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85. Hydrolysis and Photolysis of the Lampricide 2′,5-Dichloro-4′-nitrosalicylanilide (Bayer 73)

UNITED STATES DEPARTMENT OF THE INTERIOR
FISH AND WILDLIFE SERVICE

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60. Toxicity of the Lampricide 3-Trifluoromethyl-4-nitrophenol (TFM) to Nontarget Fish in Static Tests, by L. L. Marking and L. E. Olson. 1975. 27 pp.
61. Toxicity of the Lampricide 3-Trifluoromethyl-4-nitrophenol (TFM) to Nontarget Fish in Flow-Through Tests, by L. L. Marking, T. D. Bills, and J. H. Chandler. 1975. 9 pp.

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Survival of Two Species of Freshwater Clams, *Corbicula leana* and *Magnonaias boykiniana*, After Exposure to Antimycin

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Abstract

The Asiatic clam, *Corbicula leana* Prime, and a clam native to the southern United States, *Magnonaias boykiniana*, were exposed to the fish toxicant antimycin at several concentrations for various periods and then placed in an untreated earthen pond for posttreatment observation. Both species survived the concentrations and exposure periods usually used in field application. However, latent mortalities were observed in the pond 3 months after a 30-day flow-through exposure of *Corbicula* to 3.6 to 30 μg/l of antimycin. A single treatment (2 μg/l) in an earthen pond did not result in significant mortalities of *Corbicula* during 22 weeks. *Magnonaias* was more sensitive than *Corbicula* to antimycin, but both survived the maximum permissible use-pattern concentrations in flow-through tests.

Asiatic clams (several species of the genus *Corbicula*) introduced into U.S. waters have spread to many important river systems (Sinclair and Isom 1963; Keup et al. 1963; Diaz 1974). Asiatic clams are becoming so numerous that they cause problems in water intakes to industrial plants, in the pipes and plumbing of municipal water supplies, in sand and gravel operations, and as competitors with native species of clams for habitat and food.

The widespread distribution of Asiatic clams suggests hardiness and an ability to tolerate adverse environmental conditions. Sinclair and Isom (1963) indicated that these clams can survive fluctuating environmental conditions, and Chandler and Marking (1975) reported that they are more resistant than native clams to the lampricide, 3-trifluoromethyl-4-nitrophenol (TFM). Burress et al. (1976) found Asiatic clams useful in laboratory toxicity tests because they are hardy (*Corbicula leana* survived for 18 months in outdoor plastic pools with no water exchange) and can be easily collected, transported, handled, and exposed to chemicals.

Magnonaias boykiniana, the other clam used in the present studies, is native to many American river systems, including those in Georgia.

Pond studies with the fish toxicant, antimycin, showed that applications of 5 μg/l severely reduced plankton populations, whereas benthic invertebrates were not severely reduced (Callaham 1968; Callaham and Huish 1968). Field applications of antimycin in Wisconsin were reported to have resulted in delayed mortality of several species of mollusks in the East Branch, Rock River (Bratley and Mathiak 1972) and in the Ashippum River (Flowers et al. 1975). A comprehensive summary of data on the effects of antimycin on nontarget organisms prepared by Schnick (1974) indicated that the 96-h LC_{50} for antimycin against Asiatic clams was 50 μg/l.

Antimycin is generally applied at concentrations ranging from 1 to 10 μg/l in single applications for the control of undesired fish populations in ponds and lakes, or for up to 12 h in streams. The present study was designed to determine the acute toxicity and latent mortality resulting from short- and long-term exposures of the Asiatic clam, *Corbicula leana*, and a native clam, *Magnonaias boykiniana*, to antimycin. Concentrations and exposures investigated equaled or exceeded those currently used for fish control.

Materials and Methods

The clams used in this study were collected from shoals of the Flint River east of Woodbury (Upson County), Georgia. They were transported to the laboratory and kept in limed flowing water until exposed to the toxicant. Deformed organisms or any that appeared weakened by handling were discarded. Mature specimens of uniform size were selected for the tests.

Clams were exposed to antimycin (Fintrol Concentrate formulation) in limed spring water (total hardness 18–22 mg/l as $CaCO_3$ and pH 6.8) in four types of tests.

(1) Standardized static laboratory tests were conducted in three series of 19-liter glass jars containing 15 liters of water. The substrate was mud in one series and sand in a second; one series of jars contained no substrate. Ten Asiatic clams were exposed to 5, 10, or 20 μg/l of antimycin for 1 or 30 days. Antimycin was prepared in concentrated stock solutions and portions were added to the test water to obtain desired concentrations. Tests were conducted according to recommendations of the Committee on Methods for Toxicity Tests with Aquatic Organisms (1975). Surviving clams were placed in cages in untreated, previously filled earthen ponds for observation over a 112-day period.

(2) An earthen 0.04-ha pond was treated with 2 μg/l of antimycin by dispensing a solution of the toxicant into the propeller wash of an outboard motor. Mortality was recorded among the 300 stocked Asiatic clams and 85 native clams (weight range, 200–500 g) over a 22-week posttreatment observation period. The pond water had the following characteristics: total hardness as $CaCO_3$, 2.0–14.0 mg/l; pH 5.6–7.4; temperature 6.0–19.0 C; and dissolved oxygen 8.7–15.0 mg/l.

(3) Standardized flow-through toxicity tests were conducted in an apparatus similar to that described by Mount and Brungs (1967). Asiatic clams were exposed to 3.6 to 30 μg/l of antimycin in 50-liter glass aquaria for 30 days. Thirty clams were exposed to each concentration. The stock solution of antimycin was metered into a mixing box, and dilutions of the mixture yielded selected concentrations. Mortalities were recorded daily during exposure.

(4) Flow-through tests were conducted in fiberglass tanks in which 300 Asiatic and 75 or 85 native clams were exposed to 0.2 and 5 μg/l of antimycin for 12 h or to 50 μg/l for 24 h. After exposure, the clams were observed in untreated pond water for 22 weeks. Antimycin solutions were mixed in epoxy-coated steel tanks containing 3,623 liters of limed spring water and pumped through the tanks at a rate of 4.4 liters/min.

Small amounts of boiled trout chow and cereal leaves (*Daphnia* food) were offered weekly to the Asiatic clams in long-term static and flow-through exposures, and excess food was routinely siphoned off.

Survivors of each test were acclimated to holding pond temperatures and placed in cages submersed in the ponds. Each cage contained a 2-cm layer of soil substrate obtained from the holding pond. Cages were placed so that water continuously covered the substrate in the cage to a depth of 41 to 46 cm.

The clams were moved to the holding ponds and examined semimonthly after exposure. Clams that were unable to retract the foot or adduct the valves were considered dead. The period between mortality determinations was usually long enough so that only a casual observation was required because the soft tissues of dead specimens protruded from the valves. All mortality tabulations are cumulative.

Results

Corbicula survived 1-day exposures to 5, 10, and 20 μg/l of antimycin in 19-liter glass jars containing no substrate or substrates of sand or mud (Table 1). During the 29-day posttreatment holding period in jars containing untreated water, no clams died that had been exposed with no substrate, or with mud substrate; however, 70% of the clams died that had been exposed to 20 μg/l of antimycin in jars with sand substrate. During the later 112-day period in the holding pond, a few more died that had been exposed in jars with a sand substrate.

Clams exposed for 30 days to 5, 10, or 20 μg/l of antimycin were affected especially during the posttreatment observation period. By the end of the 112-day holding period in earthen ponds, all clams had died, except for 4 of 10 exposed to 5 μg/l with a sand substrate; thus exposure time was a critical factor.

Antimycin was generally not toxic in 1-day exposures, but latent mortality developed in the 30-day exposures. The 1-day exposures typify field use patterns, and 30-day exposures greatly exceed those used in fishery management.

Table 1. *Cumulative percentage mortality of* Corbicula leana *after static exposures of 1 day or 30 days to selected concentrations of antimycin. Each jar contained 10 clams.*

Substrate and concentration of antimycin (μg/l)	Exposure period (days)	Days in jars[a]	Days in untreated holding pond after exposure		
		30	28	70	112
No substrate					
0	1	0	0	0	0
0	30	0	0	10	10
5	1	0	0	0	0
5	30	10	30	80	100
10	1	0	0	0	0
10	30	50	80	100	100
20	1	0	0	0	0
20	30	50	60	100	100
Sand					
0	1	0	0	0	10
0	30	0	0	0	0
5	1	0	0	10	10
5	30	0	20	50	60
10	1	10	10	20	20
10	30	10	20	90	100
20	1	70	70	70	70
20	30	20	20	90	100
Mud					
0	1	0	0	0	0
0	30	10	10	10	10
5	1	0	0	0	0
5	30	20	20	100	100
10	1	0	0	0	0
10	30	50	50	70	100
20	1	0	0	0	0
20	30	40	40	100	100

[a]Clams exposed for 1 day were placed in 15 liters of untreated water in 19-liter jars for 29 days after exposure, and then placed in a holding pond with those exposed in jars for 30 days.

In the static pond application, in which *Corbicula* and *Magnonaias* were exposed to 2 μg/l of antimycin and the toxicant was allowed to dissipate and detoxify with time, 98% of the *Corbicula* and nearly 65% of the *Magnonaias* survived for 22 weeks after the toxicant application (Table 2).

Table 2. *Cumulative percentage mortality after 2 to 22 weeks among 300* Corbicula leana *and 85* Magnonaias boykiniana, *in a pond treated with 2 μg/l of antimycin.*

Species	Weeks			
	0	8	16	22
Corbicula	0	1.7	1.7	2.0
Magnonaias	0	22.4	32.9	35.3

Corbicula survived 30 days of exposure to antimycin concentrations of 3.6 to 30 μg/l in a standardized flow-through system (Table 3). Exceptions were a single mortality at 3.6 μg/l and two at 15.1 μg/l. These mortalities probably resulted from stresses other than the antimycin, since all clams survived at higher concentrations. Few clams died during the first 86 days after they were transferred to the holding pond, but latent mortality became significant thereafter. After 156 days, mortality ranged from 27 to 77% (Table 3). The die-off seemed to stabilize toward the end of the observation period; the increase in mortality from 128 to 156 days was minor. Again, the 30 days of continuous exposure that led to latent mortality far exceeded exposure time in field applications.

Survival of *Corbicula* exposed to 2 and 5 μg/l of antimycin for 12 h was high during exposure and during

Table 3. *Mortality among 30* Corbicula leana *during exposure for 30 days to various concentrations of antimycin in a flow-through system, and cumulative percentage mortality in a holding pond 44 to 156 days posttreatment.*

Concentration (μg/l)	Percentage mortality during exposure	Days after end of exposure period			
		44	86	128	156
0.0	0.0	0.0	0.0	0	0
3.6	3.3	3.3	10.0	47	53
4.4	0.0	0.0	3.3	27	27
5.9	0.0	0.0	0.0	23	30
8.3	0.0	0.0	3.3	50	50
12.4	0.0	0.0	3.3	50	57
15.1	6.6	6.6	16.0	63	67
20.7	0.0	0.0	3.3	63	73
24.1	0.0	0.0	3.3	67	70
30.0	0.0	3.3	6.6	77	77

the later 22-week observation period in an earthen pond; mortality ranged from only 0.7 to 2.7% for exposed clams and was 2.3% for unexposed clams (Table 4). The mortality of *Corbicula* exposed to 50 μg/l of antimycin for 24 h was slightly higher — 4% at 2 weeks and 8.7% after 22 weeks.

Magnonaias was more sensitive than *Corbicula* to antimycin in the 12-h exposures to 2 and 5 μg/l and the 24-h exposure to 50 μg/l in the flow-through tank system. Mortality was low among *Magnonaias* exposed for 12 h during the first 2 weeks of observation, but increased to 20 to 53% after 22 weeks (Table 4). During the 22-week period, however, 20% of the unexposed clams also died. The concentrations of 5 μg/l of antimycin for 12 h and 50 μg/l for 24 h killed more than half of the clams during the 22-week observation period. Despite these losses it seems clear that the population would not have been eliminated, even by 24-h exposures to 50 μg/l.

Table 4. *Cumulative percentage mortality among 300* Corbicula leana *and 75 or 85* Magnonaias boykiniana *exposed to antimycin for 12 or 24 h in flow-through tanks and moved to an earthen pond for observation for 22 weeks.*

Species and concentration (μg/l)	Exposure (h)	Weeks			
		2	8	16	22
Corbicula					
0	12	0.3	0.3	1.7	2.3
2	12	0.0	0.7	0.7	0.7
2	12	—	2.7	2.7	2.7
5	12	0.6	1.7	1.7	2.0
50	24	4.0	6.0	8.7	8.7
Magnonaias					
0	12	1.3	13.3	18.7	20.0
2	12	0.0	9.3	17.3	20.0
2	12	—	12.9	23.5	34.1
5	12	3.5	35.3	50.6	52.9
50	24	2.4	30.6	48.2	50.6

Discussion

Exposure times longer than 12 h and concentrations of antimycin higher than 10 μg/l are rare in stream treatments with antimycin. The registered label prescribes concentrations of 1 to 10 μg/l, depending on physical and chemical conditions of the water. Our exposures of 30 days were excessive. In streams the toxicant is added to flowing water, where concentrations build up to a peak of short duration and then decrease as a result of dilution and decomposition. Stream organisms are thus exposed to a slug of the toxicant passing downstream for an exposure time that varies with the species of target fish, and with environmental conditions (Gilderhus 1972).

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Chronic and Simulated Use-Pattern Exposures of Brook Trout (*Salvelinus fontinalis*) to 3-Trifluoromethyl-4-nitrophenol (TFM)

by

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Abstract

Effects of the lampricide 3-trifluoromethyl-4-nitrophenol (TFM) on brook trout (*Salvelinus fontinalis*) were compared under conditions of continuous (chronic) exposure, and under conditions simulating those used in the application of TFM in tributary streams of the Great Lakes for control of the sea lamprey (*Petromyzon marinus*). Chronic exposure of adult brock trout to concentrations of 4.0 mg/l or higher caused deleterious effects on growth, spawning, survival during spawning, and eye condition. Hatchability and viability of the eggs were reduced. Growth and survival were reduced in the fry at TFM concentrations of 1.6 mg/l or higher. No deleterious effects were noted at lower concentrations. The only effect observed in fish after simulated use-pattern exposure to TFM was a decrease in survival of adults tested at 15 C.

The lampricide 3-trifluoromethyl-4-nitrophenol (TFM) was registered in 1964 for control of larval sea lampreys (*Petromyzon marinus*) in selected tributaries of the Great Lakes. The U.S. Environmental Protection Agency (EPA) is now reviewing the registration of TFM. A substantial amount of research has been conducted on TFM toxicity and selectivity to sea lamprey larvae, as indicated by the literature reviewed by Schnick (1972). Later research on toxicity included work with algae (Maki et al. 1975), midge larvae (Kawatski et al. 1975), mayfly nymphs (Fremling 1975), aquatic invertebrates and frog larvae (Chandler and Marking 1975), and early life stages of rainbow trout (Olson and Marking 1973); other studies were on toxicity and residue dynamics in invertebrates (Sanders and Walsh 1975), and included tests on non-target fish — both static (Marking and Olson 1975) and flow-through (Marking et al. 1975).

The accumulation and elimination of TFM residues by fish exposed to TFM after a lamprey control treatment of the East Au Gres River, Michigan, were reported by Gilderhus et al. (1975). Biotransformation and elimination of TFM by fish were elucidated by Lech and Costrini (1972). Hunn and Allen (1974, 1975) reported on the factors affecting the uptake and elimination of the lampricide, and the renal excretion of the compound in fish.

The objectives of the present research were to determine the effects of TFM on brook trout (*Salvelinus fontinalis*) under continuous (chronic) exposure and under exposures simulating a use pattern to which fish would be exposed during a stream treatment. Brook trout are an important and indigenous sport fish in many of the streams treated with TFM. Factors measured were mortality, growth, and reproduction. Samples of fish were analyzed to determine accumulation and elimination of TFM after continuous and simulated use-pattern exposures.

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Materials and Methods

Chronic Study

Yearling brook trout obtained from the Manchester (Iowa) National Fish Hatchery were held in raceways at 16 C and fed a maintenance diet (Brauhn and Schoettger 1975) until the tests were started. On 14 June 1973, 12 fish were placed in each of 12 stainless steel tanks, measuring 137 cm long, 36 cm wide, and 51 cm high, in which water depth was 30 cm. Well water was delivered to the tanks at a rate of 800 ml/min per tank through a proportional diluter system modeled after Mount and Brungs (1967). Each tank was aerated with filtered air to maintain oxygen concentrations above 70% saturation. Adult fish were fed a modification (Mehrle et al. 1977) of the Oregon Test Diet (National Academy of Sciences 1973) ad libitum throughout the study. Fry were fed the commercial trout starter "Ewos."

A diluter system with the modification of McAllister et al. (1972) and a dilution factor of 0.5 between concentrations was used to continuously deliver five concentrations of TFM and a control for the chronic test. The TFM used was a 35.7% field grade formulation manufactured by the American Hoechst Chemical Company; distilled water was the carrier solvent. The measured TFM concentrations (total formulation) averaged 0.94, 1.6, 4.0, 8.8, and 16 mg/l (or 0.34, 0.57, 1.4, 3.1, and 5.7 mg/l active ingredient). Flow-splitting chambers designed by Benoit and Puglisi (1973) were used to thoroughly mix and divide each lampricide concentration for delivery to duplicate exposure tanks. The water temperature in the tanks was controlled by refrigeration units suspended in a circulating water bath. Artificial daylight was provided by the method of Drummond and Dawson (1970), and the water temperature regime and photoperiod were those recommended for brook trout tests (EPA 1972).

The study was conducted according to the recommended procedures for partial chronic tests with brook trout by EPA (1972). The fish were measured (total length) after 60 and 120 days of exposure. After the final growth determinations, the fish were thinned to five to eight per tank and two spawning substrates were placed in each tank. The excess fish above three females and two males were used for artificial reproduction (stripping of eggs and sperm). Spawning began 28 November 1974 and ended 16 January 1975. Two samples of 100 eggs from each spawning, both natural and artificial, were placed in incubator cups to determine hatchability and viability. Viability was determined 10 days after spawning by placing the eggs in 10% acetic acid for several minutes until the neural keel became visible, indicating that the embryo was developing. Upon hatching, 25 fry were randomly selected from each incubation cup, their length was determined by the photographic method of McKim and Benoit (1971), and they were transferred to growth chambers 14 cm deep, 38 cm long, and 15 cm wide, in which water was 10 cm deep. The fry were also measured photographically at 30 and 60 days, and measured directly at 90 days. Mortality of fry was recorded daily.

Use-Pattern Study

The use-pattern study was initiated a year after the chronic study, with 2-year-old fish from the same lot used in the chronic study. The procedures were essentially the same, except that the exposure of fish to TFM was designed to simulate stream treatment for lamprey control. In such treatments, TFM is metered into streams at concentrations of 2 to 20 mg/l (total formulation) for 10 to 12 h, depending on water quality and on-site bioassay. A stream is normally treated once during the summer or fall. In the simulated use-pattern study, eight fish were placed into each of six tanks. TFM was metered into two tanks for 12 h at 18 mg/l on 30 July 1974, and into two other tanks at 16 mg/l on 1 October 1974; the remaining two tanks served as controls. The diluter system used to expose the fish was that of Brungs and Mount (1967). Since egg viability and hatchability did not differ between eggs naturally and artificially spawned in the chronic study, spawning was expedited in the use-pattern study by hand stripping sexually mature brook trout. Egg viability and hatchability, and fry growth were measured as in the chronic study.

Residue Analysis

Residues of TFM were determined on both fillet and offal of four adult fish from each concentration after 137 days of continuous exposure. Six additional fish from each of the 1.6- and 4.0-mg/l exposures were transferred to uncontaminated water at 9 C to determine the elimination rate of TFM; two fish were sampled from each exposure after 3, 7, and 14 days in fresh water. A sample of eggs from each of three spawns within each TFM concentration was also analyzed. Three fish from the controls and the last exposure of the use-pattern study were sampled 45 days after that exposure. Fish and eggs were prepared for residue analysis by the method of Benville and Tindle (1970) and extracted by the column chromatography technique of Hesselberg and Johnson (1972). The extracts were analyzed by gas chromatography (Allen and Sills 1974), in which the minimum detection limit was 1 ng/g. (However, TFM residues below 10 ng/g

were not quantifiable.) Concentrations of TFM in water were periodically determined by the colorimetric method of Olson and Marking (1973).

Experimental Design and Analysis

The design of both tests was a randomized block. Growth data (length gained) were analyzed by analysis of variance (Snedecor 1965). A multiple means comparison test (least significant difference) was used to compare treatments. The effects of TFM on fish mortality and egg hatchability and viability were determined by conducting an analysis of variance on the arcsin transformation for proportions (angle = arcsin $\sqrt{\text{percentage}}$), followed by least significant difference tests.

Results

No differences in adult growth were detected after 60 days of chronic exposure to TFM. However, growth was significantly reduced ($P < 0.05$) after 120 days in fish exposed to 8.8 and 16 mg/l TFM (Table 1). Growth of adult fish under use-pattern conditions was not affected. Chronic exposure to high concentrations of TFM may have caused blindness in some adult fish. The eye was covered with an opaque layer and, in some fish, was filled with blood. After 120 days exposure to 16 mg/l TFM, 10% of the fish were affected in at least one eye; by 180 days, 42% of the fish were affected. Although none of the other fish exposed to lower concentrations showed similar effects at 120 days, a few fish exposed to 8.8 mg/l TFM developed the eye condition at 180 days.

As total spawning activity increased with time, so did the mortality of the adults exposed to 16 mg/l TFM and (to a lesser extent) of those exposed to 8.8 mg/l (Table 2). Apparently the fish died from spawning stress in addition to the TFM exposure. At death, the fish were covered with copious amounts of mucus. Under use-pattern conditions, 19% of the adult fish died during the early exposure, when the water temperature was 15 C. No mortalities occurred at 9 C, just before the fish spawned, in the late exposure.

In the chronic-exposure study, no spawns were obtained from the fish exposed to 16 mg/l TFM, and only one natural spawn occurred at 8.8 mg/l (Table 3). However, these eggs were not viable. Inasmuch as egg viability and hatchability of natural and artificial spawns were not statistically different, the viability and hatchability data were pooled (Table 4). The viability of eggs in the 4.0 mg/l TFM concentration was statistically less ($P < 0.05$) than that of eggs in the lower concentrations and controls. Hatchability of eggs at this concentration was also lower, although not significantly so. In addition, the eggs exposed to 4.0 mg/l TFM were not consistent in size, shape, and neural keel development, when compared with those exposed to lower concentrations, and the control. The TFM had no effect on viability or hatchability of eggs from adults treated in the use-pattern exposures.

Table 1. *Mean total lengths (mm; standard deviations in parentheses) of adult brook trout before, and 120 days after chronic exposure of yearlings and 100 days after use-pattern exposure of 2-year-olds, to different concentrations of TFM.*

Type of exposure, and concentration of TFM (mg/l)[a]	Length (mm) of fish: On day 0	Length (mm) of fish: On day 120 (chronic) or 100 (use-pattern)
Chronic		
0.0	213 (14)	267 (16)
0.94	215 (12)	265 (17)
1.6	218 (15)	268 (17)
4.0	213 (22)	265 (10)
8.8	220 (13)	264 (15)[b]
16.0	220 (11)	253 (18)[b]
Use-pattern		
0.0	300 (27)	316 (28)
18.0	306 (28)	324 (30)
16.0	312 (36)	320 (30)

[a] Total formulation.

[b] Length gained significantly different ($P < 0.05$) from that of controls.

Table 2. *Mortality of brook trout after chronic and use-pattern exposures to TFM.*

Type of exposure, and concentration of TFM (mg/l)[a]	Mortality (%) Adults	Mortality (%) Fry
Chronic		
0.0	0	27
0.94	0	58
1.6	0	84[b]
4.0	0	100[b]
8.8	34[b]	100[b]
16.0	68[b]	100[b]
Use-pattern		
0.0	0	14
18.0	19[b]	6
16.0	0	9

[a] Total formulation.

[b] Significantly different from controls ($P < 0.05$).

Table 3. *Production of spawn by brook trout after chronic and use-pattern exposures to TFM.*

Type of exposure, and concentration of TFM (mg/l)[a]	Natural spawns		Artificial spawns (number)
	Number	Eggs per spawn (number)	
Chronic			
0.0	7	309	2
0.94	6	294	4
1.6	8	215	3
4.0	8	236	1
8.8	1	143	0
16.0	0	—	0
Use-pattern[b]			
0.0	—	—	5
18.0	—	—	4
16.0	—	—	5

[a] Total formulation.

[b] Eggs per spawn were not considered because the fish were stripped to obtain eggs and sperm.

Table 4. *Viability and hatchability of brook trout eggs after chronic and use-pattern exposures of TFM.*

Type of exposure, and concentration of TFM (mg/l)[a]	Viability (%)	Hatch (%)
Chronic		
0.0	82	67
0.94	87	67
1.6	88	74
4.0	50[b]	43
8.8	0[b]	
16.0	—[c]	
Use-pattern		
0.0	92	82
18.0	97	88
16.0	97	85

[a] Total formulation.

[b] Significantly different from controls ($P < 0.05$).

[c] No spawns produced.

For the first 60 days of chronic exposure, no statistical difference existed in the growth of fry continuously exposed to TFM; however, at 90 days growth was significantly reduced ($P < 0.05$) in fry exposed to 1.6 mg/l TFM (Table 5). The length gained in fry exposed for 90 days to 0, 0.94, and 1.6 mg/l TFM was 35.2, 32.7, and 25.8 mm, respectively. All fry exposed to 4.0 mg/l TFM were dead within 30 days (Table 2). No significant differences in mortality of fry exposed to the two lower concentrations and controls were observed at 30 days of exposure. At 60 and 90 days, however, the mortality of fry exposed to 1.6 mg/l TFM was significantly higher ($P < 0.05$) than that of the controls. Growth and survival of fry were not affected in the use-pattern study.

Table 5. *Mean total lengths (mm; standard deviations in parentheses) of brook trout fry 90 days after chronic exposure to different concentrations of TFM or fry from parents exposed to use-pattern concentrations of TFM.*

Type of exposure, and concentration of TFM (mg/l)[a]	Length (mm) of fish:	
	On day 0	On day 90
Chronic		
0.0	14.8 (0.6)	50 (2)
0.94	14.3 (1.0)	47 (4)
1.6	15.2 (0.9)	41 (6)[b]
4.0	—[c]	—
8.8	—[d]	—
16.0	—[e]	—
Use-pattern		
0.0	16.2 (0.9)	49 (5)
18.0	16.5 (1.1)	51 (4)
16.0	16.7 (0.7)	49 (3)

[a] Total formulation.

[b] Significantly different from controls ($P < 0.05$) based on length gained.

[c] Fry died within 30 days.

[d] No eggs hatched.

[e] No spawns produced.

Table 6. *Mean concentrations of TFM (μg/g; standard error in parentheses) in fillets and offal of adult brook trout and their eggs, after continuous exposure to different concentrations of TFM for 137 days.*

Concentration of TFM (mg/l)[a]	Fillets (n = 4)	Offal (n = 4)	Eggs (n = 3)
0.0	<0.01	<0.01	<0.01
0.94	0.01 (0.003)	0.06 (0.02)	0.18 (0.02)
1.6	0.06 (0.01)	0.43 (0.10)	0.32 (0.03)
4.0	0.09 (0.01)	0.19 (0.03)	0.74 (0.17)
8.8	0.08 (0.04)	0.18 (0.05)	1.9[b]
16.0	0.11 (0.03)	0.38 (0.11)	2.8 (0.49)[c]

[a]Total formulation.

[b]n = 1.

[c]Eggs for samples were removed from females since there were no natural spawns in this exposure.

Residues of TFM in the eggs of brook trout in the chronic-exposure study averaged 0.18 μg/g in fish exposed to 0.94 mg/l TFM and 2.8 μg/g in fish exposed to 16 mg/l (Table 6). The fillet and remaining offal from adult brook trout were analyzed separately. TFM in the fillets ranged from 0.01 μg/g for fish exposed to 0.94 mg/l TFM to 0.11 μg/g for fish exposed to 16 mg/l, indicating an accumulation factor of 0.02 to 0.1, based on the active ingredient. Residues of TFM in the offal from adult brook trout varied considerably, but the indicated accumulation factor was 0.8 or less for each of the exposure concentrations. No residues were found under use-pattern conditions. Results from the residue elimination phase of the experiment indicate that the loss of TFM was rapid (Table 7).

Discussion

Chronic exposure of adult brook trout to TFM concentrations of 4.0 mg/l or less caused no significant differences in growth, but concentrations of 8.8 and 16 mg/l resulted in reduced growth and number of spawns, and adversely affected the eyes. Viability and hatchability of eggs, consistency of egg size and shape, and embryo development were affected by exposure to 4.0 mg/l of the lampricide. Some mortality occurred in the 8.8 and 16 mg/l concentrations, apparently in part as a result of the added stress of spawning. This same response was observed in adult brook trout exposed to toxaphene (Mayer et al. 1975). Effects on growth and mortality were noted in fry exposed to 1.6 mg/l or more of TFM but no significant effect was noted between fry exposed to 0.94 mg/l and controls. No significant differences were found between controls and fish exposed to use-pattern treatments, except for the increased mortality of adults.

The use pattern of TFM as a lamprey larvicide precludes long-term and continuous exposure of fish to concentrations that might cause the effects shown in this study. Gilderhus et al. (1975) showed that the concentration of TFM in water dropped rapidly after termination of the treatment of the East Au Gres River, Michigan. Coburn and Chau (1976) reported 40 μg/l of TFM in a low-flow area of a creek 3 weeks after the treatment for lamprey control. Our studies show no effect on brook trout continuously exposed to about 9.5 times this amount (0.94 mg/l or 0.34 mg/l active ingredient).

The residues in fillets and offal of adults after chronic exposure to TFM showed a low accumulation factor and TFM was rapidly eliminated when the fish were transferred to uncontaminated water. The residue elimination followed a pattern similar to that re-

Table 7. *Elimination of whole-body TFM residues (μg/g) in adult brook trout transferred to uncontaminated water after 137 days of continuous exposure to 1.6 or 4.0 mg/l TFM.*

Concentration of TFM (mg/l)[a]	Days in uncontaminated water		
	3	7	14
1.6	0.06	<0.01	<0.01
4.0	0.04	0.02	<0.01

[a]Total formulation.

ported by Sills and Allen (1975). No residues were found in adult fish at the time of spawning in the use-pattern exposure.

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Hydrolysis and Photolysis of the Lampricide 2', 5-Dichloro-4'-nitrosalicylanilide (Bayer 73)

by

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Abstract

The hydrolysis and photolysis of the lampricide Bayer 73 (aminoethanol salt of 2',5-dichloro-4'-nitrosalicylanilide) was studied by using ^{14}C-Bayer 2353 (the non-salt form of Bayer 73). No hydrolysis of ^{14}C-Bayer 2353 occurred in pond water or in distilled water buffered at pH 5.0, 6.9, or 8.7 after 56 days. During exposure to long-wave UV light, ^{14}C-Bayer 2353 degraded rapidly on silica gel thin layer chromatographic plates and on glass slides. After exposures of 24 and 168 h, less than 50 and 5%, respectively, of the remaining radioactivity was parent compound. After exposure of an aqueous solution of ^{14}C-Bayer 2353 to long-wave UV light for 14 days, only 5% of the remaining radioactivity was parent compound.

Bayer 73, the aminoethanol salt of 2',5-dichloro-4'-nitrosalicylanilide (Bayer 2353), is not only toxic to larvae of the sea lamprey (*Petromyzon marinus*) but also synergizes the activity of the lampricide 3-trifluoromethyl-4-nitrophenol (TFM) and increases its effectiveness (Howell et al. 1964).

The requirement that all piscicides used in the United States must be registered with the Environmental Protection Agency includes the development of data concerning the fate of the chemical in the environment and potential rates of hydrolysis and photolysis. In this study, we investigated the hydrolysis of ^{14}C-Bayer 2353 in water buffered at various pH's, and studied the photolysis of ^{14}C-Bayer 2353 on thin layer chromatographic plates, on glass slides, and in aqueous solutions.

General Materials and Methods

In all tests, materials that had been exposed to UV light were spotted on thin layer chromatographic (TLC) plates (silica gel, F-254, 250 μm; Brinkman Instruments, Westbury, N.Y.). The plates were developed in a solution of chloroform:methanol:ammonium hydroxide (50:20:2.5, v/v/v), dried, and placed on X-ray films. After 3 to 4 weeks of exposure, the X-ray films were developed, the R_f (distance compound moved on TLC plate/distance solvent moved on TLC plate) of each spot was determined, and corresponding radioactive spots on the TLC plates were scraped into scintillation vials for radioactive determination. Nonradioactive scrapings of TLC plates were used to determine quench and background. The scintillation cocktail consisted of Beckman TLA dissolved in toluene.

Hydrolysis of Bayer 73

To investigate the hydrolysis of Bayer 73, we used three 250-ml beakers containing 100 ml of distilled water buffered (buffer tablets, No. 13-640-304-E,K,H, Fisher Scientific, Atlanta, Ga.) to a pH of 5.0, 6.9, or 8.7, and a fourth beaker containing 100 ml of pond water (initial pH, 7.8). The pH of the buffered solutions did not change during the experiment; that of the pond water decreased from pH 7.8 to 7.0. Into each beaker we stirred 1 ml of acetone containing 5 μg of ^{14}C-Bayer 2353 (chlorosalicyclic acid ring UL-^{14}C, specific activity, 10 mCi/mmol, American Radiochemical Corporation, Sanford, Fla.). The acetone was allowed to evaporate, and the beakers were then covered with aluminum foil and kept in the dark at 20 $\pm$ 1 C. Samples (100 μl) were taken at 0, 1, 4, 7, 14, 26, and 56 days, and spotted on thin layer chromatographic plates for separation and quantification of possible degradation products.

No degradation of ^{14}C-Bayer 2353 occurred within the 56 days in either the buffered aqueous solutions or in the pond water (Table 1).

Table 1. *Amounts of ^{14}C-Bayer 2353 and ^{14}C-degradation product from TLC plates spotted with aqueous solutions of various pH values, and pond water containing ^{14}C-Bayer 2353 and aged for 0 to 56 days (values expressed as percentages of total radioactivity from each plate).*

Days	R_f[a]	Buffered water			Pond	Standard
		pH 5.0	pH 6.9	pH 8.7	(pH 7.0)	(^{14}C-Bayer 2353)
0	0	2	5	5	1	1
	0.78	98	95	95	99	99
1	0	4	6	5	1	1
	0.78	96	94	95	99	99
4	0	1	5	3	3	1
	0.78	99	95	97	97	99
7	0	1	2	2	1	1
	0.78	99	98	98	99	99
14	0	1	4	2	7	1
	0.78	99	96	98	93	99
26	0	1	5	3	6	1
	0.78	99	95	97	94	99
46	0	1	1	3	4	1
	0.78	99	99	97	96	99
56	0	6	2	2	4	1
	0.78	95	98	98	96	99

[a]0 = origin; 0.78 = parent compound, ^{14}C-Bayer 2353.

Photolysis of Bayer 73

Photolysis on Silica Gel Plates

A solution of ^{14}C-Bayer 2353 (1.31 μg in 10 μl of hexane) was spotted on 18 silica gel TLC plates, 9 of which (the controls) were wrapped in aluminum foil and 9 were left uncovered. The plates were exposed at a distance of 20.3 cm to a long-wave lamp (Blak-Ray, Model X-30, Ultra-Violet Products, Inc., San Gabriel, Calif.) with wave lengths ranging from 290 to 405 nm and a peak emission at 355 nm. The intensity at the surface of the TLC plates, measured with a Blak-Ray UV Meter (Model J221, Ultra-Violet Products, Inc., San Gabriel, Calif.) was 9,800 ergs/s per cm^2. One control and one exposed plate were removed from under the light at 0, 1, 2, 4, 6, 24, 48, 120, and 168 h and prepared for R_f determination and radiometric quantifications.

The ^{14}C-Bayer 2353 degraded rapidly on the TLC plates exposed to long-wave UV light (Table 2). About 15% of the parent compound had been degraded after 4 h of exposure, 60% after 24 h, and 95% after 168 h (Fig. 1). Most of the degraded material remained at the origin of the TLC plates; e.g., after a 168-h exposure, more than 70% of the radioactivity remained at the origin. Several other compounds with R_f values of 0.25, 0.34, 0.41, 0.71, 0.74, and 1.0 (R_f of the parent compound was 0.60) were found; their percentages increased up to 24 or 48 h of exposure and then declined. However, none of these compounds ever exceeded 8% of the total radioactivity. The only major compound other than that found at the origin, was a composite of compounds with R_f values ranging from 0.04 to 0.12. These spots were distinct, but overlapped, and could not be separated. The R_f of one other spot (0.25) corresponded to that of standard ^{14}C-chlorosalicyclic acid. This spot never exceeded 3.8% of the total radioactivity. No attempt was made to check for nonradioactive degradation products such as chloronitroaniline because the amount potentially present was very small.

Photolysis on Glass Slides

In further tests of the photolysis of Bayer 73, we streaked 10 μl (1.31 μg) of ^{14}C-Bayer 2353 on 18 glass microscope slides. The solvent was evaporated at room temperature in a fume hood, and the slides were then placed under long-wave UV as described for the TLC plates. One group of nine slides was covered with aluminum foil to serve as controls. Individual slides from each set were removed from UV exposure at 0, 1, 2, 4, 6, 24, 48, 120, and 168 h. The radioactive material was washed from the slides with 1 ml of acetone: methanol (50:50, v/v), and the wash concentrated to 0.5 ml and spotted on TLC plates for development, R_f

Table 2. *Amounts of ^{14}C-Bayer 2353 and ^{14}C-degradation products from TLC plates spotted with ^{14}C-Bayer 2353 and exposed to long-wave UV light for up to 168 h (values expressed as percentages of total radioactivity from each plate; values in parentheses are from control plates)*[a].

Time (h)	R_f 0	0.1[b]	0.25[c]	0.34	0.41	0.60[d]	0.71	0.74	Solvent front
0	0.2	0.0	0.4	0.0	0.0	99.0	0.0	0.2	0.1
1	1.9 (0.2)	1.6	0.8 (0.4)	0.9	0.8	92.7 (98.7)	0.0	0.9 (0.4)	0.4 (0.4)
2	2.7 (0.2)	2.4	0.8 (0.4)	1.5	1.3	89.3 (98.9)	0.0	1.2 (0.3)	0.9 (0.2)
4	4.2 (0.2)	3.4	0.9 (0.4)	2.4	1.6	84.5 (98.5)	1.0	1.2 (0.7)	0.9 (0.2)
6	8.5 (0.2)	5.6	2.4 (0.4)	4.5	2.6	71.4 (99.1)	1.8	1.6 (0.2)	1.5 (0.1)
24	27.7 (0.3)	10.8	3.3 (0.4)	8.0	3.9	39.9 (99.0)	2.2	2.2 (0.2)	2.0 (0.1)
48	44.0 (0.3)	11.4	3.8 (0.4)	7.5	4.0	22.7 (98.7)	2.8	2.0 (0.3)	1.6 (0.2)
120	66.9 (0.5)	10.4	3.2 (0.5)	5.2	2.9	8.0 (98.4)	1.3	1.0 (0.4)	1.0 (0.2)
168	73.7 (0.4)	11.6	2.3 (0.4)	3.9 (0.2)	1.6	4.4 (98.7)	1.1	1.0 (0.3)	0.3 (0.1)

[a]Plates exposed to 9,800 ergs/s per cm^2.

[b]A composite of spots with R_f's ranging from 0.04 to 0.12.

[c]This R_f corresponds to that of ^{14}C-chlorosalicylic acid.

[d]This R_f corresponds to that of the parent compound, ^{14}C-Bayer 2353.

determination, and radiometric quantification.

The degradation of ^{14}C-Bayer 2353 on glass slides was similar to that on TLC plates (Table 3), except that the degradation occurred more rapidly in the first 24 h. For example, after 4 and 6 h of exposure only 62.3 and 56.7% of the parent compound remained on glass slides, as compared with 84.5 and 71.4% on TLC plates. The more rapid rate of degradation could have resulted from greater penetration of UV light through the chemical on the glass slides, whereas the silica gel could have absorbed or adsorbed ^{14}C-Bayer 2353 and prevented the light from affecting all the molecules on the TLC plates. Material with an R_f of 0.1 increased during the first 24 h and then declined on glass slides whereas the same compound remained nearly constant after 24- to 168-h exposures on TLC plates. The change in the slope of the degradation curve (Fig. 1) may have resulted from incomplete elution of the radioactive compounds from the glass slides.

Photolysis in Buffered Solutions

To examine photolysis in buffered solutions, we added 1 ml of an acetone solution containing 5 μg of ^{14}C-Bayer 2353 to two 250-ml beakers containing 100 ml of distilled water buffered to pH 6.9. Depth of the solution was 4.3 cm. The beakers were placed in the dark until the acetone had evaporated. The control beaker was then covered with aluminum foil and the other was left uncovered. The beakers were placed in a constant temperature water bath maintained at 20 ± 1 C and exposed to long-wave UV light. The distance from the lamp to the surface of the solution was 20.3 cm. Samples (100 μl) were taken from each beaker at 0, 1, 4, 7, and 14 days and spotted on silica gel TLC plates for development, R_f determination, and radiometric quantification.

The ^{14}C-Bayer 2353 in the aqueous solution was also degraded by long-wave UV light (Table 4), but the degradation (which was accompanied by a reduction in pH from 6.9 to 6.7) was much slower than on the TLC plates or glass slides. However, only 49% of the parent compound remained after 7 days of exposure and only 5% after 14 days. Most of the degraded material remained at the origin as on the TLC plates and glass slides. Three other compounds with R_f values of 0.13, 0.34, and 0.41 were noticed on the X-ray films from the study of aqueous photolysis. The spot at an R_f of 0.13

Table 3. *Amounts of ^{14}C-Bayer 2353 and ^{14}C-Bayer degradation products from TLC plates spotted with eluants from glass slides. Glass slides were streaked with ^{14}C-Bayer 2353 and then exposed to long-wave UV light up to 168 h (values expressed as percentages of total radioactivity from each plate; values in parentheses are from control plates)*[a].

Time (h)	R_f 0	0.1[b]	0.25[c]	0.34	0.41	0.60[d]	0.71	Solvent front
0	0.25	0.0	0.4	0.0	0.0	98.9	0.2	0.3
1	1.4 (0.1)	2.5	1.1 (0.3)	2.2	2.6	86.7 (98.8)	1.5	1.9 (0.8)
2	3.2 (0.2)	5.0	1.5 (0.3)	2.6	2.9	81.0 (99.0)	1.4	2.4 (0.5)
4	12.8 (0.2)	9.2	(0.4)	4.8	5.8	62.3 (99.2)	2.4	2.7 (0.3)
6	13.7 (0.2)	11.2	(0.3)	6.1	7.2	56.7 (99.1)	2.7	2.2 (0.3)
24	47.5 (0.2)	12.6	(0.4)	7.3	6.2	22.9 (99.1)	2.2	1.3 (0.3)
48	56.7 (0.2)	10.3	(0.4)	5.3	5.7	19.7 (99.1)	1.7	0.4 (0.3)
120	65.9 (0.2)	9.5	(0.4)	6.2	4.8	12.8 (99.1)	0.0	0.8 (0.3)
168	83.8 (0.4)	4.5	(0.4)	4.8	3.1	3.6 (99.0)	0.0	0.1 (0.2)

[a]Glass slides exposed to 9,800 ergs/s per cm^2.

[b]A composite of spots with R_f's ranging from 0.04 to 0.12.

[c]This R_f corresponds to that of ^{14}C-chlorosalicylic acid.

[d]This R_f corresponds to that of the parent compound ^{14}C-Bayer 2353.

appeared only at the 14-day exposure and was a composite of several inseparable spots with R_f's ranging from 0.09 to 0.16.

Comparison with Other Studies

Strufe and Gönnert (1962) reported that the lowest concentration of Bayer 73 lethal to snails increased from 0.3 to about 1.8 mg/l after exposure of the chemical to UV light for 8 h. Meyling et al. (1962), who exposed 1-mg/l solutions of Bayer 73 to direct sunlight for up to 36 h, found that the concentrations of Bayer 73 in soft water dropped from 1 mg/l at 0 h to 0.9, 0.8, 0.67, and 0.56 mg/l at 8, 16, 24, and 36 h, and in hard water from 1.0 at 0 h to 0.71, 0.58, and 0.57 mg/l at 8, 12, and 16 h. Dawson (1971), who exposed Bayer 73 to UV light found that the LC_{50} to bluegills (*Lepomis macrochirus*) was 0.252 mg/l after 6 h (as compared with a control value of 0.197 mg/l) and 0.348 mg/l after 24 h (as compared with a control value of 0.250). He also reported LC_{50}'s of 0.242 for Bayer 73 exposed to sunlight for 6 h, 0.177 for shielded samples, and 0.150 mg/l for control samples.

Other workers reported no effects of UV light or sunlight on the toxicity of Bayer 73. Farringer (1972) exposed solutions of Bayer 73 to UV light for up to 96 h and found no change in toxicity of the chemical to carp (*Cyprinus carpio*). He believed that the differences between his results and those of Strufe and Gönnert (1962) may have been due to the difference in formulations. Farringer used a 99% technical formulation, whereas Strufe and Gönnert used a 70% wettable powder.

Sturrock (1974), after gathering samples of aquatic vegetation which had been sprayed with Bayer 73, reported no diminution in toxicity to snails from samples exposed to tropical sunlight for up to 8 weeks. Possibly the chemical was "protected" from UV degradation by absorption into, or adsorption onto, the plant material.

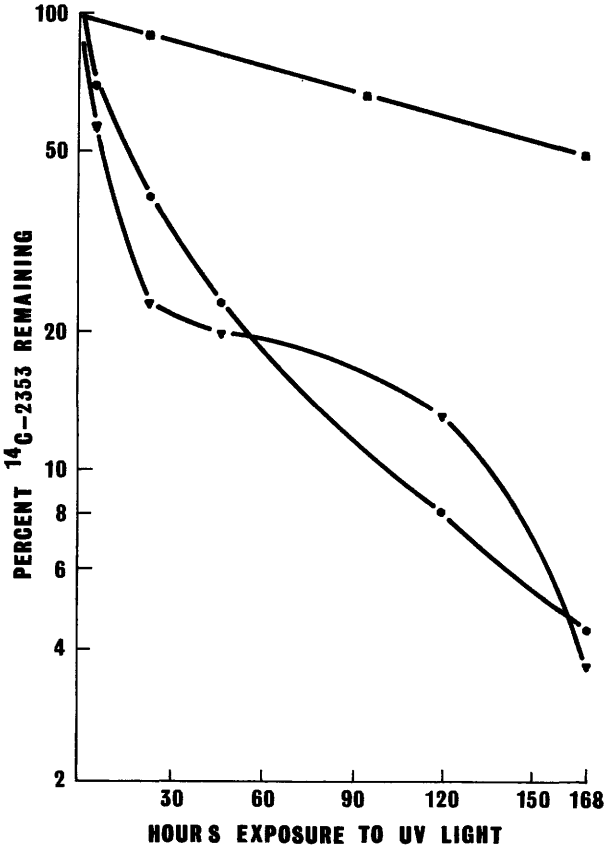

Fig. 1. Percentage of ^{14}C-Bayer 2353 remaining on thin layer plates, glass slides, and in an aqueous solution after exposure to long-wave UV light. Legend: ■ = aqueous solution, ● = thin layer plates, and ▼ = glass slides.

Table 4. *Amounts of ^{14}C-Bayer 2353 and ^{14}C-degradation products from TLC plates spotted with an aqueous solution of ^{14}C-Bayer 2353 exposed to long-wave UV light for up to 14 days (values expressed as percentages of total radioactivity from each plate)*[a].

Days and treatment	R_f				
	0	0.13[b]	0.34	0.41	0.65[c]
0					
UV	1	—	—	—	99
Control	1	—	—	—	99
1					
UV	9	—	—	—	91
Control	1	—	—	—	99
4					
UV	27	—	4	3	66
Control	2	—	—	—	98
7					
UV	41	—	6	4	49
Control	2	—	—	—	98
14					
UV	84	7	2	2	5
Control	5	—	—	—	95

[a]Solution exposed to 9,800 ergs/s per cm^2.

[b]These values are a composite of spots with R_f's ranging from 0.09 to 0.16.

[c]This R_f corresponds to that of authentic ^{14}C-Bayer 2353.

Strufe and Gönnert (1962) are the only workers known to have attempted isolation or separation of degradation products of Bayer 73; they irradiated an ethanolic solution of the chemical for several days and then used paper chromatography to separate the products. Two compounds were found, in addition to the parent compound.

In our tests the ^{14}C-Bayer 2353 was degraded by UV light into four to seven compounds.

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