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The Accumulation and Elimination of Diflubenzuron by Fish

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Diflubenzuron [1-(4-chlorophenyl)-3-(2,6 difluorobenzoyl)-urea; Dimilin; TH6040] is a new type of insecticide which inhibits chitin synthesis (POST and VINCENT 1973). It is highly effective for controlling organophosphorus-resistant strains of mosquitoes in California (SCHAEFER et al. 1975).

During 1976 extensive studies were conducted in Lake County California to evaluate the potential of diflubenzuron for control of the Clear Lake gnat, Chaoborus asticopus Dyar and Shannon. In one field treatment, a 20.6 hectare lake was treated with an amount of diflubenzuron calculated to give an initial concentration of 5 ppb (wt/volume). Water was sampled at 1 m intervals from the surface to the bottom (5 m) at 4 hr and at 1, 2, 3, 4, 7, 14, 21, 28 and 35 days post-treatment. The highest concentrations of diflubenzuron were 3-9 ppb in the upper water levels at 4 hr. At day four the diflubenzuron was evenly distributed with depth and averaged 2.6 ppb and then steadily declined to 0.4 ppb at day 35. White crappies, Pomoxis annularis Rafinesque, collected in a gill net 24 hr after treatment showed tissue residue levels of 130 ppb. Tissue residues increased daily to 355 ppb at 4 days and then steadily declined to 62 ppb at 21 days. No deleterious effects on fish were observed (APPERSON et al. 1978).

In order to understand the residue pattern in fish sampled from Lower Blue Lake, it was of interest to study the potential for accumulation and elimination of diflubenzuron in fish following exposure to treated water in the laboratory. Accumulation is defined as the residue concentration in the fish tissue divided by that in the water (both in ppb).

MATERIALS AND METHODS

Fish were field collected with a 1.2 x 9.1 m beach seine in Lake County during March, April and May 1977 and then transported in 10-gal containers (with aeration), by aircraft to Fresno. The fish were held in 20-gal aquaria and fed mosquito larvae daily prior to testing. Treat-

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ments were made applying diflubenzuron in 1 ml acetone/ℓ of water. Treated aquaria were held at 19-21°C and no food or aeration was provided during the exposure period. In elimination studies, treated fish were rinsed with tap water and then placed in a 16-ℓ rinse tank with a 225 ml/min flow-through of fresh water. At the end of each elimination study, fish were rinsed in tap water, weighed, measured, deviscerated and decaudated. The remaining tissues were extracted, subjected to clean-up by column chromatography (DIPRIMA 1976) and diflubenzuron residues were determined as described by SCHAEFER and DUPRAS (1977). In one test the skin of each treated fish was separated from the inner tissues to determine the extent to which diflubenzuron was adsorbed on the integument.

Only a few white crappies could be obtained but numerous bluegill sunfish, Lepomis macrochirus Rafinesque were caught and the latter were utilized for all but two tests. Fish residues are reported on a fresh weight basis.

The concentration of diflubenzuron remaining in the tank of water at the end of exposure periods was also determined in many of the tests. Both water and fish residues are reported in ppb.

Fortification of fish tissues with 100 ppb diflubenzuron showed quantitative recovery following clean-up and HPLC analysis. Samples of skin and inner tissues were also fortified and analyzed, as above with the same results. The minimum detection limit, using 10 g samples of fish, was 8 ppb. For water, using 600 ml samples, the minimum detection limit was 0.2 ppb (SCHAEFER and DUPRAS 1977).

The partition coefficients of diflubenzuron in a series of binary solvent systems were determined using methods similar to those described by BEROZA and BOWMAN (1965). The concentration of diflubenzuron in 1 ml of the upper phase was determined by HPLC following a single distribution between 5 ml volumes of immiscible solvents at 25°C.

Since p-chlorophenylurea forms via the hydrolysis of diflubenzuron in water, it was of interest to expose fish to 10 ppb and to compare the concentration of diflubenzuron and p-chlorophenylurea in the tank after 24 hr. A control tank (without fish) was also treated at 10 ppb and analyzed for the parent compound and primary hydrolytic product at 24 hr using methods previously described (SCHAEFER and DUPRAS 1976). The fish exposed for 24 hr were then placed in an untreated tank for an additional 24 hr and following that exposure the fish were removed and the water was analyzed for both diflubenzuron and p-chlorophenylurea.

To determine whether fish would adsorb p-chlorophenylurea directly from water, two 4-ℓ tanks were treated with 10 ppb p-chlorophenylurea; a 10 cm bluegill was placed in one and the other served as a control. After 24 hr exposure water from both tanks were analyzed for p-chlorophenylurea.

To determine whether or not exposure of fish would induce the activity of enzymes capable of metabolizing diflubenzuron, fish were held in treated tanks (10 ppb) for 24, 48 and 72 hr exposure periods. In a second experiment, fish were exposed for either 24, 48 or 72 hr but the fish were placed in freshly treated tanks after each 24 hr exposure. After the above exposure periods, the concentration of diflubenzuron in tissues were determined.

RESULTS AND DISCUSSION

When 5.0 cm fish were exposed to 10 ppb for 24 hr, white crappies showed diflubenzuron residues of 822 ppb and bluegill 848 ppb; thus an accumulation of approximately 80 fold occurred. The extent to which various size fish will accumulate diflubenzuron and the affect of volume of treated water is shown in Table 1.

TABLE 1

Residues of diflubenzuron in bluegills exposed 24 hr to water treated at 10 ppb. (A) Represents the mean fish lenght (cm), (B) the mean fish weight (ea), (C) the volume of treated water per tank (ℓ), (D) the mean tissue residue (ppb) and (E) represents the mean residual diflubenzuron concentration in water[a/] (ppb).

A	B	C	D	E
4	1.3	4	610	7.3
9	21.3	4	221	2.2
9	21.3	36	494	4.2
11	47.2	4	145	1.1

[a/] Determined after the 24 hr exposure period.

Larger fish deplete the diflubenzuron content in 4-ℓ tanks within 24 hr and larger volumes of treated water are necessary to show the accumulation potential in static tests. When 9.5 cm bluegills were exposed to concentrations of 1-10 ppb, the quantity accumulated was apparently directly proportional to concentration (Table 2).

TABLE 2

Residues of diflubenzuron in 9 cm bluegills exposed 24 hr to water treated from 1 to 10 ppb. (A) Represents conc. treated (ppb), (B) the tissue residue (ppb), (C) the final diflubenzuron conc. in water (ppb), (D) the percent of initial conc. in water at 24 hr and (E) the 24 hr accumulation.

A	B	C	D	E
1.0	14	0.2	20	14X
2.5	32	0.5	20	13X
5.0	91	1.1	22	18X
10.0	199	2.1	21	20X

Comparisons of residues in the integument and in the inner tissues of 11 cm bluegills exposed to 10 ppb for 24 hr showed 218 ppb in the skin and 232 ppb in the inner tissues. Thus, the diflubenzuron seems to be evenly distributed in these tissues.

Following a 24 hr exposure of 6 cm bluegills to 10 ppb diflubenzuron, tissues showed residues of 107 ppb; however, other lots of fish receiving the same exposure and when placed in untreated flow-through rinse tanks for 24, 48, 72 and 96 hr showed no detectable tissue residues after any of these intervals. In a second elimination study, 10 cm bluegills exposed to 10 ppb for 24 hr showed only 20 ppb and no detectable tissue residues were found in those held in the rinse tank for 48 hr or longer. Thus, diflubenzuron residues decline rapidly when the exchange equilibrium is reversed. The very rapid loss is very different than for the slow loss of biomagnified DDT from fish (HANSEN and WILSON 1970) in which a 38,000 fold biomagnification occurred and only 78-87% loss occurred after 8 weeks.

HAMELINK et al. (1971) proposed a mechanism to explain accumulation of organochlorine compounds by fish: the body load of pesticides increases as the fat content of fish increases and pesticide magnification is inverse to the water solubility of the compounds. According to their mechanism the level of accumulation corresponds to the partition coefficients of the compounds, once the fat content of the fish and the time available for equilibrium are taken into account. Fish take up compounds directly from water via the gills and in their food. The loss of compounds from contaminated fish will then depend on the reduction of residue concentration in the water.

Both diflubenzuron and DDT have low water solubilities, approximately 200 ppb for the former and

1.2 ppb for the latter. The partition coefficients for these compounds are quite different (Table 3).

TABLE 3

Solvent Combination	Partition coefficient[a] Diflubenzuron	p,p'-DDT[b]
hexane/water	0.98	
heptane/water	0.96	
iso-octane/water	0.00	
deithylether/water	1.00	
water/dichloromethane	0.00	
iso-octane/80% acetone[c]	0.54	0.93
iso-octane/dimethylformamide	0.00	0.084
iso-octane/85% dimethylformamide[c]	0.55	0.36
heptane/85% dimethylformamide[c]	0.00	
heptane/90% ethanol[c]	0.18	0.64
hexane/acetonitrile	0.00	0.38

[a] Proportion remaining in upper phase after a single partition.
[b] Data of BOWMAN and BEROZA (1965).
[c] Aqueous dilution.

Diflubenzuron partitions completely from hexane into acetonitrile while 38% of DDT remains in the less polar phase. Thus, diflubenzuron has a much greater tendency to partition into polar solvents which may partially explain its loss from fish as its concentration in water diminishes.

When 9 cm bluegills were exposed to 10 ppb diflubenzuron for 24 hr their tissues averaged 264 ppb. The water in the tanks in which the fish were exposed showed 2.6 ppb diflubenzuron and 1.1 ppb p-chlorophenylurea. The control tanks (treated at 10 ppb diflubenzuron with no fish) had aqueous residues of 8.0 ppb diflubenzuron and 3.8 ppb p-chlorophenylurea after the 24 hr holding period. Fish treated as above and then placed into untreated water for 24 hr showed only 8.0 ppb diflubenzuron in their tissues and water from the holding tank showed no detectable diflubenzuron and 0.4 ppb p-chlorophenylurea. Thus, fish eliminate diflubenzruon at a high rate and neither the parent compound nor the primary hydrolytic metobolite are important excretory products.

When 10 cm bluegills were exposed to 10 ppb p-chlorophenylurea for 24 hr, there was no uptake from the treated water; the concentration of p-chlorophenylurea remained at 10 ppb in tanks having fish as well as in the control tanks at the end of the 24 hr exposure period. Thus, this hydrolytic metabolite is apparently too polar to partition into the gills.

When 6 cm white crappies were exposed to 10 ppb diflubenzuron, tissue residues were 822, 533 and 630 ppb for exposure periods of 24, 48 and 72 hr, respectively. The reduction of tissue concentrations after 24 hr apparently is due to enzymatic degradation and continued uptake may have been limited because of depletion of diflubenzuron from the tank water. However, when 7 cm bluegills were exposed to 10 ppb diflubenzuron for 24, 48 and 72 hr and freshly treated water was provided daily, tissue residues were 158, 306 and 266 ppb for 24, 48 and 72 hr exposure, respectively. Thus, it is apparent that diflubenzuron is being metabolized by the fish at a high rate by 72 hr. The pattern observed in these studies appears to be similar to that described by MAYER (1976) for the degradation and elimination of di-2-ethylhexyl phthalate in fathead minnows, Pimephales promelas.

In summary, diflubenzuron is accumulated from water into fish tissues at levels up to 80 fold within 24 hr when fish are exposed to concentrations of 10 ppb. Within the concentration range of 1-10 ppb, the amount accumulated in a 24 hr exposure is proportional to concentration. After 24 to 48 hr exposure fish degrade and eliminate diflubenzuron and the excretory products are neither the parent compound nor p-chlorophenylurea. The amount of diflubenzuron remaining in fish tissues with time is dependent on the reduction of residue concentration in water; however, the potential for degradation and elimination is very great.

REFERENCES

APPERSON, C. S., C. H. SCHAEFER, A. E. COLWELL, G. WERNER, N. ANDERSON, E. F. DUPRAS, JR., and D. LONGANECKER: J. Econ. Entomol. (1978). In press.

BOWMAN, M. C. AND M. BEROZA: J. Assoc. Offic. Anal. Chem. 48, 943 (1965).

DIPRIMA, S. J., Analytical Methods Nos. 1, 2, 3, Research and Development Department, Thompson-Hayward Chemical Co., Kansas City, Kansas.

HAMELINK, J. L., R. C. WAYBRANT and R. C. BALL: Trans. Amer. Fish. Soc. 100, 207 (1971).

HANSEN, D. J. and A. J. WILSON, JR.: Pestic. Monit. J. 4, 51 (1970).

MAYER, F. L.: J. Fish. Res. Board Can. 33, 2610 (1976).

POST, L. C. and W. R. VINCENT: Die Naturwissenschaften, 60, 431 (1973).

SCHAEFER, C. H., W. H. WILDER and F. S. MULLIGAN III: J. Econ. Entomol. 68, 183 (1975).

SCHAEFER, C. H. and E. F. DUPRAS, JR.: J. Agr. Food Chem. 24, 733 (1976).

SCHAEFER, C. H. and E. F. DUPRAS, JR.: J. Agr. Food Chem. 25, 1026 (1977).