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DATA EVALUATION RECORD

STUDY 6

CHEM 128101 RH-5287 §165-4

FORMULATION--00--ACTIVE INGREDIENT

STUDY ID 41845006
Derbyshire, R.L., A. Jacobson, M.L. O'Dowd, and M.A. Santangelo. 1991. Metabolism of RH-5287 in bluegill sunfish. Rohm and Haas Technical Report No. 34-90-71. Unpublished study performed and submitted by Rohm and Haas Company, Spring House, PA.

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CONCLUSIONS:

Laboratory Accumulation - Fish

The fish bioaccumulation data requirement is satisfied with the data provided in this submission in conjunction with study acc. #265912 reviewed on 12/3/87.

This document was submitted by the registrant to provide additional information on the [^{14}C]residues that were detected in bluegill sunfish during 28 days of exposure to RH-5287. The concentrations of total [^{14}C]residues in the edible and nonedible tissues from fish exposed at approximately 1 ppb were reported in the document "Uptake, depuration, and bioconcentration of ^{14}C RH-5287 by bluegill sunfish (Lepomis macrochirus)", Forbis et al., EPA Acc. No. 265912); however,

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the [^{14}C]residues could not be identified because of the low dosing concentration and the small amount of tissue generated. The original study was accepted by EFGWB because the exposure concentration (1 ppb) represented actual use conditions; the registrant has, however, repeated the study using an exposure concentration of 0.99 ppb.

In order to characterize residues in the nonedible tissues, samples were extracted according to the scheme outlined in Figure 1. Samples (5 g) were extracted three times with water and once with methanol; the samples were centrifuged and the supernatants removed after each extraction. The methanol extract and the extracted tissues were analyzed for total radioactivity by LSC and LSC following combustion, respectively. The three aqueous extracts were combined, then treated with trichloroacetic acid (TCA). The resulting solution was centrifuged to remove precipitates. The supernatant was decanted, hydrolyzed with acid, and partitioned with ethyl acetate. Aliquots of the ethyl acetate extract were analyzed by LSC, HPLC, or TLC. HPLC analysis was conducted using three solvent systems of water:methanol with linear gradients of 50:50 to 5:95 or 95:5 to 5:95 and UV detection. TLC was conducted on silica gel plates (solvent system unspecified). Radioactive zones were located using a proportional scanner and quantified by scraping the silica gel from the TLC plates, eluting with methanol, and counting. The aqueous extract was analyzed for total radioactivity by LSC. The TCA-precipitated pellet was hydrolyzed with either acid or selective proteases followed by amino acid analysis, or treated with sulfhydryl reducing agent (dithiotreitol).

In order to characterize residues in the edible fish tissues, samples were analyzed according to the scheme outlined in Figure 2. Portions (3 g) of the fish tissue were extracted three times with water and once with methanol; the samples were centrifuged and the supernatants decanted after each extraction. The methanol extract and portions of the extracted tissues were analyzed using LSC and LSC following combustion, respectively. Additional portions of the extracted tissues were digested with selective enzymes, and analyzed for total radioactivity by LSC following combustion. The combined water extracts were either treated with 10% trichloroacetic acid (TCA) or subjected to freezing/thawing, and refrigerated overnight. Molecular weight determinations were made on the resulting aqueous extract by filtering through centrifugal size exclusion membranes or dialysis followed by the addition of a sulfhydryl reducing agent, dithiotreitol. The resulting dialysate and retentate were analyzed for total radioactivity by LSC and LSC following combustion, respectively. The TCA-precipitated pellet was subjected to enzyme hydrolysis, and the unextracted residues were analyzed for total radioactivity by LSC following combustion. Following enzyme hydrolysis, methanol was added to the protease soluble residue, the solution was centrifuged, and the soluble residue decanted. The methanolic solution was evaporated, water was added, and the resulting mixture was centrifuged and partitioned with ethyl ether.

The ethyl ether extract was analyzed for total radioactivity by LSC and the aqueous fraction was analyzed for amino acids and then for total radioactivity by LSC.

[^{14}C]RH-5287 and its degradates were not detected in the edible and nonedible tissues of bluegill sunfish exposed to 0.99 ppm of [^{14}C]RH-5287 in a flow-through aquarium for 28 days. In the edible fish tissues, 35.5% of the radioactivity present in the fish tissue was extracted with water (17.8% as protein and 12.9% as TCA-soluble), 9.6% was extracted with methanol, and 47.0% was not extracted (Table II). In contrast, in the nonedible tissues, 82.4% of the recovered radioactivity was extracted with water (60.6% as protein and 29.9% as TCA-soluble), 4.7% was extracted with methanol, and 15.9% was not extracted. The TCA-soluble [^{14}C]residues from the nonedible tissues were hydrolyzed, then partitioned with ethyl acetate; approximately 50% of the initial hydrolysate activity partitioned into ethyl acetate and 50% remained in the aqueous fraction (residues not further characterized; Table VII). Based on amino acid analysis and the addition of a sulfhydryl reducing agent (dithiotreitol) to the TCA-precipitated protein, the study authors stated that a significant proportion of the radioactivity involved a disulfide bond (R_1-S-S-R_2) that is most likely a cysteine adduct, as demonstrated in nonedible tissues (Figure 4). The authors concluded that the metabolism of RH-5287 in bluegill sunfish involves cleavage of the isothiazone ring, biologically or by an electrophilic or nucleophilic reaction, the formation of amino acid adducts, and incorporation into protein.

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