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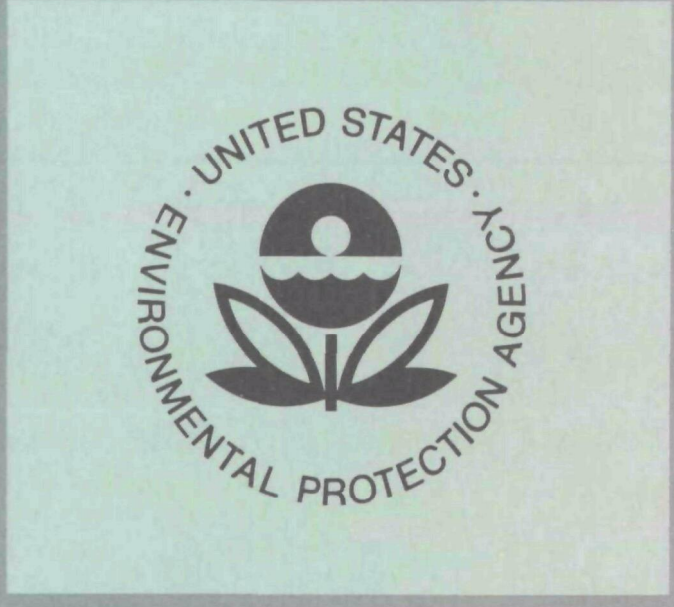
May 1976

Ecological Research Series

ACUTE TOXICITY OF CERTAIN PESTICIDES TO ACARTIA TONSA DANA



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ACUTE TOXICITY OF CERTAIN PESTICIDES TO *ACARTIA TONSA* DANA

by

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ABSTRACT

The acute toxicity to the marine copepod Acartia tonsa Dana of four technical grade insecticides was determined by bioassay using standardized procedures, homogeneous populations and constant laboratory conditions. At a water temperature of 17 ± 1°C, the 96-hour median lethal concentrations or tolerance limits for methyl parathion, Azodrin, diazinon and toxaphene were computed as 0.89 milligrams per liter, 0.24 milligrams per liter, 2.57 micrograms per liter and 7.2 nanograms per liter, respectively. Residue analysis for diazinon at zero and 96-hour exposure time revealed that the amounts of diazinon uptake by three algal organisms is greater than amounts concentrated by the copepod. The toxicity of higher concentrations above 2.0 ppm (2 milligrams per liter) has offset copepod uptake, while at lower concentrations, quantities concentrated by Acartia are negligible.

Concurrently, the world literature was surveyed for supporting toxicity data of these chemicals to closely related species.

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SECTION I

CONCLUSIONS

From experience in this study and from the work of others the following conclusions can be made:

1. It is possible to culture estuarine copepods for several generations when due attention is made to curb contamination of water and culture media from bacterial growth and other incidental contaminants.

2. Relative toxicities of pesticides can be obtained only with homogeneous populations (reasonable uniformity in age, sex, size and nutritional status of the animals exposed).

3. Stability of extrinsic factors such as temperature, photoperiod and population density are also important for interpretation of bioassay results.

4. Methods of dispensing the algal media, the water from one vessel to another, the stability of salinity levels, and knowledge of the mode of action and physical properties of the pesticides are important parameters for conducting bioassay.

5. Acute toxicities of toxaphene in the parts per trillion range, diazinon in the parts per billion range, and methyl parathion and Azodrin in the parts per million range to the copepod Acartia tonsa suggest possible pesticidal specificity.

These data augment the results obtained from pesticide residue analysis in water samples performed by the National Monitoring Program at various locations of the United States.

SECTION II

RECOMMENDATIONS

1. Reference organisms obtained from recognized reference laboratories should be used in starting cultures of Acartia, the flagellates and diatoms.

2. Acute 96-hour tests are too restrictive for sensitive species. The measurement of susceptibility or tolerance should be based on longer exposure duration, during which the peak effect of slow-acting chemicals can be detected.

3. Natural conditions should be simulated as far as possible. Natural sea water of suitable quality with the appropriate adjustment of salinity should be used as test and culture media.

4. A short term continuous flow test is preferred over a static one.

5. Exposure techniques should be standardized so that workers in various laboratories can communicate and verify their results in comparable terms.

6. Pesticide batches for bioassay should be selected from recently manufactured samples and, as far as possible, standard reference chemicals of known composition should be utilized for controls.

7. Five replicates and 20 organisms per replicate per concentration should be used to reduce individual variations in response.

8. Test mortalities of sensitive species must be given the opportunity of recovery within a reasonable period after terminating observations at any one concentration, by transferring treated individuals into food-containing test medium without the pesticide. Recovered individuals should not be counted as dead.

9. More than one closely related species, or preferably two, biologically distinct species, should be tested at the same time under similar conditions. This will allow detecting species specificity of the chemical tested. If a chemical shows irratic behavior in the dose-response curve due to some detoxification mechanisms this can be cross-checked with the other species.

SECTION III

INTRODUCTION

The scope of this contract was three-fold:

(1) to produce levels of copepod populations sufficient for toxicity bioassay (requires production of sufficient levels of three algal species as food organisms);

(2) to determine the acute and chronic toxicity of four selected pesticides and define their median lethal concentration level. In the chronic phase, to define the effect of repeated exposure to the pesticides on the growth and development of two generations of the copepod; and

(3) to review the literature for work of related scope.

Considerable effort was directed to raising several generations of the copepod, to insure the development of healthy vigorous laboratory stock, and to standardize the rearing protocol so that variations in individual response to low concentration levels were minimized.

Acute or short term toxicity tests were carried out in accordance with rigid procedures to safeguard against the influence of contamination and experimental error. Pesticide concentrations were prepared and stored in a separate building and only on dosing were they brought to the Bioassay Laboratory. Conditions of temperature, light and salinity were kept constant and uniform throughout. Data were analyzed by a computer program based on the Litchfield and Wilcoxon method of statistical analysis. Review of the literature was accomplished by consulting a wide range of abstracting journals and information retrieval systems available to private foundations, universities and government agencies.

SECTION IV

CULTURE DEVELOPMENT

SEA WATER

As provided in the contract (Article II of the schedule), artificial sea water based on a formula proposed by Kester et al (1967) was prepared. The object was to reduce travel time to the nearest ocean source, provided that artificial sea water at 20% salinity level could be produced in the laboratory that would sustain various stages of the copepod's life cycle through at least two generations. A pilot experiment was conducted to ascertain this point. Results revealed that copepods originating from natural estuarine water with a salinity level not exceeding 12% did not survive as adults on gradual adaptation up to 20% in artificial sea water. Likewise, adult copepods reared in Kester's artificial sea water at 32% at the Naval Research Laboratory, Washington, D.C., did not survive at lower salinities of natural sea water, when a few copepods were brought to our laboratory. Consequently, the artificial sea water was judged unsuitable for copepod culture development.

With similar experiments, natural sea water yielded favorable results at the conditions prevailing at our laboratory. Estuarine copepods survived and laid eggs; the eggs developed at graded levels of salinity ranging from 29% natural sea water to 11.6% (or 40% sea water). It was therefore concluded that in order to reduce rearing and sea water batch variations and insure high adult survival and reproductive rates, copepods originally obtained from the Chesapeake Bay at a salinity level of 12% could very well be adapted to higher salinities of natural sea water. In all experiments, copepods were exposed to a salinity decrease or increase of 4% at a time for a period of 24 hours (Lance 1963). Subsequent transfers of copepods into natural sea water at a salinity level of 20% gave encouraging results, thus this concentration was finally chosen for all sea water as culture and test media. Natural sea water was diluted to this salinity level by the addition of glass-redistilled water. All water batches were stored at 4°C prior to use. Diluted sea water was left overnight in an open vessel during which time 80% levels or higher of oxygen saturation concentration and temperature equilibrium were attained.

ALGAL CULTURES

Three species of marine algae, Isochrysis galbana, Rhodomonas baltica, and Cyclotella nana were grown axenically in enriched sea water medium at a salinity level of 20%. The algal medium used was the "f" medium of Guillard and Ryther (1962), and its composition is outlined in the appendix. Before autoclaving, the medium was buffered with 50 ml per liter of a 1% solution of tris (hydroxymethyl aminomethane) in glass-redistilled water, and its pH was adjusted to 7.5. All stock solutions for the algal medium were prepared with glass-redistilled water.

Final algal medium was autoclaved in quantities of 25 ml in 50 ml screw-cap test tubes fitted with Teflon-lined caps, and 100 ml quantities in Erlenmeyer flasks at a temperature of 121°C and a pressure of 15 p.s.i. for no longer than 20 minutes. Thereafter, two days were allowed for pH equilibration before inoculation of new cultures. Prior to subculture and when contamination of any culture was suspected, sterility of the cultures was checked. Sterility tests were performed by inoculating the sterility test medium with 1.0 ml of the test sample. Caps were tightened, and the inoculated tubes were stored in a dark area for one to three days (maximum of two weeks). The appearance of growth or turbulence in the test broth indicated presence of contamination in the cultures tested. Composition and preparation of the sterility test medium is described in the appendix.

Marine algal cultures were grown in sterile enriched sea water medium under constant laboratory conditions. Room temperature was 16 $\pm$ 1°C throughout, and culture tubes were placed in slightly inclined trays under continuous illumination of 200 foot-candles originating from a fluorescent, cool white light source.

Pure clones of Rhodomonas baltica (H_5), Isochrysis galbana (H_2) and Cyclotella nana (C_5) obtained from the Naval Research Laboratory formed the starting inocula. Culture tubes containing 25 ml of sterile enriched medium were inoculated weekly with approximately 1.0 ml of mature algal cultures. Larger quantities of algae were grown in Erlenmeyer flasks and in a special fill and draw-off system. Algal densities were determined by direct measurement with a Model F Coulter counter. Mature algal cultures developed 11 to 14 days post-inoculation. Average cell densities attained at the optimum stage of development for providing new inoculations and for feeding copepod cultures were approximately $2.3X10^6$ cells per ml for Rhodomonas baltica, $5.7X10^6$ cells per ml for Isochrysis galbana and $3.8X10^6$ cells per ml for Cyclotella nana (see Table 1 and Figure 1).

TABLE I: AVERAGE GROWTH RATES OF R. BALTICA (H_5), I. GALBANA (H_2), AND C. NANA (C_5) CULTURED AT HAZLETON LABORATORIES, INC., 1971-1972

Average Algal Densities (cells/ml)

	R. baltica	I. galbana	C. nana
Average initial density at inoculation	8.7×10^4	2.3×10^5	2.2×10^5
Day post-inoculation			
6	1.5×10^6	4.6×10^6	2.9×10^6
13	2.3×10^6	5.7×10^6	3.8×10^6
20	2.2×10^6	6.3×10^6	3.9×10^6
27	1.8×10^6	6.6×10^6	3.6×10^6

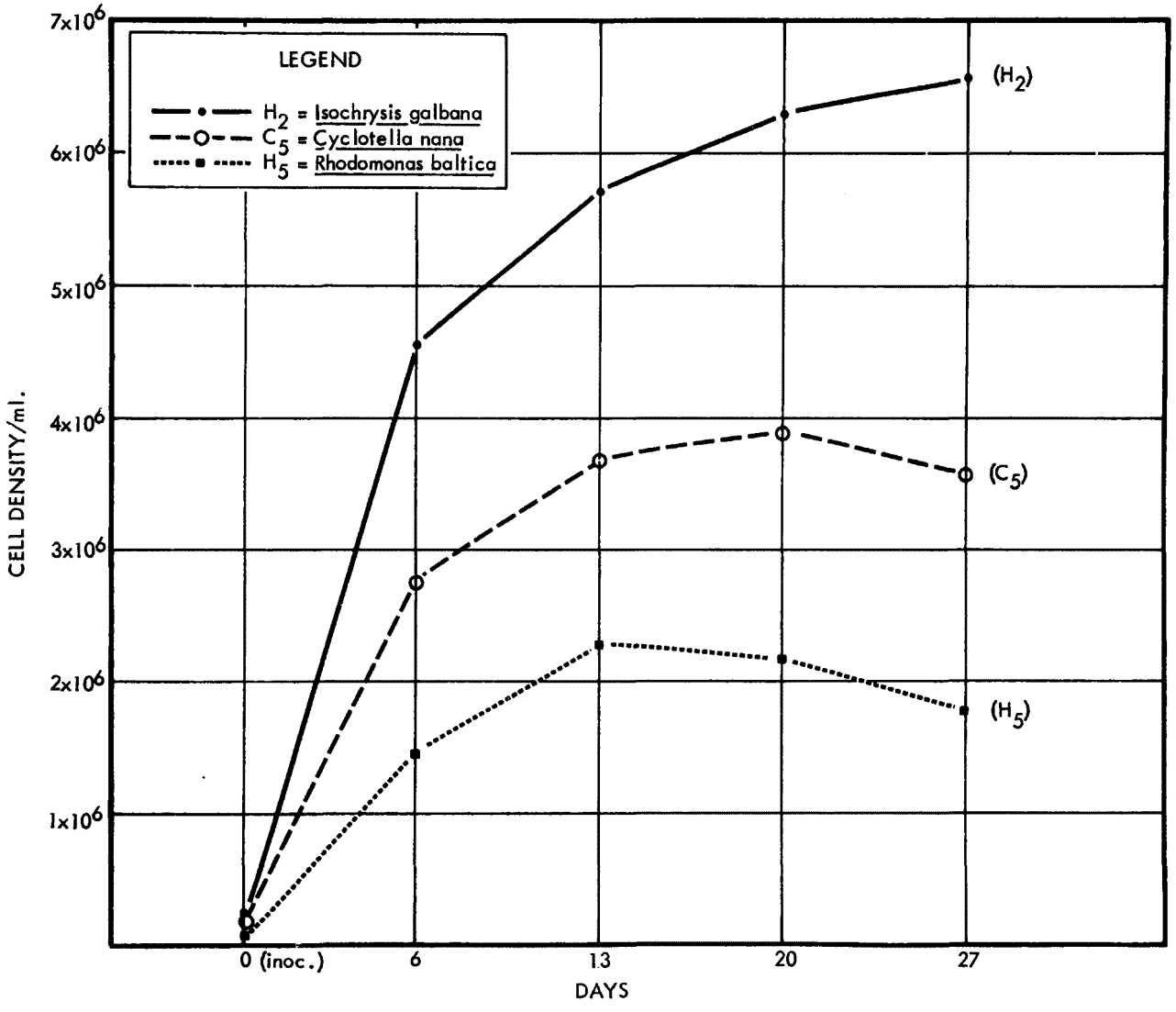


Figure 1 Growth curves of algal clones

Stock copepod cultures were fed the standard daily ration suggested by Wilson and Parrish (1971) which is a combination of the three algal species produced. Cell densities for the feeding regimen were as follows:

Species	Feeding Density
R. baltica	$3.00X10^6$ algal cells per liter of copepod culture per day
I. galbana	$5.70X10^6$ algal cells per liter of copepod culture per day
C. nana	$7.15X10^6$ algal cells per liter of copepod culture per day

The above densities were found to promote optimum copepod growth and development. Algal cultures showing any signs of contamination were immediately discarded.

COPEPODS

Acartia tonsa (Dana) was originally obtained from tows in the Chesapeake Bay, by the Chesapeake Biological Laboratory, Solomons, Maryland. Copepods were cultured in natural sea water medium at an adjusted salinity level of 20%. Stable laboratory conditions were maintained throughout the rearing period. Room temperature was 17° ± 1°C and cool white fluorescent illumination was supplied at a photoperiod of 14 hours day and 10 hours night cycle. The rearing laboratory was kept as free from contamination as possible.

All glassware was cleaned thoroughly by rinsing five times with acetone, washing in hot soapy water, rinsing with distilled water, soaking in 3N hydrochloric acid, and rinsing five times with acetone. After air drying and prior to use, dishes or vessels for copepod culture, bioassay tests or chemical samples were autoclaved. There procedures insured freedom from contamination with bacteria and adsorption of trace levels of detergents.

Copepods were cultured in 2.3 liter pyrex crystallizing dishes (190 mm x 100 mm) covered with a flat piece of glass. Approximately 200 adult copepods were cultured in one liter of sea water, and each liter of stock culture was fed a total algal cell density of $1.6X10^7$ per day comprising the three species.

Once a week, stock copepod cultures were transferred into fresh sea water medium to reduce the possibilities of accumulation of toxic metabolites and bacterial contamination. For transfers, nylon-mesh Nitex netting was used. Separation of older copepodites and adults from immatures was made with Nitex #63-T. Transfers of the entire culture or of juvenile and egg stages were made with Nitex #20-T.

Before the transfer of organisms, heavy accumulations of algae and debris were removed from the old culture dish with a dropping pipette. Used sea water medium was slowly drained off with the aid of a piece of Nitex netting, sized according to mode of transfer or separation of older stages from remaining populations and fitted snugly to the rim of the dish by a holding ring. The net holding copepods was quickly inverted and dipped into a clean sterile dish containing fresh sea water. Copepods were then immediately fed three times their normal daily algal ration (Wilson and Parrish, 1971).

Generation cages were adapted for production and age standardization of populations. Twenty to 30 adults were placed in each generation cage made of a plexiglass cylinder (125 mm X 90 mm) fitted with Nitex #63-T screen netting approximately one inch from the base. This procedure allowed eggs laid by females to drop through the netting and hatch without the possibility of cannibalism by adult copepods (Heinle, 1970). In age standardization of stock populations, adult cages were removed after 24 hours. Developing adults were then set aside for testing when gravid females appeared. At our laboratory conditions*, the average length of each developmental stage of Acartia tonsa was as follows:

Stage	Length in Days
Egg (newly oviposited)	1
Nauplius (6 instars)	7
Copepodite (6 instars)	6
Adult (until gravid)	3
Total life cycle	17

*During the course of technique development, the following references were especially useful on the biology and behavior of Acartia tonsa: Conover (1956, 1959), Lance (1965) and Heinle (1966). In the culture of copepods, emphasis was placed on some extrinsic culture requirements based on the experiences of Clarke (1959), Neunes and Pongolini (1965), Zillioux and Wilson (1966), Corkett (1967, 1968) and Heinle (1969).

SECTION V

BIOASSAY PROCEDURES

TOXICANTS TESTED

The following materials were tested: (1) 80% solution of technical grade methyl parathion; (2) 80% solution of technical grade Azodrin; (3) 97.6% solution of technical grade diazinon; and 100% technical grade toxaphene (see Appendix for further details on the toxicants).
The following procedures were followed in biological assay:

Pre-exposure

Ten adult copepods (of both sexes), of gravid age, were individually transferred by means of a capture pipette into a small beaker containing 20 ml of sea water (20% salinity level). Four such beakers were prepared for each concentration per insecticide. Prior to dose-ranging, 3 blind doses, using 10 copepods in 100 ml sea water per container at an exposure of 24 hours, were made to gain an impression of the highest dose to be finally used. Insecticide doses from which a high dose and subsequent four to six lower concentrations on a logarithmic regression scale were selected. In some instances, dose-ranging was based on half the higher dose. Higher concentrations were prepared in appropriate solvent (double-distilled water for Azodrin and acetone for other pesticides), using a precision Hamilton microliter syringe. Lower concentrations were obtained from the parent high concentration by serial dilutions. All insecticide solutions were tightly capped, sealed with Parafilm and refrigerated. Thirty minutes prior to use, they were taken out and adjusted to room temperature before dosing.

Dosing

A number of 400-ml beakers formed the test vessels for the acute test. Eighty milliliters of sea water were dispensed into each of 24 to 32 beakers. 0.1 ml of each parent concentration was added to each test beaker to produce the desired concentration level before the addition of a 20-ml sea water volume containing test organisms. $4.0X10^5$ cells per ml of test media of a ratio of 2:3.5:4.5 mixture of *Rhodomonas*, *Isochrysis* and *Cyclotella* algal species respectively were added prior to dosing. Beakers were covered with a glass plate. Comparable amounts of the solvent carrier were added to the solvent control beakers.

A parallel dish for each concentration without food organisms was similarly dosed and sent to the chemist for chemical analysis for residue determination at the conclusion of the acute test.

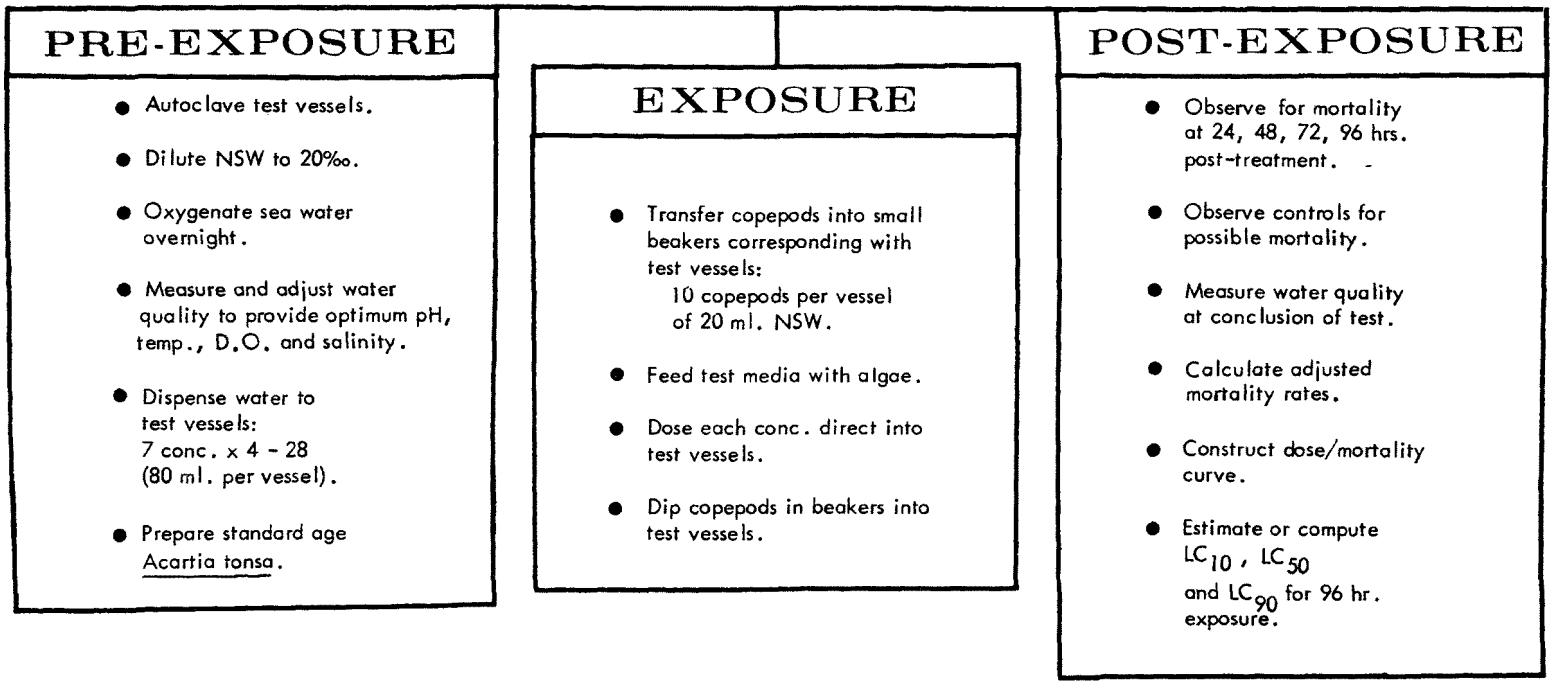
Post-Dosing Observation

Organisms were observed 24, 48, 72 and 96 hours post-exposure (times of exposure for each beaker were noted on the beaker, together with concentration level and replicate number). This included the number

of living and dead organisms in each replicate. Attempts made earlier in the tests to differentiate living from dead copepods by use of vital stains yielded inconsistent results. This method was abandoned in favor of probing with a fine needle on the thorax of dead adults to induce a response. Those exhibiting slow diving reaction, disoriented movement, tremor and discoloration were considered moribund and counted as dead individuals. Water quality measurement was made again on conclusion of the test.

LC_{50} values with confidence limits were computed, using a computer program based on the Litchfield and Wilcoxon (1949) method of statistical analysis. Figure 2 summarizes bioassay procedures.

Figure 2 Schematic plan of the procedure acute 96-hour test with pesticides



SECTION VI

RESULTS

As shown in Table 8, toxaphene is the most potent chemical of the four tested to Acartia tonsa. The LC_{50} value if 7.2 parts per trillion (nanograms/liter). Methyl parathion was the least toxic with a value of 0.89 ppm. Tables 2 through 7 give raw data, adjusted mortalities, and LC_{50} values of the four pesticides tested.

The order of toxicity from highest to lowest of the four pesticides is as follows: toxaphene, diazinon, Azodrin and methyl parathion. Azodrin seems to be about four times as toxic as methyl parathion, while diazinon is about 100-fold as toxic as Azodrin.

Unlike other cyclodiene insecticides, toxaphene is distributed throughout the animal body by the hemolymph and has no effect on the nervous system. Dehydrochlorination of this chemical under alkaline conditions of sea water may enhance its toxicity (Metcalf, 1955). Azodrin, on the other hand, is one of a few water soluble pesticides, but this solubility may enhance uptake and metabolism of sub-acute levels or may promote absorption throughout the organism and result in quicker toxic action. Chlorinated compounds, as a rule, are fat soluble, thus can be stored in fat intact at concentrations greater than the amounts ingested.

Organophosphorous compounds, such as diazinon, have different physical properties and modes of action. They hydrolyze in alkaline water media and inhibit estrases of invertebrates (Metcalf, 1955). Toxic phosphorous compounds may produce different physiological manifestations because of differences in stability, solubility and ability to inhibit the various cholinesterases and aliesterases in the animal body.

The uptake of these pesticides by both algae and copepods is an indication of their physiological tolerance and contributes to the rates affecting the process of detoxification whereby conversion to non-toxic metabolite generally occurs at sub-lethal levels (Metcalf, 1955). To calculate the amounts metabolized, either the remaining pesticide residue in the aqueous phase of the various dose levels or the residue in the organisms must be determined. Minute quantities of some pesticides in the parts per billion range cannot be readily determined with available analytical techniques from small quantities of test material. This problem must be resolved before data derived from chemical analysis can be meaningful. In the diazinon repeat test, the amounts concentrated by both algae and copepods are summarized in Table 9. Greater amounts were taken up by algae than by the copepods. The lethality of higher concentrations above 2.0 ppm (2 mg./l) has apparently offset copepod uptake, while at lower concentrations, quantities concentrated by Acartia are negligible.

Table 2: RESULTS OF ACUTE 96-HOUR EXPOSURE OF ADULT *ACARTIA TONSA* TO TECHNICAL METHYL PARATHION (10 COPEPODS EXPOSED PER REPLICATE)

A = Alive
D = Dead T = Total

Acute Toxicity Tests

exposure (series A)

dose levels	replicates	Raw data from individual replicates at post-exposure: 24 hrs. A	24 hrs. D	48 hrs A	48 hrs D	72 hrs. A	72 hrs. D	96 hrs. A	96 hrs. D	- A	- D	- A	- D	- A	- D	Percentage actual test mortalities from raw data: 24 hrs. A	24 hrs. D	48 hrs. A	48 hrs. D	72 hrs. A	72 hrs. D	96 hrs. A	96 hrs. D	- A	- D	- A	- D	- A	- D
conc. level 1 5.0 p.p.m.	1	1	9	0	10	0	10	0	10																				
	2	4	6	0	10	0	10	0	10																				
	3	1	9	0	10	0	10	0	10																				
	4	1	9	0	10	0	10	0	10																				
	T	7	33	0	40	0	40	0	40							%	82.5		100		100		100						
conc. level 2 2.5 p.p.m.	1	5	5	4	6	4	6	3	7																				
	2	7	3	6	4	5	5	4	6																				
	3	6	4	5	5	4	6	4	6																				
	4	6	4	4	6	4	6	4	6																				
	T	24	16	19	21	17	23	15	25							%	47.5		52.5		57.5		62.5						
conc. level 3 1.0 p.p.m.	1	8	2	7	3	6	4	6	4																				
	2	9	1	9	1	9	1	9	1																				
	3	8	2	7	3	3	7	3	7																				
	4	9	1	7	3	5	5	3	7																				
	T	34	6	30	10	23	17	21	19							%	15.0		25.0		42.5		47.5						
conc. level 4 0.1 p.p.m.	1	7	3	7	3	7	3	7	3																				
	2	10	0	8	2	8	2	8	2																				
	3	10	0	8	2	8	2	8	2																				
	4	9	1	8	2	7	3	7	3																				
	T	36	4	31	9	30	10	30	10							%	10.0		22.5		25.0		25.0						
conc. level 5 0.01 p.p.m.	1	10	0	10	0	8	2	8	2																				
	2	8	2	9	1	9	1	8	2																				
	3	10	0	9	1	8	2	8	2																				
	4	10	0	9	1	9	1	10	0																				
	T	38	2	37	3	34	6	34	6							%	5.0		7.5		15.0		15.0						
conc. level 6 0.001 p.p.m.	1	9	1	9	1	9	1	9	1																				
	2	10	0	10	0	10	0	9	1																				
	3	10	0	9	1	9	1	9	1																				
	4	9	1	10	0	8	2	8	2																				
	T	38	2	38	2	36	4	35	5							%	5.0		5.0		10.0		12.5						
solvent control (acetone)	1	8	2	8	2	8	2	7	3																				
	2	10	0	10	0	10	0	9	1																				
	3	10	0	10	0	9	1	9	1																				
	4	10	0	10	0	10	0	10	0																				
	T	38	2	38	2	37	3	35	5							%	5.0		5.0		5.0		12.5						
untreated control	1	10	0	8	2	8	2	8	2																				
	2	10	0	9	1	9	1	9	1																				
	3	10	0	9	1	9	1	9	1																				
	4	10	0	10	0	10	0	10	0																				
	T	40	0	36	4	36	4	36	4							%	0		10.0		10.0		10.0						

Table 3: RESULTS OF ACUTE 96-HOUR EXPOSURE OF ADULT ACARTIA TONSA TO TECHNICAL AZODRIN (10 COPEPODS EXPOSED PER REPLICATE)

A = Alive
D = Dead T = Total

Acute Toxicity Tests

exposure (series A)

dose levels	replicates	Raw data from individual replicates at post-exposure: 24 hrs. A	24 hrs. D	48 hrs A	48 hrs D	72 hrs. A	72 hrs. D	96 hrs. A	96 hrs. D	- A	- D	- A	- D	- A	- D	Percentage actual test mortalities from raw data: 24 hrs. A	24 hrs. D	48 hrs. A	48 hrs. D	72 hrs. A	72 hrs. D	96 hrs. A	96 hrs. D	- A	- D	- A	- D	- A	- D
conc. level 1 0.0001 p.p.m.	1	9	1	9	1	9	1	9	1																				
	2	10	0	10	0	9	1	9	1																				
	3	9	1	9	1	9	1	9	1																				
	4	8	2	8	2	8	2	8	2																				
	T	36	4	36	4	35	5	35	5							%	10		10		12		12						
conc. level 2 0.001 p.p.m.	1	9	1	8	2	8	2	7	3																				
	2	9	1	9	1	7	3	5	4																				
	3	8	2	8	2	8	2	6	4																				
	4	9	1	9	1	8	2	7	3																				
	T	35	5	34	6	31	9	28	12							%	12		15		22		30						
conc. level 3 0.01 p.p.m.	1	8	2	8	2	7	3	6	4																				
	2	9	1	9	1	7	3	6	4																				
	3	8	2	8	2	6	4	6	4																				
	4	8	2	8	2	7	3	7	3																				
	T	33	7	33	7	27	13	25	15							%	17		17		32		37						
conc. level 4 0.1 p.p.m.	1	8	2	7	3	6	4	5	5																				
	2	7	3	7	3	5	5	5	5																				
	3	9	1	7	3	6	4	6	4																				
	4	8	2	7	3	7	3	6	4																				
	T	32	8	28	12	24	16	22	18							%	20		30		40		45						
conc. level 5 1.0 p.p.m.	1	6	4	6	4	5	5	4	6																				
	2	7	3	6	4	4	6	4	6																				
	3	8	2	7	3	6	4	4	6																				
	4	7	3	5	5	5	5	4	6																				
	T	28	12	24	16	20	20	16	24							%	30		40		50		60						
solvent control Dis-tilled Water*	1	10	0	10	0	10	0	9	1																				
	2	10	0	10	0	9	1	9	1																				
	3	10	0	10	0	10	0	10	0																				
	4	10	0	10	0	10	0	10	0																				
	T	40	0	40	0	39	1	38	2							%	0		0		2.5		5.0						
untreated control None	1																												
	2																												
	3																												
	4																												

*Azodrin Concentrations were prepared in double-distilled water as a solvent.

TABLE 4: RESULTS OF ACUTE 96-HOUR EXPOSURE OF ADULT ACARTIA TONSA TO TECHNICAL DIAZINON (10 COPEPODS EXPOSED PER REPLICATE)

A = Alive
D = Dead
T = Total

Acute Toxicity Tests

exposure (series A)

exposure (series B)

dose levels	replicates	Raw Data From Individual Replicates Post-Exposure														Percentage Actual Test Mortalities From Raw Data											
		24 Hrs.		48 Hrs.		72 Hrs.		96 Hrs.								24 Hrs.		48 Hrs.		72 Hrs.		96 Hrs.					
		A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D
conc. level 1 — 8.0 PPB	1	7	3					3	7																		
	2	9	1					6	4																		
	3	7	3					4	6																		
	4	9	1					4	6																		
	T	32	8	29*	11*	20*	20*	17	23							%	20.0		27.5*		50.0*		57.5				
conc. level 2 — 3.2 PPB	1	7	3					5	5																		
	2	8	2					4	6																		
	3	6	4					5	5																		
	4	9	1					6	4																		
	T	30	10	28*	12*	24*	16*	20	20							%	25.0		30.0*		40.0*		50.0				
conc. level 3 — 1.6 PPB	1					6	4	6	4																		
	2					6	4	3	7																		
	3					7	3	6	4																		
	4					5	5	5	5																		
	T	32*	8*	30*	10*	24	16	20	20							%	20.0*		25.0		40.0		50.0				
conc. level 4 — 0.8 PPB	1					6	4	5	5																		
	2					9	1	7	2																		
	3					5	5	4	6																		
	4					6	4	4	6																		
	T	32*	8*	31*	9*	26	14	20	19							%	20.0*		22.5*		35		48.7				
conc. level 5 — 0.4 PPB	1	9	1	9	1	9	1	7	3																		
	2	9	1	7	3	7	3	5	5																		
	3	8	2	7	3	6	4	6	4																		
	4	8	2	7	3	6	4	6	4																		
	T	34	6	30	10	28	12	24	16							%	15.0		25.0		30.0		40.0				
conc. level 6 — 0.2 PPB	1	9	1	9	1	9	1	8	2																		
	2	8	2	8	2	7	3	7	3																		
	3	10	0	8	2	7	3	7	3																		
	4	9	1	9	1	8	2	7	3																		
	T	36	4	34	6	31	9	29	11							%	10.0		15.0		22.5		27.5				
conc. level 7 — 0.04 PPB	1	10	0	10	0	8	2	8	2																		
	2	10	0	10	0	10	0	9	1																		
	3	10	0	10	0	9	1	9	1																		
	4	9	1	9	1	9	1	7	3																		
	T	39	1	39	1	36	4	33	7							%	2.5		2.5		10.0		17.5				
solvent control	1	9	1	9	1	9	1	9	1																		
	2	10	0	9	1	9	1	8	2																		
	3	9	1	9	1	9	1	9	1																		
	4	9	1	9	1	9	1	9	1																		
	T	37	3	36	4	36	4	35	5							%	7.5		10.0		10.0		12.5				
untreated control	1	10	0	10	0	9	1	8	2																		
	2	10	0	10	0	8	2	8	2																		
	3	10	0	10	0	8	2	8	2																		
	4	10	0	10	0	9	1	9	1																		
	T	40	0	40	0	34	6	33	7							%	0.0		0.0		10.0		17.5				

*Estimated numbers

TABLE 5: RESULTS OF A REPEAT ACUTE 96-HOUR EXPOSURE OF ADULT ACARTIA TONSA TO TECHNICAL DIAZINON (10 COPEPODS EXPOSED PER REPLICATE)

A = Alive
D = Dead

Acute Toxicity Tests

exposure (series A)

dose levels	replicates	Raw Data from Individual Replicates Post-Exposure														Percentage Actual Test Mortalities from Raw Data											
		24 Hrs.		48 Hrs.		72 Hrs		96 Hrs.								24 Hrs.		48 Hrs.		72 Hrs.		96 Hrs.					
		A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D
conc. level 1 8.0 ppb	1	8	2	6	4	4	6	2	8																		
	2	8	2	7	3	6	4	3	7																		
	3	8	2	7	3	5	5	4	6																		
	4	10	0	10	0	9	1	2	8																		
	T	34	6	30	10	24	16	11	29							%	15.0		25.0		40.0		72.5				
conc. level 2 3.2 ppb	1	8	2	6	4	2	8	1	9																		
	2	4	6	2	8	2	8	1	9																		
	3	4	6	2	8	1	9	0	10																		
	4	6	4	4	6	4	6	2	8																		
	T	22	18	14	26	9	31	4	36							%	45.0		65.0		77.5		90.0				
conc. level 3 1.6 ppb	1	9	1	9	1	8	2	5	5																		
	2	9	1	7	3	7	3	4	6																		
	3	9	1	8	2	8	2	6	4																		
	4	8	2	7	3	5	5	5	5																		
	T	35	5	31	9	28	12	20	20							%	12.5		22.5		30.0		50.0				
conc. level 4 0.8 ppb	1	10	0	8	2	7	3	6	4																		
	2	10	0	9	1	8	2	6	4																		
	3	9	1	8	2	8	2	6	4																		
	4	8	2	7	3	6	4	6	4																		
	T	37	3	32	8	29	11	24	16							%	7.5		20.0		27.5		40.0				
conc. level 5 0.4 ppb	1	8	2	8	2	7	3	4	6																		
	2	9	1	8	2	7	3	6	4																		
	3	9	1	9	1	9	1	7	3																		
	4	10	0	10	0	8	2	7	3																		
	T	36	4	35	5	31	9	24	16							%	10.0		12.5		22.5		40.0				
conc. level 6 0.2 ppb	1	8	2	8	2	8	2	6	4																		
	2	8	2	8	2	8	2	8	2																		
	3	10	0	10	0	8	2	8	2																		
	4	10	0	9	1	9	1	7	3																		
	T	36	4	35	5	33	7	29	11							%	10.0		12.5		17.5		27.5				
conc. level 7 0.04 ppb	1	10	0	9	1	8	2	7	3																		
	2	9	1	8	2	6	4	6	4																		
	3	7	3	6	4	5	5	5	5																		
	4	7	3	7	3	7	3	7	3																		
	T	33	7	30	10	26	14	25	15							%	17.5		25.0		35.0		37.5				
solvent control	1	10	0	8	2	7	3	7	3																		
	2	10	0	10	0	9	1	8	2																		
	3	10	0	9	1	9	1	9	1																		
	4	9	1	9	1	9	1	9	1																		
	T	39	1	36	4	34	6	33	7							%	2.5		10.0		15.0		17.5				
untreated control	1	10	0	10	0	10	0	10	0																		
	2	9	1	9	1	8	2	8	2																		
	3	10	0	9	1	9	1	9	1																		
	4	10	0	10	0	10	0	9	1																		
	T	39	1	38	2	37	3	36	4							%	2.5		5.0		7.5		10.0				

TABLE 6: RESULTS OF ACUTE 96-HOUR EXPOSURE OF ADULT ACARTIA TONSA TO TECHNICAL TOXAPHENE (10 COPEPODS EXPOSED PER REPLICATE)

A = Alive
D = Dead T = Total

Acute Toxicity Tests

exposure (series A)

dose levels	replicates	Raw Data From Individual Replicates Post-Exposure														Percentage Actual Test Mortalities From Raw Data											
		24 Hrs.		48 Hrs		72 Hrs.		96 Hrs.								24 Hrs.		48 Hrs.		72 Hrs		96 Hrs.					
		A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D
conc. level 1 300.0 PPT *	1	6	4	6	4	6	4	2	8																		
	2	10	0	9	1	9	1	3	7																		
	3	9	1	9	1	6	4	2	8																		
	4	8	2	8	2	8	2	2	8																		
	T	33	7	32	8	29	11	9	31							%	17.5		20.0		27.5		77.5				
conc. level 2 30.0 PPT	1	5	5	5	5	5	5	3	7																		
	2	6	4	4	6	3	7	2	8																		
	3	5	5	5	5	2	8	3	7																		
	4	8	2	7	3	7	3	5	5																		
	T	24	16	21	19	17	23	13	27							%	40.0		47.5		57.5		67.5				
conc. level 3 3.0 PPT	1	7	3	6	4	6	4	6	4																		
	2	5	5	5	5	5	5	5	5																		
	3	10	0	10	0	9	1	8	2																		
	4	8	2	7	3	7	3	7	3																		
	T	30	10	28	12	27	13	26	14							%	25.0		30.0		32.5		35.0				
conc. level 4 0.3 PPT	1	9	1	9	1	8	2	7	3																		
	2	8	2	8	2	6	4	6	4																		
	3	9	1	9	1	9	1	7	3																		
	4	9	1	9	1	9	1	9	1																		
	T	35	5	35	5	32	8	29	11							%	12.5		12.5		20.0		27.5				
conc. level 5 0.03 PPT	1	9	1	8	2	8	2	8	2																		
	2	8	2	8	2	7	3	7	3																		
	3	10	0	10	0	8	2	7	3																		
	4	7	3	7	3	7	3	6	4																		
	T	34	6	33	7	30	10	28	12							%	15.0		17.5		25.0		30.0				
solvent control	1																										
	2																										
	3																										
	4																										
untreated control	1	10	0	10	0	10	0	10	0																		
	2	10	0	10	0	10	0	10	0																		
	3	10	0	9	1	9	1	9	1																		
	4	10	0	9	1	9	1	9	1																		
	T	40	0	38	2	38	2	38	2							%	0		5.0		5.0		5.0				

* Parts per trillion (nanograms/liter).

TABLE 7 - SUMMARY OF ACUTE 96-HOUR TEST AND ADJUSTED PERCENTAGE MORTALITIES[a] AND LC_{50} VALUES FOR ACARTIA TONSA EXPOSED TO METHYL PARATHION, AZODRIN, DIAZINON AND TOXAPHENE

Concentration Tested	methyl parathion			Azodrin			diazinon (Average of Two Tests)			toxaphene		
	Test M %	Adj. M %	LC_{50}	Test M %	Adj. M %	LC_{50}	Test M %	Adj. M %	LC_{50}	Test M %	Adj. M %	LC_{50}
(ppm)												
5.0	100.0											
2.5	62.5	57.1										
1.0	47.5	40.0	0.89	60.0	57.9	0.24						
0.1	25.0	14.3		45.0	42.1							
0.01	15.0	2.9		37.0	33.7							
(ppb)												
8.0							65.0	58.8				
3.2							70.0	64.7				
1.6							50.0	41.2				
1.0	12.5	0.0		30.0	26.3							
0.8							44.4	34.6				
0.4							40.0	29.4				
0.3									2.57	77.5	76.3	
0.2							27.5	14.7				
0.1				12.0	7.4							
0.04							27.5	14.7				
0.03										67.5	65.8	
(ppt[b])[b]												
3.0										35.0	31.6	
0.3										27.5	23.7	7.20
0.03										30.0	26.3	
Solvent Control	12.5			5.0			15.0					
Untreated Control	10.0			5.0						5.0		

[a]Abbott, W.S., J. Econ. Ent. 18, 265-267 (1925)

[b]Parts per trillion

TABLE 8

COMPUTED LC_{10} AND LC_{50} VALUES WITH 95% CONFIDENCE LIMITS FOR 96-HOUR EXPOSURE OF TECHNICAL METHYL PARATHION, AZODRIN, DIAZINON AND TOXAPHENE TO ACARTIA TONSA DANA (40 ANIMALS EXPOSED PER CONCENTRATION EXCEPT FOR DIAZINON[a]).

Pesticide	LC_{10}	LC_{50}
methyl parathion (80% Technical)	0.07 ppm (0.0418 - 0.1145 ppm)	0.89 ppm (0.685 - 1.163 ppm)
Azodrin (80% Technical)	0.05 ppb (0.0098 - 0.269 ppb)	0.24 ppm (0.08536 - 0.66170 ppm)
diazinon (97.6% Technical)	0.04 ppb (0.0164 - 0.0777 ppb)	2.57 ppb (1.7259 - 3.8247 ppb)
toxaphene (100% Technical)	0.0035 ppt)0.0006 - 0.0187 ppt)	7.2 ppt (3.540 - 14.636 ppt)

[a] Computation of a mean of two tests (80 animals exposed per concentration).

TABLE 9. AMOUNTS OF DIAZINON[a] UPTAKE BY COPEPODS AND ALGAE 96 HOURS POST-EXPOSURE IN REPEATED ACUTE TOXICITY TEST (ppb)

Dose Level at Exposure	Amount of Residue in H_2O (0 Hours)	Amount of Residue in H_2O (96 Hours)		Amount of Uptake (96 Hours)		
	Copepods & Algae	Copepods Only	Copepods & Algae	Copepods & Algae	Copepods Only	Algae Only
0.2	0.16	0.17	0.06	0.10	0.0	0.11
0.4	0.34	0.35	0.24	0.10	0.0	0.11
0.8	0.73	0.68	0.54	0.19	0.05	0.14
1.6	1.3	1.1	1.1	0.2	0.2	0.0
3.2	3.0	2.0	2.6	0.4	?	?
8.0	7.7	6.8	5.6	2.1	0.9	1.2

[a] LC_{50} = 2.57 ppb

[a] LC_{10} = 0.04 ppb

SECTION VII

SUPPORTING DATA

To understand the extent and importance of pesticide pollution, laboratory studies on acute and chronic toxicities are important. From a columinous literature search, it seems that estuarine organisms have received the least attention. For the purpose of this contract, we have retrieved 120 articles from the world literature dating back to 1950. We found no reference to copepod toxicity studies relevant to the pesticides being investigated. Therefore, we are including, as supporting data, some of the work relevant either to other marine invertebrates or to fresh water organisms of related scope. This review is, therefore, a selective one aimed to include only valid data that can be correlated with the results obtained in this study.

The subject of biological concentration of pesticides in aquatic organisms has been reviewed by the excellent work of Dustman and Stickel (1969), Macek (1969) and Pimentel (1971).

METHYL PARATHION

Methyl parathion is registered for use against cotton and small grain insect pests where resistance to other insecticides had developed.

The LC_{50} for blue gills was 8,500 ppb and for rainbow trout was 7,000 ppb (USDA, 1968). Early toxicity studies with Anopheles quadrimaculatus larvae gave an LC_{67} of 0.0025 ppm for 48-hour exposure (Negherbon, 1959). A recent finding (Shim & Self, 1972) indicated that the LC_{50} to Culex tritaeniorhynchus larvae was 0.54 ppb. For Daphnia magna the LC_{50} was 4.8 ppb (Frear and Boyd, 1967). Macek and McAllister (1970) gave data on the relative susceptibility of 12 fish species exposed for 96 hours to methyl parathion which were in the order of TL_{50} values of 1.1 to 3.3 ppm. The only toxicity tests with marine species were those of Eisler (1969) in which the 96-hour LC_{50} value using the sand shrimp Crangon septemspinosa was 2 ppb and with the grass shrimp Palaemonetes vulgaris the corresponding LC_{50} was 3 ppb.

AZODRIN

Azodrin, being a new organophosphorous chemical used in the control of cotton insects resistant to organochlorine pesticides, has not been implicated in the environment as much as other agricultural chemicals. Data on its toxicity to aquatic organisms is minimal. F.W.P.C.A. (1968) reported 48-hour chronic toxicity for rainbow trout as 7.0 ppm. Eichelberger and Lichtenberg (1971) stressed that Azodrin, among other organophosphorous compounds, was the most stable in raw river water, and persisted over a two-month period. This pesticide deserves further toxicity study with aquatic organisms.

DIAZINON

This is a popularly used chemical both in agriculture and in animal and human health. Its toxicity for fish is widely documented. The LC_{50} for blue gills (48-hour exposure) was 86 ppb and 170 ppb for rainbow trout (Cope, 1966). Aquatic arthropods, on the other hand, respond very quickly to trace levels of diazinon. For example, Aedes aegypti LC_{50} for 24-hour exposure was 3.3 ppb (WHO, 1970), and the water flea Daphnia pulex responded to 0.9 ppb (LC_{50} for 48-hour exposure), Sanders and Cope, 1966). Gammarus lacustris, a fresh water amphipod, responded to 200 ppm (LC_{50} for 96 hours) (Sanders, 1969).

TOXAPHENE

This insecticide is also used in agricultural pest control. Because of its high mammalian toxicity, it is restricted to a low residue tolerance level on food commodities. Its LC_{50} for 24-hour exposure for the rainbow trout was 7.6 ppb and 7.2 ppb for blue gills (USDA, 1968). This chemical has been widely tested against fresh water invertebrates and some selected data are as follows:

Species	Exposure Time (Hours)	LC_{50} (ppm)	Reference
Gammarus lacustris	96	26	Sanders, 1969
Pteronarcys californica (naiad)	96	2.3	Sanders & Cope, 1968
Daphnia magna	26	94	Frear & Boyd, 1967
Daphnia pulex	48	15	Sanders & Cope, 1966
Simocephalus serrulatus	48	19	Sanders & Cope, 1966

No references were found with effects shown in the parts per trillion dose range.

Apart from toxicity to aquatic animals, chemicals tend to accumulate in other forms of life, such as bacteria, diatoms, algae, and non-aquatic species. Studies on the interaction between pesticides and aquatic plants and animals have been increasing within the last few years. However, the pesticides under reference in this contract have not as yet been adequately studied in this regard.

SECTION VIII

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SECTION IX

APPENDIX

1. "F" ALGAL MEDIUM - COMPOSITION

Sodium Salts

$NaNO_3$	150 mg
$NaH_2PO_4.H_2O$	10 mg
$Na_2SiO_3.9H_2O$	30 mg
Fe Sequestrene	10 mg

Trace Metals

$CUSO_4.5H_2O$	0.0196 mg
$ZnSO_4.7H_2O$	0.044 mg
$CoCl_2.6H_2O$	0.022 mg
$MnCl_2.4H_2O$	0.36 mg
$Na_2MoO_4.2H_2O$	0.0126 mg

Vitamins

Thiamine. HCl	0.2 mg
Biotin	1.0 µg
B_{12}	1.0 µg

Sea Water	To 1 liter

2. STERILITY TEST MEDIUM-COMPOSITION

1 gm	Yeast extract
1 gm	Trypticase
1 ml	$FeCl_3.6H_2O$ stock solution (5 mg/ml glass redistilled water)
0.5 ml	K_2HPO_4 (anhydrous) stock solution (1 gm/100 ml glass redistilled water)
850 ml	natural sea water
50 ml	1 percent stock solution of tris (hydroxymethyl aminomethane) pH 7.5
ca. 100 ml	glass redistilled water

Preparation

Combine substances listed above and mix thoroughly. When a uniform suspension has been obtained, heat with frequent agitation until the mixture boils for one minute. Dispense 10 ml amounts per 25 ml screw-cap tube. Autoclave at 120°C, 15 psi, for 20 minutes. When sterility test medium has cooled, tighten caps and store at room temperature.

3. PESTICIDE SPECIFICATIONS

Methyl parathion (Monsanto)

Chemical name: 0,0-dimethyl 0-p-nitrophenyl phosphorothioate
Formulation: Technical, 80% in solution
Solubility: Insoluble in water; soluble in acetone and other organic solvents

Azodrin (Shell)

Chemical name: Dimethyl phosphate of 3-hydroxy-N-methyl-CIS-crotonamide

Formulation: Technical, 80% in solution

Solubility: Soluble in water, acetone and other organic solvents

Diazinon (Geigy)

Chemical name: 0,0-diethyl 0-(2-isopropyl-6-methyl-4-pyrimidinyl) Phosphorothioate

Formulation: Technical, 97.6% in solution

Solubility: Very slightly (40 ppm) soluble in water; soluble in acetone and other organic solvents

Toxaphene (Hercules, Inc.)

Chemical name: Chlorinated Camphene containing 67-69% chlorine

Formulation: 100% Technical with a chlorine content of 67-69%

Solubility: Slightly soluble in water (3 ppm) and soluble in aromatic hydrocarbons.

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(Please read Instructions on the reverse before completing)

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15. SUPPLEMENTARY NOTES

16. ABSTRACT

The acute toxicity to the marine copepod Acartia tonsa Dana of four technical grade insecticides was determined by bioassay using standardized procedures, homogeneous populations and constant laboratory conditions. At a water temperature of 17 ± 1°C, the 96-hour median lethal concentrations or tolerance limits for methyl parathion, Azodrin, diazinon and toxaphene were computed as 0.89 milligrams per liter, 0.24 milligrams per liter, 2.57 micrograms per liter and 7.2 nanograms per liter, respectively. Residue analysis for diazinon at zero and 96-hour exposure time revealed that the amounts of diazinon uptake by three algal organisms is greater than amounts concentrated by the copepod. The toxicity of higher concentrations above 2.0 ppm (2 milligrams per liter) has offset copepod uptake, while at lower concentrations, quantities concentrated by Acartia are negligible.

Concurrently, the world literature was surveyed for supporting toxicity data of these chemicals to closely related species.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Bioassay Pesticides Toxicity Marine Biology	Calanoid copepod Toxicity review Residue Analysis	6F

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