2-5-93

DATA EVALUATION RECORD

STUDY 3

CHEM 128101 RH-5287 §162-4

FORMULATION--00--ACTIVE INGREDIENT

STUDY ID 41845003
Lawrence, L.J., B. Lawrence, S. Jackson, and A. Kesterson. 1991. Aerobic aquatic metabolism of $^{13/14}$CRH-5287. Laboratory Project ID: PTRL Report No. 1291, PTRL Project No. 294, Rohm and Haas Technical Report No. 34-91-01. 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 on the Aerobic Aquatic Metabolism of $^{13/14}$C RH-5287. Performed by PTRL-East, Inc. Richmond, KY; PTRL Project #294 and 1291; Completed 5/1/92. Submitted by Rohm and Haas, Spring House, PA.
MRID #42341102

DIRECT REVIEW TIME = 17

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CONCLUSIONS:

Metabolism - Aerobic Aquatic

1. This study is not acceptable at this time and, therefore, cannot be used towards satisfying the 162-4 data reqirement.

(1)

2. In order for the registrant to satisfy the aerobic aquatic metabolism data requirement, additional information should be provided to support the "time 0" concentration of RH-5287 [such as 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]. Also, all degradates present at >10% of the applied should be identified. Comments in the COMMENTS section are also to be addressed.

Summary of Reported Results

According to the reported results, RH-5287 degrades rapidly (less than one hour) in seawater/silt loam sediment incubated under aerobic conditions. Data indicate that degradation products tend to partition (bind) strongly onto the sediment phase.

Significant amounts of $^{14}CO_2$ and volatile organics were formed during the study period, suggesting very rapid cleavage of the isothiazolone ring followed by subsequent oxidation. This was more evident at low dosages (0.05 ppm) than at higher dosages (1.0 ppm) and was attributed to the inhibitory effect of RH-5287 on microbial degradation.

Metabolites could not be characterized unequivocally, but the data suggest that the N-S is rapidly cleaved, resulting in subsequent formation of polar metabolites such as malonamic and n-octyl oxamic acid.

To further attempts to characterize bound residues were made (MRID 42341102) by exhaustive extractions of radiocarbon remaining in sediments after initial extractions, but no significant radiocarbon could be extracted. The authors conclude that the residues remaining bound to the sediment are not bioavailable.

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 had been collected from the York River, Virginia (specific site not reported). Ring-labeled [^{14}C]RH-5287 (labeled in the 3-one position, radiochemical purity 99.7%, 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 sealed with ground-glass stoppers and placed in darkened incubators at 25 $\pm$ 0 C for 30 days. Duplicate flasks were removed for analysis at 0, 1, 2, 5, 9, 15, 20, 26, and 30 days posttreatment. At each sampling interval the pH, Eh, and dissolved oxygen of the sampled flasks were measured. In

addition, two control flasks prepared and maintained in a manner identical to the study flasks were tested for pH, Eh, and dissolved oxygen at regular intervals.

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

To separate the seawater and sediment phases, samples were transferred into polypropylene bottles and centrifuged (except for the 1.0 ug/mL treatment for day 0, which was separated by vacuum filtration). 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. Extracts of the 1.0 ug/mL treatment were stored in foil-wrapped vials at 4 C prior to analysis by HPLC; extracts 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 or a C-10 Cyano column with a mobile phase of water:5% acetic acid and acetonitrile, 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.

In an attempt to characterize bound residues, (MRID 42341102) previously extrated samples (day 0 and day 30) were further extracted and characterized as humin, humic acid and fulvic acid fractions. The latter was further partitioned into acidic, neutral and basic conditions with methylene chloride.

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 in the dark at 25 $\pm$ 0 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 RH-893 HQ and CO_2 were conclusively identified. Material balances were 89.9-124.2% and 63.6-123.5 of the applied in the slurries treated at 0.05 and 1.0 ug/mL, respectively (Tables IX-X).

In sediment:seawater slurries treated at 0.05 ug/mL during the 30-day study, [^{14}C]residues in the seawater decreased from 8.5 to 1.2% of the applied (maximum day 2), extractable [^{14}C]residues in the sediment decreased from 48.2 to 13.9% (maximum day 0), and unextractable [^{14}C]residues in the sediment were 49.0-85.2% with no discernable pattern (Table XI). Volatilized CO_2 totaled 27.9% of the applied on day 30, and organic volatiles were $\leq$5% throughout the study. Only [^{14}C]residues extracted from the sediment were characterized. In duplicate samples, RH-5287 was 4.3 and 5.9% of the applied at day 0; it was not detected at any other sampling interval (Table XI). [^{14}C]Degradates, characterized as being more or less polar than RH-893, comprised up to 46.4% of the applied.

In sediment:seawater slurries treated at 1.0 ug/mL during the 30-day study (disregarding one sample at day 9 with an incomplete material balance), [^{14}C]residues in the seawater decreased from 6.0 to 0.7% of the applied (maximum day 0), extractable [^{14}C]residues in the sediment decreased from 44.5 to 20.9% (maximum day 0), and unextractable [^{14}C]residues in the sediment were 39.2 to 83.5% with no discernable pattern (Table X). Volatilized CO_2 totaled 10.0% of the applied on day 30, and organic volatiles were $\leq$3.1% throughout the study. Only [^{14}C]residues extracted from the sediment were characterized. In duplicate samples, RH-5287 was 3.5 and 5.3% of the applied at day 0; it was not detected at any other sampling interval (Table XII). One degradate,

RH-893HQ,

was 9.5 and 0.6% of the applied at day 0, 1.0% in both samples at day 5, and was not detected at any other sampling interval. Other

[^{14}C]degradates, characterized as being more or less polar than RH-893, comprised up to 38.5% of the applied (Table XII).

COMMENTS:

1. Less than 6% 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." In previously reviewed anaerobic aquatic and aerobic aquatic metabolism studies in seawater:sediment (Cranor, 1986; No MRIDs; Dynamac report dated 8/17/87), RH-5287 was 31.4 and 33.4% of the applied, respectively, at the "time 0" samplings; those studies were also faulted for failing to confirm the application rate because of poor recovery of RH-5287 at time 0. The instability of RH-5287 appears to occur only in the seawater:sediment system; RH-5287 was relatively stable in aerobic soil (30.5-47.3% of the applied was RH-5287 at 21 days posttreatment; Study 5, MRID 41845005) and was stable to photodegradation in water (half-life approximately 15 days; Study 1, MRID 41845000).

2. Degradates were not adequately characterized. The seawater was analyzed only for total [^{14}C]residues, although it contained up to 8.5% of the applied radioactivity. Extractable [^{14}C]residues from the sediment were characterized as being RH-893, more polar than RH-893, 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. However, the study authors stated that unknowns were not identified in the 1.0 ug/mL treatment because it appeared that inhibition of microbial degradation was occurring; the minimum inhibitory concentration of RH-5287 was reported to be <0.25 ug/mL. In the 0.05 ug/mL samples, "the low nominal dose rate ... made definitive identification of the metabolites detected by HPLC extremely difficult".

3. Samples were stored for an unspecified amount of time at 4 C prior to analysis. No refrigerator storage stability data was given.

4. 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.

5. The nominal treatment rates were 0.05 ug/mL or 1.0 ug/mL; using data from the LSC of the solutions used to treat the soils, the actual rates were 0.035 and 0.71 ug/mL, respectively.

6. The pH of samples decreased from 7-7.2 at the start of the experiment to 5.7-5.9 by day 30. Dissolved oxygen varied from 7.6-8.0 mg/L in sample flasks at day 1 to lows of 2.4-2.5 mg/L in 1 ug/mL flasks on day 9, 2.0-3.8 mg/L in 1 ug/mL flasks on day 20, and 2.1-4.0 mg/L in 0.05 ug/mL flasks on day 26. Eh varied from 495 to -176 (Table V).

7. The methodology in the original document stated that seawater from day 0 flasks was partitioned with methylene chloride, and that both fractions were analyzed using HPLC.

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