2-5-93

DATA EVALUATION RECORD

STUDY 2

CHEM 128101 RH-5287 §162-3

FORMULATION--00--ACTIVE INGREDIENT

STUDY ID 41845002
Lawrence, L.J., B. Lawrence, and A. Kesterson. 1991. Anaerobic aquatic metabolism of $^{13/14}$CRH-5287. Laboratory Project ID: PTRL Report No. 1313, PTRL Project No. 295, Rohm and Haas Technical Report No. 34-91-06. Unpublished study performed by PTRL East, Inc., Richmond, KY, and submitted by Rohm and Haas Company, Spring House, PA.

Kesterson, A. and Atkins, R. 1992. Supplemental Study to the Anaerobic Aquatic Metabolism of $^{13/14}$CRH-5287. Performed by PTRL-East, Richmond, KY; Completed 5/19/92.; PTRL Project 295 and 1313. Submitted by Rohm and Haas Company, Spring House, PA; Rohm and Haas Project No. 34-92-46.
MRID 42341101

DIRECT REVIEW TIME = 10

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CONCLUSIONS:

This study is not acceptable at this time and, therefore, cannot be used to satisfy 162-3 data requirements.

In order for the registrant to satisfy the anaerobic aquatic metabolism data requirement, additional information should be provided to support the "time 0" concentration of RH-5287 [such as

(1)

the purity of the test substance just prior to use, the storage stability of RH-5287, and the stability of RH-5287 (not just extraction efficiencies) in a sterile seawater:sediment system]. The registrant is also requested to address the comments in the COMMENTS section.

Summary of reported results

According to the reported results, RH-5287 rapidly degraded in a silt loam sediment/seawater system incubated under anaerobic conditions. The estimated half-life is less than 1-hour (at the first sampling interval at 1-hour posttreatment, <3% of the applied radioactivity was parent RH-5286). Data indicate that degradation products tend to partition (bind) strongly onto the sediment phase.

The degradates bound to the sediment were hard to extract (a double soxhlet extraction scheme released only 10-40% of the applied radioactivity) and of identify; they were characterized by their chromatographic behavior (polarity). The polar metabolites with retention times of 4 and 7 min have been assigned to compounds in which the ring is cleaved at the N-S bond. The two metabolites with retention time of 4 min were associated with n-octyl malonamic acid and n-octyloxamic acid (i.e., ring-opened structures in which the terminal methyl group transformed to a carboxylic acid group. The metabolites with retention times of 7 min were associated with species having a terminal aldehyde, alcohol or a beta-ketone.

Further work (supplemental study, MRID 42341101) showed that the "bound residues" (i.e., those that could not be extracted by the double soxlet extraction) could not be liberated by rigorous and exhaustive extraction procedures, indicating that the bound material would not be available for bioaccumulation.

The rapid degradation under anaerobic conditions was attributed to the large concentrations of reduced nucleophiles (H_2S/HS^- or -S-S-) and electrophiles that are likely present on such environment, which in turn catalyze the cleavage of the labile -N-S- bond.

METHODOLOGY:

Silt loam aquatic sediment from the York River (consisting of 20 g of solids and 34.1 g of seawater) and seawater (66 mL) were added to foil-wrapped sterile flasks equipped with stopcock inlet and outlet tubes (Tables I and II). Both sediment and seawater were collected from the York River, Virginia (specific site not reported), and the seawater was purged of oxygen prior to use by having nitrogen bubbled through it for 1 hour. The soils were fortified with 1.62 g glucose, and the interior of each flask was flushed with nitrogen. The flasks were sealed with ground-glass stoppers and placed in darkened incubators at 22.0- 27.5 C for 30 days.

Ring-labeled [^{14}C]RH-5287 (labeled in the 3-one position, radiochemical purity 99.8%, specific activity 55.39 mCi/g, Rohm and Haas), dissolved in acetonitrile, was added to the flasks at nominal rates of 0.05 or 1.0 ug/mL. The flasks were purged with nitrogen, stoppered, and incubated in the dark at 25.3 $\pm$ 0.4 C. Duplicate flasks were removed for analysis at 0, 1, 5, 7, 14, 29, 61, 90, 120, 180, 270, and 365 days posttreatment. At each sampling interval, the pH, Eh, and dissolved oxygen of the sampled flasks were measured. In addition, a control flask prepared and maintained in a manner identical to the study flasks was tested for pH, Eh, and dissolved oxygen at biweekly intervals.

At regular intervals, the headspaces of the flasks were flushed with nitrogen gas in order to trap volatiles in ethylene glycol and 10% sodium hydroxide solutions (Figure 1). The sample flasks were filled with nitrogen before being resealed. Aliquots of the trapping solutions were assayed for total radioactivity using LSC.

Samples were transferred into polypropylene bottles and centrifuged. Aliquots of the supernatant were analyzed by LSC; the remainder was stored at 4 C. Sediment was stored at -20 C until it was transferred to amber jars and mixed with anhydrous sodium sulfate and Quso G35 (a synthetic amorphous precipitated silica). The mixture was stored at -70 C for approximately 24 hours. The sampling flasks and centrifuge bottles were rinsed with methanol, and the rinsates were radioassayed.

Subsamples of the sediment:sodium sulfate:Quso mixture were mixed to homogeneity in a Waring blender, transferred to a Soxhlet apparatus, and extracted with methylene chloride:methanol (9:1, v:v) for approximately 48 hours. The extraction solvent was removed, radioassayed, and rotary evaporated to 3 mL. Extractions of the 1.00 ug/mL treatment were stored in foil-wrapped vials at 4 C prior to analysis by HPLC; extractions of the 0.05 ug/mL treatment were transferred to foil-wrapped vials, evaporated to dryness under nitrogen, resuspended in methanol:water (75:25, v:v), and stored at approximately 4 C until analysis by HPLC.

Subsamples of the sediment mixture were then Soxhlet extracted for approximately 24 hours. Following extraction, the methanol solvent was removed, radioassayed, and prepared for HPLC in the same manner as the methylene chloride:methanol extracts. The twice-extracted sediment was air-dried and mixed, and subsamples were analyzed by LSC following combustion.

The samples were analyzed with HPLC using a LC-18 reverse-phase column with a mobile phase of methanol:water, and with radioactivity and UV (220 nm) detection. Retention times of unknowns were compared to those of reference standards of RH-5287, RH-0244HQ, RH-0245F, RH-0206C, RH-0247R, RH-287 sulfoxide, RH-085 sulfoxide, RH-6810E, RH-16801, RH-893 sulfoxide, SW87-7564, OCPA, RH-893HQ, RH-893-I, [^{14}C]RH-893HQ, malonamic acid, malonamide, and malonic acid.

Exhaustive extraction (acid, 0.25 N HCl; base, 1 N NaOH) of sediments containing 21 and 42.6% applied radioactivity after soxlet extraction (Day 0 and Day 365, respectively) was performed at a later date, as described in the attached copies of original report.

DATA SUMMARY:

Ring-labeled [^{14}C]RH-5287 (labeled in the 3-one position, radiochemical purity 99.8%), at 0.05 and 1.0 ug/mL, appeared to degrade with a half-life of <1 hour in seawater-flooded silt loam aquatic sediment that was incubated under nitrogen in the dark at 25.3 $\pm$ 0.4 C. Based primarily on HPLC analysis of the sediment extracts and knowledge of the degradation pathway, the study authors characterized the major degradates of RH-5287 as open-ring structures resulting from breaking the N-S bond; however, no degradates other than CO_2 were conclusively identified. Material balances were 66.1-111.3% and 66.3-98.4% of the applied in the slurries treated at 0.05 and 1.0 ug/mL, respectively (Tables VI-VII).

In sediment:seawater slurries treated at 0.05 ug/mL, [^{14}C]residues in the seawater were 3.4-12% of the applied (maximum day 14), extractable [^{14}C]residues in the sediment were 5.5-37.9% (maximum day 14), and unextractable [^{14}C]residues in sediment were 37.1-74.4% (Table VI). Volatilized CO_2 totaled 9.8% of the applied on day 270, and organic volatiles were $\leq$1.0% throughout the study. Only [^{14}C]residues extracted from the sediment were characterized. In duplicate samples, RH-5287 was 1.7 and 2.2% of the applied at day 0, 4.8% in one sample at day 14, 2.6% in one sample at day 61; it was not detected at any other sampling interval (Table VIII). [^{14}C]Degradates, characterized as being more or less polar than RH-893, comprised 0.3-26.9% of the applied.

In sediment:seawater slurries treated at 1.0 ug/mL, [^{14}C]residues in the seawater were 3.2-10.8% of applied (maximum day 14), extractable [^{14}C]residues in the sediment were 17.5-43.2% (maximum day 1), and unextractable [^{14}C]residues in sediment were 21.0-61.1% (Table VII). Volatilized CO_2 was a maximum of 9.3% of the applied on day 365, and organic volatiles were $\leq$0.3% throughout the study. Only [^{14}C]residues extracted from the sediment were characterized. In duplicate samples, RH-5287 was 1.6 and 2.8% of the applied at day 0, and 0.1% in one sample at day 1; it was not detected at any other sampling interval (Table IX). [^{14}C]Degradates, characterized as being more or less polar than RH-893, comprised 1.2-26.3% of the applied (Table IX).

Exhaustive extraction showed that the bound residues were strongly bound and not available for bioaccumulation.

COMMENTS:

1. The study authors did not provide conclusive evidence that the rapid breakdown of RH-5287 was the result of metabolism in aquatic systems.

They assumed it was biological because: a) the extraction efficiency of autoclaved sediment was greater than that of nonsterile sediment; b) the extraction efficiency of the chemical from a spiked sediment/sulfate/silica mixture was greater than that from sediment alone; c) RH-5287 is stable in refrigerated acetonitrile; d) a similar half-life (<1 hour) was calculated for aerobic metabolism in seawater and; e) the photolytic breakdown of RH-5287 in distilled water had a half-life of >9 days. However, in none of these "proofs" was the biological activity in a seawater:sediment system eliminated prior to treatment and RH-5287 (not just extraction efficiency) shown to be relatively stable.

2. Dissolved oxygen in sample flasks ranged from 0.3-1.4 mg/L in sample flasks over the 365 days posttreatment, and Eh varied from -007 to -394. The pH of samples ranged from 5.3-8.6 (Table IV). The flask used for biweekly oxygen sampling had oxygen levels up to 3.2 mg/L. The maximum oxygen content of seawater which would be considered anaerobic is not stated.

3. Less than 3% of the applied radioactivity was RH-5287 at the first sampling interval; therefore, the application rate was not confirmed. The study authors stated that the "time 0" samples should be considered 1 hour posttreatment samples because it required approximately 1 hour to "inactivate the biological system" during processing and that "obtaining time points between nominal day 0 and 1 hour after initiation is impossible because of the time required to prepare the sample."

4. Storage stability data were not provided, although the samples were stored for 3 to 5 months at 4 C prior to analysis. For the attempt bound residue, identification, sediments from days 0 and 365 were used for exhaustive extraction. It is not clear if there is storage stability data to support this work, since the original study was conducted in 1991 and the exhaustive extraction in 1992.

5. The seawater was analyzed only for total [^{14}C]residues, although it contained up to 12% of the applied radioactivity. Extractable [^{14}C]residues from the sediment were characterized as being either more or less polar than RH-893; the concentrations of individual degradates were not reported. The study authors stated that "the use of RH-5287 as a marine antifoulant would result in environmental release rates of less than 1 ppb/day"; rates of 0.05 and 1.0 ug/mL were chosen to facilitate sample analysis.

6. There is a typographical error in Table IX. At 365 days, total volatile organics should be 0.2% of the applied as reported in Table VII, not 2.0% as reported in Table IX.

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