DATA EVALUATION RECORD

STUDY 3

CHEM 112701 Brodifacoum §163-1

FORMULATION--00--ACTIVE INGREDIENT

STUDY ID 42568301
Jackson, R., and B.E. Hall. 1992. Aged soil leaching of [^{14}C]brodifacoum. Laboratory Project ID: 381986. Unpublished study performed by Inveresk Research International, Tranent, Scotland, and submitted by ICI Americas Inc., Wilmington, DE.

DIRECT REVIEW TIME = 9

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CONCLUSIONS:

Mobility - Leaching and Adsorption/Desorption

1. This study can be used towards the fulfillment of data requirements.

2. Based on column leaching experiments, aged (30 days) brodifacoum residues (89-97% as brodifacoum) were relatively immobile in columns of sand, sandy clay loam, silty clay, or clay soils that were leached with 20 inches of 0.01 M calcium chloride solution. Following leaching, 78.81-94.87% of the applied radioactivity remained in the layer of aged soil and ≤0.32% was recovered in the leachate. No degradates were identified in the soil or leachate.

3. This study is acceptable and partially fulfills EPA Data Requirements for Registering Pesticides by providing information on the mobility (column leaching) of ring-labeled brodifacoum residues in columns of sand, sandy clay loam, silty clay, and clay soils. However, because so little degradation was observed, this study will be considered as fulfilling the unaged mobility data requirement. Additional data will

be needed on the mobility of brodifacoum degradates in order to satisfy the aged mobility data requirement.

4. No additional information on the mobility of aged ring-labeled [^{14}C]brodifacoum residues is required at this time. Additional data on the mobility of aged residues may be required upon the receipt of an acceptable aerobic metabolism study in which degradates of [^{14}C]brodifacoum are identified.

METHODOLOGY:

Sieved (2 mm), moistened (75% of field capacity) clay, silty clay, sandy clay loam, and sand soils from Great Britain (Appendix 5) were weighed (50 g dry weight) into Erlenmeyer flasks and treated with approximately 21 ug (0.41-0.43 ppm) of [^{14}C]brodifacoum (3-[3-(4'-bromobiphenyl-4-yl)-1,2,3,4-tetrahydro-1-naphthyl]-4-hydroxycoumarin; labeled in the phenyl ring of the hydroxycoumarin moiety; radiochemical purity >98.09%, specific activity 45.86 uCi/mg, ICI), dissolved in acetonitrile. The flasks were connected to individual continuous air-flow systems; humidified, CO_2-free air was drawn through a flask, then through two tubes of ethanolamine trapping solution. The samples were incubated in the dark at 21 $\pm$ 2 C and moistened to 75% of 0.3 bar for 30 days. The trapping solutions were collected and replaced with fresh medium at various intervals during the incubation.

Subsamples of the aged treated soils were extracted twice by shaking with methylene chloride:methanol (4:1; v:v). Portions of the extracted soils were analyzed for unextracted radioactivity using LSC following combustion. An aqueous layer that formed during the first methylene chloride:methanol extraction was analyzed using LSC. The two methylene chloride:methanol extracts were combined, and aliquots were analyzed using LSC. Additional aliquots of the methylene chloride:methanol extracts were evaporated to dryness under a stream of nitrogen, and the resulting residues were redissolved in acetone. Aliquots of the acetone solutions were analyzed for total radioactivity using LSC and for specific compounds using one-dimensional TLC on silica gel plates developed in chloroform (100%). [^{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. In order to confirm the results of the TLC analysis, aliquots of the soil extracts were further analyzed by HPLC using an Apex Silica 5 um column with a mobile phase of hexane:methylene chloride:acetic acid (75:25:0.6, v:v:v), and with UV (254 nm) and radioactivity detection. [^{14}C]Residues in the HPLC eluate were quantified by LSC and identified by comparison of the retention times of nonradiolabeled cis- and trans-brodifacoum.

Duplicate glass columns (5-cm id, 42-cm height) were packed to a height of 30 cm with untreated, air-dried, sieved (2 mm) soils; the columns were "agitated gently" during packing. The soil in each column was saturated from the bottom with a 0.01 M calcium chloride solution. Portions of the aged, treated soil was then transferred to the top of the columns of the corresponding soil type. The soil columns were leached with 20 inches (1000 mL) of a 0.01 M calcium chloride solution in 48 hours; a Hoffman clip located at the bottom of each column controlled infiltration rate, and a layer of solution constantly covered the upper soil column surface throughout the leaching period. The leachate was collected continuously in amber glass containers. Following leaching, the surface segment (the layer of aged soil) of each soil column was removed and the remainder of the column was divided into 5-cm segments.

Aliquots of the column leachates were analyzed using LSC. The soil segments were mixed, and subsamples were analyzed by LSC following combustion. The surface segment from the clay, silty clay, and sandy clay loam soil columns, and the top two segments from the sand soil columns were extracted twice by shaking with methylene chloride:-methanol (4:1; v:v), and the extracts were analyzed using LSC, TLC, and HPLC as previously described for the aged treated soil. Portions of the extracted soils were analyzed using LSC following combustion.

Aliquots of the trapping solutions used during aging were analyzed using LSC. The incubation flasks used during aging and the glass columns used during leaching were rinsed with acetone, and the rinsates were analyzed by LSC.

DATA SUMMARY:

Aged [^{14}C]brodifacoum residues were relatively immobile in columns (approximately 30-cm length) of British sand, sandy clay loam, silty clay, and clay soils that were topped with approximately 21 ug of aged [^{14}C]residues (approximately 89-97% as brodifacoum; Table 6) and leached with 20 inches of a 0.01 M calcium chloride solution during a 48-hour period. Following leaching, 78.81-96.87% of the applied radioactivity remained in the layer of aged soil and $\leq$0.32% was recovered in the leachate (Table 2). The majority of the [^{14}C]residues in the leached aged soil layer was [^{14}C]brodifacoum; two other [^{14}C]compounds, degradates "A" and "B", were present at up to 2.80 and 7.89% of the applied but were not identified (Table 7). Prior to use in the leaching experiments, the soils had been treated at 0.41-0.43 ppm with [^{14}C]brodifacoum (3-[3-(4'-bromobiphenyl-4-yl)-1,2,3,4-tetrahydro-1-naphthyl]-4-hydroxycoumarin; labeled in the phenyl ring of the hydroxycoumarin moiety; radiochemical purity >98.09%) and incubated in the dark at 21 $\pm$ 2 C and moistened to 75% of 0.3 bar for 30 days.

In duplicate sand soil columns, 79.47 and 94.86% of the applied radioactivity remained in the top soil segment, 2.00-4.73% was in the

5-cm segment directly below the layer of aged soil, and ≤0.73% was in each of the deeper 5-cm segments (Table 4). All [^{14}C]residues in the top soil segment were cis- or trans-[^{14}C]brodifacoum, at a ratio of approximately 41:51 (Table 7). [^{14}C]Residues in the leachate were 0.24-0.32% of the applied, and an additional 0.30-0.96% was recovered in an acetone wash of the glass column walls (Table 2). The material balance for the columns was 89.33 and 99.59% of the applied.

In duplicate sandy clay loam soil columns, 85.76 and 85.97% of the applied remained in the top soil segment, 0.14-0.52% was in the 5-cm segment directly below the layer of aged soil, and ≤0.77% was in each of the deeper 5-cm segments (Table 4). In the top soil segment, cis- and trans-[^{14}C]brodifacoum totaled 87.92-92.24% of the recovered (ratio approximately 51:39), degradate "A" was 1.50-2.80%, and degradate "B" was 3.44-4.33% (Table 7). [^{14}C]Residues in the leachate were 0.03-0.05% of the applied, and an additional 0.09-0.17% was recovered in an acetone wash of the glass column walls (Table 2). The material balance for the columns was 91.64 and 91.75% of the applied.

In duplicate silty clay soil columns, 78.45 and 86.44% of the applied remained in the top soil segment, 0.15-0.17% was in the 5-cm segment directly below the layer of aged soil, and ≤0.10% was in each of the deeper 5-cm segments (Table 4). In the top soil segment, cis- and trans-[^{14}C]brodifacoum totaled 83.79-88.44% of the recovered (ratio approximately 48:38), degradate "A" was 2.48-2.57%, and degradate "B" was 2.52-7.89% (Table 7). [^{14}C]Residues in the leachate were 0.13-0.21% of the applied, and an additional 0.22-0.25% was recovered in an acetone wash of the glass column walls (Table 2). The material balance for the columns was 84.87 and 92.87% of the applied.

In clay soil columns, 79.19 and 83.65% of the applied remained in the top soil segment, 0.25-0.65% was in the 5-cm segment directly below the layer of aged soil, and, with the exception of 0.97% in the 10- to 15-cm deep segment, ≤0.17% was in each of the deeper 5-cm segments (Table 4). In the top soil segment, cis- and trans-[^{14}C]brodifacoum totaled 91.09-91.62% of the recovered (ratio approximately 44:47; Table 7). Degradates "A" and "B" were 1.96 and 3.15% of the recovered, respectively, in the top soil segment from one column and were not detected in the second column. [^{14}C]Residues in the leachate were 0.07-0.14% of the applied, and an additional 0.21-0.52% was recovered in an acetone wash of the glass column walls (Table 2). The material balance for the columns was 85.09 and 87.24% of the applied.

Following 30 days of aging and prior to leaching, undegraded [^{14}C]brodifacoum was 96.79-96.96% of the recovered in the sand soil, 91.16-92.27% in the sandy clay loam soil, 88.66-88.91% in the silty clay soil, and 91.21-91.64% in the clay soil (Table 6). Three degradates, "A", "B", and "C", were 1.20-2.85%, 2.70-5.26%, and 0.91-1.81% of the recovered, respectively, in the sandy clay loam, silty clay, and clay soils, but were not identified. No degradates were

recovered from aged sand soil. At 30 days, an average 3.16% of the applied could not be extracted from the sand soil, 7.38-8.57% could not be extracted from the sandy clay loam and clay soils, and 11.76% could not be extracted from the silty clay soil (Table 1). Up to 5.53% of the applied was volatilized from the soil by 30 days (Table 1).

COMMENTS:

1. The study was carried out in British soil. Three of the four soils had organic matter contents considerably in excess of typical U.S. agricultural soils (4.1-5.5% organic matter). However, the Barassie sand contained only 0.54% organic matter. In all cases, the aged brodifacoum appeared to be immobile. Due to the consistency of these results, no additional aged column leaching studies in U.S. soils will be required at this time. However, all future soil mobility studies of brodifacoum must be carried out in U.S. soils.

2. Three unidentified [^{14}C]degradates, designated "A", "B", and "C", were isolated from the aged soil prior to leaching at $\leq$0.02 ppm. Degradates "A" and "B" were also isolated in the soil columns after leaching at maximums of 2.80% and 7.89% of the applied (0.01 and 0.03 ppm), respectively. Although the concentrations of these compounds in the aged and leached soil may be too low to permit accurate identification, the R_f values of "A" and "B" on silica gel TLC plates developed with chloroform corresponds to the R_f values of compounds designated "C" and "A" in the aerobic soil metabolism study (MRID 42579401; Study 1 of this submission). The identification of "C" and "A" (and up to 7 other compounds) has been required prior to fulfillment of the aerobic soil metabolism data requirement.

3. When additional degradates are identified in the aerobic soil metabolism study, mobility data may be required for any significant degradates not evaluated in this study.

4. Variable trace amounts of radioactivity were found throughout the segments of the leached columns and in the leachates. Most of these data values were derived from measurements which were <10 dpm above background counts; therefore, their accuracy is uncertain.

5. Extracts of the leached samples and the aged soil samples were analyzed by HPLC. The HPLC analysis was not satisfactory since no degradates were recovered and all radioactivity was attributed to brodifacoum.

6. Six 50-g samples of each soil type were treated with brodifacoum and aged. After 30 days incubation, two samples were extracted to determine residues after aging and two samples were incubated further to measure volatiles for the extra 2 days of leaching. Two samples were used to determine leaching and the apparently the entire sample (50 g) was placed on the top of the untreated columns.

7. The study author states that for leachates "the limit of reliable determination" was 0.36% of the applied radioactivity for the columns of sandy clay loam, silty clay, and clay soils, and 0.34% of the applied radioactivity for columns of sand soil assuming 250 mL of leachate per fraction and a detection limit of 30 dpm per mL solution.

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