Bull. Environ. Contam. Toxicol. (1988) 40:764-770

Environmental Contamination and Toxicology

Effects of Pulsed- and Spiked-Exposure to Methoxychlor on Early Life Stages of Rainbow Trout

Thomas A. Heming, Elizabeth J. McGuinness, Lee M. George, and Karen A. Blumhagen

Alberta Environmental Centre, Vegreville, Alberta, Canada T0B 4L0

Methoxychlor, the p,p'-methoxy analogue of DDT, has been used for more than a decade to control biting fly larvae in the Athabasca River of western Canada. Typically, two or three treatments of 200-300 μg/L for 15 min (nominal values) are used each spring and summer. Methoxychlor is relatively volatile and also binds to suspended solids and, consequently, the actual exposure regime can be expected to differ substantially from the nominal treatment regime. For example, direct measurement of methoxychlor in the Athabasca River 11 km downstream of the treatment site (nominal treatment, 199 μg/L for 15 min) found the detectable pulse of methoxychlor (≥0.03 μg/L) to last for more than an hour and to peak at 32 μg/L (McLeod 1987). By 94 km downstream, the detection time had increased to 20 h while the pulse peaked at 2.2 μg/L.

Field studies have found no evidence that these methoxychlor applications are acutely lethal to either the adults (Lockhart et al. 1977) or juveniles (McLeod 1987) of native fishes. Little is known, however, of the sublethal effects on cold-water fish, specifically on early life stages which may be especially sensitive to methoxychlor (Holdway and Dixon 1985, 1986a, 1986b).

The present studies assessed the effects of methoxychlor on the survival, growth and development of embryos and alevins of rainbow trout Salmo gairdneri. Two exposure regimes, pulsed- and spiked-exposures, were selected in an attempt to mimic environmentally realistic exposure scenarios. In the pulsed-study, fish were transferred to relatively concentrated toxicant solutions (≤580 μg/L methoxychlor) for 2 h, after which time the fish were returned to toxicant-free water. In the spiked-study, test tanks containing fish were spiked with methoxychlor to low concentrations (<30 μg/L) and the toxicant concentrations were allowed to decrease throughout the test period (68 days) due to fate processes.

Send reprint requests to T.A. Heming at University of Texas, Pulmonary Division, Route E-61, Galveston, Texas 77550.

MATERIALS AND METHODS

Pulsed-study. Effects of a 2-h pulse of methoxychlor were examined on the hatching behavior and success of rainbow trout. The fish were obtained as eyed-eggs from Rainbow Springs Hatchery, Thamesford, Ontario, and were held in commercial incubation trays. Each tray was subdivided into 12 compartments (9x10x5 cm) by plexiglass panels and contained 100 eggs per compartment. The trays were kept in darkness and were provided with flowing water (Table 1). Daily water temperature averaged (± 1 SD) 11.6 ± 0.7 °C, pH was 7.5 ± 0.1, and dissolved oxygen concentration was 9.4 ± 0.2 mg/L.

The eggs were exposed to methoxychlor when approximately 1 week prehatch, as follows. Eggs in individual compartments were transferred to nylon-mesh bags. The bags were then suspended in tanks containing 20 L of test solution at 11 °C. The bags were moved through the water column at 15-min intervals to aid water exchange. After 2-h exposure, the bags were rinsed in toxicant-free water, and then the eggs were removed from the bags and returned to the appropriate compartments of the incubation trays. The study included a control and 4 toxicant concentrations. Five bags, equivalent to 5 groups of 100 eggs each, were used with each test solution. Sanex-methoxychlor 25E (Sanex Inc., Mississauga, Ontario), a mixture of 25% methoxychlor in a proprietary emulsifier, was used as the toxicant. This emulsion was part of the same chemical allotment used to treat the Athabasca River in 1986. Fish-holding water (Table 1) was used as the diluent.

Following exposure, records were made at least once daily of the numbers of dead eggs, live hatchlings (alevins), and dead alevins. Dead fish were removed immediately. Alevin survival was monitored for 18 days posthatch.

Three days after hatching occurred, some of the resultant alevins were exposed to methoxychlor. The alevins used were naive (not previously exposed) to methoxychlor. The exposures were performed as described above for eggs. Survival of these alevins was monitored for 15 days postexposure.

Water samples for methoxychlor analysis were collected immediately after each 2-h exposure. Methoxychlor was measured

Table 1. Chemical characteristics (in mg/L) of water used in methoxychlor studies.

alkalinity	73	chromium	<0.001	manganese	<0.008
arsenic	<0.0002	cobalt	<0.001	molybdenum	<0.001
barium	0.009	copper	0.006	nickel	<0.001
beryllium	<0.001	hardness	114	selenium	<0.0002
cadmium	<0.001	iron	<0.02	vanadium	<0.003
chlorine	<0.010	lead	<0.003	zinc	0.019

by electron-capture gas chromatography (for method details, see Alberta Environmental Centre 1988). The method detection limit was 0.03 μg/L, while the mean recovery of methoxychlor (± 1 SD) from spiked samples was 110% ± 5.4.

Spiked-study. Effects of spiked-exposure were investigated on the early growth and development of rainbow trout. Fish for this study were obtained as freshly-fertilized eggs (<1 day old) from the Raven Brood Station, Caroline, Alberta. Upon receipt, the eggs were distributed to 3 experimental tanks at approximately 1,000 eggs per tank. Each tank contained 680 L of water (Table 1) under thermostatted, recirculated-flow conditions. The eggs themselves were placed in a nylon-mesh compartment (91×51×51 cm) at one end of the tank, in such a way that the entire flow of recirculated water (5 L/min) passed over them. Egg compartments were kept in darkness.

When the eggs were 1.5 days old, the tanks were spiked with Sanex-methoxychlor 25E to nominal concentrations of 0, 3 and 30 μg/L methoxychlor. Water samples for methoxychlor determination were collected 1 h after the spike. Fish samples for growth and developmental analyses were collected on test day 0 (1.5 h after spiking) and on test days 1, 2, 5, 8, and every fourth day thereafter to test day 68. Dead fish were counted and removed at least once daily. Daily water temperature averaged 10.1 ± 1.3 °C, pH was 7.8 ± 0.2, and dissolved oxygen concentration was 10.4 ± 0.3 mg/L.

When the embryos developed eye pigment, 50 eggs were transferred to a floating mesh basket (11×11×13 cm) in each egg compartment. Eggs in the floating baskets were monitored for hatching behavior (time of 50% hatch and duration of 95% hatch).

Sampled fish were preserved in 10% buffered formalin for at least 15 days. Preserved animals were blotted to remove excess surface water and weighed to the nearest 0.1 mg. Fish length was measured to the nearest 0.5 mm. As applicable, the egg capsule, yolk and tissues of each individual were separated by dissection, weighed, then dried at 60 °C to constant weight, and re-weighed. For each sampled individual, we also recorded morphological stage (Vernier 1969) and developmental index (Bams 1970).

RESULTS AND DISCUSSION

Effects of pulsed methoxychlor on the hatching behavior and success of rainbow trout are summarized in Table 2. Exposure of eyed-eggs to a 2-h pulse of methoxychlor at concentrations ≤580 μg/L had no detectable impact on egg hatchability, the timing of 50% hatch, the duration of 95% hatch, or on subsequent alevin survival. This is consistent with the 2-h pulsed threshold for hatchability effects in flagfish *Jordanella floridae* eggs (>2,580 μg/L, Holdway and Dixon 1986a) and with the 96-h LC50 for brook trout *Salvelinus fontinalis* eggs (≥50,000 μg/L, Johnson and Finley 1980).

Table 2. Effects of pulsed methoxychlor on hatching and survival of rainbow trout eyed-eggs. (mean ± 95% CL, n=5 groups of 100 eggs each).

Measured methoxychlor concentration (μg/L)	Total hatch (%)	Time of 50% hatch (days pe)	Duration of 95% hatch[a] (days)	18-day alevin survival (%)
<0.03	96±2	5.2±0.1	4.4±1.3	96±2
46	98±2	5.1±0.2	3.8±0.4	95±2
120	96±2	5.1±0.2	3.8±1.0	95±1
270	99±1	5.0±0.1	3.1±0.7	94±3
580	97±3	5.0±0.2	4.2±0.9	94±2

pe, postexposure. [a]Calculated between 2.5 and 97.5 percentile of total hatch.

Likewise, 2-h pulsed exposure of 3-day-old rainbow trout alevins to ≤402 μg/L methoxychlor had no effect on alevin survival. Survival for 15 days postexposure was (mean ± 95% CL, n = 5 groups of 100 alevins each) 96% ± 2 in the control, 94% ± 4 for fish pulse-exposed to 27 μg/L methoxychlor, 90% ± 4 for a 40 μg/L pulse, 93% ± 3 for a 203 μg/L pulse, and 92% ± 7 for a 402 μg/L pulse. These results are in keeping with the 96-h LC50 for brook trout alevins (≥400 μg/L, Johnson and Finley 1980). It is possible, nonetheless, that the test alevins were impacted sublethally. The 2-h pulsed threshold for sublethal effects to 8-day-old flagfish juveniles is 250 μg/L (Holdway and Dixon 1986b). It should be noted, however, that the validity of extrapolating the effects of a 2-h exposure of a rapidly developing fish held at 25 °C to the 2-h exposure of a slowly developing fish held at 10 °C has yet to be determined; in terms of physiological time (e.g., elapsed thermal units), 2 h at 25 °C is perhaps equivalent to 5 h at 10 °C.

Analytical constraints prevented a thorough analysis of the temporal changes in methoxychlor in spiked studies. Water samples, collected 1 h after spiking the tanks, contained <0.03, 1.0, and 9.2 μg/L methoxychlor respectively, or about one-third of the nominal spiked concentrations. The difference between nominal and measured concentrations probably reflected binding of methoxychlor to the test apparatus and to a lesser degree loss of methoxychlor to the atmosphere. Other studies conducted in our laboratory in the same experimental tanks under similar test conditions indicated the half-life of methoxychlor in our test tanks to be about 1.7 days (Alberta Environmental Centre 1988).

Initial egg viability was low in the spiked study. Examination of 50 eggs collected at experimental time 0 indicated that only 64% were viable; there was no evidence that the remainder had been fertilized. Subsequent calculations of egg survival were adjusted to take this into account.

Table 3. Effect of spiked methoxychlor on hatching, survival, yolk absorption, and growth rate of rainbow trout. (mean ± 95% CL).

	Measured methoxychlor spike (μg/L)		
	<0.03	1.0	9.2
Total hatch (%)	51	50	48
Time of 50% hatch (days postfertilization)	34.0±0.2	34.1±0.2	33.3±0.1
Duration of 95% hatch (days)	3.8	3.0	2.1
30-day alevin survival (%)	91	95	92
Yolk absorption rate[a] (mg/day)	0.30±0.05	0.32±0.04	0.33±0.04
Gross yolk conversion efficiency[b] (%)	57	55	60
Specific growth rate[a] (%/day)	6.26±1.41	6.46±1.11	6.26±1.31

[a]Calculated between hatching and maximum tissue weight.
[b]Calculated between fertilization and maximum tissue weight.

Under the present experimental conditions, methoxychlor had no detectable effect on the absorption or utilization of yolk by rainbow trout (Tables 3 and 4). Spiked exposure to ≤9.2 μg/L did not significantly alter the rate of yolk absorption, the yolk-to-tissue conversion efficiency, the rate of tissue growth, or the maximum tissue weight achieved on yolk alone. Neither development nor the composition of rainbow trout were affected (Table 4).

Likewise, exposure of freshly-fertilized eggs to a spike of ≤9.2 μg/L methoxychlor appeared to have little or no effect on egg hatchability or on subsequent alevin survival (Table 3). At the highest concentration tested, however, fish spike-exposed to 9.2 μg/L hatched significantly earlier and perhaps more rapidly than control animals. Premature hatching is a characteristic response to adverse environmental stress, including hypoxia, extremes in temperature, pH and salinity, and exposure to some xenobiotics (Rosenthal and Alderdice 1976). The biological significance of the observed difference in hatching times is questionable given that the timing of 50% hatch was shifted by less than one day (Table 3) and that no significant effects were seen at hatching in fish size, composition, or developmental stage (Table 4).

Of the ten parameters examined during pulsed- and spiked-exposures (egg hatchability, time of 50% hatch, duration of 95% hatch, alevin survival, yolk absorption rate, yolk-to-tissue conversion efficiency, tissue growth rate, morphological development, fish size, fish composition), only the hatching time of eggs spike-exposed to 9.2 μg/L methoxychlor when 1.5 days old was affected. Given the magnitude of the response, this effect is probably of minimal biological significance. Definitive

Table 4. *Effect of spiked methoxychlor on the composition and development of rainbow trout (mean ± 95% CL).*

Measured methoxychlor spike (µg/L)	Fish stage	k_D	Fish length (mm)	Total weight (mg)		Tissue dry weight (mg)	Yolk dry weight (mg)	Egg capsule dry weight (mg)	Perivitelline fluid (mg)	% water content		
				Wet	Dry					Total	Tissue	Yolk
A. AT EXPERIMENTAL TIME ZERO (1.5 days pf)												
<0.03	4±1	ND	<0.5	35.5±2.5	12.2±1.9	<0.1	12.0±1.9	1.1±0.1	1.8±1.9	64±3	ND	56±3
1.0	3±2	ND	<0.5	34.5±3.9	12.9±1.7	<0.1	11.0±1.7	0.9±0.0	1.9±1.7	64±2	ND	55±5
9.2	4±2	ND	<0.5	34.1±5.0	12.2±3.9	<0.1	11.1±3.9	1.0±0.2	1.6±0.6	64±3	ND	57±3
B. AT EYE PIGMENTATION (22-25 days pf)												
<0.03	21±1	8.2±1.1	8.2±0.6	29.0±2.8	9.6±0.6	0.7±0.3	8.1±0.6	0.8±0.4	1.9±0.6	67±1	76±9	50±8
1.0	21±0	7.9±0.9	8.7±0.8	32.1±3.3	10.3±1.1	0.8±0.3	8.6±1.1	0.8±0.1	2.6±0.6	68±1	76±8	52±1
9.2	21±0	7.9±0.4	8.9±0.6	32.4±1.9	10.5±0.8	1.1±0.3	8.6±0.8	0.7±0.1	1.9±0.3	68±1	77±3	51±3
C. AT HATCHING (33-34 days pf)												
<0.03	30±1	6.0±0.4	11.4±0.6	31.4±5.0	9.4±1.7	2.0±0.6	6.6±1.1	NP	NP	71±1	82±1	48±8
1.0	29±1	6.6±0.9	10.2±0.8	32.8±1.4	10.1±0.6	1.7±0.6	7.7±0.3	NP	NP	70±1	80±3	52±4
9.2	29±0	6.1±0.2	11.4±0.4	31.5±3.9	9.5±1.8	2.1±0.3	6.7±1.8	NP	NP	70±1	81±6	49±2
D. AT MAXIMUM TISSUE WEIGHT (54-57 days pf)												
<0.03	35±0	4.2±0.1	18.2±0.4	45.9±2.4	7.2±0.4	6.8±0.4	0.4±0.2	NP	NP	84±0	83±0	59±8
1.0	35±0	4.2±0.1	17.8±0.4	42.1±1.6	6.5±0.3	6.0±0.3	0.7±0.2	NP	NP	85±1	84±1	58±5
9.2	35±0	4.1±0.1	18.5±0.2	46.3±1.5	7.0±0.2	6.7±0.2	0.3±0.1	NP	NP	85±0	84±0	59±6

k_D, Bam's developmental index. pf, postfertilization. ND, not determined. NP, not present.

statements about the potential for adverse effects to fish due to methoxychlor application, however, require a more complete understanding of the actual exposure regimes and of the dose-response relationship during environmentally realistic exposure scenarios. The possibility of long-term effects on the reproductive characteristics of exposed fish (cf. Holdway and Dixon 1986b) cannot be overlooked, and warrents further research in cold-water species.

Acknowledgements. The authors thank the staff of the Pesticide Analysis and Research Section (Chemistry Wing) of the Alberta Environmental Centre for their technical assistance.

REFERENCES

Alberta Environmental Centre (1988) Environmental chemistry and toxicity of methoxychlor to fish. Alberta Environmental Centre Res Report:In press, Vegreville, Alberta.

Bams RA (1970) Evaluation of a revised hatchery method tested on pink and chum salmon fry. J Fish Res Board Can 27:1429-1452.

Holdway DA, Dixon DG (1985) Acute toxicity of pulsed-dosed methoxychlor to juvenile American flagfish (Jordanella floridae Goode and Bean) as modified by age and food availability. Aquat Toxicol 6:243-250.

Holdway DA, Dixon DG (1986a) Effects of methoxychlor exposure of flagfish eggs (Jordanella floridae) on hatchability, juvenile methoxychlor tolerance and whole-body levels of tryptophan, serotonin and 5-hydroxyindoleacetic acid. Wat Res 20:893-897.

Holdway DA, Dixon DG (1986b) Impact of pulse exposure to methoxychlor on flagfish (Jordanella floridae) over one reproductive cycle. Can J Fish Aquat Sci 43:1410-1415.

Johnson WW, Finley MT (1980) Handbook of acute toxicity of chemicals to fish and aquatic invertebrates. US Dept Interior, Fish Wildl Serv Res Publ 137, Washington, DC.

Lockhart WL, Metner DA, Solomon J (1977) Methoxychlor residue studies in caged and wild fish from the Athabasca River, Alberta, following a single application of black fly larvicide. J Fish Res Board Can 34:626-632.

McLeod C (1987) Athabasca River blackfly control program. Fish toxicological studies 1986. Alberta Dept Environ, Pesticide Chemicals Branch, Edmonton, Alberta.

Rosenthal H, Alderdice DF (1976) Sublethal effects of environmental stressors, natural and pollutional, on marine fish eggs and larvae. J Fish Res Board Can 33:2047-2065.

Vernier J-M (1969) Chronological table of the embryonic development of rainbow trout. Ann Embryol Morpholog 2:495-520. (Translated from French, Environ Can, Fish Mar Serv Transl Series No 3913, Ottawa, Ontario).

Received September 9, 1987; accepted January 14, 1988