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FINAL REPORT

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Adsorption-Desorption of [6a-<sup>14</sup>C]-Rotenone

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by Bottom Sediments

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Solubility @ 100°C = 15 ppm

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## SUMMARY

Solutions of  $^{14}\text{C}$ -rotenone were mixed with five types of bottom sediments to determine adsorption--desorption properties of the piscicide. Sediment samples from the Mississippi River main channel (RM 707); Mississippi River backwater (RM 704); Rice Branch Experiment Station, Arkansas; Chocolay River, Michigan, and Ford River, Michigan were selected for use in the study. The mixtures were incubated on an orbital shaker for >16 hours at pH's of 6, 7, 8, and 9 and temperatures of 5 and 20°C. After incubation, the samples were centrifuged and analyzed to determine the extent of adsorption of rotenone by sediments. Sorption coefficients ( $K_d$ ) ranged from <10 for Mississippi River main channel sediments (sand) to >100 for sediments with a high silt and organic content. Adsorption was not significantly influenced ( $p > 0.05$ ) by temperature or pH. The calculated average sorption constant ( $K_{oc}$ ) for rotenone was 1900; a value that would characterize the mobility of rotenone in soil as low to slight.

In order to determine the rate of desorption, sediments containing adsorbed rotenone were mixed with a rotenone-free buffer solution on the orbital shaker for >16 hours and analyzed as before. Rotenone was most tightly bound to those sediments that had a high silt and organic content. The average percent desorption from the sandy Mississippi River main channel sediment was 63.6%; whereas that for the silty Mississippi River backwater sediment was only 3.7%. There was no significant difference ( $p > 0.05$ ) in adsorption or desorption of  $^{14}\text{C}$ -rotenone between sterilized and unsterilized sediments at pH 7.

## INTRODUCTION

Rotenone, the active constituent of derris root, has been used extensively as an insecticide and piscicide. Its use for removing undesired fish populations in the United States began in the 1930s and it is currently the most widely used piscicide in the United States (Schnick 1974). Rotenone was registered as a fish toxicant in 1962. Since then, data requirements for the registration of pesticides have been revised several times to require more complete documentation of safety, efficacy, and persistence of chemicals in the environment. All registered pesticides are being reviewed and are now required to conform to the revised standards. One of the new data requirements for registration of a pesticide by the U.S. Environmental Protection Agency (1982) is information on the chemical's potential binding on soils (adsorption-desorption).

This study was conducted to determine the adsorptive properties of rotenone on selected bottom sediments and to determine its potential for desorption from the sediments back into solution. The influence of temperature and pH on these processes also was evaluated.

## METHODS AND MATERIALS

Chemicals

Non-labeled rotenone (lot number 1508) was obtained from S. B. Penick Corporation (Lyndhurst, New Jersey) and found to have a purity of 98% by high performance liquid chromatography (HPLC). Rotenone-6a-<sup>14</sup>C (specific

activity = 13.3 mCi/mmol, lot number 820209) was purchased from Pathfinder Laboratories, St. Louis, Missouri, and held at -20°C in a vacuum dessicator protected from light. The structure and position of the radiolabel are presented in Figure 1. Results of purity checks by thin layer chromatography (TLC) and HPLC are presented in Appendix A.

Methanol, acetone, and water used for this work were HPLC grade products obtained from J. T. Baker Chemical Company. All other reagents and buffers were ACS reagent grade.

#### Sediment

Sediment samples were selected to represent a variety of geographical locations, particle sizes, and soil types. Two samples were collected from the Mississippi River near La Crosse, Wisconsin; one was collected from the main channel at River mile (RM) 707 and the second was collected from a backwater area at RM 704. A sample of Crowley silt-loam was obtained from the Rice Branch Experiment Station, University of Arkansas, Stuttgart, Arkansas. A fourth sample was collected from the Chocoy River at Highway 28, Michigan, and a fifth sample was collected from the North Branch of the Ford River at County Road 426, Michigan.

Particle size determinations of the sediment samples were conducted according to standardized procedures for sieving and hydrometer classification (ASTM 1979). Soil classifications were provided by the University of Wisconsin Soil and Forage Laboratory, Marshfield, Wisconsin (Table 1).

### Adsorption/Desorption Procedures

A series of  $^{14}\text{C}$ -rotenone concentrations (0.1, 0.2, 0.4, 0.6, and 1.0 mg/L) in appropriate buffer solutions, a blank containing sediment, a blank without sediment, and a control containing rotenone (0.5 mg/L) without sediment were used in conducting adsorption tests. Twenty-mL buffer solutions of the chemical were mixed with 2.0 g (dry weight) of sediment in 125-mL screw-capped Erlenmeyer flasks, and then agitated in the dark on a Lab Line<sup>®1</sup> Model 3590 orbital shaker at 100 RPM for at least 16 hours to attain adsorption equilibria. Temperature ( $5 \pm 1^\circ\text{C}$  or  $20 \pm 1^\circ\text{C}$ ) was controlled by an environmental chamber and pH's were maintained using 0.1 M phosphate or carbonate buffers (Table 2). After equilibrium was reached, the mixtures were transferred to glass centrifuge tubes and centrifuged on an International Model UV centrifuge at 1,000 g (2,250 RPM) for 30 min. Constant temperatures were maintained throughout the process. After centrifugation, duplicate 250- $\mu\text{L}$  samples of the liquid phases were transferred by micropipette to scintillation vials containing 10 mL of Beckman Ready-Solv<sup>®1</sup> HP scintillation cocktail. The samples were counted to a 2% two sigma counting error or for 10 min on a Beckman Model 7500 scintillation counter programmed to correct for quench, background, and counting efficiency. An additional 15.5 mL of liquid was removed from each centrifuge tube, then 16 mL of buffered water were used to rinse the sediment back into the Erlenmeyer flask and to bring the total volume of

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<sup>1</sup>Use of trade names does not imply U.S. Government endorsement of commercial products.

liquid back to 20 mL. The flasks were again placed on the orbital shaker for equilibration (>16 hours), centrifuged, and then sampled again for scintillation counting. The resulting radioactivity was corrected for the amount of  $^{14}\text{C}$ -rotenone remaining in the 4 mL of liquid retained with the sediment to determine the amount of chemical that desorbed from the sediments.

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### Mass Balance

Representative samples of the sediment remaining in the centrifuge tube from the highest concentration in the series (1.0 mg/L) was then placed in an extraction column and extracted with 25 mL of methanol at a flow rate of less than 2 mL/min. A subsample (250  $\mu\text{L}$ ) of the methanol eluate was then counted for radioactivity to determine the amount of chemical remaining on the sediment. A summary of the adsorption/desorption procedures is given in Appendix B and equations for calculations are presented in Appendix C.

Samples of each of the five sediments were sterilized in an autoclave and comparisons of the adsorption and desorption of  $^{14}\text{C}$ -rotenone were made using sterilized and unsterilized sediments at pH 7.0. Only one concentration of rotenone (1.0 mg/L) was used in this comparison.

Representative samples were taken at the end of the 16-hour incubation/mixing period to establish that the  $^{14}\text{C}$ -rotenone was not decomposing during the course of the tests. These samples were analyzed by HPLC according to the procedures described in Appendix D.

Linear regressions with correlation coefficients were used to determine correlations between sediment characteristics and adsorption of rotenone. Paired t-tests were used to evaluate statistical significance.

## RESULTS

Preliminary studies indicated that the amount of  $^{14}\text{C}$ -rotenone in solution and the amount of  $^{14}\text{C}$ -rotenone adsorbed to sediments reached an equilibrium within 16 hours on an orbital shaker. Equilibration time for most chemicals is between 4 and 24 hours (Hance 1967). Amounts of the chemical adsorbed from solutions containing 0.1, 0.2, 0.4, 0.6, or 1.0 mg/L by sediments from the Mississippi River main channel (RM 707); Mississippi River backwater (RM 704); Rice Branch Experiment Station, Arkansas; Chocoday River, Michigan; and Ford River, Michigan, are presented in Tables 3-7. Summary data of  $^{14}\text{C}$ -activity throughout the adsorption/desorption study are given in Appendix E. The adsorption of rotenone by sediments was evaluated at buffered pH's of 6, 7, 8, and 9 and at temperatures of 5 and 20°C.

The equilibrium that developed between the amounts of chemical in solution and amounts adsorbed to sediments was represented by adsorption equilibria plots (Figs. 2-11). The concentration range of rotenone selected for this study (0.1-1.0 mg/L) brackets the levels generally used in fisheries as a piscicide. The concentrations were high enough to maintain measurable quantities in solution with even the most adsorptive sediments, and low enough ( $<10^{-5}\text{M}$ ) to avoid saturation of adsorptive sites

and resulted in relatively linear adsorption equilibria plots (Karickhoff et al. 1979). Adsorption of rotenone by sediments was not influenced by temperature or pH (Figs. 12-16).

A convenient method for summarizing adsorption data is to use sorption coefficients ( $K_d$ ) which represent the amount of chemical adsorbed by sediments divided by the equilibrium concentration in solution. The larger the sorption coefficient, the more chemical that is adsorbed from solution. Sorption coefficients for rotenone with each of the five sediment types are presented in Tables 8-12. The sorption coefficients for a series of concentrations within each set of experimental parameters were similar due to linearity of the adsorption equilibria plots. Therefore, the coefficients for each series of concentrations were averaged to further summarize the data (Table 13; Figs. 17-18). It is apparent from the sorption coefficients that temperature and pH had only minimal influence on the adsorption of rotenone compared to the effect of sediment type. Coefficients ranged from  $<10$  for Mississippi River main channel sediment (sand) to  $>100$  for sediments with high silt and organic content. Regressions of sorption coefficients with sediment characteristics indicated that the best correlation ( $r^2=0.720$ ) occurred with percent organic content. When only bottom sediment samples from rivers were used in the regression, the  $r^2$  value was 0.998.

Sorption coefficients were normalized for the organic content of the various sediments by dividing the  $K_d$  by percent organic content. This value times 100 yielded the sorption constants ( $K_{OC}$ ) which are presented

in Table 14. Wide variations in  $K_{OC}$  values for a given chemical can be expected because the range over which  $K_{OC}$  values are observed for different chemicals is 0 to  $10^6$  (Mc Call et al. 1981).

Since pH had no significant influence ( $P > 0.05$ ) on the adsorption of rotenone, sorption coefficients for the different pH's within a given temperature were averaged for each sediment type (Table 15). There was slightly more adsorption of rotenone at 5° than at 20°C with all five sediments (Fig. 19); however, the influence of temperature was not statistically significant ( $P > 0.05$ ). The calculated average sorption constants ( $K_{OC}$ ) for rotenone are presented in Table 16. Since the effect of temperature was not significant, a grand average sorption constant ( $K_{OC}$ ) was calculated for rotenone (1,900). This compares with sorption constants of 60 for 2,4D, 4,300 for trifluorolin, and 500,000 for 2,2',4,5,5'-PCB (Swann et al. 1983).

Sorption constants ( $K_{OC}$ ) can be used to classify the expected mobility of a chemical in soil. According to the classification system presented by Swann et al. (1983), the mobility of rotenone in soil would be low to slight. There is an inverse relationship between the sorption coefficient ( $K_d$ ) and leaching distance in soils. The expected leaching distance of rotenone in soils would be only 0-2 cm with most types of soils. An exception would be the sandy sediments from the Mississippi River main channel where the expected leaching distance was about 8 cm (McCall et al. 1981).

After equilibrium was established in the adsorption phase of the study, the solution was centrifuged and most of the liquid phase was removed. To determine the rate of desorption, fresh, rotenone-free buffer solution was added and the mixture was again placed on the orbital shaker until a new equilibrium was established (>16 hours). The amounts ( $\mu\text{g/g}$ ) of adsorbed  $^{14}\text{C}$ -rotenone that desorbed from each of the sediments is listed in Tables 17-21. The percent desorption (amount desorbed divided by the amount adsorbed times 100) indicates that rotenone is more tightly bound to sediments with high silt and organic content than to sandy sediments with low organic content (Figs. 20-24). The average percent desorption for rotenone from the sandy Mississippi River main channel sediment was 63.6%, whereas that for the silty Mississippi River backwater sediment was only 3.7% (Table 22).

Residues of  $^{14}\text{C}$ -rotenone bound to sediments after desorption were extracted with methanol and quantified by scintillation counting. This was then used to determine a mass balance which was reported as percent recovery of the added radioactivity. The average percent recovery  $\pm$  SE was  $94.1 \pm 3.57\%$ .

Comparisons of the adsorption and desorption of  $^{14}\text{C}$ -rotenone between sterilized and unsterilized sediments at pH 7 indicated that there was no significant difference between the two sediment preparations ( $P > 0.05$ ; Table 23). This indicates that soil bacteria did not influence the adsorption of rotenone and that decomposition or metabolism of rotenone

did not occur during the tests. This was confirmed by HPLC analysis of samples taken after incubation (Appendix D).

#### CONCLUSIONS

Sorption coefficients ( $K_d$ ) for rotenone ranged from  $<10$  for Mississippi River main channel sediment (sand) to  $>100$  for sediments with a high silt and organic content. There was relatively good correlation between sorption coefficients and percent organic content of the sediments. Adsorption was not significantly influenced by temperature or pH ( $p > 0.05$ ). The calculated average sorption constant ( $K_{OC}$ ) for rotenone was 1900. This value classifies the mobility of rotenone in soil as low to slight. Based on the inverse relationship between the sorption coefficient ( $K_d$ ) and leaching distance in soils, the expected leaching distance of rotenone in soils would be only 0-2 cm in most types of soils. An exception was the sandy sediment from the Mississippi River main channel where the expected leaching distance was about 8 cm.

Rotenone is bound most strongly to fine sediments containing high organic content; most of the adsorbed chemical is readily desorbed from sandy sediments.

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