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Acute Toxicity of Carbofuran to Adult and Juvenile Flathead Chubs

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Cross et al. (1986) and Pfleiger and Grace (1987) reported that flathead chub (*Platygobio gracilis*) populations have decreased by as much as 98% over portions of their historical range. The causes of these declines are not well understood; however, degraded habitat and water quality are suspected (Lee et al. 1980). Research has indicated that portions of the Missouri River watershed in the western Dakotas are maintaining good populations of flathead chubs (e.g., Werdon 1992); however, potentially deleterious agricultural practices in these areas during the past two decades have included increases in insecticide coverage (Facemire 1993). The impacts that these chemical applications may have on healthy fish populations is a concern to agencies attempting to assess declines of native fish stocks.

Carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranol N-methylcarbamate), an anticholinesterase carbamate, is commonly used as an insecticide, nematicide, and acaricide in agricultural practice around the world (Gupta 1994). Because of widespread carbofuran use, contamination has been found in the soil, air, food, water, and wildlife. The U.S. Fish and Wildlife Service has found carbofuran to be highly toxic to both birds and fishes due to damaging effects on the central nervous system (EXTOXNET 1993). However, Parkin and Shelton (1992) and Anton et al. (1993) both found that carbofuran is preferred over many other insecticides because it has a low persistence in most soil types (typically <60 days), breaks down in neutral or slightly alkaline water (half-life of 1 to 8 weeks depending upon water temperature), does not bind to sediments or suspended particles, and does not bioaccumulate.

In western North Dakota, carbofuran is the most broadly applied insecticide currently being utilized by farmers and ranchers for the control of grasshoppers and other insect pests (Facemire 1993). In eastern Montana and western North Dakota, carbofuran is applied multiple times with an annual application rate of 87 g per square kilometer (USGS 1997). Fish species, such as the yellow perch (*Perca flavescens*) have been reported to be sensitive to carbofuran, with lethal effects on fry occurring at concentrations as low as 147 ppb; however,

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nonlethal effects of low-level carbofuran exposure tend to be limited (Hill 1995). Many of the treated areas are also irrigated with water from the Missouri River. Return ducts filled with excess runoff water empty into riverine habitats where flathead chubs reside. Although no noticeable deleterious impacts have been recorded on flathead chub populations, the potential exists for these insecticides to become problematic with increased or careless transport and application.

The objective of this study was to evaluate the sensitivity of flathead chubs to various concentrations of carbofuran by: 1) determining the concentration at which a 50% mortality of adult and juvenile flathead chubs could be observed (LC_{50}) and 2) determining the concentration at which 50% of the flathead chubs exhibit substantial behavioral modifications (EC_{50}).

MATERIALS AND METHODS

Flathead chubs were collected in sandbar habitats from the Missouri River, North Dakota using a bag seine in September of 1997. The flathead chubs were transported to South Dakota State University where they were held in a flow-through raceway. The chubs were fed twice daily at a rate of 1.5% estimated body weight with a commercial feed (38% protein, 5% lipid; Southern States, Inc.). Flathead chubs were held for 30 d and then sorted into length groups. We determined that flathead chubs between 80- and 120-mm total length (TL) were age 1 and reproductively immature. Therefore, we classified flathead chubs between 80- and 120-mm TL as advanced juveniles. Flathead chubs greater than 120-mm were separately held for 450 d, allowing them to attain lengths exceeding 140-mm TL and ages greater than 2. The flathead chubs classified as adult varied in length from 140- to 200-mm TL and ranged in age from 2 to 4.

One hundred advanced juvenile flathead chubs were held without food for 24 h, randomly placed into one of 10 exposure chambers (10 chubs per chamber), and then acclimated for 24 h prior to carbofuran introduction. Target carbofuran concentrations were 6.00, 3.00, 1.50, 0.75, and 0.00 (control) ppm. Actual carbofuran concentrations in each exposure chamber were tested using high-pressure liquid chromatography (HPLC). The five carbofuran concentrations were each delivered in 30-min intervals to two exposure chambers with a intermittent flow-through proportional serial diluter (Lemke et al. 1978). The acute toxicity was determined using standard techniques for a 96-h intermittent flow-through bioassay (Adams 1995). We recorded the total number of mortalities and the total number of intoxicated flathead chubs immediately following exposure, and then again at 12, 24, 48, and 96 h. Intoxication counts were completed after each tank was disturbed with a probe and individual chubs had the opportunity to react. Flathead chubs that were extremely lethargic (no response, but with opercular flexing), unable to remain upright, and/or exhibited a lack of motility control were classified as intoxicated. Flathead chubs that succumbed to mortality had to pass through a period of intoxication; therefore, dead fish were also counted as intoxicated.

Seventy adult flathead chubs were held without food for 24 h, randomly placed into one of 10 exposure chambers (7 chubs per chamber), and then acclimated for 24 h prior to carbofuran introduction. Target carbofuran concentrations were 8.00, 4.00, 2.00, 1.00, and 0.00 (control) ppm. Actual carbofuran concentrations in each exposure chamber were tested using HPLC. The five carbofuran concentrations were delivered and data were recorded following the protocol described for the juvenile assessment. HPLC assessment of carbofuran concentrations revealed that our exposure cells only received approximately 60% of the calculated dose. Given that all flathead chubs in our highest carbofuran concentration did not succumb to mortality, we could not complete our analysis. Therefore, diluter concentrations were increased and we completed another 96-h bioassay for carbofuran concentrations of 8.00 and 0.00 ppm. Water samples from the second run also were tested for actual carbofuran concentrations.

The LC_{50} and EC_{50} for carbofuran on advanced juvenile and adult flathead chubs were determined using a logarithmic probability regression (PROBIT procedure; SAS 1990) on actual concentrations. This analysis technique is commonly used (e.g., Anton et al. 1993; Paul and Simonin 1995) and predicts the dose-response relation and 95% confidence intervals (CI) for the bioassay data at given probabilities ranging from 0.01 to 0.99.

RESULTS AND DISCUSSION

The HPLC analysis indicated that actual carbofuran concentrations in each exposure chamber during the advanced juvenile bioassay slightly differed from the calculated concentrations (Table 1). The logarithmic probability regression analysis indicated that the LC_{50} for carbofuran to advanced juvenile flathead chubs was 1.96 ppm (CI = 1.62, 2.36; Figure 1). The probability analysis indicated that the EC_{50} was 1.28 (CI = 0.81, 1.67; Figure 1).

The HPLC analysis indicated that actual carbofuran concentrations in each exposure chamber during the initial adult flathead chub bioassay were substantially different from the calculated values; however, calculation concentrations approximated actual concentrations during the second bioassay (Table 1). The logarithmic probability regression analysis indicated that the LC_{50} for carbofuran to adult flathead chubs was 2.64 ppm (CI = 2.07, 3.45; Figure 1). The probability analysis indicated that the EC_{50} was 1.72 (CI = 1.29, 2.09; Figure 1).

Table 1. Calculated and actual carbofuran concentrations in exposure chambers during 96-h bioassay experiments on advanced juvenile and adult flathead chubs.

Exposure chamber	Juvenile calculated ppm	Juvenile actual ppm	Adult calculated ppm	Adult actual ppm
1	6.00	6.39	8.00	5.10
2	6.00	6.44	8.00	5.02
3	3.00	3.39	4.00	2.30
4	3.00	3.38	4.00	2.22
5	1.50	1.76	2.00	1.47
6	1.50	1.73	2.00	1.81
7	0.75	0.40	1.00	0.64
8	0.75	0.40	1.00	0.64
9	0.00	<0.05	0.00	<0.05
10	0.00	<0.05	0.00	<0.05
1*	---	---	8.00	8.05
2*	---	---	8.00	7.94

*Concentrations during the second adult bioassay after adjustments to dosage calculations had been completed.

Understanding effective concentrations of toxicants is important, especially in lotic environments. Researchers have implied that many chemicals, although not directly causing death, may impact physiological functions that render the organism vulnerable to predation, impair feeding, or cause an inability to utilize optimal habitats, thus indirectly increasing mortality (e.g., Anton et al. 1993; Paul and Simonin 1995). Buckler et al. (198 1) reported that organic-based pesticides caused vertebral hemorrhaging near the dorsal tin that limited mobility and caused increased post-exposure deaths. Similar spinal dislocations were noted in 3% of the flathead chubs exposed to carbofuran. Those that survived carbofuran exposure and experienced a vertebral rupture also exhibited anterior discoloration, loss of caudal fin mobility, and were quickly colonized by the fungus *Saprolegnia* sp. in the discolored area. Secondary, or nonlethal impacts, were only noted on a small proportion of the exposed flathead chubs; therefore, we can support the statement of Hill (1995) that acute nonlethal effects caused by carbofuran are reasonably limited.

In both the juvenile and adult assessments, the EC_{50} value was approximately 65% of the LC_{50} value, suggesting a somewhat limited gradient between nonlethal and lethal effects. Also, lethal mortality probability slopes indicate that a greater increase in cabofuran concentration is required to raise the

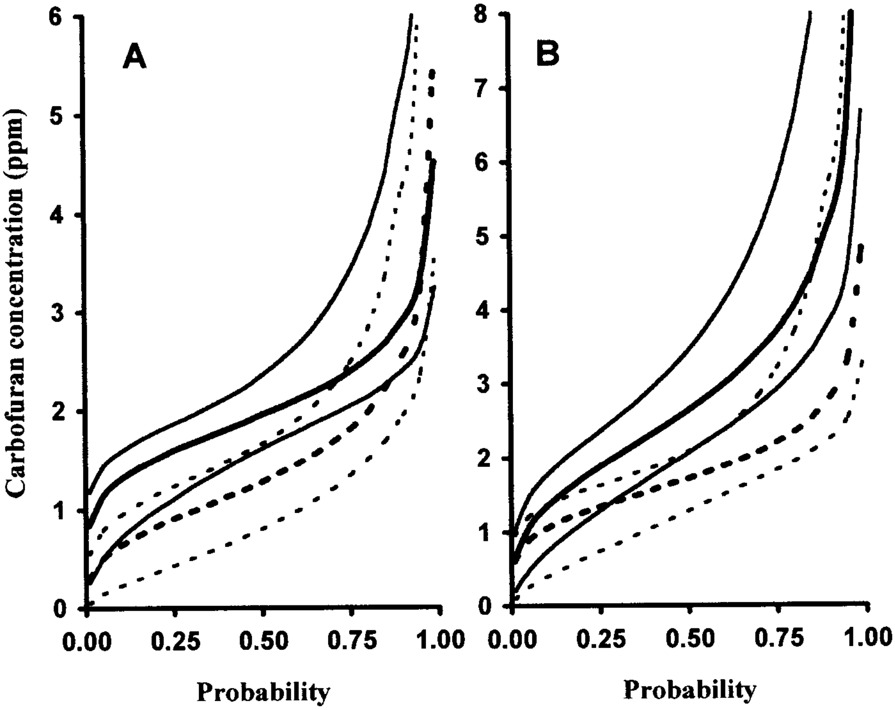

Figure 1. The probability of mortality or intoxication of advanced juvenile (A) and adult (B) flathead chubs exposed to various concentrations (ppm) of carbofuran. Solid lines are the predicted mortality probability curves and their 95% confidence intervals. The dashed lines represent the intoxication probability curves and their 95% confidence intervals.

mortality probability of adult chubs than is needed to raise the death likelihood by the same amount for juveniles. Although this study has again demonstrated that carbofuran is toxic to some fish species, the problems associated with the usage of this insecticide are fewer in comparison with other chemical applications. At LC_{50} values of 1.96 and 2.64 ppm for juvenile and adult flathead chubs, and given that this compound has a short half-life in water, tends not to bind with sediments, and does not bioaccumulate readily, carbofuran is an insecticide that should pose little threat to flathead chub populations in the Missouri River of North Dakota.

Assuming that applications of carbofuran are conducted appropriately, the chemical should degrade quickly and never be present in surface waters at concentrations potent enough to cause lethal effects. Information concerning flathead chub ability to utilize optimal habitats in lotic systems and avoid predation at various levels of carbofuran and other agricultural chemicals would be valuable. This study was completed on advanced juvenile and adult flathead chubs in a controlled setting. Eyed eggs and larval fishes of most species, especially in a natural environment, tend to be hypersensitive to many chemical compounds. Therefore, considerably lower concentrations of carbofuran could have greater detrimental effects on flathead chub populations than are indicated here.

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