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Determining the toxicity and hazard to fish of a rice insecticide

R. R. STEPHENSON*, S. Y. CHOI† AND A. OLMOS-JEREZ‡

**Shell Research Limited, Sittingbourne Research Centre, Sittingbourne, Kent ME9 8AG, UK, †Department of Agricultural Biology, College of Agriculture, Seoul National University, Suweon, Korea, and ‡Servicio Proteccion Vegetales, Apartado de Correos 125, Sillia, Valencia, Spain*

ABSTRACT. The experiments described were carried out to establish whether the effects of the insecticide, Ripcord (active ingredient cypermethrin), on fish, would limit its use in rice. Laboratory and field experiments were carried out, and the effects of cypermethrin were compared with those of a 'standard insecticide', carbofuran, widely used for pest control in rice, and a 'positive control', chlorfenvinphos, expected to be toxic to fish in rice paddies at insecticidally effective rates. Acute toxicity tests in the laboratory with the technical materials on the fish *Tilapia nilotica* showed cypermethrin, with a 96 h LC_{50} value of 2 $\mu g/\ell$, to be some 20 times more toxic than chlorfenvinphos, with a 96 h LC_{50} value of 39 $\mu g/\ell$, and some 250 times more toxic than carbofuran with a 96 h LC_{50} of 480 $\mu g/\ell$. The LC_{50} values obtained from laboratory tests with formulated products (cypermethrin EC, chlorfenvinphos granules and carbofuran granules) on *T. nilotica* and *Cyprinus carpio* were broadly in agreement with those obtained from the tests with the technical materials and confirmed *C. carpio* to have a similar susceptibility to that of *T. nilotica*. However, a field experiment carried out in paddy rice in Korea with commercial formulations and at commercial application rates showed that mortality of caged *C. carpio* was much less with cypermethrin (< 15%) than with chlorfenvinphos (97%) or carbofuran (67%). A second field experiment in Spain where cypermethrin was applied by air resulted in no mortality of caged *C. carpio* held in the paddy. It is concluded that the limited toxic effects of cypermethrin on fish in the field, when compared with those of chlorfenvinphos and carbofuran, make it acceptable for use in rice. The effects of cypermethrin were limited in comparison with those of chlorfenvinphos and carbofuran because only very low application rates of cypermethrin are needed to give pest control. The penetration of the liquid cypermethrin formulations into the water was also lower than that of the granular insecticides and, finally, cypermethrin was more rapidly lost from water. Together, these factors are sufficient to explain the minimal toxic effects of cypermethrin in the field when compared with chlorfenvinphos and carbofuran, despite its considerably higher acute toxicity determined in laboratory tests.

Introduction

Rice cultivation involves the use of large volumes of water and, as a result, rice and fish cultivation are closely linked in many areas. Even in areas where fish cultivation is not important, fish are likely to be present in the rice paddies or associated waterways. In these circumstances it is clearly desirable that pesticides used in rice should not pose a hazard to fish populations.

Laboratory studies can be used to determine the toxicity to fish of technical and formulated pesticides. If these tests indicate that the recommended application rates are unlikely to result in toxic effects on fish in the field, then further testing may not be necessary. However, if the laboratory studies indicate the possibility of toxic effects occurring in the field, then the hazard to fish can best be determined by field experiments, or the monitoring of limited 'commercial' applications. This sequential approach to hazard evaluation has been recommended by FAO (1981) and was followed by Stephenson (1982), Crossland (1982) and Crossland, Shires and Bennett (1982) when they investigated the aquatic toxicology of the pyrethroid insecticide cypermethrin. The experiments reported here pursued this sequential approach, but with the particular aim of assessing the possible hazard to fish resulting from the use of Ripcord* (cypermethrin; (*RS*)α-cyano-3-phenoxybenzyl (1 *RS*)-*cis,trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate) in rice. Laboratory and field experiments were carried out in which the effects of cypermethrin were compared with those of a 'standard insecticide', carbofuran, widely used for pest control in rice, and a 'positive control', chlorfenvinphos, expected to be toxic to fish in rice paddies at insecticidally effective rates. The formulations used were, for cypermethrin, an emulsifiable concentrate (EC), and for chlorfenvinphos and carbofuran, granules. A carbofuran granule was chosen because it is the formulation widely used in rice. The cypermethrin and chlorfenvinphos formulations used were chosen because they were considered to be those most likely to be used commercially in rice.

In the laboratory the acute toxicity of the technical and formulated insecticides to a tropical fish (*Tilapia nilotica* L.)† was determined. *T. nilotica* was used as it is a species frequently cultivated in association with rice in many parts of the world. The toxicity of the technical materials was determined in a continuous-flow test and that of the formulations in a static test. The formulations were applied to the surface of the water of the test vessels containing the fish. The application rates used encompassed commercial rates.

Two field experiments were carried out, the first in Korea, the second in Spain. The first field experiment was a replicated plot design in which the three formulated insecticides were applied to a maturing crop of paddy rice at likely or established commercial rates. During the experiment caged fish were observed for toxic effects. It had been planned to use *T. nilotica* in this study so that the results obtained would be directly comparable with those obtained from the earlier laboratory studies, but this was not possible and *Cyprinus carpio* (L.) were substituted. For this reason the acute toxicity of the formulated insecticides to *C. carpio* was determined in simple static water tests immediately prior to the field experiment.

In the second field experiment a large-scale aerial application of cypermethrin was made to paddy rice in Spain, and again the effects on caged fish (*C. carpio*) were observed.

* A Shell registered Trade Name. † Since acceptance of this paper it has come to the authors' attention that *Tilapia nilotica* L. has been renamed *Oreochromis niloticus* (Trewavas, 1983).

Materials and methods

Laboratory acute toxicity tests

Three groups of laboratory toxicity tests were carried out. In the first, the acute toxicity of technical cypermethrin, chlorfenvinphos and carbofuran to *Tilapia nilotica* was determined in 96 h continuous-flow tests. Subsequent to this the acute toxicity of the formulated materials to *T. nilotica* was determined in 96 h static water experiments. Finally, the acute toxicity of the formulated materials to *Cyprinus carpio* was determined immediately prior to the Korean field experiment using a 48 h static water test procedure.

Continuous-flow tests with technical materials

The purity of the samples of technical cypermethrin, chlorfenvinphos and carbofuran used was 98·4%, 91·3% and 99·0% respectively. The cypermethrin had a *cis*: *trans* ratio of 1:1 and the chlorfenvinphos a *Z*:*E* isomer ratio of 8·7:1.

Solutions of cypermethrin in water, for use as stock solutions in the test, were prepared by dissolving 0·25 g of cypermethrin in 250 ml of Analar acetone and pouring this on to 250 g of sieved pumice held in a glass column. The contents of the column were then evaporated to dryness and a continuous flow of water was pumped through the column. After allowing several days for stabilization the solution generated from the column and dilutions of it were used as stock solutions in the toxicity test. For chlorfenvinphos and carbofuran concentrated stock solutions were made up in Analar acetone. Before dosing, these were diluted with water so that the concentration of acetone in the test solutions was always less than 1 $\mu l/\ell$.

The *T. nilotica* used in the test were obtained from the University of Stirling, Scotland. Before use they were acclimatized to the test conditions for at least 6 days. The fish used in the tests weighed between 0·6 and 3·0 g and appeared to be healthy.

The tests were carried out in an all-glass test system in which the desired concentrations of test substance were obtained by blending together flows of diluent water, filtered (8 μm) dechlorinated laboratory mains supply, with flows of the stock solutions. The resultant flows were then passed to 20 litre glass test vessels containing the fish. In each test there was a series of test concentrations and a control which received only diluent water. Flow rates through the test vessels were in the range 45–65 ml/min, giving an 80% replacement time of 12–18 h. During the tests the temperature of the test solutions was maintained at 25 ± 2°C. The total hardness, pH and concentration of dissolved oxygen in the control and the highest test concentration were determined daily, total hardness by titration against EDTA and the pH and dissolved oxygen with electronic instruments. Water quality during the three tests was comparable and varied little. Ranges for total hardness (as $CaCO_3$), pH and concentration of dissolved oxygen were 230–270 mg/ℓ, 7·1–8·1 mg/ℓ and 7·5–8·5 mg/ℓ respectively.

Samples of the test solutions and controls were taken at intervals during the tests and the concentrations of test substance were determined. Samples for cypermethrin determination were extracted with hexane, dried by passage through anhydrous sodium sulphate and analysed by gas-liquid chromatography (glc) using electron capture detection (ecd) with a glass column packed with 3% OV1 on 100/120 mesh GCQ. Samples for chlorfenvinphos determination were either extracted with hexane, dried by passage through anhydrous sodium sulphate and analysed by glc/ecd using a glass column packed with 3% Dexsil 300 on 100/120 mesh GCQ 01, diluted 1:1 with methanol or analysed by high performance liquid chromatography (hplc) using a stainless steel column packed with Spherisorb ODS, and with 1:1 acetonitrile/water as an eluant. Samples for determination of carbofuran were passed through a column containing octadecyl silyl bonded to silica. The columns were then eluted with acetonitrile and the eluant was diluted to give 1:1 acetonitrile/water. The diluted eluants were analysed by hplc, using a stainless steel column packed with Lichrosorb RP8, 40% acetonitrile/water being used as eluant.

Ten *T. nilotica* were exposed in each test vessel; they were not fed during the test. At 3, 8, 24, 48, 72 and 96 h after their introduction the fish were examined; the number of dead was recorded and the dead were removed. The criterion of death was no opercular movement during a 15 second period of observation. No control fish died during the tests. The LC_{50} values (those concentrations lethal to 50% of the fish exposed for a defined period) were calculated by probit analysis using log-transformed analytically determined concentration values (Finney, 1971). If the data were insufficient for probit analysis LC_{50} values were estimated by graphical interpolation using log/probit graph paper (APHA, 1980).

Static water tests with formulated materials and T. nilotica

The formulations used were cypermethrin 25 g/ℓ emulsifiable concentrate (EC), chlorfenvinphos 50 g/kg granules and carbofuran 15 g/kg granules.

The *T. nilotica* were from the batch used in the continuous-flow tests. At the time of testing the fish used weighed between 0·3 and 1·4 g and had been acclimatized to the test conditions for more than 10 days.

The tests were carried out in a series of stainless steel tanks (70 × 100 × 60 cm deep) situated in a glasshouse. Twelve tanks were used for each experiment; they were filled to a depth of 30 cm with mains water and allowed to stand for 48 h before the introduction of the fish. The temperature of the water in the tanks was controlled at 24 ± 2°C. The total hardness of the water was measured at the beginning and end of the experiment and the pH and concentration of dissolved oxygen were determined daily. The water quality during the three tests was comparable and there was little variation during the tests; ranges for total hardness and pH were 240–280 mg/ℓ and 6·9–8·2 respectively. Some problems with low concentrations of dissolved oxygen were encountered in the experiment with carbofuran, when, in four of the tanks, the concentration fell to less than 4 mg/ℓ for a short period early in the experiment. This was corrected by aeration and thereafter the concentration of dissolved oxygen remained within the range found in the other experiments (6·2–8·3 mg/ℓ). There was no indication that this short period of low dissolved oxygen influenced the result of the test.

Twenty-four hours before application of the insecticides five *T. nilotica* were placed in each tank. The fish were not fed during the experiment. For each test ten tanks were randomly allocated to one of a duplicated series of five application rates that encompassed likely or known field application rates. The remaining two tanks served as controls.

The chlorfenvinphos and carbofuran granules were applied evenly on to the surface of the water by hand, and sank immediately. For cypermethrin a series of dilutions were made up in distilled water such that the application of 20 ml would give the required dosage for each tank. The diluted cypermethrin was applied to the surface of the water of the tanks using a hand-held spray gun.

For a period of 96 h after application of the insecticides the fish were observed at intervals and the number of dead was recorded. None of the control fish died.

Sub-surface water samples were taken from each tank at intervals during the experiments and analysed for cypermethrin, chlorfenvinphos or carbofuran using the methods described above.

The nominal concentrations and applied dosages which were lethal to 50% of the fish, exposed for defined periods (LC_{50} and LD_{50} values), were calculated using probit analysis or graphical interpolation as described above.

Static water tests with formulated materials and C. carpio

These experiments were carried out in the Department of Agricultural Biology of Seoul National University, Suweon, Korea. The formulations used were cypermethrin 5% EC, chlorfenvinphos 3% granule and carbofuran 3% granule.

The fish were obtained from a local commercial supplier. At the time of testing a sample of 34 fish had a mean weight of 2·1 g (SD 1·5 g). Before use the fish were held in the laboratory for at least 3 days to allow them to acclimatize to the test conditions and for any damaged or unhealthy fish to be identified and removed.

The acute lethal toxicity of the three insecticides to *C. carpio* was determined in a 48 h static water test with renewal of the test solutions after 24 h. For each test six all-glass vessels, 34 cm in diameter and 24 cm deep, were filled with 16 litre of aerated mains water. A dispersion, in water, of the insecticide to be tested was made up and added to five of the vessels to give an approximately logarithmic series of concentrations. The sixth vessel received no insecticide and served as a control. During the test the temperature, total hardness, pH and concentration of dissolved oxygen were 22–26°C, 20–25 mg/ℓ, 6·3–6·9 and 4·5–6·5 mg/ℓ respectively.

Ten *C. carpio* were introduced into each test vessel and the number of dead fish was recorded at intervals. There were no control deaths during the tests. The 24 h and 48 h LC_{50} values, based on the nominal concentrations of the active ingredient, were calculated using the methods described above.

Field experiments

Korea. The experiment site was a paddy field at the College of Agriculture, Seoul National University, Suweon, Korea. The paddy was planted with Minehikary (Japonica) rice which had been seeded on 14 April 1981 and transplanted on 26 May 1981. No fertilizer or pesticide applications had been made to the paddy after 6 July, some five weeks before the experiment began.

Five treatment regimes were used for the experiment:

1. Control—no insecticide
2. Cypermethrin—low rate—15 g a.i./ha (as 5% EC)
3. Cypermethrin—high rate—40 g a.i./ha (as 5% EC)
4. Chlorfenvinphos—1200 g a.i./ha (as 3% granule)
5. Carbofuran—1200 g a.i./ha (as 3% granule)

with three replicate plots of each treatment allocated within a randomized block design. The 15 plots required were constructed in the paddy using earth bunds and rigid plastic sheeting. Each plot was 6 m × 9 m and contained a 30 cm deep, 50 cm wide trench along one short edge. The plots were flooded to a depth of approximately 15 cm (45 cm in the trenches) one week before the application of the insecticides.

The *C. carpio* used were obtained from a local commercial supplier. At the time of the experiment a sample of 40 fish had a mean weight of 4·6 g (SD 1·3).

The fish were introduced into the plots 2 days before application of the insecticides, 50 into each plot. They were divided into five groups of ten and placed in cages previously positioned in the plot. Two sizes of wire mesh cages with narrow wood frames were used; the first were 20 cm × 60 cm × 35 cm deep and three were used in the paddy of each plot. The second type were 35 cm × 50 cm × 50 cm deep and two were placed in the trench of each plot. After their introduction the fish were checked daily for deaths and any dead found before application of the insecticides, were replaced.

The insecticides were applied on the morning of 11 August 1981 by experienced local farmers. The cypermethrin was applied by knapsack sprayer at a rate of 8·1 ℓ per plot (equivalent to 1500 ℓ/ha) and the chlorfenvinphos and carbofuran granules were applied by hand casting at a rate of 216 g per plot (equivalent to 40 kg/ha). During the application of the cypermethrin a 1·75 m high plastic sheet was erected around the plots being sprayed, to ensure that spray drift was avoided.

From 3 days before application to 7 days after application daily observations were made of the depth of water in the paddies and the rainfall. Twice daily at 0600–0700 h and at 1400–1500 h, the pH, temperature and concentration of dissolved oxygen in the paddies were measured. The total hardness of the paddy water was determined the day before application of the insecticides. Methods for the measurement of water quality were those used in the laboratory tests described above. The quantity of suspended solids in the paddy waters at the time of application was also determined. One hundred millilitres of samples of paddy water were filtered through pre-dried and weighed Whatman GF/C filter papers. The papers were re-dried and re-weighed. Daily maximum and minimum air temperatures during the experiment were obtained from the nearby Meteorological Station, Office of Rural Development.

Daily observations were made of the numbers of dead fish in the cages from the time of their introduction until 7 days after application of the insecticides.

To assess the significance of differences in the mortalities of fish under the different treatment regimes a generalized linear model (Nelder and Wedderburn, 1972) was fitted to the numbers of mortalities that occurred. A binomial error structure with a logit link was used. Several models were examined; the most appropriate one fitted the main effects of treatment regimes and situation of the cages (i.e. trench or paddy). The following comparisons were made:

1. All insecticide treatments against control;
2. Cypermethrin low rate against cypermethrin high rate;
3. Cypermethrin high rate against chlorfenvinphos and carbofuran;
4. Chlorfenvinphos against carbofuran.

In addition, mortality in the cages in trench and paddy was compared across treatments.

Spain. The experiment was carried out about 20 km south of Valencia during the week 26 July to 2 August 1982. A large plot of rice was treated with cypermethrin 1·25% (an Ultra Low Volume formulation) at 25 g a.i./ha, using aerial application by fixed-wing aircraft. In total about 250 ha of rice were sprayed at 2 ℓ/ha. The rice crop was about 50 cm high at the time of application and gave a dense cover. The water in the paddies was generally 5–10 cm deep with a slow and intermittent flow.

C. carpio for the experiment were obtained from a local hatchery. On the day before the application of the insecticide two open-topped cages, each containing 50 fish (5–10 cm long) were placed in the paddy to be treated. One cage was placed at the edge of the paddy, clear of the crop, in water 15–20 cm deep. The other was placed in a ditch draining part of the paddy where the water was somewhat deeper, 25–50 cm.

From the time of their introduction until 3 days after the application of the insecticide the cages were checked daily for dead fish; at the same time the water temperature and concentration of dissolved oxygen were recorded.

Results

Laboratory toxicity tests

Chemical analysis of test substance during the continuous-flow tests with the technical materials indicated that exposure concentrations were consistently maintained. The coefficient of variation of mean exposure concentrations was generally less than 30%. The results from these experiments have therefore been expressed in terms of the mean measured concentration of test substance. The 24 h, 48 h and 96 h LC_{50} values obtained from the continuous-flow tests (Table 1) show that technical cypermethrin, with LC_{50} values in the range 2–4 $\mu g/\ell$, was about 20 times more toxic than chlorfenvinphos, with LC_{50} values of 39–75 $\mu g/\ell$, and approximately 100–250 times more toxic than carbofuran with LC_{50} values of 480 $\mu g/\ell$.

Details of the application rates, nominal concentrations and chemically determined concentrations (as a percentage of nominal concentrations) for the static water tests with *T. nilotica* are given in Table 2. From these data it is apparent that 24 h after application of chlorfenvinphos and carbofuran granules the concentrations of chlorfenvinphos and carbofuran in the water were within 20% of the nominal values, and they remained high for the rest of the tests. On the other hand, 24 h after application of cypermethrin the concentrations of cypermethrin in the water were

TABLE 1. LC_{50} values for cypermethrin, chlorfenvinphos and carbofuran from continuous-flow tests with *T. nilotica*

	LC_{50} ($\mu g/\ell$)		
Compound	24 h	48 h	96 h
Cypermethrin	4	3	2
Chlorfenvinphos	75	57	39
Carbofuran	480	480	480

TABLE 2. Application rates, nominal concentrations and determined concentrations in the static water toxicity tests with *T. nilotica*

Cypermethrin	Application rate (g a.i./ha)		5	10	25	50	100	
	Nominal concentration (μg/ℓ)		1·7	3·3	8·3	17	33	
	Determined concentration as % of nominal	24 h	67	61	54	61	54	Mean 59
		96 h	41	25	26	36	38	Mean 33
Chlorfenvinphos	Application rate (g a.i./ha)		100	200	500	1000	2000	
	Nominal concentration (μg/ℓ)		33	67	170	330	670	
	Determined concentration as % of nominal	24 h	94	87	88	94	81	Mean 89
		96 h	104	102	100	106	104	Mean 103
Carbofuran	Application rate (g a.i./ha)		75	150	375	750	1500	
	Nominal concentration (μg/ℓ)		25	50	125	250	500	
	Determined concentration as % of nominal	24 h	94	86	82	94	81	Mean 87
		96 h	86	90	84	80	68	Mean 82

TABLE 3. LC_{50} values for the formulated materials from static water tests with *T. nilotica*

Compound	LC_{50} ($\mu g/\ell$)*		
	24 h	48 h	96 h
Cypermethrin	8	4	4
Chlorfenvinphos	47	33	<30
Carbofuran	250	200	200

* Expressed as active ingredient on a nominal concentration basis.

only about 50–70% of nominal values and by 96 h had declined to 25–40% of nominal, a loss of approximately 50%.

The LC_{50} values from these tests (Table 3) were broadly in agreement with those obtained in the continuous-flow tests, all being within a factor of three. Indeed, for cypermethrin the small differences in LC_{50} values obtained from the two tests are almost exactly explained by the fact that nominal exposure concentrations were not maintained in the static water tests (Table 2). The reason why chlorfenvinphos and carbofuran were apparently slightly more toxic when applied as granules is not clear, for they both had very similar nominal and determined concentrations in the test solutions.

The results of the static water tests with the formulated materials against *C. carpio*, carried out in Korea, are given in Table 4. These LC_{50} values, again based on nominal concentrations of active ingredient, were very similar to those obtained with *T. nilotica*, indicating no difference in susceptibility of the two species to the insecticides. Again, the results broadly confirmed the relative toxicities of the three materials previously found in both the continuous-flow and static water tests with *T. nilotica*.

TABLE 4. LC_{50} values for formulated materials from static water tests with *C. carpio*

Compound	LC_{50} ($\mu g/\ell$)*	
	24 h	48 h
Cypermethrin	10	3·4
Chlorfenvinphos	55	45
Carbofuran	280	120

* Expressed as active ingredient on a nominal concentration basis.

Field experiments

Korea. The results of the air temperature and water quality determinations are given in Table 5. Differences between plots were small and there were no marked changes in any of the parameters during the period of the experiment.

Rainfall was recorded on two occasions during the experiment, 25 mm on day 2 and 65 mm on day 6. The average depth of water in the paddies during the experiment decreased from about 14 cm on day 0 to about 10 cm on day 6. The water in the plots was soft, total hardness being less than 60 mg/ℓ as $CaCO_3$, and it had a pH of 5 to 7. The water temperatures during the experiment did not fall below 20°C and rarely exceeded 30°C. Concentrations of dissolved oxygen were always in excess of 60% of air saturation values. The concentrations of suspended solids in the water at the time of application ranged from 66–440 mg/ℓ with a mean of 205 mg/ℓ.

Of the 750 fish introduced into the cages 2 days before treatment only six died before application of the insecticides on day 0. These deaths were scattered and were attributed to handling damage. Figure 1 plots the cumulative mortality of the caged fish for each treatment regime. In the plots treated with chlorfenvinphos more than 50% of the fish had died by day 3, and by day 5 so had 50% of those in the plots treated with carbofuran. In contrast, in the control plots and the plots treated with cypermethrin, at both low and high rates, less than 10% of the fish had died by day 4. Table 6 gives details of the total mortalities on day 7. Control mortalities of fish were less than 10% in all the replicates. Under all treatment regimes, including controls, the percentage mortalities of fish held in the paddy significantly exceeded that of those held in the trench (estimated effect on logit scale = 1·69; asymptotic standard error = 0·27; $t = 6{\cdot}16$ and $P < 0{\cdot}01$).

Mortality of the fish in the plots treated with cypermethrin at the low rate was 13% and this was not significantly different ($P > 0{\cdot}05$) from that in the control plots, 6·6%. The mortality of fish in the plots treated with cypermethrin at the higher rate was 15% and this was significantly greater ($P < 0{\cdot}05$) than that in the control plots,

TABLE 5. Air temperature and water quality during the Korean field experiment

Measurements	Mean	Range†
Minimum daily air temperature (°C)	20	18–24
Maximum daily air temperature (°C)	30	26–32
Water depth (cm) day −3	13	12–15
day 0	14	13–15
day 6	10	8–11
Minimum daily water temperature (°C)	22	21–23
Maximum daily water temperature (°C)	28	25–30
Concentration of dissolved oxygen (mg/ℓ)	6·5	4·1–7·8
pH	6·3	5·6–6·8
Water hardness (mg/ℓ as $CaCO_3$) day 0	50	43–57
Suspended solids (mg/ℓ) day 0	205	66–440

† Unless otherwise stated ranges quoted are for the period day −3 to day 6.

Table 6. Mortality of caged *C. carpio* in paddies 7 days after the application of cypermethrin, chlorfenvinphos or carbofuran

		Number of dead fish													
		Control			Cypermethrin (low rate)			Cypermethrin (high rate)			Carbofuran			Chlorfenvinphos		
		1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Trench	Cage 1	0	0	1	0	1	0	1	1	1	3	5	5	9	8	9
	Cage 2	0	0	0	0	1	1	0	0	0	4	4	7	10	10	9
Trench total		0	0	1	0	2	1	1	1	1	7	9	12	19	18	18
Paddy	Cage 1	1	1	0	0	2	1	2	1	0	3	10	10	10	10	10
	Cage 2	3	1	0	3	3	1	4	0	5	9	10	6	10	10	10
	Cage 3	0	1	2	1	5	1	0	4	3	7	8	10	10	10	10
Paddy total		4	3	2	4	10	3	6	5	8	19	28	26	30	30	30
Plot total		4	3	3	4	12	4	7	6	9	26	37	38	49	48	48
Treatment total†		10_a (6·6%)			20_{ab} (13%)			22_b (15%)			101_c (67%)			145_d (97%)		

† Those treatment totals with a subscript in common are not significantly different (5% level).

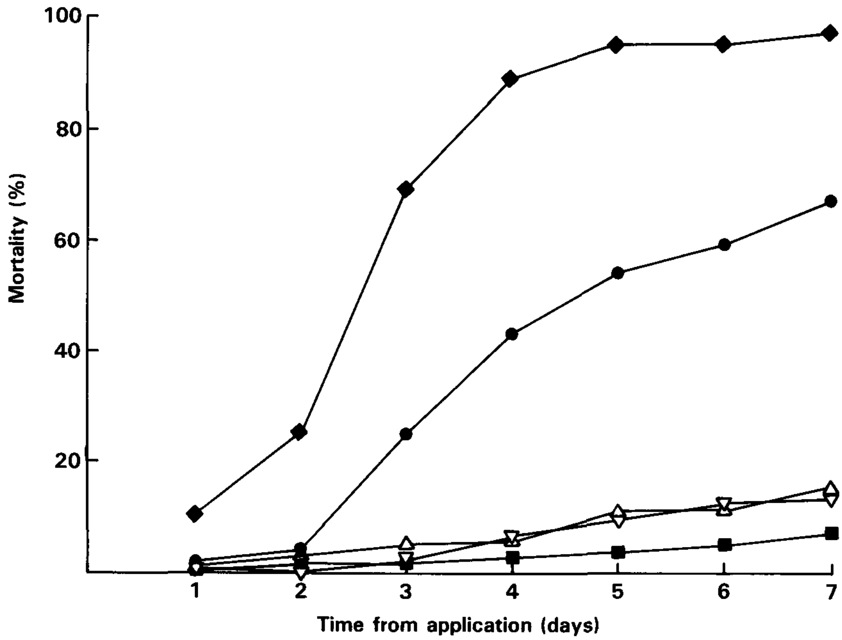

FIGURE 1. Cumulative mortality of caged *C. carpio* in paddies treated with cypermethrin (▽-high rate; △-low rate), chlorfenvinphos (◆) or carbofuran (●). ■-control.

but not significantly different ($P > 0{\cdot}05$) from that in the plots treated with cypermethrin at the low rate. Fish mortalities in the plots treated with chlorfenvinphos and carbofuran were 97% and 67% respectively and both were significantly higher than those in the control plots and the plots treated with cypermethrin at the higher rate ($P < 0{\cdot}001$).

Spain. During the experiment the water temperature and concentration of dissolved oxygen at the time of observation, 1000–1200 h, were in the range 23–26°C and 2·5–6·0 mg/ℓ respectively. There was no mortality of fish in either of the cages during the three days following the application of the cypermethrin.

TABLE 7. 96 h LD_{50} values for formulated materials from static water tests with *T. nilotica* compared with likely/accepted commercial application rates

Compound	96 h LD_{50} (g a.i./ha)	Commercial rate (g a.i./ha)
Cypermethrin	12	15–40
Chlorfenvinphos	<90	800–1200
Carbofuran	600	800–1200

Discussion

The acute lethal toxicity to fish of the active ingredients of the three insecticides used in these studies has been determined previously. Stephenson (1982) reported 96 h LC_{50} values for cypermethrin against several species of fish (*Salmo gairdneri, Salmo trutta, Scardinius erythrophthalmus* and *Cyprinus carpio*) that fell within the range 0·4–1·2 $\mu g/\ell$ and McLeese, Metcalf and Zitko (1980) gave a 96 h LC_{50} against *Salmo salar* of 2·0 $\mu g/\ell$. Laboratory data on the acute toxicity of technical chlorfenvinphos to fish are less plentiful. Lejczak (1977) found it to have a 96 h LC_{50} value of 1560 $\mu g/\ell$ against *Poecilia reticulatus* and unpublished data from this laboratory, for the same species, indicated a 96 h LC_{50} of approximately 300 $\mu g/\ell$. There are more data on the acute toxicity of technical carbofuran. Johnson and Finley (1980) give 96 h LC_{50} values for seven species of fish (*Oncorhynchus kisutch, S. gairdneri, S. trutta, Salvelinus namaycush, Pimephales promelas, Ictalurus punctatus* and *Perca flavescens*) which range from 150 to 870 $\mu g/\ell$. From the above it is apparent that the acute lethal toxicities of technical cypermethrin and carbofuran, determined in the present study, are broadly in line with the results of previous laboratory studies. For chlorfenvinphos, however, the limited data available indicate a real difference in susceptibility between *T. nilotica*, with a 96 h LC_{50} of 39 $\mu g/\ell$, and *P. reticulatus* with 96 h LC_{50} values of 300 and 1560 $\mu g/\ell$. Nevertheless, taken together, the available laboratory data show that cypermethrin is considerably more toxic to fish than either chlorfenvinphos or carbofuran.

The tests with the formulated insecticides and *T. nilotica* showed that in the laboratory their toxic effects were similar to those of the technical materials, indicating that the formulations used were not influencing the toxicity of the active ingredients, an effect which has been shown to occur with some formulations (Jarvinen and Tanner, 1982).

Before undertaking field experiments the results from the laboratory tests were examined to see if toxic effects on fish were likely to occur in rice paddies treated at commercial rates. In the field the hazard to the fish would be determined by a combination of the toxicity of the product and the exposure to it. The toxicity of the three insecticides was known for several species of fish but what the exposure would be was not known. Exposure is determined by the quantity of insecticide applied and also by the fate of that applied dosage. As the field experiments were to be carried out at commercial rates these were known and fixed. What was not known was the fate of the applied material. However, a comparison of the toxicity of the insecticides, expressed in terms of 96 h LD_{50} values (i.e. applied dosages which caused 50% mortality in laboratory tests after 96 h exposure) with commercial application rates provided a first indication as to whether, on a worst case basis, toxic effects were possible in the field. This comparison is made in Table 7, and it shows that, theoretically, in water 30 cm deep or less there was the possibility of fish deaths after commercial applications of all of the three insecticides, but more so with chlorfenvinphos than with cypermethrin or carbofuran. In the light of this, the field experiments were carried out to see if the potential hazard would be realized during normal agricultural usage of the products in rice.

In the Korean field experiment, fish mortality in the plots treated with cypermethrin at 15 g a.i./ha was not significantly greater than that in the control plots and even at 40 g a.i./ha mortality was only 9% greater than that in the control plots. Similarly, although to a lesser extent, the toxic effects of chlorfenvinphos and carbofuran were less than the laboratory results had indicated. Mortality in the plots treated with chlorfenvinphos and carbofuran reached 50% after 2–3 and 4–5 days respectively and were 97% and 67% respectively at the end of the experiment.

The limited toxic effects of cypermethrin on fish, when used at commercial rates in the field, were confirmed in the experiment in Spain when the aerial overspray of rice paddies at 25 g a.i./ha produced no mortality of caged *C. carpio.*

With cypermethrin it is clear that the spraying of liquid formulations would have resulted in a significant proportion of the insecticide being deposited on the crop and not on to the water. In addition, Crossland (1982) has shown that after the application of an EC formulation of cypermethrin to the surface of natural ponds the maximum concentration of cypermethrin found in sub-surface water was only 8–16% of the maximum theoretical concentration (i.e. the concentration that would have been achieved had all of the applied dose dispersed uniformly into the water). Crossland also showed that the concentration of cypermethrin in the sub-surface water decreased rapidly with time; within 7 days of application it was between 1% and 5% of the maximum theoretical level. Crossland considers that the low initial concentrations of cypermethrin are the result of its low water solubility and its strong affinity for surfaces, particularly those with a high content of organic matter. He also suggests that some 50% of the cypermethrin in the water was bound to suspended solids and thus was not available to the fish. The loss of cypermethrin from the sub-surface water is probably the result of several processes, including hydrolysis and biodegradation.

Both chlorfenvinphos and carbofuran were applied as granular formulations: hence, unlike the liquid formulations of cypermethrin, the bulk of the applied dosage would be expected to penetrate the rice and reach the paddy water. A secondary factor, which would aid the achievement of maximum theoretical concentrations of chlorfenvinphos and carbofuran, once the granules had reached the water, is their relatively high water solubilities (145 000 $\mu g/\ell$ and 700 000 $\mu g/\ell$) compared with that of cypermethrin (5 $\mu g/\ell$). Indeed Beynon, Edwards, Thompson and Edwards (1971) showed that even when chlorfenvinphos was applied as an EC formulation to the surface of a pond, the concentration in the water immediately after application was almost 90% of the maximum theoretical concentration. Similarly for carbofuran, Siddaramappa, Tirol and Seiber (1978) showed that when it was applied as granules to rice paddies, something in excess of 70% of the maximum theoretical concentration in water was rapidly achieved.

In conclusion, it is considered that the limited toxic effects of cypermethrin on fish in the field, when compared with those of chlorfenvinphos and carbofuran, resulted from a combination of three factors. Firstly, the low commmercial application rate required for cypermethrin to give pest control meant that the maximum theoretical concentration of active ingredient in the water after application was much lower for cypermethrin than for chlorfenvinphos or carbofuran. Secondly, the different formulations used meant that different proportions of the applied dosage would reach the water, with the use of liquid formulations favouring cypermethrin. Finally, once in the water there is evidence that cypermethrin would be lost more rapidly than chlorfenvinphos and carbofuran. Together, these factors are sufficient to explain the minimal toxic effects of cypermethrin in the field when compared with chlorfenvinphos and carbofuran despite its considerably higher acute toxicity determined in laboratory tests.

These findings on the limited toxic effects of cypermethrin extend and confirm the results of Crossland (1982) and Shires (1983), who in field experiments found that the toxic effects of cypermethrin were much less than would have been predicted from laboratory data. Crossland (1982) showed that when applied to the surface of a 1 m deep pond at 100 g a.i./ha cypermethrin EC was not lethal to fish, and Shires (1983) found LD_{50} values of 92 and 30 g a.i./ha for *S. gairdneri* and *C. carpio* when it was applied to enclosures in a pond 1 m deep. Finally, the results obtained in these studies show no reason why cypermethrin, despite its high acute toxicity to fish in laboratory studies, should be considered to pose a hazard to fish when used for pest control in rice.

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