Uptake and Accumulation of an Organochlorine Insecticide (Dieldrin) by an Estuarine Mollusc, *Rangia cuneata*

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Several studies have documented the uptake and accumulation of organochlorine insecticides from water solution by various aquatic invertebrates (BUTLER, 1966; CROSBY and TUCKER, 1971; LOWE et al., 1971; WALSH et al., 1971; WILKES and WEISS, 1971). Rangia cuneata, the marsh clam, is a common estuarine bivalve of the southern Atlantic and Gulf Coasts (ABBOTT, 1954). This clam is important both economically (HOPKINS, 1970; HOPKINS and ANDREWS, 1970) and ecologically (DARNELL, 1962, 1961, 1958) in these areas and is regularly consumed by residents of the Texas Coast.

The importance of dieldrin as a pollutant of the rivers and estuaries of the United States is well established (BREIDENBACH et al., 1967; WEAVER et al., 1965). The present study was conducted to determine the possibility of uptake and accumulation of dieldrin from dilute solution by Rangia.

Methods and Materials

Clams were collected by hand from McCollum Park on the western shore of Trinity Bay, Chambers County, Texas. Animals were selected on the basis of shell dimensions and subsequent weighings of the meat prior to analysis yielded a mean and standard deviation of 7.85 ± 1.18 grams, indicating uniformity. The water at the times of collection was shallow (1-3 ft.); salinity (as measured by refractometer) varied from 12 to 17 parts per thousand (°/₀₀); temperature range was 10-30°C; and the bottom was a mixture of soft mud and shells. Clams were generally found in small groups of 2-4 individuals lying just below the surface of the mud. After collection, the clams were packed dry in insulated containers and brought to the laboratory where they were placed in 20 gallon, glass tanks containing aerated artificial sea water made from distilled water and Instant Ocean Sea Salts (Aquarium Systems, Inc., Eastlake, Ohio). The water had a temperature of 25°C (maintained in a temperature-controlled room), a pH of 8.2, and salinity was adjusted with distilled water to 15.6 °/₀₀. The clams were allowed to remain in these holding tanks for 1 to 2 weeks to acclimate.

The experimental apparatus consisted of two 5 liter, glass tanks measuring 25x16x17.5 cm, each containing 3 liters of artificial sea water at the same salinity, temperature and pH as the holding tanks. Each tank was

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supplied with a constant flow of sea water. Dieldrin (Shell Analytical Standard) solution and acetone (Mallinckrodt nanograde acetone) control solution were added to the experimental and control tanks, respectively, through chromatographic columns, equipped with needle valve tips.

Water in the tanks was mixed by water-powered stirrers and Teflon-coated stir bars to avoid external heating of the water. Mixing was aided by an air stone in each tank. Prior to the start of the experiments, food coloring was added dropwise to both tanks so that mixing of the water could be assessed. It was found that with this combination of flow-through water, stirrers, and air stones, the water was mixed quickly and completely as indicated by the uniform distribution of food coloring throughout each tank in a matter of seconds.

Prior to the onset of each experiment, 13 clams were removed from the holding tanks. One of these was immediately shucked and frozen to be analyzed later as a background sample. Of the remaining 12 clams, 6 were placed in each tank and allowed to acclimate to the experimental conditions. The experiment was begun with the water concentration of dieldrin at 0.55 µg/L. Dieldrin solution was added to maintain water concentrations at approximately 0.55 µg/L. This low concentration was chosen because it approximates the level of dieldrin found in many bays, rivers, and estuarine areas (BREIDENBACH and LICHTENBERG, 1963; LAUER et al., 1966; WEAVER et al., 1965).

Samples of clams and water were removed from each tank after 12, 24, 36, 48, 60 and 72 hours. The animals were shucked and the meat frozen for later analysis. At 72 hours from the start of the experiment, a second background sample was taken from the holding tanks and also frozen for later analysis.

The preparation of clam tissue for chromatographic analysis was performed by a modification of the Food and Drug Administration procedure (1969) adapted from Mills (1959) involving extraction of residues from tissues with acetonitrile, partition in petroleum ether and clean-up on a Florisil (Floridin Co., Pittsburgh, Pa.) column (MILLS, 1968).

The cleaned samples were analyzed in a gas-liquid chromatograph (GLC) fitted with a 150 mCi electron-capture detector (Barber-Colman Selecta-System Series 5000, Barber-Colman, Rockford, Illinois). The GLC column used was a 2m x 4mm i.d. Pyrex glass coil, packed with 80/100 mesh Gas Chrom Q Support (Applied Science Labs, Inc., State College, Pa.), on which the liquid phase of 11% OV-17 + QF-1 (Analabs, Inc., North Haven, Conn.) was distributed. The

carrier gas used was pre-purified nitrogen and the operating temperatures were: injector, 260°C; column, 200°C; detector, 215°C.

Residue peaks were qualified by retention time relative to the standard and quantification was performed by peak height measurements (MCNAIR and BONELLI, 1969).

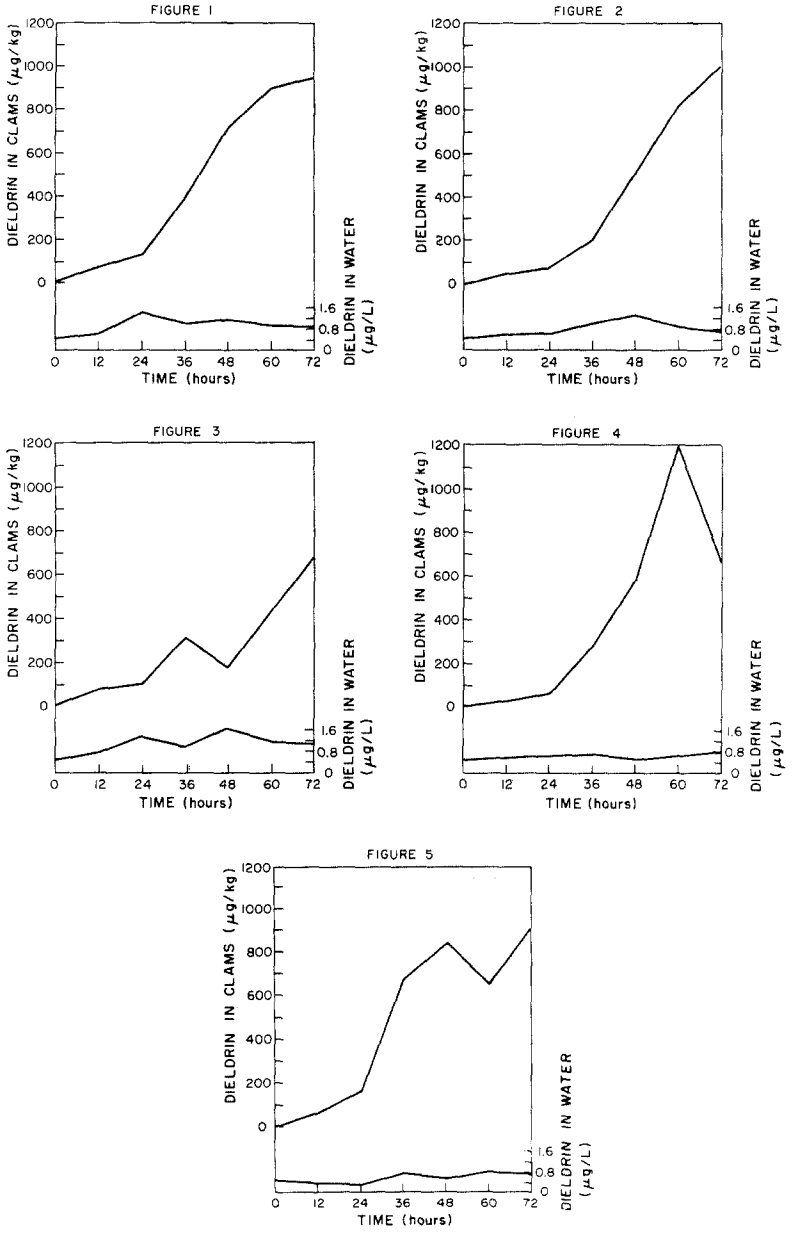
Recoveries of dieldrin from fortified clam tissue samples were consistently above 85%. Confirmation of 10 random samples was performed on a second GLC column and by "p-values."

Results and Discussion

The results of this study indicate that Rangia is capable of taking up and concentrating dieldrin in its tissues to levels far above ambient. The kinetics of dieldrin uptake in Rangia are shown in Figures 1-5 for each of the time periods sampled. Each figure represents the result of one experiment and each point, the result of one sample determination. The upper curve shows dieldrin present in Rangia tissue (scale on left) and the lower curve shows the concentration in the water (scale on right). The smallest dieldrin residue in the experimental clams was 38.2 µg/kg (water dieldrin level at 0.50 µg/L) after 12 hours of exposure. The largest residue was 1226 µg/kg (water at 0.63 µg/L) after 60 hours of exposure. The means for the 12 and 24 hour groups (67 µg/kg and 118 µg/kg, respectively) showed about a 200 fold increase above ambient, while the 36 hour groups (385 µg/kg) had an increase of almost 800 times. Maximum increase was over 2000 times ambient.

It was observed that uptake proceeds relatively slowly during the first 24 hours of exposure. This may be due to the reduced (exploratory) siphoning behavior performed by these clams when a new substance is added to their environment, or it may be due to some chemical or physical mechanism at the sites of uptake in the tissues. The rate of uptake accelerates rapidly after this point and there is no evidence for a cessation of the uptake mechanism over a period of time up to 72 hours.

The graphs do show some variations in uptake kinetics. This may be due to individual deviations from normal siphoning behavior by the clams, or experimental error since each point on the graph is based on the analysis of one clam.



Figures 1-5. Kinetics of dieldrin uptake in Rangia. Upper curves-dieldrin residues in clam tissues (µg/kg). Lower curves-dieldrin residues in exposure solution (µg/L) at time of removal of clams for analysis.

The means and standard deviations (µg/kg) of dieldrin residues in control animals and the dates of collection are shown below:

Figure 1. 6.5 ± 1.2 (Dec. 1970)
Figure 2. 15.3 ± 2.2 (Feb. 1971)
Figure 3. 16.1 ± 0.8 (Apr. 1971)
Figure 4. 14.7 ± 2.1 (July 1971)
Figure 5. 7.4 ± 2.2 (Sept. 1971)

Fluctuations in residue levels of control animals reflect seasonal variations in natural populations in the collecting area and are reported elsewhere.

The graphs also show some fluctuation in the dieldrin concentrations in the water from the desired 0.55 µg/L. However, all levels were between 0.40 and 1.67 µg/L and these differences were small compared to the high concentrations of the residues found in the experimental clams; however, the rate of concentration could be affected. None of the control water samples showed any evidence of dieldrin.

While it is not known whether or not the clams can sense the dieldrin, it is apparently not noxious enough in the concentrations used to interrupt normal siphoning; thus dieldrin is taken into Rangia and accumulated. In this way it may become a threat to the estuarine food web.

Possibly the most important result is measurable uptake and accumulation of dieldrin from extremely low levels in water which have been presumed to be safe.

References

ABBOTT, R.T.: American Seashells. New York: Van Nostrand (1954).

BREIDENBACH, A.W., C.G. GUNNERSON, F.K. KAWAHARA, J.J. LICHTENBERG, and R.S. GREEN: Public Health Service Reports, Dept. of HEW 82, 139 (1967).

BREIDENBACH, A.W., and J.J. LICHTENBERG: Science 141, 899 (1963).

BUTLER, P.A.: Trans. Amer. Fish. Soc. 95, 110 (1966).

CROSBY, D.G., and R.K. TUCKER: Environ. Sci. & Technol. 5, 714 (1971).

DARNELL, R.M.: The Amer. Midl. Nat. 68, 434 (1962).

DARNELL, R.M.: Ecology 42, 553 (1961).

DARNELL, R.M.: Publ. Inst. Mar. Sci., Univ. Texas, 5, 353 (1958).

HOPKINS, S.H.: Proc. Nat. Shell. Assn. 60, 5 (1970).

HOPKINS, S.H., and J.D. ANDREWS: Science 167, 868 (1970).

LAUER, G.J., H.P. NICHOLSON, W.J. COX, and J.I. TEASLEY: Trans. Amer. Fish. Soc. 93, 310 (1966).

LOWE, J.I., P.D. WILSON, A.J. RICK, and A.J. WILSON, JR.: Proc. Nat. Shell. Assn. 61, 71 (1971).

MCNAIR, H.M., and E.J. BONELLI: Basic Gas Chromatography. 5 ed. California: Varian Aerograph (1969).

MILLS, P.A.: J. Assoc. Off. Anal. Chemists 51, 29 (1968).

MILLS, P.A.: J. Assoc. Off. Anal. Chemists 42, 734 (1959).

U.S. DEPT. OF HEALTH, EDUC. AND WELFARE, FOOD AND DRUG ADMIN., Pesticide Analytical Manual 1 (1969).

WALSH, G.E., C.W. MILLER, and P.T. HEITMULLER: Bull. Environ. Cont. & Tox. 6, 279 (1971).

WEAVER, L., C.G. GUNNERSON, A.W. BREIDENBACH, and J.J. LICHTENBERG: Public Health Service Report, U.S. Dept. of HEW 80, 481 (1965).

WILKES, F.G., and C.M. WEISS: Trans. Amer. Fish. Soc. 100, 222 (1971).