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TOXICITY OF SELECTED CONTROLLED RELEASE AND CORRESPONDING UNFORMULATED TECHNICAL GRADE PESTICIDES TO THE FATHEAD MINNOW *PIMEPHALES PROMELAS*

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ABSTRACT

Fathead minnows were exposed to three encapsulated slow-release pesticide formulations and three corresponding technical grade products used in their manufacture. Static 4-day LC_{50} values derived from aged stock solutions indicate increased toxicity with time for encapsulated methyl parathion, diazinon and their technical grades. The technical grades, however, were more toxic than the encapsulated. Encapsulated Dursban was less toxic with time, whereas toxicity remained similar with time for the technical grade. Flow-through 4-day LC_{50} values for methyl parathion, Penncap-M, Dursban, Dursban 10 CR and diazinon are 5·36, 6·91, 0·14, 0·12 and 6·9 mg litre^{-1}, respectively.

In general, long-term toxicity was similar for both pesticide forms. Growth (weight) was the most sensitive parameter measured in the 32-day embryo–larval tests, except for the Dursban 10 CR study where it was equally as sensitive as survival. Growth effects occurred at $>0{\cdot}31-<0{\cdot}38$, $>0{\cdot}38-<0{\cdot}59$, $>0{\cdot}0016-<0{\cdot}0032$, $>0{\cdot}0022-<0{\cdot}0048$, $>0{\cdot}050-<0{\cdot}090$ and $>0{\cdot}040-<0{\cdot}076$ mg litre^{-1} for methyl parathion, Penncap-M, Dursban, Dursban 10 CR, diazinon and Knox Out 2 FM, respectively.

Water solubilities were slightly lower for the encapsulated compounds. Estimated half-lives were 18 days for methyl parathion and 15 days for Penncap-M; 41 days for Dursban and > 200 days for Dursban 10 CR and approximately 30 days for diazinon and > 230 days for Knox Out 2 FM.

INTRODUCTION

Controlled release pesticides (CRP), also called encapsulated pesticides, are a relatively recent development, although controlled release technology has been in

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use by the drug industry for many years. CRP's have been defined as the release of a pest control agent from a polymeric carrier, binder, absorbent or encapsulant at a slow continuous effective rate into the environment (Cardarelli & Walker, 1976). They were developed mainly for economic advantage—i.e. the extension of time between treatment intervals and continuous pest protection (Cardarelli & Walker, 1976). Release time can occur for months or years, depending upon the formulation. Environmental protection is another stated advantage; with CRP methods the target organisms can be controlled at chronic toxicity levels and not at acute levels as is necessary under conventional pesticide practices (Cardarelli & Walker, 1976; Janes, 1976). Human hazard is also reported to be minimised as less exposure occurs during handling of CRP's (Shaw, 1974; Cardarelli & Walker, 1976).

Some of the reasons that make CRP usage advantageous may be disadvantageous to non-target organisms. Previously non-persistent, these pesticides can now cause continuous exposure. Long-term chemical behaviour has been likened to that of a more persistent pesticide (Collins & Doglia, 1973; Marshall & Roberts, 1978). Concentrations of CR bis(tri-*n*-butyl tin) oxide recommended for chronic snail control have adversely affected behaviour and caused significant growth reduction of fish (Matthiessen, 1974).

There is a scarcity of chronic aquatic organism test data available for conventional pesticides and none except the work of Matthiessen (1974) for controlled release pesticides. CRP's and conventional pesticides are not considered different under current pesticide laws and regulations (Zweig, 1977). Toxicity and environmental studies are usually performed only on the active ingredient (technical grade).

The objectives of this study were to determine the effect of selected CRP's on fathead minnows *Pimephales promelas* when compared with that of the technical grade pesticide used in their formulation, to determine water solubility and half-life in Lake Superior water, to perform a rough qualitative analysis of degradation products and to develop test methodology for short- and long-term CRP studies. The technical grade and corresponding CRP's selected for study were methyl parathion, (*o,o*-dimethyl *o-p*-nitrophenyl phosphorothioate) 80% purity and Penncap-M®; Dursban* (chlorpyrifos) (*o,o*-diethyl *o*-(3,5,6-trichloro-2-pyridyl) phosphorothioate) 98·7% purity and Dursban 10 CR*; and diazinon (*o,o*-diethyl *o*-(2-*iso*-propyl-6-methyl-4-pyrimidinyl) phosphorothioate) 87·1% purity and Knox Out® 2 FM.

Pesticide test samples of technical and encapsulated methyl parathion were supplied by Pennwalt Corporation, Fresno, CA; technical and encapsulated Dursban by the Dow Chemical Company, Midland, MI and technical and

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encapsulated diazinon by the Pennwalt Corporation, King of Prussia, PA. Mention of trade names or commercial products does not constitute endorsement by the EPA.

MATERIALS AND METHODS

Physical conditions

All studies were conducted at the Environmental Research Laboratory—Duluth, Duluth, Minnesota.

Test water was sand-filtered Lake Superior water sterilised with ultraviolet light and warmed to approximately 25 °C by a coiled stainless steel heat exchanger located in a stainless steel headbox.

Four-day static tests were conducted with duplicates of five test concentrations and a control in 250 ml pyrex beakers that were immersed in a water bath and held at $25{\cdot}0 \pm 0{\cdot}6$ °C.

The toxicants were prepared in saturator systems (Veith & Comstock, 1975) with separate saturators used for the CRP and the technical grade. Penncap-M and Knox Out 2 FM were sorbed onto sterile glass wool that was packed in the saturator columns. Because of its large capsule size, Dursban 10 CR alone was contained within the column. In static tests the toxicant from the saturators was added to lake water to achieve the desired water exposure concentrations. Saturators were allowed to run continuously for up to 11 weeks and static 4-day LC_{50} studies were conducted at weekly intervals in order to determine the toxicity of the aged solutions.

Flow-through 4-day acute and 32-day embryo–larval studies were conducted in a mini diluter system similar to that described by Benoit *et al.* (in press) modified so that both the CRP and the technical grade pesticide could be tested simultaneously in duplicate. This was accomplished by enlarging the constant head water cell and the addition of five extra water-flow tubes; by extension of the mixing cell so that the second half was a mirror image of the first (the control segment remained unchanged); by using five extra toxicant flow booster cells and five extra toxicant flow splitter cells. Water flow from the W cells was 30 ml min^{-1}, except for the W_1 cells where it was 60 ml min^{-1}. Water flow from the C cells was 30 ml min^{-1}. The twelve experimental fish chambers on the right-hand side of the test system consisted of duplicates of a control and five concentrations of a technical grade pesticide, whereas the left-hand side of the test system contained duplicates of a control and five concentrations of the CRP. Similar saturators contained the toxicant solutions which were pumped to the W_1 cells of the continuous flow system by FMI pumps (Fluid Metering, Inc., Oyster Bay, NY) calibrated to dispense the desired toxicant flow. The experimental fish chambers held a water volume of 500 ml and the flow rate to each chamber was 15 ml min^{-1}. This maintained dissolved oxygen levels at

>75% saturation and provided a 99% replacement time for the test water in each chamber within 3 h, as determined from Sprague (1969). Fish chambers were siphoned daily, 1–2 h after the morning feeding, to remove leftover food and were brushed weekly. A constant 16-h photoperiod was maintained; daylight light intensity between the individual fish chambers varied from 28 to 50 lumens at the water surface. Waste water was filtered through dual 50 μ prefilters, then dual activated charcoal filters, before being discharged to the laboratory sewage system. Toxicants were removed from the waste water at >90% efficiency.

Biological conditions

Fathead minnow eggs less than 24 h old were obtained on spawning tiles from the Environmental Research Laboratory–Duluth Fish Culture Unit. For 4-day studies the tiles were placed in glass hatching chambers and maintained at approximately 25 °C in a water bath until hatching. In 4-day static tests ten newly hatched larvae were randomly placed in beakers of toxicant plus lake water or control beakers. In 4-day flow-through studies twenty newly hatched larvae were randomly added to each fish chamber. All 4-day studies were conducted according to the methods of the Committee on Methods for Toxicity Tests (1975). In embryo–larval studies fifty eggs were randomly assigned to embryo cups. In the methyl parathion and Penncap-M studies all embryos were hatched and fifteen of the healthiest appearing larvae were transferred to the fish chambers for the remainder of the studies. In the other studies the following procedure was used. Embryos were placed in embryo cups but 48 h later the healthy fertile embryos were randomly reduced to fifteen. After hatching all of the larvae were released to the test chambers. This procedure reduced handling just before and after hatching and made it easier to make an impartial selection of the test fish. In general, the procedures used in the embryo–larval tests followed those of the Environmental Research Laboratory—Duluth (1979). The fish were fed recently hatched brine shrimp nauplii three times daily on weekdays (twice daily during the methyl parathion studies) and twice at weekends. After 32 days' exposure the juvenile fish were killed in ice water, blotted dry and each fish weighed to the nearest milligramme.

Chemical conditions

During the studies water temperatures were maintained between 23·5 and 26 °C and were checked daily in all test chambers. Routine water chemistries were determined weekly by the methods described by the American Public Health Association *et al.* (1975). Dissolved oxygen levels ranged from 6·5 to 8·4 mg litre^{-1}. Mean total hardness, acidity and alkalinity were 45·8, 2·3 and 43·1 mg litre^{-1}, respectively; pH was between 7·4 and 7·8.

Pesticide concentrations in the fish chambers were determined weekly. The pesticides were extracted from the test water with hexane and analysed by gas chromatography. Recovery from spiked control water samples averaged 92·0 $\pm$ 7·7%

($n = 22$) for embryo–larval tests, $97{\cdot}9 \pm 6{\cdot}4\,\%$ ($n = 8$) for flow-through acute tests and $100{\cdot}5 \pm 9{\cdot}7\,\%$ ($n = 32$) for static acute tests.

Half-life studies were conducted in capped clear 3·8 litre glass jugs filled with Lake Superior water and spiked with the gramme weight of toxicant (active ingredient) needed for saturation at water solubility. The jugs were placed on a shelf under fluorescent lighting and held at room temperature ($\sim 22{\cdot}5\,^{\circ}C$). Water samples were collected weekly and analysed for pesticide content by the same method as used in the fish tests.

Potential breakdown products could not be identified using gas chromatographic techniques; therefore, water samples were extracted with methylene chloride and hexane and degradation products qualitatively identified in the static test saturator solutions or half-life studies by high performance liquid chromatography.

Statistics

Ninety-six hour LC_{50} values were determined by the Moving Average Method using an Environmental Research Laboratory—Duluth, MN computer program (C. E. Stephan, pers. comm.).

In embryo–larval tests all survival and embryo hatchability data were transformed to arcsin $\sqrt{\%}$ (Dixon & Massey, 1957). One-way analysis of variance ($p = 0{\cdot}05$) was applied to all survival, embryo hatchability and growth data to determine the pesticide effect. Dunnett's procedure (Steel & Torrie, 1960) was used to compare treatment with control means. Regression analysis was used to estimate half-life of the pesticides in lake water.

RESULTS

Water solubilities for the pesticides in the saturator systems were as follows: methyl parathion, 75·7 mg litre^{-1}; Penncap-M, 76·7 mg litre^{-1}; Dursban, 2 mg litre^{-1}; Dursban 10 CR, 1·3 mg litre^{-1}; diazinon, 40 mg litre^{-1} and Knox Out 2 FM, 34 mg litre^{-1}.

As the saturator solutions aged, both methyl parathion and Penncap-M became more toxic (Fig. 1). Static 96-h LC_{50} values decreased from 4·46 (3·27–8·20, 95 % confidence interval (CI)) to 1·22 (0·99–1·52, 95 % CI) mg litre^{-1} for the technical grade and from 8·17 (7·48–8·93, 95 % CI) to 3·47 (3·00–4·03, 95 % CI) mg litre^{-1} for the encapsulated formulation. Breakdown products qualitatively observed in both solutions were methyl paraoxon, thioate isomers (*o,o*-dimethyl *s*-4-nitrophenyl thiophosphate and *o,s*-dimethyl *o*-4-nitrophenyl thiophosphate) and *p*-nitrophenol, which was the most prominent. The toxicity of technical grade Dursban (Fig. 2) remained similar with time, with static 96-h LC_{50}'s of 0·17 (0-infinity, 95 % CI) and 0·15 (0·12–0·19, 95 % CI) mg litre^{-1}, whereas the encapsulated formulation became less toxic, the 96-h LC_{50} increasing from 0·13 (0-infinity, 95 % CI) to 0·28

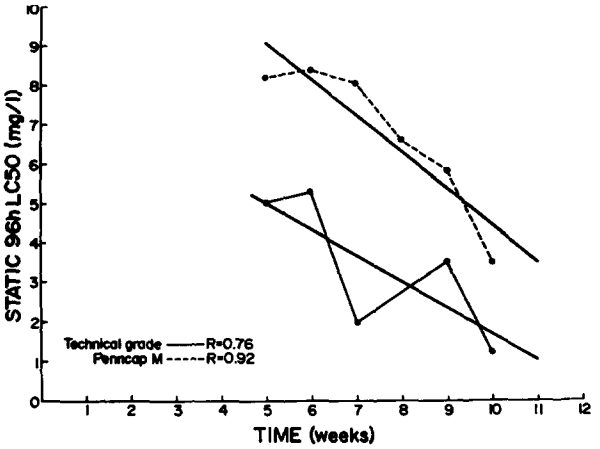

Fig. 1. Methyl parathion and Penncap-M static LC_{50} values with time and correlation coefficients (R).

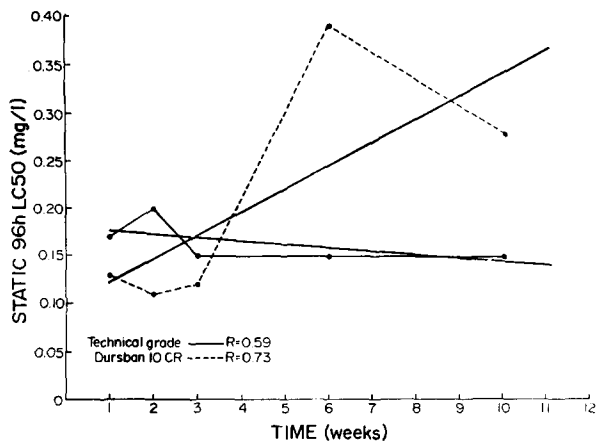

Fig. 2. Dursban and Dursban 10 CR static LC_{50} values with time and correlation coefficients (R).

(0·22–0·36, 95% CI) mg litre^{-1}. Breakdown products observed in both solutions were 3,5,6-trichloro-2-pyridinol and Dursban oxygen analogue. Diazinon became more toxic with time whereas the toxicity of Knox Out 2 FM was only slightly increased (Fig. 3). Static 96-h LC_{50} values decreased from 4·3 (3·4–5·2, 95% CI) to 2·1 (1·7–2·9, 95% CI) mg litre^{-1} for the technical grade and from 6·1 (5·0–7·6, 95% CI) to 5·1 (4·4–6·1, 95% CI) mg litre^{-1} for the encapsulated formulation. Only one breakdown product was observed in the solutions, 2-*iso*-propyl-4-methyl-6-hydroxy pyrimidine.

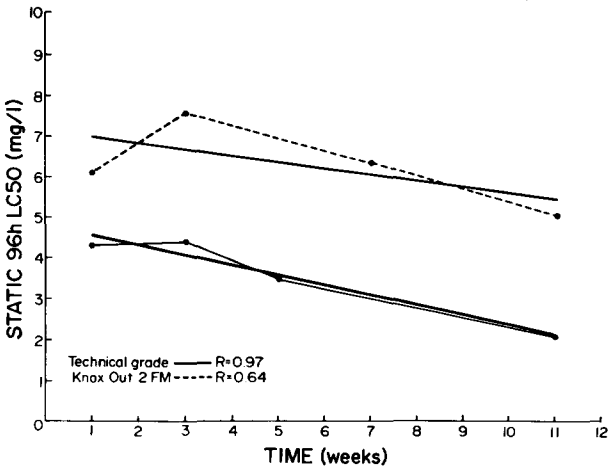

Fig. 3. Diazinon and Knox Out 2 FM static LC_{50} values with time and correlation coefficients (R).

Flow-through 96-h LC_{50} values (mg litre^{-1}) for the pesticide comparisons are: methyl parathion, 5·36 (4·93–5·82, 95% CI), Penncap-M, 6·91 (6·05–8·11, 95% CI); Dursban, 0·14 (0·12–0·16, 95% CI); Dursban 10 CR, 0·12 (0·11–0·13, 95% CI) and diazinon, 6·9 (6·2–7·9, 95% CI). No flow-through 96-h LC_{50} could be determined for Knox Out 2 FM because the release rate of the pesticide from the capsules was not rapid enough to maintain the saturator stock solution at levels needed to kill the test fish. Maximum diazinon water concentration that could be maintained with the flow-through rate used (15 ml min^{-1}) was 4·0 mg litre^{-1}.

Results of the 32-day embryo–larval study with methyl parathion and Penncap-M (Table 1) indicate that growth (weight) was a more sensitive parameter than survival. The lower chronic values (highest tested concentration not causing any adverse effect statistically different from the control at the 95% level) for the technical grade and encapsulated formulation are 0·31 and 0·38 mg litre^{-1}, respectively. The upper chronic values (lowest tested concentration causing an adverse effect statistically different from the control at the 95% level) for these same compounds are 0·38 and 0·59 mg litre^{-1}, respectively. The 'no effect' concentration for technical grade methyl parathion is between 0·31 and 0·38 mg litre^{-1} and that for Penncap-M between 0·38 and 0·59 mg litre^{-1}.

Results of Dursban and Dursban 10 CR 32-day embryo–larval studies (Table 2) demonstrated that growth was the most sensitive parameter measured for Dursban whereas growth and survival were equally sensitive for Dursban 10 CR. The lower chronic values for the technical grade and the encapsulated formulation are 0·0016 and 0·0022 mg litre^{-1}, respectively, whereas the upper chronic values are 0·0032 and 0·0048 mg litre^{-1}, respectively. Therefore, a 'no effect' concentration for the

TABLE 1
GROWTH AND SURVIVAL OF FATHEAD MINNOWS EXPOSED TO METHYL PARATHION (TECHNICAL) OR PENNCAP-M FOR 32 DAYS

Toxicant	*Measured water concentration (mg litre^{-1} ± SD)*[a]	*Number of surviving fish*	*Mean weight (mg ± 1 SD)*	*Survival (%)*
Methyl parathion (technical)	Control (ND)[b]	30	83·9 ± 17·4	100
	0·31 ± 0·09	30	74·6 ± 17·7	100
	0·38 ± 0·13	30	65·8 ± 23·7[c]	100
	0·59 ± 0·23	28	66·4 ± 28·1[c]	93·3
	0·86 ± 0·36	9	53·9 ± 32·4[c]	30·5[c]
	1.55 ± 0·55	0	0[c]	0[c]
Penncap-M	Control (ND)	30	85·6 ± 20·0	100
	0·23 ± 0·04	29	81·0 ± 18·7	100
	0·38 ± 0·09	30	74·9 ± 21·5	100
	0·59 ± 0·22	30	73·2 ± 14·5[c]	100
	0·77 ± 0·24	14	67·4 ± 21·0[c]	46·7[c]
	1·23 ± 0·38	0	0[c]	0[c]

[a] Per cent spike recovery, 97·4 ± 11·2, $n = 5$.
[b] Not detectable, <0·001 mg litre^{-1}.
[c] Values significantly different from the control.

TABLE 2
GROWTH AND SURVIVAL OF FATHEAD MINNOWS EXPOSED TO DURSBAN OR DURSBAN 10 CR FOR 32 DAYS

Toxicant	*Measured water concentration (mg litre^{-1} ± 1 SD)*[a]	*Number of surviving fish*	*Mean weight (mg ± 1 SD)*	*Survival (%)*
Dursban (technical)	Control (TR)[b]	29	147·0 ± 31·4	100
	0·0009 ± 0·0001	30	151·1 ± 32·2	100
	0·0016 ± 0·0004	28	149·1 ± 37·6	100
	0·0032 ± 0·0005	27	123·7 ± 28·7[c]	90
	0·0057 ± 0·0008	25	98·7 ± 29·2[c]	86[c]
	0·0102 ± 0·001	17	84·5 ± 24·0[c]	56·7[c]
Dursban 10 CR	Control (TR)	29	157·2 ± 33·0	100
	0·0007 ± 0·0002	30	158·1 ± 32·4	100
	0·0013 ± 0·0002	28	152·9 ± 40·8	96·7
	0·0022 ± 0·0004	27	148·0 ± 31·9	90
	0·0048 ± 0·0007	18	107·4 ± 26·4[c]	61·2[c]
	0·0086 ± 0·0008	17	82·5 ± 30·6[c]	56·7[c]

[a] Per cent spike recovery, 90·4 ± 3·8, $n = 7$.
[b] Trace (0·00007–0·0001 mg litre^{-1}).
[c] Values significantly different from the control.

technical grade is between 0·0016 and 0·0032 mg litre^{-1} whereas that for the encapsulated formulation is between 0·0022 and 0·0048 mg litre^{-1}. Although no statistically significant effects occurred at lower concentrations of either compound, the fish exposed to water concentrations lower than those where growth effects occurred exhibited unquantifiable behavioural changes when confronted with

certain external stimuli (for example, they became lethargic and extra care had to be taken to prevent the larvae from being sucked into the siphon when cleaning the fish chambers).

Results of the diazinon and Knox Out 2 FM 32-day embryo–larval studies (Table 3) also demonstrated that growth was the most sensitive parameter. The lower chronic values for diazinon and Knox Out 2 FM are 0·050 and 0·040 mg litre^{-1}, respectively, whereas the upper chronic values are 0·090 and 0·076 mg litre^{-1}, respectively. The 'no effect' concentration for the technical grade lies between 0·050 and 0·090 mg litre^{-1} whereas that for the encapsulated formulation is between 0·040 and 0·076 mg litre^{-1}. None of the six compounds tested demonstrated any adverse effect on embryo hatchability.

TABLE 3
GROWTH AND SURVIVAL OF FATHEAD MINNOWS EXPOSED TO DIAZINON OR KNOX OUT 2 FM FOR 32 DAYS

Toxicant	*Measured water concentration (mg litre^{-1} ± 1 SD)*[a]	*Number of surviving fish*	*Mean weight (mg ± 1 SD)*	*Survival (%)*
Diazinon (technical)	Control (TR)[b]	30	138·6 ± 23·9	100
	0·050 ± 0·01	29	125·3 ± 26·0	96·7
	0·090 ± 0·02	30	124·6 ± 21·5[c]	100
	0·140 ± 0·01	29	115·0 ± 22·3[c]	93·3
	0·290 ± 0·03	19	81·1 ± 22·4[c]	63·4[c]
	0·500 ± 0·06	14	72·4 ± 22·6[c]	48·6[c]
Knox Out 2 FM	Control (TR)	29	144·7 ± 29·8	100
	0·040 ± 0·05	27	134·8 ± 26·2	100
	0·076 ± 0·006	28	128·7 ± 22·6[c]	100
	0·125 ± 0·01	28	124·4 ± 29·9[c]	100
	0·260 ± 0·03	30	90·1 ± 24·1[c]	100
	0·490 ± 0·07	25	84·1 ± 26·0[c]	83·4[c]

[a] Per cent spike recovery, 90·5 ± 7·1, $n = 10$.
[b] Trace (0·00007–0·0001 mg litre^{-1}).
[c] Values significantly different from the control.

The estimated half-life for technical grade methyl parathion in Lake Superior water is 18 days and a similar half-life of 15 days was estimated for Penncap-M. However, it took 70 days for Penncap-M to be reduced greater than 90%, whereas the technical grade had a greater than 90% loss in 43 days. A 41-day half-life was determined for technical grade Dursban. No definite half-life could be determined for Dursban 10 CR in the time allotted (200 days). No appreciable decrease in water concentration occurred after equilibrium with the lake water was reached within 8 weeks at ~0·20 mg litre^{-1}; therefore, the half-life can only be stated as > 200 days. However, when a half-life for Dursban 10 CR was determined for a previously saturated solution already at water solubility (toxicant saturator solution), the half-life was only about 14 days longer than that for the technical grade (55 compared with 41 days). The half-life of Knox Out 2 FM was considerably longer than that for

technical grade diazinon (>230 compared with 30 days). As with Dursban 10 CR, no appreciable decrease in water concentration occurred once the compound had reached an equilibrium with the lake water after 1 week, at 34 mg litre^{-1}.

A general summary of the data is presented in Table 4. In general, long-term effects occurred at similar water exposures whether the compound was encapsulated or not. Methyl parathion was the only compound where the technical form was

TABLE 4
METHYL PARATHION, PENNCAP-M, DURSBAN, DURSBAN 10 CR, DIAZINON AND KNOX OUT 2 FM DATA SUMMARY

Toxicant	*Water solubility (mg litre^{-1})*	*Toxicity of aged saturator solutions with time*	*Flow-through 96 h LC_{50} (mg litre^{-1})*	*32-day embryo–larval growth effects (mg litre^{-1})*	*Half-life in Lake Superior water (days)*
Methyl parathion (technical)	76	Greater (4·46–1·22)[a]	5·36	>0·31 <0·38	18
Penncap-M	77	Greater (8·17–3·47)	6·91	>0·38 <0·59	15
Dursban (technical)	2·0	Similar (0·17–0·15)	0·14	>0·0016 <0·0032	41
Dursban 10 CR	1·3	Less (0·13–0·28)	0·12	>0·0022 <0·0048	>200 55[b]
Diazinon (technical)	40	Greater (4·3–2·1)	6·90	>0·050 <0·090	30
Knox Out 2 FM	34	Slightly greater (6·1–5·1)	—[c]	>0·040 <0·076	>230

[a] Static ninety-six hour LC_{50} (mg litre^{-1}), stock solution aged 1–11 weeks.
[b] Half-life for a water solution at solubility (~1 mg litre^{-1}).
[c] No LC_{50} could be determined because of the slow release rate from the capsules, maximum water concentration maintained was only 4 mg litre^{-1}.

slightly more toxic. Ninety-six hour acute toxicity, in general, was greater for the technical grades except for Dursban, which was slightly less toxic than Dursban 10 CR. Ageing of the saturator solutions increased methyl parathion and diazinon toxicity whilst the toxicity of technical Dursban remained similar and Dursban 10 CR became less toxic. Water solubility was slightly lower for the encapsulated compounds with the exception of Penncap-M, which was similar to that of the technical grade. Half-lives for both methyl parathion compounds were also similar whereas, in the other two comparisons, the encapsulated formulation was much more persistent.

DISCUSSION

Methyl parathion and Penncap-M

Water solubilities observed for methyl parathion and Penncap-M are slightly higher than those observed by others. According to the US Environmental Protection Agency (1975), the solubility in water at 25 °C is 55–60 mg litre^{-1} and

Smith *et al.* (1978) stated that it was 50 mg litre^{-1}. In the present study solubility was about 76 mg litre^{-1}. The difference might be due to different water characteristics or perhaps because the saturators warmed the solutions to about 35 °C. Both compounds rapidly entered solution, most probably because the outside walls of the capsules are coated with technical grade methyl parathion which helps to prevent loss of methyl parathion from the encapsulated formulation during storage (Anon., 1976). This excess coating of technical grade methyl parathion could also help explain the similar half-lives observed for the two compounds.

Static studies indicated increased toxicity with time for both compounds. The technical grade was more toxic than the encapsulated formulation and this was probably caused by a difference in the ratio of breakdown products to parent compound. The encapsulated solution should have a higher amount of parent compound present at any given time, and the degradation products are generally considered more toxic than methyl parathion itself (US Environmental Protection Agency, 1975). The primary breakdown product observed for both compounds was *p*-nitrophenol, which is the probable cause of the yellow coloration of the saturator solutions (Smith *et al.*, 1978). Only a trace of methyl paraoxon was identified. Initial static 96-h LC_{50} values were lower than those from flow-through 96-h tests, possibly because the stock solutions had aged for 1 week before the static tests were conducted, whereas the flow-through studies were conducted about 3 days after the saturators were started. The encapsulated formulation was 45–60 % less toxic than the technical grade in static tests, but only 22 % less toxic in the flow-through acute tests, probably because there was less build up of degradation products in the flow-through tests. Some 96-h LC_{50} values for methyl parathion and fathead minnows from the literature are 10·4 mg litre^{-1} (Henderson & Pickering, 1958), 8·0 mg litre^{-1} (Pickering *et al.*, 1962) and 8·9 mg litre^{-1} (Macek & McAllister, 1970). These values are slightly greater than in the present study. This difference, however, could easily be caused by fathead minnow variability, different test water characteristics, or the fact that in this study newly hatched larvae were exposed, whereas in the other studies older fish were tested.

Embryo–larval results also demonstrated slightly greater toxicity (19–35 %) for the technical grade. Here again, it was probably related to the amount of degradation products present. The half-lives for both compounds were similar; however, the encapsulated formulation persisted about 27 days longer. Smith *et al.* (1978) demonstrated that the half-life for methyl parathion will vary from 8 to 38 days, depending upon sunlight during the various seasons of the year. They also calculated a half-life of 89 days for methyl parathion in aqueous solution at 25 °C and below pH 8.

Dursban and Dursban 10 CR

Water solubilities observed for Dursban and Dursban 10 CR (2·0 and 1·3 mg litre^{-1}) are similar to those observed by others (Gray, 1965; Martin, 1971; Dow Chemical Company, 1976*a*). Marshall & Roberts (1978) stated that

chlorpyrifos (Dursban) is only slightly soluble in distilled water (0·4 mg litre^{-1} at 23 °C). The encapsulated formulation was slightly less water soluble than the technical grade and this could have happened because the polyethylene capsules prevented a rapid enough release of the pesticide. Hughes *et al.* (1980) observed that polyethylene-lined pools had a great affinity for Dursban and that residues were maintained for long periods of time.

Static studies demonstrated that the toxicity of the technical grade remained similar over time whereas toxicity of the encapsulated formulation decreased. This was most probably caused by the formation of 3,5,6-trichloro-2-pyridinol: both the oxygen analogue and 3,5,6-trichloro-2-pyridinol were qualitatively observed but the parent compound and the oxygen analogue are considered more toxic than 3,5,6-trichloro-2-pyridinol (Marshall & Roberts, 1978). The same authors state that the oxygen analogue is unstable in water and is unlikely to be encountered. Hurlbert *et al.* (1970) have stated that all Dursban analogues are unstable and that they break down into compounds not readily detectable.

Flow-through 96-h and 32-day studies indicate that both the technical grade and the encapsulated form have similar toxicity. Perhaps a better range for an embryo–larval 'no effect' concentration would therefore lie between 0·0022 and 0·0032 mg litre^{-1}. No literature values (acute or chronic) are available for fathead minnows except for one 72-h LC_{50} value (1·0 mg litre^{-1}, Dow Chemical Company, 1976*a*). Based upon the results in the present study, fathead minnows are apparently not as sensitive to Dursban as are other fish species. Bluegills *Lepomis macrochirus* have a 96-h LC_{50} of 0·0036 mg litre^{-1} (Macek *et al.*, 1972) and the 96-h LC_{50} for striped bass *Morone saxatilis* is 0·00058 mg litre^{-1} (Korn & Earnest, 1974). These acute values are similar to the fathead minnow chronic values observed for this study.

The half-lives for Dursban and Dursban 10 CR already in solution at water solubility (1–2 mg litre^{-1}) are 41 and 55 days, respectively, and are similar to those observed by others. Meikle & Youngson (1978) determined a half-life of 22·8 to 62·7 days for chlorpyrifos (Dursban) in buffered distilled water at 25 °C and pH 8·1, 6·9 and 4·7. Marshall & Roberts (1978) summarised the data from a number of studies and stated that the reported half-life of Dursban associated with hydrolysis in relatively pure waters ranges from 10 to 100 days at temperatures between 15 and 35 °C and pH in the range of 5 to 9. They stated that in natural waters the apparent half-life is actually much shorter. When encapsulated Dursban (1·5 mg litre^{-1}, active ingredient) was added to Lake Superior water in the present half-life study it took about 8 weeks to achieve an equilibrium or plateau, near 0·20 mg litre^{-1}, and no decrease in water concentration had occurred in 200 days. This is similar to results observed by Mulla *et al.* (1973) and Lawson *et al.* (1973), who observed that a dosage of 1·0 mg litre^{-1} in distilled water took 8 weeks to peak at 70 μg litre^{-1} and, after 16 weeks, was still at 60 μg litre^{-1}. Marshall & Roberts (1978) have stated that controlled release formulations can produce chronic exposure patterns analogous to

those associated with the more persistent organochlorine insecticides. The recommended dosage rate for Dursban 10 CR is 1·5 mg litre^{-1} active ingredient in the receiving water (Dow Chemical Company, 1976*b*; Rathburn, 1979). Use of the controlled release Dursban 10·6 % AI has been demonstrated to control mosquitos for 22 weeks (Nelson & Evans, 1974; Evans *et al.*, 1975; Nelson *et al.*, 1976). Dosage rates were 1 and 2 mg litre^{-1} AI; mean measured water concentrations were about 0·0009 amd 0·0014 mg litre^{-1}, respectively. Therefore, a dosage of 1·5 mg litre^{-1} should provide an average water concentration of about 0·0011 mg litre^{-1}. Evans (1977) reported that a dosage of controlled release Dursban at 0·5 to 1·0 mg litre^{-1} AI could control mosquitos for up to 52 weeks. Mean measured water concentrations for the two dosages were 0·00019 and 0·00052 mg litre^{-1}, respectively, and the highest water concentration reached was 0·001 mg litre^{-1}. Brown *et al.* (1976) have reported a growth effect on phytoplankton at 0·0012 mg litre^{-1} technical grade Dursban. Thirugnanam & Forgash (1977) exposed the mummichog *Fundulus heteroclitus* to Dursban at water concentrations as low as 0·001 mg litre^{-1} and observed cumulative acetylcholinesterase inhibition with time. This indeed indicates a potential for chronic effects on non-target aquatic organisms. An embryo–larval chronic level for Dursban 10 CR and fathead minnows in the present study would be about 0·0032 mg litre^{-1} (geometric mean of the lower and upper chronic endpoints). This value is similar to the average expected water concentrations that can be maintained over long periods of time. Non-quantifiable behavioural changes, however, were observed at exposures as low as 0·0007 mg litre^{-1}, and it is possible that a full chronic study with Dursban 10 CR and fathead minnows could demonstrate a statistically significant adverse effect at lower concentrations.

Diazinon and Knox Out 2 FM

Water solubilities observed for technical grade and encapsulated diazinon, 40 and 34 mg litre^{-1}, respectively, are similar to those in the literature (British Crop Protection Council, 1971; Ciba-Geigy, 1975). The slightly lower water solubility for the encapsulated formulation is probably related to the holding capability of the polyethylene capsules.

Static 96-h LC_{50} values demonstrated increased toxicity with time for both compounds. Only one breakdown product peak was observed by liquid chromatography, 2-*iso*-propyl-4-methyl-6-hydroxy-pyrimidine. Diazinon is said to hydrolyse to 2-*iso*-propyl-4-methyl-6-hydroxy-pyrimidine (Gomaa *et al.*, 1969), which is considered less toxic than the parent compound (Ciba-Geigy, 1975; Meier *et al.*, 1979). The diazinon oxygen analogue, diazoxon, is considered more toxic (Ciba-Geigy, 1975) but is reported to be unstable in water (Gomaa *et al.*, 1969).

The flow-through fathead minnow 96-h LC_{50} value (6·9 mg litre^{-1}) for technical grade diazinon is similar to those reported by other researchers. Allison & Hermanutz (1977) determined a flow-through 96-h LC_{50} for fathead minnows of

7·8 mg litre^{-1} (mean of three studies; 6·8, 6·6 and 10·0 mg litre^{-1}). Meier *et al.* (1979) reported a 96-h LC_{50} of 10·3 mg litre^{-1} for fathead minnows in a static test, whereas Dennis *et al.* (1979), also using static tests, observed 96-h LC_{50} values between 5·6 and 10 mg litre^{-1} for a 2% dust formulation and 3·7 mg litre^{-1} for a 48·2% emulsifiable concentrate. In the current study no 96-h LC_{50} could be determined for Knox Out 2 FM at the flow-through rates used, this perhaps being an indication that the encapsulated formulation would not be as great a problem in a moving body of water. However, problems could potentially arise when these capsules settled in a quiet, slow moving area. Some acute values for other species are available for Knox Out 2 FM. The 96-h LC_{50}'s for rainbow trout *Salmo gairdneri* and bluegills are 60·3 and 28·6 mg litre^{-1}, respectively, whereas the 48-h LC_{50} for the water flea *Daphnia magna* is 0·005 mg litre^{-1} (G. D. Curl, pers. comm.). Values for the same species with the technical grade are: rainbow trout, 1·35 mg litre^{-1}, bluegill, 0·12 mg litre^{-1}, and daphnids, 48-h EC_{50}, 0·002 mg litre^{-1} (Meier *et al.*, 1979); bluegill, 0·17 and 0·53 mg litre^{-1} for 2% dust and 48·2% emulsifiable concentrate, respectively (Dennis *et al.*, 1979) and bluegill, 0·46 mg litre^{-1} (Allison & Hermanutz, 1977). A possible explanation for the lower toxicity of the encapsulated formulation mentioned above is that the values were calculated with nominal water concentrations based upon the active ingredient contained in the capsules and not what was actually measured in the water. In the present study it took longer than 1 week for Knox Out 2 FM to reach water solubility levels of 34 mg litre^{-1}.

Embryo–larval upper and lower chronic values are similar for the technical grade and encapsulated formulation, this indicating similar toxicity. Results of the embryo–larval study, however, are not consistent with those of Allison & Hermanutz (1977). These authors conducted a full chronic fathead minnow study and observed significant scoliosis and reproductive effects at the lowest concentration tested (0·0032 mg litre^{-1}). It was also stated that related effects on progeny appeared to have been caused by exposure of parent fish and not by exposure following fertilisation. Growth was affected only at 0·229 mg litre^{-1} and above. In the present study significant growth reduction occurred at 0·076 mg litre^{-1}. In another diazinon chronic study with flagfish *Jordanella floridae* Allison (1977) reported a reduction in larval growth as the most sensitive chronic effect at 0·014 mg litre^{-1}. Goodman *et al.* (1979) chronically exposed sheepshead minnows *Cyprinodon variegatus* to diazinon and demonstrated a statistically significant reduction in fecundity at the lowest concentration tested—0·00047 mg litre^{-1}—but survival of parent fish and survival and growth of progeny in a subsequent 28-day test were unaffected at concentrations as high as 0·0065 mg litre^{-1}. Apparently, for some pesticides and certain fish species, the embryo–larval stage may not be the most sensitive. Some of the toxicity related to diazinon may have been caused by an impurity, sulfotepp. This impurity has been demonstrated to be a hundred times more toxic than technical standard diazinon and has been observed in diazinon formulations at 0·2 to 0·7% (Meier *et al.*, 1979). In the present study sulfotepp was present at less than 0·5% in the technical grade diazinon.

The half-life for technical grade diazinon in Lake Superior water was approximately 30 days whereas that for the encapsulated formulation was extended to more than 230 days. The half-life for the technical grade in other studies has ranged from 14 to 184 days (Gomaa *et al.*, 1969; Cowart *et al.*, 1971; Ciba-Geigy, 1975).

CONCLUSIONS

In these studies the toxicity of the controlled release pesticides appears to be similar to that of the technical grade. Slight differences occur, perhaps related to degradation and parent compound ratios or the affinity of the polyethylene capsules for the pesticide. The danger of controlled release pesticides to the environment appears to be their extended persistence—and thereby potential for chronic effects on non-target aquatic life. Effects can occur at water concentrations expected to be present in the environment. If recommended land usage patterns indicate a potential for contamination, studies to determine possible chronic adverse effects on sensitive non-target organisms should be required before such encapsulated compounds are registered.

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