

US EPA ARCHIVE DOCUMENT

75-90% of the accumulated [¹⁴C]residues eliminated from the fish tissues by day 14 of the depuration period. The exposure phase of the study was stopped after 25 days because the fish exhibited increasing mortality and abnormal behavior, including loss of equilibrium, quiescence, and lying on the bottom of the chambers.

3. This study is acceptable and fulfills EPA Data Requirements for Registering Pesticides by providing information on the accumulation of ring-2-labeled [¹⁴C]molinate in laboratory fish.
4. No additional information on the accumulation of molinate in laboratory fish is needed at this time.

METHODOLOGY:

Bluegill sunfish (*Lepomis macrochirus*; mean length and weight 60 mm and 8.3 g, respectively) were held in culture tanks on a 16-hour photoperiod for ≥ 14 days prior to the initiation of the study. During the study, the fish were fed a dry pelleted food daily equivalent to approximately 3% of their body weight.

Flow-through aquatic exposure systems were prepared using two 100-L aquaria. Aerated well water (15-20°C, pH 7.8-8.3, dissolved oxygen content 9.2-10.1 ppm; Table 1) was provided to each aquarium at a rate of approximately 6.8 turnovers per day. The aquaria were immersed in a water bath and maintained at $22 \pm 2^\circ\text{C}$. One aquarium was continuously treated with ring-2-¹⁴C-labeled molinate (radiochemical purity 99%, specific activity 10.5 mCi/mmol, source unspecified) using a flow-through proportional diluter system at 0.10 ± 0.012 ppm; the second aquarium served as an untreated control. The test aquaria were allowed to equilibrate for 24 hours before the bluegill sunfish (120) were transferred into each aquarium. During the exposure period, water and fish (6, 15, or 25; Table 2) samples were collected at 4 hours and at 1, 3, 7, 14, 21, and 25 days from both the treated and control aquaria. After 25 days, both aquaria were drained of water twice to a depth of 3 inches, then filled with approximately 70 L of pesticide-free well water; the fish remained in the aquaria during this procedure. During the 14-day depuration period, water and fish (6 or 15; Table 2) were collected on 1, 3, 7, 10, and 14 days from each aquarium. Additional samples of water were collected on days 0, 21, and 25 of the exposure period and day 14 of the depuration phase for possible metabolite identification.

Aliquots of the water samples were quantified for total [¹⁴C]residues using LSC; the detection limit was 0.002 ppm. Pooled samples (3 fish) of edible (body, muscle, skin, skeleton) and nonedible (fins, heads, internal organs) tissues, and whole fish (3 fish) were homogenized with dry ice and analyzed for total radioactivity by LSC following combustion. Recovery efficiencies from fortified fish samples averaged 95-96% for all tissue types (Table 7). The [¹⁴C] detection limits were 0.0805 ppm in edible tissues, 0.0843 ppm in nonedible tissues, and 0.0828 ppm in whole fish. Homogenized edible and nonedible fish tissues were stored frozen (duration of storage and storage conditions not specified) before metabolite identification.

Homogenized edible and nonedible tissues from fish sampled on days 21 and 25 of the exposure period and from day 14 of the depuration period were analyzed for molinate and its degradates. The fish tissues were extracted twice with acetonitrile by shaking for 2 hours, followed by a third acetonitrile extraction with refluxing for 4 hours. The extracted tissue was then air-dried and analyzed for total radioactivity by LSC following combustion. The extracts were combined, and aliquots were analyzed for total radioactivity by LSC; the ^{14}C -extractable residues from the depuration samples were insufficient to proceed with characterization. The remaining combined extracts from the exposure period were evaporated to dryness under a nitrogen stream and redissolved in 10 mL of methylene chloride:cyclohexane (50:50, v:v). Aliquots of the concentrates were analyzed for total radioactivity by LSC; the remainder was filtered through a gel permeation chromatographic (GPC) column (S-X3 packing) with methylene chloride:cyclohexane (50:50, v:v). Aliquots of the eluate were analyzed by LSC; the remaining solution was evaporated under a nitrogen stream, taken up in acetone, and aliquots were analyzed by LSC. Additional aliquots of the acetone solutions were analyzed using two-dimensional TLC on preparative silica gel plates developed in hexane:acetone (1:1, v:v) in the first direction and toluene:ether (2:3, v:v) in the second direction. Unlabeled reference compounds (Table II) were cochromatographed with the extracts and visualized under UV light. Following development, radioactive zones on the plates were located using autoradiography and UV fluorescence of the reference compounds. Radioactive areas were quantified by scraping the radioactive areas from the plates and analyzing the scrapings by LSC. Recoveries for control edible and nonedible tissue samples fortified with 1.70 or 9.72 ppm of molinate averaged 88.3% following extraction and 92.6% following GPC concentration (Table V). Method recoveries for treatment samples through the extraction and clean-up phases ranged from 90 to 109% and averaged 100% (Table V).

DATA SUMMARY:

[^{14}C]Molinate residues accumulated in nonedible, edible, and whole fish tissues from fish that were exposed to ring-2-labeled [^{14}C]molinate (radiochemical purity 99%) at 0.10 ppm in flow-through aquaria for 25 days. The maximum bioconcentration factors were 29x for edible tissues (body, muscle, skin, skeleton), 140x for nonedible tissues (fins, heads, internal organs), and 72x for whole fish (Table 3). The maximum concentrations of total [^{14}C]residues were 3.0 ppm for edible tissues (day 21), 14 ppm for nonedible tissues (day 3), and 7.2 ppm for whole fish (day 3, Table 3). Based on TLC analyses of the 21- and 25-day edible and nonedible tissue samples, molinate was 0.52-0.68 ppm in the edible tissues and 4.74-4.81 ppm in the nonedible tissues (Table VII). Five degradates were identified:

carboxymolinate, at 0.77-0.87 ppm in edible and 0.11-0.46 ppm in nonedible tissues;

4-ketomolinate, at 0.13-0.18 ppm in edible and 0.02-0.07 ppm in nonedible tissues;

4-hydroxymolinate, at 0.14-0.16 ppm in edible and 0.09-0.23 ppm in nonedible tissues; and

molinate sulfoxide, at 0.14-0.27 ppm in edible and 1.18-1.32 ppm in nonedible tissues;

molinate sulfone, at 0.05-0.06 ppm in edible and 0.21-0.76 ppm in nonedible tissues (Table VII).

Unextracted [¹⁴C]residues comprised 0.89-0.98 ppm in the edible tissues and 1.87-2.46 ppm in the nonedible tissues (27.1-31% and 16-26% of the total radioactivity in the tissue, respectively; Table IV). "Immobile" (presumably [¹⁴C]compounds remaining at the origin on the TLC plates) residues were 0.04-0.14 ppm in the edible tissues and 0.13-0.32 ppm in the nonedible tissues (2-7% and 2-5% of the extracted radioactivity, respectively; Table VII).

[¹⁴C]Residues in whole fish stabilized by 1 day after treatment was initiated (Table 3; Figure 2). Depuration was initially rapid, but by day 14 of the depuration period, only 75-90% of the accumulated [¹⁴C]residues were eliminated from the fish tissues (Table 4; Figure 2).

Throughout the study, the temperature of the treated water was 21- 22°C, the pH ranged from 7.9 to 8.2, and the dissolved oxygen content ranged from 6.1 to 8.7 mg/L. Total [¹⁴C]residues in the water ranged from 0.080 to 0.12 ppm during the exposure period (Table 3).

COMMENTS:

1. The exposure phase of the study was terminated after 25 days because of the fish exhibited increasing mortality and abnormal behavior, including loss of equilibrium, quiescence, and lying on the bottom of the chambers. A total of four fish died; the remaining fish were "normal" at the termination of the study. The study author provided no explanation of these observations or any possible effects on the outcome of the study.

A preliminary 7-day dynamic toxicity study had been conducted to determine the acute toxicity of molinate to bluegill sunfish. The 7-day LC₅₀ level was estimated to be 8.2 mg/L and the 7-day no-observed effect concentration was 5.3 mg/L. In view of these results, the registrant chose an exposure level of 0.10 ppm (1/82 of the 7-day LC₅₀) for the bioaccumulation study. This is comparable to previously reviewed ecotoxicity studies. In an acceptable acute toxicity test conducted for 96 hours in a flow-through system, the LC₅₀ ranged from 18-32 mg/L (MRID 43337602). However, in a static test (MRID 40098001), the LC₅₀ was 0.32 mg/L.

2. The 21- and 25-day fish samples collected for degradate identification purposes were analyzed to confirm that the concentration of [¹⁴C]residues in the samples had not changed during storage prior to analysis; however, it is not known if specific compounds within the samples were stable. The in-life portion of the study was conducted in April and May, 1986; at this point, the homogenized tissue samples were stored frozen until the metabolite characterization was performed September, 1986 - January, 1987. However, storage conditions were not specified.

3. Metabolite characterization was performed using a single 2-dimensional TLC system and identified only by comparison to cochromatographed reference standards. It is preferable that specific compounds isolated by TLC be identified by a confirmatory method such as MS.
4. Radioactive residues in the water were not characterized. The study author stated that additional water samples were collected on days 21 and 25 of the exposure period and on day 14 of the depuration period for possible metabolite characterization; no data were provided for these analyses and there was no evidence that the samples were ever analyzed. However, since molinate is stable to photodegradation and hydrolysis (MRIDs 41599301 and 40817901, respectively) and there were approximately 6.8 turnovers of water volume per day, it is probable that the majority of [¹⁴C] residues in the water were parent material.
5. Control data from analyses of untreated water and fish were not provided.
6. The study author stated that the dissolved oxygen concentrations, which ranged between 6.1 and 8.7 mg/L, stayed between 66 and 95% saturation at 20°C, respectively, and were considered adequate for testing.