PMSP/ISB

0498

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

NOV 21 1985

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: EPA Reg. No. 6704-Q: Rotenone; Evaluation of Analytical Methods Used in Uptake and Elimination Studies with Fish. Accesss. No. 258460. RCB No. 1365

FROM: J. Garbus, Chemist
Residue Chemistry Branch
Hazard Evaluation Division (TS-769)

THRU: A. R. Rathman, Section Head
Special Registration Section I
Residue Chemistry Branch
Hazard Evaluation Division (TS-769)

TO: W. Miller, PM-16
Registration Division (TS-767c)

W. H. Gingerich and J. J. Rach of the National Fishery Research Laboratory, U. S. Fish and Wildlife Service, La Crosse, Wisconsin have submitted a final report entitled: "Accumulation and Elimination of [6a-^{14}C]-Rotenone by Bluegills." RCB has been requested to comment upon the acceptability and validity of the analytical methods used in this study.

Rotenone, 1,2,12,12a tetrahydro-8,9-dimethoxy-2-(1-methylethenyl)[1]benzopyrano[3,4b]furo(2-3-h)-benzopyran-6(6aH)-one, is extracted from cube', derris, and timbo roots. It is used as a selective contact insecticide and as a piscicide in eradicating or managing fish populations. The active material is rapidly degraded by sunlight, air, and water. No tolerances are established for rotenone.

Although rotenone is widely used in fish management programs, little is known as to its bioaccumulation and elimination by fish. The present study was undertaken to determine the rate of uptake and depuration of rotenone in fish and to determine the nature and amount of rotenone metabolites in fish tissues.

The initial experiment reported here was to determine the 24 hour and 96 hour LC_{50}'s for rotenone for bluegills. These were determined to be 10.9 micrograms/L and 14.0 micrograms/L, respectively. On this basis. 5.0 micrograms/L was chosen as the concentration in the water to be used with bluegills in the bioaccumulation and elimination studies. In these experiments, rotenone in water was determined by the method of Dawson et al., Trans. Amer. Fish Soc. 112: 725-727 (1983).

The accumulation-elimination experiments were conducted by continuously exposing bluegills to water containing 5 micrograms/L of 6a-^{14}C rotenone. (We believe that either the position of the labeled carbon is incorrectly shown in the structural diagram that is given or that the nomenclature of the labeled rotenone is incorrect. If the position is correctly shown, then the labeled carbon is 12a, not 6a.) The fish were exposed to the chemical in the aquarial water for 30 days and to rotenone-free water for an additional 21 days.

Three water samples were collected each day to determine rotenone concentrations in the aquaria. Groups of 5 fish were sampled for rotenone uptake at 6, 12, and 18 hours and at 1, 3, 7, 10, 14, 22, 30, 31, 33, 37, 40, 44, and 51 days. A separate group of ten fish were sampled on day 30 to allow for the determination of rotenone metabolites in the tissues.

Water samples were added directly to a high aqueous capacity scintillation cocktail. Fish were electrocuted, dried, and dissected into head, viscera, and carcass. Samples of these tissues were dried, oxidized to CO_2 and dissolved in the scintillation cocktail. Sample vials were counted for radioactivity and after the appropriate corrections and calculations expressed as micrograms rotenone.

The results indicate that rotenoid radioactivity rapidly accumulated in bluegills during the first 72 hours of exposure and then remained nearly constant for the ensuing 27 days of exposure. Elimination of radioactivity after cessation to exposure occured in two phases, an initial rapid decline with a calculated half-life of 25.8 hours and a slower secondary phase with a calculated half-life of 578 hours. Bioconcentration factors (BCF) were calculated for the whole body as 315, 165 for the fish head, 3,550 for the viscera, and 128 for the carcass.

We note that recovery studies were not conducted nor were studies carried out to determine the limits of sensitivity. However, for the purposes of this experiment, i. e., to determine the uptake and elimination of rotenone and to determine bioaccumulation factors for various tissue, we do not consider such validation studies as necessary. For example, if all individual results were to be corrected for recoveries, or if the method were to be made more sensitive, the overall pattern of uptake and elimination and the BCF's would not be altered.

We conclude the methods employed for the uptake and elimination studies are adequate and acceptable.

A third set of experiments was carried out to identify and quantify the radioactive rotenoid residues in fish tissues. Samples of fish tissues containing ^{14}C-rotenone residues were obtained on the 30th day of exposure, freeze dried, and extracted, first with acetone and then with methanol. The organic solvent phases were

evaporated to dryness and residues partitioned into water/diethyl ether. The organic phases were evaporated to dryness, redissolved in cyclohexane/dichloromethane, and cleaned up by gel-permeation chromatography. Residues remaining in the aqueous phases were concentrated by reverse phase chromatography. Fractions from these procedures containing radioactivity were evaporated to dryness, taken up in methanol, filtered through Milipore, and subjected to reverse phase HPLC on a C_{18} column. One ml fractions were collected and counted for radioactivity by LSC.

The results indicated that rotenone is readily transformed by bluegills after its uptake. This was demonstrated by the observation that acetone removed all of the radioactivity from tissues spiked with ^{14}C rotenone but extracted less than 50% of the residual radioactivity from the tissues of bluegills exposed to rotenone in water. The chromatographic results showed that rotenone, per se, accounted for 20% of the residual radioactivity, at most. Another major portion of the radioactivity coeluted with 6',7'-dihydro-6',7'-dihydroxyrotenolone, accounting for 25% of the activity in the carcass. Fifty to seventy per cent of the radioactivity remained unidentified. Treatment of aqueous tissue extracts with beta-glucuronidase and aryl sulfatase did not enhance the radioactivity in less polar fractions. Viscera, the tissue showing the fastest rates of uptake and elimination, contained the greatest percentage of polar, unidentified metabolites. The conclusion is drawn that metabolism to more polar products is a prerequisite for elimination.

As in the uptake and depuration studies, recoveries and limits of sensitivities were not determined. However, we conclude that for the purposes for which this experiment were done, the methods employed were satisfactory and the results, valid.

Conclusion:

After reviewing the methods employed in these studies and the results, we conclude that the methods are satisfactory and that the results appear to be valid.

cc: S.F., R. F., Circ., EEB, Reviewer, PMSD/ISB
RDI:ARR:11/20/85:RDS:11/20/85
TS-769C:RCB:JG:jg:557-1864:CM#2:Rm.708:11/21/85