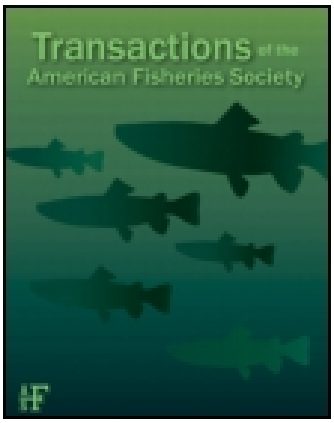

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A Method for Establishing Acceptable Toxicant Limits for Fish—Malathion and the Butoxyethanol Ester of 2,4-D

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A Method for Establishing Acceptable Toxicant Limits for Fish—Malathion and the Butoxyethanol Ester of 2,4-D

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ABSTRACT

Two 10-month exposures of malathion and butoxyethanol ester of 2,4-D under continuous-flow conditions were made to determine the effect of these pesticides on reproduction and growth of fathead minnows, *Pimephales promelas* Rafinesque. Results show that 1/45 and 1/19 of the 96-hour median tolerance limit (TL_m) concentrations of malathion and butoxyethanol ester, respectively, will not harm growth and reproduction of fathead minnows during continuous exposure. We suggest that these two fractions applied to TL_m concentrations determined for other species and other types of water should estimate accurately safe concentrations for other fishes to these pesticides.

RATIONALE OF THE EXPERIMENTS

In the many articles on toxicity of pollutants to fishes, one is impressed by the absence of suggestions for permissible concentrations based upon quantitative experimental data. Enactment of water pollution control legislation in 1965 (P. L. 89–234) suggests that some type of "standards" will be forthcoming in the near future. Persons concerned with the well-being of fishes will be among those who must decide upon acceptable concentrations for specific water uses. The concern herein discussed is for the specific use of fish production. As used in this paper, fish production refers to rate of growth and reproduction of the fish in question.

Two major problems hinder rapid progress toward agreement on permissible levels for aquatic life. One is the application of laboratory data to field conditions in the many instances in which adequate field studies cannot or will not be made. The second, and possibly more important, difficulty is the inability of professional personnel to agree on the type of effect to be measured to assess damage.

Obviously, the problem of applying laboratory findings to field situations will always exist as long as laboratory experiments are used. If it is true, however, that acceptable concentrations will have to be established solely from laboratory data because of practical considerations of time and expense, then the pollution biologist should conduct only the most meaningful laboratory tests available. This implies that the results of laboratory experiments should be verified when possible by field observations.

To determine acceptable concentrations of toxic materials from laboratory tests, the investigator must choose a measurable effect that will indicate when the fish are in an unacceptable environment. Some of the measurable effects that have been used are death, behavioral and physiological change, growth rate, and tainting of the flesh. Persons concerned with pollution abatement often encounter authorities who are not willing to agree that the environment is deterimental to the fish just because an environment produces a change from normal. Pollution biologists must have quantitative data to prove that the observed change resulting from exposure is ecologically detrimental to fish or the harvestable crop. An exposure causing death is obviously significant, but even the best fish physiologist would have difficulty establishing that a 10% reduction in the hematocrit would result in an undersirable effect on a population. Even a reduction in growth during a 30-day exposure might be explained as a transient effect that would be insignificant over a long period. The measured effect used to evaluate the significance of an exposure should, therefore, be readily relatable to those factors important in maintaining an acceptable commercial or sport fishery.

We propose the use of a "laboratory fish production index" (LFPI) as the measure of effect to furnish a first approximation as to whether an environment is unacceptable

to fish. For the index, exposure data would be gathered over at least one generation and would reflect effects on growth, reproduction, spawning behavior, viability of eggs, and growth of the fry. It seems logical that a fish production rate not greatly different from that present in an uncontaminated environment would indicate that the tested environment should be judged acceptable. Therefore, the "maximum acceptable toxicant concentrations" would be established on the basis of chronic exposures using LFPI as the measure of effect.

Obviously, the LFPI does not take into account tainting, behavioral change that could alter fishing success, shifts among dominant species, accumulation of unacceptable residues in flesh, or sensitivity of food organisms; and it is not certain that all adverse effects will be apparent in a one-generation test. We contend, however, that the LFPI is both more inclusive and more practical to determine than any other measure of effect currently in use. In addition, results from laboratory exposures in which the LFPI is used as the measure of effect would probably require less extrapolation for use in field application. In the field one would find difficulty in rejecting a specified permissible concentration if the *species of concern* produced adequate poundage per acre and the fish were normal in size and quality.

Even though maximum acceptable toxicant concentrations as established by the LFPI may be very useful, many species of fish cannot be subjected to this type of test. Also, there is not enough time to test even important species of fish against most of the important pollutants, especially when the effect of environmental variables on toxicity is considered. We propose that maximum acceptable toxicant concentrations for species that can be tested in the laboratory be used to calculate "application factors" for estimating "safe concentrations" for other species that cannot be laboratory tested. Henderson's (1957) broader approach toward application factors incorporated this same idea. Our use of chronic exposure and the LFPI incorporates many considerations suggested by him and we are proposing that the application factor for a particular toxicant be calculated by dividing the concentration not inhibiting reproduction or growth by the acute (48- or 96-hour) TL_m value. The resulting fraction is then multiplied by a TL_m value for fish and waters for which long-term testing cannot be done to establish an estimate of the safe concentration for other species for the pollutant of concern. In this way one can utilize the direct and interpretable LFPI data and the ease of acute tests. Application factors derived for one species and one kind of water have not yet been shown to be applicable to other species and water. This approach, however, seems to have a more logical basis than some commonly suggested, arbitrary factor such as 10. In the past many investigators have avoided the use of chronic (long-term) toxicity tests because such tests are difficult, expensive, and time-consuming. Most researchers either have concentrated on determining 24- to 96-hour TL_m values and then extrapolating these results to estimate the "safe" concentrations or have used more sensitive physiological and behavioral measurements and speculated about the significance of the changes observed to the well-being of the fish.

It is the purpose of this paper to report the methods and the results of two 10-month laboratory exposures under flow-through conditions carried out to establish (1) the practicability of using the LFPI as the measure of effect of exposure and (2) the determination of an application factor for the species and toxicants tested. To overcome deficiencies extant in the tests, suggested improvements are given. Finally, we acknowledge that, until the validity of applying an application factor derived for one species and one kind of water to other species and waters is established, and until some field observations are completed, the suggested approach must be tentative.

INTRODUCTION

Malathion was chosen as one of the first two toxicants to be studied because of the large amount in use, its low mammalian toxicity, and its relatively high toxicity to fish. Its persistence is apparently very dependent on conditions, pH, but in general it is

more stable than many other organophosphates. Johnson (1955) found hatchery pond water to be as toxic 14 days after treatment as on the first day of treatment. Johnson, Krog, and Poland (1963) list the consumption of malathion in the United States as 6 to 12 million pounds per year.

Pickering, Henderson, and Lemke (1962) point out the great variability in the sensitivity of various fish species to organophosphate insecticides. There is then no need to review here the toxicity of malathion to many species except to mention the wide range in concentrations that are acutely toxic to fish. Pickering *et al.* (1962) report a 96-hour TL_m of 12.5 mg/l for fathead minnows. The lethal level is 0.1 mg/l at 55 F for rainbow trout and as low as 0.02 mg/l for bluegills at 85 F (U. S. Dept. Interior, 1963). Anderson (1960) reported for *Daphnia* that 0.0009 mg/l was the concentration that produced immobilization in 50 hours.

The second toxicant tested was the butoxyethanol ester of 2,4-D. It is used extensively for aquatic vegetation control; according to Smith (1963) 87.5 tons of a 20% active ingredient formulation of this ester was used during a 1-month period on 2 Tennessee Valley Authority reservoirs. Static tests with fathead minnows using 2,4-D acid without solvents resulted in no observable effect at 500 mg/l for 96 hours.

The reported toxicities for 2,4-D compounds are so variable because of the particular form (ester, acid, etc.), solvents, and emulsifers that a summary of toxicity data is difficult. Beaven, Rawls, and Beckett (1962) found no acute toxicity of 30, 60, and 120 lb/acre (acid equivalent) of this ester to oysters, crabs, clams, sunfish, and perch. As Springer (1957) points out, some, and sometimes nearly all, toxicity of a formulation may be due to emulsifiers and solvents. According to Hughes and Davis (1963) the 96-hour TL_m of 2,4-D herbicides ranges from approximately 1 mg/l for esters to 900 mg/l for alkanolamine salts of 2,4-D.

METHODS

We began the chronic tests in December, continuously exposing 1-inch fathead minnows, *Pimephales promelas* Rafinesque, to a series of concentrations of malathion and butoxyethanol ester of 2,4-D (hereinafter referred to as BEE). Exposure was continued until the end of the following September when spawning decreased markedly and, when by gross observation, no additional spawning was expected from fish that had not previously spawned. There were four test concentrations and a control, all in duplicate for both pesticides. Initially, 10 fish were placed in each tank; and in March the number was reduced to five because ten were thought to be too many for satisfactory egg production. We wanted two males and three females in each tank; however, sexing was difficult at that time of year, and the desired sex ratios were not obtained in every case.

The tanks were rectangular and were made of Type 304 stainless steel. These were 24 × 6 × 8 inches deep with glass ends sealed in place with aquarium cement. Water depth was maintained at 3 inches to provide a water volume of approximately 7 liters. For spawning sites, cement-asbestos tiles, 3 inches in diameter, were cut into 3-inch lengths and each piece was then cut in half to give a semicircular cross section. Two such pieces were placed side by side near each end of the tanks so that the underside of the tile could be seen through the glass ends.

Ten tanks were placed on each of 2 aluminum tables in the laboratory, and two 40-watt, white fluorescent bulbs were placed 2 feet above the water surface. The programmed photo period was 12 hours until 1 June, when it was increased to 16 hours for the duration of the test. Initially, plastic curtains were placed over the ends of each tank to avoid disturbance to the minnows by laboratory activity. By experience, we found that this was not necessary, and they were removed to make observation easier.

Since there were only five fish in each chamber, nearly every spawning could be recorded, but, undoubtedly, some eggs disappeared before they were observed. Markens (1934) noted that the first eggs produced were often eaten but, contrary to his findings, we found no spawning during the night. Fry from two egg masses from each tank were

reared to 6 weeks of age in order to check for gross abnormalities. These fry were obtained by putting the tile holding the eggs in a separate container, flowing test water over them, and placing an air stone beside the eggs to supply oxygen and keep the eggs clean. Occasionally, those fry that hatched from eggs left with the males would escape being eaten, but usually all were eaten immediately after hatching. The fry that were reared for 6 weeks in separate chambers (receiving the tank effluent) were then released into the test tanks where they grew rapidly; some were nearly 1 inch long when the test was terminated.

No growth data were obtained on either the adults or fry because of the danger of excessive handling stress on the fish. Recent experience indicates that such data can be obtained up to sexual maturation when growth rates may change. We have found that a second generation can be obtained in a 1-year test if eggs are produced early enough and that 10 fish can breed successfully in these chambers.

A serial dilution system like the one described by Mount and Warner (1965) was used to maintain the pesticides at the desired concentration by continuous renewal of the test water. The malathion was added to the diluter as a 100 mg/l aqueous solution by means of the chemical metering apparatus described in the above reference. Sufficient acetone and Triton X-100[1] were added to aid in dissolving the malathion in deionized water (malathion is soluble up to 145 mg/l).[2] In the final test water, the highest concentrations of acetone and Triton X-100 were 2 mg/l and 0.03 mg/l, respectively. Equal amounts of each were added to the control in a similar manner. In tests (unpublished) at this laboratory the 96-hour TL_m's for acetone and Triton X-100 are 10,700 mg/l and 6 mg/l, respectively.

The BEE was added to the serial diluter by a microinjector designed in the laboratory. Basically, a lever arm, activated by the incoming water and connected through a gear train, injected 1.5 μl of the BEE-acetone solution from a 5-ml glass syringe. The stock solution was made by diluting 17 g of BEE and 2 g of Triton X-100 to a volume of 100 ml in acetone. The highest concentrations of acetone and Triton X-100 in the water were approximately 7.0 mg/l and 0.2 mg/l, respectively. Equal amounts of each were added to the control as described for malathion.

The experimental malathion, furnished by American Chemical Paint Company, contained 95% active ingredient. This company also furnished reference standards of malaoxon and malathion diacid. The BEE, from an experimental sample from American Chemical Paint Company,[3] contained 90% ester. The 2,4-D acid and 2,4-dichlorophenol came from Matheson, Coleman, and Bell.

The pesticides and certain decomposition products were measured on a gas chromatograph equipped with an electron-capture detector and a ⅛-inch by 5-foot-length aluminum column packed with 5% SF-96 on 60/80 mesh Chromosorb W. The measurement of the unaltered pesticides in test water was accomplished by the extraction of water samples with hexane and the injection of an aliquot of the hexane extract onto the gas chromatograph. Peak heights were compared to those of standards run at the same time.

Since both of these pesticides are organic esters, a two-part procedure was developed to allow for measuring hydrolysis products as well as unchanged pesticides. In the first part of the procedure, the unchanged pesticide was extracted from the water sample (pH = 7 to 8) with 30-ml portions of dichloromethane by gentle shaking for 30 seconds and extracting 3 times. The combined extracts were evaporated to dryness on a steam bath after the addition of 2 ml of a 1% solution of polyethylene glycol 600 in dichloromethane (Johnson, 1964). The insecticides

[1] Mention of product does not constitute endorsement by the Federal Water Pollution Control Administration.

[2] Anon. Mosquito control with malathion insecticide. Tech. Bulletin, Cyanamid.

[3] We have since been informed that the company has changed its name to Amchem Products, Inc., and that in the past BEE was produced from essentially pure n-butoxyethanol. Recently the company began producing BEE from a mixture of n-butoxyethanol and iso-butoxyethanol. We found no difference in 96-hour TL_m's between the two compositions, that we used in our tests, and the new one.

were dissolved in 5 ml of heptane and measured on the gas chromatograph as before.

In the second part of the procedure, the hydrolysis product of the insecticide was extracted from the same water sample in the same manner after acidification with 1 ml of concentrated HCl per 100 ml water. Polyethylene glycol was added to the combined extracts and they were evaporated to dryness. Five ml of a methanol-ether solution (Erickson and Hield, 1962) and 1 ml of diazomethane solution (Yip, 1964) were added, and the solvents were allowed to evaporate at room temperature. The residue was dissolved in 5 ml of heptane and examined on the gas chromatograph. Recoveries from samples spiked with malathion, BEE, and 2,4-D acid were not obviously different from 100%. Malaoxon and 2,4-dichlorphenol cannot be measured by these procedures.

PHYSICAL AND CHEMICAL CONDITIONS

Initially, the dilution water for the tests was carbon-filtered Cincinnati tap water that had been held approximately 60 days in an open, outdoor, concrete pool. In April, before spawning began, we changed to spring water (also detained in the pool) from the Newtown Fish Farm because there was less chance that undesirable substances would be present (Cincinnati tapwater is from the Ohio River). The two types of water were similar in characteristics. During the 10-month tests, the pH of the water ranged from 7.1 to 8.4, and the total hardness, from 112 to 198. *Daphnia* and other invertebrates were abundant from time to time, and there was a moderate production of phytoplankton. A varying photosynthetic rate caused the pH fluctuation. Each chamber received 25 ml of water per minute and this volume furnished a supply of live food supplemental to the dry trout diet that was fed *ad libitum*. No attempt was made to remove the profuse growth of green algae and diatoms from the bottom and sides of the test chambers, but uneaten food and excrement were removed when necessary.

Temperature was not controlled because earlier studies indicated that the fish did not spawn as readily if constant warm temperatures were maintained. From December until the end of March the mean daily temperature was very close to 15 C; the temperature then rose slowly through April and May, and by 15 June the average daily temperature was 24 C. The mean daily temperature from 15 June until the end of September was 24 to 25 C, with maximum temperature of 26 C during the warmer afternoons. The maximum diurnal temperature fluctuation was 3 C. Temperatures were continuously recorded in one chamber; checks made at various times by means of an 11-channel recorder indicated less than 1 C variation among all chambers.

Results of analyses for six commonly measured chemical characteristics of the test water were as follows: (1) dissolved oxygen, 5 to 10 mg/l; (2) pH, 7.4 to 8.4; (3) methyl orange alkalinity, 40 to 148 mg/l as $CaCO_3$; (4) total versenate hardness, 111 to 192 mg/l as $CaCO_3$; (5) conductivity, 320 to 470 μmhos; and (6) acidity, 0 to 4 mg/l as $CaCO_3$. Procedures described in Standard Methods (1960) were used throughout.

Tables 1 and 2 list the nominal and measured values of malathion, BEE, and 2,4-D acid. Within the first month, the rate of degradation (presumably hydrolysis) of malathion had stabilized, and several checks showed that the degradation occurred in the test chamber and not in the water stock solution. The influent to the test chambers when measured was always near the calculated concentration. The test for hydrolysis products of malathion gave one peak with methylation and none without. The retention time was about halfway between that of malathion and methylated malathion diacid. Thus, this substance was presumed to be malathion monoacid; and, assuming it has about the same electron-capture ability as malathion, it was present in significant quantity. Malathion monoacid and malathion diacid are common metabolites of malathion (O'Brien, 1960). There was also degradation of BEE to 2,4-D acid as shown in Table 2. The hydrolysis of 2,4-D esters has been found in plants (Erickson, Brannaman, and Coggins, 1963) and water (Aly and Faust, 1964). These results show that the exposures were continuous, but not constant, and that in both studies there was a simultaneous chronic ex-

TABLE 1.—*Nominal and measured concentrations of malathion in "grab" samples taken from the test chambers*

Nominal concentration (mg/l)	Measured concentration (mg/l)		
	Mean	Range	Number of measurements
1.00	0.58	0.32 – 0.94	24
0.33	0.20	0.10 – 0.38	22
0.11	0.07	0.03 – 0.12	23
0.04	0.03	0.002– 0.04	23

TABLE 2.—*Nominal and measured concentrations of BEE and 2,4-D acid in "grab" samples from the test chambers*[1]

Nominal concentration (mg/l)		Measured concentration (mg/l)					
		Mean		Range		Number of measurements	
BEE	Acid	BEE	Acid	BEE	Acid	BEE	Acid
1.8	0	0.31	0.80	T–0.68[2]	0.33 – 1.35	22	14
0.6	0	0.06	0.20	T–0.22	0.05 – 0.53	24	16
0.2	0	0.03	0.04	T–0.09	0.01 – 0.10	23	16
0.07	0	0.01	0.01	T–0.04	0.002– 0.03	21	16

[1] To convert the BEE to the acid equivalent, multiply the BEE concentration by 0.67.
[2] T = <0.01.

posure to the unchanged pesticide and to its hydrolysis product.

The calculated dilution error of both serial diluters was ± 3% or less for each concentration. There was more error in the daily delivery of the chemical metering apparatus. For malathion the daily calculated concentration was between 86 and 100% of the mean for 90% of the days, and similarly for BEE, the concentrations were between 95 and 100% of the mean on 90% of the days. In both cases the distribution is skewed toward the low side because a high delivery rate was unlikely because of the physical arrangement (Mount and Warner, 1965). The analyses of grab samples indicate much greater variability than can be attributed to mechanical errors; thus the delivery system variation was insignificant.

RESULTS

A. Malathion.—During the first 7 weeks of exposure, 20% of the fish in the 0.58 mg/l concentration died. One fish in the 0.20 mg/l concentration died after showing typical symptoms of malathion toxicity (flexed fins, crooked back, and hemorrhaging). After 4-months' exposure (when the number of fish in each chamber was reduced to five from the original 10) 16 fish survived in the 0.58 mg/l concentration, but four of these were deformed and sickly. Apparently, 0.58 mg/l is very near the maximum concentration in which prolonged survival is possible.

Spawning began on 13 May. In Table 3 the sex ratios and spawnings per female for the fish in the 10 malathion test chambers are listed. There is no consistent relationship between concentration and number of spawnings. One important observation is that, at the mean concentration of 0.58 mg/l (which killed or crippled 40%), some fish survived and were able to spawn, although all the eggs produced could easily have come from one female. The eggs hatched normally, and the fry produced were the same as the controls in appearance, behavior, and growth. Apparently there was no effect at 0.07 mg/l, and judging from behavior, external sexual development, and ovarian egg size, there was no effect at 0.20 mg/l except the single death noted above. Measurements of length and visual estimation of the condition of the fish indicated no effect on growth or adult size.

Eggs produced by unexposed fish were placed in egg cups (Pickering and Vigor, 1965) under the influents to the high concentration and the control tanks. Survival of the eggs in all four cups was 90% or better even though the malathion concentration in the influent was approximately 1.0 mg/l. Both groups of fry survived equally well for 14 days.

Histological examinations of liver and kidneys were made on the adults by means of haemotoxylin and eosin stains. No obvious pathology was observed except for kidney degeneration in one fish from the 0.58 mg/l concentration.

B. BEE.—No mortality occurred among the fish exposed to BEE. In Table 4, the spawning data for the BEE exposures are listed and as with the malathion data, the relationship between concentration and spawning is not consistent. The fish in all tanks appeared to be normal in behavior and in sexual development. Spawning began on 27 April, and fry hatched

TABLE 3.—*Sex ratios and spawnings per female for the malathion exposure*

Mean water concentration (mg/l)	Tank	Number females	Number males	Observed spawnings/ female
0.58	6	3	2	1.3
0.58	6A	2	3	0
0.20	7	1	4	0
0.20	7A	2	3	0.5
0.07	8	3	2	3.3
0.07	8A	4	1	4.0
0.03	9	4	2	0.8
0.03	9A	3	2	2.7
Control	10	2	3	5
Control	10A	3	2	1

TABLE 4.—*Sex ratios and spawnings per female for the BEE exposure*

Mean water concentration (mg/l)		Tank	Number females	Number males	Observed spawnings/ female
BEE	Acid				
0.31	0.80	1	3	2	0
0.31	0.80	1A	2	3	1
0.06	0.20	2	4	1	3.7
0.06	0.20	2A	1	4	4
0.03	0.04	3	4	1	2.8
0.03	0.04	3A	2	3	0
0.01	0.01	4	3	2	5
0.01	0.01	4A	3	2	1
Control	Control	5	4	1	3.8
Control	Control	5A	3	2	2

from eggs of exposed fish survived as well as those from the controls and grew normally for 6 weeks. At the end of the test there were numerous young-of-the-year nearly 1 inch long in several of the chambers. There were well-developed eggs in the ovaries of many of the females from all tanks.

Spawning behavior was observed in both tanks in which successful egg production did not occur. Based on ovary condition, the females appeared to be ready to spawn at the end of the test. As with malathion, no effect on the final size of the adult fish was evident.

Normal eggs placed under the influent to the high concentration (nearly all was in the ester form) tanks did not survive 48 hours, an indication of rather high toxicity of the ester to the eggs. Again the survival of the eggs and fry up to 14 days was 90% or better in both control groups.

Histological examinations of gills, liver, and kidney from adult fish were made, again employing haematoxylin and eosin stains. No abnormalities were observed in any of the fishes. Since there were no deaths in any concentration and no observable deleterious effects at any time during the exposure, one must conclude that there was little, if any, effect of these exposures insofar as the LFPI is concerned.

DISCUSSION

The results of these tests are not as clear-cut as one would wish, but they do indicate concentrations that should be reasonably acceptable for this species in the absence of other pollution. The ability of some fish to reproduce, apparently normally, at malathion concentrations that killed others seems unusual but probably is to be expected for compounds that are not highly persistent and are readily metabolized. Based on these data, 0.2 mg/l malathion should not reduce production rate of fathead minnows; but, as stated earlier, this concentration would be acutely toxic to bluegills. The maximum acceptable malathion concentration for fathead minnows is between 0.20 mg/l and 0.58 mg/l. From the BEE data, one can conclude that 0.3 mg/l of ester and 0.8 mg/l of the acid would not reduce the production rate of fathead minnows. It is important, however, to emphasize that approximately 1.5 mg/l of the ester killed eggs in 48 hours, so the maximum acceptable BEE concentration for fathead minnows is between 0.3 mg/l and 1.5 mg/l.

Static tests of acute toxicity as prescribed by Standard Methods (1960) were made for malathion and BEE with adult fathead minnows at 23 C in the same type of water as used for the chronic tests. The 96-hour TL_m for malathion was 9 mg/l, 45 times higher than the 0.2 mg/l that apparently had no effect during the chronic exposure. The application factor calculated from the TL_m value and the concentration not affecting the LFPI after prolonged exposure is 1/45. Until otherwise indicated, an application factor for determining the acceptable concentration of malathion for more sensitive fishes could be set at 1/45 of the 96-hour TL_m as determined in the water and with the species in question. (Since 0.58 mg/l was obviously harmful, the true value is between 1/45 and 1/15). The numerical value of this factor would have to be reduced if

other types of pollution are present or if food organisms are especially sensitive.

A 96-hour TL_m for the BEE determined in static tests in a similar manner was 5.6 mg/l. This is 19 times higher than the 0.3 mg/l considered to be acceptable as determined from this study (disregarding the acid present because of its low toxicity). Since eggs were killed in 48 hours at approximately 1.5 mg/l of ester, the 0.3 mg/l concentration cannot be greatly different from an inimical concentration. The application factor for this ester is estimated at $\frac{1}{19}$ of the 96-hour TL_m value and the true value between $\frac{1}{19}$ and $\frac{1}{4}$. In addition to the problem of the range of toxicities of the various forms of 2,4-D, there could be a tainting problem from the phenolic compounds associated with BEE. Of course, this factor does not apply to acceptable concentrations for preventing tainting of fish flesh.

One can derive from these results a first approximation of acceptable concentrations of malathion and BEE for fathead minnows in clean water. Remaining unknown are the effects of small amounts of other toxicants, the possibility that intermittent exposures may be more damaging than continuous ones, and other considerations mentioned earlier. One very important unknown is whether an application factor derived in the case of malathion for a tolerant species (fatheads) would be accurate for a very sensitive species (bluegills). If the same factor does apply, assuming a TL_m value of 0.02 mg/l, an acceptable concentration for malathion for bluegills would be approximately 0.4 μg/l. We hope to determine the factor for bluegills experimentally in the near future. Until evidence is available, we see no reason why the factor based on acutely and chronically toxic concentrations should change with species sensitivity.

CONCLUSION

Several improvements in experimental methods are suggested from the data obtained in these studies. Even though 50 fish were used for each test, unfavorable sex ratios in some chambers and other unknown causes resulted in a lack of spawning in those chambers where the toxicant concentration was obviously not the cause. One cannot expect to measure reproduction as precisely as death because many other factors are involved, especially behavior. Larger numbers of fish are required to reduce the danger of unfavorable sex ratios and perhaps minimize other problems. Growth rate during the first months of exposure, before spawning occurs, should be measured to get some quantitative measure of growth. More quantitative data on hatching success, and fry growth and survival would place interpretation of the data on a more objective basis.

This study does demonstrate that knowledge and instrumentation are far enough advanced to make long-term exposures feasible. Perhaps others will direct their attention to this neglected area of chronic conditions in fish toxicology, an area effectively used in mammalian toxicology with relatively few animals. This type of test is direct and requires less extrapolation than any other type of laboratory test now in use. If the chronic exposure approach were used to evaluate sensitive physiological measurements, such tests might be highly useful and practical for assessing significant damage. But until such measurements are understood and interpreted, they are of limited value for practical application to pollution problems. We hope some effort will be directed toward the use of more interpretable effects by other investigators in the near future, but in the last analysis, the validity of any approach will be established only by field observations in actual situations.

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