DATA EVALUATION RECORD

STUDY 3

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ACCEPTABILITY OF STUDY:

This study is upgradeable to acceptable upon submission of data to identify the unknown metabolite represented by "Peak B" (see deficiency #2 below). The study only partially fulfills the data requirement for bioaccumulation in fish, because the test species, rainbow trout, is not relevant to the likely use sites (California and Louisiana rice-growing areas). Additional studies on other fish species (bluegill sunfish and channel catfish) are required.

ABSTRACT

Laboratory Accumulation - Fish

The fish accumulation of cyhalofop-butyl was studied using alpha-phenoxy ring-labeled [U-^{14}C]cyhalofop-butyl (DE-537 n-butyl ester; (R)-2-(4-(4-cyano-2-fluorophenoxy) phenoxy)propionate, –butyl ester; DE-537 nBE; radiochemical purity 99.7%), at nominal concentrations of 0.5 and 5.0 ng/mL. However, the actual concentrations of cyhalofop-butyl in the water decreased substantially during the first seven days of exposure, then stabilized at approximately 27-36% and 18-27% of the nominal for the 0.5 and 5.0 ng/mL exposures, respectively, for the remainder of the 28-day exposure period. The average measured exposure concentrations during days 7-28 were 0.19 and 1.10 ng cyhalofop-butyl/mL for the 0.5 and 5.0 ng/mL exposure aquaria, respectively. Total [^{14}C]residues in the exposure water were 0.56 ± 0.02 and 5.23 ± 0.28 ng/mL for the 0.5 and 5.0 ng/mL exposure aquaria, respectively. Cyhalofop-butyl hydrolyzed to its acid equivalent,

(R)-2-(4-(4-cyano-2-fluorophenoxy)phenoxy)propanoic acid (DE-537 acid; XDE-537 acid),

in the exposure water; DE-537 acid was present in the exposure water at 63-73% and 73-82% of the nominal 0.5 and 5.0 ng/mL concentrations, respectively, from 7 to 28 days of the exposure period. Following the exposure period, fish were transferred to clean aquaria for an 8-day (0.5 ng/mL exposure study) or 14-day (5.0 ng/mL exposure study) depuration period.

Radiolabeled residues accumulated in rainbow trout that were exposed to [^{14}C]cyhalofop-butyl at nominal concentrations of 0.5 and 5.0 ng/mL under flow-through conditions. Bioconcentration factors (BCF) were calculated for total [^{14}C]residues from the ratio of total [^{14}C]residues in the various fish tissues versus the average concentration of cyhalofop-butyl in the exposure water from 7 to 28 days (0.19 or 1.10 ng/mL).

In the **0.5 ng/mL exposure study**, BCF values for total [^{14}C]residues were 576, 27, and 507 mL/g for the nonedible, edible, and whole fish tissues, respectively. Mean total [^{14}C]residues were highest in the nonedible tissue (105 ± 18.7 ng cyhalofop-butyl equivalents/g) compared to edible (5.0 ± 0.9 ng cyhalofop-butyl equivalents/g) and whole fish (97.5 ± 15.8 ng cyhalofop-butyl equivalents/g) tissues. Maximum total [^{14}C]residues were 135, 5.9, and 113 ng cyhalofop-butyl equivalents/g for the nonedible, edible, and whole fish tissues, respectively, on exposure day 14. Accumulation plateaus were reached by 3.4-5.3 days. Depuration was rapid, with elimination half-lives of <1.2 days. At the end of the 8-day depuration period, total [^{14}C]residues in the nonedible, edible, and whole fish tissues were 6.7, 0.4, and 4.6 ng cyhalofop-butyl equivalents/g, respectively. [^{14}C]Residues were not characterized in fish tissues.

In the **5.0 ng/mL exposure study**, BCF values for total [^{14}C]residues were 672, 27, and 436 mL/g for the nonedible, edible, and whole fish tissues, respectively. Mean total [^{14}C]residues were highest in the nonedible tissue (823 ± 253 ng cyhalofop-butyl equivalents/g) compared to edible (30.5 ± 4.6 ng cyhalofop-butyl equivalents/g) and whole fish (698 ± 223 ng cyhalofop-butyl equivalents/g) tissues. Maximum total [^{14}C]residues were 1211, 36.8, and 993 ng cyhalofop-butyl equivalents/g for the nonedible, edible, and whole fish tissues, respectively, on exposure day 7. Accumulation plateaus were reached by 5.3-9.0 days. Cyhalofop-butyl was not present in most fish samples, and represented <1% of the total extractable radioactivity in samples in which it was present. DE-537 acid,

the taurine conjugate of DE-537 acid, and

an unidentified degradate designated "Peak B"

were present in the whole fish tissue at maximums of 335-337, 521-719, and 158-396 ng cyhalofop-butyl equivalents/g, respectively, on exposure day 7, and were 177-194, 101-104, and 41-42, respectively, on exposure day 28. [^{14}C]Residues were characterized in the muscle fillets only on exposure day 10; DE-537 acid accounted for all of the [^{14}C]residues, 36-39 ng cyhalofop-butyl equivalents/g, on day 10. Depuration was rapid, with elimination half-lives of <2.1 days. At the end of the 14-day depuration period, total [^{14}C]residues in the nonedible and whole fish tissues were 18.3 and 10.6 ng cyhalofop-butyl equivalents/g, respectively, and were not detected in the edible tissue.

BCFs for cyhalofop-acid, based on data from Tables 5 and 11, were 86 on day 7, 60 on day 14, and 46 on day 21. Lipid-normalized values (based on 2.42% lipid content for exposed fish) were 3560 mL/g, 2470 mL/g, and 1890 mL/g, respectively.

MATERIALS AND METHODS

The fish accumulation of cyhalofop-butyl was studied using alpha-phenoxy ring-labeled [U-^{14}C]cyhalofop-butyl (DE-537 n-butyl ester; (R)-2-(4-(4-cyano-2-fluorophenoxy) phenoxy)propionate, n-butyl ester; DE-537 nBE; radiochemical purity 99.7%; specific activity 30.1 mCi/mmole; pp. 10-11), at nominal concentrations of 0.5 and 5.0 ng/mL. The low concentration exposure study was conducted from March 30, 1999 to May 5, 1999, and the high concentration exposure study was conducted from May 25, 1999 to July 6, 1999 (p. 13).

For use in the study, juvenile rainbow trout (*Oncorhynchus mykiss* Walbaum) were received from Stoney Creek Trout Farms in Grant, MI, and maintained in holding tanks at approximately 13 °C (p. 13). The fish were fed a standard laboratory fish diet containing approximately 19% protein and 8% lipid by weight at a rate of 1-2% of total fish biomass per day (p. 15). The fish were held in the tanks until they reached approximately 1.0-2.0 g body weight (p. 13). The fish were acclimated for at least 14 days prior to study initiation. Fish that appeared unhealthy or diseased were not used in the study (p.13).

Flow-through aquatic exposure systems were prepared using four 43-L glass aquaria (two exposure plus one control for each exposure; p. 13). The dilution water used in the study was from Lake Huron (p. 12); the water was sand-filtered, dechlorinated through activated carbon, and irradiated with UV light prior to use in the study. Water quality parameters of the supply water were as follows for the 0.5 and 5.0 ng/mL exposure studies:

	0.5 ng/mL exposure	5.0 ng/mL exposure
pH	6.6-7.7	6.7-6.9
alkalinity (mg/L as $CaCO_3$)	24-38	27-32
hardness (mg/L as $CaCO_3$)	52-62	54-60
conductivity (μmhos/cm)	130-157	132-152

Three days prior to the introduction of the fish into the aquaria, the exposure tanks were treated with alpha-phenoxy ring-labeled [U-^{14}C]cyhalofop-butyl, dissolved in dimethylformamide, at nominal concentrations of 0.5 and 5.0 ng/mL (pp. 13-14). The high-concentration dose solution was prepared by diluting [^{14}C]cyhalofop-butyl with non-radiolabeled cyhalofop-butyl (purity 99.5%; p. 12). The test compound was introduced into the exposure water by injection with a syringe pump equipped with a gas-tight, glass syringe. Control aquaria were treated only with dimethylformamide, at the same concentration delivered to the exposure aquaria (0.1 mL/L). The aquaria were maintained at 13 ± 2 °C, and the dilution water was continuously supplied at an average measured flow rate of 178 mL/min (258 L/day; equivalent to approximately 6 turnovers/day).

Following the 3-day equilibration period, rainbow trout (150 for the 0.5 ng/mL exposure and 225 for the 5.0 ng/mL exposure) were placed into the exposure and control aquaria (p. 14). The mean weight of the fish (based on analysis of 10 fish) were 1.4540 ± 0.2952 g for the 0.5 ng/mL exposure and control aquaria, and 1.2689 ± 0.4875 g for the 5.0 ng/mL exposure and control aquaria. The initial fish:water ratios were 0.8 g fish/L/day and 1.1 g fish/L/day for the 0.5 and 5.0 ng/mL exposures, respectively. The exposure period for all four aquaria was carried out for 28 days. The aquaria were maintained under fluorescent lighting with a light/dark cycle of 16/8 hours per day (p. 12). During the exposure period, four fish were collected from the exposure and control aquaria at 0 (pre-exposure), 2, 5, 7, 14, 21, and 28 days to determine total radioactivity in the whole fish (p. 14). Four additional fish were collected from the aquaria at 2, 7, 14, 21, and 28 days to determine total radioactivity in the muscle and nonedible tissues (head, skin, viscera, and skeleton). To determine the distribution of [^{14}C]residues in whole fish, six fish were collected from the 0.5 ng/mL exposure aquarium at the end of the 28-day exposure period, and four fish were collected from the 5.0 ng/mL exposure and the corresponding control aquaria at 2, 7, 14, 21, and 28 days; the fish were extracted and analyzed by HPLC (p. 15). Additionally, ten fish were collected from the high-concentration exposure aquarium on day 10, dissected into muscle, and the muscle fillets were extracted and analyzed by HPLC to determine the distribution of [^{14}C]residues.

Following the 28-day exposure period, the remaining fish were transferred from the test aquaria to clean aquaria for an 8-day (0.5 ng/mL exposure study) or 14-day (5.0 ng/mL exposure study) depuration period (p. 15). The dilution water was continuously supplied at a rate of 258 L/day. During the depuration period, four fish each were collected from the 0.5 ng/mL exposure and control aquaria at 0.2, 1, 3, and 8 days, and from the 5.0 ng/mL exposure and control aquaria at 0.2, 1, 3, 7, 10, and 14 days to determine total radioactivity in the whole fish. Four additional fish were collected from the aquaria at 1, 3, and 8 days (0.5 ng/mL exposure study), and at 1, 3, 7, and 14 days (5.0 ng/mL exposure study) to determine total radioactivity in the dissected muscle and nonedible tissues. To determine the distribution of [^{14}C]residues in whole fish, four fish were collected from the 5.0 ng/mL exposure and the corresponding control aquaria at 1, 3, 7, and 14 days, extracted, and the extracts were analyzed by HPLC.

Total radioactivity in the dilution water of the exposure and control aquaria was determined daily during the exposure phase of the study by direct sampling (p. 16). Water samples were collected for the determination of total radioactivity through 2 days of clearance for the 0.5 ng/mL exposure study and through 5 days of clearance for the 5.0 ng/mL exposure study. At each sampling interval, six water samples (4-mL aliquots) were collected from the exposure aquaria and two water samples (4-mL aliquots) were collected from the control aquaria. Samples were analyzed for total radioactivity by LSC. The limits of detection were 0.01 and 0.05 ng cyhalofop-butyl equivalents/mL for the 0.5 and 5.0 ng/mL exposure studies, respectively. The distribution of [^{14}C]residues in the water was determined weekly by HPLC. The limits of detection were 0.04 and 0.2 ng cyhalofop-butyl equivalents/0.5 mL for the 0.5 and 5.0 ng/mL exposure studies, respectively (p. 25). Prior to analysis, the water samples were transferred to a glass vial containing acetonitrile with 0.25% phosphoric acid (yielding a final solution of approximately 10:90 acetonitrile:dilution water); the acetonitrile was spiked with non-radiolabeled cyhalofop-butyl and its acid equivalent, DE537 acid, to track elution using a UV detector (p. 16). Dilution water samples were analyzed by "HPLC Method A" using the following conditions (pp. 21-23):

Column	4.6 mm x 250 mm Zorbax SB-C_8; 5-μm particle
Solvent A	Acetonitrile:methanol (plus 1% acetic acid; 50:50, v:v)
Solvent B	Distilled, deionized water (plus 1% acetic acid)
Mobile phase gradient (A:B)	40:60 (0-2 min), ramp to 90:10 (2-12 min), hold at 90:10 (12-20 min)
UV detector	LDC Sprectromonitor 3200 (ELC-0352); 254 nm
Radiochemical detector	Radiomatic Series A-200 (ELC-0015) with, 0.2-mL Ysi Flow Cell
Flow rate	1.0 mL/min
Approximate retention times	Cyhalofop-butyl - 24 minutes DE537 - 20 minutes

Throughout the study, the water temperature and flow rate of each aquaria were monitored daily, and dissolved oxygen and pH were measured at a minimum, on sampling days (p. 15).

Fish tissue subsamples (0.5 g for edible tissue and 3 to 4 segments of 0.1-0.5 g each for whole fish and nonedible tissue) were analyzed for total radioactivity following tissue solubilization (p. 18). The tissues were solubilized in Soluene-350, and analyzed for total radioactivity by LSC. The limits of detection were 0.1-0.2 and 0.6-0.9 ng/g for the 0.5 and 5.0 ng/mL exposure studies, respectively (p. 25). To determine the distribution of [^{14}C]residues in tissue samples, the samples were homogenized (while frozen) in two repetitive extractions with acetonitrile plus 1% acetic acid (p. 19). The combined extracts were concentrated on a Speed-Vac, reconstituted with water:methanol:acetonitrile (plus 1% phosphoric acid; 38.5:38.5:23, v:v:v), and analyzed by LSC and HPLC. The limits of detection were 0.04 and 0.2 ng/0.5 mL for the 0.5 and 5.0 ng/mL exposure studies, respectively (p. 25). Total [^{14}C]residues remaining in the extracted tissue solids were determined by LSC following solubilization. Fish tissue samples were analyzed by "HPLC Method B" using the following conditions (pp. 22-23):

Column	4.6 mm x 250 mm YMC ODS-AQ; 5-μm particle
Solvent A	Acetonitrile:methanol (plus 1% acetic acid; 50:50, v:v)
Solvent B	Distilled, deionized water (plus 1% acetic acid)
Mobile phase gradient (A:B)	5:95 (0-4 min), ramp to 40:60 (4-6 min), hold at 40:60 (6-10 min), ramp to 90:10 (10-20 min), hold at 90:10 (20-35 min)
UV detector	LDC Sprectromonitor 3200 (ELC-0352); 254 nm
Radiochemical detector	Berthold LB507B, 150-μL Flow Cell
Flow rate	1.0 mL/min
Approximate retention times	Cyhalofop-butyl - 29 minutes DE537 - 25 minutes

To characterize [^{14}C]residues present in the fish tissues at >50 ng/g, representative extracts of the whole fish tissue were analyzed by HPLC/MS with negative ion electrospray ionization and simultaneous radioactivity detection (p. 23). HPLC conditions were essentially the same as those previously described for the analysis of fish tissues. Detailed HPLC and MS operating parameters were reported on pages 23-24.

Fish were collected from each aquaria (both exposure and control) for percent lipid determinations at 0 (pre-loading), 2, and 28 days, and clearance day 8 for the 0.5 ng/mL exposure study, and at 0 (pre-loading) and 28 days, and clearance days 1 and 14 for the 5.0 ng/mL exposure study (p. 19). The lipid determination was a gravimetric technique employing an isopropanol:hexane extraction system. The mean lipid values (g lipid/g whole fish expressed as a percent) were 3.01 ± 0.99% and 2.81 ± 0.75% for the 0.50 and 5.0 ng/mL studies, respectively for control and exposed fish combined(Table 12, p. 51).

In a method validation and storage stability study of cyhalofop-butyl in water, a 400-mL volume of dilution water was fortified to contain 200 dpm/mL of cyhalofop-butyl (p. 17). The water was maintained at 12°C, and samples were collected within 10 minutes and at 6, 24, and 48 hours following spiking. Recoveries of total [^{14}C]residues ranged from 195 to 199 dpm/mL (98-100% of target). However, cyhalofop-butyl decreased from 100% to 63.9% of the recovered [^{14}C]residues following 48 hours; the remaining radioactivity was present as DE-537 acid.

In a method validation study of cyhalofop-butyl and DE-537 acid in fish tissues, edible, nonedible, and whole fish tissues were fortified separately with cyhalofop-butyl and DE-537 acid at approximately 35000 dpm of radioactivity (p. 20). Recoveries of cyhalofop-butyl and DE-537 acid from solubilized edible, nonedible, and whole fish tissues ranged from 90.2 to 98.1%.

In a frozen storage stability study of fortified whole fish tissues, recoveries of cyhalofop-butyl and DE-537 acid from whole fish extracts following 43-50 days of storage (-10°C) were 94.1-94.2% and 102% of the initial radioactivity, respectively (p. 36; Table 13, p. 52).

RESULTS/DISCUSSION

Radiolabeled residues accumulated in rainbow trout that were exposed to alpha-phenoxy ring-labeled [U-^{14}C]cyhalofop-butyl [(R)-2-(4-(4-cyano-2-fluorophenoxy) phenoxy)propionate, –butyl ester; radiochemical purity 99.7%] at nominal concentrations of 0.5 and 5.0 ng/mL, under flow-through conditions. The actual concentrations of cyhalofop-butyl in the water were 92-97% of their respective nominal concentrations at the initiation of each study, decreased substantially during the first seven days of exposure, then stabilized at approximately 27-36% and 18-27% of the nominal for the 0.5 and 5.0 ng/mL exposures, respectively, and remained constant for the remainder of the 28-day exposure period (Table 5, p. 44). The average measured exposure concentrations during days 7-28 were 0.19 and 1.10 ng cyhalofop-butyl/mL for the 0.5 and 5.0 ng/mL exposure aquaria, respectively (Table 6, p. 45). Total [^{14}C]residues in the exposure water were 0.56 ± 0.02 ng/mL (range of 0.49-0.60 ng/mL) for the 0.5 ng/mL exposure aquarium and 5.23 ± 0.28 ng/mL (range of 4.71-5.86 ng/mL) for the 5.0 ng/mL exposure aquarium (Table 4, p. 43). Cyhalofop-butyl hydrolyzed to its acid equivalent,

> (R)-2-(4-(4-cyano-2-fluorophenoxy)phenoxy)propanoic acid (DE-537 acid; XDE-537 acid; Appendix A1, p. 72),

in the exposure water; DE-537 acid was present in the exposure water at 63-73% and 73-82% of the nominal 0.5 and 5.0 ng/mL concentrations, respectively, from 7 to 28 days during the exposure period (Table 5, p. 44). [^{14}C]Residues were not detected in the exposure water by day 2 of the depuration period (Table 4, p. 43).

In the **0.5 ng/mL exposure study**, mean total [^{14}C]residues were highest in the nonedible tissue (105 ± 18.7 ng cyhalofop-butyl equivalents/g) compared to edible (5.0 ± 0.9 ng cyhalofop-butyl equivalents/g) and whole fish (97.5 ± 15.8 ng cyhalofop-butyl equivalents/g) tissues (Table 7, p. 46). Maximum total [^{14}C]residues were 135, 5.9, and 113 ng cyhalofop-butyl equivalents/g for the nonedible, edible, and whole fish tissues, respectively, on exposure day 14. Accumulation plateaus were reached by 3.4-5.3 days (Table 9, p. 48; Figures 8-9, pp. 60-61).

Bioconcentration factors (BCF) for total [^{14}C]residues were 576, 27, and 507 mL/g for the nonedible, edible, and whole fish tissues, respectively. BCF values were calculated for total [^{14}C]residues from the ratio of total [^{14}C]residues in the various fish tissues versus the average concentration of cyhalofop-butyl in the exposure water from 7 to 28 days (0.19 ng/mL). Depuration was rapid, with elimination half-lives of <1.2 days (Table 9, p. 48). At the end of the 8-day depuration period, total [^{14}C]residues in the nonedible, edible, and whole fish tissues were 6.7, 0.4, and 4.6 ng cyhalofop-butyl equivalents/g, respectively (Table 7, p. 46). The mortality rates for the exposure and control aquaria were each 2.7% (4 mortalities from each tank/150 fish loaded per tank; p. 15).

In the **5.0 ng/mL exposure study**, mean total [^{14}C]residues were highest in the nonedible tissue (823 ± 253 ng cyhalofop-butyl equivalents/g) compared to edible (30.5 ± 4.6 ng cyhalofop-butyl equivalents/g) and whole fish (698 ± 223 ng cyhalofop-butyl equivalents/g) tissues (Table 8, p. 47). Maximum total [^{14}C]residues were 1211, 36.8, and 993 ng cyhalofop-butyl equivalents/g for the nonedible, edible, and whole fish tissues, respectively, on exposure day 7. Accumulation plateaus were reached by 5.3-9.0 days (Table 10, p. 49; Figures 10-11, pp. 62-63). Cyhalofop-butyl was not present in most fish samples, and represented <1% of the total extractable radioactivity in samples in which it was present (p. 33). DE-537 acid,

the taurine conjugate of DE-537 acid, and

an unidentified degradate designated "Peak B"

were present in the whole fish tissue at maximums of 335-337, 521-719, and 158-396 ng cyhalofop-butyl equivalents/g, respectively, on exposure day 7, and 177-194, 101-104, and 41-42, respectively, on exposure day 28 (Table 11, p. 50). [^{14}C]Residues were characterized in the muscle fillets only on exposure day 10; DE-537 acid accounted for all of the [^{14}C]residues, 36-39 ng cyhalofop-butyl equivalents/g, on day 10.

Bioconcentration factors (BCF) for total [^{14}C]residues were 672, 27, and 436 mL/g for the nonedible, edible, and whole fish tissues, respectively (Table 10, p. 49). BCF values were calculated for total [^{14}C]residues from the ratio of total [^{14}C]residues in the various fish tissues versus the average concentration of cyhalofop-butyl in the exposure water from 7 to 28 days (1.10 ng/mL). Depuration was rapid, with elimination half-lives of <2.1 days. At the end of the 14-day depuration period, total [^{14}C]residues in the nonedible and whole fish tissues were 18.3 and 10.6 ng cyhalofop-butyl equivalents/g, respectively, and were not detected in the edible tissue (Table 8, p. 47). The mortality

rates for the exposure and control aquaria were 6.2% (14 mortalities/225 fish loaded) and 2.7% (6 mortalities/225 fish loaded), respectively (pp. 15-16).

BCFs for cyhalofop-acid, based on data from Tables 5 and 11, were 86 on day 7, 60 on day 14, and 46 on day 21. Lipid-normalized values (based on 2.42% lipid content for exposed fish) were 3560 mL/g, 2470 mL/g, and 1890 mL/g, respectively.

During the exposure and depuration periods (both studies), the temperature was 12.3-13.3 °C, the pH was 6.9-7.2, and the dissolved oxygen content was 8.1-8.5 mg/L (Table 3, p. 42); ranges are based on the mean values for each aquaria (exposure and control).

DEFICIENCIES/DEVIATIONS

1. The cyhalofop-butyl acid equivalent, DE-537 acid, was the primary [^{14}C]residue present in the exposure aquaria following exposure day 7 (Table 5, p. 44); [^{14}C]residues in the exposure water were not characterized between 0 and 7 days. The study authors stated that the increased degradation of the parent in the exposure aquaria was caused, at least in part, by a biological or enzyme-mediated process likely associated with the fish (p. 29). This conclusion was based on two observations: (i) the ratio of cyhalofop-butyl to DE-537 acid was much greater in the mixing cell than in the exposure water (following day 7) and (ii) the concentration of the parent in the day 0 (pre-loading) exposure aquaria represented >90% of the total radioactivity following a 3-day equilibration period, then decreased rapidly once the fish were loaded into the tank, The study authors added that previous fish toxicity studies conducted with cyhalofop-butyl have shown that the parent is unstable in aqueous solution, and quickly hydrolyzes (half-life <1 day) to DE-537 acid (p. 10).

2. Extractable [^{14}C]residues present at >50 ppb were not identified, as required by Subdivision N guidelines. The degradate designated as "Peak B" was present in the whole fish tissue at a maximum of 158-396 ng cyhalofop-butyl equivalents/g at exposure day 7 (Table 11, p. 50). This was 16-27% of total ^{14}C activity.

3. The study authors stated that it was difficult to precisely define the time in which the accumulation plateaus were reached for the various tissue types because the total [^{14}C]residues in the fish tissues were variable over time (p. 30).

4. Reported bioconcentration factors were based on the total [^{14}C]residues present in the fish tissues. The study authors stated that the estimated BCF for cyhalofop-butyl, which accounted for <1% of the total [^{14}C]residues detected in the fish, was 4-7 mL/g (p. 33). BCFs for the "acid" metabolite were calculated by the reviewer to be 46-86 mL/g.

5. The study authors stated that the molar equivalent concentrations of cyhalofop-butyl were maintained at their intended nominal concentrations, despite the instability of the parent in the aqueous systems (p. 9).

6. The study authors stated that six fish were collected from the 0.5 ng/mL exposure aquarium at the end of the 28-day exposure period to determine the distribution of [^{14}C]residues in whole fish (p. 15); however, the data were not reported. The reviewer noted that total [^{14}C]residues in the whole fish were 87.5 ng cyhalofop-butyl equivalents/g on exposure day 28 (Table 7, p. 46).

7. The study authors stated that only two fish per sampling day were processed to characterize [^{14}C]residues, and that typically only one representative control fish per sampling day was solubilized from each tissue type for the determination of total radioactivity (pp. 18-19). The additional control fish collected at each sampling interval was not fully analyzed.

8. The 96-hour LC_{50} for cyhalofop-butyl was reported as >490 ng/mL for rainbow trout and 760 ng/mL for bluegill sunfish (p. 11).

9. Reported lipid-normalized BCFs for total [^{14}C]residues in whole fish were 16800 and 15500 mL/g lipid for the 0.50 and 5.0 ng/mL exposure studies, respectively (p. 36).

10. Juvenile rainbow trout were used in this study. Subdivision N guidelines list bluegill sunfish and channel catfish as the preferred species for fish bioaccumulation studies. A study of bioaccumulation in koi carp was mentioned (p. 33, p. 38, ref. 14).

11. Results from an inorganic and organic analysis of the dilution water was presented in Tables 1-2 (pp. 40-41).

12. The study authors stated that there was no discernible difference in growth between control and exposed fish at either test concentration (p. 27).

13. Representative HPLC chromatograms of the dilution water and the whole fish extracts indicated good separation of peaks (Figures 4-5, 12-14, pp. 56-57, 64-66).

14. The water solubility of cyhalofop-butyl was reported as 0.4 mg/L in pH 7 buffer (p. 10).

15. Good Laboratory Practice Compliance and Quality Assurance statements were provided with this study.

ATTACHMENT 1
Data Critical to the Study Interpretation

THE FOLLOWING ATTACHMENT IS NOT AVAILABLE ELECTRONICALLY
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Table 3. The Bioconcentration of DE-537 N-Butyl Ester by the Rainbow Trout *Oncorhynchus mykiss* Walbaum. Water Quality Conditions during Uptake and Clearance Phases. Data are Means and (Ranges) of Measured Values.

	Exposure Level of DE-537 n-butyl Ester			
	Low-Concentration		High-Concentration	
Parameter	Control Aquarium	Exposure Aquarium	Control Aquarium	Exposure Aquarium
Temperature	12.4 °C (11.9-13.0)	13.2 °C (12.2-14.0)	12.3 °C (11.9-12.6)	13.3 °C (12.2-14.1)
Dissolved Oxygen	8.5 mg/L (7.3-9.9)	8.3 mg/L (7.6-9.6)	8.3 mg/L (6.8-10.1)	8.1 mg/L (6.8-9.5)
pH	7.2 (6.4-7.5)	7.1 (6.2-7.5)	6.9 (6.7-7.0)	6.9 (6.1-7.3)
Water Flow	177 mL/min (172-182)	177 mL/min (174-180)	179 mL/min (170-185)	177 mL/min (166-182)

Table 4. The Bioconcentration of DE-537 N-Butyl Ester by the Rainbow Trout, *Oncorhynchus mykiss* Walbaum. Total ^{14}C Residue Levels in the Exposure and Clearance Water Samples.

		Low-Concentration [a]		High-Concentration [b]	
Phase	Study Day	DPM/mL	ng/mL	DPM/mL	ng/mL
Pre-Exposure	-3	91	0.49	168	4.21
	-2	99	0.53	200	5.01
	-1	100	0.53	170 †	4.26
	0; Pre-loading	105	0.56	208	5.21
Exposure	0; Post-loading	92	0.49	194	4.86
	1	103 †	0.55	193 †	4.84
	2	102 †	0.55	192 †	4.81
	3	107	0.57	191	4.79
	4	104	0.56	202	5.06
	5	101	0.54	209	5.24
	6	104	0.56	211	5.29
	7	104	0.56	217	5.44
	8	108	0.58	188	4.71
	9	103	0.55	215	5.39
	10	107	0.57	227	5.69
	11	110	0.59	222	5.56
	12	112	0.60	228	5.71
	13	106	0.57	203	5.09
	14	102	0.55	210	5.26
	15	106	0.57	210	5.26
	16	105	0.56	212	5.31
	17	101	0.54	214	5.36
	18	105	0.56	212	5.31
	19	105	0.56	234	5.86
	20	103	0.55	208	5.21
	21	102	0.55	201	5.04
	22	100	0.53	205	5.14
	23	101	0.54	199	4.99
	24	102	0.55	209	5.24
	25	107	0.57	209	5.24
	26	102	0.55	221	5.54
	27	105	0.56	205	5.14
	28	101	0.54	216	5.41
	Average =	**104**	**0.56**	**209**	**5.23**
	Std. Dev. =	**4**	**0.02**	**11**	**0.28**
	Rel. SD (95% CI) * =	**8%**	**7%**	**11%**	**11%**
Clearance	Cl 0.5	3	0.02	9	0.23
	Cl 1	ND ††	ND	4	0.10
	Cl 2	ND	ND	ND	ND
	Cl 3	Not Sampled		ND	ND
	Cl 4	Not Sampled		ND	ND
	Cl 5	Not Sampled		ND	ND

Control Aquariums <2 DPM/mL (<0.01 ng/mL Low Exp; <0.05 ng/mL High Exp)

[a] Low-Concentration Study: Specific Activity of 187 DPM/ng

[b] High-Concentration Study: Specific Activity of 39.9 DPM/ng

† Aquarium was sampled twice on days denoted by †; values represent the mean of two sampling times.

* Rel. SD (95% CI) = (t-value x Std. Dev.)/Average; t-value = 2.048 (28 degrees of freedom)

†† Not Detected; Limit of Detection = 2 DPM/mL; (0.01 ng/mL Low Exp; 0.05 ng/mL High Exp)

Table 5. The Bioconcentration of DE-537 N-Butyl Ester by the Rainbow Trout, *Oncorhynchus mykiss* Walbaum. Characterization of Exposure Water Samples by HPLC Radioassay.

% of Total Radioactivity in Low-Level Aqueous Sample

Exposure Day #	Exposure Aquarium		Mixing Chamber	
	DE-537 nBE	DE-537 acid	DE-537 nBE	DE-537 acid
0; Pre-loading	92	8	Not analyzed	
7	36	63	Not analyzed	
8	35	65	89	11
14	35	65	88	12
21	27	73	80	20
27	35	65	74	26
Average:	**43%**	**57%**	**83%**	**17%**
Std Deviation:	±24%	±24%	±7%	±7%

Average Percent DE537nBE in Exp Aq from Days 7-27: 34% ±10%
Interpolated Linear Decline in Percent DE537nBE from Days 0-7: 8.2% per day

% of Total Radioactivity in High-Level Aqueous Sample

Exposure Day #	Exposure Aquarium		Mixing Chamber	
	DE-537 nBE	DE-537 acid	DE-537 nBE	DE-537 acid
0; Pre-loading	97	3	100	0
7	22	78	97	3
14	27	73	90	10
21	18	82	89	11
27	18	82	74	26
Average:	**36%**	**64%**	**90%**	**10%**
Std Deviation:	±34%	±34%	±10%	±10%

Average Percent DE537nBE in Exp Aq from Days 7-27: 21% ±4%
Interpolated Linear Decline in Percent DE537nBE from Days 0-7: 10.9% per day

Table 6. The Bioconcentration of DE-537 N-Butyl Ester by the Rainbow Trout, *Oncorhynchus mykiss* Walbaum. Calculated Concentration of ^{14}C DE-537nBE (as ng DE537nBE equivalent/mL) in the Exposure Water.

	Low-Concentration		High-Concentration	
Exposure Day	**Percent DE537nBE** (from HPLC Radioassay)	**Calculated † DE537nBE** (ng DE537nBE/mL)	**Percent DE537nBE** (from HPLC Radioassay)	**Calculated † DE537nBE** (ng DE537nBE/mL)
0	92	0.52	97	5.07
1	84 *	0.47	86 *	4.50
2	76 *	0.43	75 *	3.92
3	68 *	0.38	64 *	3.35
4	60 *	0.34	53 *	2.77
5	52 *	0.29	42 *	2.20
6	44 *	0.25	31 *	1.62
Days 7 - 28	34 **	0.19	21 **	1.10

† **Calculated DE537nBE Conc. = Average Total Aqueous ^{14}C (as ng/mL equiv.) x % DE537nBE**
Study Average equiv. Aqueous DE537nBE (from Total ^{14}C): 0.56 ng/mL for low-level study
5.23 ng/mL for high-level study

* **Interpolated values; % DE537nBE not measured on these days.**
assumes linear decline in Percent DE537nBE through days 0 - 7
8.2% per day for low-level study; 10.9% per day for high-level study

** **Average of Days 7 - 27 HPLC Radioassays (n=5 low-level; n=4 high-level).**

Table 7. The Bioconcentration of DE-537 N-Butyl Ester by the Rainbow Trout *Oncorhynchus mykiss* Walbaum. Total ^{14}C Residues in Solubilized Whole Fish, Muscle and Remainder Tissue from the Low-Concentration Study.

Exposure Day	Mean [a] Fish wt (g)	Average ng Total ^{14}C (as DE-537 nBE)/g tissue					
		Whole Fish [b]		Muscle [b]		Remains [b]	
2	0.9357	110	(30)	5.4	(27)	97.5	(20)
5	0.8595	105	(30)	Not Analyzed		Not Analyzed	
7	1.1553	98.3	(19)	5.6	(28)	108	(22)
14	1.1067	113	(66)	5.9	(60)	135	(22)
21	1.0575	71.2	(33)	3.6	(10)	97.1	(23)
28	1.3640	87.5	(47)	4.7	(30)	85.6	(5)
Average (±Std. Dev.) Total ^{14}C Exposure Phase		**97.5 ±15.8**		**5.0 ±0.9**		**105 ±18.7**	
Clearance							
0.2	1.3498	57.1	(12)	Not Analyzed		Not Analyzed	
1	1.3238	38.4	(14)	1.2	(30)	50.2	(59)
3	1.2416	6.2	(11)	0.3	(27)	9.8	(36)
8	1.6310	4.6	(41)	0.4	(25)	6.7	(35)

[a] Average weight of whole fish sampled from exposure aquarium.

[b] Expressed as average ng ^{14}C DE-537 nBE equivalent/g of tissue (% Relative Standard Deviation).

Table 8. The Bioconcentration of DE-537 N-Butyl Ester by the Rainbow Trout *Oncorhynchus mykiss* Walbaum. Total ^{14}C Residues in Solubilized Whole Fish, Muscle and Remainder Tissue from the High-Concentration Study.

Exposure Day	Mean [a] Fish wt (g)	Average ng Total ^{14}C (as DE-537 nBE)/g tissue: Whole Fish [b]		Muscle [b]		Remains [b]	
2	1.8472	701	(19)	31.7	(20)	821	(25)
5	1.5777	918	(16)	Not Analyzed		Not Analyzed	
7	1.4701	993	(48)	36.8	(5)	1211	(52)
14	1.3102	623	(43)	30.9	(14)	750	(36)
21	1.6563	406	(28)	24.1	(17)	505	(9)
28	1.5377	545	(13)	29.1	(6)	827	(36)
Average (±Std. Dev.) Total ^{14}C Exposure Phase		**698**	**±223**	**30.5**	**±4.6**	**823**	**±253**
Clearance							
0.2	1.0255	263	(24)	Not Analyzed		Not Analyzed	
1	1.6359	196	(46)	2.5	(47)	400	(59)
3	1.5764	158	(87)	2.2	(NA)	76.9	(86)
7	1.7556	42.7	(50)	1.1	(NA)	22.6	(35)
10	1.6969	14.1	(20)	Not Analyzed		Not Analyzed	
14	1.9765	10.6	(25)	Not Detected [c]		18.3	(28)

[a] Average weight of whole fish sampled from exposure aquarium.

[b] Expressed as average ng ^{14}C DE-537 nBE equivalent/g of tissue (% Relative Standard Deviation).

[c] Not Detected in Solubilized Muscle Tissue; Limit of Detection = 0.9 ng DE537nBE equiv./g muscle.

Table 9. The Bioconcentration of DE-537 N-Butyl Ester by the Rainbow Trout, *Oncorhynchus mykiss* Walbaum. Kinetic Parameters Estimated by Two-Compartment Modeling of Total ^{14}C Uptake/Clearance in Rainbow Trout from Low-Level Exposure.

Parameter	Units	^{14}C-Whole Fish [a]	^{14}C-Muscle [a]	^{14}C-Remainder [a]
K_1	$mL\text{-}g^{-1}\text{-}day^{-1}$	441	15	501
(95% Confidence Limit)		(±187)	(±10)	(±208)
k_2	day^{-1}	0.870	0.563	0.870
(95% Confidence Limit)		(±0.330)	(±0.362)	(±0.332)
BCF [b]	mL/g	507	27	576
(95% Confidence Range)		(212-1160)	(5.4-124)	(244-1320)
$t_{1/2}$ [c]	days	0.8	1.2	0.8
(95% Confidence Range)		(0.6-1.3)	(0.7-3.4)	(0.6-1.3)
$t_{ss95\%}$ [d]	days	3.4	5.3	3.4
(95% Confidence Range)		(2.5-5.6)	(3.2-15)	(2.5-5.6)

[a] Mean Exposure Concentration of 0.19 ng DE537nBE/mL (average of days 7-28).
[b] Total ^{14}C Bioconcentration Factor (BCF) = K_1/k_2.
[c] Elimination half-life was calculated from: $t_{1/2} = \ln 2/k_2$.
[d] Time to reach 95% of steady-state was calculated from: $t_{ss95\%} = -(\ln 0.05)/k_2$.

Table 10. The Bioconcentration of DE-537 N-Butyl Ester by the Rainbow Trout, *Oncorhynchus mykiss* Walbaum. Kinetic Parameters Estimated by Two-Compartment Modeling of Total ^{14}C Uptake/Clearance in Rainbow Trout from High-Level Exposure.

Parameter	Units	^{14}C-Whole Fish [a]	^{14}C-Muscle [a]	^{14}C-Remainder [a]
K_1	$mL\text{-}g^{-1}\text{-}day^{-1}$	146	15	225
(95% Confidence Limit)		(±56)	(±6.9)	(±108)
k_2	day^{-1}	0.335	0.563	0.335
(95% Confidence Limit)		(±0.046)	(±0.130)	(±0.061)
BCF [b]	mL/g	436	27	672
(95% Confidence Range)		(236-699)	(12-51)	(295-1220)
$t_{1/2}$ [c]	days	2.1	1.2	2.1
(95% Confidence Range)		(1.8-2.4)	(1.0-1.6)	(1.8-2.5)
$t_{ss95\%}$ [d]	days	9.0	5.3	9.0
(95% Confidence Range)		(7.9-10)	(4.3-6.9)	(7.6-11)

[a] Mean Exposure Concentration of 1.10 ng DE537nBE/mL (average of days 7-28).
[b] Total ^{14}C Bioconcentration Factor (BCF) = K_1/k_2.
[c] Elimination half-life was calculated from: $t_{1/2} = \ln 2/k_2$.
[d] Time to reach 95% of steady-state was calculated from: $t_{ss95\%} = -(\ln 0.05)/k_2$.

Table 11. The Bioconcentration of DE-537 N-Butyl Ester by the Rainbow Trout, *Oncorhynchus mykiss* Walbaum. Extraction Efficiency, HPLC Distribution of ^{14}C Activity, and Levels of Radiolabeled Residues Detected in Extracts of Whole Fish and Muscle Tissue; High-Concentration Exposure.

		Extraction [a]	% HPLC Distribution [b]			Total Extr. ^{14}C Act. [c]	Equiv Conc. of Residue (ng/g) [d]		
Day #	Tissue	Efficiency	Taurine Conj.	Peak B	DE537 acid	(ng/g)	Taurine Conj.	Peak B	DE537 acid
Exposure Phase									
2	Whole Fish-a	67.5	27.3	24.2	46.7	420	115	102	196
2	Whole Fish-b	88.0	41.2	23.4	35.4	590	243	138	209
7	Whole Fish-a	87.1	49.5	27.3	23.2	1452	719	396	337
7	Whole Fish-b	91.2	51.4	15.6	33.0	1014	521	158	335
14	Whole Fish-a	84.4	21.5	32.0	46.0	410	88	131	189
14	Whole Fish-b	87.0	45.0	13.2	41.8	591	266	78	247
21	Whole Fish-a	84.0	27.0	8.0	64.3	252	68	20	162
21	Whole Fish-b	87.7	35.2	7.9	53.4	400	141	32	214
28	Whole Fish-a	85.3	32.1	13.0	54.5	324	104	42	177
28	Whole Fish-b	86.8	29.9	12.2	57.6	337	101	41	194
10	Muscle-a	92.4	ND [e]	ND	100	36	ND	ND	36
10	Muscle-b	93.2	ND	ND	100	39	ND	ND	39
Clearance Phase									
CL-1	Whole Fish-a	82.3	49.7	28.5	21.8	266	132	76	58
CL-1	Whole Fish-b	79.5	56.8	21.2	22.0	158	90	33	35

[a] Extraction Efficiency = ^{14}C in Solvent Extract/(^{14}C in Solvent Extract + "non-extractable" ^{14}C bound to solubilized tissue solids) x 100.

[b] These values represent the amount of activity attributed to ^{14}C-labeled residues in HPLC fractions relative to summed activity from all of the collected fractions. Summing the three values may not add up to 100% because in some runs other small (<5% of total activity) radiolabeled peaks were detected.

[c] Total extractable ^{14}C activity, expressed as ng DE537nBE equivalent per gram fish tissue.

[d] Residue values are expressed as ng DE-537 nBE equiv/g fish tissue.

[e] ^{14}C Activity Not Detected in HPLC Radioassay; Limit of Detection = 0.2 ng DE537nBE equivalent per fraction.

Table 12. The Bioconcentration of DE-537 N-Butyl Ester by the Rainbow Trout, *Oncorhynchus mykiss* Walbaum. Lipid Content of Fish from Low- and High-Concentration Exposures.

		% Lipid		
Exposure Level	Study Day	Control Fish	Exposure Fish	Overall [a]
Low-Concentration	Day 0 Pre-exposure	4.44	NA [b]	4.44 ± 0.26 (n=2)
	Exposure Day 2	2.13	2.20	2.16 ± 0.40 (n=4)
	Exposure Day 28	2.82	2.56	2.69 ± 0.20 (n=4)
	Clearance Day 8	2.66	2.86	2.76 ± 0.21 (n=4)
	Mean ± Std. Dev.	3.01 ± 1.00	2.54 ± 0.33	3.01 ± 0.99
High-Concentration	Day 0 Pre-exposure	3.93	NA	3.93 ± 0.45 (n=2)
	Exposure Day 28	2.68	2.06	2.37 ± 0.65 (n=4)
	Clearance Day 1	2.08	3.01	2.54 ± 0.58 (n=4)
	Clearance Day 14	2.56	2.20	2.38 ± 0.79 (n=4)
	Mean ± Std. Dev.	2.81 ± 0.79	2.42 ± 0.51	2.81 ± 0.75

[a] Overall mean and standard deviation values calculated from the full complement of fish used to determine lipid content on a given sampling day, usually four fish sampled (2 control; 2 exposure).

[b] NA = Not applicable.

Table 13. The Bioconcentration of DE-537 N-Butyl Ester by the Rainbow Trout, *Oncorhynchus mykiss* Walbaum. Storage Stability of DE537 acid and DE537nBE in Whole Fish Extracts after approximately 50 days of Frozen Storage.

43-day Stability of DE-537 acid

Sample ID	Date Analyzed		Total ^{14}C Activity		HPLC Radioassay (Purity) [a]	
			Total ^{14}C Activity (DPM/mL)	Percent of Initial	% DE537 acid	Percent of Initial
DE537 acid Spike A	3/2/99	i	146728	--	98.8%	--
DE537 acid Spike A	4/14/99	r	149001	**102%**	99.7%	**101%**
DE537 acid Spike B	3/2/99	i	148419	--	97.8%	--
DE537 acid Spike B	4/14/99	r	151519	**102%**	99.7%	**102%**

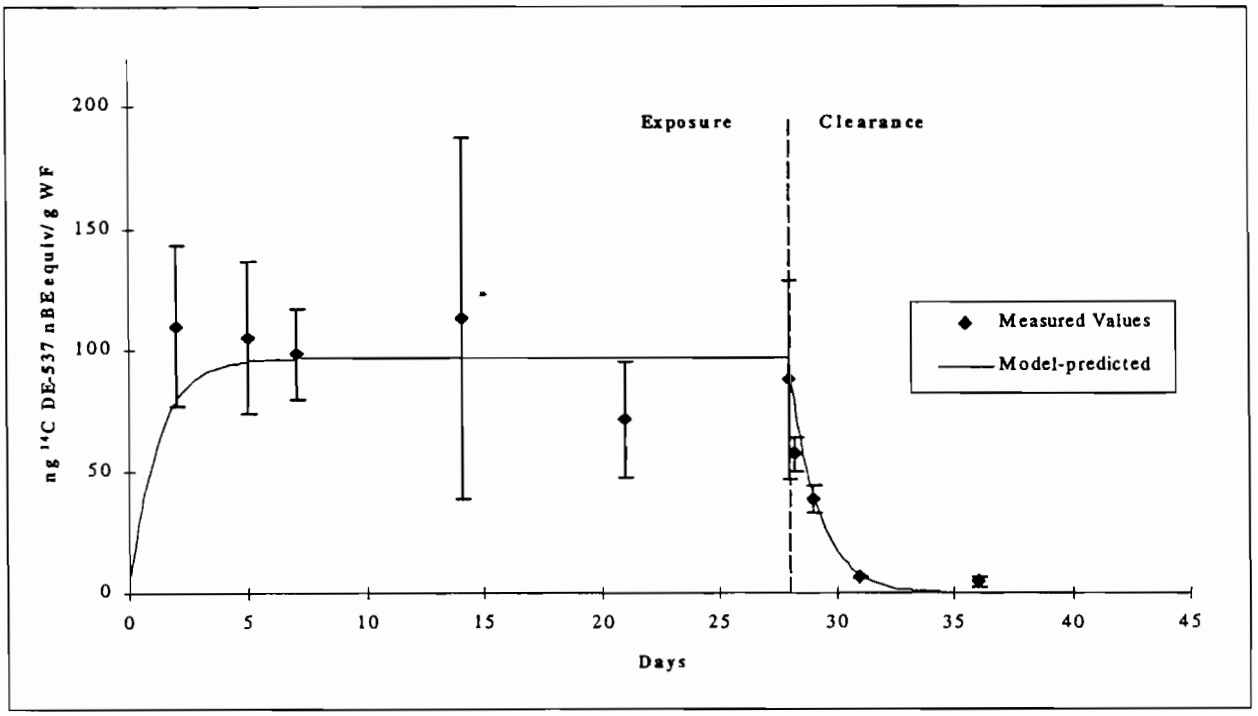
50-day Stability of DE-537 n-butyl Ester

Sample ID	Date Analyzed		Total ^{14}C Activity		HPLC Radioassay (Purity) [a]	
			Total ^{14}C Activity (DPM/mL)	Percent of Initial	% DE537nBE	Percent of Initial
DE537nBE Spike A	2/23/99	i	142484	--	99.7%	--
DE537nBE Spike A	4/14/99	r	134085	**94.1%**	99.7%	**100%**
DE537nBE Spike B	2/24/99	i	125420	--	99.5%	--
DE537nBE Spike B	4/14/99	r	118094	**94.2%**	99.6%	**100%**

[a] Percentage of Detectable Radioactivity (from HPLC radioassay) attributed to analyte of interest.

"i" denotes initial analysis of a sample
"r" denotes repeat analysis of a sample

Figure 8. The Bioconcentration of DE-537 N-Butyl Ester by the Rainbow Trout, *Oncorhynchus mykiss* Walbaum. Two-Compartment Pharmacokinetic Modeling of Total ^{14}C Residue Data in Whole Fish, Low-Level Exposure; Comparison of Constant Exposure Concentration (top), and Varying Exposure Water Concentration (bottom).



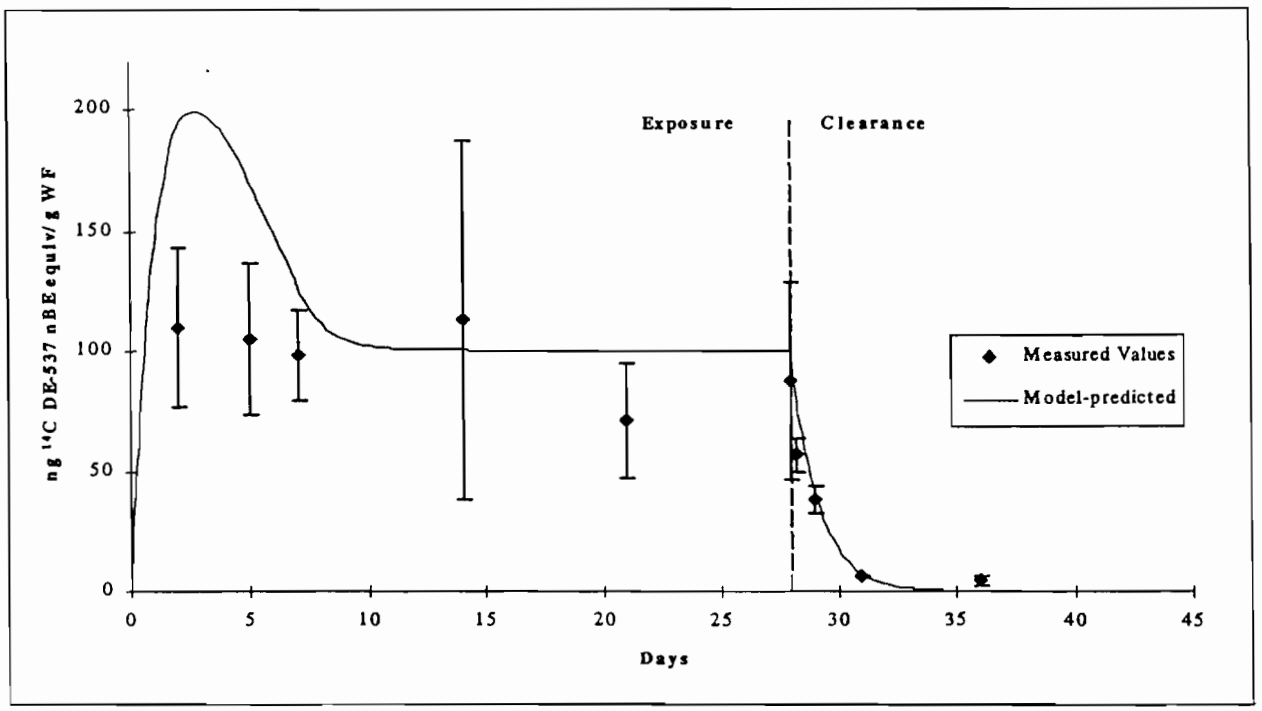
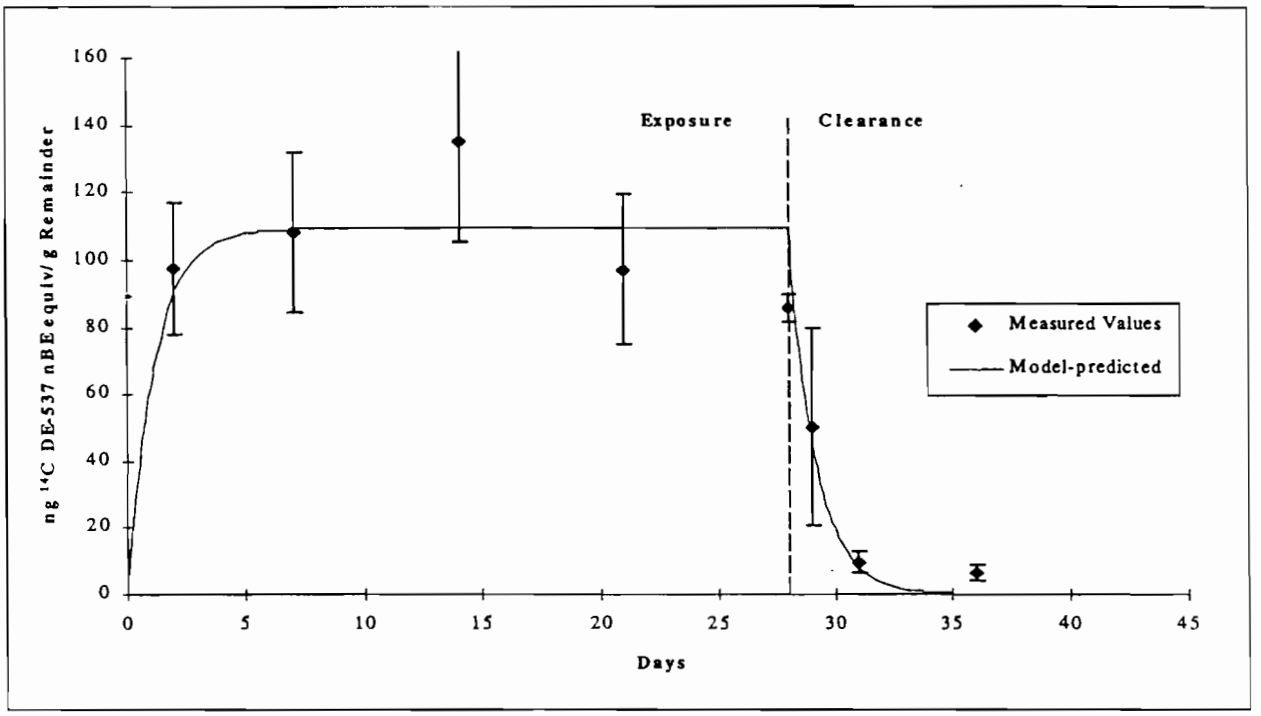


Figure 9. The Bioconcentration of DE-537 N-Butyl Ester by the Rainbow Trout, *Oncorhynchus mykiss* Walbaum. Two-Compartment Pharmacokinetic Modeling of Total ^{14}C Residue Data (Low-Level Exposure) in Remainder Tissue (top), and Muscle (bottom); Constant Exposure Concentration.



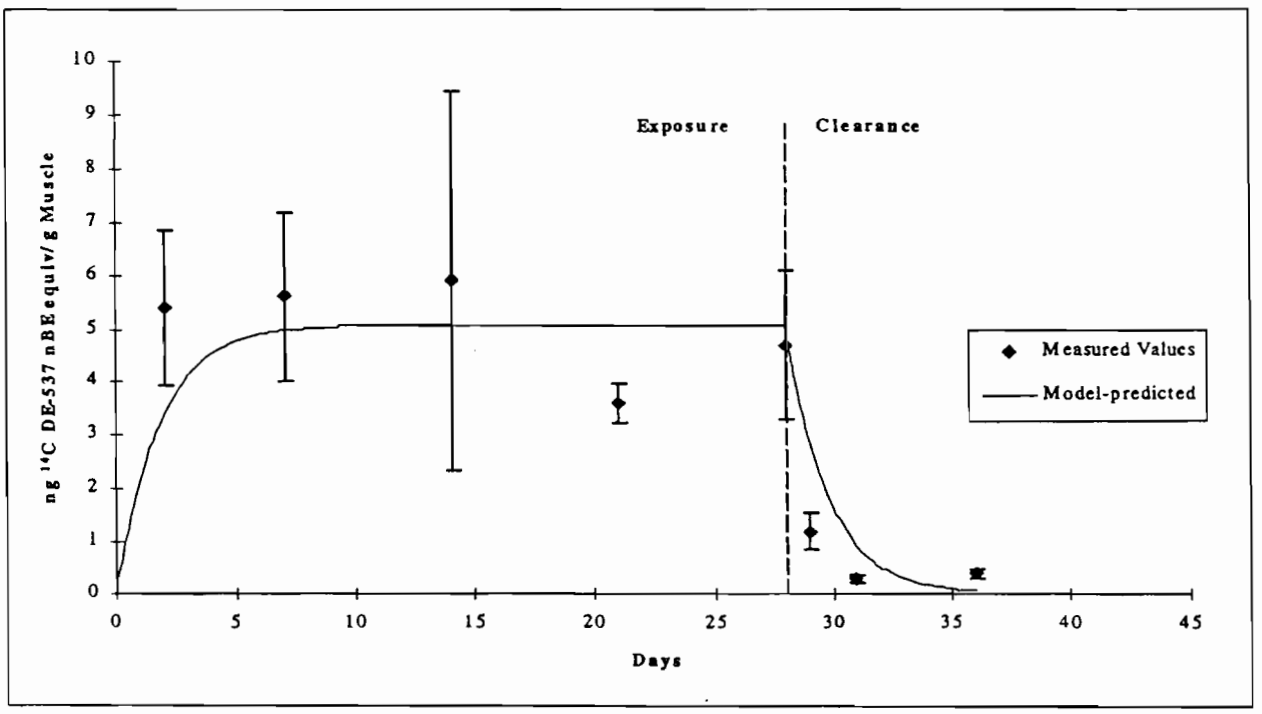


Figure 10. The Bioconcentration of DE-537 N-Butyl Ester by the Rainbow Trout, *Oncorhynchus mykiss* Walbaum. Two-Compartment Pharmacokinetic Modeling of Total ^{14}C Residue Data in Whole Fish, High-Level Exposure; Comparison of Constant Exposure Concentration (top), and Varying Exposure Water Concentration (bottom).

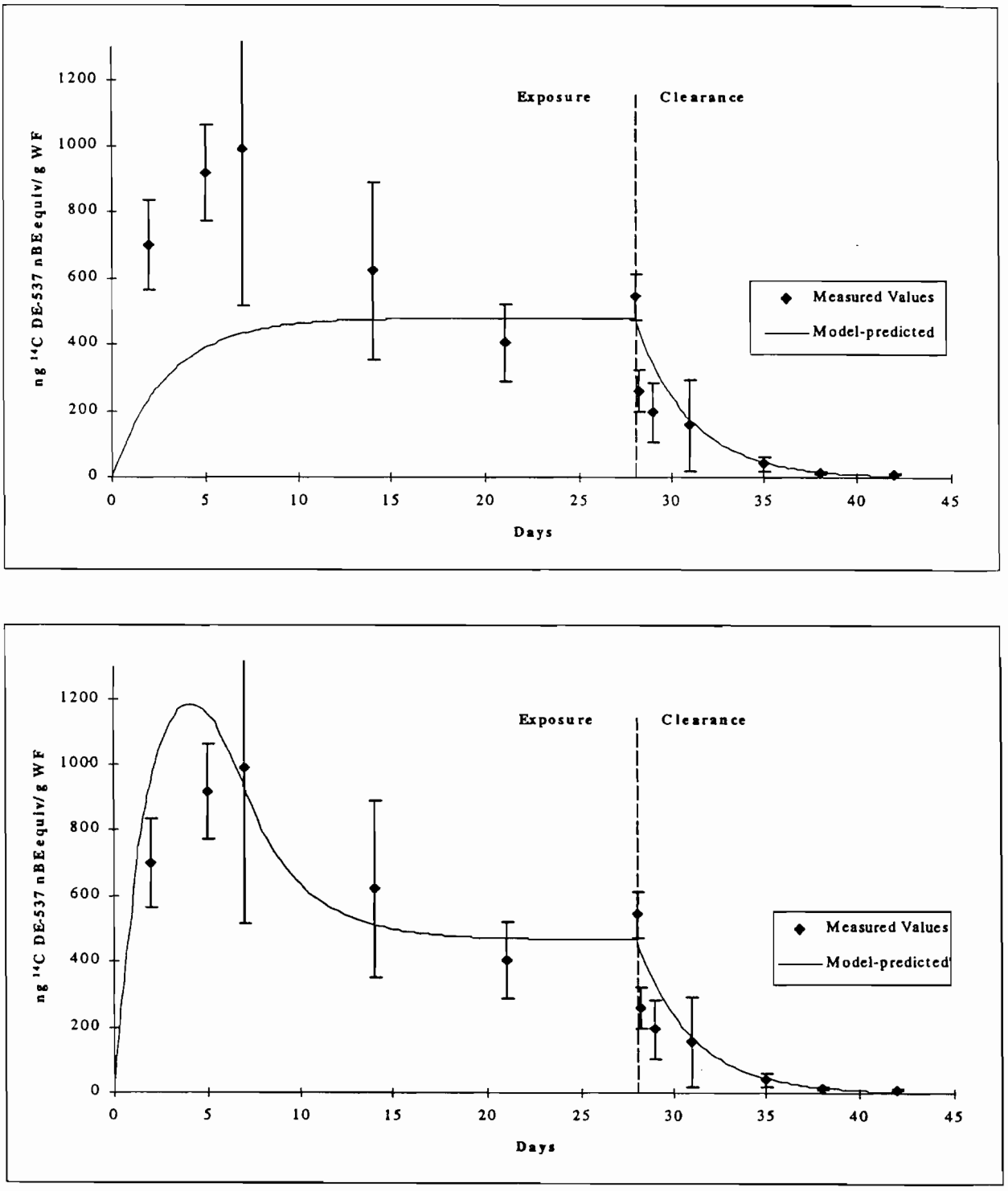


Figure 11. The Bioconcentration of DE-537 N-Butyl Ester by the Rainbow Trout, *Oncorhynchus mykiss* Walbaum. Two-Compartment Pharmacokinetic Modeling of Total ^{14}C Residue Data (High-Level Exposure) in Remainder Tissue (top), and Muscle (bottom); Constant Exposure Concentration.