Biomagnification of Dieldrin Residues by Food-Chain Transfer from Clams to Blue Crabs under Controlled Conditions

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Analyses of field-collected estuarine species have demonstrated an increase in organochlorine insecticide residue concentrations correlated with increasing trophic levels of organisms (JENSEN et al. 1969; ROBINSON et al., 1967; WOODWELL et al., 1967). Subsequent laboratory studies have confirmed the hypothesis of uptake and accumulation of insecticides from water (PETROCELLI et al., 1973; GRZENDA et al., 1971, 1970; GAKSTATTER, 1968) and that of "biological magnification" (REINERT, 1972; JOHNSON et al., 1971; METCALF et al., 1971). However, there are relatively few published data of the direct, controlled transfer of these compounds in a food chain comprised of organisms consumed by man.

The significance of dieldrin in the environment is demonstrated by its ubiquitous occurrence (RISEBROUGH et al., 1968; TATTON and RUZICKA, 1967; TARRANT and TATTON, 1968), its toxicity to aquatic species and wildlife (TARZWELL and HENDERSON, 1957; KATZ, 1961; TUCKER and CRABTREE, 1970) and its persistence (NASH and WOOLSON, 1967).

The ecological and commercial significance of the blue crab, Callinectes sapidus and the marsh clam, Rangia cuneata are well known and documented (TAGATZ and HALL, 1971; U.S. DEP'T. OF COMMERCE, 1972; HOPKINS, 1970; HOPKINS and ANDREWS, 1970). DARNELL (1962, 1961, 1958) found Rangia to be an important food source for a variety of fin-fishes and invertebrates, including blue crabs.

This study was designed to test the possibility of the biological magnification of dieldrin residues in a two-part food chain consisting of clams and blue crabs.

Materials and Methods

Rangia were collected by hand from the large population extant at a water depth of 2-3 feet along the shoreline just above Grassy Point in Hynes Bay of the San Antonio Bay System, Texas. The clams were packed dry in insulated containers and brought to the laboratory where they were placed into 20 gallon glass tanks containing aerated seawater prepared by dissolving Instant Ocean Synthetic Sea Salts in distilled water and diluting to the desired salinity. The water temperature was 20-22° C, pH was 8.2 and salinity was 15°/$_{oo}$. Clams were held under these conditions for 2 weeks prior to experimen-

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tation. Blue crabs were collected from crab traps in the mouth of Hynes Bay off McDowell Point, transferred to double-thickness brown paper bags and placed on ice until arrival in the laboratory where they were placed in holding tanks containing 10°/$_{oo}$ salinity seawater and maintained there for a 2 week acclimation period. Under these conditions, mortalities due to collection and subsequent transfer to laboratory conditions were minimal. Because it is difficult to maintain a large group of blue crabs together in a single aquarium for any length of time due to their cannibalistic behavior, an apparatus was constructed based on a design of COOK (1972) which would allow separation of individual crabs in a recirculating seawater system (Fig. 1). Crabs were placed, one to a compartment, in the holding boxes shown in the figure and six of these boxes were stacked as indicated. The seawater (10°/$_{oo}$ S) in the reservoir was pumped into the top left compartment (No. 1) by means of a submersible pump and flowed by gravity through the boxes as indicated by the arrows. After leaving the last compartment (No. 11), the seawater passed through a filter of washed whole Rangia shell, crushed oyster shell, and activated charcoal, and back into the reservoir. Salinity was checked daily and maintained by the addition of distilled water when necessary. Crabs were held in the feeding apparatus for several days prior to the commencement of an experiment to allow them to acclimate to conditions.

Since the uptake and accumulation of dieldrin from dilute seawater solutions by Rangia has been demonstrated (PETROCELLI et al., 1973) the emphasis of this study centered on the transfer of dieldrin residues from experimentally contaminated clams to blue crabs by feeding. Rangia were placed in covered, glass aquaria which contained aerated seawater, at the same temperature, pH and salinity as the holding tanks and with dieldrin in solution at a concentration of 5.5 µg/l. After 36 hours of exposure, clams were removed and shucked directly into a Waring Blendor cup. Meats were blended at high speed until homogeneous in appearance (5 min.), then poured into cocktail ice cube trays and frozen at -15° C until required. Control cubes were prepared by blending unexposed Rangia and freezing as described for experimental cubes. Prior to crab feeding experiments, samples of each group of cubes were analyzed by electron-capture gas-liquid chromatography (GLC) for dieldrin residue levels.

Experiments were begun with the feeding of control crabs (No. 1, 3, 5, 7, 9, 11) with a 3 gm uncontaminated cube and the experimental crabs (No. 2, 4, 6, 8, 10, 12) with a 3 gm dieldrin-contaminated cube. Crabs were fed once each day at noon by forceps and accepted food readily. These low levels of feeding insured that crabs were hungry at feeding time and observations indicated that there was very little spill-over

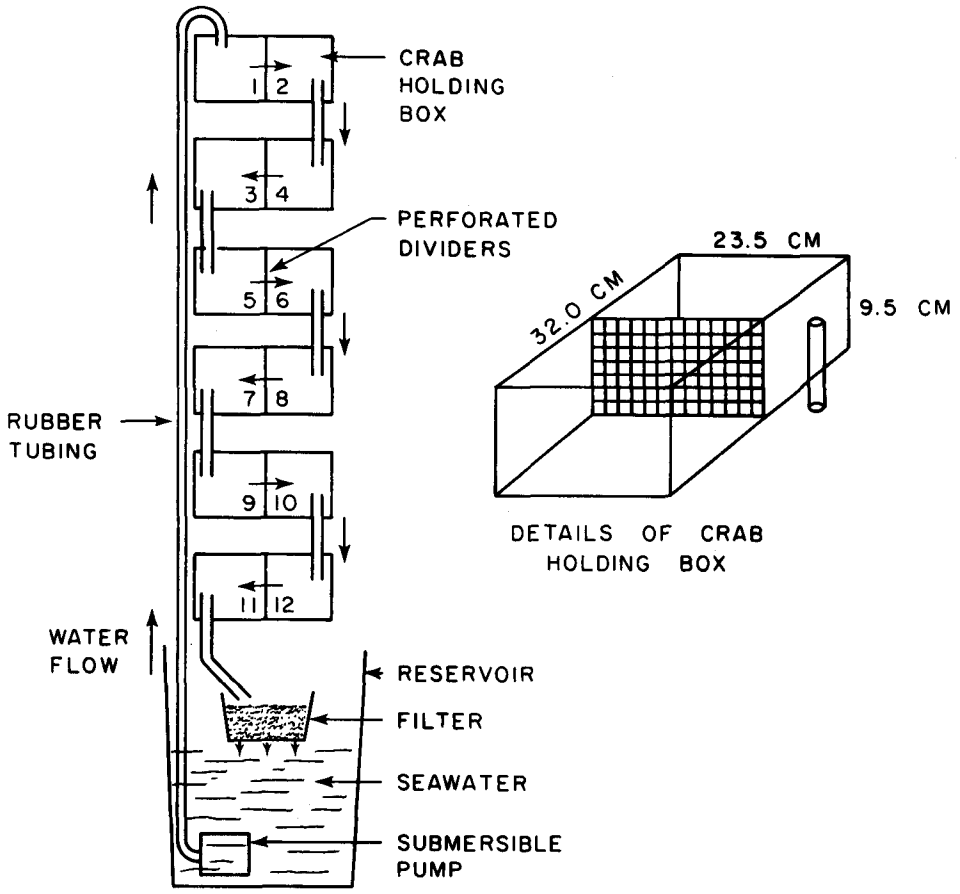

Fig. 1. Recirculating seawater system for the maintenance and feeding of blue crabs.

of food fragments between compartments. Nitex screening on box dividers prevented movement of larger particles of food between adjacent compartments and subsequent analyses showed no cross contamination.

After the appropriate number of days of feeding, crabs were removed from compartments one at a time, immersed in crushed ice to decrease biological activity, wrapped in aluminum foil and killed by placement in a freezer at -15° C where they remained until analyzed. Samples of freshly prepared seawater and water samples from compartments 1, 2, 11, 12 and the reservoir were taken during the experiment and analyzed along with the crab tissues.

Dieldrin residues were extracted from tissue samples in acetonitrile and partitioned in petroleum ether. Clean-up of extracts was performed on a Florisil column (PESTICIDE ANALYTICAL MANUAL, 1969). Water samples were extracted with petroleum ether and did not require clean-up. Analyses were performed on a Barber-Colman Selecta-Series 5000 gas-liquid chromatograph fitted with an electron-capture detector. The GLC column was packed with 80/100 mesh Gas Chrom Q support on which the liquid phase of 3% OV-1 was distributed. The carrier gas was pre-purified nitrogen and the operating temperatures were, injector, 200° C, column 180° C, detector 210° C. Identification of dieldrin residues was made by measurement of retention time relative to a dieldrin standard and quantification was performed by peak height measurements. Confirmation of residues was made on a second GLC column and by "p-values" (PESTICIDE ANALYTICAL MANUAL, 1969). Recoveries of dieldrin from fortified samples were consistently above 85% and reported concentrations are not corrected for recovery.

Results and Discussion

Analysis of the two batches of dieldrin-contaminated clam cubes yielded dieldrin concentrations of 193 and 181 µg/kg. Uncontaminated cubes contained 0 or trace (< 1.0 µg/kg) residues. Callinectes fed the contamined clam meat concentrated dieldrin residues to 4.7 and 6.8 times the daily dose after 10 days of feeding and 3.9 times after five days (Tables 1, 2, 3). There was no dieldrin detected in any water samples taken from the various compartments or from the reservoir of the crab feeding apparatus. Calculations of the percent dieldrin retained in crab tissues through feeding on clam meat were made by dividing the total number of micrograms (µg) of dieldrin actually taken up in feeding (mean total µg dieldrin in experimental clams - mean total µg dieldrin in controls) by the number of µg of dieldrin actually fed. That is, if experimental crabs contained 4.10 µg dieldrin and controls, 1.40 µg, then the total number of µg taken up in feeding would be 4.10 - 1.40 = 2.70,

Table 1.

The total content and weight specific concentration of dieldrin in tissues of blue crabs fed contaminated clam meat.

All crabs fed 3 gm of clam meat/day

Experimental crabs (2,4,6,8,10,12): Fed dieldrin contaminated meat at 193 µg/kg for 10 days = 0.579 µg/day = 5.79 µg total.

Control crabs (1,3,5,7,9,11): Fed clean crab meat for same length of time as experimental crabs.

Crab	Total Dieldrin (µg)	Dieldrin residue conc. (µg/kg)
1	0.86	5.9
2	5.04	28.4
3	1.62	10.5
4	3.17	21.8
5	0.83	5.8
6	3.04	19.2
7	1.63	8.0
8	3.83	23.8
9	1.11	6.1
10	4.59	26.6
11	2.37	8.4
12	4.95	31.9
$\bar{X}$ + S.D.		
Control	1.40 ± 0.59	7.5 ± 1.9
Experimental	4.10 ± 0.88	25.3 ± 4.6

Crabs incorporated: 4.10 - 1.40 = 2.70/5.79 = 47%

Magnification factor: 2.70/0.579 = 4.7 times daily dose

Table 2.

The total content and weight specific concentration of dieldrin in tissues of blue crabs fed contaminated clam meat.

All crabs fed 3 gm meat/day.

Experimental crabs (2,4,6,8,10,12): Fed dieldrin contaminated meat at 181 µg/kg for 10 days = 0.543 µg/day = 5.43 µg total.

Control crabs (1,3,5,7,9,11): Fed clean crab meat for same length of time as experimental crabs.

Crab	Total Dieldrin (µg)	Dieldrin residue conc. (µg/kg)
1	0.57	6.9
2	4.13	203.9
3	0.33	4.5
4	4.33	52.3
5	0.42	5.1
6	2.69	18.4
7	0.80	5.5
8	4.19	50.7
9	0.16	2.8
10	4.24	68.3
11	0.37	3.2
12	5.11	186.0
$\bar{X} \pm$ S.D.		
Control	0.44 ± 0.22	4.7 ± 1.5
Experimental	4.12 ± 0.79	96.6 ± 78.1

Crabs incorporated: 4.12 - 0.44 = 3.68/5.43 = 68%

Magnification factor: 3.68/0.543 = 6.8 times daily dose.

Table 3.

The total content and weight specific concentration of dieldrin in tissues of blue crabs fed contaminated clam meat.

All crabs fed 3 gm clam meat/day

Experimental crabs (2,4,6,8,10,12): Fed dieldrin contaminated meat at 193 µg/kg for 5 days = 0.579 µg/day = 5.79 µg total.

Control crabs (1,3,5,7,9,11): Fed clean crab meat for same length of time as experimental crabs.

Crab	Total Dieldrin (µg)	Dieldrin residue conc. (µg/kg)
1	0.37	3.9
2	2.71	30.7
3	0.38	5.9
4	2.51	29.3
5	0.16	2.9
6	2.45	41.5
7	0.17	3.1
8	2.13	37.3
9	0.10	2.4
10	2.44	27.0
11	0.25	2.4
12	2.76	31.1
$\bar{X}$ + S.D.		
Control	0.24 ± 0.11	3.4 ± 1.3
Experimental	2.50 ± 0.23	32.8 ± 5.5

Crabs incorporated: 2.50 - 0.24 = 2.26/2.90 = 78%

Magnification factor: 2.26/0.579 = 3.9 times daily dose.

and the percent of dieldrin incorporated would be equal to the total number of µg taken up in feeding divided by the total number of µg fed over the duration of the experiment, 2.70/5.79 = 47%. The magnification factor was calculated by dividing the total number of µg taken up in feeding by the daily feeding concentration, 2.70/0.579 = 4.7 times greater than the daily dose. These concentration factors are of the same order of magnitude as those found by ODUM et al. (1969) in their study of fiddler crabs which concentrated DDT residues by a factor of 3 after feeding on contaminated detritus for 10 days.

When analyzing these food chain data, it must be realized that the animals were fed small amounts of food contaminated by dieldrin at relatively low levels over a short period of time. Therefore, under natural conditons in the field, animals consuming contaminated food for several months or years may have much higher concentration factors. This type of short-term laboratory study can only serve to indicate that such magnification is possible and give a general picture of the magnitude.

Magnification of organochlorine insecticide residues has several implications for the species involved and also for man. Estuarine organisms are exposed to these compounds in water, in prey organisms, in sediments and in detrital material. These insecticides are selectively retained in the tissues of these species and their levels tend to be "enriched" with time. Man, in consuming these species, incurs an increasing body burden of these compounds. Since very little is known regarding effects of sublethal concentrations of these insecticides on the physiology of estuarine organisms and on man, it is not yet possible to predict the consequences of residues found in animal tissues. Current research in our laboratory is attempting to assess the effects of sublethal concentrations of a variety of pollutants on several estuarine species.

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