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Acute and Chronic Toxicity of the Herbicide Stam®M-4 in Field and Laboratory Exposures

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Abstract. Propanil (3′,4′-dichloropropionanilide), the active ingredient in the herbicide Stam®M-4, is possibly the most extensively used herbicide for rice production in the world. Propanil and its metabolites are transported within characteristic ditch ecosystems in the production landscape of northeast Arkansas. Runoff from these ditch ecosystems is further transported to a river or other water body supplied by the Mississippi River Alluvial aquifer. Forty-eight–hour acute toxicity tests with *Ceriodaphnia dubia* (cladoceran) and *Pimphales promelas* (fathead minnow) were conducted on stormwater runoff, laboratory synthetic water, and irrigation (ground) water. No effects on survival were observed in this study following 48-h toxicity testing with the stormwater. Survival studies indicated assimilative capacity in irrigation (ground) water as opposed to laboratory synthetic water. Mean 48-h LC_{50}s of *C. dubia* increased from 2.94 mg/L Stam®M-4 in laboratory synthetic water to 8.01 mg/L Stam®M-4 in irrigation water. Likewise, *P. promelas* mean 48-h LC_{50}s increased from 23.76 (laboratory synthetic water) to 33.52 mg/L Stam®M-4 (ground water). In 7-d chronic tests, there was an increase in mean LC50s of *C. dubia* when comparing synthetic water to irrigation water (0.48 to 1.24 mg/L Stam®M-4, respectively). *P. promelas*, however, had less tolerance for Stam®M-4 in irrigation water (4.45 mg/L) than in synthetic water (5.93 mg/L) in 7-d chronic toxicity tests. Forty-eight–hour toxicity tests indicate that ground water affords organisms some assimilative capacity that laboratory synthetic water does not. Since herbicides and most other pesticides are manufactured to elicit rapid responses, 48-h toxicity results best describe potential nontarget organism effects in aquatic ecosystems.

Stam®M-4 (active ingredient propanil) targets barnyard grass (*Echinocloa crusgalli*) and broadleaf weeds and is used in the postemergent treatment of rice (*Oryza sativa*). In 1992, the average propanil application rate in Arkansas was 7.85 L per ha, at a cost of $53.38 per ha, on an estimated 87% of rice acreage (Spradley 1992). Jackman (1994) determined that over two million kilograms of propanil were applied in Arkansas alone in 1992. Due to the massive volume of propanil applied in Arkansas, the leading rice producing state in the United States and Delta region, site-specific studies upon biological responses are needed to evaluate the effects of the chemical upon ecosystem services.

Propanil initially degrades into DCA (3,4-dichloroaniline) and propionic acid (Bartha and Pramer 1967; Chisaka and Kearney 1970). Studies have shown continued degradation into TCAB (3,3′,4,4′-tetrachloroazobenzene) and TCAOB (3,3′,4,4′-tetrachloroazoxybenzene) (Bartha and Pramer 1967; Hill *et al.* 1981; Di Muccio *et al.* 1984). Since propanil has a field half-life of only one day, many researchers have not concentrated on the parent compound. Instead they focused their attention on the metabolites DCA, TCAB, and TCAOB. There is great concern regarding TCAB and TCAOB because of embryolethal, teratogenic, and possible genotoxic effects shown in toxicity tests (Pothuluri *et al.* 1991).

The purpose of this study was to compare toxicity techniques and results involving lentic mesocosm testing as part of the chemical registration process with field validation of a broadleaf herbicide, Stam®M-4 utilizing irrigation water in actual application. While mesocosm tests can closely represent actual field conditions, they do not adequately demonstrate the purpose or usefulness of surrounding drainage ditches. This study incorporated research upon a trade chemical for weed control in rice, instead of only active ingredients. Additionally, this study addressed the appropriateness of regulatory tier testing and specific organisms used in actual field conditions and related responses to those of other indicator organisms used in protecting Arkansas' water resources through biological criteria. Mesocosm studies have depended on responses from larger bluegill fish, while more sensitive early life stage responses from fathead minnows and cladocera (as used in this study) are used in assessing most biological criteria. Toxicity testing was used to determine if Stam®M-4 caused damage to organisms located within or surrounding the agriecosystem. After ambient water analysis, laboratory observations aided in determining what concentration of Stam®M-4 would establish a first level of effect in standardized test organisms. An evaluation of ecological benefits that currently exist in the rice production

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field is offered to provide evidence of management practices that are known to reduce nonpoint pollution.

Materials and Methods

Field studies were conducted on a 36-ha rice farm (T & J Farms, Inc., Corning, Arkansas) approximately 2 km south of the Arkansas–Missouri border (T21;R3E;S28) within the Delta. Rice and soybeans have been the primary production crops on this specific field (Amagon silt-loam soil), which has been in agricultural production since 1954. For this study, Stam®M-4 (43.5% propanil, Rohm & Haas, Co., Philadelphia, PA, USA) was the only chemical applied to the field throughout the growing season. The study field was aerially treated with 1.54 L/ha Stam®M-4 on 13 May 1995. Less than ten hours later, ambient water samples were collected from surrounding drainage ditches, since field water was not available. Two ambient water samples were taken within the field on 04 June 1995. Following significant rainfall (at least five centimeters) on 13 June, 22 June, 25 June, and 8 July 1995, stormwater runoff exiting the field was collected in addition to water from the surrounding ditches. Ambient water samples were also collected from within the field during both the late season and field drainage periods of rice production. All samples were transferred to the Arkansas State University Ecotoxicology Research Facility (ASU ERF) within 1 h of collection. Upon arrival, samples were prepared for immediate testing.

Acute and Chronic Toxicity Tests

All ambient water samples were subjected to acute, static 48-h toxicity tests using cladocera and *P. promelas* (US EPA 1993). Upon receiving samples at the ASU ERF, ambient water was filtered using Nitex® mesh to exclude any collected macroorganisms. Following laboratory screening tests, acute, static 48-h definitive tests were conducted using the same organisms. Concentrations of Stam®M-4 (0.1–100 mg/L) were mixed with both site irrigation (ground) water and laboratory synthetic (moderately hard) water immediately before addition of test organisms. Following the series of acute toxicity tests, 7-d chronic toxicity tests were conducted upon the same organisms with similar concentrations of the spiked synthetic and site waters following US EPA (1989) protocol. Both acute and chronic LC_{50} values were obtained for Stam®M-4 spiked site and synthetic water.

Analytical Methodology

Stam®M-4 spiked samples of both synthetic and site water were analyzed using a Dionex® high-pressure liquid chromatograph. Separations used a Zorbax®SB-C8 bonded phase along with an unacidified acetonitrile/acidified water mobile phase. Water was acidified to pH 2 with HCl. The Zorbax®SB-C8 column (4.6 × 150 mm, 5 µm) was used at a pump pressure of 300 psi, and samples were measured at a wavelength of 254 nm. The calibration curve for the standard solutions had a r^2 value of 0.9806. Replicated samples were measured for the presence of both propanil (active ingredient of Stam®M-4) and 3,4-dichloroaniline (a metabolite of propanil). Individual samples of 10 µl were injected into the Zorbax®SB-C8 column with a gradient time of 15 min. Analytical standards were 99% pure (ChemService, West Chester, PA, USA).

Statistical Analysis

Nominal concentrations of Stam®M-4 spiked waters and mortality data were used to calculate the 48-h and 7-d LC_{50} by US EPA Probit© analysis or trimmed Spearman-Karber, utilizing a personal computer.

Results and Discussion

Acute Toxicity

Ambient water samples taken from standing ditch and river water on 13 May 1995 showed little effect upon the test organisms (cladocera and fathead minnows) following 48-h acute testing (Table 1). Likewise, ambient water samples collected from actual rice field sites (Figure 1) throughout the growing season yielded similar results. Collected stormwater samples from four separate rain events failed to demonstrate any effects upon the test organisms. Following a series of screening tests, acute 48-h LC_{50} comparisons were conducted between laboratory synthetic water (moderately hard) and site irrigation water, both spiked with Stam®M-4. Both test organisms, *C. dubia* and *P. promelas,* demonstrated an increased mean LC_{50} in site water as opposed to synthetic water (Table 2).

Chronic Toxicity

As with laboratory acute testing with spiked waters, *C. dubia* exhibited an increased chronic mean LC_{50} value in site water as compared to synthetic water (Table 3). Conversely, the *P. promelas* chronic mean LC50 value actually increased in synthetic water (5.93 mg/L Stam®M-4) as opposed to site water (4.45 mg/L Stam®M-4).

Analytical Chemistry

High-Pressure Liquid Chromatography (HPLC) was used to determine specific concentrations of both propanil and 3,4 DCA within Stam®M-4 spiked samples of both synthetic and site water (Table 4). Average recovery of propanil was 73%. With the exception of the targeted concentration of 1.000 mg/L Stam®M-4, propanil concentrations increased with an increase in targeted Stam®M-4 concentration.

Results from this study indicate that field stormwater runoff and discharged water associated with rice irrigation produced no acute toxicity to exposed organisms. Furthermore, toxicity comparisons using site and synthetic water revealed that site water was a functional component of the agriecosystem's assimilative capacity. After completing HPLC analysis, 48-h LC_{50} values for *P. promelas* expressed as measured technical grade propanil were 8.99 mg/L. These results were comparable to those published for *P. promelas* by Call *et al.* (1983). Because Stam®M-4 is the actual chemical applied to fields by farmers, it was necessary to report its specific effects in ambient water as well as laboratory waters.

Few studies have been published concerning collection of ambient ditch and field water for bioassay evaluation. Only three studies dealing with rice ecosystem and runoff have been published (Nakamura 1982; Watanabe *et al.* 1984, 1985). None of these studies dealt with the herbicide Stam®M-4. Similarly, no known studies have been published regarding the effects of drainage ditches upon the reduction of herbicide toxicity. The majority of studies dealing with propanil (active ingredient of Stam®M-4) have been conducted in laboratory microcosms with water (Isensee *et al.* 1981; Call *et al.* 1983) or in only soil

Table 1. 48-h acute survival of standard test organisms to selected field water samples and stormwater ($n = 2$ cladoceran; $n = 4$ fish)

Date	Event	Sample Site	Organism	Percent Survival
051395	Stam®M-4 application	1	*Daphnia pulex*	90
			P. promelas	95
		2	*D. pulex*	100
			P. promelas	85
060495	Early growing season	1	*D. pulex*	100
			P. promelas	100
		3	*D. pulex*	100
			P. promelas	100
061395	First storm event	4	*C. dubia*	100
			P. promelas	100
		5	*C. dubia*	100
			P. promelas	100
062295	Second storm event	4	*C. dubia*	100
			P. promelas	95
		5	*C. dubia*	100
			P. promelas	100
062595	Third storm event	1	*C. dubia*	100
			P. promelas	95
		2	*C. dubia*	100
			P. promelas	90
		4	*C. dubia*	100
			P. promelas	95
		6	*C. dubia*	100
			P. promelas	100
070995	Fourth storm event	2	*C. dubia*	100
			P. promelas	95
		4	*C. dubia*	100
			P. promelas	95
		5	*C. dubia*	100
			P. promelas	100
072495	Mid-growing season	3	*P. promelas*	100
		7	*P. promelas*	100
		8	*P. promelas*	100
072495	Mid-growing season	9	*P. promelas*	100
082195	Field drainage	3	*C. dubia*	100
			P. promelas	100
		7	*C. dubia*	100
			P. promelas	100
		8	*C. dubia*	100
			P. promelas	100
		9	*C. dubia*	100
			P. promelas	100

Survival of cladoceran and fathead minnows were determined from 20 exposed organisms

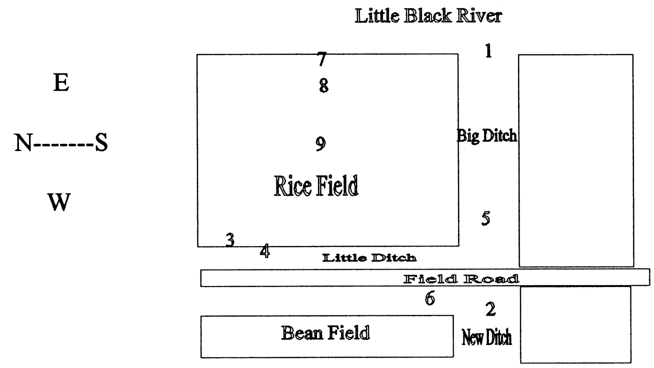

Fig. 1. Field study plot and sampling sites, Clay County, Arkansas, 1995

Table 2. 48-h acute LC_{50} values for standard test organisms in moderately hard synthetic and groundwater mixed with Stam®M-4 ($\alpha = 0.05$) ($n = 2$)

			95% Confidence Intervals	
Organism	Water	LC_{50} mg/L	Upper Limit	Lower Limit
P. promelas	MH synthetic	20.13	23.74	17.07
		27.38	33.23	22.57
	$\bar{x}$	23.76		
	Groundwater	33.23	36.58	30.19
		33.81	38.70	29.54
	$\bar{x}$	33.52		
C. dubia	MH synthetic	3.98	5.81	2.72
		1.89	2.45	1.46
	$\bar{x}$	2.94		
	Groundwater	5.71	6.57	4.95
		10.31	14.11	8.82
	$\bar{x}$	8.01		

(Bartha *et al.* 1967; Kearney *et al.* 1969; Chisaka and Kearney 1970).

Comparisons between site and synthetic water acute *C. dubia* mean LC_{50} values from Stam®M-4 bioassays revealed that tests using site water produced twice the LC_{50} values of synthetic water. Similarly, *P. promelas* acute mean LC_{50} values increased nearly 10 mg/L between synthetic and site water. Simply stated, organisms in the site ground water could withstand a greater amount of Stam®M-4 than could organisms in synthetic water. According to Cairns (1977), assimilative capacity is the ability of an ecosystem to cope with certain levels of discharged wastes without suffering significant deleterious biological effects. This research has shown, through replicated testing procedures, that organisms in natural site water are able to tolerate conditions associated with greater amounts of the introduced herbicide than can organisms in laboratory synthetic water. The state of the natural water reduced the bioavailability of the toxicant, something that could not be reproduced in the synthetic water. Assimilative capacity functioning within the agriecosystem has been supported as the reason for these test observations.

Chronic impairment tests were also conducted upon *P. promelas* and *C. dubia*, but with slightly different results. Differences between mean LC_{50} values for both *P. promelas* and *C. dubia* were much less than in observed acute tests. In the chronic *P. promelas* tests, the mean 7-d LC_{50} value Stam®M-4 in synthetic water (5.93 mg/L) was slightly greater than in tests using site water (4.45 mg/L Stam®M-4). Mean 7-d LC_{50} values for *C. dubia* were slightly greater in site water (1.23 mg/L Stam®M-4) rather than synthetic water (0.48 mg/L Stam®M-4).

It is pertinent to mention that farmers are stewards of the ecosystem services that we all enjoy—provision of food, breathable air, and potable water (Cairns and Niederlehner 1994). Often, political boundaries overlap into the field of agriculture to serve as a guardian for human health interests. Farmers are recognizing the need to take the initiative to provide scientific information as to site-specific effects of agriculture, as evidenced in the cooperation with this study. Studies that reveal the ecological resources at work to degrade,

Table 3. 7-d chronic LC_{50} values for site (groundwater) and synthetic water (moderately hard) mixed with Stam®M-4 ($\alpha = 0.05$) ($n = 2$)

Organism	Water	LC_{50} mg/L	95% Confidence Intervals Upper Limit	Lower Limit
P. promelas	MH synthetic	8.49	9.38	7.68
		3.37	4.26	2.66
	$\bar{x}$	5.93		
	Groundwater	7.69	8.92	6.63
		1.22	—	—
	$\bar{x}$	4.45		
C. dubia	MH synthetic	0.37	0.66	0.10
		0.59	0.94	0.37
	$\bar{x}$	0.48		
	Groundwater	1.92	2.58	1.42
		0.55	0.77	0.39
	$\bar{x}$	1.24		

Table 4. Measured 3,4 DCA and propanil levels from Stam®M-4 spiked synthetic and site waters ($n = 3$)

Stam®M-4 Nominal Concentrations mg/L	Measured Synthetic Water 3,4 DCA mg/L	Propanil mg/L	Site Water 3,4 DCA mg/L	Propanil mg/L
Control	<0.086	0.029	<0.086	0.012
0.100	0.020	0.022	1.116	1.018
1.000	0.128	0.132	0.394	19.546
4.000	0.634	0.490	0.537	1.412
7.000	0.146	2.573	1.551	2.065
10.000	0.168	4.338	0.133	4.510
25.000	0.199	8.993	0.056	11.528
50.000	<0.086	20.189	0.189	21.591
75.000	0.410	31.063	0.129	39.591
100.000	0.280	40.241	0.167	55.268

transform, and recycle metabolites and nutrients provide a clearer vision of tangible returns from management practices.

The various toxicity values from site testing related the importance of comparing techniques used for regulatory purposes to feasible application in the agriculture receiving system. These tests allow a better estimate of the predictive chemical effects upon the surrounding environment, since each site has specific characteristics (clay content, drainage ditches, etc.) that may enhance the assimilation of chemical applications.

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