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# Toxicity of 3,4-Dichloroaniline to Fathead Minnows, *Pimephales promelas,* in Acute and Early Life-Stage Exposures

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3,4-Dichloroaniline (3,4-DCA) is a chemical which may enter surface waters as a contaminant in applications of agricultural herbicides, as a metabolite of several herbicides, or in industrial effluents from dye manufacturing plants. 3,4-DCA is an intermediate in the synthesis of certain urea and acylanilide herbicides, such as diuron, linuron, neburon, and propanil (DiMuccio et al. 1984). These herbicides are degraded to 3,4-DCA by microorganisms in the soil or water (Bartha and Pramer 1967; Deuel et al. 1977; El-Dib and Aly 1976).

In water, 3,4-DCA is resistant to biodegradation (El-Dib and Aly 1976; Wolff and Crossland 1985). However, it is dissipated from water by phototransformation (Wolff and Crossland 1985), and has a half-life in shallow ponds ranging from about 2 to 6 d (Deuel et al. 1977; Wolff and Crossland 1985). 3,4-DCA has been detected in various surface waters in western Europe (Wegman and deKorte 1981), and in flooded rice field water (Deuel et al. 1977).

The toxicity of 3,4-DCA was studied here as part of a cooperative effort with the Environmental Research Laboratory-Duluth (U.S. EPA) and its quantitative structure-activity relationship (QSAR) testing program, in which approximately 600 organic chemicals have been tested for their acute toxicities to fish. Selected compounds from various chemical groups have also been tested for their subchronic (early life-stage) toxicities for the purpose of comparing acute and subchronic toxicities. 3,4-DCA is of particular interest due to its toxicity at low concentrations in extended exposures.

## MATERIALS AND METHODS

Three separate acute toxicity tests were conducted with fathead minnows (Pimephales promelas) ranging in age from 28 to 34 d. Mean fish weight was 0.113 ± 0.05g, 0.220 ± 0.075g, and 0.077 ±

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0.035g for the three tests. Continuous-flow diluter systems (Benoit et al. 1982) were used with a dilution factor of 0.65 between five successive exposures ranging in concentration from 2.6 to 15.7 $mg \cdot L^{-1}$. Each chamber contained 20 fish.

Dilution water was untreated Lake Superior water in two tests and dechlorinated water from the City of Superior, WI, municipal supply in the third test. The source of the municipal supply is a series of shallow wells beneath Lake Superior. Control tanks received dilution water only. Mean water temperatures in the three tests were 25.1 ± 1.1, 25.0 ± 1.4, and 24.5 ± 0.6° C. Mean values for pH, EDTA hardness and total alkalinity in Lake Superior water were 7.6, 43.5 $mg \cdot L^{-1}$ and 40.2 $mg \cdot L^{-1}$ as $CaCO_3$, respectively; and in dechlorinated city water were 7.2, 50.2 $mg \cdot L^{-1}$ and 41.6 $mg \cdot L^{-1}$ as $CaCO_3$, respectively. Dissolved oxygen levels as percent of saturation were maintained at 73.7 ± 3.9, 75.0 ± 2.1, and 83.6 ± 4.9% for the three tests, with overall ranges from 68.1 to 91.7%.

Early life-stage tests were conducted in flow-through diluter systems (Mount and Brungs 1967) with dilution factors of 0.5 and 0.6. The tests began with the introduction of fertilized eggs (<24h) into each incubation cup. Two incubation cups were used in each exposure, each containing 40 eggs (Test 1) or 67-78 eggs (Test 2). Egg cups, with Nytex® mesh screen bottoms, were suspended from a rocker arm assembly and moved slowly up and down in the exposure chamber to enhance water movement around the eggs. Upon hatching (4-5 d), 30 fry were removed from the incubation cups and placed into the exposure chambers for the duration of the exposure. Fry were fed newly hatched brine shrimp (Artemia sp.) 2-3 times daily in equal amounts to all chambers. Exposure chamber volumes were approximately 4.9 and 5.8 L in the first and second tests, respectively. A photoperiod of 16L:8D was used, with mean light intensities at the water surface of 28 ± 6 and 52.4 ± 20.6 ft-candles in first and second tests, respectively.

The first early life-stage test was conducted with Lake Superior water as dilution water, having the following measured characteristics: temperature, 26.0 ± 0.5 C; pH, 7.6 ± 0.0; EDTA hardness, 44.4 ± 1.1 $mg \cdot L^{-1}$ as $CaCO_3$; and total alkalinity, 43.0 ± 3.4 $mg \cdot L^{-1}$ as $CaCO_3$. Dissolved oxygen as percent saturation averaged 73.4 ± 7.6%, with a range from 51.8 to 83.6%. The second test used dechlorinated Superior municipal water as dilution water having the following characteristics: temperature, 24.9 ± 0.9 C; pH, 7.4 ± 0.2; EDTA hardness, 50.7 ± 1.1 $mg \cdot L^{-1}$ as $CaCO_3$; and total alkalinity, 41.7 ± 2.1 $mg \cdot L^{-1}$ as $CaCO_3$. The mean dissolved oxygen level was 78.2 ± 9.0%, with a range from 58.4 to 88.3%.

3,4-Dichloroaniline was supplied by Aldrich Chemical Co., Milwaukee, WI (98% purity). Concentrations were measured daily in all chambers in the acute tests and twice weekly in all chambers in the early life-stage tests. Water samples were collected near chamber mid-depth, the 3,4-DCA extracted with organic solvent (hexane, methylene chloride, or toluene), and the extracts

analyzed by gas-liquid chromatography with electron-capture detection. Mean recoveries of spiked water samples in each of the five tests ranged from 78.2 to 122%. Concentrations were corrected for recovery separately in each test. The mean percent agreement between samples collected in duplicate ranged from 93.5 to 99.6% for the five tests.

Mortalities were recorded at 24, 48 and 96 h in acute tests, and median lethal concentration ($LC_{50}$) was estimated by the trimmed Spearman-Karber method (Hamilton et al. 1977). Early life-stage test endpoints of fry length and weight were analyzed by one-way ANOVA and Dunnett's procedure (Steel and Torrie 1980). Data on percent hatch, percent dead or abnormal fry, and percent fry survival were arcsin transformed prior to analysis by ANOVA.

## RESULTS AND DISCUSSION

The acute toxicity of 3,4-DCA to fathead minnows (Table 1) is similar in the guppy (Poecilia reticulata), for which 96 h $LC_{50}$'s of 9.0 and 8.7 $mg \cdot L^{-1}$ were reported in tests conducted in freshwater (Adema and Vink 1981). A 14 d $LC_{50}$ of 6.3 $mg \cdot L^{-1}$ was also obtained with the guppy (Hermens et al. 1984). The molluscs, Lymnaea stagnalis and Dreissena polymorpha, were somewhat more tolerant of 3,4-DCA when tested at the life-stages of the first cleavage egg (48 h $LC_{50}$ >32 $mg \cdot L^{-1}$) or adult ($LC_{50}$ = 22 $mg \cdot L^{-1}$) (Adema and Vink 1981). They also reported a 48 h $LC_{50}$ of 12 $mg \cdot L^{-1}$ for adults of Daphnia magna and a 48 h $LC_{50}$ of 0.23 $mg \cdot L^{-1}$ for larval daphnids. $EC_{50}$'s (48 h) of 0.290 and 0.440 $mg \cdot L^{-1}$ were reported for neonates of Daphnia magna and Daphnia longispina, respectively (Crossland and Hillaby 1985).

Table 1. $LC_{50}$ and 95% confidence intervals for 3,4-dichloroaniline and fathead minnows (Pimephales promelas) at selected intervals, $mg \cdot L^{-1}$

| | 24 h | 48 h | 96 h |
|---|---|---|---|
| Test 1 | 9.03 (8.55-9.53) | 8.88 (8.36-9.43) | 6.99 (6.55-7.47) |
| Test 2 | 11.3 (9.92-12.8) | 10.0 (9.65-10.4) | 8.06 (7.26-8.95) |
| Test 3 | 12.0 (11.5-12.5) | 9.24 (8.43-10.1) | 7.70 (7.03-8.43) |

The first early life-stage test was repeated due to the unusually small size of the control fish (Table 2). No apparent reason was found for the small size of these fish. Due to the high mortality level (100% at 157 $\mu g \cdot L^{-1}$), and severe impact upon growth at 86.1 $\mu g \cdot L^{-1}$ and 45.1 $\mu g \cdot L^{-1}$, the highest exposure of Test 2 was selected to be near 45 $\mu g \cdot L^{-1}$.

Egg hatchability was not affected by any of the exposures in either early life-stage test. Newly hatched fry were alive and

Table 2. Hatchability, development, growth and survival of fathead minnows (Pimephales promelas) exposed to 3,4-dichloroaniline in two early life-stage tests

| Parameter | Mean 3,4-dichloroaniline concentration ± s.d. ($\mu g \cdot L^{-1}$) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Test 1 | 0.0 (±0.0) | - - | - - | 15.1 (±2.6) | 26.0 (±4.0) | 45.1 (±3.8) | 86.1 (±7.9) | 157 (±22.6) |
| Mean percent hatch[a/] | 83.8 | - - | - - | 90.0 | 88.8 | 88.7 | 95.0 | 90.6 |
| Mean percent dead or abnormal fry[b/] | 0.0 | - - | - - | 0.7 | 2.8 | 2.8 | 4.6 | 3.5 |
| Mean percent survival through 28-d[c/] | 91.6 | - - | - - | 73.4 | 43.3 | 5.0* | 5.0* | 0.0* |
| Mean standard length (mm) | 17.8 | - - | - - | 19.4[d/] | 15.7** | 8.7** | 9.3** | - - |
| Mean wet weight (g) | 0.096 | - - | - - | 0.132[e/] | 0.084 | 0.017* | 0.020* | - - |
| Mean dry weight (g) | 0.021 | - - | - - | 0.029[e/] | 0.017 | 0.002* | 0.002* | - - |
| Mean total biomass/chamber (g) | 2.722 | - - | - - | 2.866 | 1.091 | 0.026* | 0.030* | - - |
| Test 2 | 0.0 (±0.0) | 5.10 (±1.90) | 7.10 (±2.08) | 14.8 (±4.96) | 23.3 (±7.45) | 51.1 (±8.94) | - - | - - |
| Mean percent hatch[a/] | 89.3 | 94.2 | 92.3 | 89.0 | 76.8 | 85.8 | - - | - - |
| Mean percent dead or abnormal fry[b/] | 0.0 | 3.0 | 2.2 | 0.8 | 3.8 | 13.9* | - - | - - |
| Mean percent survival through 28-d[c/] | 100.0 | 100.0 | 95.0 | 100.0 | 33.4** | 3.4** | - - | - - |
| Mean standard length (mm) | 20.3 | 19.9 | 19.3** | 19.3** | 15.2** | 13.0** | - - | - - |
| Mean wet weight (g) | 0.150 | 0.145 | 0.130** | 0.131** | 0.071** | 0.054** | - - | - - |
| Mean dry weight (g) | 0.030 | 0.030 | 0.026** | 0.025** | 0.014** | 0.008** | - - | - - |
| Mean total biomass/chamber (g) | 4.486 | 4.352 | 3.714 | 3.920 | 0.706** | 0.054** | - - | - - |

a/ Live fry/total eggs.
b/ [Dead plus abnormal (deformed) fry]/total fry at time of transfer from egg cups 5 days after initial exposure of eggs.
c/ Based on 30 fry transferred to duplicate exposure chambers.
d/ Significantly longer than controls ($p<0.05$ from two-tailed Dunnett's test).
e/ Significantly heavier than controls ($p<0.01$ from two-tailed Dunnett's test).
*, ** Significantly different from controls at $p<0.05$ and $p<0.01$, respectively.

normal in appearance by gross examination at day 5 when fry were transferred from incubation cups to the exposure chambers. One exception was noted at the highest exposure in Test 2. However, exposures up to three times higher had no apparent effect in Test 1. A mean concentration of 23.3 $\mu g \cdot L^{-1}$ in Test 2 did not affect the survival or appearance of fry immediately after hatching, but did affect all other test endpoints. Fish survival was not affected at exposures of 14.8 $\mu g \cdot L^{-1}$ or lower. Several of the surviving fish at exposures of 23.3 and 51.1 $\mu g \cdot L^{-1}$ of Test 2 were observed to have swollen bodies with what appeared to be reddish zones of hemorrhaging along the visceral mass. A few fish had either an accumulation of reddish pigmented wastes or a zone of hemorrhaging in the vent area. These observations were also made in some of the fish exposed to 14.8 $\mu g \cdot L^{-1}$, but to a lesser extent than at higher exposures.

Length and weight were reduced at all Test 2 exposures above 7.10 $\mu g \cdot L^{-1}$, but were not affected at 5.10 $\mu g \cdot L^{-1}$. Mean total biomass per chamber, a parameter which integrates survival and weight effects, was reduced at the two highest exposures of Test 2. Based upon reduced growth in Test 2, the maximum acceptable toxicant concentration (MATC) for 3,4-DCA was found to lie between 5.10 and 7.10 $\mu g \cdot L^{-1}$. In Test 1, the MATC was based upon reduced length, and was somewhat higher (15.1-26.0 $\mu g \cdot L^{-1}$) due to the smaller size of the control fish.

Exposure of juvenile rainbow trout (Salmo gairdneri) weighing 4.5-5.9 g to a mean 3,4-DCA concentration of 39 $\mu g \cdot L^{-1}$ for 28 d did not cause mortalities or reduce wet weight, but did reduce length and relative growth rate (NO Crossland, unpublished manuscript). A concentration of 19 $\mu g \cdot L^{-1}$ did not result in significant effects upon growth.

The MATC for Daphnia magna was between 10 and 20 $\mu g \cdot L^{-1}$ based upon reduced reproduction (Crossland and Hillaby 1985). Adema and Vink (1981) reported an MATC of 5.6-10 $\mu g \cdot L^{-1}$ for Daphnia magna and an MATC of 3.2-10 $\mu g \cdot L^{-1}$ for the marine polychaete Ophryotrocha diadema, both based upon inhibited reproduction. They also reported a 7 mo $EC_{50}$ of 45 $\mu g \cdot L^{-1}$ based upon reproduction for the crustacean Chaetogammarus marinus.

Some organisms have exhibited more tolerance to 3,4-DCA. Adema and Vink (1981) observed no effect in a 16 d exposure of the freshwater mollusc Lymnaea stagnalis at 130 $\mu g \cdot L^{-1}$, or in a 4 wk exposure of the marine crustacean Artemia salina at 32 $\mu g \cdot L^{-1}$. The 4 wk reproduction $EC_{50}$ for Artemia was 100 $\mu g \cdot L^{-1}$. No effect upon mortality, growth or malformations occurred in a 3 mo exposure of the fish, Pleuronectes platessa, to 32 $\mu g \cdot L^{-1}$, while the 3 mo $LC_{50}$ was 180 $\mu g \cdot L^{-1}$ (Adema and Vink 1981).

An acute-chronic ratio (ACR) greater than 1,200 was obtained in this study when the geometric mean of the MATC for Test 2 was taken as a chronic point estimate. Kenaga (1982) reviewed the

literature on acute and chronic toxicities to aquatic organisms, and only 2 of 135 chemicals had ACR's which exceeded 1,000. One of these was the herbicide propanil, with an ACR of 18,100. Technical grade propanil was used in the study reported (Call et al. 1983b), and caused symptoms in the fish (e.g. swollen bodies and apparent abdominal hemorrhaging) very similar to those observed with 3,4-DCA. It is possible that some of the observed toxicity of propanil may have been due to 3,4-DCA or to measured trace amounts of 3,3',4,4'-tetrachloroazobenzene present as a contaminant of the herbicide. It is also possible that 3,4-DCA may have contributed to the relatively high ACR of 435 for the herbicide diuron (Call et al. 1983a), either as an impurity in the herbicide or as a metabolite.

An ACR as large as 1,200 for an industrial organic chemical is unusual. The majority of industrial organics tested to date have been found to have a nonspecific anesthetic effect upon aquatic animals, with ACR's averaging about 10-12 (Call et al. 1985; Kenaga 1982). Therefore, 3,4-DCA must operate by a different and more toxic mode of action. 3,4-DCA may result in irreversible cell damage which causes delayed chronic effects (Kenaga 1982). It would be of interest to see if other halogenated anilines are also highly toxic in extended exposures.

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