Friesen*, M.K. 1979. Toxicity testing using eggs of the burrowing mayfly Hexagenia rigida (Ephemeroptera = Ephemeridae), with methoxychlor as toxicant. Proc. Fifth Annual Aquatic Toxicity Workshop, Hamilton, Ontario, Nov. 7-9, 1978. Fish. Mar. Serv. Tech. Rep. 862, pp. 266-277.

ABSTRACT

Eggs of H. rigida, in two different stages of development, were exposed to methoxychlor and start of hatch, hatch rate, and success of hatch were monitored to determine lethal and sublethal effects. Bioassays were conducted at 24+2°C, at which temperature hatch begins nine to ten days after release from the female. "Early-stage" eggs were exposed to concentrations ranging from 0.09 mg/L to 0.7 mg/L for ten and a half days using Red River water or reconstituted water for incubation and as diluent. In addition, "mid-stage eggs" (fresh and stored) were exposed to similar concentrations using reconstituted water only. Effects ranged from partial suppression of hatch at the lowest concentration to total suppression at the highest concentration, with the incipient LC50 values estimated to be 0.12 mg/L in Red River water and $<$0.09 mg/L in reconstituted water. Mid-stage eggs stored at 8°C for ten weeks were somewhat more tolerant to methoxychlor than fresh mid-stage eggs (estimated incipient LC50 values of 0.07 mg/L and 0.10 mg/L. respectively). Stored eggs may be of value in toxicity testing because thay can be held nearly year round (up to ten months) with minimal maintenance requirements, and are available for toxicity testing on short notice.

Key Words: Eggs, bioassays, methoxychlor, hatching, embryonic development, aquatic, insect

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INTRODUCTION

Toxicity studies on mayflies have been largely confined to the nymphs, probably because this is the most important stage in the aquatic food web. However, all aspects of the animal's life cycle should be considered in order to adequately evaluate the effects of a toxicant on the survival of an organism. McCart et al. (1977) studied the effects of methanol on development and hatching of the eggs of the mayfly Ephemerella sp. and Friesen (in preparation) the effects of saline groundwater on egg viability of the burrowing mayfly Hexagenia rigida (McDunnough). This latter study and a study on the relationship between temperature and duration of egg development of H. rigida (Friesen et al. in press) showed that the egg stage is sensitive to chemical and physical parameters, and that eggs are convenient to use in bioassays. The temperature study also showed that eggs about mid-way through embryonic development could be stored successfully at a low temperature (8°C) for up to ten months: hence, a source of eggs, collected during the summer at peak emergence and mating periods, could be available for use in toxicity testing throughout most of the year.

Methoxychlor, a chlorinated hydrocarbon, used as a larvicide in the control of blackflies and other pests, has been shown to affect non-target organisms, including mayfly nymphs, at treatment levels (Flannagan et al. in press). Literature on the effects of methoxychlor on environmental quality has been reviewed by Gardner and Bailey (1975).

The objective of the present study was to determine the sensitivity of developing eggs of H. rigida to methoxychlor, including eggs which had been stored at 8°C for ten weeks.

MATERIALS AND METHODS

Eggs of ten female imagines collected on 16 July 1978 at the Red River at Winnipeg, Manitoba were dissected into reconstituted water, pooled and aliquots put into acetone-hexane washed 90 mm diam. glass Petri dishes. Incubation temperature during the bioassay was 24±2°C. At this temperature hatch is expected to start about nine to ten days after release of the eggs (Friesen et al. in press). "Early-stage" eggs (just released from the female and in which only yolk is visible) were exposed to nominal methoxychlor concentrations of 0.1, 0.5 and 1.0 mg/L for ten and a half days. Red River water (filtered through 0.22μ filters) or reconstituted water, was used as diluent. Other eggs were allowed to develop for four and a half days in reconstituted water to a "mid-stage" of development (embryonic form just becoming discernible). One batch of dishes with mid-stage eggs (referred to as fresh mid-stage eggs) were exposed to the above concentrations for six days. A second batch was transferred to 8°C. After ten weeks this latter batch of dishes (referred to as stored mid-stage eggs) was transferred to 24±2°C, and after two hours was exposed to the same concentration for seven days.

Eggs were examined for first hatch and for five days thereafter at 12 hour intervals. They were checked once again, about two weeks later when no further hatch was expected (eggs were either deteriorating internally or had turned dark). Embryonic development was noted two times; once four and a half days after eggs were released and later when no further hatch was expected to occur. There were three dishes per treatment with 123 to 836 eggs per dish. Counts were made at x10 magnification and embryonic observations at x25. The reconstituted water, a defined culture medium, was made up from salts dissolved in double distilled water modified from the method described by Proyosoli et al. (1970). Chemical characteristics of Red River water and reconstituted water are given in Table 1.

The emulsifiable concentrate from which methoxychlor concentrations were made up contained approximately 20% active ingredient (R. Sebastian personal communication). Initial and final samples (from one dish per concentration)were analysed according to the method described by Solomon and Lockhart (1977). The same methoxychlor solutions used in the early-stage eggs were used for the fresh mid-stage egg exposures, and initial concentrations in the latter case were, unfortunately, not determined.

Criteria used for test measurements were; day of first hatch, hatch rate (cumulative percent hatch of total number of eggs which would finally hatch per concentration per day), and final percent hatch (percent hatch of total number of eggs per concentration). Final percent hatch and standard deviations were calculated using arcsin transformed data. Final percent mortalities due to treatment were calculated using Abbott's formula (Abbott 1925), where adjustments are made for non-treatment related mortality. This is a correction formula commonly used in insect studies when mortality (in this case non-hatch) may be due to factors other than the treatment e.g. unfertilized eggs (Philip Barker personal communication). Incipient LC50's ("the lethal concentration for 50 percent of individuals on long exposure") were estimated graphically by plotting the percent mortality due to treatment versus concentration (see Sprague 1969). Initial concentrations of methoxychlor were used for the estimations.

RESULTS

Initial methoxychlor concentrations were appreciably lower than expected and decreased considerably over the exposure period (especially at the higher concentrations), however, they were initially and remained in a graded series (Table 2). Reduction in methoxychlor levels in the Red River water was considerably higher than in reconstituted water.

Hatch rates did not show considerable differences between treatments and are not shown. Day of first hatch, final percent hatch and standard deviations, and final percent mortality due to treatment are given in Table 3. Hatch did not occur, or was delayed and reduced in varying degrees in the two higher concentrations in all cases. Eggs incubated with Red River water as

Table 1: Chemical characteristics of Red River water (filtered) and reconstituted water.

	Red River Water	Reconstituted Water
	mg/L	mg/L
Cl	35.5	143
SO4	99.0	29.0
Na	31.7	21.6
K	6.6	32.3
Mg	26.3	9.1
Ca	82.6	71.7
Mn	0.02	<0.04
Fe	0.02	<0.04
pH	8.4	6.2
Conductivity	640	610

Table 2: Initial and final methoxychlor concentrations in mg/liter of solutions in which H. rigida eggs were exposed continuously in Red River water or reconstituted water, and which fresh and stored mid-stage eggs were exposed in the latter part of embryonic development.

RED RIVER WATER:

Nominal concentrations of methoxychlor in mg/	DAY 0	DAY 10.5
0.1	0.09	0.04
0.5	0.24	0.06
1.0	0.55	0.08

RECONSTITUTED WATER:

Nominal concentrations of methoxychlor in mg/ Continuous Exposure	DAY 0	DAY 10.5
0.1	0.06	0.06
0.5	0.31	0.21
1.0	0.66	0.26
Fresh Mid-Stage Eggs Exposure		DAY 6
0.1	-	0.04
0.5	-	0.22
1.0	-	0.32
Stored Mid-Stage Eggs		DAY 7
0.1	-	0.05
0.5	0.35	0.24
1.0	0.87	0.32

Table 3: Day of first hatch at 24°C, mean and standard deviations of final hatch (of total number of eggs) per treatment, and final percent mortality due to treatment of H. rigida eggs exposed to methoxychlor continuously in Red River water or reconstituted water, and of fresh and stored mid-stage eggs exposed to methoxychlor in reconstituted water.

TREATMENT (3 replicates) Nominal** Concentration Methoxychlor in mg/liter	DAY OF FIRST HATCH (at 24°C)	MEAN AND STANDARD DEVIATION OF FINAL PERCENT HATCH	FINAL PERCENT MORTALITY DUE TO TREATMENT
RED RIVER DILUENT			
continuous exposure;			
control	9.5	42.6±0.01	0.0
0.1	10.0	32.0±1.7	23.8
0.5	10.5	1.0±2.8	93.4
1.0	10.5	0.3±0.8	98.1
RECONSTITUTED WATER			
continuous exposure;			
control	9.5	43.1±0.2	0.0
0.1	9.5	5.7±3.0	82.7
0.5	no hatch	0.0	100.0
1.0	no hatch	0.0	100.0

Continued...

Table 3: (Concluded)

TREATMENT (3 replicates) Nominal** concentration methoxychlor in mg/liter	DAY OF FIRST HATCH (at 24°C)	MEAN AND STANDARD DEVIATION OF FINAL PERCENT HATCH	FINAL PERCENT MORTALITY DUE TO TREATMENT
RECONSTITUTED WATER			
fresh mid-stage eggs exposed;			
control	9.5	41.3+0.2	0.0
0.1	10.0	24.4+4.9	36.3
0.5	no hatch	0.0	100.0
1.0	no hatch	0.0	100.0
stored mid-stage eggs exposed;			
control	10.0	38.1+0.5	0.0
0.1	10.0	21.0+0.4	44.6
0.5	10.5	10.9+7.2	61.6
1.0	11.5	0.3+0.0	99.8

**See Table 2 for actual concentrations

diluent did not seem to be affected as much as eggs incubated with the reconstituted water. Hatching characteristics of eggs exposed continuously to methoxychlor were similar to mid-stage eggs (exposed only in latter part of development) except that there was a higher mortality at 0.1 mg/L. Stored mid-stage eggs seemed to be somewhat less sensitive to methoxychlor than fresh mid-stage eggs.

When embryonic development was observed on day four and a half, eggs (in a proportion similar to that in the controls) were at similar stages of development in all treatments. By the later observation time, development had ceased in eggs (which had shown some development but had not hatched) at advanced stages of development in all concentrations with treatment related mortality (whether eggs had been exposed continuously or only in the latter period of development).

The estimated incipient LC50 values for eggs exposed to methoxychlor continuously with Red River was 0.12 mg/L, with artificial water was <0.06 mg/L, for fresh mid-stage eggs was 0.07 mg/L, and for stored mid-stage eggs was 0.10 mg/L (Fig. 1).

DISCUSSION

The initial concentrations of methoxychlor were lower than expected probably because of adsorption of methoxychlor to the glass surfaces of the preparation containers and Petri dishes (J. Solomon personal communication). The lower methoxychlor levels found in Red River water compared to the reconstituted water may have been due to the adsorption of methoxychlor onto extremely fine particulate matter. Merna and Eisele (1973) in a laboratory experiment on breakdown rate of methoxychlor in water suggested that the initial high disappearance rate which they observed may have been due to adsorption of methoxychlor onto particles which settled out and were consequently missed in the sampling procedure.

Hatching characteristics of eggs exposed continuously to methoxychlor and those exposed only in the latter period of development were similar. In the temperature study (Friesen et al. in press) and the saline groundwater study (Friesen in preparation) early-stage eggs showed a greater sensitivity than did mid-stage eggs. The observations of embryos in the present study indicated that the most sensitive period to methoxychlor occurred in advanced stages of development and perhaps methoxychlor, which seems to act on the nervous system (see Gardner and Baily 1975), has no target tissue until the nervous system of the embryo becomes developed. Although eggs were exposed to methoxychlor not less than six days the sensitive period may be much shorter and it would be important to know when the target tissue is developed sufficiently to be affected and determine exposure times with no adverse effects.

The estimated LC50's are higher than proposed levels for the Great Lakes (0.04 mg/L) (Great Lakes Quality Board 1976) but are lower than maximum levels (0.3 mg/L to 0.4 mg/L) used in the treatment of some rivers for blackflies (Fredeen 1974). These LC50 values, particularly for the Red River water,

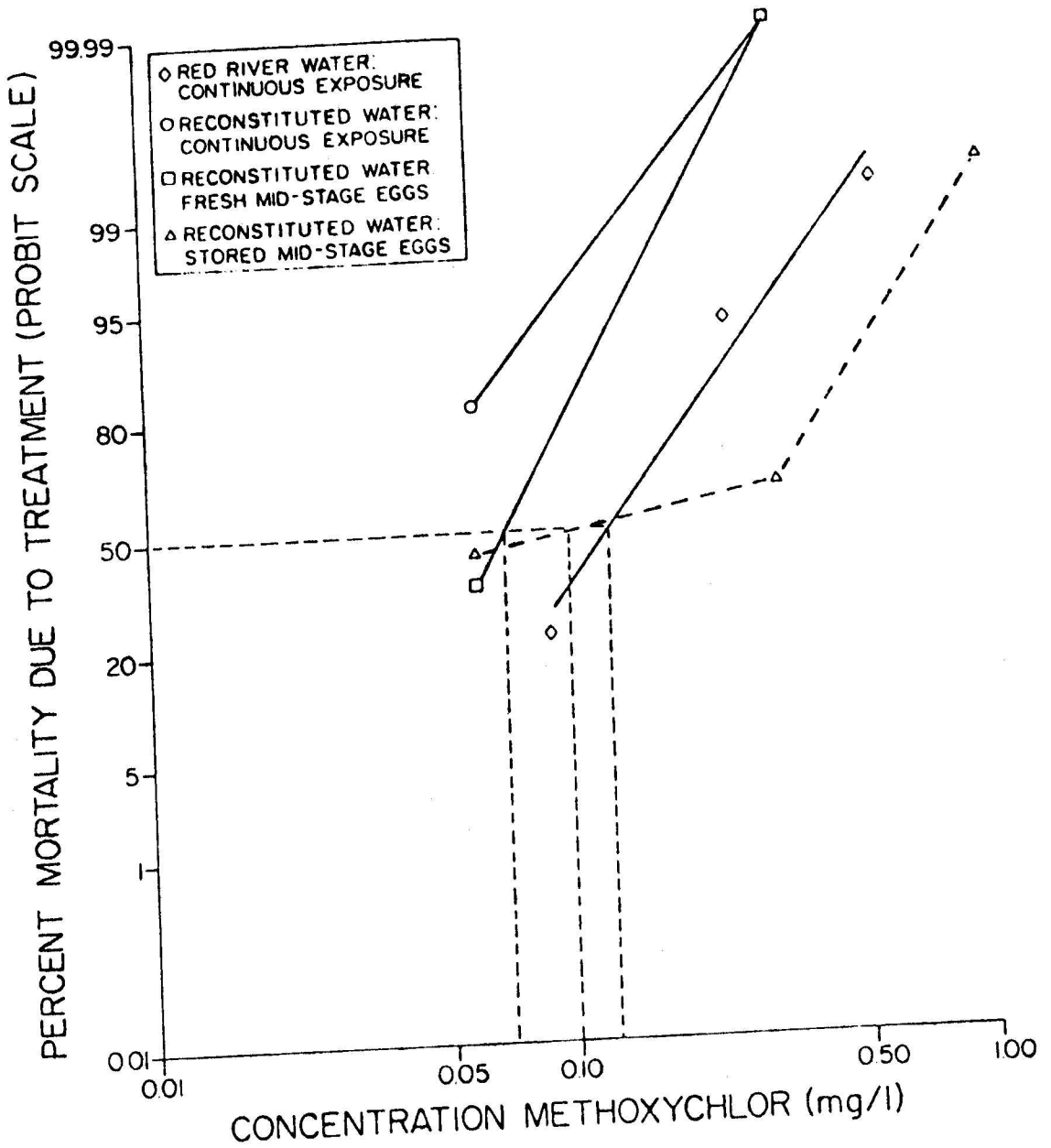

Fig. 1. Estimation of incipient LC50's (smaller dotted lines) of H. rigida eggs exposed to methoxychlor: final percent mortality due to treatment on probit scale versus concentration of methoxychlor on log scale.

are conservative values since they were estimated using the initial concentration levels which decreased considerably over the exposure period and were probably lowest during the most sensitive period of development. Although field conditions are obviously different from this laboratory study, the estimated LC50 values are near enough to levels which may and do occur in the aquatic environment that possible adverse effects of methoxychlor on the egg stage cannot be eliminated. Information on the relative tolerance of the nymphal stage is needed to adequately evaluate the toxic effects of methoxychlor on this organism.

The reason for the apparent decreased sensitivity in stored mid-stage eggs is not known. Clubb et al. (1975) found that the stonefly nymphs Arcynopteryx collected after November were more tolerant to cadmium than those collected prior to November and suggested that this might have been due to the insects being in a more advanced stage of development. This is probably not the case here. The study done by Friesen et al. (in press) indicates that development does not occur at 8°C. This is also supported in the present study since stored mid-stage eggs took half a day longer to hatch than did fresh mid-stage eggs when total time at 24°C is considered.

This study(and the saline groundwater study (Friesen in preparation)) has shown that eggs are sensitive to chemicals and are convenient to work with as bioassay material. Although results of stored mid-stage eggs may not necessarily be extrapolated to a field situation, eggs held in this manner may prove useful as a screening test in the evaluation of the toxicity of a chemical because of ease in maintenance over prolonged periods and availability on short notice.

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