987

low concentrations (<0.1-1.8 µg l^{-1}) of mecoprop in drain and streamwater, but subsequently no more was detected and only negligible amounts were present at any time in sediments and fish. Rainfall intensity during this period was <5.0 mm day^{-1} (mean = 1.5 mm day^{-1}).

Calculations based on estimated flowrates at site A show that <1% of the total applied mecoprop appeared in the stream during the experiment, of which >95% entered the stream within 30 h of rainfall commencement. As little biodegradation, volatilisation or co-distillation would be expected within this period, it is assumed that most of the mecoprop migrated to below the 1m soil horizon. The current version of the model, which includes degradation parameters, underestimated the rate of mecoprop removal from surface soil and overestimated its appearance in the stream. This is probably due in part to the assumed loss of mecoprop to deeper soil layers, but further development of the model is clearly needed.

2. UTILITY OF SMALL POND MESOCOSMS FOR PESTICIDE HAZARD ASSESSMENT

Pesticide hazard assessment in water requires information on the predicted environmental concentration (the PEC) and on the 'safe' or no effect concentration for aquatic life (NOEC) to establish if there is a significant safety margin between them. In practice, the NOEC is estimated by extrapolating from acute toxicity data, although wide error bands can result (Suter et al., 1985). For many pesticides, however, the safety margin is so large that these uncertainties do not invalidate the hazard assessment. Accurate hazard predictions may be more difficult if the safety margin is small or non-existent. The usual response to this situation (Crossland, 1988) is to do further tests, using additional species and more sensitive end-points (e.g. effects on growth and reproduction). This narrows the confidence interval of the predicted NOEC, thus giving a more reliable assessment, but whether the laboratory-derived NOEC is similar to the 'true' NOEC for natural ecosystems is questionable.

It was originally accepted that pesticide impact studies on aquatic communities would be more sensitive than single-species laboratory tests. Some early work (e.g. Hurlbert et al., 1972) using replicated small ponds did indeed show that direct impacts on one species might have many indirect effects on others. The induction of phytoplankton blooms and consequent asphyxia of fish following the insecticidal elimination of crustacean grazers (Hurlbert, 1975; Crossland, 1984) is one example. Clearly, single-species tests could never reveal such effects. However, a major difficulty with using complex pond or stream systems (e.g. Solbé, 1988) lies in their largely unavoidable variability resulting from poorly-controlled water quality factors and dissimilar starting conditions. Furthermore, multispecies tests often do not provide increased sensitivity because processes such as selection for resistance, rapid biodegradation, and general background 'noise' (produced by such variables as the trophic status of the contributing species) reduce the impact of the pollutant (Slooff, 1985; Kooijman, 1985). Also, most ecosystems have a considerable redundancy which damps out the functional impact of losing a few species (Perry et al., 1987), an effect which ameliorates the impact of actual pollution incidents. Lastly, the enormous expense and practical difficulty of tests with complex experimental ecosystems, allied to the absence of well-defined end-points, precludes their use for regulatory purposes.

There is, nevertheless, still a need to show that single-species tests can forecast the environmental NOEC. Successful forecasts of this type have been published (Cairns & Cherry, 1983; Crossland & Wolff, 1985; Larsen et al., 1986) and are implied in many literature reviews for the setting of

water quality standards. One approach has been to use replicated enclosures of natural lentic ecosystems in order to minimise initial variability and simplify sampling (reviewed in EIFAC, 1983). Although Sanders (1985) suggested that such enclosures should be large (> 1-2000 m^3 in eutrophic systems) so as to minimise sidewall effects, much smaller enclosures (1 m^3) have been used successfully (Stephenson & Kane, 1984) to demonstrate secondary ecological perturbations during 50 days after treatment with methyl parathion and linuron. Small enclosures have also been used to show that short-term exposures of fish to cypermethrin give less-than-predicted mortality due to adsorption of the pesticide by particulates (Shires, 1983).

It is acknowledged that use of small enclosures to validate the predictions of laboratory tests shows considerable promise. In 1987, we began to develop a system based on 16 enclosures in a concrete pond at the Langford Treatment Works of the Essex Water Company. In April 1987, the pond was lined to a depth of 30-60 cm with settling pond sediment, filled to 1 m with water from the River Chelmer, seeded with fresh pond sediment, and matured for 10 days. The enclosures are open-ended 2 m cubes made from 'Stokbord' reconstituted plastic sheet (12 mm thick) joined by aluminium alloy edge sections. Initial surveys confirmed that the zooplankton populations were developing well and the enclosures were then placed in the pond at the end of April, pushing them firmly into the substrate. Seventeen sticklebacks (Gasterosteus aculeatus; 6 ♂ 11 ♀) in breeding condition were then added to each enclosure as a top zooplankton predator.

In 1987, we investigated the effects of 2 concentrations of tributyltin oxide (TBT) applied as a solution in acetone at weekly intervals for 20 weeks from mid-June. TBT was chosen both because there is already a considerable body of ecotoxicological data (Waldock et al., 1987a), and its presence in some freshwaters (Waldock et al., 1987b) has generated interest in its impact on freshwater ecosystems. Zooplankton population structure was examined 5 times during the treatment period. Colonisation of passive invertebrate samplers, and growth and reproductive success of the fish were also studied. The main aim has been to define optimum sampling strategies to reveal significant effects on major groups (e.g. fish), but insufficient data are yet available from which to draw firm conclusions. So far the most important lesson is that simple experiments of this type are nevertheless expensive and difficult to maintain and evaluate, reinforcing the view that they are currently unsuitable for routine regulatory purposes. A simplification of the criteria for 'damage' is required.

3. IMPACT OF CONTAMINATED SEDIMENT ON COLONISING ORGANISMS

One way of minimising variability in multispecies tests is to study recolonisation of contaminated systems from which all organisms have been removed, so ensuring that starting conditions are virtually identical in all treatments. This approach is particularly appropriate for studies of aquatic sediments in which animals can be killed in advance by freezing. The contaminated, invertebrate-free sediment is exposed in open-topped boxes to colonisation by aquatic biota in either true field situations, or in the laboratory using an unfiltered water supply from a nearby natural waterbody. The latter approach, although not universally successful, has been used effectively for the study of drilling muds (Blackman et al., 1988 a & b), while the former has been pioneered by Tagatz (Tagatz & Deans, 1983) and others (Arnoux et al., 1985). Tagatz et al. (1987), for example, showed that fenvalerate reduces the number of macrofaunal species colonising sand-filled boxes placed sub-tidally in an estuary. One limitation of this technique, which uses solid-sided exposure boxes, is that it primarily

involves colonisation by planktonic dispersal stages, excluding immigration by adult burrowing organisms. To overcome this, mesh-sided boxes were used to study colonisation by benthic meiofauna (Decker & Fleeger, 1984), but similar methods have not been used for macrofauna. This paper briefly describes a marine sediment bioassay being developed at this laboratory.

The experiments were done on the Maplin Sands near Shoeburyness, intertidal muddy-sand flats supporting extensive beds of eelgrass (Zostera marina). In the laboratory, invertebrates in the surface sediment (top 20 cm) were killed by two cycles of freezing (-20°C) and thawing and a 1 m^3 subsample blended for 1 h in a cement-mixer with British National Oil Corporation (BNOC) diesel-based drilling mud (DBM) (900 mg kg^{-1} dry wt. as DBM equivalents). This mud was identical to that used by Blackman et al., (1988 a). Three further 1 m^3 subsamples were blended with finely-divided tributyltin copolymer antifouling paint, scoured from a dried paint film with a device similar to that used for scrubbing yachts. Nominal TBT treatment rates (based on an analysis of the added paint) were 0.1, 1.0 and 10 mg kg^{-1} dry wt.

The four treated sediments, plus an untreated control, were re-laid at Maplin Sands in April 1987 in trenches (3 m long, 20 cm deep, 30 cm wide) lined with 5 mm polythene mesh held down with steel pegs. Each end of the trench was blocked with a vertical roofing slate. At intervals between April and September 1987, benthic macro-fauna were sampled by collecting 4 replicate sub-samples (approx. 6 l each) of sediment sliced from the end of each trench, after which the slate was moved up to the exposed face and the excavation backfilled with clean sediment. Each sub-sample was washed through a 0.5 mm sieve, and the retained macrofauna preserved in 5% formol-saline containing eosin or rose bengal stain, until identified. Sediment samples were taken at intervals from a range of depths and frozen to await diesel or TBT analysis. At each visit, the total number of polychaete lugworm (Arenicola marina) casts visible on the surface of each trench were counted.

During much of the experiment, all treatments except 0.1 mg TBT kg^{-1} markedly reduced colonisation by the common polychaete Scoloplos armiger

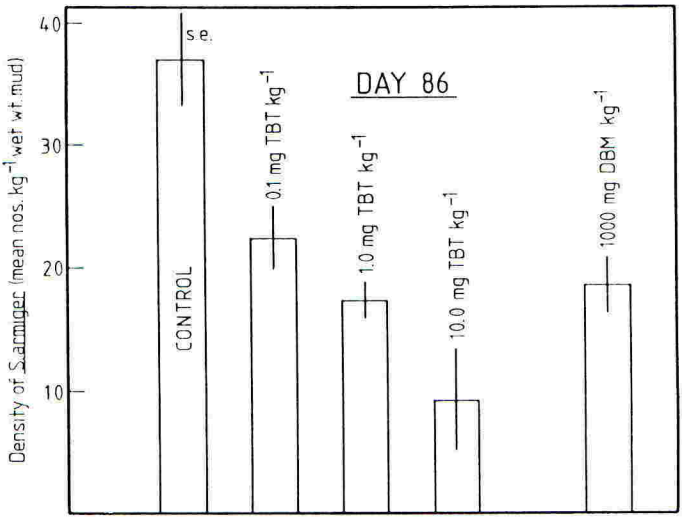

Fig. 3 Density of the polychaete Scoloplos armiger in contaminated sediments after colonisation for 86 days. Vertical bars represent the standard error of the mean

(Orbiniidae), and reduced or inhibited the casting activity of A. marina (Arenicolidae). Population densities of S. armiger after exposure of the treated sands for 86 days (Fig. 3) illustrate that the effect of TBT was related to concentration. The demonstrated effect of 0.1 mg TBT kg^{-1} was not evident at other sampling times. The DBM and 10 mg TBT kg^{-1} treatments completely inhibited casting in A. marina for the entire experiment, while in the 1.0 mg TBT kg^{-1} treatment it was reoccurring by September. In addition, all treatments retarded colonisation by the amphipod crustacean Urothoe poseidonis, and the effects of TBT were again concentration-related.

There were no discernible effects on other crustacea, annelids, hydrobiid gastropods or bivalve molluscs. Analysis of the sediments for diesel revealed that concentrations in the top 2 cm, to which most species are confined, fell rapidly. However, deeper layers remained contaminated (ca 60% of the initial concentration) throughout the experiment. Thus, it may be significant that the three affected species are burrowers, while most of the others live close to the surface. Blackman et al , (1988a) observed colonisation of their DBM-contaminated tanks once the oil concentration in surficial sediment declined.

The effects of TBT in sediment contrast markedly with those in laboratory studies with TBT in the absence of sediment (Waldock et al., 1987a) where bivalve molluscs are very susceptible and polychaetes and crustacea are not susceptible. This may be due to mechanisms such as differential availability (ingestion on particulates rather than absorption from solution) and avoidance, but it illustrates the value of this type of field test. Improvements to the method are being made and will be tested in 1988. It is hoped to extend the technique to assess the biological impact of the disposal of dredge spoils containing a variety of contaminants.

DISCUSSION

These examples of field methods for investigating the behaviour and effects of pesticides illustrate our belief that such methods should be used primarily to validate models and predictions from laboratory techniques, rather than as ends in themselves. Their complexity and expense exclude their use for primary hazard assessment, but they give important insights into the strengths and limitations of laboratory methods, and thus improve prediction of potential problems arising from the use of new pesticides.

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REFERENCES

Anon. (1986). Water pollution from farm wastes 1985. England and Wales. London: Water Authorities Association, 24 pp.

Arnoux, A.; Stora, G.; Diana, C. (1985). In situ experimental study of the evolution and recolonization of polluted sediments. Marine Pollution Bulletin 16, 313-318.

Blackman, R. A. A.; Fileman, T. W.; Law, R. J.; Thain, J. E. (1988a). The effects of oil-based drill-muds in sediments on the settlement and development of biota in a 200-day tank test. Oil and Chemical Pollution 4, 1-19.

Blackman, R. A. A.; Law, R. J.; Thain, J. E. (1988b). The effects of new oil-based drill-muds in sediments on the settlement and development of biota in an improved tank test. Oil and Chemical Pollution in press.

Burns, L. A.; Clive, D. M; Lassiter, R. R. (1982). Exposure analysis modelling system (EXAMS): User manual and system documentation. Report no. EPA-600/3-82-023, Athens, Georgia; U.S. Environmental Protection Agency.

Cairns, J.; Cherry, D. S. (1983). A site-specific field and laboratory evaluation of fish and Asiatic clam population responses to coal fired power plant discharge. Water Science and Technology 15, 10-37.

Croll, B. T. (1986). The effects of the agricultural use of herbicides on fresh waters in Effects of land use on fresh waters. Agriculture, forestry, mineral exploitation, urbanisation. J. F. de L. G. Solbe (ed.) Chichester: Ellis Horwood Ltd., pp. 201-209.

Crossland, N. O. (1984). Fate and biological effects of methyl parathion in outdoor ponds and laboratory aquaria. II. Effects. Ecotoxicology and Environmental Safety 8, 482-495.

Crossland, N. O. (1988). Validating the hazard assessment process. Chemistry and Industry (1), 23-24.

Crossland, N. O.; Wolff, C. J. M. (1985). Fate and biological effects of pentachlorophenol in outdoor ponds. Environmental Toxicology and Chemistry 4, 73.

Department of the Environment (1986). River quality in England and Wales 1985. London : Her Majesty's Stationery Office.

Department of the Environment (1987). Survey of potentially dangerous substances in U.K. waters. Report by SAC Scientific. London: DOE ref. no. PECD 7/7/201.

Donigan, A. S.; Beverlein, D. C.; Davis, H. H.; Crawford, M. H. (1977). Report no. EPA-600/3-77-098, Athens, Georgia ; U.S. Environmental Protection Agency.

EEC (1975). Council directive of 16 June 1975 concerning the quality required of surface water intended for the abstraction of drinking water in the Member States. Official Journal of the European Communities, L194, 26-31, 75/440/EEC.

EIFAC (1983). Revised report on fish toxicity testing procedures. European Inland Fisheries Advisory Commission, Technical Paper no. 24, Revision 1. Rome : FAO, 37 pp.

Frank, R.; Braun, H. E.; Holdrinet, M. V. H.; Sirons, G. J.; Ripley, B. D. (1982). Agriculture and water quality in the Canadian Great Lakes Basin; V. Pesticide use in 11 agricultural watersheds and presence in stream water, 1975-1977. Journal of Environmental Quality. 11, 497-505.

Friesen, M. K.; Galloway, T. D.; Flannagan, J. F. (1983). Toxicity of the insecticide permethrin in water and sediment to nymphs of the burrowing mayfly Hexagenia rigida (Ephemeroptera; Ephemeridae). Canadian Entomologist 115, 1007-1014.

Haith, D. A. (1980). A mathematical model for estimating pesticide losses in runoff. Journal of Environmental Quality 9, 428-433.

Hurlbert, S. H. (1975). Secondary effects of pesticides on aquatic ecosystems. Residue Reviews 57, 81-148.

Hurlbert, S. H.; Mulla, M. S.; Willson, H. R. (1972). Effects of an organophosphorus insecticide on the phytoplankton, zooplankton, and insect populations of fresh-water ponds. Ecological Monographs 42, 269-299.

Knisel, W. G. (1982). USDA, national modelling projects; Why, what and how. Tifton, Georgia : USDA-ARS, pp. 85-101.

Kooijman, S. A. L. M. (1985). Toxicity at population level in Multispecies toxicity testing. J. Cairns (ed.) SETAC Special Publication, Oxford : Pergamon Press, pp. 143-164.

Larsen, D. P.; De Noyelles, F.; Stay, F; Shiroyama, T. (1986). Comparisons of single-species, microcosm and experimental pond responses to atrazine exposure. Environmental Toxicology and Chemistry 5, 179-190.

Mackay, D.; Paterson, S.; Cheung, B.; Neely, W. B. (1985). Evaluating the environmental behaviour of chemicals with a level III fugacity model. Chemosphere 14, 335-374.

Perry, J. A.; Troelstrup, N. H.; Newsom, M.; Shelley, B. (1987). Whole ecosystem manipulation experiments : the search for generality. Water Science and Technology 19, 55-71.

Sanders, F. S. (1985). Use of large enclosures for perturbation experiments in lentic ecosystems ; a review. Environmental Monitoring and Assessment 5, 55-99.

Shires, S. W. (1983). The use of small enclosures to assess the toxic effects of cypermethrin on fish under field conditions. Pesticide Science 14, 475-480.

Solbé, J. (1988). Ecotoxicological testing and observations on pollutants in natural, semi-natural and artificial systems. Chemistry and Industry (1), 16-22.

Slooff, W. (1985). The role of multispecies testing in aquatic toxicology in Multispecies toxicity testing. J. Cairns (ed.), SETAC Special Publication, Oxford ; Pergamon Press, pp. 45-60.

Spencer, W. F.; Cliath, M. M.; Blair, J. W.; LeMert, R. A. (1985). Transport of pesticides from irrigated fields in surface run-off and tile drain waters. Conservation Research Report, Agricultural Research Service (U.S.) 31, 71 pp.

Suter, G. W.; Barnthouse, L. W.; Breck, J. E.; Gardner, R. H.; O'Neill, R. V. (1985) in Aquatic Toxicology and Hazard Assessment. R. D. Cardwell, R. Purdy and R. C. Bahner (eds.) American Society for Testing and Materials STP 854. pp. 400-413.

Tagatz, M. E.; Deans, C. H. (1983). Comparison of field - and laboratory - developed estuarine benthic communities for toxicant exposure studies. Water, Air and Soil Pollution 20, 199-209.

Tagatz, M. E.; Stanley, R. S.; Plaia, G. R.; Deans, C. H. (1987). Responses of estuarine macrofauna colonising sediments contaminated with fenvalerate. Environmental Toxicology and Chemistry 6, 21-25.

Waldock, M. J.; Thain, J. E.; Waite, M. E. (1987 a). The distribution and potential toxic effects of TBT in UK estuaries during 1986. Applied Organometallic Chemistry 1, 287-301.

Waldock, M. J.; Waite, M. E.; Thain, J. E. (1987 b). Changes in concentrations of organotins in U.K. rivers and estuaries following legislation in 1986. Proceedings of the Oceans 87 Symposium, 4, Halifax, Nova Scotia, 28 Sept.-1 Oct. 1987. Piscataway : IEEE, and Washington D. C. : Marine Technology Society, 1352-1356.

Wauchope, R. D. (1978). The pesticide content of surface water draining from agricultural fields - a review. Journal of Environmental Quality. 7, 459-472.

Williams, R. J.; Bird, S. C. (1987). Total impact of pollutants in river basins: Phase 2. Wallingford: Institute of Hydrology/Welsh Water Authority progress report, DOE contract PECD 7/7/170, 36 pp.

ASSESSING THE EFFECTS OF HERBICIDES ON AQUATIC FLORA

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ABSTRACT

The impact of herbicides on the flora of freshwater habitats is related to standard, short term and long term effects with an emphasis on the long term effect. This is the result of any form of impact which delays the process of recolonisation longer than that estimated for a standard effect. Current methods of assessing both standard and long term effects are considered with an emphasis on the problems encountered in aquatic systems. The dissipation of herbicides throughout a water body and the problems in establishing control sites makes experimental design very difficult. Submerged and floating plants can be difficult to assess and careful consideration must be given to differentiating between the effect of the chemical and other factors which could bring about long term changes. Methods for assessing submerged and floating plants are considered briefly.

INTRODUCTION

There is much concern about the impact of pesticides in the environment. The aquatic environment is particularly sensitive because water has a wide range of uses including for drinking and because polluting substances can be transported by water away from the site of pollution. Pesticides can be polluting substances if they enter the water accidentally and have undesirable side effects. Herbicides are the most widely used form of pesticide and the purpose of this paper is to discuss and evaluate critically the methods of assessing the impacts of herbicides on aquatic flora with particular reference to any long term effects. In order to demonstrate that a herbicide has had an effect on aquatic flora, it is necessary to show that a change has taken place in the plant community and that the change was caused either directly or indirectly by the herbicide.

Herbicides enter the aquatic environment either by deliberate application, to control aquatic weeds, or by accident as run-off, drift, seepage or spillage of herbicides intended for terrestrial use. Whichever the source, the effect on the aquatic flora is potentially the same. Either the herbicide is present at a phytotoxic concentration and in contact with the plant for sufficient time to be absorbed in toxic amounts or it is not. Both the concentration and the exposure time are important in determining the effect on the plants. If it is phytotoxic then it may kill some or all of the plant species or, at lower concentrations, it may limit or modify their growth.

Herbicides are used in many countries for aquatic weed control and the methods of assessing their immediate impact on the weeds are well documented (Brooker and Edwards, 1975; Newbold, 1975; Robson and Barrett, 1977; Pierterse and Murphy, in press). However, it should be emphasised that qualitative and quantitative techniques adapted from terrestrial methods are more difficult to apply in aquatic situations than on dry land. These techniques are discussed later in the paper and are basically the same whether used to assess either short or long term impacts.

The assessment of the impact of herbicides on aquatic plants is a very wide subject which, for the purposes of this paper, must be defined and limited. The range of aquatic flora includes algae and vascular plants which normally grow in freshwater although they may occasionally be found on banks adjacent to the water. The phrase 'long term impact' is more difficult to define in biological terms because there is no satisfactory definition of a 'standard' or 'short term' impact. For the purposes of this paper, we shall define a standard impact on a single species as the time which it would take for that species to recolonise and regrow to its original biomass after a sudden, complete kill of all vegetative growth including the roots and rhizomes. Recolonisation occurs from propagules or by reinvasion from untreated areas. Thus, a short term impact would occur, for example after cutting as the plants regrow from undamaged roots and rhizomes. A standard impact would be the result of complete mechanical or chemical removal of the weed, provided that the habitat remained otherwise unaltered and the herbicide did not persist. A long term impact could be the result of any form of control which delayed the process of recolonisation longer than a standard impact. Thus dredging, for example, could have such an impact by removing the silt beds in which the plants grow. Biological control agents would also tend to have a long term impact if they persisted after the initial removal or reduction in the biomass of weed. Herbicides could have a long term impact if they were sufficiently persistent to affect germinating propagules or reinvading vegetation; were continually leaching into the water at phytotoxic concentrations; were applied at regular and repeated intervals more frequently than the intervals of a standard impact; or had an indirect effect which altered the environment.

These definitions can be extended to cover aquatic ecosystems as a whole. The effects of total removal of all weed (i.e. non-selective control) could extend considerably the standard impact period. If the water body, from which the weed had been removed, were an old, diverse ecosystem then a succession of species similar to that found in a newly created water body would develop before the water body returned to the original state. The more advanced the ecosystem, then the longer would be the time taken for full recovery. This is not a long term impact in the sense of a persistent herbicide, simply a natural succession of plants exploiting the conditions created by the removal of an existing botanical community. Even selective control of a limited range of species might be expected to produce a delayed recovery if it allowed other competitive species to invade and impede the recovery of the target weeds. The

standard impact period would also depend on the size and shape of the affected area since this would affect the ease with which reinvasion could occur.

The standard impact period resulting from control of a single species or a whole plant community will also depend on environmental conditions. If these are highly suitable for the growth of weeds, then the period of recovery is likely to be short. Whereas, at the limit of the range of a species, recovery will be much slower, or may be delayed indefinitely until the return of some chance set of conditions which had originally allowed the species to colonise the area. On the other hand, where weed control operations have been regularly carried out, the species most able to tolerate these operations will be dominant, and recovery to the original community will be more rapid. The standard impact period will also depend on the time of year when the plants are killed and on the seasonal growth of the plants.

The impact of a pesticide could be caused either by direct toxicity to the plant community or by an indirect effect resulting from the initial changes when the chemical entered the water. These could include changes in the invertebrate or fish populations caused either by direct toxicity or by loss of habitat. The fish or invertebrate communities can have a direct influence on the range and quantity of plant species and the time taken for them to recolonise the water. Although they are outside the scope of this paper, any assessment of herbicide impact should include a study of the interactions between fish, invertebrates and plants.

There are three approaches to the methodology for studying the long term impacts of herbicides on aquatic flora. The first is based on measurement of changes in the plant communities within a habitat over a period of time. The second is to focus on the distribution and autecology of species selected as indicator species. The third is to determine the no-effect level of the herbicide on indicator species under laboratory conditions and to monitor the residue levels in water and hydrosoils. It is also necessary to establish the half life of the herbicide under field conditions so that some estimate of the persistence of the chemical in an active form can be made.

The paper also gives some consideration to the choice and application of practical methods for assessing submerged and floating aquatic macrophytes.

METHODS OF ASSESSMENT

Measurement of the plant communities within a habitat in which changes are measured over a period of time.

a. Methods based on investigations of limited duration

Investigations of the environmental effects of herbicides have traditionally been based on field work restricted to one, two and occasionally three years duration. Typically, the chemical is introduced into the water subsequently after a short period of preliminary observation (Brooker & Edwards, 1973 a & b). The effect of the chemical is then observed usually over the next two seasons. This approach often incorporates a control section of water in which the chemical has not been applied (Way et al. 1971).

Separating off sections of the same piece of water (Robson et al., 1978), or establishing separate control units (Way et al. 1971; Marshall, 1981, 1984) is difficult. In the former case problems arise in preventing the movement of the herbicide into the control sections. In the latter there are two fundamental obstacles. Firstly, the control unit, say a small lake, may be different from the treated small lakes, albeit in an apparently small way (Way et al. 1971; Marshall, 1981, 1984). After treatment these small differences can become significant especially if plant species, present only in small amounts and restricted to the treated units, develop into significant stands (Newbold, 1974).

The environmental components typically included in these studies are water chemistry, an evaluation of the aquatic macrophyte community and/or the aquatic fauna. A variety of measures have been used for the assessment of the aquatic flora and a range of techniques have been developed by which to make the measurement. These include presence-absence/frequency estimations, measurements of density, cover, biomass/standing crop and productivity. The Department of the Environment (1987) provide a useful account of a number of these estimations and measures as used in surveys and Vollenweider (1974) reviews methods for assessing primary production in aquatic environments.

Most of the investigations following this limited duration model have been primarily concerned with the standard and/or short term effects of the chemical, and in the case of aquatic herbicides include some measure of efficacy.

In order that investigations of limited duration might yield useful information on the long term effects, attention must be directed at other components of the habitat. What is the effect of the chemical on the propagative potential of the different species? This is especially relevant for the seeds, spores, winter buds, turions and other such propagules. Are they inactivated by the chemical? Is their dormancy altered in any way? Likewise, does the effect of the chemical interfere with the production of seeds?

The importance of propagule banks in the hydrosoil has been recognised for a number of species, for example Chara (Wade & Edwards, 1978), Callitriche truncata (Wade, Vanhecke & Barry, 1986). These are typically primary colonisers and if a chemical introduced into the water had an effect on the propagules of such a species, long term perturbations could be expected.

Such research is equally important in determining the ways in which plants respond differentially to a chemical and the consequent effects on competition between species; and the effects of herbicides on the flowering, fruiting and ripening processes. Does the chemical inhibit or otherwise interfere with such elements of the plant's physiology? Does this have an effect on the competitive ability of the plant? There are large gaps in our knowledge about these effects, some of which could be filled by including appropriate field observations and laboratory studies in investigations of herbicidal activity of limited duration.

b. Methods based on surveillance over longer periods

In contrast to investigations into the specific effects of known treatments/exposure to herbicides and other pesticides on the aquatic flora, surveillance can be undertaken to detect the long term impact of such chemicals.

Such an approach can have advantages including less intensive use of manpower, detection of chemicals which might have gone unnoticed and greater perception of impact on the aquatic plant community. Disadvantages include the obsequious effects of other changes in the environment, some of which can be very subtle, e.g. climatic change, alterations in nutrient balance or change in the aquatic biota.

The surveillance can be forward or backward looking. Nature reserves based on exhausted peat and gravel excavations are being established in Hatfield Chase, South Humberside/Yorkshire. Regular surveillance of the aquatic flora of these artificial lakes could be established to ensure that there are no long term effects on their aquatic macrophyte communities from the herbicides and other pesticides used in the surrounding agricultural land. Such surveillance should be based on a simple methodology, e.g. a species list with DAFOR (dominant, abundant, frequent, occasional or rare) rating repeated at least annually. Repeat visits should be made on the same month(s). If effort is available vegetation maps are informative but it is necessary to remember that these must be repeated annually.

Retrospective surveys will depend on an ability to describe the aquatic flora as it was and related changes in species composition to known changes in the presence of pesticides in the water. Wade (1981) demonstrated the potential for such surveys though data were not available for all the herbicides approved for use in or near water limiting the value of this approach. The review highlighted the importance of recording the vegetation of sites treated with herbicide before application, preferably to include summer and spring seasons. Wade & Edwards (1980) used documentary records and data from herbarium specimens to reconstruct the aquatic flora of a series of drainage channels in South Wales over the period 1840 to 1976. The study enabled the effects of the aquatic herbicides 2,4,-D and dalapon to be compared with other environmental changes which had taken place since 1840. Similar work has been undertaken by Driscoll (1982). Wade (1979) elaborates on the value of the data in herbaria for making such reconstructions.

The main disadvantages with this approach is that the impact of the herbicide will only be one of a number of factors affecting the plant communities in a given water body. This problem has been described for terrestrial situations, e.g. differentiating between the effects of seed cleaning and herbicides on arable weeds. Wade (1981) cites a variety of environmental factors which need to be considered should this approach be adopted.

The distribution and autecology of macrophytes selected as indicator species

The changes in the distribution of a number of terrestrial species associated with agricultural land have been useful indicators of the long term effects of chemicals used by farmers. Species of aquatic plants should be examined to determine their potential as such indicators. A number of Potamogeton species could be valuable in this context. (C.D. Preston, personal communication).

More attention needs to be paid to the surveillance of the national distribution of aquatic macrophyte species to provide a sound base for detecting long term trends due to herbicides. The distribution of these species has been neglected for a number of reasons. Recording aquatic plants is not easy and there is a need for special equipment ranging from a pair of waterproof boots to a boat and from a weed grapnel to SCUBA equipment. Aquatic plants often vary in their growth forms and usually lack flowers and this poses identification problems. The situation is changing however, and the potential of aquatic plants is being exploited (Department of the Environment, 1987). There is increasing interest in aquatic plant distribution stimulated by the need to manage these plants more selectively, as evidenced by publications such as Spencer-Jones & Wade (1986), "Aquatic plants - a guide to recognition", and specialist texts, such as Moore (1986) and the Potamogeton handbook currently in preparation by the Botanical Society of the British Isles.

Determination of the no-effect level of the herbicide on indicator species and monitoring the residue levels in water and hydrosoils.

The emphasis of herbicide studies typically centres on target species and data on potentially valuable indicator species are limited. The no-effect levels need to be determined for selected herbicides through laboratory studies. Such investigations should be coupled with studies of the effect of the herbicides on key aspects of the plant's physiology, e.g. germination and flowering as described above. These concentrations provide an important part of the information needed to determine whether or not a particular chemical is likely to produce long term effects.

The other important piece of information is the concentration of that chemical which will be encountered in the aquatic habitat. Generally speaking this can be in the water or the hydrosoil and measurements need to be made of these levels at appropriate intervals after the chemical has been applied. The situation will not necessarily be as straightforward as a single application and particular attention needs to be paid to the effects of repeated applications or prolonged exposure to the herbicide, the significance of which is stressed by a number of workers (Johnannes et al.,1975; Robson et al., 1978; Wade, 1981.

Consultation between appropriate agencies should be encouraged with the aim of producing a short list of candidate species for more detailed investigation of distributional ecology. It is recommended that autecological studies subscribe to the criteria laid down by the British Ecological Society for their Biological Floras (Barry & Wade, 1986) and coupled with laboratory investigations into the effect of selected herbicides on the species under consideration. Such a research programme lends itself to a co-operative venture between public and private sectors.

PRACTICAL METHODS OF ASSESSMENT

The techniques used to assess changes in aquatic flora under field conditions are based mainly on those developed for terrestrial use. There are a number of factors which can influence the choice appropriate to individual situations. These include the depth, velocity and clarity of the water which affect the ability of the researcher to work in, or see through, the water. Growth seasons, distribution and morphology of the aquatic plants can also influence the choice of sampling technique. Some water plants are free floating and can drift with the wind or water currents and some species of algae can control their depth in the water and rise or descend depending on the light and carbon dioxide availability. Flood and drought conditions can also influence the distribution and condition of aquatic plants and can affect timing and accuracy of sampling.

The first step in assessing an impact on plant communities is the identification of the species. This can be difficult because of the plasticity of many species which have different growth forms under different growing conditions and because of the reluctance of aquatic plants to produce flowers. Algae are particularly difficult to identify and, even with a microscope, can present problems.

Submerged and floating plants can present special difficulties. The extent of plant cover can be assessed by mapping transect or quadrats, by using echosounding, SCUBA diving or, more traditionally, wading or working from a boat. The chief problem with these methods is fixing the position of a sample point relative to a known point on land. Aerial photography overcomes this problem but is expensive and for submerged plants is reliant on clear water. Cover, density and species diversity estimates can be made using quadrats laid out in an appropriate arrangement.

Biomass estimates are the basis of other assessments of plant populations. These can be particularly difficult in aquatic habitats especially in deep or turbid water where quadrats disappear from sight and cut weed drifts away from the sample area. Cropping floating and submerged plants inevitably stirs up the sediments and rapidly reduces visibility. Filamentous algae are especially difficult to sample using these methods. Grapnel, grab and rake samples can be used to overcome some of these problems but are variable in result depending on the growth form of the species, the substrate and the skill of the operator to take standard samples. SCUBA diving can provide another solution to some of these problems but requires trained operators and can be expensive. Even floating species such as water-lilies present difficulties if rhizomes and roots are to be sampled.

Emergent plants are much easier to deal with and methods devised for terrestrial systems are usually applicable. Significant problems arise however when trying to make comparisons based on cover estimates between stands of erect/emergent species and submerged and floating species.

More detailed information about methods used for assessing aquatic plants can be found in Vollenweider (1974), Wood (1975), Murphy, Hanbury & Eaton (1981), Wade and Bowles (1981), Hanley (1982) and Department of the Environment (1987).

DISCUSSION

In the simplest form, the effect of a herbicide on a plant is to kill or damage it. Even when a herbicide has been used deliberately to control water weeds, it may take days, weeks or even months for the direct effects to become apparent. A gradual recovery will then take place until the species has re-established itself. In an experiment, the degree of control and the rate of recovery can be compared with control and standard treatments (i.e. treatments known to have no long term impact). In theory, these effects could be determined by replicated trials with adequate controls to ensure statistical validity of the results. In practice, it is difficult to see how field experiments, based solely or mainly on biological evaluation, could be set up to test the impact of every new herbicide, as well as all the existing ones, under a range of environmental conditions. Herbicide experiments in the aquatic field situation are difficult to set up, even more difficult to replicate in statistical terms, subject to large variability and unpopular with water authorities and other water users. To do so simply to test the possible environmental impact of a pesticide which is not intended for use in water would be almost impossible. Probably the best compromise is to use artificially created ponds such as those described by Newbold (1974) or Crossland (1988).

If the long term impact is to be assessed, it is necessary first to determine the length of a standard impact and then to maintain the experiment long enough to measure the difference in recovery time between the standard and the test chemical. If, however, the length of these two impacts could be defined, then a number of advantages might accrue. The major one would be that any impact, whether short or long term, could be expressed as a fraction or multiple of the standard impact. This could be used in helping to define either the harmful or beneficial effects of a herbicide in the aquatic environment depending on whether it was an accidental pollution or a deliberate introduction for weed control. These assessments could only be determined by deliberately constructed experiments. It is doubtful if the results of an accidental spillage or slow leaching of a pesticide into a water course could be assessed in the same way. In these instances, determination of the pesticide residue, coupled with laboratory studies on test species, would be a more reliable method of assessing the impact.

It is clear that the time of recovery, i.e. the impact period, cannot be defined simply in terms of months or years without defining a range of parameters including such ill defined values as the closeness to the ecological limits in which the test species is growing. While it may be possible to measure the impact period of a herbicide by field experiment using the techniques described above, the value obtained will only apply to that particular site, and to be accurate, only on that particular occasion. It would also be necessary to include a "standard treatment" to determine the normal impact period so that the test treatment could be assessed as short, normal or long term in its impact.

Since the time taken for recovery of a plant community can be so variable and it is impossible to create reliably replicated or controlled experiments, it is almost impossible to assess a long term impact by field assessment of the plant community. A more realistic approach, particularly for the majority of herbicides which might only enter water by accident, is to determine the no-effect level of the chemical on a range of test species under laboratory conditions and the half life of the chemical in the field. From these data, the impact period could be predicted.

There are two other aspects which relate to the assessment of pesticide impacts in the aquatic environment. The first is that there is not definition of what is an acceptable or an unacceptable impact so that, even if the total extent and duration of an impact could be assessed, the decision as to its acceptability is arbitrary. The second is that many water courses are man-made for a particular function so that an impact which is unacceptable in one water body might be acceptable or even, highly desirable, in another. Until the requirements and objectives of all interested parties in a particular water body could be agreed and ranked in order of priority, no decision can be made sensibly as to the acceptability or otherwise of a pesticide impact.

REFERENCES

Barry, R. & Wade, P.M. (1986) Callitriche truncata Guss. Biological Flora of the British Isles. J. Ecol., 74, 289-294.

Brooker,M.P. & Edwards, R.W., 1973a, Effects of the herbicide paraquat on the ecology of a reservoir. Freshwater Biology, 3, 157-175.

Brooker,M.P. & Edwards, R.W., 1973b, Effects of the herbicide paraquat on the ecology of a reservoir.II.Community metabolism. Freshwater Biology, 3, 157-175.

Brooker, M.P. & Edwards, R.W., 1975. Aquatic herbicides and the control of water weeds. Water Reasearch, 9, 1-15.

Crossland, N.O., 1988 Experimental design of pond studies. In this volume.

Department of the Environment, 1987. Methods for the examination of waters and associated materials: Methods for the use of aquatic macrphytes for assessing water quality. 1985-86. H.M.S.O. London. 176pp.

Driscoll, R. 1982. The dyke vegetation at Oby and Thurn: a comparison of late 19th century and recent records. Trans. Norfolk Norwich Nat. Soc, 26, 43-49.

Hanley, S. 1982. A technique for surveying aquatic plant populations using an echo-sounder. Proc.EWRS 6th Symp. Aquatic Weeds, 1982, 171-176.

Johannes, H., Heri, W., & Reyneart, J. 1975. Le triazone nella lotta contro le alghe e le piantw vascolari sommerse. Notizaria sulle Malattie delle piante, 92/93, 39-59.

Marshall, E.J.P., 1981. The ecology of a land drainge channel. 1. Oxygen balance. Water research, 15, 1075-1085.

Marshall, E.J.P., 1984. The ecology of a land drainage channel. 1, Biology, chemistry and submerged weed control. Water Research, 18, 817-825.

Moore,J.A., 1986. Charophytes of Great Britain and Ireland. B.S.B.I. Handbook 5, B.S.B.I., London.

Murphy, K.J., Hanbury, R.G. & Eaton, J.W. 1981. The ecological effects of 2-methylthiotriazine herbicides used for aquatic weed control in navigable canals. 1. Effects on aquatic flora and water chemistry. Arch. Hydrobiol., 91, 294-331.

Newbold, C., 1974. The ecological effects of the herbicide dichlobenil within pond ecosystems. Proc. EWRC 4th Int. Symp. Aquatic Weeds, Vienna, 37-52.

Newbold, C., 1975., Herbicides in aquatic systems. Biol. Conserv., 7, 97-118.

Pierterse, A. & Murphy, K.J. (eds) (in press). Aquatic plant management. Oxford University Press.

Robson, T.O. & Barrett, P.R.F., 1977. Review of effects of aqautic herbicides. In: Perring, F.H. & Mellanby, K., (eds) Ecological effects of pesticides. Linnean Society Symposium Series, 5,111-118.

Robson, T.O., Fowler, M.C. & Hanley, S. 1978. Observations on a lake treated with terbutryne in three alternate years. Proc. EWRS. 5th Int. Symp. Aquatic Weeds, Amersterdam, 303-313.

Spencer-Jones, D. & Wade, P.M. 1986. Aquatic plants: a guide to recognition. I.C.I. Farnham, 169pp.

Vollenweider, R.A. 1974. A manual on methods for assessing primary production in aquatic environments. I.B.P. Handbook 12, Blackwell Scientific Publications, Oxford.

Wade, P.M., 1979. The importance of plant collections in a study of the ecology of the aquatic flora of the Monmouthshire levels. Mus. J., 78, 171-173.

Wade, P.M., 1981. The long term effects of aquatic herbicides on the macrophyte flora of freshwater habitats - a review. Proc. Aquatic Weeds and their Control, Oxford, 233-240.

Wade, P.M. & Bowles, 1981. A comparison of the efficiency of freshwater macrophyte surveys carried out from underwater with those from the shore or a boat. Progress in Underwater Science, 6, 7-11.

Wade, P.M. & Edwards, R.W. 1980. The effect of channel maintainance on the aquatic macrophytes of the drainage channels of the Monmouthshire Levels, South Wales, 1840-1976. Aquatic Botany, 8, 307-322.

Wade, P.M., Vanhecke, L. & Barry, R. 1986. The importance of habitat creation, weed management and other habitat disturbance to the conservation of the rare aquatic plant, Callitriche truncata Guss. Proc. EWRS/AAB 7th Symp. Aquatic weeds, Loughborough, 389-394.

Way, J.M., Newman, J.F., Moore, N.W. & Knaggew, F.W. 1971. Some ecological effects of the use of paraquat for the control of weeds in small lakes. J. appl. Ecol., 8, 509-532.

Wood, R.D. 1975. Hydrobotanical methods. University Park Press, Baltimore.

PROCHLORAZ - ENVIRONMENTAL IMPACT OF PRODUCTION EFFLUENT ON MACOMA BALTICA IN THE RIVER MERSEY

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ABSTRACT

Schering Agrochemicals Limited are building a new manufacturingplant for the fungicide prochloraz, 1-N-propyl-N - [2-(2,4,6-trichlorophenoxy)ethyl]carbamoylimidazole, at their production site at Widnes on Merseyside. As a condition of the planning consent, Schering have been required to undertake a programme of environmental monitoring in the River Mersey since the latter is the end point for aqueous effluent. Although the effluent will be subjected to a waste treatment regime, it is still possible that small quantities of intermediates and impurities from the production process could be discharged into the river

Since the River Mersey is a key site for migrating wading birds, the environmental impact assessment has focussed specifically on Macoma balthica, a shellfish species known to be a major food source for estuarine birds. Two 'marker' compounds have been selected from the prochloraz effluent stream and one of them has been evaluated in the laboratory to determine if bio-accumulation in Macoma is likely to occur. Analytical methods for the 'marker' compounds have also been developed and 'background' levels in both Macoma and river water have been evaluated over a six month time course prior to production start-up. Following commencement of manufacture, analysis of water and Macoma samples will continue to determine if bioaccumulation takes place.

INTRODUCTION

A large area of the River Mersey estuary is designated as a site of special scientific interest. It was first notified in 1951 under the National Parks and Access to the Countryside Act, 1949. The area was revised and extended in 1984 under section 28 (1) of the Wildlife and Countryside Act, 1984.

The estuary has been recommended for designation as a Ramsar site by the Nature Concervancy Council (NCC). The site is so named following an international convention held at Ramsar, Iran in 1971. The UK Government signed the Convention in 1973 thus requiring that it designates sites to be included in a list of wetlands of international importance. The Mersey estuary fulfils the criteria since its wetland supports a large overwintering population of wildfowl and waders. In 1980-1981 the estuary had the highest monthly count of wildfowl of any British site (57,700 birds) with species such

as Pintail, Teal, Shelduck and Wigeon being recorded. In 1982-1983 a monthly count of waders totalled 26,593 birds, including species such as Dunlin, Curlew, Redshank and Golden Plover (NCC, personal communication, 1984). Fish and invertebrate species are also increasing in numbers with over 30 species of fish currently inhabiting the estuary (North West Water Authority, personal communication, 1985).

To maintain and improve this estuarine environment it has become essential to control the level, and nature of, any polluting imputs to the estuary particularly since the numbers of incidents of bird mortalities has been linked to the bioaccumulation of toxicants in their prey species (Wilson et al, 1986). It is considered that the majority of harmful pollutants are generated by the chemical and petrochemical industries which utilise the estuary. Consequently, considerable attention is now given to determining the ecotoxicological properties of waste water.

In 1985 Schering Agrochemicals Limited made a planning application to construct a new synthesis plant for the fungicide prochloraz at its manufacturing site at Widnes, Cheshire. A map of the relevant section of the River Mersey estuary is given in figure 1. As a condition of approval, and prior to permitting discharge of waste water components into the Mersey estuary, via a sewage treatment work, North West Water Authority (NWWA) requested that a study be made of the bioaccumulation potential of these components. This report describes the investigations which are being carried out.

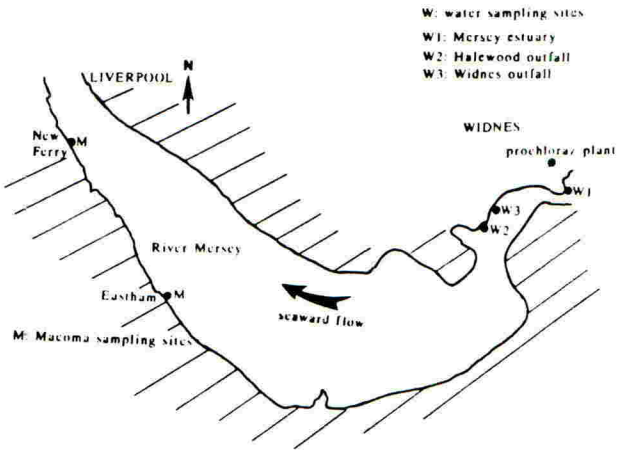


Figure 1 Sketchmap of River Mersey estuary

Experience with the synthetic process suggested that a wide range of by-products could be present in the waste waters, albeit at low levels. Consequently, two 'marker' compounds were selected on the basis of their contrasting chemical properties. Equally important, neither 'marker' was expected to be present in the waters of the River Mersey from pollutant sources other than prochloraz manufacture. Whilst estuarine birds feed on a range of aquatic species, Macoma balthica was selected as being one of the major food sources and hence a suitable biological 'target'.

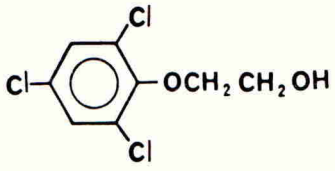
Thereafter it was agreed that analytical procedures would be developed for the two 'marker' compounds and subsequently applied to samples of river water and Macoma. Residue levels would be determined both prior to start-up of the production plant and for 12 months afterwards. Moreover a [^{14}C] laboratory bioaccumulation study would be carried out in Macoma using one of the marker compounds.

MATERIALS AND METHODS

Marker Compounds

The two marker compounds selected were BTS 3037 (2-2,4,6-trichlorophenoxyethanol) and BTS 42 825 (1,2-bis(2,4,6-trichlorophenoxy) ethane).

BTS 3037



BTS 42 825

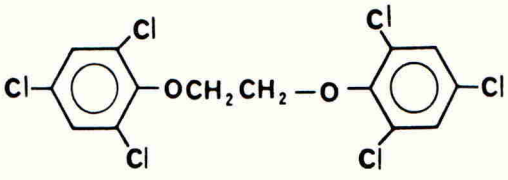


Figure 2 Structure of BTS 3037 and BTS 42 825

Sampling Sites

Macoma samples were obtained for residue analysis from two sites on the River Mersey:-

I New Ferry, Birkenhead, Merseyside, NGR SJ 340 862 which is outside the SSSI boundary

AND

II Eastham Locks, Eastham, Merseyside, NGR SJ 373 809, which is adjacent to the SSSI site.

The Macoma used in the bioaccumulation sites were obtained from a 'neutral' site at Snettisham, Norfolk NGR TF 647 336.

Water samples were obtained from three sites:-

I Mersey estuary - Widnes, west bank landing stage, NGR SJ 511 836, sampled at slack water, high tide - to provide background data on river pollution.

II Halewood Sewage Treatment Works, Cheshire, discharge to river outfall - to provide information on residue levels in waste being discharged by an established contract manufacturer currently synthesising prochloraz on batch production basis.

III Widnes Sewage Treatment Works, Cheshire, discharge to river outfall - to provide background residue data prior to the commencement of prochloraz manufacture.

Analytical Methods

I Water

Water samples taken from both the Mersey estuary and from the Halewood Sewage Works outfall were analysed for levels of the two marker compounds, BTS 3037 and BTS 42 825, using the following procedure.

Aliquots of each sample (250ml) were transferred to a 500ml roundbottomed flask connected to a 100ml liquid/liquid extractor primed with water (50ml) and hexane (10.0ml) and heated under reflux for four hours to extract each of the components using the principle of steam distillation. After cooling, an aliquot of the hexane layer (1.0ml) was pipetted into a 20ml glass vial followed by addition of the GC marker solution (1.0ml of a solution containing 0.5µg/ml DDE (1,1-dichloro-2,2- bis(4-chlorophenyl)ethylene) and 10.0ug/ml (N-butyl-2-chlorobenzoate) NB2CB in hexane. A series of calibration standards were similarly prepared by pipetting volumes of 0.05, 0.1, 0.2, 0,3 and 0.5ml from a solution containing 1.0ug/ml of both BTS 3037 and BTS 42 825 into separate 20ml vials and adding the GC marker solution (1.0ml)

Calibration standards and sample extracts were then injected into a capillary gas chromatograph under the following conditions:

Instrument:	Varian 6000 fitted with 63Ni constant current electron capture detector.
Column:	Fused silica capillary, SPB-5 bonded phase, 30m x 0.25mm i.d., 0.25mm df (Supelco 2-4034).
Carrier:	Nitrogen at approximately 0.5ml/min.
Detector Make-up:	Nitrogen at 75ml/min.
Split Flow:	20ml/min (split ratio approximately 40:1).
Oven:	200°C for 6 min, then programmed at 10°C/min to 270°C for 15 min.
Injector:	Split mode, 210°C.
Detector:	280°C.
Injection Volume:	2 µl.
Retentions:	NB2CB 5.4 min
	BTS 3037 6.6 min
	DDE 12.9 min
	BTS 42 825 23.2 min

From each chromatograph, the peak height ratios of BTS 3037/NB2CB and BTS 42 825/DDE were calculated. Using calibration curves of peak height ratio versus weight of BTS 3037 and BTS 42 825, respectively, concentrations of the two compounds in each sample extract were obtained.

The method is sensitive to 0.001mg/l (1ppb, w/v) for each component.

II Macoma

The method determined BTS 3037 only.

After removal of shells, Macoma samples (10g) were weighed into a macerating flask and homogenised for 5 minutes with water (25ml). The extracts were then transferred into a 500ml round-bottomed flask, rinsing with water to a volume of approximately 150ml. Analysis then proceeded as for water samples, except that smaller capacity (25ml) liquid/liquid

extractors were used, primed with water (10ml) and hexane (5.0ml). After extraction, 3.0ml aliquots were removed and added to GC marker solution (1.0ml) for GC determination.

A determination limit of 0.01mg/kg BTS 3037 in Macoma was obtainable by this approach.

A typical chromatogram showing the retention times of the marker compounds and the reference standards is given in Figure 3.

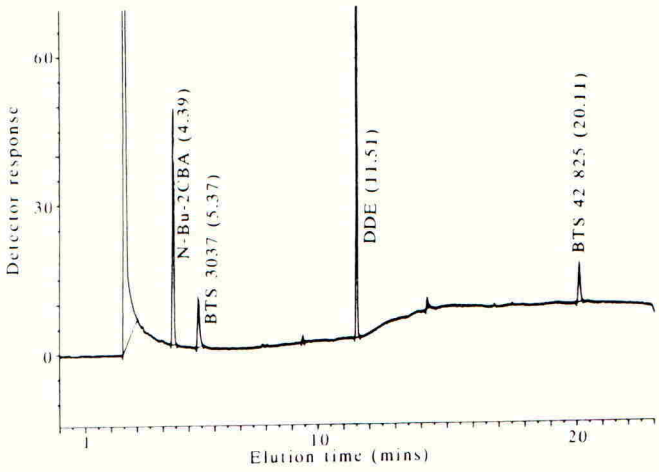


Figure 3 Chromatogram showing the relative retention times of BTS 3037, BTS 42 825 and the reference standards

Bioconcentration Study

I Preliminary Investigation

A preliminary acute toxicity study was carried out in order to set the dose level for the bioconcentration study. In that study using static conditions, Macoma were exposed to BTS 3037, in artificial sea water, at concentrations up to approximately 8mg/l. This was the maximum solubility attainable with the use of carrier solvents DMSO and Tween 80.

No significant effects of the chemical were seen within the usual time frame of 96 hours. However, exposure was continued for up to 28 days to determine the longer term effects, if any, of holding the Macoma under test conditions; none were observed.

As a toxic effect was not observed in the preliminary investigation, the concentration of BTS 3037 selected for the bioconcentration study was the mean value measured at the Halewood Sewage outfall over a period of 2 months. This concentration was 0.13mg/l.

II Bioconcentration Study

Macoma balthica were collected from mudflats at Snettisham, Norfolk and acclimatised to artificial sea water in the laboratory for several days before being transferred to the test vessels. The test vessels were 7 litre capacity all glass tanks. Macoma were allocated at random, 50 individuals per tank.

Three tanks contained artificial sea water and carrier solvents (Tween 80 and DMSO 50:50 v/v at a concentration of 0.2ml/l), and three tanks contained artificial sea water plus [^{14}C]-BTS 3037, in carrier solvents, at a nominal concentration of 0.13mg/l. Peristaltic pumps were used to supply fresh solutions of the appropriate composition, to each tank at a rate of 10ml/minute. An overflow device on each tank maintained solutions at a constant volume of 5 litres. All tanks were gently aerated throughout the incubation period, and daily checks, were made of oxygen concentration and pH in each tank. Temperature was continuously monitored throughout the study in one of the control tanks. The concentration of BTS 3037 in the treatment tanks was measured daily by radio-counting and HPLC.

On days 1, 3, 7, 10, 12, 15 and 17 Macoma were sampled from each tank for analysis to determine the amount of radioactivity accumulated in both the tissue and shell.

RESULTS

Residue Study

Weekly samples of water taken from the Mersey estuary and the Halewood Sewage Works outfall between January and March, 1987, were analysed as described previously, giving the results as shown in Table 1.

Table 1 Analysis of Water

	Residue Level (mg/l)			
	Halewood Outfall		Mersey Estuary	
Date Sampled	BTS 3037	BTS 42 825	BTS 3037	BTS 42 825
28/01/87	0.18	0.02	ND	ND
	0.39	0.02		
	0.32	0.02		
04/02/87	0.05	0.02	ND	ND
11/02/87	0.14	0.05	ND	<0.001
18/02/87	0.11	0.03	0.003	0.002
25/02/87	0.01	0.02	<0.001	<0.001
04/03/87	0.06	0.01	<0.001	<0.001
11/03/87	0.05	0.004	<0.001	<0.001
19/03/87	0.09	0.02	ND	ND
26/03/87	0.05	0.02	ND	ND
27/03/87	0.13	0.03	ND	ND

ND denotes a non-detectable residue

Residues in the estuary water were essentially non-detectable throughout the monitoring period, with maxima of 0.003mg/l BTS 3037 and 0.002mg/l BTS 42 825 found on 18 February.

Residues in the outfall water ranged between 0.01 and 0.14mg/l BTS 3937 with lower levels 0.004 to 0.05 mg/l, of BTS 42 825. The only result to exceed the maxima of these ranges was a mean value of 0.30mg/l BTS 3037 (from 3 replicate analyses) found on 28 January 1987.

Several Macoma samples were taken from sites along the Mersey estuary between January and July, 1987. Results of BTS 3037 analyses are shown in Table 2; none of the residues exceeded the determination limit of 0.01mg/kg.

Table 2 Analysis of Macoma

Date Sampled	Site	BTS 3037 Residue Level (mg/kg)
23/01/87	New Ferry	<0.01
04/04/87	New Ferry	<0.01
07/05/87	New Ferry	<0.01
29/06/87	New Ferry	<0.01
17/07/87	New Ferry	<0.01
17/07/87	Eastham Locks	<0.01

Bioaccumulation Study

I Exposure Phase

The mean measured concentration of [^{14}C]-BTS 3037 in the treatment tanks during the bioconcentration phase was 0.1395mg/l. The concentration of [^{14}C]-BTS 3037 in Macoma tissues reached a plateau at around Day 10 (Figure 4). The mean measured concentration in the Macoma tissue (obtained from summation at plateau of the four samples analysed on Days 10 to 17 inclusive) was approximately 5.9µg/g fresh weight of tissue. Only negligable [^{14}C]-residues were detected in the shell.

The bioconcentration factor (BCF) was determined to be 42X

II Depuration Phase

On Day 17, the remaining shellfish in the treatment tanks were transferred to clean tanks with untreated artificial sea water supplied at a nominal flow rate of 10ml/min for the depuration phase. The radiochemical was depurated rapidly with approximately 50% loss of the residue in 3 days and an estimated 80% loss in 7 days. Depuration was terminated after 6 days due to deterioration in the health of both the control shellfish and those in the depuration vessels which was in no way related to the treatment (Figure 4).

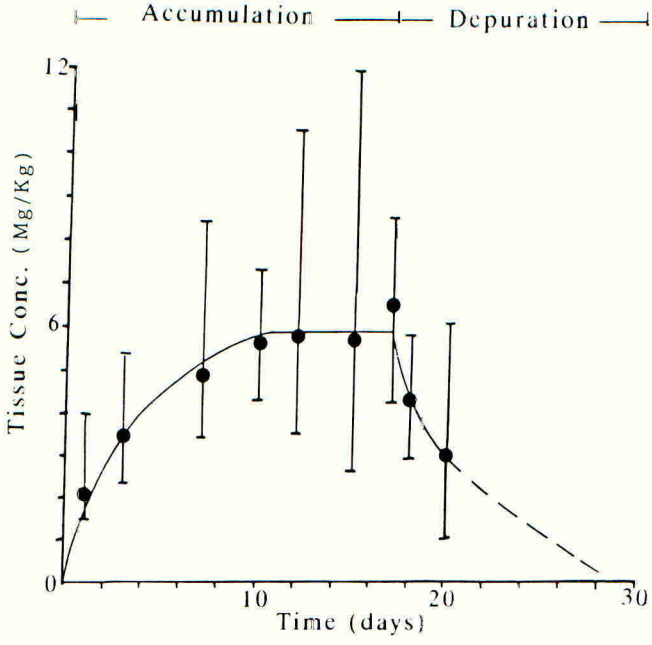


Figure 4 Accumulation and depuration of [^{14}C] BTS 3037 in Macoma balthica

DISCUSSION AND CONCLUSION

To-date negligible residues of the two marker compounds BTS 3037 and BTS 42825 have been seen in the water of the Mersey estuary unless sampled adjacent to the outfall from an existing contract manufacturer. Equally important Macoma taken from two sites have been clear of BTS 3037 residues. Background monitoring has restarted (March 1988), with the addition of water samples being taken from the outfall of the Widnes Sewage Treatment Works. The latter are considered relevant since ultimately waste water from the new prochloraz plant will enter the Mersey via the treatment plant.

The bioaccumulation study indicated that there will be no significant accumulation of BTS 3037 in Macoma, thereby presenting little or no risk to wildfowl in the Mersey Estuary. Nevertheless as a confirmatory measure it is proposed that samples of both river water and Macoma will continue to be analysed at regular intervals for up to one year post-start-up of the production facility.

REFERENCES

Nature Conservancy Council, personal communication, 1984

North West Water Authority, personal communication, 1985

Wilson, K. W., Head, P. C. and Jones, P. D., (1986) Mersey estuary (U.K.) bird mortalities - causes, consequences and correctives. Water Science Technology, 18, 171-180.

EXPERIMENTAL DESIGN OF POND STUDIES

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ABSTRACT

The design of pond studies depends on their objectives. In order to obtain new basic information concerning biological effects of pesticides on ecosystems, some degree of system replication is essential to demonstrate that observed changes are caused by treatments. Replication is particularly important to demonstrate causal relationships between treatments and secondary effects such as those on predator/prey interactions, algal blooms and dissolved oxygen concentrations. However, replication of treatments may not be the best strategy to assess the potential hazard of a new pesticide to aquatic environments. Potential hazard of a pesticide is critically dependent on dispersion and degradation and study of these under field conditions does not require replication of treatments. Further, important effects, such as toxicity to fish, can usually be assessed satisfactorily in unreplicated studies, especially if two or more treatment levels are investigated.

INTRODUCTION

Pond studies with pesticides are an established method for evaluating environmental risks associated with compounds that pose a potential hazard to aquatic environments (Boyle, 1985; Crossland and Wolff, 1988; Giddings *et al.*, 1984). Their most important advantage, compared with laboratory or microcosm studies, is that it is possible to study effects of pesticides on aquatic organisms under realistic conditions of exposure to the pesticide. In outdoor aquatic environments pesticides are subject to various transport and degradation processes which can profoundly affect exposure. It is often difficult, or impossible, to assess the exposure of aquatic organisms from laboratory data, either because of difficulties in measuring physicochemical parameters of the pesticides or because of uncertainties in estimating effects of environmental parameters on rates of transport and degradation. Often, these difficulties and uncertainties can be resolved by studies in outdoor, experimental ponds. Furthermore, once the effect of the environment on transport and degradation processes is clearly understood, it is possible to extrapolate from a pond study to other kinds of aquatic environments, using mathematical models (Crossland *et al.*, 1986).

Replication of treatments is essential to demonstrate the link between treatments and relatively small changes in population densities or secondary effects such as those on predator-prey interactions, algal blooms, dissolved oxygen concentrations and fish growth. Replication is particularly important when fundamental insights into the interactions that may occur in complex natural ecosystems are sought. Detailed studies of this kind have been published (Crossland and Hillaby, 1985; Giddings *et al.*, 1984; Hall *et al.*, 1970; Hurlbert *et al*, 1972; Mauck *et al.*, 1976; Papst and Boyer, 1980). Such studies have established that secondary effects can occur if the zooplankton and aquatic insect populations are affected by pesticides. The nature of many of these secondary effects and the reasons for their occurrence are now well established and need not be investigated for each new pesticide.

As far as exposure is concerned there is very little variation between ponds within a block of experimental ponds, irrespective of whether the chemical is transported by evaporation or sorption, or degraded by bio-degradation or photodegradation. This is not surprising as transport and degradation rates are affected by environmental parameters (e.g. windspeed, sunlight, temperature) that do not vary substantially within the boundaries of an experimental site. Thus, there is little value in replicating treatments merely to study spatial variation in exposure. This would require chemical analysis of a relatively large number of samples to acquire relatively little useful information. Detailed studies of the distribution of residues between water, sediment and the biota within only one or two ponds are of more value. Detailed temporal studies often yield much valuable information. Samples should, therefore, be taken in a time series so that the data can be fitted to equations describing changes in the mass balance, thus permitting analysis of process kinetics.

In unreplicated studies statistical methods cannot differentiate between changes caused by natural phenomena and those caused by the pesticide. However, changes caused by acute toxicity of pesticides

are, generally, all-or-nothing, i.e. if a particular species is susceptible its population declines dramatically and suddenly. Such changes can be demonstrated and attributed to the treatment without the use of statistics.

In this paper some replicated pond studies are used to illustrate the effects of transport and degradation processes on the concentration-time (ct) profiles of various chemicals in pond water and to illustrate the absence of significant variation between ponds. A pond study with a new pyrethroid insecticide is described in detail to illustrate the data obtained using a very simple, unreplicated design.

MATERIALS AND METHODS

Field work was carried out in a series of 12 ponds in the flood plain of the river Sherway at Grigg Farm, Headcorn, Kent. Each pond was 10 m long, 5 m wide and 1.0 m deep and separated from its neighbours by concrete dividing walls. The short (5 m) sides and bottoms were composed of alluvial silt, clay and organic matter. Taking into account depth, slope of the banks and irregularities in the rectangular shape, the volume of each pond was calculated to be 40 m^3. When not in use, the ponds were interconnected by pipes and the water occasionally pumped between them to promote the uniform distribution of materials and organisms. Shortly before starting experiments the connecting pipes were closed.

In most of the experiments chemicals were distributed beneath the surfaces of the ponds using a knapsack sprayer fitted with a boom and nozzles. In one experiment, with a pyrethroid insecticide, the chemical was sprayed over the surface of ponds with the spray boom held c. 20 cm above the water. Full details of methods used for chemical and biological analyses of pond samples and methods used for monitoring environmental parameters are given elsewhere (Crossland, 1984; Crossland and Bennett, 1984; Crossland and Wolff, 1985; Crossland *et al.*, 1987).

RESULTS

Transport and degradation processes

Replicated pond experiments were carried out with 2,5,4′-trichlorobiphenyl (3-CB), pentachlorophenol (PCP) and parathion-methyl (MEP) using three replicates of each treatment. Concentrations of chemicals recovered in water from treated ponds are given in Table 1. Almost identical ct profiles were obtained for 3-CB in three different ponds. Approximately 90% of 3-CB was lost by evaporation and 10% by sorption onto sediment. Three very similar ct profiles were obtained for PCP which was lost relatively quickly (half life 2–4 d) by phototransformation. Three very similar ct profiles were also obtained for MEP which was lost by degradation by sediment bacteria.

Table 1
Concentrations (ug l^{-1}) of 2,5,4'-trichlorobiphenyl (3-CB), parathion-methyl (MEP) and pentachlorophenol (PCP) in samples of pond water.

Time (days)	3-CB			PCP			MEP		
	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
0	14*	14*	14*	60*	60*	60*	100*	100*	100*
1	8.0	8.5	7.7	38	35	30	96	84	89
2				30	31	30	88	85	79
4/5	3.0	3.0	2.9				85	85	75
7/8	1.7	1.8	1.8	8	9	10	69	73	69
14–16	0.7	0.8	0.8				40	48	43
28–30	0.2	0.2	0.2				8	20	13

*nominal concentrations

Clearly, the rate of loss of chemicals from the water was primarily determined by environmental parameters that did not vary substantially between ponds. Further, these examples cover most of the major routes for transport and degradation of pesticides in aquatic environments (evaporation, sorption, biodegradation and phototransformation). Thus, there are unlikely to be substantial differences between loss rates of chemicals from the water of different experimental ponds within a block, whatever loss mechanisms may be involved.

An unreplicated pond study

Three adjacent ponds were chosen for this experiment. Pre-treatment examinations and collections of invertebrates were carried out to ensure that the physico-chemical and biological characteristics of the ponds were similar. One of the ponds was treated with the pyrethroid insecticide fenpropathrin (RSα-cyano-3-phenoxybenzyl 2,2,3,3-tetramethyl-1-cyclopropane carboxylate) at a 'high' dose, (equivalent to an overspray with 100 g a.i. ha^{-1}), one at a 'low' dose (equivalent to 10 g a.i. ha^{-1}) and the third pond remained untreated.

Twenty small rainbow trout, *Salmo gairdneri* Richardson, were placed in a wire mesh cage in each pond to determine the effect of fenpropathrin on fish mortality, residues at death and uptake and depuration of residues in fish exposed to sub-lethal concentrations.

Concentration-time profiles for fenpropathrin in the water column and residues in fish were investigated in both of the treated ponds. Residues in fish were also investigated in both of the treated ponds. Fenpropathrin residues in surface water and sediment were determined only for the pond treated with 100 g ha^{-1}.

Effects on zooplankton and macroinvertebrates were monitored by taking samples at weekly intervals from two weeks before until two months after treatment. Effects on phytoplankton were monitored by measuring chlorophyll *a* concentrations in the water column at approximately weekly intervals.

Differences between treatments for populations of all major groups of invertebrates were subjected to one-way analysis of variance followed by Dunnett's test. A significance level of 1% was chosen to separate the control and treatment means. This, rather than the more widely used 5% level was chosen because of the lack of replication. Differences between control and treatment means, even at the 1% level, can be interpreted as either treatment effects or as random events attributable to natural variation between ponds. However, by using a 1% level of significance, the chances of incorrect interpretation of the results were reduced.

In the surface film of the pond treated with 100 g ha^{-1}, the concentration of fenpropathrin was 5,800 $\mu g\ l^{-1}$ 2 h after treatment, decreasing to 230 $\mu g\ l^{-1}$ 24 h after treatment. Maximum concentrations in subsurface water, 6 h after treatment, were 9.5 $\mu g\ l^{-1}$ and 1.1 μg^{-1} in high dose and low dose treatments, respectively. Fenpropathrin was lost from the water column relatively quickly with a pseudo-first-order rate constant of 0.25 d^{-1}, equivalent to a half-life of 2.8 days. No fenpropathrin was found in the sediment (limit of detection 10 $\mu g\ kg^{-1}$).

There were no deaths of fish in the control pond or in that treated with a low dose of fenpropathrin. In the pond treated with a high dose all the fish died one to three hours after treatment. Residues of fenpropathrin in these fish were 0.3 – 0.4 mg kg^{-1}. The cage in this pond was restocked with 20 rainbow trout seven days after treatment and residues of fenpropathrin decreased from 0.11 mg kg^{-1}, seven days after their introduction to the pond, to close to the limit of detection (0.02 mg kg^{-1}) 14 days later. These residue levels indicated a relatively low potential for bioaccumulation and were consistent with the relatively high water solubility of this compound.

Twenty-seven groups of macroinvertebrates were identified in sweep-net samples. There was no indication of any effects on molluscs or oligochaetes. Both treatment levels had severe effects on aquatic insects and mites. Dytiscid beetles, notonectids and corixids were dead or dying very soon after spraying. Significant mortality ($p < 0.05$) of mayfly larvae and water mites occurred in both treatments. There was no evidence of any effect of the low dose treatment on dragonfly and damsel fly larvae and there were too few of them in the high dose treatment to permit assessments. Neither treatment had affected

the benthic macroinvertebrates. Most of the aquatic insect fauna had recovered eight weeks after treatment, although the population of water mites in the high dose treatment was still significantly less than in the control.

The most abundant zooplankters were *Daphnia* spp. (mainly *D. longispina*), *Diaptomus* sp., *Cyclops* sp. and the nauplii of *Diaptomus* and *Cyclops*. Populations of Daphnia spp. (Fig. 1) were severely affected by both treatments. In the low dose treatment, populations were reduced to zero for a period of three weeks and then recovered rapidly to pre-treatment levels four to six weeks after treatment. In the high dose treatment, populations were reduced to zero for four weeks and then recovered to pre-treatment levels from five to seven weeks after treatment. In both treatments the recovering *Daphnia* populations overshot an equilibrium density level before decreasing to pre-treatment levels.

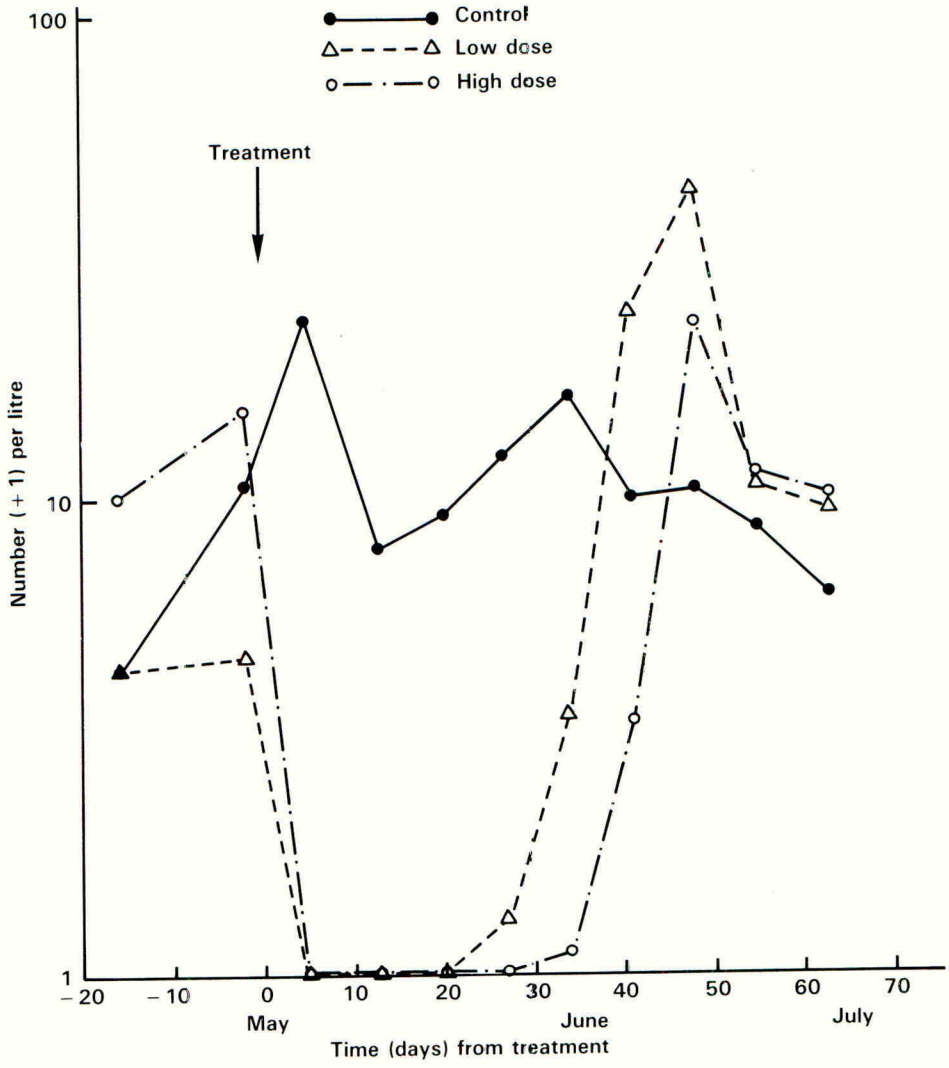

Fig. 1 Effect of fenpropathrin on *Daphnia spp.*

Populations of Diaptomus sp. in both treatments were reduced to very low levels until the last sample, 63 days after treatment.

Populations of *Cyclops* sp. were reduced to zero for a period of four weeks in the high dose treatment. Thereafter, there was gradual recovery, although numbers remained relatively low. The low dose treatment appeared to cause a small, significant ($p < 0.01$), mortality from which the population recovered within a week or two. A population explosion of *Cyclops* then occurred, after which their numbers decreased gradually to pre-treatment levels nine weeks after treatment.

Populations of nauplii were reduced to zero for a period of four weeks following treatment with a high dose. Their numbers then recovered during the period five to nine weeks after treatment. The low dose had a less severe effect and numbers of nauplii recovered more quickly.

Concentrations of chlorophyll a in the untreated pond varied from 0.5 to 6.2 $\mu g\ l^{-1}$. Those in the low dose treatment were generally similar except for a rise to 25.5 $\mu g\ l^{-1}$ 20 days after treatment. Following treatment with a high dose, there were two peaks, of 18.2 and 28.3 $\mu g\ l^{-1}$, representing blooms of phytoplankton 20 and 55 days after treatment. In the control and the low dose treatment, the dissolved oxygen (DO) concentrations showed a typical seasonal pattern, decreasing slowly and steadily from 9 – 10 mg l^{-1} in April/May to 5 – 6 mg l^{-1} in June/July, reflecting the seasonal change in the ratio of photosynthesis : respiration. In the high dose treatment there was a similar seasonal pattern between April and early June but on 16 June and 15 July, (34 and 63 days after treatment) the DO concentration was depressed to 4 mg l^{-1}, following collapse of the phytoplankton blooms.

DISCUSSION

In the unreplicated pond study with fenpropathrin most of the surface-applied insecticide was subsequently found in subsurface water. This was consistent with the fact that the water solubility (330 $\mu g\ l^{-1}$) is substantially greater than concentrations that were expected in the water (15 and 1.5 $\mu g\ l^{-1}$), assuming complete and uniform dispersion into subsurface water. Fenpropathrin was lost from the water column relatively quickly (half-life 2.8 days) with no indication of accumulation in sediment. The probable loss mechanism was biodegradation by sediment bacteria. Residues in fish also decreased relatively quickly, indicating a low potential for bioaccumulation.

Effects of fenpropathrin on fish and aquatic invertebrates were consistent with the results of laboratory toxicity tests and ct profiles for fenpropathrin in pond water. For example, in the laboratory the 96 h LC_{50} was 2.3 $\mu g\ l^{-1}$ for rainbow trout. Mortality of rainbow trout in the ponds was observed when water concentrations were higher than this value but not when water concentrations were less than 2 $\mu g\ l^{-1}$. Secondary effects were similar to those reported in the literature for ponds treated with other insecticides.

These findings, together with data obtained in the laboratory, provide a reasonable basis for estimating the risks to aquatic organisms in the event of contamination of surface waters by fenpropathrin.

REFERENCES

Boyle, T.P. (Ed.). *Contaminants in Aquatic Ecosystems*. American Society for Testing and Materials, Philadelphia, 1985.

Crossland, N.O. (1984). Fate and biological effects of methyl parathion in outdoor ponds and laboratory aquaria II. Effects. *Ecotoxicology and Environmental Safety, 8*, 482 – 495.

Crossland, N.O. and Bennett, D. (1984). Fate and biological effects of methyl parathion in outdoor ponds and laboratory aquaria I. Fate. *Ecotoxicology and Environmental Safety 8*, 471 – 481.

Crossland, N.O., Bennett, D. and Wolff, C.J.M. (1987). Fate of 2,5,4′-trichlorobiphenyl in outdoor ponds and its uptake via the food chain compared with direct uptake via the gills in grass carp and rainbow trout. *Ecotoxicology and Environmental Safety 13*, 225 – 238.

Crossland, N.O., Bennett, D., Wolff, C.J.M. and Swannell, R.P.J. (1986). Evaluation of models used to assess the fate of chemicals in aquatic systems. *Pesticide Science 17*, 297 – 304.

Crossland, N.O. and Hillaby, J.M. (1985). Fate and effects of 3,4-dichloroaniline in the laboratory and in outdoor ponds: II. Chronic toxicity to *Daphnia* spp. and other invertebrates. *Environmental Toxicology and Chemistry 4*, 489 – 499.

Crossland, N.O. and Wolff, C.J.M. (1985). Fate and effects of 3,4-dichloroaniline in the laboratory and in outdoor ponds: I. Fate. Environmental Toxicology and Chemistry 4, 481 – 487.

Crossland, N.O. and Wolff, C.J.M. (1988). Outdoor ponds: their construction management and use in experimental ecotoxicology. In *The Handbook of Environmental Chemistry*, Vol. 2D, Hutzinger, O., Ed., Springer-Verlag, Berlin.

Giddings, J.M., Franco, P.J., Cushman, R.M., Hook, L.A., Southworth, G.R. and Stewart, A.J. (1984). Effects of chronic exposure to coal-derived oil on freshwater ecosystems: II. Experimental ponds. *Environmental Toxicology and Chemistry 3*, 465 – 488.

Hall, D.J., Cooper, W.E. and Werner, E.E. (1970). An experimental approach to the production dynamics and structure of freshwater animal communities. *Limnology and Oceanography, 15*, 839 – 928.

Hurlbert, S.H., Mulla, M.S. and Willson, H.R. (1972). Effects of an organophosphorus insecticide on the phytoplankton, zooplankton and insect populations of freshwater ponds. *Ecological Monographs, 42*, 269 – 299.

Mauck, W.L., Mayer, F.L. Jr. and Holz, D.D. (1976). Simazine residue dynamics in small ponds. *Bulletin of Environmental Contamination and Toxicology, 16*, 1 – 8.

Papst, M.H. and Boyer, M.G. (1980). Effects of two organophosphorus insecticides on the chlorophyll a and pheopigment concentrations of standing ponds. *Hydrobiologia, 69*, 245 – 250.

TESTING INSECTICIDES FOR USE IN RICE/FISH CULTIVATION

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ABSTRACT

In order to evaluate the hazard of a new insecticide to fish in rice paddies a series of laboratory and field tests was carried out in the UK and West Java.

In the laboratory, 96 h LC_{50} values for rainbow trout (*Salmo gairdneri*) revealed that an SC formulation of the pyrethroid insecticide alphacypermethrin was much less toxic to fish than an EC formulation. Small scale enclosure tests carried out in the UK in an outdoor pond confirmed the lower hazard of the SC formulation.

Subsequently the acute toxicity of the SC formulation of alphacypermethrin to fish was assessed in the laboratory and in the field in West Java. In the field, small rice paddies stocked with common carp (*Cyprinus carpio*) and Java carp (*Puntius gonionotus*) were treated with a 'standard' insecticide or with alphacypermethrin SC; only the 'standard' insecticide caused significant fish mortality. Finally, experiments in which alphacypermethrin SC was used under a full season commercial spray regime revealed no adverse effects on fish growth or productivity.

The utility of this approach to testing rice insecticides is discussed.

INTRODUCTION

The cultivation of rice in paddies involves the use of large volumes of water and in some parts of the world this has led to the simultaneous use of paddies for rice and fish cultivation. Because of this close association, it is important that pesticides used in rice should not pose a hazard to fish.

Laboratory studies can be used to determine the toxicity of technical and formulated pesticides to fish. If these tests indicate that there is a sufficient margin of safety between toxic concentrations and concentrations which might be achieved in shallow waters oversprayed at recommended application rates, then further testing may not be necessary. However, if the laboratory studies indicate that there is even a possibility of toxic effects in the field, the hazard to fish will need to be examined further. This more precise assessment of hazard to fish can best be achieved by a series of field experiments.

The sequential approach to hazard evaluation was recommended by FAO (1981) and has been widely used in recent years. Stephenson (1982), Crossland (1982) and Crossland *et. al.* (1982) described how such an approach was used to assess the hazard of the synthetic pyrethroid cypermethrin (RIPCORD*) to the aquatic environment. More recently Stephenson (1984) described a series of studies with cypermethrin aimed at assessing its acute toxic hazard to fish when it was used for pest control in rice. The sequence of studies with cypermethrin involved acute toxicity testing of the technical material in the laboratory, testing of formulated material in indoor tanks and finally cage tests in paddy rice. The present paper describes how this approach has been developed and extended to assess the hazard to fish in rice paddies of a novel particulate formulation of the pyrethroid insecticide alphacypermethrin (FASTAC*).

INITIAL STUDIES IN THE UK

Laboratory tests

The acute toxicity of technical alphacypermethrin to the rainbow trout (*Salmo gairdneri*) was determined at Sittingbourne Research Centre in a semi-static water test with 12 hourly renewal of the test media

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made up in filtered (8 μm) mains tap water. Ten *S. gairdneri* (mean weight 3.3 g) were exposed to each of a series of concentrations of alphacypermethrin in 40 l glass aquaria at 15°C. Analysis of the fresh test media and the test media immediately prior to renewal (12 h later) by glc-ecd indicated that initial concentrations were approximately 80% of nominal values and that concentrations fell by some 25% during the 12 hours between renewals. The 96 h LC_{50} value for alphacypermethrin, based on nominal exposure concentrations, was calculated to be 2.8 μg l^{-1}. This high acute toxicity to fish is a characteristic shared by other synthetic pyrethroids (Hill, 1987).

Subsequent laboratory tests with two formulations of alphacypermethrin, an emulsifiable concentrate (EC) and a suspension concentrate (SC), revealed marked differences in their acute toxicity to *S. gairdneri*. In 96 h static (without renewal of the test media) water tests ten *S. gairdneri* (1 – 5 g) were exposed in 20 l of filtered (8 μm) mains tap water to each of a series of concentrations of the two formulations at 15°C. There were clear differences in the LC_{50} values for the two formulations (Table 1). The SC formulation with a 96 h LC_{50} of 240 μg a.i. l^{-1} was some 50 times less toxic than the EC formulation, which with a 96 h LC_{50} of 5 μg a.i. l^{-1} was of similar toxicity to the technical material.

TABLE 1
Acute toxicity of an EC and an SC formulation of alphacypermethrin to *S. gairdneri*

	LC_{50} μg ai l^{-1}			
	24h	48h	72h	96h
SC formulation	> 500	380	270	240
EC formulation	5	5	5	5

Field experiments

The difference in the toxicity of the two formulations revealed in the laboratory tests was further explored in a field study carried out in enclosures in a small pond. The methods used were as described by Shires (1983 and 1985) and involved introducing a series of open-ended stainless steel enclosures with a capacity of ~ 1 m^3 into a mature experimental pond located near Headcorn, Kent, UK. The enclosures were pushed into the pond sediment, forming an effective seal, and the tops left open to the air. Each enclosure therefore had a sediment/water interface and a water/air interface. Twenty rainbow trout (~ 5 g) were introduced into each enclosure. Four dosages of each formulation were tested by spraying diluted formulation onto the water surface of different enclosures using a hand-held aerosol sprayer. The fish were then monitored for mortality for eight days. Temperatures during the experiment were low (~ 6°C). Table 2 summarises the results of the experiment and shows that the SC formulation, with only 5% mortality at an application rate of 300 g ai ha^{-1}, had much less effect than the EC formulation, which caused mortality at 30 g ai ha^{-1} but not at 10 g ai ha^{-1}.

TABLE 2
Mortality (%) of *S. gairdneri* after eight days in pond enclosures treated with either the SC or the EC formulation of alphacypermethrin

	Dose rate (g ai ha^{-1})			
	10	30	100	300
SC formulation	0	0	0	5
EC formulation	0	30	100	100

These data from laboratory and field tests in the UK indicated that the SC formulation of alphacypermethrin should provide a good margin of safety for fish present in paddy rice. In view of this a further series of experiments was carried out in West Java to fully assess the effects of the SC formulation on fish under conditions more relevant to its use as a rice insecticide. Simultaneous studies on the effects of the SC formulation on important rice pests and beneficial organisms were carried out (Shires, 1986).

STUDIES IN WEST JAVA

Laboratory tests

The acute toxicity of the two formulations to *Cyprinus carpio* (common carp) and *Puntius gonionotus* (Java carp) was determined in 96 h semi-static water tests with 24 hourly renewal of the test media. For each test substance seven glass vessels were filled with 20 l of aerated tap water and quantities of a dispersion of one of the formulations added to six of them, the seventh received no test substance and served as a control. Ten *C. carpio* (3.5 – 4.0 g) or ten *P. gonionotus* (0.3 – 0.5 g) were introduced to each test vessel. The contents of the vessels were aerated and during the tests water temperatures ranged from 24 to 30°C. Under the conditions of these tests *C. carpio* and *P. gonionotus* were more susceptible to both the EC and SC formulations than had been *S. gairdneri* in the tests carried out in the UK and, *P. gonionotus* appeared to be more susceptible than *C. carpio* (Table 3). However the 24 h and 96 h LC_{50} values for both species still indicated a marked difference in the toxic effects of the two formulations with the SC being 8 to 35 times less toxic than the EC, depending on the species tested and length of exposure (Table 3).

TABLE 3
Acute toxicity of an EC and a SC formulation of alphacypermethrin to *C. carpio* and *P. gonionotus*

	LC_{50} (μg ai l^{-1})			
	C. carpio		*P. gonionotus*	
	24h	96h	24h	96h
SC formulation	460	11	20	3.2
EC formulation	4.5	0.8	0.7	0.4

In another experiment carried out in a series of 12 outdoor tanks the toxic effects of the SC formulation of alphacypermethrin were compared with those of two standard insecticides, one an EC and the other a granule (G), both widely used for pest control in rice. The bottoms of the tanks (215 × 75 cm) were partially covered with coarse gravel and then filled with tap water to a depth of 20 cm. For each insecticide three application rates were used, the recommended commercial rate (proposed rate for alphacypermethrin SC) and 1/2 and 1/4 of this rate. There was also a control tank for each insecticide which received no treatment.

Fifteen *C. carpio* (mean weight 6.3 g) were introduced into each tank. The fish were fed daily during the test and water temperature ranged from 23 – 26°C. In two of the three control tanks no mortality occurred; in the other, 3 fish died over the period day 5 – 7 (Table 4). Mortality in the tanks treated with alphacypermethrin did not exceed 20%, the same as the highest control mortality and was not dose-related. In all of the tanks treated with the standard EC and in the tank treated with the commercial rate of the standard granule there was a high mortality.

TABLE 4
Mortality of *C. carpio* 7 days after the application of insecticides to outdoor tanks

Application Rate	% Mortality after 7 days		
	Alphacypermethrin SC	Standard EC	Standard granule
Control	20	0	0
1 × Commercial Rate	7	100	87
½ × Commercial Rate	20	100	7
¼ × Commercial Rate	0	100	13

Note:
Commercial rate for alphacypermethrin SC = 15 g ai ha^{-1}; standard EC = 250 g ai ha^{-1}; standard granule = 510 g ai ha^{-1}.

These results indicated that the SC formulation of alphacypermethrin was unlikely to be toxic at commercial rates in the field and a series of field experiments was therefore carried out to see if this was the case.

Field Experiments

Acute toxicity study
This study was designed to assess the acute toxic effects of the SC formulation of alphacypermethrin on fish under field conditions in rice paddies. The experiment was carried out in a series of purpose-built paddies using both caged and free fish, and compared the acute lethal effects of alphacypermethrin SC with that of the standard EC used in the outdoor tank tests. The experiment was carried out 12 days after transplantation when the rice was at an early stage of development.

Each plot of paddy rice was 5m × 5m and had a diagonal trench across it which was 20 cm deeper than the rest of the plot and approximately 50 cm wide. On the day prior to treatment with the insecticides the water depth in the plots was adjusted to 10 cm and the plots sealed for the 7 days of the study. There were three replicate plots of each of the following treatments:

Control –	no insecticide
Alphacypermethrin SC	7.5 g ai ha^{-1}
Alphacypermethrin SC	15 g ai ha^{-1}
Alphacypermethrin SC	30 g ai ha^{-1}
Standard EC	200 g ai ha^{-1}

The rates for alphacypermethrin were chosen to bracket the likely commercial rate of 15 g ai ha^{-1}. All applications were made at 500 l ha^{-1} by knapsack sprayer.

Prior to application of the insecticides 4 fish cages were placed in the trench in each plot. Two cages in each plot contained 20 *C. carpio* (2.5 – 5.0 g) and two 20 *P. gonionotus* (1.0 – 4.5 g). In addition 30 *C. carpio* and 30 *P. gonionotus* were released to swim freely in each plot. The plots were checked at least twice daily and fish found dead recorded. Water temperatures during the experiment were 25 – 34°C.

The percentage mortality of the caged and free fish at the end of the experiment is given in Table 5. Results for the two species were similar. For both, only the standard EC caused significant mortality, 70% or greater in all cases except for the free *P. gonionotus* where only 36% died. The mortality of caged or free fish in the plots treated with alphacypermethrin SC only exceeded 7% in one case (when it was 15%) and generally was less than 5%.

TABLE 5
Mortality of *C. carpio* and *P. gonionotus* during the seven days after application of insecticides in an experiment in Bogor, West Java

Treatment	Mean mortality (%)			
	Caged fish		Free fish	
(g ai ha^{-1})	*C. carpio*	*P. gonionotus*	*C. carpio*	*P. gonionotus*
Control	1	1	1	1
Standard EC (200)	71*	70*	82*	36*
Alphacypermethrin SC (7.5)	7	3	2	0
Alphacypermethrin SC (15)	15	5	1	1
Alphacypermethrin SC (30)	2	4	0	1

* Significantly higher mortality than in the control.

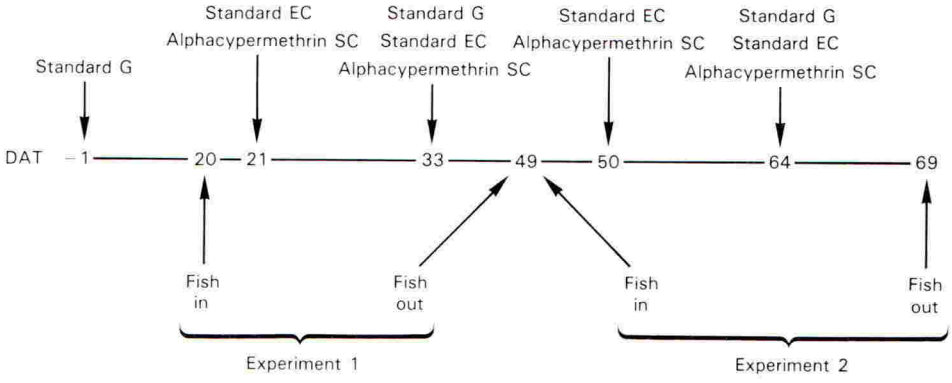
The lack of a significant effect of the SC formulation of alphacypermethrin was encouraging, particularly because the conditions under which the experiment was carried out were such as to have maximised the hazard. The application took place early in the growing season when crop- cover was minimal; the water was only some 10 cm deep; the plots were sealed during and after the insecticide applications and the *C. carpio* used were small. In the light of these promising results a further set of experiments was carried out the following year, again in West Java.

Effects on growth and productivity

In these experiments, the effects of the alphacypermethrin SC and two widely used rice insecticides (the standard EC and the standard granule used in the outdoor tank experiment) on the survival and growth of *C. carpio* were examined under a season-long, commercially recommended spray regime (Fig. 1).

FIGURE 1

Spray regime used to study effects on fish survival and growth in two experiments



DAT = Days after transplantation

As in the previous field study a replicated experiment design was used, four plots of paddy rice (5m × 10m) were treated with each insecticide and four remained untreated as controls. Each insecticide was applied at the recommended rate (see Table 4) following a pre-established commercial spray regime.

Two experiments were carried out. During each, the plots received two applications of either alphacypermethrin SC or the standard EC and one application of the standard granule (Fig. 1). The alphacypermethrin SC and the standard EC were applied by calibrated knapsack sprayers and the standard granule by hand.

Twenty weighed *C. carpio* were released into each of the plots, 20 days after transplantation of the rice for the first experiment and 49 days after transplantation for the second. The mean weight of the fish in the first experiment was 7.6 g and in the second 6.0 g. The depth of the water in the plots at the time of spraying was less than 10 cm and the subsequent flow of water into the plots was limited to that required to replace losses resulting from evaporation and leakage. The plots were checked daily for dead fish and at the end of each experiment the plots were drained down and the surviving fish collected and weighed.

The mean values for mortality of *C. carpio* assessed on the basis of dead fish found during the 48 h following application of the insecticides are given in Table 6. Only the standard EC posed an acute lethal hazard to the fish, neither of the other treatments causing any significant mortality during the 48 h after treatment.

TABLE 6

Mean mortality (%) of *C. carpio* in the 48 hours after application of insecticides at varying numbers of days after transplanting rice (DAT). No application of granules of the standard insecticide were made 21 DAT or 50 DAT. This trial took place at Pusakanagara, West Java

Treatment	Mean mortality %			
	Experiment 1		Experiment 2	
(g ai ha^{-1})	(21 DAT)	(33 DAT)	(50 DAT)	(64 DAT)
Control	0	0	0	0
Alphacypermethrin SC (15)	1	0	0	0
Standard EC (250)	18	0	35	0
Standard G (517)	–	0	–	0

At the end of each experiment a significant proportion of the fish introduced had not been recovered as corpses during the experiment or as live fish at the end. This proportion ranged from 36% to 71%. These fish are thought to have been taken by predators, probably snakes, considerable numbers of which were found when the plots were drained. Despite the loss of these fish interpretation of the data on fish growth and productivity is clear. None of the treatments had a deleterious effect on fish growth (Table 7), indeed in the first experiment the fish from plots treated with the alphacypermethrin SC had grown significantly more than those in the control plots. However, the total weight of fish harvested from the plots treated with the standard EC was markedly less than that from the control plots, plots treated with alphacypermethrin SC or those treated with the standard granule. This was due to the fish mortality caused by the applications of the standard EC.

TABLE 7

Growth of *C. carpio* in paddies treated with insecticides at varying numbers of days after transplanting rice (DAT), following a commercial spray regime. The mean weight of the fish at the start was 7.6 g in Experiment 1 and 6.0 g in Experiment 2.

Treatment	Experiment 1 (20 – 49 DAT)		Experiment 2 (49 – 60 DAT)	
	Mean wt.	Total wt.	Mean wt.	Total wt
(g ai ha^{-1})	(g)	(g)	(g)	(g)
Control	23	800	14	330
Alphacypermethrin SC (15)	30*	1100	17	450
Standard EC (250)	29	410	–	–
Standard G (517)	21	1100	16	440

* Significantly different from control ($p < 0.05$)

CONCLUSION

Taken together, the results of this series of experiments in the UK and West Java provide convincing evidence of the lack of hazard of alphacypermethrin SC to fish in rice paddies. What is more, the experiments indicate the value of a step-wise approach to evaluating the hazard posed by pesticides to fish in rice paddies. The initial experiments in the UK demonstrated that alphacypermethrin SC was less toxic to rainbow trout than alphacypermethrin EC. This was confirmed in simple enclosure experiments in the field.

Subsequent experiments in the laboratory and in the field in West Java confirmed that *C. carpio* and *P. gonionotus* were also less susceptible to alphacypermethrin SC than to alphacypermethrin EC. Field experiments were then used to show that there were no acute lethal effects on these fish when paddies were sprayed with alphacypermethrin SC at double the proposed commercial rate under conditions which posed maximum hazard. Finally, experiments under a season-long commercial spray regime showed no effects of alphacypermethrin SC on fish growth or productivity.

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REFERENCES

Crossland, N.O. (1982) Aquatic toxicology of cypermethrin. II. Fate and biological effects in pond experiments. *Aquatic Toxicology 2*, 205 – 222.

Crossland, N.O.; Shires, S.W; Bennett, D. (1982) Aquatic toxicology of cypermethrin. III. Fate and biological effects of spray drift deposits in freshwater adjacent to agricultural land. *Aquatic Toxicology 2,* 253 – 270.

FAO (1981) Second Expert Consultation on Environmental Criteria for Registration of Pesticides. FAO *Plant Production and Protection*, Paper 28 FAO, Rome.

Hill, I.R. (1985) Effects on non-target organisms in terrestrial and aquatic environments in *The Pyrethroid Insecticides* Ed. J.P. Leahey, Taylor and Francis, London and Philadelphia.

Shires, S.W. (1983) The use of small enclosures to assess the toxic effects of cypermethrin on fish under field conditions. Pesticide Science *14*, 475 – 480.

Shires, S.W. (1985) Toxicity of a new pyrethroid insecticide, WL85871, to rainbow trout. *Bulletin of Environmental Contamination and Technology 34*, 134 – 137.

Shires, S.W. (1986) An integrated approach to developing a new rice insecticide. *Proceedings of the Second International Conference on Plant Protection in the Tropics*, Genting Highlands, Malaysia 198 – 201.

Stephenson, R.R. (1982) Aquatic toxicology of cypermethrin. I. Acute toxicity to some freshwater fish and invertebrates in laboratory tests. *Aquatic Toxicology 2*, 175 – 185.

Stephenson, R.R. (1984) Determining the toxicity and hazard to fish of a rice insecticide. *Crop Protection 3*, (2), 151 – 165.

DISCUSSION

P. Mineau: Whereas you may not need any replication for compounds with half lives of two to four days, do you think that, for compounds which also have 100% differences in their half-lives but which have much longer half lives, that you do need replicates?

R. Stephenson, Shell Research Ltd: If one had a substance wwith a long half life then one may well want to look at that aspect of experiments. I would suggest that, in the current climate, there will not be many agricultural chemicals which have long half lives.

H. Crick, Aberdeen University: To follow that question up, a quick calculation shows that, on the basis of 100% difference in half life, after 30 days there will be an order of magnitude difference in the residues in the water. This could be important, so lack of replication will preclude good measures of variability and proper assessment of the compound's stablity will be impossible.

R. Stephenson: One must look at it as you are suggesting. One takes an upper limit, predicted on the sort of error that you suggest will occur, and then decides whether or not that causes any concern. We have sufficient experience to indicate that variability is not likely to be much greater than a factor of two. This can be used to judge whether or not it is going to give you a problem. I would not argue, on any grounds, that it is always appropriate to use unreplicated pond studies. It is matter of recognising that they have a role to play, and not dismissing them as something which can't give useful information. One must decide whether or not a replicated pond study is needed in the first instance, with limited knowledge of treatment concentrations to use or of the required sampling frequency. An unreplicated pond study leads you into setting up a better replicated study, if this proves necessary. In many instances, it will give sufficient information to judge the effect of the pesticide or to identify a particular aspect to be studied in more detail.

R. Brown: Could you comment on how you identify potential side-effects of insecticides in wide scale rice/fish culture? What is the relative importance of the effects of the insecticide on the fish food compared to the direct toxicity in the fish?

R. Stephenson: Firstly, one does not want to kill the fish directly, because that results in no fish. That is the most important criterion. Whether or not the pesticide will have an indirect effect in terms of fish growth is secondary. It is often quite difficult to detect. Many fish grown in paddy systems do not feed on only invertebrates or algae but will feed on what is there. One, therefore, has a complex system to deal with.

R. Brown: Do you have any idea of the mechanism behind the safety factor between the two formulations?

R. Stephenson: It is associated with aspects of formulation. We know from previous experience that when pyrethroids are absorbed to particulate organic matter they are "less available" to fish and the mechanism is based on that observation.

B. Bagnall: Dr Matthiessen, in aquatic studies would you recommend that we should be looking at normal dose rates, those several times higher than normal or fractions of normal? We wonder sometimes if we should be looking

for spillage factors, where dose rates might be very high. On the other hand should we be looking at fractions of normal doses, such as might arise from spray drift?

P. Matthiessen, MAFF Fisheries Laboratory: I can only give my personal view, rather than an official Ministry response. My view is that we do have to take spillage factors into account in certain circumstances. An example of this might be TBT. I know TBT has now been banned for use in small vessels but it is still used in timber treatment. There have been a number of large fish kills in the last few years caused by spillage of TBT, and dieldrin, contained in different treatment products. There would seem to be a lack of knowledge on what happened in those circumstances in the aquatic environment. I would argue that apparently excessive doses might be worth investigating, especially with regard to decay from high doses and seeing at what stage re-invasion takes place.

M. Greaves, Long Ashton Research Station: Dr Matthiessen, you said that you could not account for a very large proportion of the herbicide that entered the soil. You assumed it had gone straight down to ground water. Presumably, it comes out of ground water at some time in the future. There is evidence that in some of our rivers there is a continuous exposure of the flora and fauna to low levels of herbicides. Would you like to speculate on the environmental impact of that? It seems to me to be a potentially severe long-term exposure and we haven't discussed many real long-term impacts in the proceedings of the last day or so.

P. Matthiessen: I think you are right. That could be one source of the herbicides we see very widely in surface waters. But I do not know any more than that. I do not know how herbicides behave in ground water, whether they are still susceptible to degradation, for instance, under aquifer conditions or whether or not they can reside there for a long time and then reappear in surface waters.

M. Greaves: What about the biological response to long-term exposure to very low doses? Can you speculate about that?

P. Matthiessen: It depends on the compound you are looking at; I don't think you can generalise. As far as we can tell, the compounds which have been very widely found in surface waters are not a threat to aquatic life. However, that is an outcome of luck rather than judgement on the part of pesticide authorities. Maybe in the future we need to think more carefully about possible routes from aquifers into surface water.

P. Matthiessen: Can I ask about the prochloraz work? We are interested in possible routes of uptake of pollutants. We found dramatic differences in suceptibility when animals were under semi-natural conditions compared to conditions where they were exposed to solutions of TBT in the laboratory. I wonder if, in the prochloraz work, the *Macoma* were exposed to a solution of prochloraz or to naturally-contaminated sediment?

L. Somerville, Schering Agrochemicals: The *Macoma* in the laboratory were exposed to one of the marker compounds which, of course, was not prochloraz. But it was exposed in solution.

P. Matthiessen: Do you think that under conditions of sediment contamination we might get a different pattern of uptake?

L. Somerville: It is always possible, because these animals are filter feeders, that if the chemical was absorbed it might affect the *Macoma* differently. This, however, raises questions of what is the right sediment particle size and how do you contaminate it with the right concentration of chemical? We are not attempting to do anything very technical in the laboratory, rather we are trying to gain data that will, if possible, provide explanations for the findings of detailed analyses after the plant comes on stream.