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Responses of Non-Target Aquatic Organisms to Aqueous Propanil Exposure

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Propanil (3',4'-dichloropropionanilide) is one of the world's most widely used rice herbicides, and it is extensively used in Arkansas, the leading rice producer in the United States (Webster and Gunnel1 1992). On average, the United States has applied approximately five kg/ha/year to about 70-100% of rice hectareacreage for the past two decades (US EPA 1987; Schlenk and Moore 1993). Arkansas, in 1992 alone, applied over 2.7 million kg of propanil (Jackman 1994). It is important to understand the toxicity of such herbicides to non-target aquatic organisms because of the amounts of pesticide field application and the risk of mixture with water exiting fields. During agricultural applications, aerial drift or accidental spills may expose nearby non-target areas such as ponds, rivers, lakes, wetlands, etc. to herbicides. Predictions of possible impacts upon the diverse range of species found in these ecosystems are usually drawn from a somewhat limited number of toxicity tests with standardized organisms. Comparative toxicity tests should use species of different feeding preferences, habitats, physiology, and size to determine a toxicant's effects (Rodgers *et al.* 1997). The relative sensitivities of five freshwater aquatic test species to propanil were determined in aqueous laboratory exposures to provide a wider range of response data inclusive of amphibians, insects, and crustacea. Test species utilized in this study were a cladoceran (*Ceriodaphnia dubia*), an epibenthic amphipod (*Hyalella azteca*), a larval midge (*Chironomus tentans*), the fathead minnow (*Pimephnles promelas*), and an amphibian (*Xenopus laevis*). Data generated from such comparative toxicity experiments may be used for future assessments of potential effects on non-target organisms following accidental (or intentional) exposures. Comparative slopes that are specific for each test organism can also offer resolution of risks associated with the recent movement toward using more concentrated pesticide products.

MATERIALS AND METHODS

Test organisms were cultured at the Arkansas State University Ecotoxicology Research Facility. Static 48-h acute toxicity experimental exposures were conducted with the organisms (US EPA 1993). *C. dubia* used for testing were < 24 hr old, while *H. azteca* were 7 -10 d old. Third instar (14 d) *C. tentans* were

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tested in addition to 7 and 6 d old *P. promelas* and *X. laevis*, respectively. Toxicity experiments were conducted at 25 ± 1°C under a 16:8 h photoperiod. Five *C. dubia* and five *H. azteca* were exposed in each of four replicate test chambers per propanil concentration. Ten *P. promelas* were placed in each of two replicate test chambers, while ten *C. tentans* and ten *X. laevis* were placed in each of four replicate test chambers. Test chambers were 250-ml wide-mouth borosilicate glass beakers. Organisms were not fed during experimental exposures. Laboratory synthetic water was used as both control and dilution water. Water chemistry parameters measured included dissolved oxygen, pH, temperature, conductivity, alkalinity, and hardness (APHA 1992) (Table 1).

Table 1. Range of laboratory synthetic water characteristics used in propanil toxicity experiments.

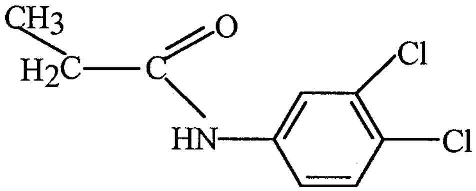
Parameter	Unit	Synthetic water
Dissolved oxygen	mg/L	≥ 6.0
PH	s.u.	7.2-8.2
Temperature	°C	24-26
Conductivity	μmhos / cm	319-343
Alkalinity	mg/L as $CaCO_3$	60-64
Hardness	mg/L as $CaCO_3$	90-100

Propanil stock concentrations were prepared by dissolving known quantities of Stam M-4™ (Rohm and Haas Company, Philadelphia, PA) (43.5% active ingredient propanil) (Table 2) into one liter of Milli-Q™ water. Aqueous exposure concentrations ranging from 0.1 - 100 mg/L (expressed as nominal concentrations) were prepared by dissolving quantities of the stock solution into laboratory synthetic water. A previous study by Moore and Farris (1997) indicated that mean percent recovery of propanil in aqueous exposures was 32%.

Organism survival data and exposure concentrations were used to calculate exposure-response curves, slopes, LC50s, as well as upper and lower thresholds. Upper threshold was defined as the concentration exhibiting a saturation of response (100% mortality). Lower threshold was defined as that concentration where approximately 110-20% mortality was observed. If controls exhibited any substantial or unacceptable mortality (>10%), that specific experiment was ruled invalid. Point estimates (LC_{50}) were calculated using either Probit or Trimmed Spearman-Karber analyses (Hamilton *et. al* 1977).

Table 2. Physical properties of propanil.

Structure[1]



Water solubility[1]	225 mg/L
Molecular weight[1]	218.08 g
Specific gravity[1,2]	1.25 g/cm^3
Melting point[2,3]	92-93°C;91-93°C
Vapor pressure[4]	9 x 10^{-5} mmHg
K_{oc}[5]	188
Persistence in soil ($T_{1/2}$)[5,6]	1-3 days

[1]EXTOXNET 1993
[2]CHEMFINDER 1997
[3]Windholz 1976
[4]Worthing 1987
[5]USDA 1990
[6]WSSA 1989

RESULTS AND DISCUSSION

The microcrustacean *C. dubia* was the most sensitive organism tested, with a 48-hr LC_{50} of 1.65 mg/L propanil (Table 3). Rohm and Haas (1991) reported a *Daphnia magna* 48-hr LC_{50} of 0.14 mg/L using technical-grade propanil. *H. azteca,* an epibenthic invertebrate, was nearly four times less sensitive than the *C. dubia,* with a 48-hr LC_{50} of 6.58 mg/L propanil. The vertebrates *X. laevis* and *P. promelas* demonstrated similar responses to aqueous exposures of propanil, with 48-hr LC_{50}s of 8.17 and 8.64 mg/L, respectively. Call *et al.* (1983) reported a 48-hr LC_{50} for *P. promelas* and propanil of 10.2 mg/L, which corresponded well with results from this study. 96-hr LC_{50}s for other fish species (*Oncorhynchus mykiss, Lepomis machrochirus,* and *Ictalurus punctatus*) exposed to propanil ranged from 2.3 - 6 mg/L (Rohm and Haas 1991; Meister 1992; and Schlenk and Moore 1993). The 48-hr LC_{50} for *C. tentans,* a benthic invertebrate, was 17.09 mg/L propanil, approximately two times higher than those for both *X. laevis* and *P. promelas* and therefore represented the least sensitive organism tested.

Table 3. Propanil 48-h LC50 (95% C.I.) values, confidence intervals and exposure-response slopes for five aquatic test organisms (n=3).

Test Species	LC50 (C.I.) Threshold (mg/L)	Slope (% mortality / (mg/L)	Upper Threshold mg/L)	Lower (mg/L)
Ceriodaphnia dubia	1. 52 (1.23, 1.88)	60	1.50	4.35
C. dubia	1.69 (1.49, 1.92)			
C. dubia	1.73 (1.18, 2.53)			
Hyalella azteca	5.64 (3.96, 8.03)	17	5.00	
			13.05	
H. azteca	7.03 (5.67, 7.85)			
H. azteca	7.07 (6.40, 7.80)			
Xenopus laevis	8.13 (7.61, 8.70)	23	8.05	8.70
X. laevis	8.15 (8.09, 8.21)			
X. laevis	8.23 (8.19, 8.28)			
Pimephales promelas	8.27 (7.24, 9.07)	7	6.50	13.92
P. promelas	8.76 (7.43, 10.30)			
P. promelas	8.90 (7.80, 9.77)			
Chironomus tentans	15.50 (13.9, 17.2)	5	12.5	43.5
C. tentans	16.20 (14.7, 18.0)			
C. tentans	19.60 (17.2, 22.4)			

Moore *et al.* (1998) discussed the utility of reporting both point estimates (LC_{50}s) and exposure-response slopes (potency estimations). By reporting these slopes, predictions of potential organism effects could be made over a greater range of possible concentrations. As evidenced in this study, the amphibian *X. laevis* was of intermediate sensitivity among organisms tested; however, any incremental increase of less than 1.0 mg/L above the lower threshold concentration (approximately 8.05 mg/L propanil) will result in the upper threshold concentration (100% mortality) (Table 3). These data would have been masked if only point estimates had been reported.

Lower threshold responses of organisms to propanil followed a pattern similar to the potency relationships. Lower thresholds for *C.dubia, H. azteca, P. promelas, X. laevis,* and *C. tentans* were 1.5, 5.0, 6.5, 8.05, and 12.5 mg/L propanil, respectively. Upper thresholds of response (100% mortality) observed for *C. dubia, X. laevis, H. azteca, P. promelas,* and *C. tentans* were 4.35, 8.7, 13.05, 13.92, and 43.5 mg/L propanil, respectively.

This research focuses on the importance of validation data for 1) extrapolation of laboratory toxicity data to different aquatic systems (Moore *et al.* 1998), 2) providing information for risk assessments for more concentrated pesticide formulations, and 3) providing evidence to protect organisms not included in earlier pesticide databases (i.e. amphibians, epibenthic crustaceans, etc.). By continuing to examine not only toxicity point estimates, but also exposure-response slopes, researchers can better predict potential effects on a wide range of non-target organisms. Such information could be beneficial to risk assessors, pesticide manufacturers, or any other interested persons, since it would allow a more informative decision concerning potential effects on aquatic biota to be made.

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