INSTITUTE OF APPLIED SCIENCES

THE UNIVERSITY OF THE SOUTH PACIFIC

AQUATIC TOXICITY TESTS USING THE GUPPIES *Poecilia mexicana* AND *Poecilia reticulata*: DEVELOPMENT OF A REGIONALLY APPROPRIATE LC_{50} BIOASSAY FOR WASTE WATERS IN SOUTH PACIFIC COUNTRIES.

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by

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1 INTRODUCTION

1.1 Background

Increasing industrial development, population growth and urban concentration in South Pacific countries have caused an increase in pollution. Serious water pollution, especially marine pollution, is one of Oceania's most pressing environmental concerns (Dahl, 1984; Fuavao and Morrison, 1992). The main contaminants of water in the South Pacific are sewage-related pollutants, industrial wastes from engineering and food process industries, waste from logging and mining, agricultural chemicals and pesticides (Fuavao and Morrison, 1992). Pollution effects are most severe in lagoons but domestic water supplies are also deteriorating.

"An increase of pollutants in the aquatic environment will result in the degradation or stabilisation of ecosystems to levels where it is impossible to maintain the natural resources on which most of the Island communities depend. Furthermore, increase in pollutants will also affect the health of humans and other organisms" (Naidu *et al.*, 1989). Therefore, we need to monitor and assess pollution.

1.2 Bioassays complement chemical analysis

There are two major classes of methods for determining the toxicity of a suspected pollutant: by direct chemical determinations and by bioassay. The two approaches are complementary.

In chemical measurement, the analyst determines the concentrations of toxic elements or compounds suspected to be present and compares the values with concentrations at which harmful effects are known to occur. The method is quantitative and repeatable within the limits of analytical technology for substances whose harmful components are well known.

The toxic components of many complex industrial wastes are, however, poorly known. A very large number of chemical components may be present making complete analysis for all potentially harmful compounds difficult or impossible. Therefore, bioassays are often used to determine the overall hazard of pollutant mixtures. In a bioassay, some living system is challenged by exposure to the pollutant under standard conditions. The responses of the living system are the measure of toxicity (Elder, 1990). Observed responses may be behavioural, physiological, developmental, genetic, reproductive - in fact anything that can be measured objectively. Bioassay is most often used as a screening tool with direct chemical methods to follow for more precise identification of the causes of toxicity.

1.3 The acute lethal aquatic toxicity test: LC_{50}

The most common whole-animal aquatic toxicity test is the 50% lethal concentration test (LC_{50}), which determines the concentration of a toxicant that would kill 50% of the test population in a prescribed time, most often set at 96hr. The terms toxicity test and bioassay are used interchangeably. Toxicity tests and bioassays have increasingly gained recognition in the determination of pollutant effects on aquatic life. They complement chemical analysis.

LC_{50} tests are fundamental in pollution control regulations of many industrialised nations. Most of these nations are in the northern hemisphere temperate zone. In tropical countries, the biological species named in standard LC_{50} test protocols are not readily available, nor are the standard conditions of temperate zone tests appropriate. The problem of transferring LC_{50} tests developed for marine waters of Europe and North America to other regions is not trivial even for New Zealand, a developed nation in the temperate South Pacific (Auckland Regional Council, 1992).

In order to calibrate a toxicity test, organisms are subjected to a series of reference toxicants, over a specified time period, usually 96hr. We wish to calibrate LC_{50} tests on regional species using well-known reference toxicants, so that regional tests may be compared to world standards.

Toxicity tests demonstrate how organisms respond to contaminants, integrate the effects of combinations of contaminants including those that are not detected by established analytical methods and serve as biological measurements of the effects of different levels of contaminants on the biota (Nunogawa *et al.*, 1970; Elder, 1989; Auckland Regional Council, 1992; Mayer and Ellersieck, 1986). Hence the 96hr LC_{50} has become a fundamental procedure in pollution assessment of many industrialised nations. Biological species used include algae, shrimps, urchin, fish, insects and gastropods (Auckland Regional Council, 1992; Mayer and Ellersieck, 1986; Canada Environmental Protection Series, 1990 (hereafter referred to as Canada, 1990)).

1.4 The concept of probit analysis

To estimate LC_{50}, populations of a test organism are exposed to a graded series of concentrations of a chemical or mixture. The test concentrations are chosen to bracket LC_{50}; the median lethal concentration is then determined by interpolation. The plot of cumulative mortality versus concentration is generally sigmoid (Figure 3), with a low plateau of zero mortality, rise beginning at some threshold concentration and a rise to the upper plateau of 100% mortality. Probit analysis transfers the sigmoid relationship to a linear one which is more tractable statistically (Auckland Regional Council, 1992; Greenberg *et al.*, 1992).

1.5 Purpose and Scope

Governments, industry and the general population are increasingly aware of the need to defend limited and fragile natural resource systems from the effects of development. It is timely to establish standard LC_{50} tests in the South Pacific. Existing standard test organisms could be imported and cultured, but it is preferable to develop an appropriate regional test, using locally available organisms.

The first phase in this development was to explore the suitability of a number of common organisms for laboratory experimentation. We sought regional analogues for the common temperate test organisms. Then selected species would be challenged with graduated concentrations of reference toxicants so that their responses could be compared with those of the known test species.

The project reported here is a first step toward the establishment of a standard LC_{50} test for use in Fiji and the South Pacific.

2 MATERIALS AND METHODS

2.1 Choice of test organisms

We sought local species that were easily captured or reared in captivity, reasonably representative of a variety of important natural resources and amenable to laboratory experimentation using simple, small apparatus.

Vittozzi and DeAngelis (1991) carried out an extensive literature search for data on the acute toxicity of organic chemicals to species recommended by OECD for toxicity testing. The species were: trout (*Oncorhynchus mykiss*, formerly *Salmo gairdneri*), bluegill (*Lepomis macrochirus*), guppy *(Poecilia reticulata)* and carp (*Cyprinus carpio*).

The fish specified in LC_{50} bioassays in marine waters by the US Environmental Protection Agency (USEPA) are the Sheepshead Minnow, *Cyprinodon variegatus*, the Inland Silversides, *Menidia berylina* and the topsmelt, *Atherinops affinus*. In Canada, the threespine stickleback, *Gasteroceus aculeatus* and juvenile rainbow trout, *Oncorhynchus mykiss* are used in freshwater, and freshwater results for rainbow trout are used to estimate the toxicity of effluents discharged to saltwater. We sought fish that appeared likely to be analogues of one or another of these species. Australia and New Zealand as yet have no standard acute lethality test using fish.

Our initial candidate fish species were tilapia for freshwater; the common Fiji ditch guppy for fresh to estuarine waters; and the neon damselfish for fully marine water.

The three authors have worked sequentially on this project : Eliesa in 1994, Singh in 1995 and Sulu in 1996. Because of limitations in resources, we performed most experiments with ditch guppies. These were easiest to obtain, but they also seemed the most likely to be useful in a wide variety of salinity and temperature conditions. We performed a few preliminary experiments with neon gobies and none with tilapia.

2.2 Description of test organisms used

2.2.1 Poecilia mexicana

P. mexicana are live bearing fishes of the family Poecilliidae (Migdalski and Fichter, 1976). The species, commonly called the Amazon shortfin molly is native to Central America (Carlson, 1975). It has been introduced into many parts of the world to control mosquitoes. There is apparently no record of its introduction into Fiji, but the species is abundant in drainage ditches around the University of the South Pacific (USP) and Laucala Bay, Suva. The largest observed was 70mm. *P. mexicana* are excellent aquarium fishes (see Figure 1).

2.2.2 Poecilia reticulata

P. reticulata, formerly *Lebistus reticulatus* (Hoedeman, 1975) are also live bearers of the family Poecilliidae. They are native to northern South America and nearby Islands of the West Indies. Males are extremely variable in colour, particularly on their fins. Their dorsal and anal fins also vary greatly in shape. *P. reticulata* are commonly called guppies. The guppies have also been widely introduced throughout the world for mosquito control (Migdalski and Fichter, 1976).

Guppies are commonly found in the Mamulele and Gilbert camp areas of Honiara, Solomon Islands. Females can grow to a maximum of 60mm. Males are usually shorter (see Figure 2).

It is possible that they were introduced into the Solomon Islands by the Americans during World War II to combat malaria spreading mosquitoes (J. Seeto, 1996, pers. comm.).

2.3 Capture and adaptation

P. mexicana used in all the experiments were captured from the storm drain between the School of Social and Economic Development (SSED) and the School of Pure and Applied Science (SPAS) Suva, Fiji.

Poecilia reticulata were obtained from streams at Mamulele and Gilbert Camp in Honiara, Solomon Islands.

The fish were concentrated using a 5mm mesh seine net and transferred with a 1mm mesh dipnet to buckets partially filled with the ambient water. In the laboratory they were transferred to clean tap water that had been dechlorinated by standing overnight in an open 40L container. The fish were allowed to acclimate for 24hr. Atmospheric temperature within the laboratory in the course of the experiments ranged diurnally from 25°C early in the morning to a high of 33°C in the mid afternoon. Stocking density ranged from 1 to 5 fish per litre of water. Water was aerated during acclimation period. Fish length used in all the experiments ranged from 20 - 60mm with most test animals in the range 35 - 50mm for *P. mexicana* and an average length of 30mm for *P. reticulata*. The fish were not exposed to direct sunlight but laboratory windows admitted light in the daytime. Trials by Eliesa in 1994 and Singh in 1995 indicated that *P. mexicana* can survive in excess of 10 days without food and aeration under these conditions. *P. reticulata* has a 95% survival rate under laboratory conditions without food and minimal aeration for 8 days.

Tests have shown that *P. mexicana* can be transferred from freshwater to estuarine salinity (about 28‰) through a series of four increasing salinities within 8hr with no apparent acute stress or mortality during subsequent holding to 72hr.

P. reticulata can survive transfer from freshwater to a salinity of about 30‰ through a series of four increasing salinities over a 96hr period and without acute stress or mortality during subsequent holding for a further 96hr without food and minimal aeration. Direct transfer from freshwater to saltwater resulted in 100% mortality within 3hr.

2.4 General experimental procedures

Aquatic toxicity tests were conducted using static aquaria.

Following capture and acclimation, fish were distributed into aquaria filled with 10 litres of either dechlorinated tap water or fresh seawater from either the USP jetty (Suva) or IMR shoreline (Solomon Islands) with toxicant dose pre-mixed. Attempts were made to make sure there was an uniform body size distribution among all the tests.

For efficiency and conservation, each test began with a preliminary range finding test. A few animals (usually four) were used at each of a widely graduated range of concentrations to locate the approximate LC_{50}.

Definitive tests were then conducted using 10 fish per 10L, at each of a narrow range of concentrations, bracketing the approximate LC_{50}.

Fish were counted daily and scored for mortality. Dead animals were removed, measured and disposed. No later analysis of the organisms was done.

Probit analysis of these results was done by plotting the mortality in each concentration against the concentrations using probit papers obtained from UNEP/FAO/IAEA (1989). LC_{50} values were estimated from these plots.

2.5 Probit analysis

Probit analysis transforms mortality so that the ordinate is on a probability scale while the abscissa is on a logarithmic scale. A best fit line is drawn through these points. LC_{50} is determined as the concentration corresponding to the intersection of the best fit line with 50% mortality. The best fit line can be determined by statistical methods (USEPA (1994) or other computer statistical package); or graphically using probit paper (Anderson, 1996, pers. comm.; Greenberg *et al.*, 1992; UNEP, 1989). We have applied the graphic approach (graph paper from UNEP, 1989).

2.6 Reference toxicant selection

Reference toxicants were selected according to guidelines set by Canada (1990). Criteria for selection prepared by Singh in 1995 are set out in Appendix 1. The regional availability of chemicals was also considered.

The organic reference toxicant selected for freshwater and saltwater was phenol. Zinc sulfate was selected as an inorganic reference toxicant for use in

soft freshwater, but zinc sulfate is not suitable for use in hardwater or saltwater. Potassium chromate was selected as an inorganic reference toxicant for use in saltwater and hardwater.

2.6.1 Phenol (C_6H_5OH)

Phenol is a common industrial chemical (Nunogawa, *et al.*, 1970), that has been commonly used as a reference toxicant and has satisfied criteria set out by many authors (example, Nunogawa *et al.*, 1970; Elder, 1990; Canada, 1990). Preliminary tests have shown that phenol is a reliable reference toxicant. Phenol toxicity is not affected by water hardness or pH and remains stable in solution for up to four days. Phenol is reported to have given reproducible results under typical laboratory conditions (Canada, 1990). Phenol crystals melt at room temperature, hence must be weighed with deliberate speed. Phenol is a very strong oxidising agent, hazardous to humans and must be handled very carefully.

2.6.2 Zinc sulfate ($ZnSO_4$)

Zinc sulfate has been commonly used in bacteriocides (Mayer and Ellersieck, 1986). Zinc sulfate is a good reference toxicant in water with pH less than 7.5. Preliminary tests conducted at the USP Marine Studies, Suva have shown that zinc sulfate gives reproducible results in dechlorinated tapwater with a hardness of 40mg/L. However zinc sulfate is not suitable for use in salt water and in hardwater (Honiara). We observed that zinc sulfate precipitates in saltwater and hardwater giving a cloudy solution. Precipitation reduces toxicity (Canada, 1990). High concentrations of up to 60mg/L in saltwater and 20mg/L in hardwater does not result in any mortality.

2.6.3 Potassium chromate (K_2CrO_4)

Potassium chromate gives reproducible results in waters with pH of 7 or more (Canada, 1990). Tests we have conducted show that potassium chromate is suitable for use in saltwater. Potassium chromate is carcinogenic and must be handled carefully.

3 RESULTS

3.1 Results for Amazon shortfin molly, *Poecilia mexicana*, from Fiji

3.1.1 Poecilia mexicana with zinc sulfate in freshwater

Table 1 shows preliminary test results obtained by Eliesa in 1994. Table 2 shows initial definitive tests obtained by Singh in 1995 while Table 3 shows further definitive tests conducted by Sulu in 1996. The LC_{50} based on Eliesa's results was 10.0mg/L^{-1} (Figure 4), while Singh and Sulu obtained 15.0mg/L^{-1} and 9.2mg/L^{-1} respectively (Figures 5,6).

3.1.2 Poecilia mexicana with phenol in freshwater

Singh conducted preliminary tests and initial definitive tests for *P. mexicana* in freshwater with phenol in 1995 (Tables 4, 5). Sulu conducted definitive tests in 1996 (Table 6). In Sulu's test, mortality was higher in 35mg/L^{-1} than in 45mg/L^{-1}. Disease, water quality and random effects may have contributed to this anomaly. Singh established the 96 hr LC_{50} at 36mg/L^{-1} (Figure 8) while Sulu obtained 40mg/L^{-1} (Figure 9).

3.1.3 Poecilia mexicana with phenol in saltwater

Table 7 shows the mortality of *P. mexicana* in saltwater with phenol. Random effects probably caused the apparent low mortality in 36mg/L^{-1} compared to 33, 34 and 35mg/L^{-1}. Probit analysis established the 96hr LC_{50} at 32mg/L^{-1} (Figure 10).

3.1.4 Poecilia mexicana with potassium chromate (K_2CrO_4) in saltwater.

Table 8 shows the data for *P. mexicana* in saltwater with K_2CrO_4. 96hr LC_{50} obtained by probit analysis of these data was 155mg/L^{-1} (Figure 11).

3.2 Results for guppies, *Poecilia reticulata*, from Solomon Islands

3.2.1 Poecilia reticulata with zinc sulfate in freshwater

Toxicity tests were conducted in Honiara for *P. reticulata* in the concentration range 0mg/L^{-1} - 17.5mg/L^{-1}. Due to the extreme hardness of water, the toxicity of zinc sulfate was affected by precipitation. There were no mortalities.

3.2.2 Poecilia reticulata with phenol in freshwater

Results for tests in freshwater are shown in Table 9. The lowest concentration that killed any fish was 38mg/L^{-1}. At 46mg/L^{-1} a few died. Extrapolation of this data gave an approximate LC_{50} at 52mg/L^{-1} (Figure 12). Further tests are required to bracket and establish the 96hr LC_{50} for phenol in freshwater.

3.2.3 Poecilia reticulata with phenol in saltwater

Range finding tests for *Poecilia reticulata* in saltwater with phenol gave 100% mortality at 60mg/L^{-1}. Time limitations did not allow definitive tests.

4 DISCUSSION

The 96hr LC_{50} for *Poecilia mexicana* in zinc sulfate with freshwater ranged between 10mg/L^{-1} and 15mg/L^{-1}. Mayer and Ellersieck (1986) reported that the 96hr LC_{50} for cut throat trout in freshwater with zinc sulfate is in the range 6mg/L - 8mg/L. Their results are comparable to ours for *P. mexicana*, though apparently the trout are somewhat more sensitive.

The 96hr LC_{50} for *P. mexicana* in freshwater with phenol was in the range 36mg/L^{-1} to 40mg/L^{-1}. Nunogawa *et al.* (1970) reported 96hr LC_{50} for phenol in freshwater to be 26mg/L^{-1} for *Gambusia affinis* and 31mg/L^{-1} for *Lebistus reticulatus* (*P. reticulata*). Our results and those of Nunogawa *et al.* (1970) are within reasonable agreement.

It is notable that *P. mexicana* was three to four times more sensitive to phenol in saltwater than to phenol in freshwater.

Our result for *P. mexicana* with potassium chromate in saltwater was 155mg/L^{-1} Canada (1990) reported 96hr LC_{50} of fish species in general to be in the range 38mg/L^{-1} to 214mg/L^{-1}. Our results are within the range obtained by others (Table 11).

We cannot yet make a complete comparison between the toxicity responses of two species investigated. Further tests are required on *P. reticulata*.

A major hindrance to the tests conducted in Honiara on *P. reticulata* was the water quality. Water sources in Honiara are bore holes drilled into rocks. The water had a very low oxygen content (according to SIWA, 0.08mg/L^{-1}). Aeration was required to prepare the water for experiments, following high mortalities in early control populations. Total hardness of water in Honiara was 214 to 217.5mg/L^{-1} (Solomon Islands Water Authority (SIWA), 1996). This was significantly higher than Suva water which had a total hardness of 40mg/L^{-1} (C. Mulder, 1996, pers. comm.). The hardness of the water reduced the solubility of zinc sulfate, hence lowering its' apparent toxicity. To successfully carry out tests in Honiara, rainwater or deionised water should be used.

Time limitation did not permit remedial measures to be taken on the Honiara experiments. The basic reported here will be valuable for the design of later experiments.

5 CONCLUSIONS

Poecilia mexicana and *P. reticulata* are suitable organisms for the bioassay of wastewater in the South Pacific. They are both suitable for tests in either freshwater or saltwater.

The 96hr LC_{50} values obtained in our tests were comparable to those by other experimenters using temperate zone species. The LC_{50} tests explored in this research are now ready, with few additional experiments, for application in the South Pacific region.

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8. TABLES

Time, hr	Concentrations (mgL^{-1})				
	0	5	10	15	20
0	0	0	0	0	0
2	0	0	0	0	0
24	0	0	0	0	2
48	0	0	0	3	6
72	0	0	3	6	8
96	0	0	5	7	10
120	0	4	7	8	10

Table 1. Mortality versus time for *Poecilia mexicana* in freshwater with zinc sulfate.

Time, hr	Concentrations (mgL^{-1})							
	0	5	7.5	10	12.5	15	17.5	20
0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	2	2
48	0	1	1	0	0	1	3	3
72	0	2	1	1	2	2	5	6
96	0	2	1	2	3	4	7	8

Table 2. Mortality versus time for *Poecilia mexicana* in freshwater with zinc sulfate.

Time, hr	Concentrations (mgL^{-1})										
	0	7.5	8.5	9.5	10.5	11.5	12.5	13.5	14.5	15.0	16.0
0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
24	0	0	0	0	1	0	0	0	0	1	1
48	0	0	0	0	3	0	3	3	5	8	9
72	0	0	0	5	6	5	7	7	10	10	10
96	0	1	1	7	8	10	10	10	10	10	10

Table 3. Mortality versus time for *Poecilia mexicana* in freshwater with zinc sulfate.

Time, hr	Concentrations (mgL^{-1})							
	0	20	30	40	50	60	70	80
0	0	0	0	0	0	0	0	0
2	0	0	0	0	3	7	8	7
24	0	0	2	3	8	10	10	10
48	0	0	3	4	10	10	10	10
72	0	1	3	4	10	10	10	10
96	0	1	3	4	10	10	10	10

Table 4. Mortality versus time for *Poecilia mexicana* in freshwater with phenol.

Time, hr	Concentrations (mgL^{-1})						
	0	20	25	30	35	40	45
0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	1
24	0	0	1	1	2	2	4
48	0	0	2	3	4	5	7
72	0	1	2	4	4	5	8
96	0	1	2	4	4	5	8

Table 5. Mortality versus time for *Poecilia mexicana* in freshwater with phenol.

Time, hr	Concentrations (mgL^{-1})						
	0	20	25	30	35	40	45
0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0
24	0	0	0	0	0	0	3
48	0	0	0	0	10	5	8
72	0	0	0	0	10	5	8
96	0	0	0	0	10	5	8

Table 6. Mortality versus time for *Poecilia mexicana* in freshwater with Phenol.

Time, hr	Concentrations (mgL^{-1})									
	0	30	31	32	33	34	35	36	37	38
0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0
24	0	0	3	3	6	7	9	5	9	10
48	0	1	3	3	7	8	10	6	9	10
72	0	1	3	3	7	8	10	6	9	10
96	0	1	3	3	7	8	10	6	9	10

Table 7. Mortality versus time for *Poecilia mexicana* in saltwater with phenol.

Time, hr	Concentrations (mgL^{-1})										
	0	120	130	140	150	160	170	180	190	200	210
0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0	0	0	0
48	0	0	2	0	1	2	3	1	2	2	4
72	0	1	3	2	3	5	4	5	9	10	8
96	0	1	4	2	4	9	7	5	9	10	9

Table 8. Mortality versus Time for *Poecilia mexicana* in saltwater with potassium chromate.

Time, hr	Concentration (mgL^{-1})				
	0	34	38	42	46
0	0	0	0	0	0
2	0	0	0	0	1
24	0	0	0	1	2
48	0	0	1	2	2
72	0	0	1	2	2
96	1	0	1	2	2

Table 9. Mortality versus time for *Poecilia reticulata* in freshwater with phenol.

Time, hr	Concentrations (mgL^{-1})			
	0	44	52	60
0	0	0	0	0
2	0	0	0	0
24	0	3	4	4
48	0	8	10	9
72	0	9	10	9
96	0	9	10	9

Table 10. Mortality versus time for *Poecilia reticulata* in saltwater with phenol.

SUMMARY TABLE COMPARING 96hr LC_{50} BY DIFFERENT RESEARCHERS						
Researcher	Year	Media	Location	Species	Toxicant	96 hr LC_{50}, mg/L^{-1}
Sulu, R.J.	1996	Freshwater	USP, Fiji	*P. mexicana*	$ZnSO_4$	9.9
Singh, V.V.	1995	Freshwater	USP, Fiji	*P. mexicana*	$ZnSO_4$	10.0
Eliesa, W.	1994	Freshwater	USP, Fiji	*P. mexicana*	$ZnSO_4$	15.0
Sulu, R.J.	1996	Freshwater	USP, Fiji	*P. mexicana*	C_6H_5OH	40.0
Singh, V.V.	1995	Freshwater	USP, Fiji	*P. mexicana*	C_6H_5OH	42.0
Sulu, R.J.	1996	Saltwater	USP, Fiji	*P. mexicana*	C_6H_5OH	32.0
Mayer and Ellersieck	1986	Freshwater	USA	Cut throat trout	$ZnSO_4$	6-8
Nunogawa *et al.*	1970	Freshwater	Hawaii	*Gambusia affinis*	C_6H_5OH	26
Nunogawa *et al.*	1970	Freshwater	Hawaii	*P. reticulata*	C_6H_5OH	31
Sulu, R.J.	1996	Saltwater	USP, Fiji	*P.mexicana*	K_2CrO_4	155
Canada Environment Protection Series	1990	Freshwater	Canada	Fish generally	K_2CrO_4	38-214

Table 11. Summary of LC_{50} data comparable to the present experiments.

ORGANIC REFERENCE TOXICANTS				
CRITERIA	4 Chloro-phenol	Dodecyl Sodium Sulphate (DSS)	Phenol	Sodium Pentachlorophenate (NaPCP)
Detection of abnormal organism	0	No	Yes	Yes
Established toxicity database	No	Yes	Yes	Yes
Readily available in pure form	Yes	Yes	Yes	Yes
Soluble	Yes	Yes	Yes	Yes
Stable in solution	Yes	No	No	Yes
Stable shelf life	Yes	Yes	Yes	Yes
Limited intra-laboratory effect	Yes	Yes	Yes	E
Easily analysed	Yes	Yes	Yes	Yes
Suitable for marine environment	0	No	Yes	Yes
Suitable for freshwater	0	No	Yes	Yes
ph effects	0	0	Yes	No
Total Score	5	3	7	8

Key O - Too few data
E - Conflicting reports

Scoring
Yes - +1; No - -1; E - No Score; O - No Score
After applying the primary selection criteria, reference toxicants scoring 7 or more were further evaluated for safety, cost and regional availability.

Table 12. Selection criteria for reference toxicants.

INORGANIC REFERENCE TOXICANTS							
CRITERIA	$CdCl_2$	K_2CrO_4	$CuSO_4$	KCl	$AgNO_3$	NaCl	$ZnSO_4$
Detection of abnormal organism	O	E	No	O	O	E	Yes
Established toxicity database	Yes	Yes	Yes	No	Yes	Yes	Yes
Readily available in pure form	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Soluble	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Stable in solution	Yes	Yes	Yes	Yes	E	Yes	Yes
Stable shelf life	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Limited intra-laboratory effect	O	Yes	Yes	Yes	No	Yes	Yes
Easily analysed	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Suitable for marine environment	No	Yes	No	Yes	No	No	No
Suitable for freshwater	No	No	No	Yes	No	Yes	Yes
pH effects	No	No	No	Yes	No	Yes	Yes
Total Score	3	4	3	8	1	8	7

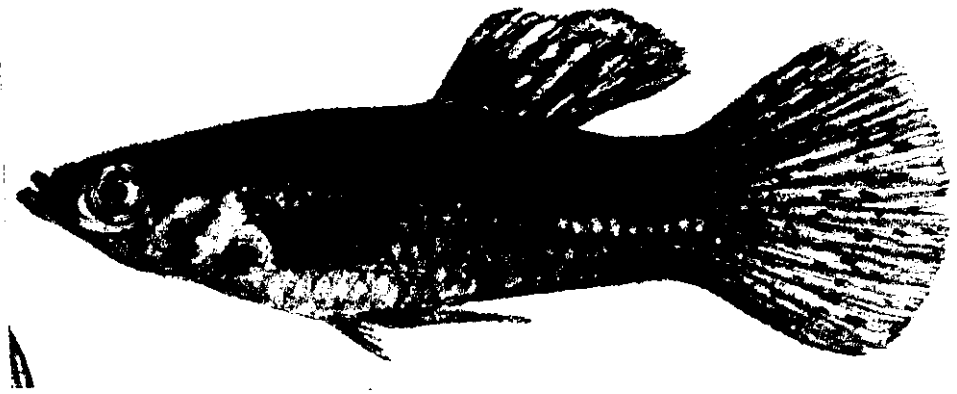
Key O - Too few data
E - Conflicting reports

Scoring
Yes - +1; No - -1; E - No Score; O - No Score
After applying the primary selection criteria, reference toxicants scoring 7 or more were further evaluated for safety, cost and regional availability.

(From Canada, 1990, pg 51-66)

Table 12. (continued).

9 FIGURES



Shortfin molly, *Poecilia mexicana* (Midgalski and Fichter, 1976).

Figure 1. ***Poecilia mexicana***

Common guppies, *Poecilia reticulata*, shorter coloured ones are males while the longer uncoloured ones are females. (Axelrod et. al., 1986).

Figure 2. ***Poecilia reticulata***

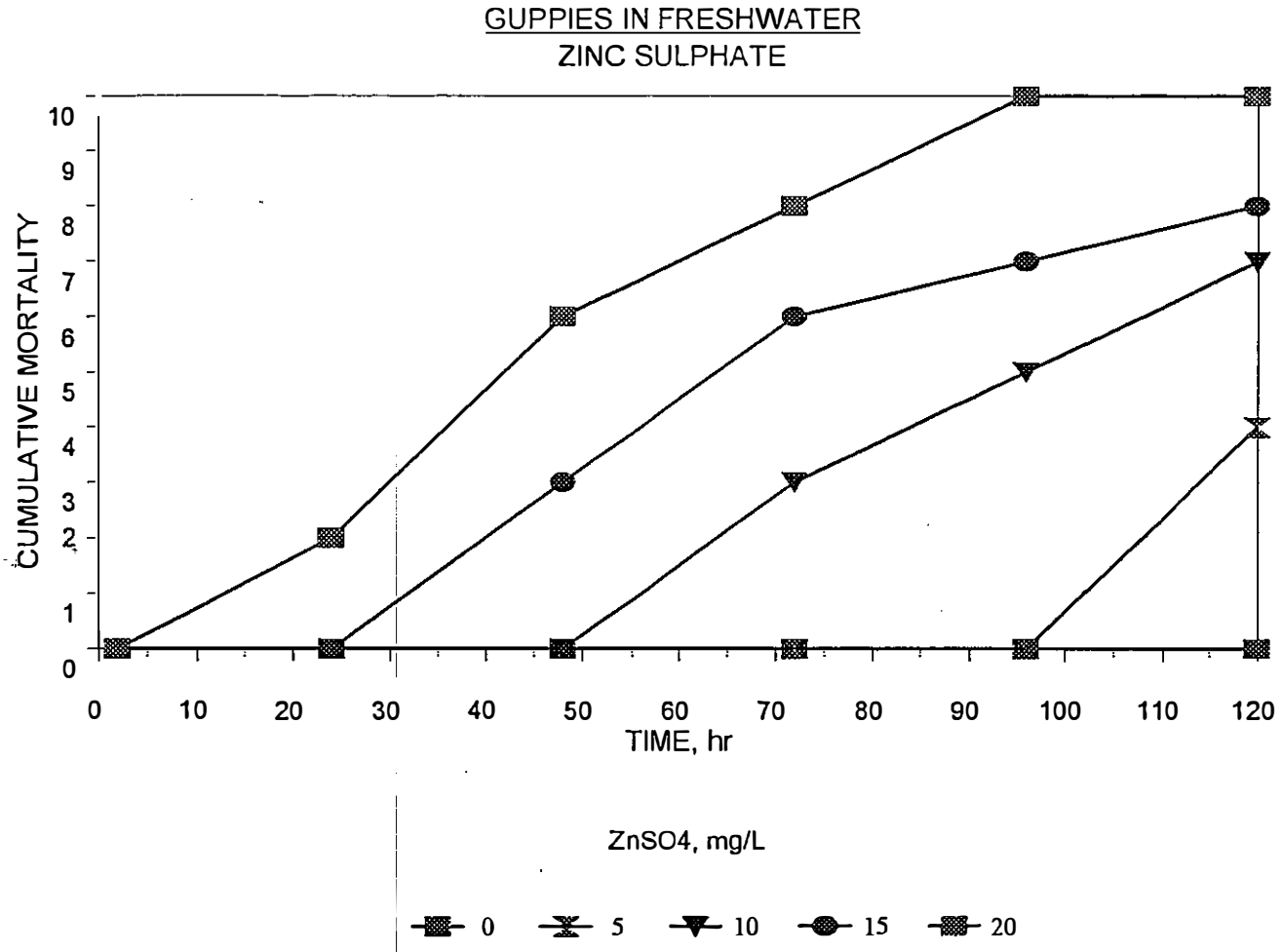


Figure 3. Example mortality/time plot, showing sigmoid response (Ref. Table 1)

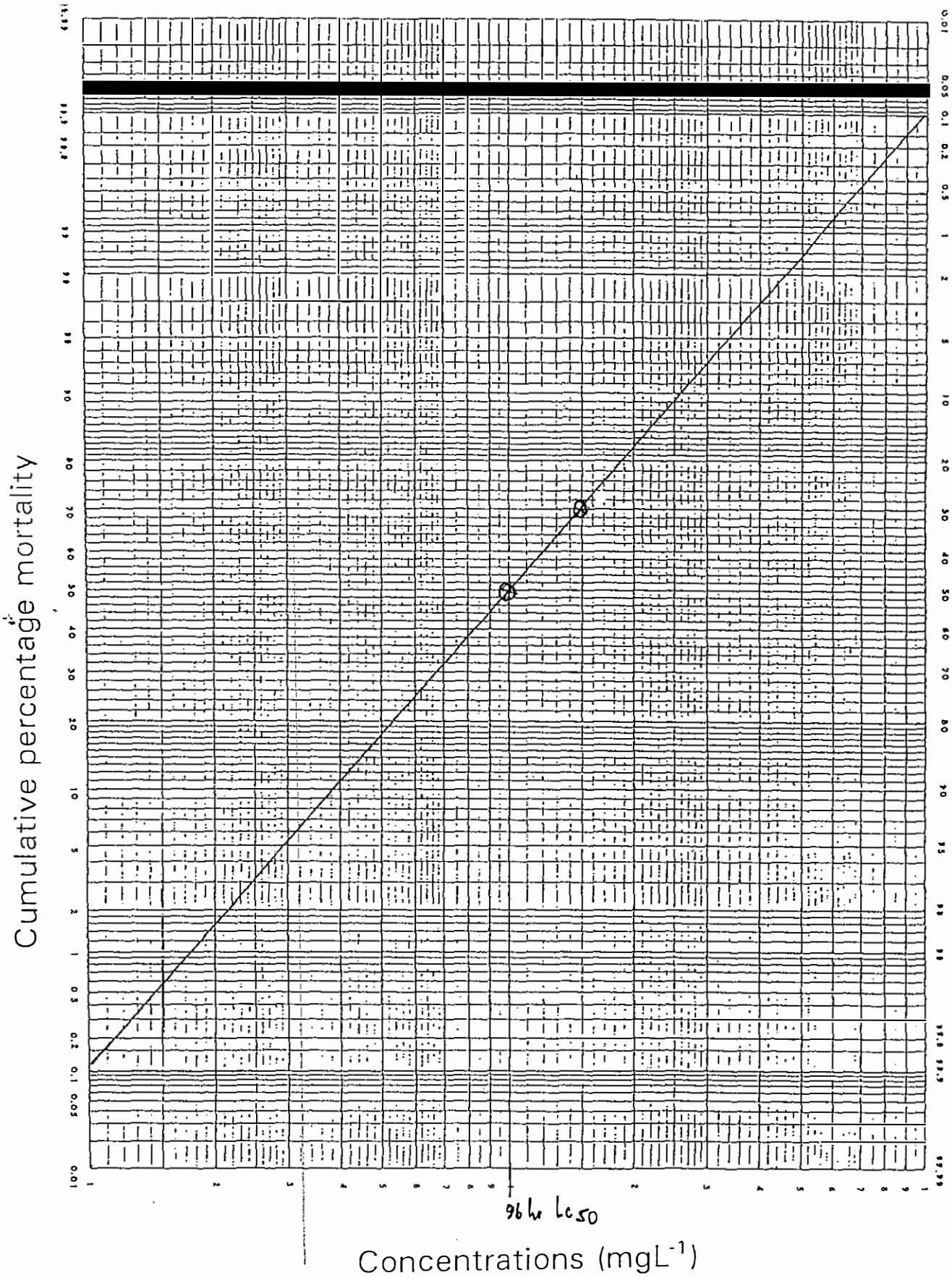


Figure 4. **LC_{50} graph for *Poecilia mexicana* in freshwater with zinc sulfate (ref. Table 1)**

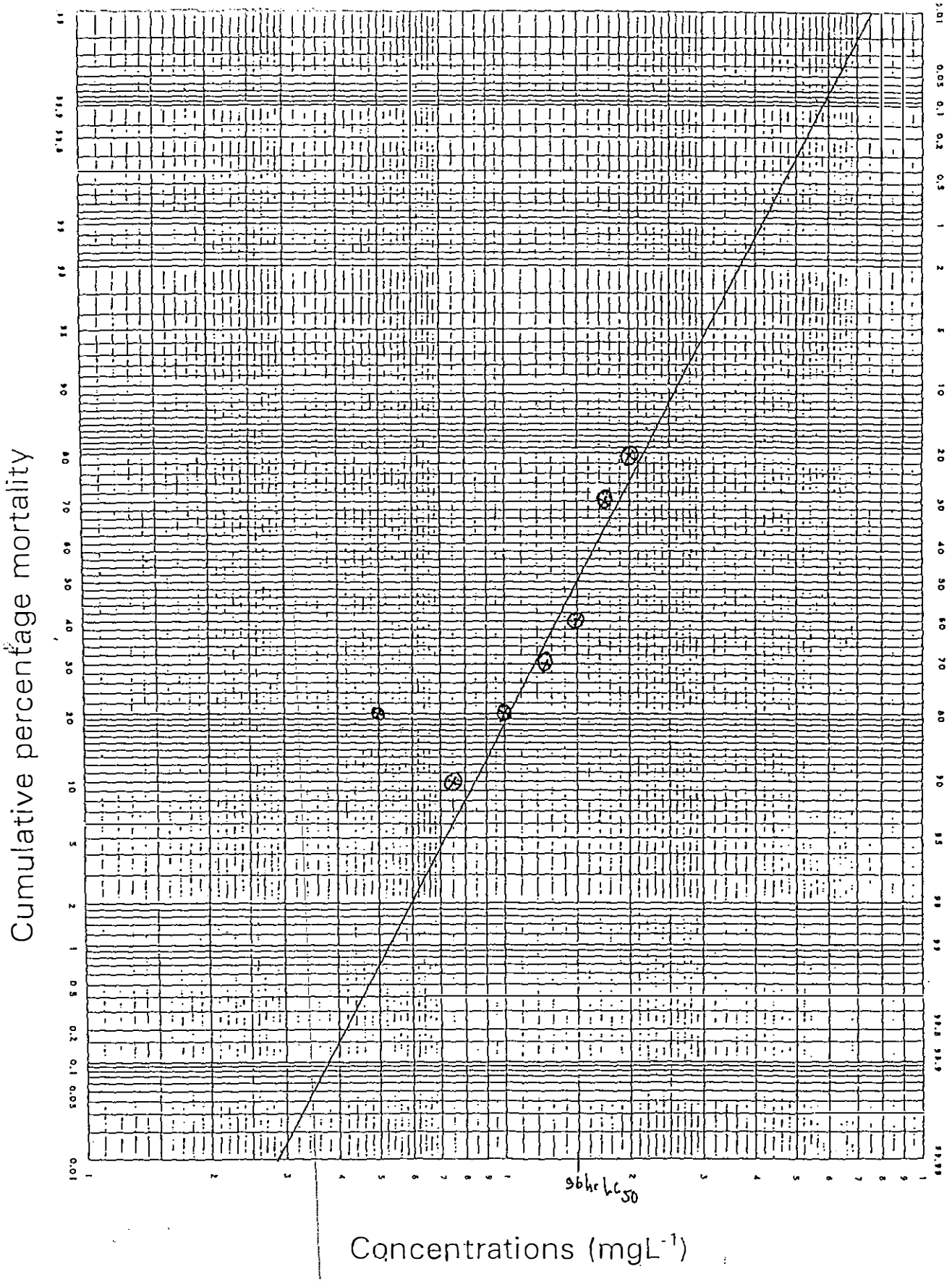


Figure 5. LC_{50} graph for ***Poecilia mexicana*** in freshwater with zinc sulfate (ref. Table 2)

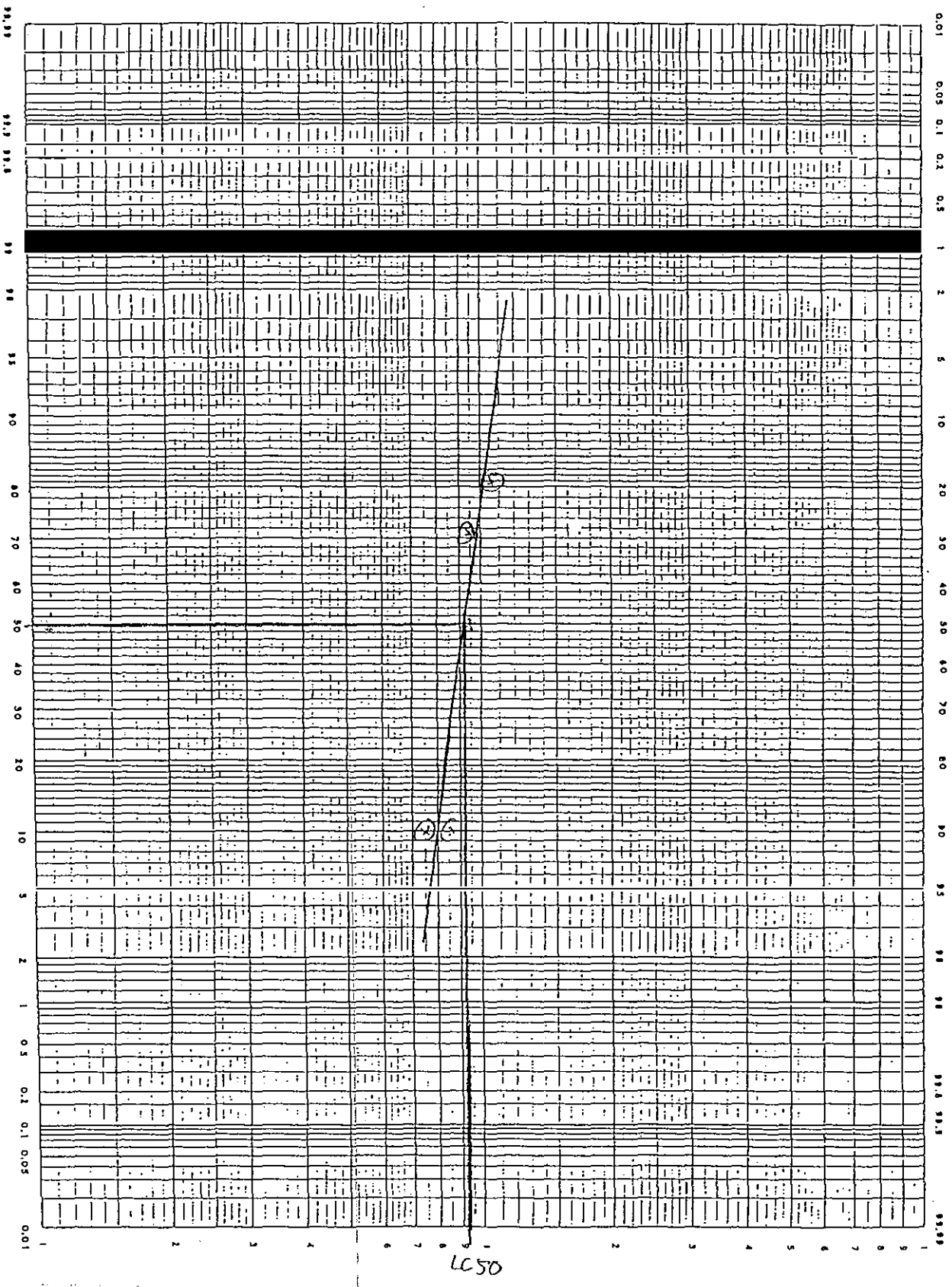


Figure 6. LC_{50} graph for *Poecilia mexicana* in freshwater with zinc sulfate (ref. Table 3)

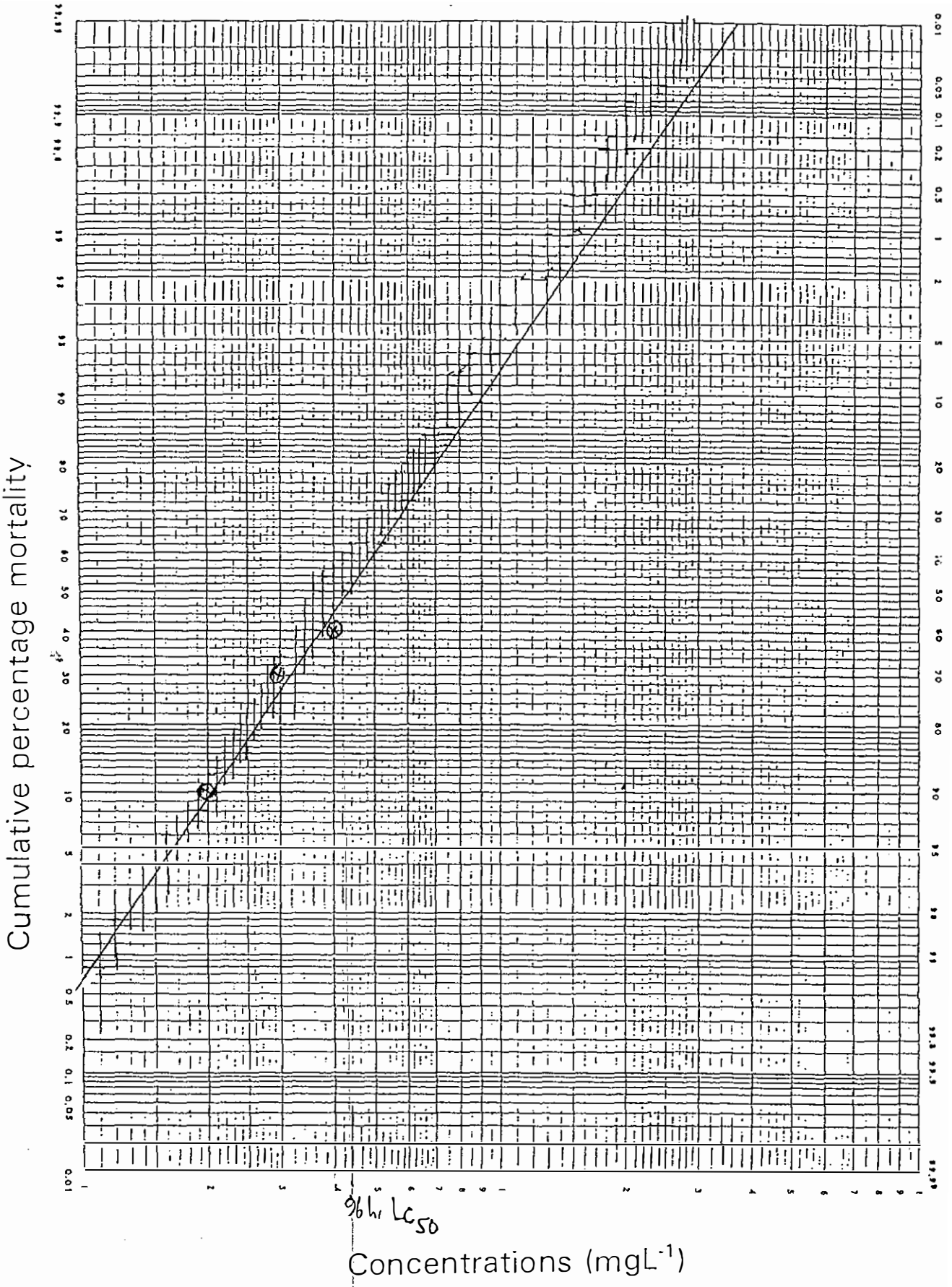


Figure 7. LC_{50} graph for *Poecilia mexicana* in freshwater with phenol (ref. Table 4)

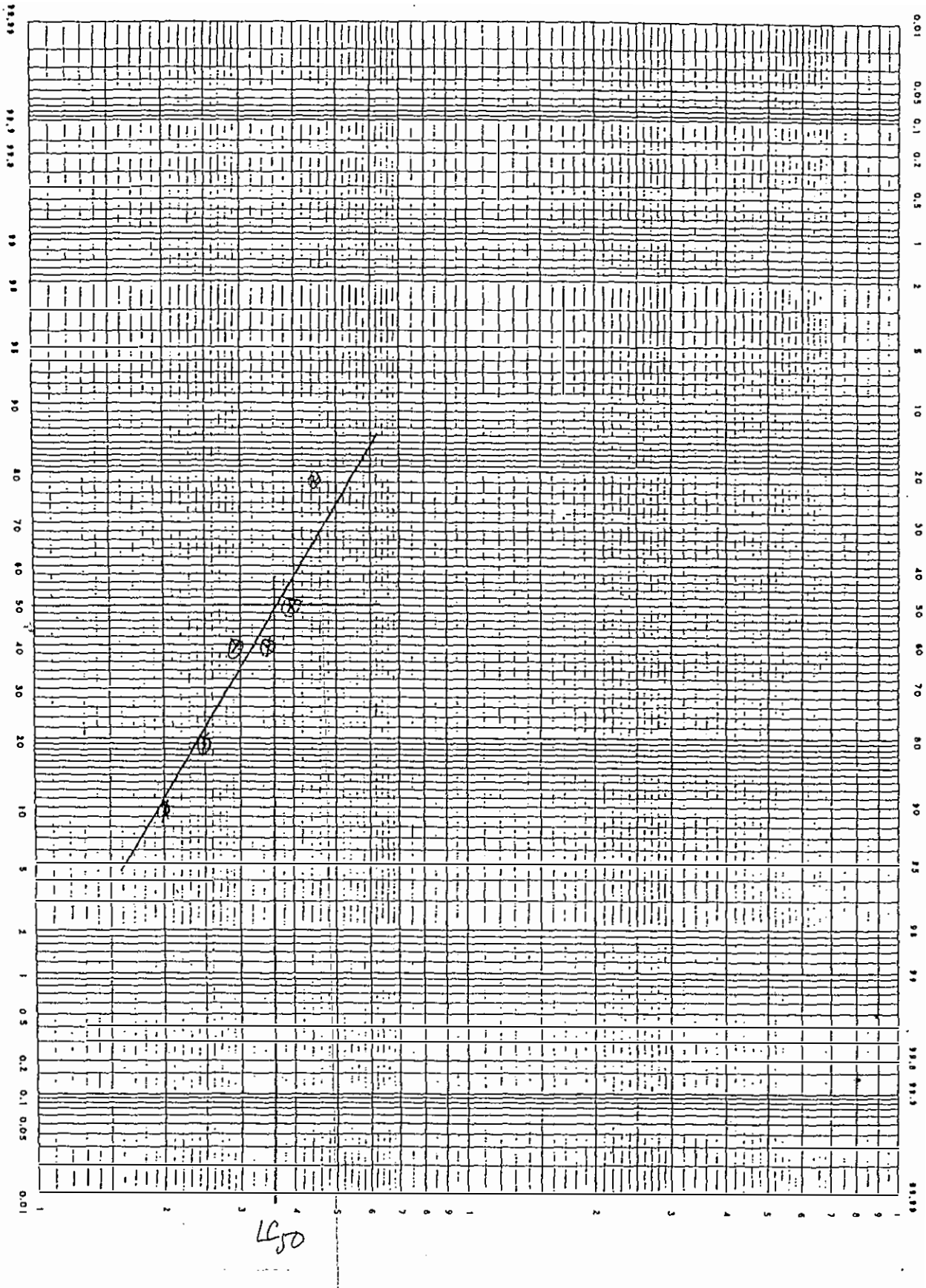


Figure 8. LC_{50} graph for *Poecilia mexicana* in freshwater with phenol (ref. Table 5)

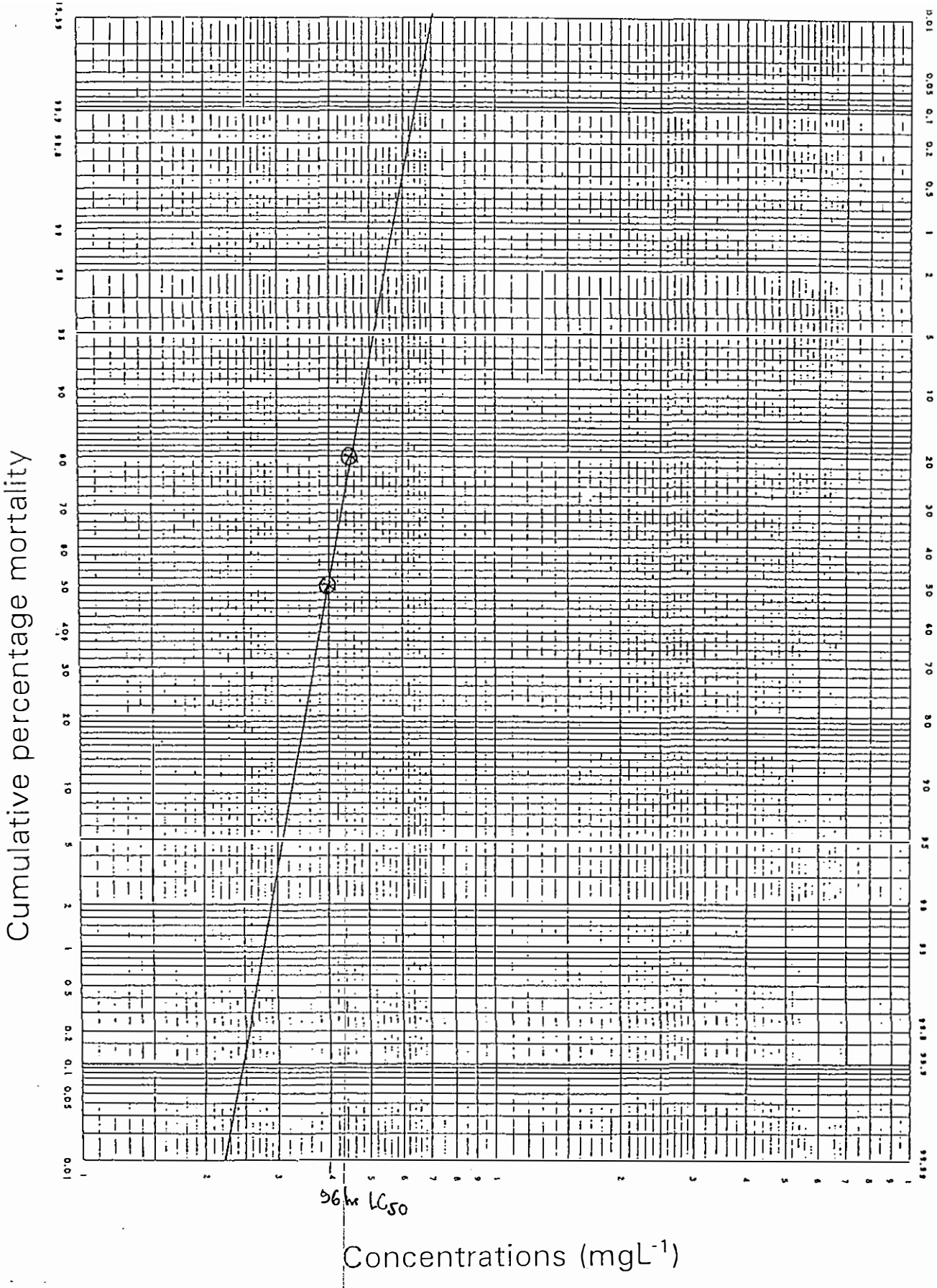


Figure 9. LC_{50} graph for *Poecilia mexicana* in freshwater with phenol (ref. Table 6)

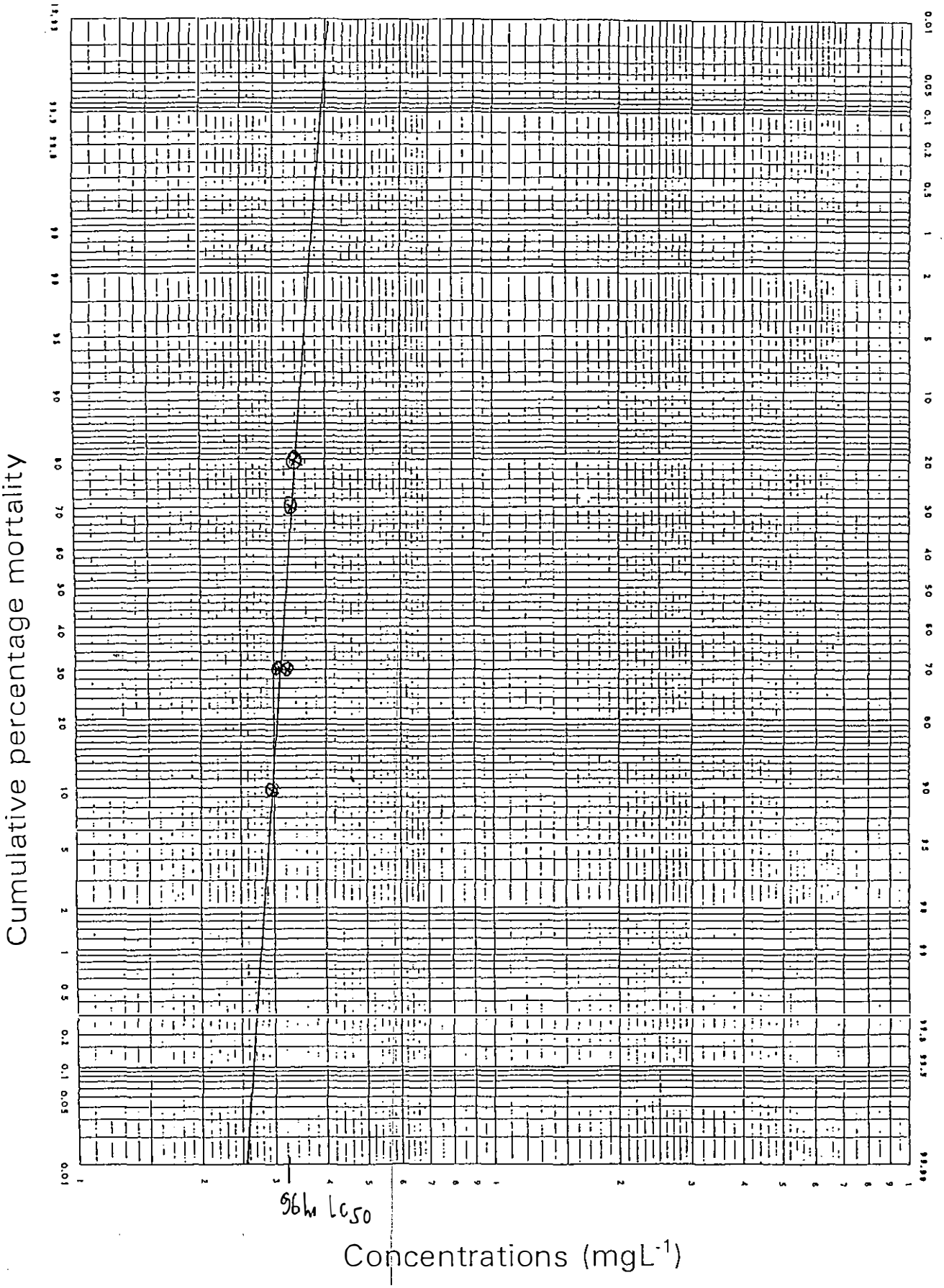


Figure 10. LC_{50} graph for *Poecilia mexicana* in saltwater with phenol (ref. Table 7)

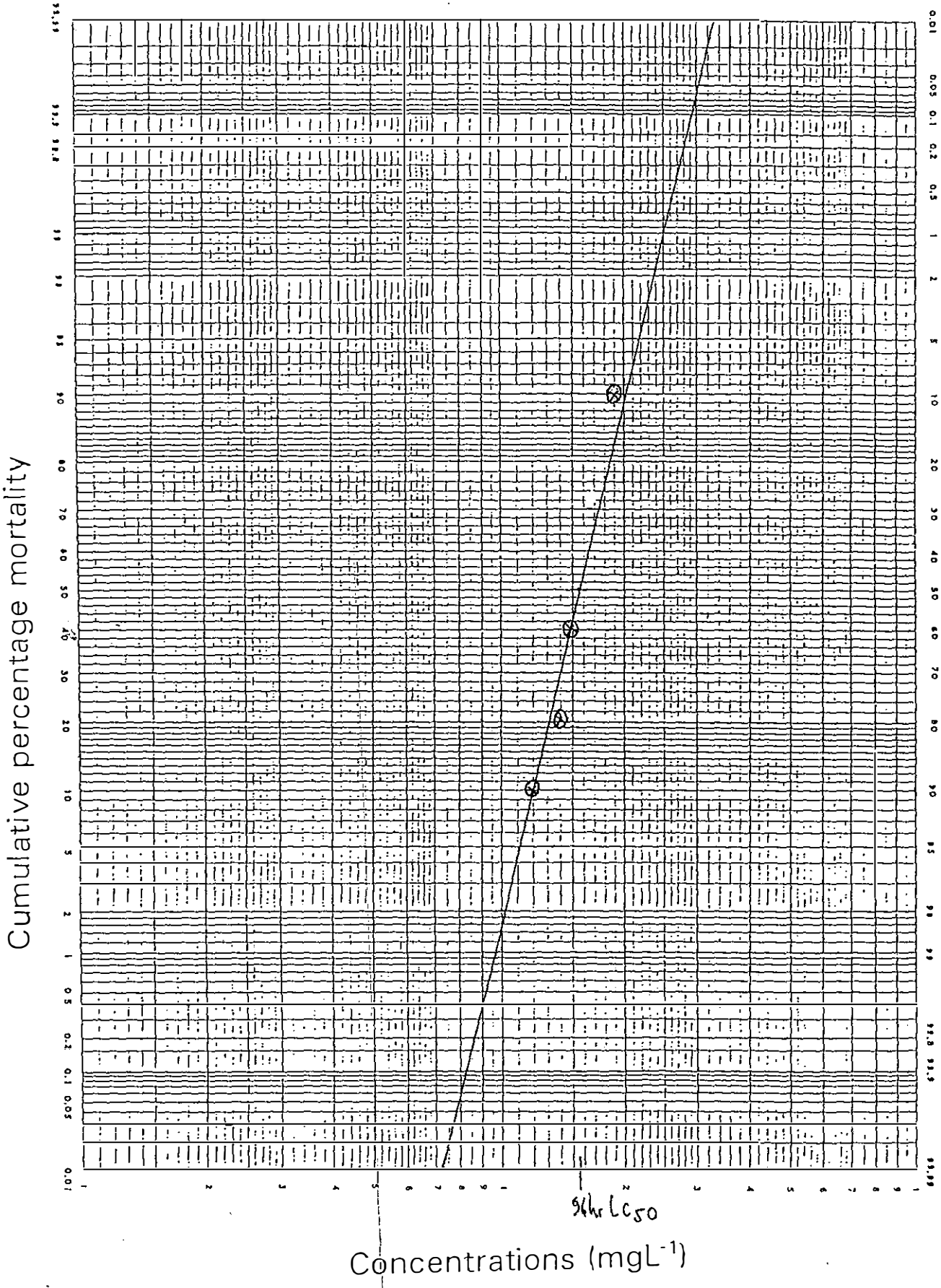


Figure 11. LC_{50} graph for *Poecilia mexicana* in saltwater with potassium chromate (ref. Table 8)

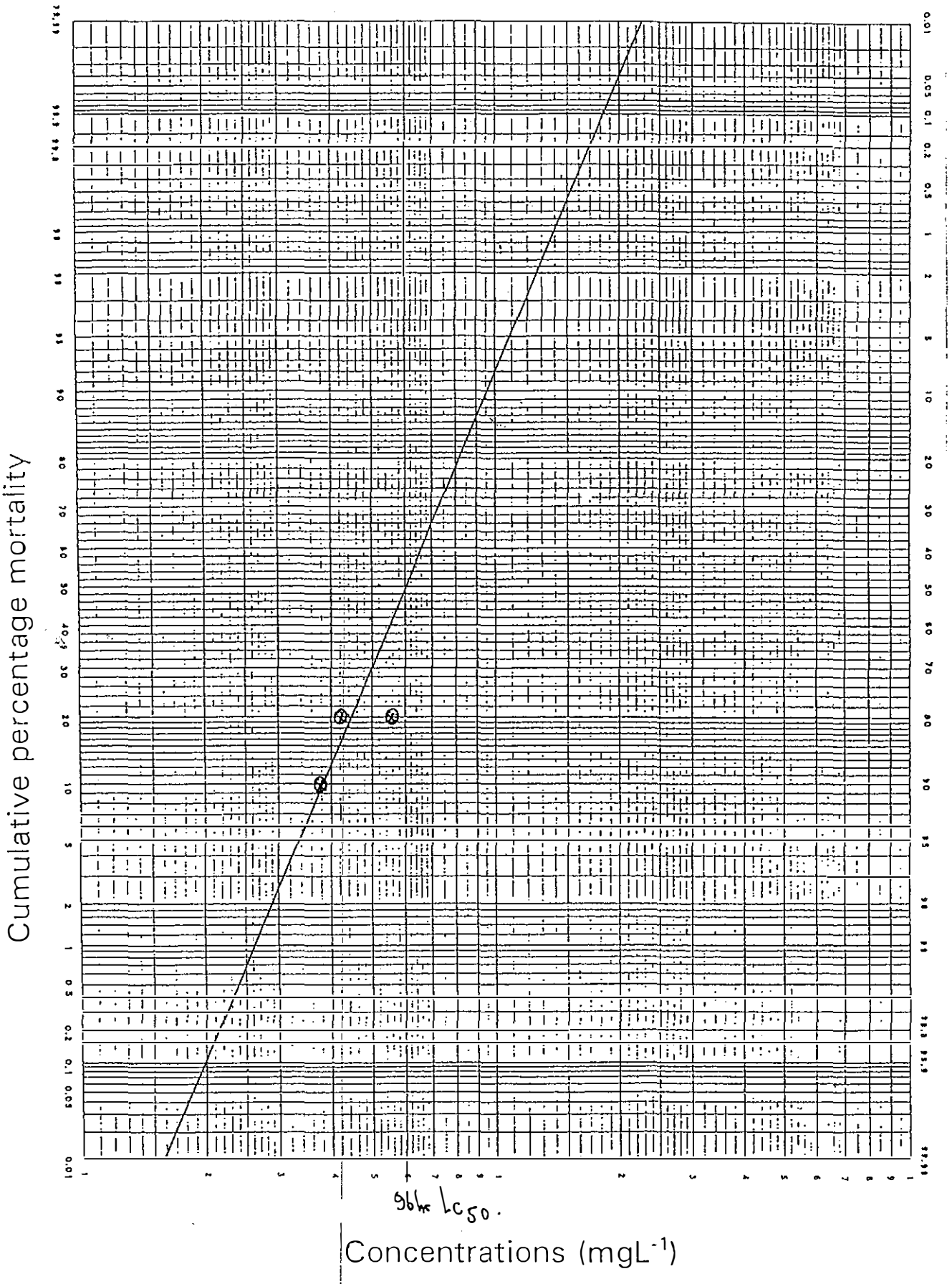


Figure 12. LC_{50} graph for *Poecilia mexicana* in freshwater with phenol (ref. Table 9)