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Toxicity of Carbofuran to Selected Macroinvertebrates in Prairie Ponds

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Because of the extensive use of insecticides and the increasing popularity of aerial application across much of the northern prairies of North America, concern has been expressed that the innumerable small water bodies commonly referred to as prairie potholes which dot the prairie agricultural landscape may become contaminated by either direct overflight spraying or drift deposit from spraying slough borders (Grue et al. 1986; Sheehan et al. 1987). A large proportion of North America's waterfowl relies on prairie potholes as a source of high-protein invertebrate food throughout the breeding and brood-rearing portions of their annual cycle (Swanson and Meyer 1973; Bellrose 1980). Accidental contamination could potentially reduce their invertebrate standing crops and thus seriously degrade their value for waterfowl.

In recent years, the carbamate insecticide, carbofuran (2,3-dihydro-2-2 dimethyl-7-benzofuranyl methylcarbamate) has been one of the most widely-used insecticides on the Canadian prairies, especially for grasshopper (Acrididae: Orthoptera) control. However, the effects of carbofuran on aquatic macroinvertebrates appears limited to laboratory or field studies of its acute toxicity to Daphnia (Yu et al. 1974; Hartman and Martin 1985; Johnson 1986), midge larvae (Diptera: Chironomidae) (Mulla and Khasawinah 1969; Karnak and Collins 1974; Johson 1986) and mosquito larvae (Lancaster and Tugwell 1969).

In this study, we assessed carbofuran-induced mortality in selected groups of macroinvertebrates known for their importance as waterfowl foods by confining the animals in small cages in prairie ponds and subsequently treating the ponds with carbofuran.

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MATERIALS AND METHODS

Eight farm ponds located about 90 km N of Edmonton, Alberta (51°14' N, 113°36'W) and excavated in the 1950's were used in 1986. In 1987, the study was conducted within twenty-one 1.8 m diameter X 0.5 m deep polyethylene enclosures which were placed in one semi-permanent prairie pothole located near Sherwood Park, Alberta (53°33'N, 113°15'W) at depths ranging from 40 to 45 cm. Some characteristics of the study ponds are outlined in Table 1.

Table 1. Selected characteristics of the study ponds.

Year	Pond[a]	pH	Conductivity (µmhos/cm)	Turbidity (NTU)	Diss. O_2 (mg/L)	Sediment Org.Mat. (%)
1986	T1	8.4	1686	5.8	7.5	6.5
	T2	8.8	953	25.0	8.8	4.1
	T3	8.9	404	6.6	7.4	3.6
	T4	8.5	427	5.3	7.1	4.6
	C1	10.1	341	1.3	10.8	---
	C2	10.1	329	3.4	12.0	---
	C3	8.9	744	6.9	8.6	---
	C4	9.8	326	3.3	9.6	---
1987	Sherwood Park[b]	9.0	431	4.3	9.4	20.0

a - T1 to T4 refer to treatment ponds and C1 to C4 to control ponds.
b - Values for Sherwood Park pond based on means of measurements within each of the 21 enclosures.

In 1986, adult *Gammarus lacustris* (Gammaridae:Amphipoda), late-instar larvae of *Chironomus tentans*, (Chironomidae: Diptera), and nymphs of the damselfly genus *Enallagma* (Coenagrionidae: Zygoptera) were collected from nearby ponds and stored in large buckets which contained pond water one day before the tests began. Several midge larvae were returned to the laboratory where they were allowed to emerge so that the adults could be used to confirm the species identification. On July 23, eight *G. lacustris*, six *C. tentans*, or six *Enallagma* were caged in a series of 500 mL plastic containers, one taxon per cage. Mosquito netting (mesh size=1 mm) was placed over the top of each container with an elastic band to hold it in place. For each taxon, six containers were placed on the bottom of each pond at randomly selected shallow sites (25-75 cm deep), another six at randomly selected deep sites (76-125 cm deep) of two control and two treatment ponds. Each cage contained a small amount of sieved sediment (mesh size=500 µm) and aquatic macrophytes collected from the respective ponds. Following placement of the cages (July 23), carbofuran (Furadan 480 Flowable) was applied to the two treatment ponds from a canoe using a backpack sprayer. This process, including the placement of another series of containers containing the above taxa, was repeated on July 30 using two other treatment and control ponds.

Sixteen and 124 hr after spraying, water samples were collected from a depth of 15 to 30 cm at 4 sites in each treatment pond, stored in 2-L glass jars, acidified with H_2SO_4, placed over ice in a cooler and returned to the laboratory where residues were extracted with dichloromethane following addition of NaCl and analyzed using reverse-phase high pressure liquid chromatography with an ultra-violet detector. Standards of carbofuran were analyzed so the water samples could be quantified. Blanks, spikes, and duplicates were analyzed on a 10% basis. The recovery from water samples was 110±11% (mean±CV). Analysis of duplicates indicated reasonable precision (average CV=13%). The detection limit was 0.5 μg/L.

The containers were retrieved from each pond 72 to 96 hr after treatment and their contents gently washed through a 500 μm sieve, a procedure that permitted the counting of live and dead organisms. An animal was considered to be alive if it moved when touched. For each of the six cages per taxon in each depth zone in each pond, averages of the proportions remaining alive were calculated.

Data were arc-sine transformed. A split-plot design (Steele and Torrie 1960) incorporating the main plot effect of treatment and the subplot effects of depth and treatment-depth interaction was used to evaluate the effects of carbofuran at the two depths on the proportions of each of the groups of macroinvertebrates which remained alive. When significant interaction effects were found, the simple effects were analyzed using protected LSD tests (Snedecor and Cochran 1980). Methods for obtaining the appropriate standard errors and, where applicable, weighted t-values were outlined by Steele and Torrie (1960).

In 1987, adult and nearly full-grown Hyalella azteca (Talitridae: Amphipoda), late-instar larvae of the genus, Limnephilus (Limnephilidae: Trichoptera) and C. tentans larvae were collected from ponds near the study pond one day before treatment. Species identification was confirmed for C. tentans as in 1986. On July 15, 10 H. azteca, ten C. tentans larvae, or eight Limnephilus larvae were placed in containers, one taxon per container, as described above. For each taxon, containers were randomly assigned to 21 polyethylene enclosures, one cage per taxon per enclosure. Seven enclosures were designated as controls (receiving no carbofuran), seven as 5-μg/L and seven as 25-μg/L enclosures. Immediately after the placement of the containers, the enclosures were treated with the appropriate amount of carbofuran as Furadan 480 Flowable.

Retrieval and counting of live and dead organisms followed the procedures outlined above. A Kruskal-Wallis test was used to determine whether there were differences among treatment levels in the median proportions surviving for each taxon. Where significant differences were found, a multiple comparisons procedure was used to compare each of the carbofuran concentrations with the control.

RESULTS AND DISCUSSION

In 1986, mean concentrations of carbofuran in the treatment ponds 16 h after spraying were 9, 14, 32, and 32 µg/L in ponds T1 to T4 respectively; after 124 hr, concentrations had declined to 3, 4, 3, and 12 µg/L, a rate of decline that is consistent with that found for carbofuran in alkaline water (Chapman and Cole 1982).

Since the experiment began on July 23 for four ponds and on July 30 for the other four, the effect of date was initially included in the analysis. Neither date nor its interaction with treatment were significant for any of the taxa ($P>0.4$, 1,4df, in all cases). Therefore, ponds were pooled across dates and the analyses repeated.

For both G. lacustris and C. tentans, the effect of carbofuran varied with depth ($P<0.04$, 1,6df, both taxa). For both taxa, survival in the shallow zone was significantly reduced in treatment ponds when compared to control ponds (Table 2). Survival of C. tentans larvae in deep zones was also significantly lower in treatment ponds when compared to control ponds although the magnitude of the reduction was not as great as in the shallow zones (Table 2). In treated ponds, survival of G. lacustris was significantly lower in shallow zones than in deep zones; however, in deep zones, their was no significant difference in their survival between treatment and control ponds (Table 2). Survival of Enallagma damselflies also appeared to be lower in treated than in control ponds (Table 2), especially in ponds T1 and T4 (Table 2) where initial carbofuran concentrations were 9 and 32 µg/L respectively. However, overall survival did not differ significantly between treatment and control ponds ($F=2.9$, $P=0.14$, 1,6df) nor was it influenced significantly by the interaction between treatment and depth ($F=0.7$, $P=0.43$, 1,6df).

For the treatment ponds, confidence limits for survival rates of all three taxa were very wide (Table 2), probably due, in part, to the differences among ponds in carbofuran concentrations. Survival of both G. lacustris and C. tentans was low in ponds T3 and T4, both of which

Table 2. Percentages of animals surviving after 72-96 hr of exposure in cages placed in shallow and deep zones of control and carbofuran-treated ponds.

	% Survival					
	G. lacustris		*C. tentans*		*Enallagma*	
Pond	Shallow	Deep	Shallow	Deep	Shallow	Deep
C1	100	94	72	58	61	78
C2	88	90	89	53	73	70
C3	80	88	67	59	63	53
C4	79	81	64	42	56	80
Mean[a]	87Aa	88Aa	73Aa	53Aa	63Aa	70Aa
95%CL[a]	(61-99)	(79-95)	(53-90)	(40-65)	(51-75)	(50-88)
T1	0	65	3	44	31	11
T2	44	98	11	22	75	75
T3	0	82	3	6	45	67
T4	4	11	8	6	28	17
Mean[a]	12Ba	64Ab	6Ba	20Ba	44Aa	42Aa
95%CL[a]	(9-49)	(5-98)	(1-14)	(0-51)	(13-78)	(1-91)

a - Means and confidence limits have been backtransormed from arcsine (square-root (proportion)).
For each taxon, means of treated and control ponds that are followed by similar upper case letters are not significantly different ($P>0.05$).
For each taxon, means of shallow and deep zones which are followed by similar lower case letters are not significantly different ($P>0.05$).

Table 3. Percentage (mean±SE, median, n=7) of animals surviving in cages 72-96 hr after enclosures were treated with carbofuran.

	Treatment Level		
Species	Control	5 µg/L	25 µg/L
H. azteca	92.8±2.9, 90	67.1±8.4, 70	11.4±7.0, 0
C. tentans	72.9±6.4, 80	72.9±4.2, 70	27.1±6.8, 30
Limnephilus	79.0±4.5, 75	72.9±6.4, 71	0.0±0.0, 0

received carbofuran loadings of approximately 32 µg/L. Survival was higher in pond T2 which received only about 14 µg/L. However, the lack of a well-defined relationship between survival and concentration in this study suggests that other factors, perhaps limnological in nature, may have modified the carbofuran-induced mortality rates to these taxa.

The significant interaction effect between treatment and depth on the survival of *G. lacustris* and *C. tentans* imply that depth, or some correlate of depth, may influence the survival of certain benthic macroinvertebrates in ponds exposed to a given range of carbofuran concentrations. This result was unexpected because of the relatively high water solublity of carbofuran (415 ppm, Kenaga and Goring 1980) coupled with the shallowness of the ponds (mean depth < 2.0 m for all ponds) and wind-induced mixing which led to the expectation that it would disperse rapidly and thoroughly throughout the water column, thus exposing macroinvertebrates to more or less equal concentrations of carbofuran throughout each pond.

In this study, differences in the survival of G. lacustris between depths may have been influenced by the concentrations of carbofuran in the treatment ponds. These differences were greatest in ponds T1 and T2 in which initial carbofuran levels were lowest (9 and 14 µg/L respectively) as well as in pond T3 in which the initial concentration was high (32 µg/L), but degradation rapid (only 3 µg/L after 124 hr). In pond T4, in which initial concentration was high (32 µg/L), and degradation, slow (12 µg/L remained after 124 hr), survival was equally reduced in the shallow and deep zone. For C. tentans, the apparent influence of depth on survival was not clear-cut. Only in pond T1 was survival noticeably reduced in the shallow zone when compared with the deep zone (Table 2). While the effect of depth on the survival of benthic macroinvertebrates in carbofuran-exposed ponds remains speculative, it is evident from the results of this study that within-pond variation in survival can be quite high. Such variability may have implications for the survival of mobile benthic invertebrates if they are able to detect and actively avoid potentially lethal concentrations of a pesticide.

In 1987, carbofuran was not detected in water samples taken from control enclosures 2 hr after treatment. The mean (±SD) concentration of carbofuran in water samples from a subsample of 5-µg/L enclosures was 6.3±1.5 µg/L (n=3) while in the 25-µg/L enclosures, it was 22.5 µg/L (range: 13-32 µg/L, n=2). Significant differences among treatment levels were found for all three taxa (Kruskal-Wallis: $P<0.002$ in all cases). For H. azteca, the proportions of animals which survived were significantly different ($P<0.01$) in both the 5- and 25-µg/L enclosures when compared with the controls. Median survival was only slightly reduced in the 5-µg/L enclosures, while in the 25-µg/L enclosures the effects were more evident (Table 3). For both C. tentans and Limnephilus larvae, survival was significantly reduced ($P<0.01$) only in the 25-µg/L enclosures when compared with the controls. The median percentages of C. tentans larvae that survived were similar in the control (80%) and 5-µg/L enclosures (70%), while in the 25-µg/L enclosures, it was much lower (30%) (Table 3). The median percentages of Limnephilus larvae that survived were 79, 73, and 0% in the control, 5- and 25-µg/L enclosures respectively (Table 3). Survival of C. tentans in the 5-µg/L enclosures was higher than expected based on the laboratory-derived LC50 of 1.6 ppb (Karnak and Collins 1974), possibly as a result of the rapid degradation of carbofuran in the alkaline pond water (Table 1) or interpopulation differences in resistance.

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