DATA EVALUATION RECORD

STUDY 2

CHEM 112701 Brodifacoum §162-1

FORMULATION--00--ACTIVE INGREDIENT

STUDY ID 42579401
Hall, B.E., and I. Priestly. 1992. Brodifacoum: Metabolism in soil under aerobic conditions. Laboratory Project ID: 381441. Unpublished study performed by Inveresk Research International, Tranent, Scotland, and submitted by ICI Americas Inc., Wilmington, DE.

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CONCLUSIONS:

Metabolism - Aerobic Soil

1. This study can be used to estimate the persistence of brodifacoum in soil.

2. Brodifacoum degraded with a half-life of 157 days in sandy clay loam soil incubated in the dark at 21 C and 75% of 0.33 bar moisture capacity. No volatile degradates other than $^{14}CO_2$ were identified; $^{14}CO_2$ comprised 36% of the applied radioactivity at 52 weeks posttreatment.

3. This study is scientifically sound, but up to eleven [^{14}C]compounds other than [^{14}C]brodifacoum were isolated from the soil extracts at 2.07 to 17.34% of the applied (0.008 to 0.067 ppm), but were not identified.

METHODOLOGY:

Sieved (2 mm) sandy clay loam soil (63.1% sand, 16.5% silt, 20.4% clay, 4.24% organic matter, pH 7.1, CEC 13.56 meq/100 g), was weighed (50 g dry weight) into 250-mL Erlenmeyer flasks and moistened with deionized water to 75% of 0.33 bar. After 5 days of acclimatization, the soil was treated at 0.38 ppm with [^{14}C]brodifacoum (3-[3-(4'-bromobiphenyl-4-yl)-1,2,3,4-tetrahydro-1-naphthyl]-4-hydroxycoumarin; uniformly labeled in the phenyl ring of the hydroxycoumarin moiety; radiochemical purity 98.2%, specific activity 45.86 uCi/mg, ICI), dissolved in acetonitrile. The soils were mixed by tumbling, then the sample flasks were connected to a continuous air-flow system. Humidified, CO_2-free air was drawn through separate flasks, and the gases leaving the flasks were combined and passed sequentially through a polyurethane foam plug and ethanediol, 0.5 M sulfuric acid, and ethanolamine trapping solutions. The samples were incubated in the dark at 19-22.5 C, and the moisture content of the soils was maintained at 75% of 0.33 bar for the duration of the experiment. The foam plugs and trapping solutions were replaced weekly for the first 8 weeks, then either biweekly or when samples were removed for analysis through 52 weeks. Duplicate flasks of soil were collected for analysis immediately posttreatment; at 3, 7, 14, and 28 days; and at 8, 13, 17, 26, 39, and 52 weeks.

The soil samples were extracted twice with methylene chloride:-methanol (4:1; v:v), first by shaking overnight on an orbital shaker, then for 2-3 hours using a wrist-action shaker. Following each extraction, the slurries were centrifuged and the supernatant removed. Portions of the extracted soils were analyzed for unextracted [^{14}C]residues using LSC following combustion. Aliquots of the individual extracts from the 0-, 3-, and 7-day posttreatment samples were analyzed for total radioactivity using LSC; the two extracts from each sample were then combined for further analysis. For samples from later intervals, the two extracts were pooled into a single sample prior to analysis using LSC. Aliquots of the combined extracts were evaporated to dryness under a stream of nitrogen, and the resulting residues were redissolved in acetone and analyzed using one-dimensional TLC on silica gel plates developed in chloroform (100%; Solvent System 1), dioxan:petroleum ether (30:70, v:v; Solvent System 2), or toluene:propan-2-ol:acetic acid (9:1:1, v:v:v; Solvent System 3). All sample extracts were analyzed using Solvent System 1; extracts of samples from 0 days through 17 weeks were analyzed using Solvent System 2; and extracts of samples from 28 days and 17 through 52 weeks were analyzed using Solvent System 3. [^{14}C]Residues on the plates were located and quantified using a linear scanner, and were identified by comparison to the location of unlabeled brodifacoum and 4-hydroxycoumarin reference standards that had been cochromatographed with the samples and located using UV (254 nm) detection.

After the soil samples were removed, the incubation flasks were rinsed with acetone and the rinsate was analyzed by LSC. The polyurethane foam plugs were rinsed with acetonitrile, and the

rinsates were analyzed for total radioactivity using LSC. Aliquots of the trapping solutions were analyzed using LSC.

DATA SUMMARY:

[^{14}C]Brodifacoum (3-[3-(4'-bromobiphenyl-4-yl)-1,2,3,4-tetrahydro-1-naphthyl]-4-hydroxycoumarin; uniformly labeled in the phenyl ring of the hydroxycoumarin moiety; radiochemical purity 98.2%), at 0.38 ppm, degraded with a registrant-calculated half-life of 157 days (22.5 weeks) in silty clay loam soil that was incubated in the dark at 21 $\pm$ 2 C and moistened to 0.75% of 0.33 bar for 1 year. In duplicate samples, [^{14}C]brodifacoum declined from 88.77-97.48% of the applied at 0 days posttreatment to 54.38-56.13% at 17 weeks, 38.60-40.23% at 26 weeks, and 15.96-17.91% at 52 weeks (sum of cis and trans isomers in Table 2; TLC with chloroform solvent). The trans isomer of [^{14}C]brodifacoum degraded more rapidly than the cis isomer; the ratio of cis- to trans-[^{14}C]brodifacoum changed from 41:52 immediately posttreatment to 12:5 at 52 weeks.

Using three different TLC solvent systems, possibly eleven different [^{14}C]compounds were isolated from soil extracts but were not identified. Using chloroform as the solvent, degradate "A" (R_f 0.05) was a maximum of 3.94% of the applied; degradate "B" (R_f 0.09) was a maximum of 3.50%; degradate "C" (R_f 0.20) was a maximum of 2.07%; degradate "D" (R_f 0.43) was a maximum of 7.72%; and degradate "E" (R_f 0.73) was a maximum of 16.07% (Table 2). Using dioxan:petroleum ether (30:70) as the solvent, degradate "F" (R_f 0.30) was a maximum of 8.38% of the applied; degradate "G" (R_f 0.35) was a maximum of 4.33%; degradate "H" (R_f 0.44) was a maximum of 3.92%; and degradate "I" (R_f 0.10) was a maximum of 4.51% (Table 3). Using toluene:-propan-2-ol:acetic acid (9:1:1) as the solvent, degradate "J" (R_f 0.52) was a maximum of 3.77% of the applied and degradate "K" (R_f 0.69) was a maximum of 17.34% (Table 4).

Unextracted [^{14}C]residues in the soil increased from 2.60-3.91% of the applied immediately posttreatment to 11.12-13.00% at 28 days and a maximum of 23.29-30.15% at 39 weeks (Table 1). $^{14}CO_2$ was 21.41% of the applied at 26 weeks and 35.80% at 52 weeks; other volatile [^{14}C]compounds totaled 1.85% of the applied at 52 weeks. During the study, material balances ranged from 96.63 to 108.88% of the applied.

COMMENTS:

1. Using three different TLC solvent systems, eleven [^{14}C]compounds other than [^{14}C]brodifacoum were isolated from the soil extracts at 2.07 to 17.34% of the applied (0.008 to 0.067 ppm, respectively). No attempt was made to identify the compounds that were isolated; therefore it was uncertain if the same compounds were isolated in

more than one solvent system; the study authors suggested that degradates "E" and "K" may be the same compound. The only degradate for which a reference standard was cochromatographed was 4-hydroxycoumarin, which was never isolated. Subdivision N guidelines specify that degradates present at $\geq$0.01 ppm should be identified.

2. The degradates were not identified and a degradation pathway was not established. Depending on the proposed degradation pathway, additional information may be required using brodifacoum in which other portions of the molecule carry the radiolabel.

2. Radioactivity in the ethanolamine traps was assumed to be $^{14}CO_2$; it did not appear that confirmatory techniques such as precipitation with barium chloride were performed.

3. In an attempt to extract additional radioactivity from the soil, portions of methylene chloride:methanol-extracted soil from the 8-, 13-, and 17-week sampling intervals were extracted once with methanol and twice with methanol:water, each time by shaking overnight on a wrist-action shaker. The extracts were analyzed for total radioactivity using LSC. An additional 1.20-2.31% of the applied was extracted with methanol, and an additional 0.77-1.34% was extracted with methanol:water.

4. In an ancillary experiment to determine microbial viability, additional flasks of soil were either treated with unlabeled brodifacoum or were left untreated. These samples were incubated with the soil treated with [^{14}C]brodifacoum. The populations of microbes in the soil were measured at 26 and 53 weeks posttreatment, and no significant difference was observed between the treated and untreated soils.

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