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DATA EVALUATION RECORD

STUDY 5

CHEM 112602 Cimectacarb §165-4

FORMULATION--00--ACTIVE INGREDIENT

STUDY ID 41869543
Fackler, P.H. 1991. CGA-163935 - Flow-through bioconcentration study with bluegill sunfish (Lepomis macrochirus) for metabolite characterization and identification. Laboratory Project ID: SLI Report No. 91-203654; Sponsor Protocol No. 184-90. Unpublished study performed by Springborn Laboratories, Inc., Wareham, MA, and Ciba-Geigy Corporation, Greensboro, NC, and submitted by Ciba-Geigy Corporation, Greensboro, NC.

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CONCLUSIONS:

Laboratory Accumulation - Fish

1. This study provides only supplemental information and cannot be used towards the fulfillment of the data requirement at this time.

2. Cimectacarb residues accumulated in bluegill sunfish exposed to 1.4 ppm of cimectacarb for 14 or 28 days. The maximum bioconcentration factors were 1.9x, 9.9x, and 5.5x for edible, nonedible, and whole

fish, respectively (See Table I). Two degradates identified in the edible and nonedible fish tissues were 4-(cyclopropyl-hydroxy-methylene)-3,5-dioxocyclohexane carboxylic acid (CGA-179500) and 6-cyclopropyl-6-hydroxyl-2-methyl-4-one-hex-2,5-dienoic acid (Peak B).

3. This study is scientifically sound, but does not meet Subdivision N guidelines for the following reasons:

   [^{14}C]Residues in the fish tissues were incompletely characterized. One HPLC peak (Peak A) contained up to 1.6 ppm of radioactivity, but was described only as having been "shown to be composed of several components, including CGA-179500".

   The fish were sampled only twice, after 14 and 28 days of exposure to the [^{14}C]cimectacarb.

   There was no depuration phase.

4. Since the fish were only sampled twice and since there was no depuration period, this study alone is inadequate to provide information on the accumulation of total [^{14}C]cimectacarb residues in fish. However, this study, in conjunction with the bioaccumulation study (Study 4 in this report, MRID 41869542), can be used to fulfill the data requirements for laboratory accumulation in fish if additional information about "Peak A", such as the approximate number of compounds migrating with "Peak A" and their relative concentrations, is provided by the registrant. Any [^{14}C]compounds migrating in "Peak A" and present at $\geq$0.05 ppm must be identified.

METHODOLOGY:

Juvenile bluegill sunfish (Lepomis macrochirus; mean length and weight of 47 mm and 1.25 g, respectively), were held in culture tanks on a 16-hour daylight photoperiod for $\geq$14 days prior to the initiation of the study. During this period, a flow-through aquatic exposure system was prepared using one 500-L aquarium. Aerated well water (pH 6.7-7.0, dissolved oxygen content 73-83% of saturation, total hardness 36-37 mg/L as $CaCO_3$, alkalinity 24-26 mg/L as $CaCO_3$) was provided to the aquarium at a rate of 8.4-9.2 turnovers per day. The water in the aquarium was maintained at 17 C. The fish were fed a dry commercial fish meal, ad libitum, daily except during the 24 hours prior to the initiation of the study.

Bluegill sunfish (1000) were transferred into the aquarium, which was continuously treated at 1.4 ppm with ring-labeled [1,2,6-^{14}C]cimectacarb (radiochemical purity 99.9%, specific activity 65.0 uCi/mg, Ciba-Geigy) plus unlabeled cimectacarb (purity 92.2%) dissolved in acetone. During the study, the fish were fed a dry commercial fish meal twice daily at a total rate of 1.5% of their total biomass, except during the 24 hours prior to sampling. On day 14 of the exposure period, 500 fish were sampled. An additional 500

fish from the holding tank were placed into the aquarium on day 14 of the exposure period, and separated from the existing fish population by a nylon screen basket. On day 28 of the exposure period, all of the fish were removed, and separated into two groups: fish that had been exposed for 28 days and fish that had been exposed for 14 days. Three 5-mL water samples were collected on days 0, 1, 3, 7, 10, 14, 17, 21, and 28 of the exposure period.

Water samples were analyzed for total [^{14}C]residues using LSC. The detection limit was 0.011 ppm. Triplicate aliquots of the 17-day water samples were analyzed by HPLC to verify the concentration of [^{14}C]cimectacarb in the exposure tank. HPLC analysis was conducted using a PRP-1 C_8 column, with a mobile phase of acetonitrile:HPLC grade water:phosphoric acid (80:20:0.1, v:v:v) and UV detection (280 nm). Recovery efficiencies from freshwater samples fortified with 0.505, 1.01, or 10.1 ppm of cimectacarb and analyzed by HPLC ranged from 96.1 to 104% of the applied (Table 1A).

To analyze for total radioactivity in the fish samples, three fish were removed on day 14 and ten fish were removed on day 28 (five exposed for 14 days and five for 28 days). These fish samples were analyzed by Springborn Laboratories (SLI). The fish were dissected into edible (muscle or fillet and bones) and nonedible (heads, fins and viscera) tissues; the tissues were air-dried for $\geq$24 hours and analyzed for total [^{14}C]residues using LSC following combustion. The detection limits varied, ranging from 0.056 to 0.20 ppm for edible tissues and 0.079 to 0.20 ppm for nonedible tissues.

In order to characterize radioactivity in the fish samples, additional fish tissue samples were analyzed by Ciba-Geigy. Samples of the edible and nonedible tissues were homogenized, then extracted and analyzed according to the schemes outlined in Figure 2. The homogenized tissues were extracted with acetonitrile:water (8:2, v:v) by stirring for approximately 30 minutes, and then were centrifuged for 10 minutes. The liquid was decanted and filtered; the filter cake was extracted once (twice for the 28-day edible tissue sample) with acetonitrile:water (8:2, v:v). The filtrates were combined, and aliquots were analyzed by LSC. Additional aliquots of the filtrates were concentrated (method unspecified), then partitioned once with warm hexane. The hexane and aqueous fractions were analyzed for total radioactivity by LSC. The hexane fractions were further analyzed by two-dimensional TLC on silica gel plates developed in toluene:acetone:formic acid (75:25:1, v:v:v) and chloroform:methanol:formic acid:water (75:20:4:2, v:v:v:v). Radioactive zones were located using autoradiography, and reference standards were visualized under UV light. Residues in the sample extracts were further analyzed for cimectacarb and its degradates using GC/MS and FAB/MS. The extracted fish tissues were analyzed for unextracted radioactivity by LSC following combustion.

The aqueous fractions from the 28-day edible and nonedible tissues were used for degradate identification. Aliquots of the nonedible

tissues were concentrated by rotary evaporation and analyzed by HPLC using a Hibar Lichrosorb RP-18 column, with mobile phase gradients of acetonitrile and acidified water and UV (254 nm) and solid flow cell detection. The HPLC methods (Methods 9-13) used for characterization, isolation, and purification of degradates are outlined in Table 1B. The aqueous fraction of the 28-day nonedible tissues was purified by HPLC Method #9; Peaks 1, B, and A were collected individually for further purification. The aqueous fraction from the 28-day edible tissues was used to isolate Peak 3, which was purified by HPLC Method #9. Peaks 1, B, and 3 were purified twice using HPLC Method #9. Peak 1 was further purified using HPLC Method #10, and Peak B was further purified using HPLC Method #11 followed by Method #12. Final purification of Peak 3 was conducted using HPLC Method #13. Peaks 1 and 3 were then partitioned once with chloroform, and the chloroform extracts were analyzed for total radioactivity by LSC, then concentrated for MS analyses. Peaks 1 and 3 were concentrated (method unspecified) and analyzed by GC/MS; Peak B was analyzed by FAB/MS. Additional aliquots of the aqueous fractions were analyzed by two-dimensional TLC as described for the hexane fractions.

DATA SUMMARY:

[^{14}C]Cimectacarb residues accumulated in edible and nonedible tissues of bluegill sunfish that were exposed to [1,2,6-^{14}C]cimectacarb (radiochemical purity 99.9%) at 1.4 ppm for 14 or 28 days. Registrant-calculated bioconcentration factors (BCFs) were 1.3-1.9x for edible tissues, 6.2-9.9x for nonedible tissues, and 3.5-5.5x for whole fish (based on Tables I and II for Springborn Laboratories); BCFs were higher in fish exposed for 14 days than in fish exposed for 28 days. The maximum mean concentrations of total [^{14}C]residues, from fish exposed for the first 14 days of the study, were 2.67 ppm for edible tissues (muscle or fillet and bones), 14.0 ppm for nonedible tissues (head, fins, viscera, and tails), and 7.84 ppm for whole fish (Table II-SLI).

Additional samples were analyzed separately in order to isolate and identify [^{14}C]residues in the fish tissues. Total [^{14}C]residues were 3.02-3.20 and 36.33-59.39 ppm in edible and nonedible fish tissues (viscera only), respectively, and >90% of the recovered residues were extractable with acetonitrile (Table II-Ciba-Geigy). Based on the averaged results from TLC and HPLC analyses, undegraded [^{14}C]cimectacarb (CGA-163935) was 1.50-1.59 and 2.20-2.94 ppm in edible and nonedible tissues, respectively;

> 4-(cyclopropyl-hydroxy-methylene)-3,5-dioxocyclohexane carboxylic acid (CGA-179500)

was 1.17-1.30 and 27.45-46.68 ppm in edible and nonedible tissues, respectively; and

6-cyclopropyl-6-hydroxyl-2-methyl-4-one-hex-2,5-dienoic acid (Peak B)

was 0.04-0.09 and 1.05-1.43 ppm in edible and nonedible tissues, respectively (Tables III-V). HPLC Peak A, which eluted with the solvent front and contained 0.09-0.10 ppm in the edible tissues and 1.24-1.60 ppm in the nonedible tissues, was "shown to be composed of several components, including CGA-179500". Additional uncharacterized extractable [^{14}C]residues ("Others") totaled 0.01-0.59 ppm in the fish tissues (Table IV). Unextracted [^{14}C]residues comprised ≤9.7% of the recovered radioactivity in the edible and nonedible tissues (Table II-Ciba-Geigy).

Throughout the study, the temperature of the water was 17-18 C, the pH ranged from 6.9 to 7.6, and the dissolved oxygen content ranged from 6.3 to 9.2 mg/L during the exposure period. Total [^{14}C]residues in the water, which were identified in the day 17 sample as consisting only of cimectacarb, ranged from 1.23 to 1.61 ppm during the exposure period (Table I).

COMMENTS:

1. Study 4 in this report (MRID 41869542) provided information on the accumulation of total [^{14}C]cimectacarb residues in fish, but because the fish tissue samples partially degraded before residue characterization was initiated, the study could not be used to fulfill data requirements. Therefore, the registrant conducted this study (Study 5, MRID 41869543) to obtain fresh fish tissue samples for metabolite identification; since Studies 4 and 5 were meant to be considered together, the experimental design for this study included only two fish samplings (days 14 and 28) and did not include a depuration period. However, [^{14}C]residues in the fish tissues were incompletely characterized. One HPLC peak (Peak A) contained up to 1.6 ppm of radioactivity, but was described only as having been "shown to be composed of several components, including CGA-179500".

   This study, in conjunction with the bioaccumulation study (Study 4), can be used to fulfill the data requirements for laboratory accumulation in fish if additional information about "Peak A", such as the approximate number of compounds migrating with "Peak A" and their relative concentrations, is provided by the registrant. Any [^{14}C]compounds migrating in "Peak A" and present at ≥0.05 ppm must be identified.

2. [^{14}C]Residues in edible tissues analyzed by Ciba-Geigy were approximately one-third higher than those obtained at SLI. The author suggested that the differences may have been due to different tissue preparation and combustion methods used by the two laboratories. SLI used intact fresh tissues from individual fish that were air dried for ≥24 hours prior to combustion; the radioactivity determinations were not adjusted for recovery. In

contrast, Ciba-Geigy homogenized the frozen tissue samples in granulated dry ice prior to combustion; the radioactivity determinations were corrected for recovery.

In addition, comparison of nonedible tissue concentrations obtained from the two analytical laboratories cannot be made directly. Nonedible tissues were defined by SLI as viscera, heads, fins, and tail, whereas Ciba-Geigy defined nonedible tissues as viscera only.

3. The study author calculated mean steady-state bioconcentration factors by dividing the mean measured equilibrium [^{14}C]concentration for each tissue type by the mean measured water concentration for the entire exposure period. It is more typical to divide the mean measured concentration of [^{14}C]residues in the fish tissue by the mean measured water concentration up to and including the respective sampling day during the exposure period, so that the maximum mean bioconcentration factor for each tissue can be determined.

4. According to the study author, sufficient tissues were obtained from fish exposed for the first 14 days for identification purposes; therefore, the second set of tissues from fish exposed for the second 14 days of the 28-day study were not analyzed by SLI.

5. The detection limits for the water and fish samples varied, and were dependent upon counting efficiency, sample size, and background levels of radiation for the liquid and combusted samples.

6. During the exposure period, the fish were reported to appear healthy and exhibited normal behavior. There were two deaths among a total of 1500 exposed fish.

7. There was no control group of untreated fish, based on the information provided in the methodology of the original document.

8. The metabolic pathway for cimectacarb in bluegill sunfish is provided in Figure 19.

9. The nominal test concentration was approximately 1/100 of the 96-hour LC_{50} value (140 ppm) for bluegill sunfish.

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