1980

The Effects of Cis-Chlordane and Dieldrin on the Short Food Chain: *Artemia* to Winter Flounder

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THE EFFECTS OF CIS-CHLORDANE AND DIELDRIN ON THE
SHORT FOOD CHAIN: ARTEMIA TO WINTER FLOUNDER

BY

SCOTT MCLEAN

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE
REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE
IN
FOOD SCIENCE AND TECHNOLOGY

UNIVERSITY OF RHODE ISLAND

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MASTER OF SCIENCE THESIS
OF
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1980

Abstract

Live Artemia sp. nauplii were contaminated with the pesticides cis-chlordane and dieldrin singly and in combination at two concentrations. The bioconcentration factors were 88.29 ± 13.09 and 128.03 ± 24.78 , for cis-chlordane and dieldrin, respectively. Winter flounder, Pseudopleuronectes americanus, post-larvae, fed the contaminated nauplii accumulated the pesticides. The resulting bioaccumulation factors were 0.67 ± 0.33 for cis-chlordane and 0.38 ± 0.16 dieldrin. Metabolism of cis-chlordane by the Artemia sp. is indicated by the presence of oxychlordane. However, metabolism of dieldrin in the Artemia sp. is not clearly implied. Cis-chlordane was only metabolized in the winter flounder when high levels of dieldrin were present. The rate of dieldrin metabolism in the winter flounder increased with an increase in its body concentrations.

Analysis of the lipids and fatty acid methyl ester profiles of the Artemia sp. suggest that an increase in lipid metabolism occurs with pesticide contamination. The winter flounder total lipid decreased during the experimental period for all the treatment groups, including the controls, suggesting a condition of inadequate food supply. No change in the lipids was attributed to the pesticides.

The increase in length of the fish was less in the

flounder fed the combined pesticides treatment Artemia sp. than in the control or singly contaminated Artemia sp., suggesting that cis-chlordane and dieldrin acted in combination to reduce growth.

No mortalities occurred among the winter flounder during the experimental period. This demonstrates that dieldrin and cis-chlordane probably were not solely responsible for mortalities observed in test animals fed San Pablo Bay and Utah Artemia sp. in previous studies (Beck et al., 1980; Johns et al., 1980; Klein-MacPhee et al., 1980; Klein-MacPhee, unpublished data).

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PREFACE

**This thesis has been prepared according to the
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MANUSCRIPT

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THE EFFECTS OF CIS-CHLORDANE AND DIELDRIN ON THE

FOOD CHAIN: ARTEMIA TO WINTER FLOUNDER

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Live Artemia sp. nauplii were contaminated with the pesticides cis-chlordane and dieldrin singly and in combination at two concentrations. The bioconcentration factors were 88.29 ± 13.09 and 128.03 ± 24.78 , for cis-chlordane and dieldrin, respectively. Winter flounder, Pseudopleuronectes americanus, post-larvae, fed the contaminated nauplii accumulated the pesticides. The resulting bioaccumulation factors were 0.67 ± 0.33 for cis-chlordane and 0.38 ± 0.16 dieldrin. Metabolism of cis-chlordane by the Artemia sp. is indicated by the presence of oxychlordane. However, metabolism of dieldrin in the Artemia sp. is not clearly implied. Cis-chlordane was only metabolized in the winter flounder when high levels of dieldrin were present. The rate of dieldrin metabolism in the winter flounder increased with an increase in its body concentrations.

Analysis of the lipids and fatty acid methyl ester profiles of the Artemia sp. suggest that an increase in

lipid metabolism occurs with pesticide contamination. The winter flounder total lipid decreased during the experimental period for all the treatment groups, including the controls, suggesting a condition of inadequate food supply. No change in the lipids was attributed to the pesticides.

The increase in length of the fish was less in the flounder fed the combined pesticides treatment Artemia sp. than in the control or singly contaminated Artemia sp., suggesting that cis-chlordane and dieldrin acted in combination to reduce growth.

No mortalities occurred among the winter flounder during the experimental period. This demonstrates that dieldrin and cis-chlordane probably were not solely responsible for mortalities observed in test animals fed San Pablo Bay and Utah Artemia sp. in previous studies (Beck et al., 1980; Johns et al., 1980; Klein-MacPhee et al., 1980; Klein-MacPhee, unpublished data).

Introduction

The brine shrimp, Artemia sp., are organisms of widespread distribution, occurring in over 150 known locations around the world. They are one of the most important sources of live foods used by aquaculturists (Sorgeloos and Persoone, 1975) and are also used by aquatic research culturists as food for many species of biological and toxicological test animals prior to and during

experiments (Beck et al., 1980).

In accordance with the decisions of the International Symposium on Artemia salina (1979) and the Artemia Reference Center, Ghent, Belgium the species name of the brine shrimp will be Artemia sp. until further taxonomic research concludes otherwise (compare, International Symposium on Artemia salina, Corpus Christi, Tx, 1979).

Biological performance studies utilizing several geographically different strains of brine shrimp have been carried out at the Environmental Protection Agency's Environmental Research Laboratory in Narragansett, R.I. (EPA-ERL-N). Differences in growth and survival of test organisms was attributed to the strain of Artemia sp. used.

Larvae of two species of crab, Rhithropanopeus harrisii and Cancer irroratus, proceeded through normal zoeal development and metamorphosed to first crab stage successfully when reared on nauplii hatched from cysts from four localities, Macau, Brazil; Shark Bay, Australia; Margherita di Savio, Italy; and San Francisco Bay, California, USA (Johns et al., 1980). However, when the crabs were fed Artemia sp. hatched from cysts from Great Salt Lake, Utah (Utah), or San Pablo Bay, California (SPB), abnormal development occurred, usually ending in mortality during metamorphosis to the first crab stage. Klein-MacPhee et al. (1980) showed that winter flounder, Pseudopleuronectes americanus, post-larvae cultured with Artemia sp. from Utah and SPB also had significantly greater

mortality than winter flounder grown on the other strains. In another experiment, P. americanus post-larvae were fed Brazil Artemia sp. nauplii through metamorphosis and then the fish were changed to SPB nauplii. Mortality in the experimental fish was first observed on the thirteenth day of being fed the SPB diet and peaked on day 20, when the study was terminated (Klein-MacPhee, unpublished data). Likewise Atlantic silversides, Menidia menidia, exhibited increased mortality when fed the Utah and SPB brine shrimp nauplii (Beck et al., 1980).

Biochemical analyses of the Artemia sp. strains were performed in the laboratories of the Department of Food Science and Technology, Nutrition and Dietetics at URI.

Analysis of protein and amino acid quality in the five strains of Artemia sp. by Seidel et al. (1980) showed no obvious cause for the observed mortalities. Soejima et al. (1980) found no obvious difference in the carotenoid content, but did detect the presence of chlorophyll in the SPB Artemia sp.

Analysis of the lipid fraction in various strains of Artemia sp. by Schauer et al. (1980) showed that both of the strains which caused mortalities have lower levels of specific, essential fatty acids than the other strains. They suggested that the deficiency in the fatty acids may not have been sufficient to cause the observed mortalities but may have been capable of producing a nutritional stress which could have been aggravated by some toxic compound or

other stressful condition.

Olney et al. (1980), using multi-residue pesticide analysis, was able to demonstrate that DDT probably was not the causal agent of mortality. The Italian strain, showed excellent growth and survival although it had the highest level of DDT (395 ppb) of the five strains analyzed. This finding is contrary to that of Bookhout and Costlow (1970). They reported detecting DDT at concentrations of 7050 ppb in Utah nauplii, and suggested that the insecticide was the causal agent of mortality observed in the crab larvae fed the Artemia sp.. Olney et al. (1980) did not detect this level of DDT in the Utah Artemia sp., and therefore could not support the conclusions of Bookhout and Costlow (1970).

Cis-chlordane and dieldrin residues, both considered toxic to fish, were shown to be present in SPB and Utah Artemia sp. (Olney et al., 1980). Although the concentrations are relatively low, it is felt that either through the action of bioaccumulation or in concert with the suggested fatty acid deficiency, these two pesticides might exert a toxic stress on the test organism.

To test the bioaccumulation hypothesis, Artemia sp. nauplii were contaminated in the laboratory with cis-chlordane and dieldrin. They were then fed to winter flounder post-larvae. The results of this research are presented in this paper.

Materials and Methods

Culture of Artemia

Seawater was prepared at the EPA-ERL by filtration through a 0.45 micron filter and irradiation with ultra-violet light.

All organic solvents used in this experiment were pesticide analytical grade reagents.

The pesticides, cis-chlordane and dieldrin, were provided by the EPA Pesticide and Toxic Substances Repository at Research Triangle Park, North Carolina. Solutions of 1000 ng/mL (ppb) were made in acetone. One serial dilution was made for each pesticide to 100 ng/mL.

Brazilian Artemia sp. cysts (Aquaria Products, lot 10) were provided by the Artemia sp. Reference Center, Ghent, Belgium. The Artemia sp. were hatched as follows: A 4 L separatory funnel containing 3 L of filtered seawater and 15 mL of Artemia sp. cysts was vigorously aerated and maintained at 25° C for 28 hours (Johns et al., 1980). The aeration was stopped after 28 hours and the stage I nauplii sank to the bottom of the funnel from which they were drained into a sieve (Beck et al., 1980). The nauplii were rinsed with distilled water and the excess water was blotted from the sieve.

Ten liter erlenmeyer flasks containing 500 mL seawater were treated as follows; 1 no treatment (biological control), 2 1 mL acetone (acetone control), 3 0.5 mL 100 ppb cis-chlordane + 0.5 mL acetone (LC), 4 0.5 mL 1000 ppb cis-chlordane + 0.5 mL acetone (HC), 5 0.5 mL 100 ppb

dieldrin + 0.5 mL acetone (LD), 6 0.5 mL 1000 ppb dieldrin + 0.5 mL acetone (HD), 7 0.5 mL 100 ppb cis-chlordane + 0.5 mL 100 ppb dieldrin (LC/LD), 8 LC/HD, 9 HC/LD, 10 HC/HD. The concentration of pesticide in the water was 0.1 ppb at the low level and 1.0 ppb at the high level. Treatment numbers and corresponding contamination levels are presented in Table 1.

One gram lots of stage I nauplii were transferred to each of the contamination flasks. The flasks were aerated to provide mixing of the flask contents.

Contamination was terminated after 24 hours. Each flask was drained through a sieve. The *Artemia* sp. were rinsed with distilled water and blotted dry. Enough Artemia sp. to feed the winter flounder were weighed and placed in a 20 mL screw-cap vial and the vial was filled with seawater. The vials were transported to the EPA-ERL where the flounder culture facilities were located.

The contamination procedure was repeated every 2 days to provide live, contaminated nauplii to the winter flounder every 2 days. The contaminated nauplii in excess of those used to feed the fish were accumulated in composite samples of each treatment group for later biochemical analysis.

Winter Flounder Culture

Winter flounder, Pseudopleuronectes americanus, larvae were provided by Dr. Klein-MacPhee of the EPA-ERL. They had

been spawned on February 7, 1980 and cultured as described by Klein-MacPhee et al. (1980). When large enough to ingest Artemia sp. nauplii, the fish were fed uncontaminated, 24 hour old Brazil Artemia sp.. As the flounder grew, 4 - 10 day old Artemia sp. (Brazil) cultured at the EPA-ERL-N were fed to them (Klein-MacPhee, personal communication).

Winter flounder, 107 days old, were provided from the above culture for the pesticide study. At this age the fish were post-metamorphosed flat-fish and many were developing pigmentation.

The experimental fish were selected randomly from the common culture tanks. Each fish was measured for standard length, from tip of the lower jaw to base of the caudal fin rays. No attempt was made to secure wet weights since previous experience had shown that high mortalities would ensue. Twenty fish were stocked in each of the 10 treatment tanks. Seventeen more fish were sacrificed immediately and the wet weights, dry weights and lengths were measured.

The treatment tanks were black polyethylene dish pans (34.0 x 29.5 x 15.0 cm) containing 6 L of seawater as described by Klein-MacPhee et al. (1980). The tanks were maintained in a running seawater table at ambient ocean temperatures (16.3° C to 19.6° C). The photoperiod was 12 hours light: 12 hours dark.

The winter flounder were acclimated for 5 days to a live Brazil nauplii diet. A feeding rate of 0.6 g nauplii per tank added every 2 days was chosen initially. This rate

allowed for the complete consumption of all the Artemia sp.

Upon initiation of the experiment, each tank was fed with the appropriate Artemia sp. contamination group at a rate of 0.6 g nauplii/tank every 2 days. The tanks were siphoned daily to remove fecal detritus. The lost water was replaced with new seawater.

On experimental day 11, the fish, which were usually docile and lying on the tank bottom, were observed swimming in the water column and gulping air through the air-water interface. The dissolved oxygen was measured in the tanks and it was found to be below optimum for the the fish (Klein-MacPhee, personal communication). Thereafter, the tanks were aerated and one-third of the water was changed every 2 days.

The feeding rate of the first 4 feedings was 0.6 g nauplii/tank every 2 days. The subsequent 8 feedings were at rate of 0.8 g nauplii/tank every 2 days when it was found the fish would consume more nauplii. The total weight of nauplii fed to each tank was 8.80 g. The last feeding occurred on day 24 of the experiment.

The feeding experiment was terminated on day 25. The length and wet weight of each fish was measured and each fish was individually freeze-dried for 24 hours in a Virtis Unitrap freeze-drier. Dry weights were then determined, and the fish pooled into treatment groups for later analysis.

Length versus dry weight graphs were plotted for the initial and treatment group fish. Change in length was

determined by matching the longest final length with the longest initial length and taking the difference.

Biochemical Analysis - Pesticides

All biochemical analyses were performed in duplicate on the composite Artemia sp. samples and pooled fish samples.

A schematic flow diagram for the chlorinated hydrocarbon analysis is presented in Figure 1.

Chlorinated hydrocarbons were extracted using the Enos micro-method as modified by Wilson (in Warlen, 1974). A preweighed sample not exceeding 1.5 g was ground 3 times with 10 mL portions of acetonitrile in a 20 mL Potter Elvehjem tissue homogenizer. After each grinding the liquid and tissue were separated by filtration.

The 30 mL of acetonitrile was quantitatively transferred to a 250 mL separatory funnel and 25 mL of petroleum ether (PE) was added together with 100 mL of distilled water. The separatory funnel was inverted several times and the phases were allowed to separate. Sodium chloride was added if an emulsion formed. The aqueous (lower) phase was drained and discarded. The PE phase was washed twice with 100 mL distilled water which was also discarded. The PE was decanted to a 25 mL Kuderna-Danish (KD) concentrator tube with micro-snyder condenser and evaporated to approximately 1 mL.

For every 5 samples that were analyzed, one reagent blank was processed to determine the contamination introduced by the procedure.

Lipid and other co-extractants partitioned with the chlorinated hydrocarbons into the PE were removed from the sample using the modified micro-alumina chromatography techniques described by Holden and Marsden (1969). The sample was chromatographed with 3 g of Woelm alumina, activity grade 3, packed in a glass column (1 X 20 cm). The eluting solvent was 2% dichloromethane (DCM) in PE. One 20 mL fraction was collected and it was evaporated as described above. Hexane, 2 mL, was added and the sample was evaporated to near dryness. This insured the complete removal of DCM.

Silicic acid micro-columns were used to chromatographically separate the PCB interference from the pesticides. Glass columns (1 X 20 cm) were packed with 2.5 g of silicic acid (deactivated with water, 4% by weight) and washed with DCM as described by Bidleman et al. (1978). The concentrated samples were chromatographed on these columns. The PCBs were eluted first with 35 mL PE and the pesticides were then eluted with 20 mL DCM. The fractions were concentrated as before.

Pesticide identification and quantification were performed using dual column electron capture gas chromatography (ECGC) as described by Olney et al. (1980). A Tracor MT-220 equipped with two Ni-63 detectors and two

180 X 0.4 cm glass columns packed with 1.5% OV-17/1.95 QF-1 and 4% SE-30/6% QF-1 on 100/120 mesh Supelcon AW-DCMS (Olney et al., 1980) was operated isothermally and the temperatures were: injector = 250° C, column = 200° C, and detector = 350° C. The nitrogen carrier gas was maintained at a flow rate of 60 mL/min. No purge gas was used.

Biochemical Analysis - Lipids

Artemia and winter flounder samples were kept at -20° C until lipid analysis.

Not more than 1.0 g of sample was extracted 3 times with 20 mL portion of PE in a 20 mL Potter Elvehjem tissue homogenizer. The PE and tissue were separated by filtration. The 60 mL lipid extract was transferred to a round bottom flask and partially evaporated with a rotary vacuum evaporater. The lipid solution was quantitatively transferred to a pre-weighed, pear-shaped flask and evaporated to dryness. The lipids were weighed gravimetrically, transferred in benzene to a screw-cap vial and stored under nitrogen at -20° C until further preparation.

Fatty acid methyl esters (FAME) were prepared by saponifying the crude lipids with 0.5 N methanolic KOH for 40 minutes at 100° C. The saponified material was transferred into hexane by acidification with 6 N HCl and centrifugation at 3000 rpm for 4 minutes. The free fatty

acids were then methylated with 14 % boron trifluoride-methanol for 8 minutes at 100°C (Morrison and Smith, 1964).

Identification and quantification of the FAME was performed with a Varian-Aerograph model 1200 GLC equipped with a flame ionization detector (FID) and fitted with a 210 X 0.32 cm stainless steel column packed with 10% SP-2330. The GLC was operated isothermally and the conditions were: injector = 225° C, oven = 200° C and detector = 250° C. The carrier gas was nitrogen and the flow rate was 40 mL/min. Identification was accomplished using authentic standards to provide accurate retention time and peak area/ug response values.

Experimental Design and Data Analysis

One run of the winter flounder feeding experiment was performed. It consisted of the 10 treatments described previously. A two-way analysis of variance (ANOV) as described by Snedecor and Cochran (1967) was used to separate the analytical variation from the variation attributable to the pesticide contamination. However, because the individual fish were so small (approximately 0.02 g/fish dry wgt.), pooled samples were used for all biochemical analyses. Therefore biological variation could not be separated from the analytical or treatment variation. Furthermore, because only one experiment was performed,

experimental variation could not be tested.

The variables tested by ANOV were percent lipid, total FAME, individual FAME percents and increase in length. These variables were tested in both the *Artemia* sp. and winter flounder.

Statistical analyses were performed by the University of Rhode Islands Academic Computer Center utilizing the S.A.S. '79 statistical package. Variables with statistically significant variation beyond that attributable to replication had their means ranked in increasing order and the means were then separated with Duncan's Multiple Range Test.

None of the lipid data showed statistically significant variation ($p = 0.05$) which could be interpreted as following a particular trend. Some of the variables do appear to follow trends, however, they are not statistically significant at the 0.05 confidence level.

The differences in the change in length of the winter flounder were statistically significant between treatments at the 0.05 confidence level.

The bioconcentration factors (BCF) were calculated from the following equation:

$$BCF = \frac{C_A}{C_W} \quad \text{where: } C_A = \text{concentration (ng/g) of pesticide in the Artemia}$$

$$C_W = \text{concentration (ng/mL) of pesticide in the water}$$

The bioaccumulation factors (BAFs) were calculated from the following equation:

$$BAF = \frac{C_{WF}}{C_A} \quad \text{where: } C_{WF} = \text{concentration (ng/g) of pesticide in the winter flounder}$$

$$C_A = \text{concentration (ng/g) of pesticide in the } \underline{\text{Artemia}}$$

The percent uptake values for Artemia sp. were calculated according to the following equation:

$$\text{Percent Uptake} = \frac{\text{Total ng of pesticide in } \underline{\text{Artemia}}}{\text{Total ng of pesticide presented in the water to 1 g of } \underline{\text{Artemia}}} \times 100$$

The percent uptake values for the winter flounder were calculated using the following equation:

$$\text{Percent Uptake} = \frac{\text{Total ng of pesticide in all fish of one treatment}}{\text{Total ng of pesticide in 8.80 g of } \underline{\text{Artemia}}} \times 100$$

Results and Discussion

Bioconcentration of Pesticides in Artemia

Cis-chlordane, oxychlordane and dieldrin were the only chlorinated hydrocarbon pesticides detected in the experimental Artemia sp. and they were found only in those groups specifically contaminated. Polychlorinated biphenyls (PCBs), primarily of the Aroclor 1242 type, occurred in all treatment groups at similar levels (approximately 5 ppb). The source of the PCBs was not determined, however several possible sources do exist. It has been demonstrated that by pumping PCB contaminated air through water, the water will become contaminated to a level sufficient to allow bioconcentration to occur in plankton (Scura and Theilacker, 1977). PCB could also be introduced in the water secured at the EPA laboratory.

Artemia bioconcentrated cis-chlordane and dieldrin from the contaminated water. The pesticide concentrations found in the Artemia sp., the exposure concentrations, the bioconcentration factors (BCFs) and the percent uptake of the pesticides are presented in Table 2.

The dieldrin BCFs were significantly larger ($\bar{x}=128.0$, $s=36.68$) than the cis-chlordane values ($\bar{x}=88.26$, $s=16.33$). Vieth et al. (1979) suggests that a polar function, such as the epoxide function on dieldrin, would reduce the the BCF of the compound. This was not the case here, cis-chlordane, which has no polar function, did not concentrate as greatly as dieldrin.

The variation of the estimates for the dieldrin and cis-chlordane BCFs indicate that for these experimental conditions, the values are relatively accurate. They are somewhat less than expected when compared to the values found in the literature. Vieth et al. (1979) suggests that generally the BCFs of halogenated compounds should be greater than 5000. Epifanio (1973), on the other hand, found that Artemia sp. nauplii hatched and maintained in water containing 0.5 ppb dieldrin for 36 hours had a BCF of 426. Our results are more similar to those reported by Epifanio.

Oxychlordane was detected at approximately 3% of the cis-chlordane levels in those Artemia sp. treated with high levels of cis-chlordane. Oxychlordane is a metabolic degradation product usually associated with trans-chlordane in animals (Brown, 1978; Feroz and Khan, 1979); however it has been formed from cis-chlordane in the laboratory (Schwenmer et al., 1970). Its presence indicates that metabolism of cis-chlordane was occurring in the HC treatment groups. It was not detected in the low cis-chlordane Artemia sp., but its concentration may have been below the limit of detection for the procedure. Metabolism of cis-chlordane in Artemia sp., and perhaps other invertebrates, may be important because higher animals do not appear to have this ability (Brown, 1978). Ecologically, an invertebrate's ability to metabolize cis-chlordane could provide a biological means for reducing

the environmental levels of this persistent compound.

Cis-chlordane BCFs are significantly lower in the combined treatment groups 8 through 10 ($\bar{x}=77.4$, $s=7.84$) than groups 3, 4, or 7 ($\bar{x}=99.1$, $s=15.51$). Either cis-chlordane assimilation from the environment was lower in these groups or metabolism and subsequent depuration was greater. The previous observation of oxychlordane production supports the metabolism viewpoint. If the lower cis-chlordane BCFs in the combined treatment groups are indicative of a higher level of metabolism, this could mean that the mixed-function oxidase (MFO) activity has been synergistically induced to higher levels in the combined treatment groups.

Cis-chlordane metabolism, as shown by oxychlordane production alone, is evidence of MFO activity. Increased MFO activity in the combined groups shows the effect of dieldrin, a known inducer of the MFO in mice and rats (Triolo and Coon, 1966; Chadwick et al., 1975), on the MFO of Artemia sp. It should be noted that a high concentration of dieldrin and/or cis-chlordane is required to produce this synergistic effect. This suggests that a threshold concentration of pesticide may be necessary to induce the Artemia sp. MFO and that groups 8 - 10 have surpassed that threshold.

The bioconcentration of pesticides in the Artemia sp. from the aqueous environment produced higher body concentrations than did accumulation of the pesticides from the diet. In a preliminary experiment, Artemia sp. were fed

a defatted rice bran diet contaminated with 0.1 and 1.0 ppb cis-chlordane and dieldrin for 14 days, but the Artemia sp. produced by the procedure also had much lower body concentrations of the pesticides. The bioaccumulation factors (BAFs) were consistently less than one. This finding is in agreement with the general belief that contamination from the aqueous environment is more significant than contamination via the food web (Macek et al., 1979; Veith et al., 1979; Epifanio, 1973). Veith et al. (1979) go on to say that only if the food contaminant concentration is very great and the water levels very small will the food make a large and significant contribution to the overall contaminant concentration of the organism.

The Artemia sp. were produced prior to the feeding experiment and frozen until needed because of the greater amount of time and logistical support (i.e. daily feeding and cleaning of the cultures) needed for contamination. The winter flounder, when fed the thawed product, did not eat them. Thus the diet was not only difficult to produce but it was also undesirable to the fish.

Lipid Analysis of the Artemia

Lipid analyses were performed on the Artemia sp. because one of the hypotheses concerning the cause of mortality in the Artemia sp. is based on a biochemical deficiency in the Artemia sp. and its interaction with pesticides. Specifically it has been suggested that fatty

acid deficiencies in Artemia sp., particularly in the w3 polyunsaturated fatty acids (PUFA), would produce a stress in the fish which the pesticide contamination could aggravate, resulting in mortality (Schauer, et al., 1980). It has been reported that pesticides affect the metabolism of lipids (Tinsley, 1966; Tinsley and Lowry, 1972; Phillips and Buhler, 1979) and it has also been shown that the nutritional status of an organism affects the toxicity of a compound (McLean and McLean, 1966; Tinsley, 1966; Krijnen and Boyd, 1971; Miranda and Webb, 1972; Mehrle et al., 1974; Addison and Zinck, 1977; Mehrle et al., 1977; Walton et al., 1978).

The lipid and FAME results for the Artemia sp. are presented in Table 3.

The percent lipid and percent FAME are lower in the pesticide contaminated Artemia sp. than the controls, although the differences are not statistically significant. Except for group 8 (LC/HD), the percent lipid values steadily decrease from treatment 3 to 10. Pesticide contamination at levels found in this experiment may act to stimulate the Artemia sp. metabolism and cause an increase in lipid metabolism, thereby reducing the percent lipid. High cis-chlordane treatment groups have lower percent lipids than the low cis-chlordane groups. The MFO require energy, NADPH and NADH if they have been induced and the lipids may be metabolized to provide them, along with providing energy for growth and maintenance.

The percent FAME decrease slightly, though not significantly, in the contaminated nauplii, more so in groups 6 - 10. This may be evidence of the effect of high dieldrin or combined pesticides on increasing the metabolic activity of the Artemia sp..

The FAME profiles do not contain any consistent evidence of effects of pesticide contamination.

Bioaccumulation of Pesticides in Winter Flounder

Chlorinated hydrocarbons, other than those already mentioned, were not detected in any of the winter flounder treatment groups. Cis-chlordane was found in all the treatment groups, including the controls and the single contaminant dieldrin groups. In those cases where cis-chlordane was not specifically fed, it was detected at an average level of 2.76 ppb. This value was used when correcting the cis-chlordane concentrations prior to calculation of the bioaccumulation factors (BAFs) and the percent uptake. Table 4 contains the results showing the pesticide concentrations in the fish, BAFs and percent uptake.

The BAFs for all the winter flounder groups are less than one except group 4 (HC). The fact that the BAFs are so small is significant because biomagnification, defined by Macek et al. (1979) as the increase of contaminant concentration from one trophic level to the next higher trophic level, does not appear to be occurring in this

experiment.

The dieldrin BAFs and percent uptake values are inversely proportional to the dieldrin diet concentrations. This suggests that the fish MFO may have been induced more by the high dieldrin concentrations than the low, resulting in increased metabolism and depuration of dieldrin and lower apparent accumulation factors. These results also suggest that a threshold level of dieldrin may be required to stimulate the MFO. Dieldrin depuration has been shown to occur in the channel catfish, Ictalurus punctatus (Argyle et al., 1975) and lake trout, Salvelinus namaycush (Rienart et al., 1974).

Although cis-chlordane did accumulate in the winter flounder, no distinct pattern was evident concerning its uptake. The BAF for the HC group (1.28) is nearly twice as large as the next largest value (HC/LD=0.74) and almost four times larger than the smallest value (0.24 of the LC group). No reason for this accumulation pattern has been developed.

The residual level of cis-chlordane detected in all the experimental fish had either been accumulated from the feed used prior to the experiment or it had been concentrated from the water. No determination of its source was attempted however.

Oxychlordane was detected in all the HC treatment groups, at approximately 3% of the cis-chlordane concentrations. Its presence can be explained in two ways. Either oxychlordane was accumulated from the Artemia sp.

which have previously been shown to be contaminated with it or it is a metabolic degradation product being formed from cis-chlordane present. Feroz and Khan (1979) have showed that cis-chlordane is particularly stable in the goldfish, Carassius auratus. Therefore, oxychlordane was probably accumulated from the diet.

Lipids and FAME of the Winter Flounder

Several differences exist between the initial winter flounder lipids and the treatment flounder lipids as seen in Table 5. It is important to compare the treatment fish to the initial fish because the treatment fish reflect the quality of the diet.

The treatment winter flounder, when compared to the initial fish, showed a reduction of from 62% in groups 1 and 2, to 80% in group 10 of their total percent lipid and a reduction of 59% in group 5, to 90% in group 10, in their percent FAME. These comparisons suggest that the treatment fish did not receive enough caloric input in the diet to satisfy the demands for maintenance and growth. This caused the fish to use their stored lipids in addition to the dietary lipids as an energy source. It has been suggested that these final percent lipid and FAME levels indicate that the fish were in a form of lipid stress (P.S. Schauer, personal communication).

The percent FAME are consistently lower in groups 6 through 10 of the winter flounder. The inherent

cis-chlordane residue may be a factor in this observation. The fish metabolism may have been stimulated by the cis-chlordane introduced to the fish prior to the experimental period. Cis-chlordane presented to the fish in the treatment Artemia sp. might then not cause any greater metabolic increase. However, dieldrin might stimulate it, but only if a threshold is attained or in concert with higher levels of cis-chlordane. Hence, low dieldrin does not cause the metabolism of fatty acids while high dieldrin and combination groups do.

The FAME profiles of the initial and treatment winter flounder do not resemble each other. This is due more to the abnormal nutritional state of the fish, i.e. low caloric intake, than to the pesticide contamination. Because the fish was not receiving enough energy input in the diet, it was catabolizing lipid from both the diet and its inherent stores. However, specific fatty acids (16:0, 18:0, 20:3w3 and 20:5w3) were apparently spared probably for use in the functional lipids. This is shown by the relative increase in these FAME percent values. Other fatty acids, i.e. 16:1w7 and 16:2w4, were catabolized for the required energy.

It is significant that the winter flounder had such low lipid levels and that they were metabolizing what they had, in addition to the pesticide loads they were acquiring in the experiment. Phillips and Buhler (1979) suggest that increased fat metabolism may increase the organism's vulnerability to dieldrin. This is because dieldrin is

stored in the lipid and as the lipid level decreases the dieldrin will eventually be released to circulate to the body and express its toxicity and/or be metabolized and depurated. Addison and Zinck (1977) showed that the dehydrochlorination of DDT is inversely proportional to the lipid pool size. By removing the storage sites of the contaminant, more of it becomes free to affect the organism and be affected by it.

Growth and Survival of Winter Flounder

No mortalities occurred in any winter flounder treatment group during the experimental period. The amounts of cis-chlordane and dieldrin in the low concentration Artemia sp. were within one order of magnitude of the strains, SPB and Utah, which were being simulated by this experiment. The levels of contamination in the high concentration Artemia sp. were an order of magnitude greater when compared in this context. The winter flounder did accumulate some of the pesticides which demonstrates that assimilation did occur. Because SPB Artemia sp. were not fed simultaneously in this experiment, these results cannot be correlated to the previous biological studies performed at the EPA. However, it can be said conclusively that the pesticides, cis-chlordane and dieldrin, when fed in the diet to post-larval winter flounder at apparently background levels and at levels elevated above background levels, do

not cause mortalities. Epifanio (1973) showed that 213 ppb of dieldrin in a diet did not cause mortalities in crabs fed the diet.

The change in length of the fish from the beginning to the end of the experimental period was found to be significantly different at the 5% confidence limit between the treatment groups by ANOV. Ranking of this data confirmed that there was an effect due to the pesticides (Table 6). The Duncan's Multiple Range Test showed that the combined high treatment group (HC/HD) caused the smallest increase in length and that this treatment was statistically different from the remaining groups. The other combined treatment groups were clustered together as having the next smallest increase in length. The single contaminant group, HD, stood alone, followed by the remainder of the treatment groups, all of which were ranked together.

These results suggest that cis-chlordane and dieldrin act in combination to reduce the increase in length of the young winter flounder. Dieldrin, when fed in the diet, has been shown to reduce the growth in the channel catfish (Argyle et al., 1975). These results demonstrate that combining cis-chlordane with dieldrin produces a greater reduction in change in length than dieldrin alone, while cis-chlordane alone does not affect the change in length. Low levels of dieldrin alone do not appear to affect the change in length. Acetone, however, did reduce the increase in length somewhat.

The reduction in growth by nearly 75% (treatment 1 versus treatment 10) has ecological significance. With the growth rate reduced, the fish may be removed from its predator-prey niche. The food it relies on to grow may not be available to it in the new time frame produced by the new growth rate. O'Connors et al. (1978), after showing that PCBs and dieldrin both suppressed the growth of certain algae, suggest that this could cause a shift in the food web dynamics. Smaller algae might bring in a different primary consumer which would change the entire structure of the food web. A slower growing flounder may find itself with a different food supply. The most obvious effect a change in growth rate will have on the fish is to change its survivability as a prey of other species, either by increasing the number of predators or by increasing the length of time a specific predator has to prey on it.

Conclusions

The Artemia sp. bioconcentrated the pesticides to levels somewhat lower than previous research would lead one to expect. Oxychlordan was detected in the high cis-chlordane treatment Artemia sp. suggesting a degree of cis-chlordane metabolism. There appears to be a threshold concentration required to induce dieldrin metabolism. A slight combined effect of the pesticides on the induction of the MFO is suggested.

The winter flounder bioaccumulate the pesticides from

the diet. The evidence suggests that biomagnification is not occurring. Some dieldrin metabolism seems to exist. The results are conflicting concerning the metabolism of cis-chlordane.

The percent lipid and percent FAME of the Artemia sp. were generally lower in the treatment groups than in the controls. No obvious trends in the FAME profiles were observed. The winter flounder seemed to be lipid stressed, yet this did not hamper survival, even when the fish were stressed with pesticides.

Cis-chlordane and dieldrin did not cause mortalities in the winter flounder when they were fed in the diet. Growth did decrease in the winter flounder of the combined treatment groups over the single treatment and control groups as a result of the pesticide contamination.

These experiments were performed to simulate the conditions of previous growth studies at the EPA-ERL using Artemia sp. nauplii as the food. The conditions were not strictly the same as the winter flounder were older in this experiment than those previously reported (Klein-MacPhee et al., 1980; Klein-MacPhee, unpublished data). The Artemia sp. also were 24 hours older. In retrospect a negative control consisting of SPB Artemia sp. nauplii should have been maintained with the rest of the treatments. However, the research did show that the pesticides cis-chlordane and dieldrin probably were not the causal agents of mortality in the previous growth and survival studies. It has also been

demonstrated that live Artemia sp. nauplii can be contaminated to desired concentrations easily and quickly for use in toxicological assays where a contaminated food for larval and juvenile stages of aquatic animals might be desired.

FIGURE 1: Pesticide Analytical Scheme

TABLE 1: *Artemia* sp. Contamination Treatments, Amounts of Contamination, Contamination Water Concentrations and Treatment Designations.

TREATMENT NUMBER	TREATMENT	CONTAMINATION	CONCENTRATION	DESIGNATION
1	Biological Control	None	0	Cont.
2	Acetone Control	1 mL acetone	0	Ace. Cont.
3	Low Cis-chlordane	0.5 mL 100 ppb cis-chlordane 0.5 mL acetone	0.1 ppb	LC
4	High Cis-chlordane	0.5 mL 1000 ppb cis-chlordane 0.5 mL acetone	1.0 ppb	HC
5	Low Dieldrin	0.5 mL 100 ppb dieldrin 0.5 mL acetone	0.1 ppb	LD
6	High Dieldrin	0.5 mL 1000 ppb dieldrin 0.5 mL acetone	1.0 ppb	HD
7	Low Cis-chlordane Low Dieldrin	0.5 mL 100 ppb cis-chlordane 0.5 mL 100 ppb dieldrin	0.1 ppb 0.1 ppb	LC/LD
8	Low Cis-chlordane High Dieldrin	0.5 mL 100 ppb cis-chlordane 0.5 mL 1000 ppb dieldrin	0.1 ppb 1.0 ppb	LC/HD
9	High Cis-chlordane Low Dieldrin	0.5 mL 1000 ppb cis-chlordane 0.5 mL 100 ppb dieldrin	1.0 ppb 0.1 ppb	HC/LD
10	High Cis-chlordane High Dieldrin	0.5 mL 1000 ppb cis-chlordane 0.5 mL 1000 ppb dieldrin	1.0 ppb 1.0 ppb	HC/HD

TABLE 2: Pesticide Concentrations (ng/g), Bioconcentration Factors (BCFs) and Percent Uptake of the Pesticides in the Artemia sp. Treatment Groups.

TREATMENT GROUP	Art 1 Art 2	Art 3	Art 4	Art 5	Art 6	Art 7	Art 8	Art 9	Art 10
TREATMENT	Cont.	LC	HC	LD	HD	LC/LD	LC/HD	HC/LD	HC/HD
Pesticide Conc. in Water (ng/mL)									
Cis-chlordane	0	0.1	1.0	0	0	0.1	0.1	1.0	1.0
Dieldrin	0	0	0	0.1	1.0	0.1	1.0	0.1	1.0
ng Exposure									
Cis-chlordane	0	50	500	0	0	50	50	500	500
Dieldrin	0	0	0	50	500	50	500	50	500
Pesticide Conc. in <u>Artemia</u> sp. (ng/g)									
Cis-chlordane	0	10.8* 2.45**	92.7 6.80	0	0	9.65 1.72	7.44 0.91	78.2 7.47	79.6 11.9
Dieldrin	0	0	0	12.0 0.74	170. 63.5	13.4 1.99	115. 52.1	9.70 0.50	131. 37.9
BCFs of <u>Artemia</u> sp.									
Cis-chlordane	0	108.	92.7	0	0	96.5	74.4	78.2	79.6
Dieldrin	0	0	0	120.	171.	134.	115.	96.9	131.
Percent Uptake									
Cis-chlordane	0	21.7	18.5	0	0	19.3	14.9	15.6	15.9
Dieldrin	0	0	0	24.1	34.2	26.8	23.1	19.4	26.2

* Means of the two analytical replicates

** Standard deviation of the two means

TABLE 4: Pesticide Concentrations (ng/g), Bioaccumulation Factors (BAFs) and Percent Uptake of the Pesticides in the Winter Flounder Treatment Groups.

TREATMENT GROUP	WF 1 WF 2	WF 3	WF 4	WF 5	WF 6	WF 7	WF 8	WF 9	WF 10
TREATMENTS	Cont.	LC	HC	LD	HD	LC/LD	LC/HD	HC/LD	HC/HD
Pesticide Conc. in <u>Artemia</u> sp. (ng/g)									
Cis-chlordane	0	10.3	92.7	0	0	9.65	7.44	78.2	79.6
Dieldrin	0	0	0	12.0	171.	13.4	115.	9.70	131.
Pesticide Conc. in Fish (ng/g)									
Cis-chlordane	3.11* 1.66**	6.66 2.32	121. 16.3	2.41 1.08	2.75 0.57	8.30 1.62	5.27 4.06	62.1 37.7	53.2 6.39
Dieldrin	0	0	0	7.73 1.90	57.2 0.22	5.29 1.98	24.4 8.27	4.27 3.12	30.2 10.3
BAFs									
Cis-chlordane	0	0.24	1.28	0	0	0.57	0.37	0.74	0.63
Dieldrin	0	0	0	0.65	0.36	0.39	0.25	0.43	0.23
Percent Uptake									
Cis-chlordane	0	10.4	35.0	0	0	18.6	10.3	19.0	18.4
Dieldrin	0	0	0	20.6	8.50	11.8	5.88	11.0	6.66

*Means of the analytical replicates

**Standard deviations of the means

TABLE 3: Quantities of Lipid, Total FAME and FAME Profiles of Artemia Treatment Groups.
 Lipid expressed as g/100 g tissue. Total FAME expressed as g/100 g tissue.
 Individual FAME expressed as g/100 g Total FAME.

TREATMENT GROUP	Art 1 Art 2	Art 3	Art 4	Art 5	Art 6	Art 7	Art 8	Art 9	Art 10
TREATMENT	Cont.	LC	HC	LD	HD	LC/LD	LC/HD	HC/LD	HC/HD
FAME									
14:0	3.71	3.66	3.81	3.82	3.58	3.50	3.70	3.73	3.71
16:0	12.8	12.2	12.5	11.9	12.6	11.6	12.3	11.6	11.3
16:1w7	12.6	13.7	14.2	13.9	13.9	13.8	14.3	13.9	12.9
16:2w6	2.60	2.90	2.91	2.93	2.76	3.02	2.88	3.22	2.82
18:0	4.02	4.18	4.14	4.01	4.93	3.91	4.72	3.71	4.08
18:1w9	31.7	31.3	32.4	33.1	32.0	31.9	32.7	32.6	29.1
18:2w6	13.0	10.4	10.1	10.1	9.67	10.5	10.2	10.2	9.17
18:3w3/20:1	6.31	6.13	6.22	6.73	6.58	6.38	6.68	6.90	6.03
20:2w6/20:3w6	1.06	2.76	0.35	0.36	0.21	1.81	0.48	0.16	0.23
20:3w3/20:4w6	5.90	6.13	6.37	6.52	6.56	6.50	6.11	6.69	5.72
20:5w3	6.55	6.53	6.92	7.02	7.21	6.81	6.20	7.33	5.92
Lipid	1.58	1.25	1.16	1.03	1.09	1.18	1.40	1.01	0.87
Total FAME	0.33	0.31	0.24	0.28	0.22	0.15	0.21	0.24	0.24

TABLE 5: Quantities of Lipid, Total FAME and FAME Profiles of the Winter Flounder Treatment Groups. Lipid expressed as g/100 g tissue. Total FAME expressed as g/100 g tissue. Individual FAME expressed as g/100 g Total FAME.

TREATMENT GROUP	Initial Fish	WF 1 WF 2	WF 3	WF 4	WF 5	WF 6	WF 7	WF 8	WF 9	WF 10
TREATMENT	None	Cont.	LC	HC	LD	HC	LC/LD	LC/HD	HC/LD	HC/HD
FAME										
14:0	2.15	3.91	4.93	4.83	4.14	5.06	4.11	3.20	2.91	4.29
16:0	13.2	21.0	17.2	15.1	13.7	18.8	18.0	17.7	20.0	19.6
16:1w7	17.1	11.7	13.2	12.9	13.4	13.4	10.2	9.09	11.3	15.0
16:2w4	3.51									
18:0	4.46	11.8	10.5	8.91	8.93	10.6	12.1	11.0	12.8	8.75
18:1w9	34.9	29.5	29.5	30.2	29.7	32.6	29.2	28.8	27.6	32.6
18:2w6	7.89	7.07	7.17	8.32	8.85	7.11	5.44	5.97	6.89	7.05
18:3w3/20:1	4.61	3.16	2.31	3.15	4.09	1.21	0.81	1.09	4.02	2.06
20:2w6	0.29									
20:3w3/20:4w6	4.41	8.24	10.7	11.4	10.0	8.85	13.4	13.2	9.72	9.19
20:5w3	4.45	5.32	4.49	5.07	5.17	6.15	6.82	5.49	4.83	2.58
22:5w3	0.26									
Lipid	1.96	0.76	0.28	0.63	0.53	0.31	0.29	0.66	0.25	0.23
Total FAME	0.244	0.058	0.062	0.054	0.092	0.024	0.024	0.034	0.028	0.022

TABLE 6: Increase in Length (cm) Ranked and Separated
Using Duncan's Multiple Range Test.

TREATMENT NUMBER	TREATMENT	INCREASE IN LENGTH (cm)
10	HC/HD	0.74 ^{a*}
7	LC/LD	1.40 ^b
9	HC/LD	1.43 ^b
8	LC/HD	1.50 ^b
6	HD	2.02 ^c
2	Ace. Cont.	2.83 ^d
4	HC	3.31 ^{d,e}
3	LC	3.45 ^{d,e}
5	LD	3.70 ^e
1	Cont.	4.11 ^e

* Values with the same letters in the superscript are similar, using the Duncan's Multiple Range Test.

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APPENDIX A

Literature Review

The Artemia Problem

Brine shrimp, Artemia sp., are one of the most important sources of live foods used by aquaculturists (Sorgeloos and Persoone, 1975), and are used by aquatic research culturists as food for many species of biological and toxicological test organisms prior to and during experimental periods (Beck et al., 1980). They are organisms of widespread distribution, occurring in over 150 known locations around the world. Historically, Artemia sp. from different geographical locations have been considered equivalent as nutritive sources for aquatic organisms. However, this assumption has been shown to be untrue.

Biological performance studies utilizing several geographically different strains of brine shrimp have been carried out recently at the EPA Environmental Research Laboratory (ERL) in Narragansett, R. I. Differences in growth and survival of the test organisms was attributed to the strain of Artemia sp. used.

Larvae of two species of crab, Rhithropanopeus harrisii and Cancer irroratus proceeded through normal zoeal development and metamorphose to the first crab stage successfully when reared on nauplii hatched from cysts from four localities, Macua, Brazil; Shark Bay, Australia; Margherita di Savio, Italy; and San Francisco Bay, California, USA (Johns et al., 1980). However, when these two crab species were fed either Artemia sp. hatched from Great Salt Lake, Utah (Utah), or San Pablo Bay, California

(SPB) cysts, abnormal development occurred, usually ending in mortality during the metamorphosis to the first crab stage.

Klien-MacPhee et al. (1980) showed that winter flounder, Pseudopleuronectes americanus cultured with Artemia sp. from Utah and SPB sustained significantly greater mortality than those flounder grown on the other strains. Atlantic silversides, Menidia menidia, also exhibited increased mortality when fed the Utah and SPB brine shrimp nauplii (Beck et al., 1980).

There have been other reports of lower survival and growth of test organisms cultured with the Utah strain. Larvae of the prawn, Palaemon serratus developed abnormally and expired during metamorphosis when reared on Artemia sp. from Utah (Forester and Wickins, 1967; Reeve, 1969; Little, 1969; Wickins, 1972). Other crustaceans, Cancer magister (Reed, 1969), Rhithropanopeus harrisii, Hexapanopeus angustifrons and Libinia emarginata (Bookhout and Costlow, 1970), responded in a similar manner when fed brine shrimp from Utah. Shelbourne (1966, unpublished report to the British Whitefish Authority) working with the larvae of sole, Solea solea and Slobodkin (1968) working with plaice, Pleuronectes platessa, larvae both reported reduced survival of the larvae when fed Utah Artemia sp. nauplii.

Bookhout and Costlow (1970) attributed the increased mortality of their test animals to secondary poisoning caused by high levels of DDT (7050 ppb) found in the Utah

brine shrimp. Wickins (1972), analyzing samples of Utah Artemia sp. collected the same year, was unable to find excessive amounts of DDT. After extensive analysis of the lipid fraction and trace minerals, Wickins concluded that there must be some biochemical deficiency in the brine shrimp, thus providing an incomplete nutrient source for the prawn. Shelbourne (in Provasali, 1979) and Oppenhiemer (in Provasali, 1979) both suggest that a biochemical deficiency in the Utah Artemia sp. is the causal agent of the observed mortalities.

Biochemical analysis of the Artemia sp. strains tested at the EPA-ERL was performed in the laboratories of the Department of Food Science and Technology, Nutrition and Dietetics at URI.

Analysis of protein and amino acid quality in five strains of Artemia sp. by Seidel et al. (1980) showed no obvious cause for the mortalities observed upon feeding the brine shrimp to the test organisms. Soejima et al. (1980) found no obvious difference in the carotenoid content, the main pigment being canthaxanthin. He did however detect the presence of chlorophyll in the SPB Artemia sp..

Analysis of the lipid and fatty acid composition by Schauer et al. (1980) found that both strains which caused mortalities may be deficient in specific essential fatty acids, while the other strains were not. They proposed that the lack of in these fatty acids may not be sufficient to cause the observed mortalities but might be capable of

producing a nutritional stress which could be aggravated by some toxic compound or other stressful condition.

Olney et al. (1980), using multi-residue pesticide analysis was able to show that DDT probably was not the causal agent for mortality. The Italian strain showed excellent growth and survival but had the highest level of DDT contamination (395 ppb) of the strains tested. This conclusion, contrary to that of Bookhout and Costlow (1970), suggests that DDT was not the primary problem. However, Olney et al. (1980) did not rule out entirely the possible effects of other pesticides or chlorinated hydrocarbons.

Winter Flounder

The winter flounder, Pseudopleuronectes americanus, is one of several test animals routinely cultured at the EPA-ERL in Narragansett. The eggs and larvae of P. americanus have been used as bioassay organisms (Voyer et al., 1977). Their spawning season generally runs from January through May and each female produces an average of a half million eggs (Klien-MacPhee, 1978). The relatively high rate of survival through metamorphosis, approximately 50 to 60% (Klien-MacPHEE, 1978), allows for the use of the larvae from a single female for an experiment. This means a relatively homogenous group of animals is available for testing, an important factor in the toxicological evaluation of compounds (Matsumura, 1975).

Winter flounder are an economically important species (Howe and Coates, 1975). They spend their larval development time in estuaries (Klien-MacPhee, 1978). It has been shown that juvenile forms of aquatic animals are generally more susceptible than adult forms to chlorinated hydrocarbon contamination, particularly pesticides (Butler, 1968; McKim, 1977). Finally, estuaries act as a funnel that a water borne pesticide will pass through on its way to the ocean. This funneling action concentrates the watershed runoff of the pesticides in the estuary, particularly during those seasons when aquatic organisms are the most susceptible, i.e. the spring and the summer. Therefore knowledge of the effects of pesticides on winter flounder is of importance.

Chlorinated Hydrocarbons

Chlorinated hydrocarbon residues are probably the most persistent chemical compounds man has added to the environment. The fate of these compounds and their resultant degradation products has been studied intensively. Woodwell et al. (1971) proposed that the final resting place of DDT residues is the ocean and that they are transported there via the air and via surface run-off into rivers, then to estuaries and finally the ocean. Because of this method of introduction of chlorinated hydrocarbons to the water and the possible accidental direct spraying or spillage of these

compounds into water systems, Holden (1973) suggests that research into toxicities, effects, bioaccumulation patterns, etc. of these compounds in aquatic organisms is of primary importance.

The toxicity, bioaccumulation, biodegradation and physiological effects of the DDT family have been well documented (Macek, 1968a and 1968b; Buhler, 1969; Macek and Korn, 1970; Johnson et al., 1971; Warlen, 1974; Sanborn et al., 1975; Harding and Vass, 1977; Harding and Vass, 1979). Cyclodienes, such as dieldrin and cis-chlordane, are generally more toxic than DDT to insectan fish food species (Brown, 1978). Endrin, sometimes used as a piscicide, has been shown to reduce growth in rainbow trout, Salmo gairdneri (Grant and Mehrle, 1975). Polychlorinated biphenyls (PCBs) have been shown to cause several different effects from decreasing ecdysis in the fiddler crab, Uca pugilator (Fingerman and Fingerman, 1979), to decreasing egg hatchability, mean time to hatching, and alevin survival of coho salmon, Oncorhynchus kisutch (Halter and Johnson, 1974).

Technical chlordane is a commercial insecticide that is a mixture of chlorinated cyclodienes containing more than twenty-six compounds. Approximately 43% of this mixture is composed of chlordane (1,2,4,5,6,7,8,8-octachloro-2,3,3a,4,7,7a-hexahydro-4,7-methanoindene) of which two configurational isomers, cis- and trans-chlordane exist (Cochrane and Greenalgh, 1975). It is a persistent stomach and contact poison to most species of insects and it shows a

marked residual toxicity (Spencer, 1973). It is considered a broad spectrum insecticide for home and industrial use (Spencer, 1973). The most common target species include corn insects, ants and termites (Brown, 1978). It is considered a moderately toxic insecticide with an acute oral LD50 of 457 - 590 mg/Kg (rat), with heptachlor and cis-chlordane considered the most toxic isomers to mammals. Cis-chlordane is considered the most toxic isomer to insectan fish food species (Brown, 1978).

Cis-chlordane is stable in the environment and in the fish (Feroz and Khan, 1979). Its high toxicity to fish is attributed to its stability (Feroz and Khan, 1979). Generally trans-chlordane is metabolized to oxychlordane and eventually excreted from the body (Schwenmer et al., 1970; Roberts et al., 1977). Feroz and Khan (1979) showed a high level of chlordane is present in the gall-bladder and the bile in Cichlasoma sp. and indicated that this may be a major path of excretion.

Dieldrin (1,2,3,4,10,10-hexachloro-exo-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo,exo-5,8-dimethanonaphthalene), HOED, is a chlorinated cyclo diene insecticide. It is a persistent contact and stomach poison (Spencer, 1973) used to control cotton, soil and other crop pests. It is considered of moderate toxicity with an acute LD50 of 100 mg/Kg (rat) (Spencer, 1973). Its use and the use of aldrin were prohibited in 1974 in the USA because they were believed to be carcinogens.

In the environment and the organism aldrin is transformed to dieldrin, a more toxic substance (Brown, 1978). Dieldrin is stable in the aqueous environment (Lu et al., 1975). Crabs remove dieldrin very rapidly from the water (Petrocelli et al., 1975). Secondary poisoning in crabs eating flesh contaminated with dieldrin has been shown by Brown (1978). He states that dieldrin is toxic to fish when water concentrations are at ppb levels. Two crabs, Leptodius floridanus and Panopeus herbstii could not complete larval development and died when water concentrations of dieldrin exceeded 10 ppb (Epifanio, 1971). Crabs generally depurate dieldrin rapidly (Petrocelli et al., 1975).

Bioconcentration, Bioaccumulation and Depuration

Two mechanisms are present in nature which allow for aquatic organisms to accumulate chlorinated hydrocarbons. One, bioconcentration, is defined as the accumulation of a dissolved substance in aquatic organisms by transport of the chemical through the respiratory surfaces and/or the epidermal membrane (Veith et al., 1979). This definition excludes the accumulation by consumption of and assimilation from contaminated foods.

Chlorinated hydrocarbons such as PCBs, DDT, chlordane and dieldrin are lipophilic. It has been shown that these lipophilic compounds behave in nature in a manner that is

correlated with their N-octanol/water partition coefficient (Branson et al., 1975; Chiou et al., 1977; Clayton et al., 1977), i.e. they are hydrophobic and therefore partition into the lipid fraction of the organism. Branson et al. (1975) suggest that the partition coefficient may be an accurate indicator of a residue's potential to accumulate. Southworth et al. (1978) agrees with this hypothesis and goes on to suggest that short-term biological tests, i.e. days in length, are as accurate as long-term, i.e. weeks, in determining the bioconcentration potential of a residue.

The second mechanism, bioaccumulation, is defined as the accumulation of chemical residues by ingestion of and assimilation from contaminated food, as defended by Macek and Korn (1970). This hypothesis was supported at first because evidence in nature indicated that animals at higher trophic levels had higher concentrations of the chlorinated hydrocarbons, as shown by the Clear Lake example (Cremlyn, 1978). Early research had indicated that bioaccumulation of chlorinated hydrocarbons from the food was probably more significant than bioconcentration from the environment. Macek and Korn (1970) reported that uptake of DDT from food was 10 times greater than from the water in the rainbow trout. It has been shown that the amount of chlorinated hydrocarbons accumulated by oral ingestion is dose dependent (Robinson, 1967; Buhler et al., 1969; Macek and Korn, 1970; Warlen, 1974; Warlen et al., 1977).

Macek et al. (1979) recently showed that

bioaccumulation is only greater for DDT and its analogues, when the only source of contamination is the food.

Bioconcentration, according to them, is the predominant pathway if there is any contamination in the environment.

Epifanio (1973) showed that uptake of dieldrin from the water was 19.1 times faster than from the food for the larvae of Leptodius floridanus.

Depuration is a process that occurs simultaneously with bioconcentration and bioaccumulation by which the organism detoxifies and excretes the various pollutants it accumulates. Depuration is constantly occurring and therefore must be taken into account when attempting to determine bioconcentration factors. Several researchers have developed models which accommodate depuration (Branson et al., 1975; Southworth et al., 1978; Norstrom et al., 1976; Harding and Vass, 1979; Veith et al., 1979). Half-lives of several chlorinated hydrocarbons, in aquatic organisms, have been determined (Reinart et al., 1974; Warlen et al., 1977; Gakstatter and Wiess, 1967). Macek et al. (1979) state that half-life determination is an important measure of the bioaccumulation potential of a compound.

Invertebrates play three significant roles in the accumulation and maintenance of organochlorine residues in the environment (Johnson et al. 1971). They rapidly concentrate the pesticide from the environment, thereby preventing its dispersion or diffusion. They raise low environmental levels to relatively high body levels. They

finally pass the contamination to an animal at a higher trophic level that would not necessarily have encountered the contaminant.

The lipid content of an organism is an important factor when considering the bioconcentration potential of a compound. The lipid pool of an individual is a reservoir into which the organochlorine pesticides partition. The persistence of chlordane in the northern redhorse sucker, Moxostoma macrolepidum, was shown to be directly related to the lipid content of the individual fish (Roberts et al., 1977). They go on to suggest that as the lipid pool decreases in size those compounds that can be metabolized are, while those that can not be metabolized are concentrated to higher levels. This may be a mechanism of toxicity (Schauer, 1979).

APPENDIX E

Speculative Discussion Winter Flounder Lipid Results

The lipid and FAME data for the winter flounder (Table 5) do not clearly show a pesticide effect on lipid metabolism. However, they may contain information concerning the nutritional requirements for lipids and the metabolism of lipids in flounder.

There was a substantial decrease of percent lipid and percent FAME in the experimental fish when compared to the initial fish. This suggests that either these compounds were being diluted due to a high rate of growth or that the lipids were being metabolized due to a low dietary lipid intake. Because the growth was not excessive during the experimental period, it will be assumed for the rest of this discussion that the flounder were receiving a quantitatively low diet.

The evidence indicates that the fish were in an abnormal nutritional state. It has been shown that when a fish is nutritionally satisfied, the fatty acid profile for the fish resembles that of its food supply (Lee et al., 1967; Owen et al., 1972; Seidel et al., 1980). Since a comparison fatty acid profiles treatment fish and the treatment Artemia sp. shows a general lack of similarity, the fish were probably in an abnormal nutritional state. It has been suggested that the excessively low lipid levels, in fact, are evidence of lipid stress (P.S. Schauer, personal communication).

It is assumed that when an animal requires more energy

than is provided in the diet it will metabolize adipose tissue, mainly composed of triglycerides, that has been stored during times of excess. Lipids associated with functional components will be preferentially conserved in this situation.

Steelhead, Salmo gairdneri, fry have been shown to retain long-chain polyunsaturated fatty acids (PUFA) and the saturated fatty acids 16:0 and 18:0 (Hayes et al., 1973). Turbot, Scophthalmus maximus, have been shown to conserve PUFA when placed on a fat free diet and the PUFA are preferentially allocated to the phospholipids at the expense neutral lipids (Covey et al., 1976). Phospholipids are a functional lipid associated with membrane systems. Brockerhoff et al. (1968) showed that phospholipids are composed of PUFA, usually esterified to the internal position of the glycerol backbone, a saturated or monoenoic fatty acid esterified to one external positions (sn-1) and the phosphate group located at the other external position (sn-3, in stereospecific nomenclature).

If the winter flounder are truly stressed, as the evidence suggests, then the observed fatty acid profiles may be composed of those fatty acids associated with functional lipids. Closer inspection FAME profiles should reveal information pertaining to the fatty acid requirements and metabolic pathways of lipids in the stressed fish.

Conservation of some fatty acids by the treatment fish

seems to have occurred. The fatty acids 16:0, 18:0, 20:3w3/C20:4w6 and 20:5w3 appear in higher relative proportions in the treatment than in the initial fish. This indicates that the fish were preserving these fatty acids instead of metabolizing them. This is not unreasonable considering the importance of fatty acids to the phospholipid composition. This also suggests that these fatty acids may be required to some extent by the flounder. Hayes et al. (1973) showed steelhead, Salmo gairdneri, fry tend to accumulate these fatty acids during development. While turbot can utilize high levels of 18:3w3 for growth, a better growth conversion was produced when the fish were fed low levels of long-chain PUFA w3 series (Leger et al., 1979). Owen et al. (1975) suggest that turbot are unable to desaturate dietary fatty acids, therefore making the supplementation of PUFA in their diet necessary.

An interesting association exists between the 20:3w3/C20:4w6 and the 18:3w3 fatty acids. The treatment fish had higher levels of 20:3w3 than the initial fish. This indicates that the fish were preferentially conserving 20:3w3 and/or the 18:3w3 was being elongated. Chain elongation occurs to a limited extent in turbot (Cowey et al., 1976). The loss of 18:3w3 suggests that it was occurring in these flounder also.

Metabolism of fatty acids 16:1w7 and 16:2w4 appears to have occurred in the treatment fish. The relative levels of

acids are lower in the treatment fish than the initial fish. The slight increase in the percentage of 14:0 may indicate its importance to some functional system. However, there is no evidence in the literature to support this view.

The fatty acid 18:1w9 composed a quarter to a third total FAME profile fish. Although it did decrease slightly in the treatment fish, the drop was relatively small. This indicates that it was probably being conserved

Although the winter flounder feeding experiment was not designed to provide information about the lipid requirements fish, it appears that some conclusions can be drawn about the relative importance of certain fatty acids to the flounder. Conservation and perhaps selective accumulation of 16:0 and 18:0 has occurred. Conservation and possibly synthesis of 20:3w3 from 18:3w3 occurred. The conservation of 20:5w3 was evident also. This information suggests that the PUFA are a necessary component fish and are conserved, accumulated and synthesized when possible. Chain elongation of 18:3w3 to 20:3w3 might be happening. However, there is no evidence of desaturation to 20:5w3. Finally, the fact that acids 16:0 and 18:0 are conserved rather than metabolized for energy indicates a possible relationship to the PUFA and phospholipid composition.

It is interesting to note that the fish, which were stressed nutritionally were still able to survive the abnormally high pesticide loads. This emphasizes the

importance of continued research in the field of nutrition and its effects on toxicology.

APPENDIX C

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