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ABSTRACT

Laboratory Accumulation - Fish

The fish accumulation of BAS 510 F in rainbow trout was studied using [diphenyl-U-^{14}C]BAS 510 F [2-chloro-*N*-(4'-chlorobiphenyl-2-yl)-nicotinamide; radiochemical purity >98%] at a nominal concentration of 20 µg/L and [pyridine-^{15}N, 2-^{13}C]BAS 510 F (chemical purity >97%). Exposure aquaria were treated with [^{14}C] or [^{15}N, 2-^{13}C] BAS 510 F at a nominal concentrations of 20 and 200 µg/L, respectively, and were maintained under flow-through conditions. Both exposure studies were conducted using 35-day exposure and 14-day depuration periods.

In the **20 µg/L exposure study**, mean total [^{14}C]residues in the water during the accumulation phase were 20.7 µg equivalents/L. All radioactivity present in the water was BAS 510 F. The concentration of total radioactivity in fish tissues reached steady state within 1.4-2.5 days. Mean total [^{14}C]residues at steady state were highest in the nonedible tissue compared to the edible and whole fish tissues. Maximum total [^{14}C]residues at steady state were 0.863 µg equivalents/g for the edible tissue (day 35), 2.073 µg equivalents/g for the nonedible tissue (day 28), and 1.395 µg equivalents/g for the whole fish tissue (day 28). BAS 510 F was the major radioactive component recovered from the edible, nonedible, and whole fish tissues.

The registrant-calculated bioconcentration factors (BCF) for total [^{14}C]residues were 36X, 84X, and 57X in edible, nonedible, and whole fish tissues, respectively. Depuration was rapid, with [^{14}C]residues accumulated in nonedible tissues eliminated with a model-derived half-life of 0.8 days. Mean total [^{14}C]residues on day 14 of the depuration period were 0.068 µg/g for the nonedible fish tissue.

In the **200 µg/L exposure study**, mean total [^{14}C]residues in the water during the accumulation phase were 198.9 µg equivalents/L. All radioactivity present in the water was BAS 510 F. The concentration of total radioactivity in fish tissues reached steady state within 1.5-3.3 days. Mean total [^{14}C]residues at steady state were highest in the nonedible tissue compared to the edible and whole fish tissues. Maximum total [^{14}C]residues at steady state were 10.23 µg equivalents/g for the edible tissue, 27.51 µg equivalents/g for the nonedible tissue, and 17.12 µg equivalents/g for the whole fish tissue, all on day 35 of exposure. BAS 510 F was the major radioactive component recovered from the edible, nonedible, and whole fish tissues.

The registrant-calculated bioconcentration factors (BCF) for total [^{14}C]residues were 44X, 105X, and 70X in edible, nonedible, and whole fish tissues, respectively. Depuration was rapid, with [^{14}C]residues accumulated in nonedible tissues eliminated with a model-derived half-life of 1.0 day. Mean total [^{14}C]residues on day 14 of the depuration period were 0.76 µg/g for the nonedible fish tissue.

Study Acceptability: This study is classified acceptable and satisfies the guideline data requirement for a laboratory accumulation in fish study.

MATERIALS AND METHODS

The fish accumulation of BAS 510 F was studied using [diphenyl-U-^{14}C]BAS 510 F; [2-chloro-*N*-(4'-chlorobiphenyl-2-yl)-nicotinamide; radiochemical purity >98%, specific activity 376000 dpm/µg; Batch No. 641-2017; p. 13] at a nominal concentration of 20 µg/L and [pyridine-^{15}N, 2-^{13}C]BAS 510 F (chemical purity >97%; Batch No. 640-3009; p. 13), at a nominal concentration of 200 µg/L; corresponding measured concentrations were 20.7 and 198.9 µg/L, respectively (pp. 11, 13, 28). The exposure study was conducted from June 17, 1999 to August 6, 1999 (p. 12).

For use in the study, juvenile (4 weeks) rainbow trout (*Oncorhynchus mykiss* Walbaum) were received from Selcoth Fish Farm in Moffat, Scotland (p. 15). Fish were maintained in holding tanks and acclimated for 14 days at 13.6-15.4°C (p. 16). Fish were fed daily with Salmon Fry Diet, Nutra Fry during the holding and test periods. A light/dark cycle of 16/8 hours was maintained throughout the study. Mains water used in this study was dechlorinated by continuous aeration, and passed through a particle filter, an activated charcoal filter, and a UV sterilizer prior to use in the study (p. 15). Water quality parameters were as follows for both exposure studies (Appendix 7, pp. 83-90):

pH	7.3-7.9
Alkalinity (mg/L as $CaCO_3$)	30
Hardness (mg/L as $CaCO_3$)	60-72
Conductivity (mS)	0.16-0.21
Dissolved oxygen (mg/L)	9.3-10.3

Three days prior to the introduction of the fish into the aquaria, the exposure tanks were treated with [U-^{14}C]BAS 510 F at a nominal concentration of 20 µg/L, or with [^{15}N, 2-^{13}C]BAS 510 F at a nominal concentration 200 µg/L; in both studies, the test compound was dissolved in dimethylformamide (p. 20). A contingency aquarium was treated with [^{15}N,2-^{13}C]BAS 510 F at a nominal concentration of 200 µg/L for 14 days. The test compound was introduced into the exposure water by injection with a syringe pump (p. 17). Control aquaria were treated only with dimethylformamide, at the same concentration delivered to the exposure aquaria (0.1 g/L). The aquaria were maintained at 15 ± 2°C, and the dilution water was continuously supplied at an average measured flow rate of 200-216 mL/min (*ca.* 5 turnovers/day) during the exposure and depuration periods for both test concentrations (p. 17, Appendix 10, pp. 97-98).

Flow-through aquatic exposure systems were prepared using 60-L glass aquaria (39 cm x 55 cm x 46 cm; p. 17). Following the equilibration period, approximately 150 fish were placed into both of the bioaccumulation exposure aquaria and the control aquarium, and 50 fish (body weight *ca.* 1 g) were placed into the contingency aquarium (p. 20). The aquaria were maintained under fluorescent lighting using a 16-hour light/8-hour dark photoperiod (p. 16). The test fish weighed approximately 1 g at study initiation (body

weight range of a representative sample was 0.551–0.931 g at 3 days prior to study initiation; p. 15). Both exposure studies were conducted using a 35-day exposure period; five fish were collected from the treated and control aquaria on days 0, 1, 2, 4, 7, 14, 21, 28, and 35 days. For each sampling interval, the sampled fish were used to determine total radioactivity in the whole fish tissues (p. 20). An additional 15 fish from each exposure aquarium were collected at 14, 21, and 28 days to characterize [14C]residues. Also, four fish were sampled at 3 hours and day 3 as a contingency to optimize modeling; however, these data were deemed unnecessary and were not utilized. Fifty fish were collected from the contingency aquarium on exposure day 14 and were replaced with 55 fish that were then sampled at day 28; however, these fish were not analyzed as part of the study. Water samples (15-40 mL) were collected from the bioaccumulation aquaria on the last two days of the equilibration period and daily during the accumulation period to determine total [^{14}C]residues (p. 20). Additional water samples (250 mL) were collected from the bioaccumulation aquaria once during equilibration and at 0, 7, 14, 21, 28, and 35 days to characterize [^{14}C]residues in the water. Throughout the study, the water temperature, dissolved oxygen, pH, conductivity, and flow rate of each aquarium were monitored daily (pp. 16-17).

Following the exposure periods, the fish remaining in each of the test aquaria were transferred to clean aquaria for a 15-day depuration period (p. 20). Four fish were collected from the bioaccumulation aquaria at 1, 2, 4, 7, 10, and 14 days during the depuration period to determine total [^{14}C]residues. Water samples were collected from the bioaccumulation exposure aquaria at 1, 2, 4, 7, and 14 days during the depuration period to determine total [^{14}C]residues.

At each sampling interval during the exposure period, aliquots (1 mL) of water were collected from the exposure and control aquaria and analyzed for total radioactivity using LSC (p. 21). Water samples collected to characterize [^{14}C]residues were passed through a separate 47-mm C_{18} solid phase extraction disc under vacuum (p. 22). The discs were allowed to dry and [^{14}C]residues were eluted once with methanol followed by three times with acetone. The eluents were combined, concentrated under a stream of nitrogen, and reconstituted in water:acetonitrile (4:1, v:v) prior to HPLC analysis. Only extracts containing >10% of the total radioactivity in the initial sample were analyzed by HPLC. Water extracts were analyzed using HPLC System 3, LC 1 and LC 2. Analytical conditions for HPLC System 3 were as follows (p. 23):

Column	YMC J'sphere ODS H80, 250 x 4.0 mm; 4-µm particle
Mobile phase	Acetonitrile:water with 2.5% sulphuric acid (6:4, v:v)
Gradient	Isocratic
UV detector	Hewlett-Packard, model 1050
Radiochemical detector	Berthold LB 507A
Flow rate	1 mL/min

All fish sampled were separated into edible (muscle) and nonedible (viscera) tissues (p. 21). Subsamples of the dissected fish tissues were finely chopped using scissors, and analyzed for total radioactivity by LSC following combustion. Mean combustion efficiencies for each set of analyses were >96%. Separate tissue pools of the dissected edible and nonedible tissues used to characterize [^{14}C]residues were composited by sampling interval (14, 21, and 28 days), and subsamples (6-12 g) were minced to a paste using scissors (p. 22). Subsamples of fish tissue homogenate (5-10 g) were extracted twice with methanol, centrifuged, and analyzed by LSC. An aliquot (10 mL) of the methanol extract was concentrated and reconstituted in methanol:HPLC solvent (1:2, v:v) (p. 114). Extracts containing >10% of the total radioactivity in the initial sample were analyzed by HPLC. Fish tissue extracts were analyzed using HPLC Systems LC 1 and LC 2; selected extracts were also analyzed using LC 3 to resolve polar metabolites (p. 35). Analytical conditions for each system were as follows (Appendix 12, pp. 113-114):

	LC 1		LC 2		LC 3	
Column	YMC Carotenoid 5 µm; 250 x 4.6 mm id		Hypersil Green ENV, 5 µm; 150 x 4.6 mm id		Hypersil Green ENV, 5 µm; 150 x 10 mm id	
Solvent A	Formic acid in water		Formic acid in water		Formic acid in water	
Solvent B	Acetonitrile		Acetonitrile		Acetonitrile	
Mobile phase gradient	Time (min) 0-15 15-30 30-75 75-80 80-80.1 80.1-90	%B 0-20 20 20-100 100 100-0 0	Time (min) 0-80 80-85 85-90 90-90.1 90.1-95	%B 0-45 45-100 100 100-0 0	Time (min) 0-80 80-85 85-90 90-90.1 90.1-95	%B 0-45 45-100 100 100-0 0
Flow rate	1.0 mL/min		1.0 mL/min		4.0 mL/min	

The identity of BAS 510 F was confirmed in selected fish extracts using ESI/MS/MS (p. 32).

Ten fish were collected on exposure days 0, 14, and 28 from the control aquarium to determine percent lipid during the exposure period (p. 24). Lipid determination was performed using a gravimetric technique involving a chloroform:methanol extraction system. Lipid values (g lipid/g whole fish expressed as a percent) were 2.38-3.20%, 5.41-5.87%, and 3.65-4.34% in the edible, nonedible, and whole fish tissues, respectively (Table 9, p. 50).

In a preliminary study to determine the adsorption of BAS 510 F to test apparatus, aquaria, and fish diet, systems were exposed to 20 µg/L of [^{14}C]BAS 510 F dissolved in 0.1 g/L dimethylformamide for 19 hours (p. 27). There was no evidence of BAS 510 F adsorbing to glassware or of binding to fish diet over a 2-hour incubation period.

Water samples and fish extracts were stored frozen (-20°C), shipped and analyzed within 4 months after collection (p. 24; Appendix 12, p. 117). BAS 510 F appeared stable in water during the exposure period; therefore, a storage stability study was not conducted.

RESULTS/DISCUSSION

The fish accumulation of BAS 510 F in rainbow trout was studied using [diphenyl-U-^{14}C]BAS 510 F [2-chloro-*N*-(4'-chlorobiphenyl-2-yl)-nicotinamide; radiochemical purity >98%] at a nominal concentration of 20 µg/L and [pyridine-^{15}N, 2-^{13}C]BAS 510 F (chemical purity >97%) at a nominal concentration of 200 µg/L (pp. 11, 13). Actual mean concentrations of BAS 510 F in the water were 20.7 µg/L in the [U-^{14}C]BAS 510 F exposure aquarium, and 198.9 µg/L in the [^{15}N, 2-^{13}C]BAS 510 F exposure aquarium (p. 28). Under flow-through aquarium conditions, radiolabeled residues accumulated in fish that were exposed to BAS 510 F. However, >90% of the accumulated residues were eliminated following 1.4-3.3 days of depuration. Both exposure studies were conducted using 35-day exposure periods and 14-day depuration periods.

In the **20 µg/L exposure study**, mean total [^{14}C]residues at steady state (4 days) were 0.856 µg/g for whole fish tissues (Table 8, p. 46). Maximum mean total [^{14}C]residues were 2.073 µg/g (day 28), 0.863 µg/g (day 35), and 1.395 µg/g (day 28) for the nonedible, edible, and whole fish tissues, respectively. Based on HPLC analyses of fish extracts, BAS 510 F in edible tissue comprised 89.7%, 90.0%, and 95.4% of the HPLC distribution on exposure days 14, 21, and 28, respectively; in nonedible tissue, BAS 510 F comprised 80.1%, 71.9%, and 86.4% of the HPLC distribution on exposure days 14, 21, and 28, respectively. All remaining [^{14}C] activity was attributed to degradates; M510F01 was a maximum of 19.9 and 8.5% in nonedible and edible tissues, respectively, and M510F05 was ≤5.3% (Table 12, p. 55).

Registrant-calculated bioconcentration factors were 36X for the edible, 84X for the nonedible, and 57X for the whole fish tissues (Table 10, p. 51). Depuration was rapid, with [^{14}C] residues accumulated by exposure day 35 eliminated with a model-derived half-life of less than 1 day. Mean total [^{14}C]residues on day 14 of the depuration period were 0.023, 0.068, and 0.043 µg equivalents/g for the edible, nonedible, and whole fish tissues, respectively (Table 8, p. 47). The control aquarium had seven mortalities and the exposure aquarium had 10 mortalities (Appendix 11, p. 99).

During the exposure and depuration periods, the temperature ranged from 13.6-14.8°C; the pH ranged from 7.5 to 7.9; and the dissolved oxygen content ranged from 9.6 to 10.3 mg/L (Appendix 7, pp. 83-90).

In the **200 µg/L exposure study**, mean total [^{14}C]residues at steady state (4 days) were 10.39 µg/g for whole fish tissues (Table 8, p. 48). Maximum mean total [^{14}C]residues were 10.23 µg/g, 27.51 µg/g, and 17.12 µg/g for the nonedible, edible, and whole fish tissues, respectively, on exposure day 35. Based on HPLC analyses of fish extracts, BAS 510 F in edible tissue comprised 95.8%, 89.3%, and 97.0% of the HPLC distribution on

exposure days 14, 21 and 28, respectively; in nonedible tissue, BAS 510 F comprised 86.1%, 85.0%, and 84.9% of the HPLC distribution on exposure days 14, 21 and 28, respectively. All remaining [^{14}C]activity was attributed to degradates, with M510F01 at a maximum of 7.7% and 5.8% in nonedible and edible tissue, respectively and M510F05 at ≤3.7% (Table 12, p. 55).

Registrant-calculated bioconcentration factors were 44X for the edible, 105X for the nonedible, and 70X for the whole fish tissues (Table 10, p. 51). Depuration was rapid, with [^{14}C]residues accumulated by exposure day 35 eliminated with a model-derived half-life of ≤1 day. Mean total [^{14}C]residues on day 14 of the depuration period were 0.22, 0.76, and 0.46 µg equivalents/g for the edible, nonedible, and whole fish tissues, respectively (Table 8, p. 49). The control aquarium had seven mortalities and the exposure aquarium had 19 mortalities (Appendix 11, p. 99).

During the exposure and depuration periods, the temperature ranged from 13.2-14.4°C; the pH ranged from 7.3 to 7.8; and the dissolved oxygen content ranged from 9.3 to 10.2 mg/L (Appendix 7, pp. 83-90).

DEFICIENCIES/DEVIATIONS

1. Juvenile rainbow trout were used in this study. Subdivision N Guidelines specify that bluegill sunfish and channel catfish are the preferred species for fish bioaccumulation studies.

2. The exposure concentrations were <10% of the known 96-hour LC_{50} concentration for rainbow trout, as required by Subdivision N Guidelines. The 96-hour LC_{50} for BAS 510 F in rainbow trout was reported as 46.4 mg/L; effects were observed at 2.15 mg/L (p. 18).

3. Fish in the 200 µg/L aquarium swam close to the bottom of the aquarium beginning on day 32 of the exposure period (Appendix 11, p. 99). A few fish exhibited a loss of equilibrium or lethargy and a loss of appetite. These effects gradually decreased during the depuration period and after day 10, no abnormal behavior was observed. The study authors noted that most fish sacrificed on day 35 had enlarged and darkened livers and gall bladders, and enlarged and discolored gastrointestinal tracts. Similar but less pronounced effects were observed during the depuration period. These effects were not observed in the control fish.

4. Whole fish were not analyzed during the study. Instead, whole fish tissue concentration data were calculated based on the edible and nonedible tissue concentrations (Table 8, pp. 45-49). It is preferred that separate fish be utilized for the analysis of whole fish tissues.

5. The study authors concluded that BAS 510 F initially metabolizes to form M510F01, which is the oxygenated form, and in a separate pathway, conjugates with cysteine to form M510F05 (p. 10).

6. Results from inorganic and organic analyses of the dilution water are presented in Appendix 5 of the study report (pp. 80-81).

7. The length and weight of the collected fish were reported in Appendix 3 (pp. 71-77).

8. The solubility limit of BAS 510 F in water is 4.2-6.0 mg/L, based on information supplied by the Sponsor (p. 18).

9. Method detection limits were not reported. Method detection limits should be reported to allow the reviewer to evaluate the adequacy of the method used.

10. The study was according to OECD Guideline No. 305. Signed and dated Good Laboratory Practice, Quality Assurance, and No Data Confidentiality were submitted with this study.

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