3-11-2002

DATA EVALUATION RECORD

STUDY 13

CHEM 120603 Tetraconazole §165-4
CAS No. 112281-77-3
FORMULATION–00–ACTIVE INGREDIENT

STUDY ID 44751320

Wyness, L. E. 1995. Tetraconazole: flow-through fish bioconcentration test with *Oncorhynchus mykiss*. Laboratory Project ID: 1320/3-1018. Unpublished study performed by Corning Hazleton (Europe), Harrogate, North Yorkshire, England; and submitted by Sostram Corporation, Roswell, GA

DIRECT REVIEW TIME =

REVIEWED BY: Andrew Glucksman SIGNATURE:
TITLE: Staff Scientist DATE:

EDITED BY: Kathleen Ferguson SIGNATURE:
TITLE: Senior Staff Scientist DATE:

EDITED BY: Joan Harlin SIGNATURE:
TITLE: Senior Staff Scientist DATE:

ORG: Dynamac Corporation
Rockville, MD
TEL: 301/417-9800

APPROVED BY: Iwona. Maher
TITLE: Chemist
ORG: ERBI/EFED/OPP
TEL: 703/605-0569

SIGNATURE: 3-11-2002

ABSTRACT

Laboratory Accumulation - Fish

1. This study is scientifically valid and provides upgradable marginally supplemental information that [^{14}C]tetraconazole residues (uncharacterized) accumulated in rainbow trout that were exposed to triazole ring-labeled [U-^{14}C]tetraconazole [(±)-2-(2,4–dichlorophenyl)-3-(1H-1,2,4-triazol-1-yl)-propyl 1,1,2,2-tetrafluoroethyl ether; radiochemical purity >99.2%] at nominal concentrations of 4 or 40 µg/L for 6 days under

(1)

flow-through aquarium conditions. Maximum bioconcentration factors were 24-31x for the edible tissue, 58-61x for the nonedible tissue, and 39-42x for whole fish. At 4 µg/L, maximum mean concentrations of [^{14}C]residues were 0.103 µg/g in the edible tissue, 0.263 µg/g in the nonedible tissue, and 0.167 µg/g in whole fish after 6 days of exposure. At 40 µg/L, maximum mean concentrations of [^{14}C]residues were 1.429 µg/g in the edible tissue, 2.686 µg/g in the nonedible tissue, and 1.930 µg/g in whole fish after 5 days of exposure. More than 98% of the accumulated [^{14}C]residues were eliminated from fish tissues by day 2 (4 µg/L dose) or day 5 (40 µg/L dose) of the depuration period.

2. This study does not meet Subdivision N Guidelines for the fulfillment of EPA data requirements on the flow-through fish bioconcentration for the following reasons:

   A. Data on the characterization of residues in fish tissue were not provided;

   U. Only one tetraconazole ring was labeled (triazole ring); and

   B. The growth/weight patterns of the fish throughout the study were not provided

   Upon the registrant submission of the metabolic profiling results the study may be upgraded to supplemental.

3. No further information is needed on the flow-through fish bioconcentration study of tetraconazole at the present time.

MATERIALS AND METHODS

Prior to the initiation of treatment, rainbow trout (*Oncorhynchus mykiss*; length 4.8-5.7 cm, weight 1.26-2.17 g) were maintained in filtered tap water (12.9-13.2°C, pH 7.0-7.4, hardness 47.4-52.3 mg/L as $CaCO_3$, dissolved oxygen 73-101% air saturation, residual chlorine 0.01-0.07 mg/L; Appendix 2, p. 55) under a light/dark cycle of 16 hours/8 hours per day for >14 days (p. 17).

Flow-through aquatic exposure systems were prepared using three 122-L glass tanks (control, low dose, and high dose). Each aquarium contained 100 L of filtered tap water (14.3-14.7°C, pH 7.4-7.6, hardness 47.5-49.1 mg/L as $CaCO_3$, dissolved oxygen 86-95% air saturation; Table 2, p. 30). The water was continuously supplied to the tanks at a measured flow rate of 280 mL/minute (~4 turnovers/day). The two exposure tanks were treated at nominal concentrations of 4 or 40 µg/L with triazole ring-labeled [U-^{14}C]tetraconazole [(±)-2-(2,4–dichlorophenyl)-3-(1H-1,2,4-triazol-1-yl)-propyl 1,1,2,2-tetrafluoroethyl ether; radiochemical purity >99.2%, specific activity 5.0552 MBq/mg; Sostram Corp., Batch 144, Roswell, GA; pp. 15, 23], dissolved in methanol. The control tank was treated only with methanol. The test compound (or pesticide-free carrier solvent) was introduced into the exposure water (or control tank) by variable speed peristaltic

pumps (p. 18; diagram Appendix 3, p. 57). Water quality measurements (temperature, dissolved oxygen, pH) were taken daily throughout the study (p. 16).

Rainbow trout (100) were placed into each of the three aquaria. During the study, the fish were fed daily with commercially prepared food (Paul's No. 4 Crumb) at 2% wet body weight per day (p. 18; Appendix 2, p. 54). At approximately 1 hour after feeding, the tanks were cleaned of debris using a siphon tube. The tanks were subjected to a light/dark cycle of 16 hours/8 hours and maintained at approximately 14°C throughout the exposure period.

During the exposure period, water samples were collected from the treated and control aquaria prior to treatment (-0.1 days) and 0.2, 0.4, 0.8, 1.5, 2, 3, 4, 5, and 6 days after the initiation of treatment for the determination of total [^{14}C]residues (pp. 18-19). Four fish were collected from each treated aquarium at 0.2, 0.4, 0.8, 1.5, 3, 4, 5, and 6 days to determine total [^{14}C]residues. Additional fish were collected from each treated aquarium at 5 and 6 days for [^{14}C]residue characterization; characterization data were not submitted in this MRID. Four fish were collected from the control aquarium at the start and end of the exposure period and analyzed for total radioactivity. Upon removal fish were weighed and dissected into edible (body muscle, skin, skeleton) and nonedible (fins, head, abdominal cavity contents) tissues (p. 20). Tissue samples that were not analyzed immediately were stored at approximately -20°C for <3 months (p. 14).

Following the 6-day exposure period, a 10-day depuration period was initiated by transferring the fish to clean tanks pre-filled with pesticide-free tap water (p. 18). Water samples were collected from each aquarium on each day of the depuration period for the determination of total [^{14}C]residues. During depuration, four fish were collected from each treated aquarium at 0.5, 1, 2, 5, and 10 days, and from the control aquarium at 10 days for determination of total [^{14}C]residues. Additional fish were collected from the control and treated aquaria on the last day of the depuration period for [^{14}C]residue characterization.

For each sampling interval, three water samples (1 mL) were analyzed for total radioactivity by LSC (p. 87); the limit of detection was reported as twice background (p. 21). Selected water samples (0, 1 and 6 days) of ~1 L each were collected and analyzed by reverse-phase HPLC under the following column operating conditions (pp. 16, 41):

Column	Spherisorb ODS2, 250 x 4.6 mm, 5µm
Detectors	Radiodetection and UV (254 nm)
Mobile Phase	Phase A - water:trifluroacetic acid (99.8:0.2, v:v); Phase B - methanol:acetonitrile (18:82, v:v)
Gradient	30:70 (v:v, A to B)
Retention Time: Tetraconazole	4-5 minutes

HPLC chromatogram for the radiochemical purity check of the test substance prior to dosing,

(3)

show a retention time for tetraconazole of approximately 4-5 minutes). The single peak observed in treated water samples, accounting for >99% of the radioactivity, coincided with this retention time. Representative chromatograms for treated water samples are depicted in Figures 6 and 7 (pp. 46, 47).

Samples of the fish tissues were macerated with scissors. Duplicate subsamples (80-150 mg) were weighed into glass scintillation vials and solubilized in 1 mL of Soluene®-350 by sonication. After solubilization, the samples were thoroughly mixed with 10 mL of Hionic-Fluor® scintillation fluid and analyzed for total radioactivity by LSC (p. 20).

RESULTS AND DISCUSSION

[^{14}C]Residues accumulated in the tissues of rainbow trout that were exposed to triazole ring-labeled [U-^{14}C]tetraconazole (radiochemical purity >99.2%) at nominal concentrations of 4 and 40 µg/L for 6 days under flow-through aquarium conditions. All data are reported in tetraconazole equivalents.

At 4 µg/L, maximum bioconcentration factors were 24x for the edible tissue, 61x for the nonedible tissue, and 39x for whole fish (Table 6, p. 34). Maximum mean concentrations of [^{14}C]residues were 0.103 µg/g in the edible tissue, 0.263 µg/g in the nonedible tissue, and 0.167 µg/g in whole fish after 6 days of exposure (Table 4, p. 32). By day 1 of the depuration phase, mean accumulated [^{14}C]residues had decreased approximately 95-95%, to 0.004 µg/g in the edible tissue, 0.013 µg/g in nonedible tissue, and 0.008 µg/g in whole fish (Table 4, p. 32). By day 2, [^{14}C]residues were not detected in the edible tissue and 0.004 and 0.002 µg/g in nonedible tissue and whole fish, respectively. [^{14}C]Residues were not detected in any fish samples at 5 days..

At 40 µg/L, maximum bioconcentration factors were 31x for the edible tissue, 58x for the nonedible tissue, and 42x for whole fish (Table 7, p. 35). Maximum mean concentrations of [^{14}C]residues were 1.429 µg/g in the edible tissue, 2.686 µg/g in the nonedible tissue, and 1.930 µg/g in whole fish after 5 days of exposure (Table 5, p. 33). By day 1 of the depuration phase, mean accumulated [^{14}C]residues had decreased approximately 95-96%, to 0.049 µg/g in the edible tissue, 0.126 µg/g in nonedible tissue, and 0.082 µg/g in whole fish (Table 5, p. 33). By day 5, [^{14}C]residues were 0.011 µg/g in the edible tissue, 0.003 µg/g in nonedible tissue, and 0.008 µg/g in whole fish.

The concentration of total [^{14}C]residues (as parent equivalents) in the exposure aquaria water was 3.93-4.63 µg/L (mean 4.29 µg/L) or 41.3-54.8 µg/L (mean 46.0 µg/L; Table 3, p. 31). Greater than 99% of the recovered residues in the aquaria water were identified as tetraconazole during the exposure period (p. 23; Figures 6 and 7, pp. 46-47).

Mortalities were observed at 7 (1 fish), 9 (1 fish), and 11 (5 fish) days at the low concentration, and 3, 4, 5, and 11 (1 fish each) days at the high concentration. None of the surviving fish

exhibited symptoms of toxicity (p. 23). Since the deaths at the highest dosing occurred during expose and deaths in the low dose occurred during depuration, it is possible that the deaths were due to treatment. There were no signs of toxicity or mortalities among the 100 control fish during the study.

During the exposure period, the temperature of the exposure water was 14.3-14.7°C, the dissolved oxygen content was 86-88%, and the pH was 7.4 (Table 2, p. 30).

DEFICIENCIES/DEVIATIONS

1. Data on the characterization of residues in fish tissue were not provided. The study author stated that metabolic profiling was carried out under a separate study number (p. 19). These data should be submitted to the Agency for review.

2. Subdivision N Guidelines require that the exposure period extend for 28 days or until an accumulation plateau is reached. In the subject study, the exposure period was 6 days. However, mean [^{14}C]residues reached an apparent plateau after ~3 days for both exposure concentrations.

3. It was not clear from the study whether tetraconazole was allowed to equilibrate in the aquaria prior to the introduction of the fish.

4. The study was not conducted according to U.S. GLP; however, the study was conducted in accordance with the UK Principles of Good Laboratory Practice, The UK Compliance Programme, (Department of Health, London, 1989), and the OECD Good Laboratory Practice in the Testing of Chemicals (Final Report ISBN 92-64-12367-9, Paris, 1982; p. 3).

5. The bioaccumulation of tetraconazole was studied in rainbow trout. Subdivision N Guidelines specify the use of bluegill sunfish or channel catfish for bioaccumulation in fish studies. Rainbow trout are generally much more sensitive to contaminants than sunfish or catfish. It is likely that the study could have been conducted at a higher dose rate if the more tolerant species had been used.

6. Subdivision N Guidelines state that the concentration of the test substance in the water must not exceed 10% of the 96-hour LC_{50} of the test species, but should be high enough to facilitate chemical identification of residues in fish. The nominal exposure concentrations used in this study, 4 and 40 µg/L, are respectively 1/1200 and 1/120 of the reported 96-hour LC_{50} value for tetraconazole to rainbow trout (4.8 mg/L; p. 15). At the low and high concentrations, 7% and 4% of the fish, respectively, died during the study. However, the deaths among the low dose fish occurred during depuration, while those among the high dose fish occurred during exposure except for one fish that died during

depuration. None of the surviving fish exhibited symptoms of toxicity (p. 23). There were no signs of toxicity or mortalities among the control fish throughout the study.

7. The growth/weight patterns of the fish throughout the study were not provided. The size of the fish used in the study was determined using a sample of ten test fish collected at the start of the exposure period (p. 17). The fish had a mean length of 5.4 cm (range 4.8-5.7 cm) and a mean weight of 1.79 g (range 1.26-2.17 g).

8. Tetraconazole contains more than one ring structure; only the triazole ring was labeled in the current study.

9. Total [^{14}C]residues in whole fish were calculated by the registrant by summing the radioactivity in edible and nonedible tissues, accounting for the weights of the respective tissues (p. 24).

10. The water solubility of tetraconazole was reported as 159 mg/L at 23°C (p. 15). The vapor pressure was reported as 1.4×10^{-4} Pa at 20°C.

(6)