

Toxicity of a Mixture of 2,4-Dichlorophenoxyacetic Acid and Monosodium Methanearsonate to the Red Swamp Crawfish, *Procambarus clarkii*

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Abstract: 2,4-dichlorophenoxyacetic acid and monosodium methanearsonate are often sold in commercial herbicide mixtures. Toxicity studies have been performed for each herbicide individually, but there is a dearth of information concerning the toxicity of these herbicides in a mixture. The following study examined the toxicity of a mixture of these two herbicides in the red swamp crawfish, *Procambarus clarkii*. 96-hour acute toxicity assays were performed to determine whether surfactant significantly altered the toxicity of these herbicides individually or in combination. Marking's additive index was calculated to identify the interactions of the herbicide mixture. Surfactant was observed to significantly increase the toxicity of 2,4-dichlorophenoxyacetic acid and the toxicity of the herbicide mixture. The herbicide mixture alone displayed half the toxicity of the individual herbicides, but the mixture with surfactant was twice as toxic as the individual herbicides. The synergistic action of surfactant may be attributed to increased pesticide absorption across biological membranes. 2,4-dichlorophenoxyacetic acid and surfactant may also compromise gill function, increasing the sensitivity of the crawfish to herbicide toxicity. The antagonistic effects of the herbicide mixture in the absence of surfactant may be caused by competition of both herbicides for the same sites of activity.

Key words: crawfish, 2,4-dichlorophenoxyacetic acid, monosodium methanearsonate, herbicide mixture, acute toxicity, Marking's Additive Index

Introduction

When precipitation washes herbicides from soil and foliage surfaces into waterways in close proximity to sprayed areas, nontarget organisms may be directly affected through exposure or indirectly affected through consumption of exposed organisms. One possible nontarget organism that may be affected is the red swamp crawfish *Procambarus clarkii*. Crawfish are an important human food source in Louisiana as well as an important part of the food chain for many native animals.

Two herbicides of particular local interest are 2,4-dichlorophenoxyacetic acid (2,4-D) and monosodium methanearsonate (MSMA). MSMA is widely used to

clear weeds from non-cropland areas such as public rights-of-way; it is also used to control weed growth in sugar cane, a major money crop in Louisiana. 2,4-D is also applied to keep public rights-of-way clear, as well as being used to treat approximately 60% of sugarcane acreage in Louisiana to prevent severe losses to weed invasion during crop establishment [1].

Because of the rapid microbial decomposition 2,4-D undergoes when applied to soil rather than sprayed directly onto foliage, it may be paired with another herbicide such as MSMA to control germinating annuals [2]. This well illustrates the field practice of using multiple herbicides to increase cost effectiveness and effectiveness to target vegetation while supposedly

reducing negative impacts on nontarget species and the environment in general [3]. But the use of herbicide mixtures can also potentially introduce new elements of toxicity to nontarget organisms. Mixed herbicides may combine to form another potentially harmful compound. Alternatively, the toxic effects produced by each individual herbicide may be enhanced by the herbicides in combination. Even when not directly applied in a mixture, herbicides may undergo subsequent mixing due to environmental factors.

Data are available regarding the effects of 2,4-D and MSMA individually on red swamp crawfish, but there is little information available regarding the combined effects of these two herbicides on this species. The following study examined the potential changes in toxicity, uptake, and excretion of a mixture of 2,4-dichlorophenoxyacetic acid (2,4-D) and monosodium methanearsonate (MSMA) in the red swamp crawfish.

Materials and Methods

Red swamp crawfish were obtained from the KJEAN seafood company of New Orleans, Louisiana. Crawfish were fed oatmeal three times per week and acclimated in holding tanks for 1 - 2 weeks before being used in bioassays.

The relatively high solubilities of 2,4-D and MSMA made possible the use of the static with renewal method of acute toxicity testing, in which test crawfish were moved to fresh dechlorinated water containing the same concentration of herbicide as the original tank after 24 hours to minimize waste buildup, oxygen depletion, and degradation of the herbicides.

The acute toxicity bioassays were performed using 2,4-D dimethylamine salt (active ingredient 38.8% 2,4-D) and MSMA (active ingredient 46.33% As). Each herbicide was tested with and without added surfactant to determine whether their toxicity was significantly altered by the presence of surfactant. Acute toxicity tests were then performed on mixtures of equal concentrations of 2,4-D and MSMA, with and without surfactant. In accordance with the manufacturer's instructions for the normal use of Syndets (Non-Ionic) Surfactant, polyethylene glycol alkyl ether, the surfactant was added to each herbicide/surfactant bioassay tank at 0.5% the volume of the herbicide dose.

For each 96-hour test, eight to ten randomly selected crawfish were placed into a series of five 12-liter glass tanks filled with dechlorinated water, into which the test substance(s) was added. Crawfish were also added to a control tank of dechlorinated water. Following each 24-hour period, crawfish mortality in each tank was recorded and water samples were collected to confirm that the dose levels for each tank remained constant. Laboratory lighting was programmed for a 12-hour photoperiod (8 AM to 8 PM).

A range-finding process was initially performed to determine what concentrations should be targeted for

toxicity testing. The range of concentrations tested was narrowed with each assay until the concentration of herbicide(s) at which 50% of the test subjects died after 96 hours (96-hour LC₅₀) was identified. This bioassay was repeated to confirm the statistical precision of the experimental data.

To measure actual 2,4-D concentrations, water samples were analyzed with an Agilent 1100 Series HPLC (high pressure liquid chromatography) system with G1312A binary pump, G1322A vacuum degasser, G1316A thermostated column compartment, G1313A autosampler, G1315B diode array detector, and Agilent ChemStation software. An LC-18 column obtained from Supelco scientific products was used for the analysis, with a mobile-phase composition of 40% 25mM KH₂PO₄ acidified to pH 2 and 60% acetonitrile.

To measure actual MSMA concentrations as total arsenic, water samples were digested in a CEM MDS-2000 microwave to reduce interference from organics and to convert the arsenic to a form that could be analyzed by ICP. Nine milliliters of sample and one milliliter of concentrated HNO₃ were initially heated to 160 ± 4°C or 70 psi in 10 minutes. The temperature of the samples was then raised to 165 -170°C (or 85 psi) for 10 minutes. Analysis of samples for arsenic concentration was performed using an Agilent Technologies 7500 series inductively coupled plasma mass spectrometer (ICP-MS) with Plasma chromatographic software. Since the molecular weight of arsenic (74.9216) is 46.33 % that of MSMA (161.7), the concentrations of arsenic obtained through ICP analysis was considered to be 46.33% of the actual concentration of MSMA. To correct for the dilution during microwave digestion, ICP results for water samples were multiplied by 1.11.

All acute toxicity data was processed using the EPA Probit Analysis program version 1.3 [4], which used the probit method to transform observed crawfish mortalities to the 96-hour LC₅₀ for each assay. Probit analysis results were compared using the Student-t test in the following pairs to determine whether observed differences in mortalities were statistically significant:

- 2,4-D versus 2,4-D with surfactant.
- MSMA versus MSMA with surfactant.
- 2,4-D/MSMA versus 2,4-/MSMA/surfactant.

To determine whether the effects of the two herbicides in a 50:50 mixture were additive, synergistic, or antagonistic, the following equation for Marking's additive index was used [5]:

$$\frac{Am}{Ai} + \frac{Bm}{Bi} = S$$

where, *A* and *B* were the individual herbicides, *i* and *m* were the individual and mixture LC₅₀s, respectively, and *S* was the sum of activity.

Marking places an additive index value of 0 at $S = 1$, a sum of activity at which the mixture components would be equitoxic (a demonstration of additive toxicity).

If $S \leq 1.0$, the additive index = $\frac{1}{S} - 1.0$ (synergistic toxicity).

If $S \geq 1.0$, the additive index = $S(-1) + 1$ (antagonistic toxicity).

The magnification factors for mixture toxicity were referenced from Marking's chart of index values and corresponding magnification factors.

Results

Results of the acute toxicity bioassays are summarized in Table 1. Acute toxicity bioassays for 2,4-D yielded an average 96-hour LC_{50} of 185 mg/L (95% confidence limits of 75 mg/L, 343 mg/L). This was observed to be much lower than the expected 96-hour LC_{50} of 750 mg/L \pm 6.4 mg/L [3]. Acute toxicity bioassays for 2,4-D / surfactant yielded an average 96-hour LC_{50} of 108 mg/L (95% confidence limits of 34 mg/L, 203 mg/L). Statistical comparison of these two bioassays confirmed that surfactant has a significant effect on the acute toxicity of 2, 4-D ($p=0.022$).

The 96-hour LC_{50} s for the MSMA bioassays were as follows:

1. 1,025 mg/L (no confidence limits were calculated by the probit software)
2. 1,100 mg/L (used from Anderson et al. [6] due to a shortage of crawfish).

This yielded an approximate average 96-hour LC_{50} of 1063 mg/L. Acute toxicity bioassays for MSMA / surfactant yielded an average 96-hour LC_{50} of 1099 mg/L (95% confidence limits of 639 mg/L, 1510 mg/L). Statistical comparison of these two bioassays confirmed that surfactant does not have a significant effect on the acute toxicity of MSMA ($p = 1$).

Acute toxicity bioassays for the 50:50 mixture of 2,4-D and MSMA yielded an average 96-hour LC_{50} of 342 mg/L (95% confidence limits of 247 mg/L, 429 mg/L). Acute toxicity bioassays for a 50:50 mixture of 2,4-D and MSMA with surfactant yielded an average 96-hour LC_{50} of 43 mg/L (95% confidence limits of 12 mg/L, 92 mg/L). Statistical comparison of these two bioassays confirmed that surfactant has a significant effect on the acute toxicity of a 2,4-D / MSMA mixture ($p=0.001$).

Table 1: Summary of acute toxicity bioassay results

<i>Herbicide Treatment</i>	<i>Ratio</i>	<i>96-hr. LC_{50} (mg/L)</i> <i>(95% confidence limit)</i>	<i>Is the Surfactant Effect</i> <i>Significant? (Yes or No)</i>	<i>MAI^a</i>
2,4-D	--	185 (75-343)	--	
MSMA	--	1063 (--)	--	
2,4-D and MSMA	1	342 (247-429)	--	-1.1736 ^b
2,4-D + surfactant	--	108 (34-203)	Yes	
MSMA + surfactant	--	1099 (639-1510)	No	
2,4-D and MSMA+ surfactant	1	43 (12-92)	Yes	3.2810 ^c

^a Marking's Additive Index [5]

^b Antagonistic effect

^c Synergistic effect

According to Marking's additive index, the herbicide mixture without surfactant displays antagonistic toxicity, with an additive index of -1.1736 , or $1/2$ of that expected when looking at the individual herbicide toxicities. The herbicide mixture with surfactant displays synergistic toxicity, with an additive index of 3.2810 , or two times that expected when looking at the individual herbicide toxicities.

Discussion

High doses of 2,4-D can alter enzyme activity and disrupt liver function, as well as causing alterations in gill structure that can lead to respiratory stress [7-9]. This added stress and interference with the processes of metabolism and detoxification would be expected to enhance the toxicity of whatever herbicide 2,4-D was combined with. But the toxicity of the 2,4-D/MSMA herbicide mixture depends upon whether or not surfactant is added to the mixture. The synergistic relationship between the two herbicides mixed together with surfactant was at least in part due to surfactant's ability to facilitate absorption of the herbicides into tissues. Greater absorption equals greater toxicological impact from each herbicide.

The two herbicides mixed together without surfactant displayed an antagonistic relationship. The mixture's toxicity was lowered by approximately one-half of the sum of the toxicities of the individual herbicides. The two herbicides may compete for the same sites of activity on a molecular level, or one herbicide may alter the number or activity of the critical sites involved.

The natural assumption when dealing with different chemicals mixed together would be that a mixture's toxicity equals the sum of the toxicities of its parts. But as this experiment has shown, mixture toxicity is far more complicated than simple addition. Greater toxicity may be due not only to the toxic effects of the herbicides themselves but to supposedly inert additives such as surfactant.

Alternatively, a mixture may be less toxic than the sum of its parts due to antagonistic interactions between its components. The examination of the effects of herbicide runoff cannot be determined by an examination of the individual components analyzed. Any opportunities for synergism or antagonism must be identified for an accurate understanding of mixture toxicity.

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