EFFECTS OF PESTICIDES ON EMBRYONIC DEVELOPMENT OF CLAMS AND OYSTERS AND ON SURVIVAL AND GROWTH OF THE LARVAE

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ABSTRACT

Fifty-two compounds were tested for their effects on embryos of the hard clam, *Mercenaria mercenaria*, and the American oyster, *Crassostrea virginica*, and on their larvae. The pesticides included 17 insecticides, 12 herbicides, one nematocide, four solvents, and 18 miscellaneous bactericides, fungicides, and algicides.

Most of the compounds affected embryonic development more than survival or growth of larvae. Some, however, drastically reduced growth of larvae at concentrations that had relatively little effect on embryonic development. It is necessary, therefore, to evaluate the effects of pesticides on all stages of the life cycle of an organism before the pesticide can be considered safe. Nevertheless, differences in toxicity to bivalve larvae among compounds of each category of pesticide are large enough that it should be possible to select compounds to control pest species without serious damage to commercial shellfish.

The extensive use in recent years of highly persistent pesticides for control of certain insects and undesirable plants, not only on agricultural lands but also on recreational areas, lakes, streams, and marshes, has made imperative an evaluation of the effects of these compounds on fish and wildlife. The eventual goal is to control undesirable species, with the least harm to the desirable members of the ecosystem. Attainment of this goal requires extensive knowledge of how each pesticide affects each species or representative species of the system. Also, the pesticide must be highly specific or be applied so that its dispersion is strictly limited.

Pesticides may enter the habitat of shellfish in several ways. One is by being carried there in runoff water from treated land areas. Cottam (1960) stated that 2 to 3 billion pounds (9.07 to 13.61 x 10^8 kg.) of pesticides are used annually in the United States on about 100 million acres (40.5 x 10^6 ha.) of land. Thimann (1964) stated that the United States used 175,000 short tons (158,760 metric tons) of insecticides in 1962 and about half that much of fungicides and herbicides. Doudoroff, Katz, and Tarzwell (1953) made laboratory tests of soils collected from toxaphene-treated fields and concluded that stream waters can be made toxic to fish by the drainage from such fields. Such runoff water may carry the pesticides in solution, adsorbed on suspended particles, or incorporated in plants and animals in the water. Certainly, large quantities of the pesticides that leach from the soil must eventually reach coastal marine waters and sediments.

A second, more direct, and perhaps more easily regulated method by which pesticides may enter the estuarine environment is the use of insecticides and herbicides on salt marshes and estuaries to control mosquitoes and undesirable plants. In some regions large areas are sprayed near shellfish beds. Pesticides used in this way probably create higher concentrations of the active ingredients in the estuarine water than are achieved by any other method.

Loosanoff, MacKenzie, and Shearer (1959 [2]), Loosanoff et al. (1960), and Loosanoff (1961) proposed the use of several pesticides for the control of certain predators and competitors of

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[2] Loosanoff, V. L., C. L. MacKenzie, Jr., and L. W. Shearer. 1959. Use of chemical barriers to protect shellfish beds from predators. Bur. Commer. Fish. Biol. Lab., Milford, Conn., Bull. 6, 23: 1-11.

commercial shellfish. The methods of application proposed by these authors, however, were designed to restrict the dispersal of the pesticide. Compounds relatively insoluble in water were dissolved in polychlorinated benzenes, which are themselves virtually insoluble in water—a characteristic that further limited the solubility of the pesticide. Moreover, the polychlorinated benzenes, which are heavier than sea water, were mixed with dry sand to anchor the pesticide to the bottom of the particular shellfish bed treated. This essentially two-dimensional treatment of limited areas with a control pesticide had little or no effect on pelagic or planktonic organisms.

Butler, Wilson, and Rick (1962) presented data on the effects of some pesticides on adult oysters and Davis (1960) reported the effects of 31 compounds, including several types of pesticides, on fertilized eggs and larvae of bivalves. The authors of the two papers considered the effect of pesticides on growth to be the most sensitive index for these mollusks.

The highest concentration of any pesticide that can be considered "safe" for use in waters in which valuable species of bivalves reproduce is the highest concentration that has no appreciable effect on survival of the developing embryo or on growth and survival of the fully formed veliger larvae. It is also necessary to determine the concentrations tolerated by spawning individuals and by organisms that serve as food for larval and adult bivalves (Ukeles, 1962).

A distinction is made between effects on development of the embryo and on survival and growth during the larval stage because tolerances of these two pelagic stages to a given toxicant are often markedly different. Growth of the veliger larvae, moreover, may be drastically retarded at concentrations of toxicants too low to cause direct mortality of either embryonic or larval stages. Such a retardation of growth, however, serves to prolong the pelagic life of the larvae and, thus, increases the chance for their loss through predation, disease, and dispersion.

This report summarizes the data obtained at the Bureau of Commercial Fisheries Biological Laboratory in Milford concerning the effects of various compounds used in control of various types of undesirable organisms, on the development of fertilized eggs of hard clams, *Mercenaria mercenaria*, and American oysters, *Crassostrea virginica*, and on the survival and growth of the larvae. The data, unfortunately, are not complete for all of the compounds tested. The work on pesticides at the laboratory in Milford has been terminated by transfer of pesticide work to the Bureau's Biological Laboratory at Gulf Breeze, Fla. In the early experiments the effects of the compounds on development of fertilized eggs were not determined and many were tested in only a single experiment. Furthermore, for some experiments in which growth of larvae in control cultures was not satisfactory, we can give only the data on development of fertilized eggs. The effects of a number of these pesticides on some of the algal foods of bivalve larvae have also been determined (Ukeles, 1962).

METHODS

Methods for spawning oysters out of season and standard methods for culturing the larvae have been described in detail by Loosanoff and Davis (1963). These methods were followed throughout the present series of experiments.

In most experiments all pesticides were tested at concentrations of 0.25, 0.50, 1, 2.5, 5, and 10 p.p.m. (parts per million), with duplicate cultures at each concentration. If a toxic range was not established in the first experiment, these concentrations were increased or decreased by a factor of 10 in the next experiment. Usually, however, the range of 0.25 to 10 p.p.m. included concentrations that had no effect and concentrations that caused 100 percent mortality. Stock solutions of water-soluble pesticides were made up in water; all others were made up in acetone, except for a very few that were insoluble in either water or acetone. The latter were used as water suspensions.

For observations on development of embryos, fertilized eggs were introduced into the test concentrations soon after release and fertilization, usually when the eggs were in the two-cell stage of development. Quantitative samples were taken 48 hours later to determine the percentage of the fertilized eggs in each culture that had developed to normal straight-hinge veliger larvae.

For tests to determine the effect of compounds on survival and growth of veliger larvae, we used cultures of 2-day-old larvae that had been reared to the straight-hinge stage under normal conditions. These larvae were then reared, in the different concentrations of substances being tested, for a period of 10 days for clam larvae or 12 days for oyster larvae. Thus, when the quantitative samples were taken at the end of the period, the larvae were 12 and 14 days old, respectively. These periods represent the normal time of setting for the two species under good environmental conditions at the temperatures used ($24° \pm 1°$ C.).

We fed the test larvae a mixture of live flagellates, generally at a rate of 0.01 ml. of packed cells to each 1-liter culture per day. Sea water and test compound were renewed in the cultures every second day. The contents of a culture vessel were washed onto a stainless steel screen that retained the larvae but allowed the sea water and dissolved pesticide to pass through. The Pyrex[3] culture vessels used in the experiments were then thoroughly washed before the larvae and the fresh solution of pesticide in sea water were again added. The renewal of the culture medium every second day in this manner minimized the buildup of harmful metabolites and made it possible to maintain accurately the concentration of test compound at the desired level.

Quantitative samples, consisting of 1.6 percent of the total larval population of a culture, were taken at the end of the 2-day and the 12- or 14-day experimental periods and preserved for microscopic examination. We determined the survival by counting the number of larvae that had been living at the time of preservation. Survival values (table 1, cols. 1, 2) are expressed as a percentage (R) of the survival in control cultures and were calculated as follows:

$$R = \frac{\text{Average number of larvae in experimental cultures}}{\text{Average number of larvae in control cultures}} \times 100$$

Growth, or increase in mean lengths in the 12- and 14-day experiments (table 1, col. 3), is expressed as a percentage (G) of the increase in mean length of larvae in control cultures and was calculated as follows:

$$G = \frac{\text{Mean length of larvae in experimental cultures—mean length at 2 days}}{\text{Mean length of larvae in control cultures—mean length at 2 days}} \times 100$$

Mean length was determined by measuring the maximum length of the shell, parallel to the hinge line, of 100-oyster larvae or of 50-clam larvae from the preserved samples. The expression of results as percentages of the increase in mean length and survival of larvae in control cultures made possible the direct comparison of data from the different experiments.

Measurements of effects of toxicants on growth and survival of larvae are subject to considerable error due to random sampling error and variations introduced by the slight uncontrolled environmental differences between cultures. In addition, changes in sea water and food quality between successive experiments undoubtedly caused variation in observed effects. These factors must be taken into consideration in judging reliability of the data and in ascertaining safe and harmful levels of the test compound.

The standard error in measurements of growth is considerably greater for oyster larvae than for clam larvae. A length-frequency distribution of clam larvae receiving a given treatment is highly kurtotic, whereas that for oyster larvae exhibits this central tendency to a much lesser degree. Figure 1 shows the 95 percent confidence limits of the mean ($\pm 2\ SE_m$) in microns, with $N=100$ for oyster larvae and $N=50$ for clam larvae, at the various mean lengths encountered in our experimental cultures.

After considering the variations encountered, in addition to sampling errors, we believe our

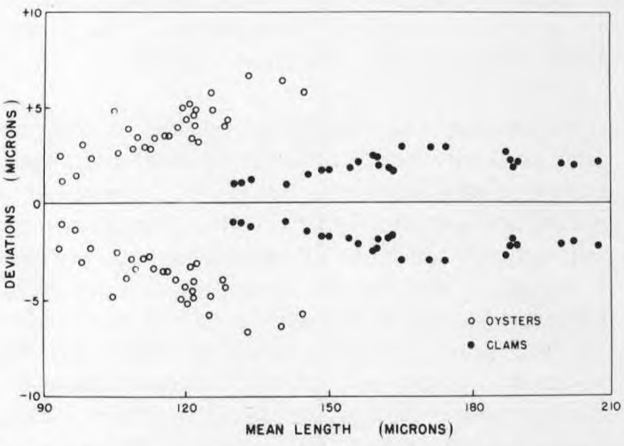

FIGURE 1.—The 95 percent confidence limits of measurements of mean lengths of oyster and clam larvae of different sizes. Values indicate $\pm 2\ SE_m$. $N=100$ for oyster larvae; $N=50$ for clam larvae.

[3] Trade names referred to in this publication do not imply endorsement of commercial products.

transformed values (G) for measurements of growth are generally accurate to about ±10 percent for oyster larvae and to about ±5 percent for clam larvae. The confidence limits for oyster larvae were not narrowed appreciably even by increasing N to 300; therefore, it was considered impractical to increase the number of measurements of oyster larvae sufficiently to reduce the error to that of measurements of clam larvae.

Errors involved in our techniques for determining numbers of larvae developing from fertilized eggs to straight-hinge larvae or the number surviving in growth experiments have been found to be about ±10 percent.

In any event, the effects of test compounds on larval growth and survival are readily distinguished, from random variation, by the regular stepwise reduction at each successive increase in concentration of the test chemical.

EFFECTS OF DIFFERENT COMPOUNDS ON EMBRYOS AND LARVAE

Table 1 shows the relative percentage of fertilized oyster and clam eggs that developed through normal embryonic stages into straight-hinge larvae, the relative percentage of larvae that survived, and the relative percentage increase in mean length when exposed to various pesticides and chemicals. We calculated the relative percentages, as has been previously stated, by using the survival and rate of growth of larvae in the control cultures of each experiment as 100 percent. The values given (except where noted) are averages for duplicate cultures at each concentration in each experiment. When more than one experiment was run, we combined the results of all experiments.

Some compounds were more toxic to embryos than to larvae (despite the much shorter exposure period of the embryos), although the reverse is generally indicated in our tests. The differences between the tolerance of developing embryos and larvae of oysters to the same pesticide are strikingly evident from the effects of the weedicides, Amitrol and Endothal (table 1). Embryos developed normally in higher concentrations of Amitrol (500 p.p.m.) than those at which larvae showed good growth (100 p.p.m.). In contrast, eggs could tolerate only 10 p.p.m. of Endothal, whereas larvae showed about normal growth at concentrations as high as 50 p.p.m.

The compounds we used in our tests differed widely in chemical composition and presumably have different modes and sites of action. It is not too surprising, therefore, that although the toxic levels of most compounds are about the same for clams and oysters, some are appreciably more toxic to one than to the other. The tolerance of oyster larvae to Endothal, for example, was considerably greater than that of clam larvae (although oyster embryos were slightly less tolerant than were clam embryos). Oyster larvae showed fair survival and normal growth at 25 p.p.m., whereas this concentration caused 100 percent mortality of clam larvae. In general, however, the rates of growth of clam larvae are less affected by toxicants than growth of oyster larvae.

As has been reported previously (Davis, 1960), some of the lower concentrations of certain compounds significantly accelerated growth of larvae (notably Sevin, Endothal, 2-4-D salt, phenol, and Sulmet—table 1). Although the reasons for this phenomenon are not clear, we believe it is the result of the bacteriostatic or, possibly, chelating effect of these compounds. Because growth of clam larvae is less affected by bacterial and algal toxins than is growth of oyster larvae, any bacteriostatic or chelating effect these compounds might have would be expected to have a less marked effect on growth of clam larvae than on growth of oyster larvae.

Synergistic Action of Solvent With Some Compounds

With certain pesticides, the solvent may act as a synergist and increase the toxicity of the compound, but with other pesticides the same solvent may show no such action. Acetone appeared to act as a synergist with Co-Ral but not with Di-Syston and Phygon. In experiments when the stock solutions of these three water-insoluble compounds were made up in acetone (appendix), the pair of control cultures receiving 100 p.p.m. acetone (the maximum concentration used in any of the experimental cultures) showed no significant reduction in growth or survival of either clam or oyster larvae. Survival and growth of clam larvae receiving Di-Syston and Phygon decreased progressively as the concentrations of these compounds increased, just as it did in various concentrations of the water-soluble toxicants (table 1). The toxic effects of Co-Ral, however, show a definite break in the middle of the series that corresponds to the break in acetone concentrations.

To obtain the six concentrations of these acetone-soluble compounds, from 0.25 to 10 p.p.m., we used two stock solutions. The concentrations of 0.25, 0.5, and 1 p.p.m. of toxicants were obtained by increasing volumes of the less concentrated stock solution so that the concentrations of acetone were 25, 50, and 100 p.p.m. The concentrations of 2.5, 5, and 10 p.p.m. were achieved by increasing volumes of the more concentrated stock solution, again giving concentrations of acetone of 25, 50, and 100 p.p.m.

The stepwise decrease in survival and growth of clam larvae at 0.25, 0.5, and 1 p.p.m. Co-Ral, followed by better survival and growth at 2.5 p.p.m. and stepwise reduction at 5 and 10 p.p.m. indicates that the action of Co-Ral was being synergized by the acetone solvent (the action of Di-Syston and Phygon was not). The effect of Aldrin on clam embryos shows some evidence of similar synergism.

Variable Effects of Endrin and Dieldrin

Results of different experiments with these two compounds varied considerably even when acetone stock solutions were used. The results given for endrin and dieldrin, therefore, are the average values of a number of experiments but in some experiments tolerances were significantly below these averages. We assume that the variation in the several experiments was caused by differences in particle size and degree of suspension attained in the test culture, since these compounds are essentially insoluble in water. We would expect field observations on commercial applications to yield conflicting data, depending upon the degree of dispersion attained when the pesticide reaches the water.

Possible Indirect Effect of Compounds Through Food Chain

Ukeles (1962) showed that the tolerance for pesticides of some of the best algal foods for bivalve larvae was considerably lower than the tolerances of the larvae. Therefore, even a concentration of a pesticide that showed no effect on eggs or larvae might, indirectly, if used in the field, markedly reduce the growth of bivalve larvae by killing or preventing reproduction of the algae that serve as foods.

We believe the results given in table 1 are, at least primarily, the direct effect of these compounds on the embryos or larvae themselves because we are not dependent upon reproduction of the algae in our larval cultures. We add the food cells to our experimental cultures daily, and the pesticide would have an indirect effect through the food chain only if it destroyed the food cells. The concentration of Sulmet (sodium sulfamethazine) used routinely as a bactericide in our larval cultures, for example, is sufficient to inhibit or prevent reproduction of the algae used for food, yet it has no adverse effect on growth of larvae under our laboratory conditions of feeding.

Significance of TL_m Values

In table 2 we have listed the 24-hour TL_m (the concentration in p.p.m. that would cause an approximate 50-percent reduction in the number of eggs developing into normal straight-hinge larvae) for oyster and clam eggs. Also listed are the 12-day TL_m for clam larvae and the 14-day TL_m for oyster larvae. We believe the TL_m values listed are of value only for rough comparisons of toxicity because some compounds drastically reduce the rate of growth of larvae at concentrations too low to cause appreciable mortality or may kill embryos at lower concentrations than are required to affect growth or survival of larvae. Both endrin and dieldrin, for example, had 14-day TL_m's for oyster larvae greater than 10 p.p.m., yet either of these compounds, at concentrations of only 1 p.p.m., reduced the rate of growth of these larvae drastically. Other compounds, such as Nemagon, Aldrin, and toxaphene, permitted development of embryos at considerably higher concentrations than those at which the larvae could survive and grow. Conversely, other compounds, such as griseofulvin (on clams) and Endothal (on oysters), almost completely stopped embryonic development at concentrations too low to affect seriously survival and growth of the larvae.

In comparison with the TL_m values given for other species, the rankings of Amitrol, Endothal, Omazene, and Phygon are the same for clam and oyster larvae as Bond, Lewis, and Fryer (1960) found for largemouth bass, *Micropterus salmoides*, and two species of salmon, *Oncorhynchus kisutch* and *O. tshawytscha*. The median tolerance limits for the least tolerant stages in the life cycle of clams and oysters for these compounds, however, were lower than the values given for fish. The 96-hour TL_m for bluegills, *Lepomis macrochirus*, to Co-Ral and Di-Syston, reported by Henderson, Pickering, and Tarzwell (1960), was also higher than that for the least tolerant stages of clams.

NEED FOR FURTHER STUDY

The examples cited indicate the need for evaluating all aspects of toxicity on rapidly growing and changing animals at each stage of their life cycle. The high tolerance of bivalve larvae to some of these pesticides also suggests that compounds can be chosen to control pest species without serious damage to commercial shellfish. Within the series of insecticides, for example, Davis (1960) showed that DDT was much more toxic to oyster larvae than lindane. Similarly, within the series of herbicides tested, Amitrol was "safe" at 100 p.p.m., whereas MCPA caused a significant reduction in the rate of growth of oyster larvae at all concentrations above 0.25 p.p.m. We believe, as Thimann (1964) suggested, that emphasis should be placed on developing "substances whose action is selective and on those which decompose quickly."

TABLE 1.—*Percentage of eggs of American oyster and hard clam that developed normally, percentage of larvae that survived, and percentage increase in mean length, in the presence of different concentrations of chemicals* [1]

Compound	Concentration	Oysters			Clams		
		Eggs developing	Survival of larvae	Increase in length of larvae	Eggs developing	Survival of larvae	Increase in length of larvae
Insecticides:	*P.p.m.*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Aldrin (2 experiments; acetone solution)	0.25				96	[2] 75	[2] 20
	.50				90	[2] 37	[2] 9
	1.00				71	0	
	2.50				86	0	
	5.00				83	0	
	10.00				64	0	
Co-Ral (2 experiments; acetone solution)	.0025	89	74	83			
	.0050	100	66	105			
	.01	103	87	92			
	.025	111	91	88			
	.050	98	90	72			
	.10	55	95	61			
	.25	0	104	49	95	87	89
	.50	0	99	38	97	72	67
	1.00	0	75	17	99	44	39
	2.50				88	74	57
	5.00				87	52	44
	10.00				42	5	14
DDT (1 experiment; water suspension)	.025		80	54			
	.050		0				
Dicapthon (1 experiment; water solution)	.10				112	91	89
	.20				91	88	87
	1.00				95	89	59
	2.00				60	94	65
	10.00				0	0	
Dieldrin [3] (4 experiments; 1 water suspension and 3 acetone solution)	.025	95	69	95			
	.05	75	68	79			
	.10	74	66	67			
	.25	67	58	30			
	.50	60	59	42			
	1.0	29	63	35			
	2.5	46	91	37			
	5.0	40	84	27			
	10.0	31	80	13			
Dipterex (1 experiment; water solution)	.025			67			
	.050			78			
	1.00		50	64			
Di-Syston (2 experiments; acetone solution)	.025	110	103	106			
	.050	98	99	116			
	.100	96	115	117			
	.25	103	97	101	99	91	98
	.50	82	91	80	92	81	96
	1.00	75	76	51	80	67	74
	2.50	53	64	9	75	2	14
	5.00	56	34	2	52	0	
	10.00	21	0		16	0	
Endrin [4] (5 experiments; 2 water suspension and 3 acetone solution)	.025	103	79	111			
	.050	91	67	70			
	.100	92	70	61			
	.25	52	67	38			
	.50	58	66	20			
	1.00	44	50	30			
	2.5	44	78	35			
	5.0	42	79	12			
	10.0	48	83	11			

See footnotes at end of table.

TABLE 1.—*Percentage of eggs of American oyster and hard clam that developed normally, percentage of larvae **that survived**, and percentage increase in mean length, in the presence of different concentrations of chemicals* [1]—Continued

Compound	Concentration	Oysters: Eggs developing	Oysters: Survival of larvae	Oysters: Increase in length of larvae	Clams: Eggs developing	Clams: Survival of larvae	Clams: Increase in length of larvae
	P.p.m.	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Insecticides—Continued							
Guthion (1 experiment; water solution)	.25	91	------	------	106	81	95
	.50	65	------	------	109	81	95
	1.00	0	------	------	31	38	98
	2.50	------	------	------	------	------	------
	5.00	0	------	------	0	0	------
	10.00	0	------	------	0	0	------
Lindane (1 experiment; water solution)	.25	127	------	------	97	117	86
	.50	114	------	------	112	102	98
	1.00	84	------	------	111	100	101
	2.50	------	------	------	------	------	------
	5.00	82	------	------	106	103	107
	10.00	43	------	------	60	67	71
Malathion (2 experiments; acetone solution)	.25	104	90	86	------	------	------
	.50	95	88	90	------	------	------
	1.00	101	66	77	------	------	------
	2.50	89	52	74	------	------	------
	5.00	85	20	72	------	------	------
	10.00	42	3	41	------	------	------
N-3452 (1 experiment; water solution)	.50	0	0	------	------	------	------
	1.0	0	0	------	------	------	------
	2.5	0	0	------	------	------	------
	5.0	0	0	------	------	------	------
N-3514 (1 experiment; water solution)	1.0	0	0	------	0	5	30
	10.0	0	0	------	0	0	------
Parathion (1 experiment; water solution)	.025	------	------	103	------	------	------
	.050	------	------	87	------	------	------
	1.00	------	------	22	------	------	------
Sevin (3 experiments; 1 water solution and 2 acetone solution)	.02	88	111	109	85	------	------
	.025	------	------	------	97	------	------
	.050	------	------	------	100	------	------
	.10	96	113	119	77	------	------
	.20	104	117	124	91	------	------
	1.00	106	119	106	94	------	------
	2.00	90	135	72	77	103	92
	2.50	------	------	------	64	------	------
	4.00	11	0	------	48	85	30
	5.00	------	------	------	0	------	------
	10.00	0	0	------	[2] 41	------	------
TEPP (1 experiment; water solution)	1.00	100	101	80	------	------	------
	2.50	91	93	70	------	------	------
	5.00	100	101	61	------	------	------
	10.00	74	90	41	------	------	------
Toxaphene (2 experiments; acetone solution)	.25	------	------	------	84	33	21
	.50	------	------	------	91	11	5
	1.00	------	------	------	51	0	------
	2.50	------	------	------	39	0	------
	5.00	------	------	------	37	0	------
	10.00	------	------	------	0	0	------
Herbicides:							
Amitrol (2 experiments; water solution)	2.50	101	99	117	------	------	------
	5.00	99	94	111	------	------	------
	10.00	103	100	96	------	------	------
	25.00	95	80	117	------	------	------
	50.00	104	80	112	------	------	------
	100.00	104	96	116	------	------	------
	250.00	104	51	71	------	------	------
	500.00	94	5	36	------	------	------
	1,000.00	2	0	------	------	------	------
Amitrol-T (1 experiment; water solution)	.25	93	91	116	------	------	------
	.50	100	89	107	------	------	------
	1.00	48	97	109	------	------	------
	2.50	93	96	105	------	------	------
	5.00	89	104	99	------	------	------
	10.00	88	78	108	------	------	------
2-4-D ester (1 experiment; acetone solution)	.025	------	95	99	------	------	------
	.05	------	97	95	------	------	------
	.10	------	80	89	------	------	------
	.25	90	102	70	------	------	------
	.50	89	98	100	------	------	------
	1.00	80	0	------	------	------	------
	2.50	89	------	------	------	------	------
	5.00	85	------	------	------	------	------
	10.0	27	------	------	------	------	------

See footnotes at end of table.

TABLE 1.—*Percentage of eggs of American oyster and hard clam that developed normally, percentage of larvae that survived, and percentage increase in mean length, in the presence of different concentrations of chemicals* [1]—Continued

Compound	Concentration	Oysters: Eggs developing	Oysters: Survival of larvae	Oysters: Increase in length of larvae	Clams: Eggs developing	Clams: Survival of larvae	Clams: Increase in length of larvae
	P.p.m.	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Herbicides—Continued							
2-4-D salt (2 experiments; water solution)	.025		84	148			
	.05		98	194			
	.10		93	134			
	.25	84	92	124			
	.50	91	89	111			
	1.0	82	102	119			
	2.5	78	103	83			
	5.0	79	112	86			
	10.0	88	97	77			
	25.0	29	104	62			
	50.0	32	52	39			
	100.0	0	45	8			
Diuron (1 experiment; water solution)	.25				92	128	99
	.50				91	112	101
	1.00				81	127	94
	5.00				0	[5] 100	62
EMID (4 experiments; water solution)	.25	103	99	81			
	.50	101	92	99			
	1.0	107	95	87			
	2.5	101	106	94			
	5.0	101	106	82			
	10.0	90	98	78			
	25.0	2	61	47			
	50.0	0	11	1			
	100.0	0	0				
Endothal (2 experiments; water solution)	.25	95	97	124	98	100	104
	.50	108	98	148	86	104	108
	1.00	101	97	129	85	103	104
	2.50	91	111	130	98	92	99
	5.00	102	109	129	93	91	91
	10.0	94	109	119	89	60	64
	25.0	57	74	96	76	0	
	50.0	1	48	90	51	0	
	100.0	0	2	18	0	0	
Fenuron (2 experiments; water solution)	.025				100	92	[6] 76
	.050				100	53	[6] 64
	.20				100	69	[6] 38
	.25				[7] 91	86	[7] 110
	.50				[7] 92	[7] 75	[7] 115
	1.00				[6,7] 98	[7,8] 95	[7,8] 119
	2.00				[6] 97	[6] 0	[6] 0
	4.00				[6] 98	[6] 0	[6] 0
	5.00				[7] 95	[7] 115	[7] 109
	10.00				[6] 115	[6] 0	[6] 0
MCPA (2 experiments; water solution)	.25	100	75	69			
	.50	103	89	99			
	1.00	99	89	110			
	2.50	94	88	86			
	5.00	96	80	77			
	10.00	80	89	61			
	25.00	0	64	30			
	50.00	0	8	14			
	100.00	0	0				
Monuron (1 experiment; water solution)	.25				93	120	114
	.50				99	122	109
	1.00				91	128	86
	5.00				92	111	93
Neburon (1 experiment; water solution)	2.4				0	0	
	4.8				0	0	
Silvex (1 experiment; acetone solution)	.025		89	128			
	.05		101	108			
	.1		99	91			
	.25	100	102	94			
	.50	81	85	38			
	1.0	75	0				
	2.5	78					
	5.0	56					
	10.0	22					
Nematocide:							
Nemagon (2 experiments; acetone solution)	.25				100	116	75
	.50				98	97	30
	1.00				100	14	21
	2.50				98	<1	4
	5.00				95	0	
	10.00				86	0	
Solvents:							
Acetone (1 experiment; confirmed by others)	10.0	162			91	100	110
	25.0				107	100	104
	50.0	122			98	100	91
	100.0	158			93	100	91
	250.0					100	87

See footnotes at end of table.

TABLE 1.—*Percentage of eggs of American oyster and hard clam that developed normally, percentage of larvae that survived, and percentage increase in mean length, in the presence of different concentrations of chemicals* [1]—Continued

Compound	Concentration	Oysters: Eggs developing	Oysters: Survival of larvae	Oysters: Increase in length of larvae	Clams: Eggs developing	Clams: Survival of larvae	Clams: Increase in length of larvae
	P.p.m.	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Solvents—Continued							
Allyl alcohol (1 experiment; water solution)	.25	—	—	—	95	0	—
	.50	—	—	—	76	0	—
	1.0	—	—	—	51	0	—
	2.5	—	—	—	0	0	—
	5.0	—	—	—	0	0	—
	10.0	—	—	—	0	0	—
Orthodichlorobenzene (1 experiment; acetone solution)	.25	—	—	—	107	121	95
	.50	—	—	—	94	111	98
	1.0	—	—	—	99	123	92
	2.5	—	—	—	94	107	97
	5.0	—	—	—	106	107	94
	10.0	—	—	—	79	82	86
Trichlorobenzene (1 experiment; acetone solution)	1.0	59	—	—	72	108	102
	10.0	21	—	—	58	69	104
Bactericides, fungicides, algicides, miscellaneous:							
Chloramphenicol (1 experiment; water solution)	.1	—	—	—	108	97	111
	.2	—	—	—	111	104	117
	1.0	—	—	—	106	84	104
	2.0	—	—	—	100	87	101
	10.0	—	—	—	107	90	81
	100.0	—	—	—	30	0	—
Delrad (1 experiment; water solution)	.01	—	94	89	—	117	96
	.05	—	9	50	—	[2] 88	42
	.1	—	0	—	—	0	—
	.2	—	0	—	—	0	—
Dowicide A (1 experiment; water solution)	.25	—	—	—	104	70	114
	.50	—	—	—	86	136	119
	1.00	—	—	—	102	0	—
	2.50	—	—	—	100	0	—
	5.00	—	—	—	94	0	—
	10.00	—	—	—	55	0	—
Dowicide G (1 experiment; water solution)	.25	—	—	—	0	0	—
	.50	—	—	—	0	0	—
	1.00	—	—	—	0	0	—
	2.50	—	—	—	0	0	—
	5.00	—	—	—	0	0	—
	10.00	—	—	—	0	0	—
Griseofulvin (1 experiment; water solution)	.025	—	—	—	—	106	101
	.050	—	—	—	—	94	100
	.10	—	—	—	—	99	109
	.25	—	—	—	36	101	98
	.50	—	—	—	0	105	103
	1.00	—	—	—	0	86	101
	2.50	—	—	—	0	—	—
	5.00	—	—	—	0	—	—
	10.00	—	—	—	0	—	—
PVP-Iodine (2 experiments; water solution)	.25	—	—	—	—	99	106
	.50	—	—	—	—	103	101
	1.00	—	—	—	—	108	102
	2.50	—	—	—	108	116	100
	5.00	—	—	—	103	85	97
	10.00	—	—	—	94	92	93
	25.00	—	—	—	1	82	56
	50.00	—	—	—	0	1	9
	100.00	—	—	—	0	—	—
Nabam (1 experiment; water solution)	.5	0	—	—	0	118	9
	1.0	0	—	—	0	102	8
	2.5	0	—	—	0	0	—
	5.0	0	—	—	0	0	—
	10.0	0	—	—	0	0	—
Nitrofurazone (1 experiment; water solution)	2.50	—	—	—	104	94	97
	5.00	—	—	—	111	101	83
	10.00	—	—	—	111	101	75
	25.00	—	—	—	119	87	53
	50.00	—	—	—	103	93	45
	100.00	—	—	—	102	80	23
Omazene (2 experiments; water solution)	.025	87	107	122	101	93	104
	.05	79	103	123	97	91	104
	.10	34	84	112	21	93	98
	.25	0	76	22	0	90	74
	.50	0	0	—	0	12	33
	1.00	0	0	—	0	0	—

See footnotes at end of table.

TABLE 1.—*Percentage of eggs of American oyster and hard clam that developed normally, percentage of larvae that survived, and percentage increase in mean length, in the presence of different concentrations of chemicals* [1]—Continued

Compound	Concentration	Oysters: Eggs developing	Oysters: Survival of larvae	Oysters: Increase in length of larvae	Clams: Eggs developing	Clams: Survival of larvae	Clams: Increase in length of larvae
	P.p.m.	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Bactericides—Continued							
Pentachlorophenol (1 experiment; acetone solution)	.025	--	115	104	--	--	--
	.05	--	86	80	--	--	--
	.10	--	0	--	--	--	--
	.25	0	0	--	--	--	--
	.50	0	0	--	--	--	--
	1.00	0	--	--	--	--	--
	2.50	0	--	--	--	--	--
	5.00	0	--	--	--	--	--
Pentachlorophenyl acetate (1 experiment; acetone solution)	.025	--	41	60	--	--	--
	.05	--	0	--	--	--	--
	.10	--	0	--	--	--	--
	.25	0	0	--	--	--	--
	.50	0	0	--	--	--	--
	1.00	0	--	--	--	--	--
	2.50	0	--	--	--	--	--
	5.00	0	--	--	--	--	--
Phenol (1 experiment; water solution)	.025	103	--	--	111	121	109
	.05	110	--	--	101	131	114
	.10	112	--	--	126	143	115
	.20	102	--	--	105	130	119
	1.0	103	--	--	99	137	125
	2.0	94	--	--	113	166	128
	10.0	102	--	--	95	149	55
	100.0	5	--	--	0	0	--
Phygon (2 experiments; acetone solution)	.00025	110	106	100	97	99	99
	.0050	101	113	76	91	99	99
	.01	69	95	39	65	104	96
	.025	0	79	20	1	94	76
	.050	0	32	9	0	58	31
	.10	0	0	--	0	34	21
	.25	--	--	--	0	0	--
	.50	--	--	--	0	0	--
	1.00	--	--	--	0	0	--
Roccal (1 experiment; water solution)	.1	--	--	--	97	80	86
	.2	--	--	--	45	0	--
	1.0	--	--	--	0	0	--
Rosin Amine D (1 experiment; acetone solution)	.025	--	5	--	--	--	--
	.05	--	0	--	--	--	--
	.10	--	0	--	--	--	--
	.25	0	0	--	--	--	--
	.50	0	0	--	--	--	--
	1.00	0	--	--	--	--	--
	2.50	0	--	--	--	--	--
	5.00	0	--	--	--	--	--
Sulmet (1 experiment; water solution of tinted veterinary formula)	.1	--	--	--	103	97	109
	.2	--	--	--	108	113	112
	1.0	--	--	--	111	103	106
	2.0	--	--	--	105	106	87
	10.0	--	--	--	95	113	83
	100.0	--	--	--	107	98	37
Sulmet (1 experiment; water solution of new untinted 1962 formulation)	50.00	--	90	150	--	111	97
	100.00	--	95	155	--	90	100
	150.00	--	94	148	--	--	--
	200.00	--	108	138	--	114	101
	300.00	--	108	131	--	111	100
	400.00	--	110	121	--	108	98
	500.00	--	112	92	--	110	96
	600.00	--	101	72	--	--	--
	1,000.00	--	--	--	--	98	85
TCC (1 experiment; water solution)	.0025	--	--	--	--	106	91
	.005	--	--	--	--	92	48
	.01	--	--	--	--	19	5
	.025	--	--	--	69	89	18
	.05	--	--	--	0	7	6
	.1	--	--	--	0	0	--
	.25	--	--	--	0	--	--
	.50	--	--	--	0	--	--
	1.00	--	--	--	0	--	--
TCP (1 experiment; water solution)	.025	--	100	116	--	--	--
	.05	--	94	129	--	--	--
	.10	--	96	117	--	--	--
	.25	96	87	116	--	--	--
	.50	62	99	114	--	--	--
	1.00	2	85	14	--	--	--
	2.50	20	--	--	--	--	--
	5.00	0	--	--	--	--	--
	10.00	0	--	--	--	--	--

[1] Relative percentages calculated by using survival and growth rates of larvae in control cultures.

[2] 1 experiment only.

[3] Results with Dieldrin very erratic (see text).

[4] Results with Endrin very erratic (see text).

[5] 1 culture only; duplicate 97 percent mortality.

[6] 1st experiment.

[7] 2d experiment.

[8] 97 percent mortality in 1st experiment.

[9] 1 culture only; duplicate 100 percent mortality.

TABLE 2.—*Estimated concentrations (TL_m values), calculated by interpolation from data in table 1, at which 50 percent of the eggs of oysters and clams develops normally or 50 percent of the larvae survives*

Compound	Oysters		Clams	
	48-hr. TL_m eggs	14-day TL_m larvae	48-hr. TL_m eggs	12-day TL_m larvae
Insecticides:				
Aldrin			>10.00	0.41
Co-Ral	0.11	>1.00	9.12	5.21
DDT		.034		
Dicapthon			3.34	5.74
Dieldrin	.64	>10.00		
Dipterex		1.00		
Di-Syston	5.86	3.67	5.28	1.39
Endrin	.79	>10.00		
Guthion	.62		.86	.86
Lindane	9.10		>10.00	>10.00
Malathion	9.07	2.66		
N-3452	<.50	<.50		
N-3514	<1.00	<1.00	<1.00	<1.00
Parathion				
Sevin	3.00	3.00	3.82	>2.50
TEPP	>10.00	>10.00		
Toxaphene			1.12	<.25
Herbicides:				
Amitrol	733.70	255.44		
Amitrol-T	>10.00	>10.00		
2-4-D ester	8.00	.74		
2-4-D salt	20.44	64.29		
Diuron			2.53	>5.00
EMID	16.82	30.00		
Endothal	28.22	48.08	51.02	12.50
Fenuron			>10.00	>5.00
MCPA	15.62	31.30		
Monuron			>5.00	>5.00
Neburon			<2.4	<2.4
Silvex	5.90	.71		
Nematocide: Nemagon			10.00	.78
Solvents:				
Acetone	>100.00		>100.00	>100.00
Allyl alcohol			1.03	<.25
Orthodichlorobenzene			>100.00	>100.00
Trichlorobenzene	3.13		>10.00	>10.00
Bactericides, fungicides, algicides, miscellaneous:				
Chloramphenicol			74.29	50.00
Delrad		.031		.072
Dowicide A			>10.00	.75
Dowicide G			<.25	<.25
Griseofulvin			<.25	<1.00
PVP-Iodine			17.10	34.94
Nabam	<.50		<.50	1.75
Nitrofurazone			>100.00	>100.00
Omazene	.078	.34	.081	.378
Pentachlorophenol	<.25	.071		
Pentachlorophenyl acetate	<.25	<.025		
Phenol	58.25		52.63	55.00
Phygon	.014	.041	.014	1.75
Roccal			.19	.14
Rosin Amine D	<.25	<.025		
Sulmet, tinted			>100.00	>100.00
Sulmet, untinted	>600.00	>600.00	>1,000.00	>1,000.00
TCC			.032	.037
TCP	.60	>1.00		

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APPENDIX

TABLE A-1.—*Source, solubility, and chemical names of compounds tested*

Common name	Chemical name	Solubility in p.p.m.	Source
Insecticides:			
Aldrin	Hexachlorohexahydro-endo, exo-dimethanonaphthalene.	Insoluble in water; 1,590,000 in acetone	Shell Chemical Corp.
Co-Ral	O,O-diethyl O-3-chloro-4-methyl-2-oxo-2H-1-benzopyran-7-yl phosphorothioate.	Insoluble in water; soluble in acetone	Chemagro Corp.
DDT	Dichlorodiphenyltrichloroethane	0.0002 in water (0.2 as colloid); 590,000 in acetone.	
Dicapthon	O-O-dimethyl-O(2 chloro-4 nitrophenyl) phosphorothioate.	Very low solubility in water; very low solubility in acetone.	American Cyanamid Co.
Dieldrin	Hexachloroepoxyoctahydro-endo, exo-dimethanonaphthalene.	Insoluble in water; 540,000 in acetone	Shell Chemical Corp.
Dipterex	O,O-dimethyl-1-hydroxy-2,2,2-trichloroethyl-phosphonate.	130,000 in water	Chemagro Corp.
Di-Syston	O,O-diethyl S-2-(ethylthio)ethyl phosphorodithioate.	25 in water; soluble in acetone	Do.
Endrin	Hexachloroepoxyoctahydro-endo, endo-dimethanonaphthalene.	"Insoluble" in water; "moderately" soluble in acetone.	Shell Chemical Corp.
Guthion	O,O-dimethyl S-4-oxo-1,2,3-benzotriazino-3(4H)-ylmethyl phosphorodithioate.	33 in water	Chemagro Corp.
Lindane	1,2,3,4,5,6-hexachlorocyclohexane	10 in water; 440,000 in acetone	Niagara Chemicals Division, Food Mach. & Chem. Corp.
Malathion	O,O-dimethyl dithiophosphate of diethyl mercaptosuccinate.		American Cyanamid Co.
N-3452	Alkyl (C_8–C_{18}) dimethyl benzyl ammonium chloride.		Niagara Chemicals Division, Food Mach. & Chem. Corp.
N-3514	2-chloro-1-nitropropane	8,000 in water	Do.
Parathion	O,O-diethyl O-p-nitrophenyl thiophosphate.	20 in water	Chemagro Corp.
Sevin	1-naphthyl-N-methylcarbamate	1,000 in water; 300,000 in acetone	Union Carbide Chem. Co.
TEPP	Tetraethyl pyrophosphate	Miscible in water (hydrolyzes); miscible in acetone.	Niagara Chemicals Division, Food Mach. & Chem. Corp.
Toxaphene	Mixture of polychloro bicyclic terpenes with chlorinated camphene predominating.	1.5 in water; 4,500,000 in acetone	Hercules Powder Co.
Herbicides:			
"Amitrol" Aminotriazole	3-amino-1H-1,2,4-triazole	Very soluble in water	
Amitrol-T	3-amino-1H-1,2,4-triazole with ammonium thiocyanate.	do	
2-4-D ester	Butoxyethanol ester of (2,4-dichlorophenoxy) acetic acid.	"Insoluble" in water	Thompson Hayward Chemical Co.
2-4-D salt	Dimethylamine salt of (2,4-dichlorophenoxy)acetic acid.	Very soluble in water	Do.
Diuron	3-(3,4-dichlorophenyl)-1,1-dimethylurea	42 in water	E. I. duPont de Nemours & Co.
EMID	2,4-dichlorophenoxy-acetamide	Very soluble in water	
Endothal	Disodium 3,6-endoxohexahydrophthalate	280,000 in water	Pennsalt Chemical Co.
Fenuron	3 phenyl-1,1-dimethylurea	2,900 in water	E. I. duPont de Nemours & Co.
MCPA	Dimethylamine salt of (2-methyl-4-chlorophenoxy) acetic acid.	Very soluble in water	American Chemical Paints Co.
Monuron	3-(p-chlorophenyl)-1, 1-dimethylurea	230 in water	E. I. duPont de Nemours & Co.
Neburon	1-n-butyl-3-(3,4-dichlorophenyl)-1-methylurea.	4.8 in water	Do.
Silvex	Butoxyethanol ester of (2,4,5-trichlorophenoxy)propionic acid.	0.014 percent in water; 15.2 percent in acetone.	
Nematocide:			
Nemagon	1,2-dibromo-3-chloropropane	1,000 in water; very soluble in acetone	Shell Chemical Corp.
Solvents:			
Acetone	Acetone	Miscible in water	Mallinckrodt Chemical Works.
Allyl alcohol	Propen-1-ol-3	do	Shell Chemical Corp.
Orthodichlorobenzene	o-dichlorobenzene	130 in water; miscible in acetone	Niagara Chemicals Division, Food Mach. & Chem. Corp.
Trichlorobenzene	Trichlorobenzene	25 in water; miscible in acetone	Do.
Bactericides, fungicides, algicides, miscellaneous:			
Chloramphenicol	(Chloromycetin)D-(-)-threo-2-dichloroacetamido-1-p-nitrophenyl-1,3-propanediol.	2,500 in water; very soluble in acetone	Parke, Davis & Co.
Delrad	Dehydro-abietylamine acetate	Very soluble in water	Hercules Powder Co.
Dowicide A	O-phenylphenol, sodium salt	1,220,000 in water	Dow Chemical Co.
Dowicide G	Sodium pentachlorophenate, technical	330,000 in water	Do.
Griseofulvin	7-chloro-2',4,6-trimethoxy-6'-methylspiro[benzofuran-2(3H),1'-[2]cyclohexene]-3,4-dione.	10.0 in water	McNeil Laboratories, Inc.
PVP-Iodine	Polyvinylpyrrolidone-iodine complex	Very soluble in water	Antara Chemicals Division, General Analine & Film Corp.
Nabam	Disodium ethylenebis(dithiocarbamate)	do	Niagara Chemicals Division, Food Mach. & Chem. Corp.
Nitrofurazone	5-nitro-2-furaldehyde semicarbazone	238 in water	Hess Clark Division, Vick Chemical Co.
Omazene	Copper dihydrazinium sulfate	"Very slightly" soluble in water	Olin Mathieson Chemical Corp.
Pentachlorophenol	Pentachlorophenol	80 in water; very soluble in acetone	Monsanto Chemical Co.
Pentachlorophenyl acetate	Pentachlorophenyl acetate		Niagara Chemicals Division, Food Mach. & Chem. Corp.
Phenol	Phenol	82,000 in water; very soluble in acetone	Mallinckrodt Chemical Works.
Phygon	2,3-dichloro-1,4-naphthoquinone	"Insoluble" in water	Niagara Chemicals Division, Food Mach. & Chem. Corp.
Roccal	Alkyl (C_8H_{17}–$C_{18}H_{37}$)dimethylbenzyl-ammonium chloride.	10 percent solution; very soluble in water.	Sterwin Chemicals, Inc.
Rosin Amine D	Rosin amine D (technical grade of dehydroabietylamine).	"Insoluble" in water; very soluble in acetone.	Hercules Powder Co.
Sulmet (tinted veterinary soluble)	(Sodium sulfamethazine)sodium(4,6-dimethyl-2-sulfanilamidopyramidine).	Very soluble in water	Agricultural Division, American Cyanamid Co.
Sulmet (untinted soluble)	do	do	Do.
"TCC"	3,4,4'-trichlorocarbanilide	"Insoluble" in water; 40,000 in acetone	Monsanto Chemical Co.
2,4,5 TCCPA	Propylene glycol to butyl ether esters of 2-(2,4,5-trichlorophenoxy)propionic acid.		

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