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TOXICITY OF 2-*SEC*-BUTYL-4,6-DINITROPHENOL (DINOSEB) AND MONOSODIUM METHANEARSONATE (MSMA), INDIVIDUALLY AND IN A MIXTURE, TO CHANNEL CATFISH (*ICTALURUS PUNCTATUS*) AND FATHEAD MINNOWS (*PIMEPHALES PROMELAS*)

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Abstract—The toxicity of technical-grade dinoseb, technical monosodium methanearsonate (MSMA), several formulations of each and a mixture of the two were tested using channel catfish (*Ictalurus punctatus* Rafinesque) and fathead minnows (*Pimephales promelas* Rafinesque) under standard conditions at 12°C in soft, reconstituted water. The 96-h LC50s for technical dinoseb were 0.058 mg/L for channel catfish and 0.088 mg/L for fathead minnows. Differences among Vertac General®, Vertac Selective®, Premerge 3®, Gebutox® and technical dinoseb did not affect dinoseb toxicity to either fish species. The 96-h LC50s for technical MSMA were 2,390 mg/L for channel catfish and 1,210 mg/L for fathead minnows. Formulation had a measurable effect on MSMA toxicity, with the Daconate® and Daconate 6® formulations nearly an order of magnitude more toxic than technical MSMA and Bueno 6® to both fish species. The mixture or joint toxicity of technical dinoseb and technical MSMA was greater than additive (synergistic), with additive toxicity indices for channel catfish and fathead minnows of 0.51 and 5.78, respectively.

Keywords—Dinoseb MSMA Formulation Mixture

INTRODUCTION

2-*Sec*-butyl-4,6-dinitrophenol (dinoseb) and monosodium methanearsonate (MSMA) are contact herbicides; the former is used to control broadleaf weeds and the latter to control grasses. Both are currently used for specific agricultural and ornamental weed control throughout the United States.

Dinoseb is a nitrogenated phenol available in different salt forms depending on the intended use. It exists as a dark brown solid or a dark orange liquid, depending on the ambient temperature. The phenol form of dinoseb (Vertac General®, Caldon®, Sinox General®) is used as a postemergent, general contact herbicide and as a desiccant. The ammonium salt (Vertac Selective®, Sinox W®) is applied for postemergent control of broadleaf weeds. The alkanolamine salt (Premerge 3®) is applied as a preemergent treatment of upper soil layers to kill germinating weeds and as a postemergent spray [1]. The triethanolamine salt (Gebutox®) is used as an insecticide in dormant-fruit sprays to control scale insects and mites (Hoechst Co. product label). Dinoseb is degradable in soils; however, it shows phytotoxicity for two to four weeks after application [2].

MSMA (Arsonate Liquid®, Bueno 6®, Daconate®, Daconate 6®) is a selective postemergent contact herbicide used to control grasses. MSMA is commercially available as an aqueous solution in the technical form (Arsonate Liquid) or as MSMA plus a surfactant (Daconate, Daconate 6 and Bueno 6). MSMA is not appreciably degraded in soils but becomes biologically unavailable through soil surface adsorption and ion exchange [2].

In areas where dinoseb and MSMA are applied, drift from aerial spraying or runoff could pose a threat to aquatic resources. Furthermore, since both chemicals are occasionally applied to the same areas for weed control, they could simultaneously enter an aquatic system and produce unexpected joint effects.

This study was conducted to determine the toxicities of dinoseb and MSMA, several different formulations of each, and their mixture to two warm-water species of fish, channel catfish (*Ictalurus punctatus* Rafinesque) and fathead minnows (*Pimephales promelas* Rafinesque). These species were selected because of their widespread use in

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comparative toxicity testing and because their natural ranges coincide with most areas where these herbicides are applied.

MATERIALS AND METHODS

Bioassay procedures

Reconstituted soft water conditions [3] of 12°C, hardness of 44 mg/L as $CaCO_3$, alkalinity of 33 mg/L as $CaCO_3$ and pH of 7.4 were maintained throughout the study. Technical dinoseb (95.0% active ingredient (a.i.)), Vertac General (55.0% a.i.), Vertac Selective (13.7% a.i.), Premerge 3® (50.7% a.i.), and Gebutox (24.0% a.i.) were used as the formulations for dinoseb testing. Stock solutions were made by dissolving the chemical in 5 ml acetone, then diluting to volume with deionized water. Acetone concentrations did not exceed 0.1 ml/L in any exposure vessel.

Arsonate Liquid as technical MSMA (51.0% a.i.), Bueno 6 (48.3% a.i.), Daconate (35.2% a.i.) and Daconate 6 (48.3% a.i.) were used as the formulations for MSMA testing. All formulations tested were in aqueous solution and no solvents or carriers were used in making stock or test solutions. All toxicants were administered into the test vessels by direct pipetting of stock solutions or, in the case of the large doses of MSMA, by direct addition of the chemical. One-year-old fathead minnows and channel catfish were obtained from cultures at the U.S. Fish and Wildlife Service National Fishery Research Laboratory.

Prior to testing, all fish were held in isolation and fasted for 96 h to permit bowel evacuation and to minimize biochemical oxygen demand (BOD) loading in experimental vessels. Fish were placed in 18-liter test vessels 24 h before the addition of the toxicant(s) to allow for acclimation to the test conditions. Each vessel contained 15 liters of reconstituted water and all fish were incinerated upon termination of the experiment.

Ten fish were placed in each test vessel, unless the loading rate exceeded 1.0 g/L [3]. All tests, except for preliminary ones, were replicated so that 20 fish were used per treatment level. Ten fish per vessel were used and each vessel was duplicated per treatment level for fathead minnows. With channel catfish, five fish per vessel were used because of loading limitations and each treatment level had four replicate vessels. Data from each treatment were pooled and used in the LC50 statistical analysis.

Testing began after the 24-h acclimation period by adding toxicant, adequately stirring and then recording mortality at predetermined time intervals during the 96-h test period. The water temperature in the test aquaria was maintained at 12°C by a constant-temperature waterbath. Fish that died during the test were removed to minimize BOD effects on the remaining fish in the vessel.

Preliminary testing experiments were conducted to establish the range in which the LC50 was found. Mortality was recorded at 0.25, 0.50, 1, 3, 6, 24, 48, 72 and 96 h. Behavioral responses to the toxicant including swimming abnormalities, pigmentation and integument changes; respiratory, alimentary and sensitivity responses were also noted.

Definitive testing was conducted with 10 vessels using nine concentrations and one control. Percent mortality was recorded at 1, 3, 6, 24, 48, 72 and 96 h and was used to calculate LC50s.

Mixture testing was performed with the same protocol used in the definitive tests, except that both chemicals were added to the test chambers. It was assumed that the chemicals would show additive toxicity and that the highest concentration in the mixture test was the LC50 amount of dinoseb and MSMA. For example, with channel catfish, the high concentration was 0.058 mg/L for dinoseb and 2,390 mg/L for MSMA mixed in the test vessel. The ratio of the amount of dinoseb to MSMA (0.058:2390 mg/L or one LC50 equivalent:one LC50 equivalent) was maintained in each vessel used for the mixture. The mixture LC50 for each chemical was calculated from the concentrations of each chemical used in the mixture toxicity test. The mixture LC50s for dinoseb and MSMA were 0.034 and 1,420 mg/L, respectively, for channel catfish. These values and the individual chemical LC50s of 0.058 and 2,390 mg/L for dinoseb and MSMA using channel catfish were used to calculate the additive index value.

Data analyses

LC50s and statistical analyses among LC50s were calculated by the Litchfield and Wilcoxon method [4] using a p value of 0.05. Mixture index determinations were performed using the method of Marking and Dawson [5]. The criteria for the mixtures to be additive, greater than additive (synergistic) or less than additive (antagonistic) were as follows: If the index value was near zero and the 95% confidence limits overlapped zero, the mixture was additive. If the index value and its 95% confidence limits were greater than and did not overlap zero, then the mixture was greater than

additive (synergistic). If the index value and 95% confidence limits were less than and did not overlap zero, then the mixture was less than additive (antagonistic).

Chemical analyses

Samples from exposure vessels were randomly collected periodically from each test batch throughout the experiments for verification of pesticide concentrations. For dinoseb, one high-concentration vessel was sampled twice and analyzed in triplicate to determine if chemical degradation was occurring during the test period. No degradation analysis was performed for MSMA.

Dinoseb. Dinoseb absorbances were measured by UV-visible spectrophotometry. The analysis was performed on a Beckman DU-6 UV-visible spectrophotometer at a wavelength of 376 nm. Standard solutions were prepared by dissolving analytical dinoseb in methanol and diluting to volume with distilled water. Sodium hydroxide was added in the stock solutions for the standard curve, which converted dinoseb to its sodium salt and produced a yellow color (in-house method No. 70205, Vertac Chemical Corp., Memphis, TN). The estimated detection limit for the method was 0.1 mg/L, which was identical to the detection limit provided by the manufacturer. Recoveries from solution averaged 99% for the duration of the study. Stock solution concentrations and test vessel concentrations were 91 to 105% (average 99%) of the predicted concentrations. Test vessel concentrations were calculated using the analyzed stock concentration values and applying the dilution series used for mixing the test vessel solutions.

MSMA. MSMA concentrations were verified with the silver diethyldithiocarbamate method No. 307B [6]. Standards and samples were digested with 10 ml concentrated nitric acid and 2 ml 1:1 concentrated sulfuric acid (personal communication, Dr. E. A. Woolson), then analyzed for absorbance at 535 nm with a path-length of 1 cm. Concentrations were derived from a standard curve using digested standards. The average percent recovery from digestion was 78%, with values ranging from 71 to 87%. A sample of MSMA was spiked with 2 μg arsenic and the percent recovery was 74%, which fell in the range of recoveries. The estimated detection limit for this analysis was approximately 3 μg MSMA as arsenic. MSMA test vessel concentrations were 94 to 116% (average 105%) of the predicted concentrations. MSMA concentrations were measured directly from test solutions.

Materials

Dinoseb samples were obtained from the Vertac Chemical Corporation (Memphis, TN) and Hoechst Aktiengesellschaft (Frankfurt, F.R.G.). MSMA samples were obtained from the SDS Biotech Corporation (Painesville, OH). One-year-old fathead minnows and channel catfish were supplied by the LaCrosse National Fishery Research Laboratory. Testing was conducted at the LaCrosse Laboratory using their test vessels, reconstituted water system, waterbaths and analytical equipment.

RESULTS AND DISCUSSION

Dinoseb toxicity

Dinoseb and its formulations had 96-h LC50s ranging from 0.028 to 0.058 mg/L for channel catfish and from 0.088 to 0.15 mg/L for fathead minnows. The LC50s for technical dinoseb were 0.058 mg/L for channel catfish and 0.088 mg/L for fathead minnows (Table 1). LC50s of 0.067 mg/L for lake trout (*Salvelinus namaycush*) and 0.044 mg/L for cutthroat trout (*Salmo clarki*) at 10°C were reported by Woodward [7]. In contrast, Call et al. [8] found a 96-h LC50 for dinoseb to 30-d-old fathead minnows of 0.7 mg/L in Lake Superior water. The difference between the two values, 0.7 mg/L measured by Call et al. and 0.088 mg/L found in this study, may be due to three factors: greater alkalinity (42 mg/L as $CaCO_3$), higher temperature (23–28°C) and younger fish in the Call et al. study [8]. The difference in alkalinity was slight; however, the temperature and age differences were

Table 1. 96-h LC50s (and 95% confidence intervals) for dinoseb and its formulations to channel catfish and fathead minnows at 12°C in soft, reconstituted water[a]

Formulation	Channel catfish	Fathead minnows
Technical dinoseb (95% a.i.)	0.058 (0.052–0.063)	0.088 (0.078–0.098)
Vertac General® (55.0% a.i.)	0.054 (0.016–0.18)	0.090 (0.076–0.11)
Vertac Selective® (13.7% a.i.)	0.042 (0.036–0.048)	0.093 (0.075–0.11)
Gebutox® (23.5% a.i.)	0.053 (0.049–0.057)	0.10 (0.087–0.13)
Premerge 3® (50.7% a.i.)	0.028 (0.024–0.033)	0.15 (0.11–0.20)

[a]All concentrations (mg/L) based on the active ingredient (a.i.) of the formulations.

substantial. Of these two factors, temperature was the most likely cause of the difference.

MSMA toxicity

MSMA and its formulations had 96-h LC50s ranging from 385 to 2,460 mg/L for channel catfish and from 448 to 1,290 mg/L for fathead minnows (Table 2). These results did not agree in any respect with those reported in the literature. According to Johnson and Finley [9], 96-h LC50s for a formulation containing 34.8% MSMA and 37.7% surfactant, similar to Daconate used in this study, were 4.6 mg/L for fathead minnows at 18°C and 9.3 mg/L for channel catfish at 17°C (after concentrations were adjusted to account for the active ingredient). The 96-h LC50s for Daconate were 448 mg/L for fathead minnows and 385 mg/L for channel catfish at 12°C in this study, a difference of nearly two orders of magnitude. Temperature or synergistic effects from the surfactant could have been a factor. In Johnson and Finley's study, cutthroat trout at 10°C showed a 96-h LC50 of more than 100 mg/L total material, a result that was much closer to the results obtained in this study. Further tests would be needed to determine if temperature was responsible for this difference between results.

Formulation testing

Formulation variations did not substantially affect toxicity among the dinoseb herbicides tested. The LC50s for both fish species were generally similar for the different formulations. The toxicity of Premerge 3, the alkanolamine salt, was lower than those of the other formulations to fathead minnows. Premerge 3 and Vertac Selective, the ammonium salt, were slightly more toxic to channel catfish than the other formulations.

Formulation noticeably affected toxicity among the MSMA herbicides tested. Arsonate Liquid and Bueno 6 produced similar LC50s, whereas Daconate and Daconate 6 were nearly an order of magnitude more toxic than Arsonate Liquid (technical MSMA) and Bueno 6 to both species. The vast difference in toxicity between Arsonate Liquid and Bueno 6 as compared with Daconate and Daconate 6 implies that the surfactants in the latter formulations may have contributed to toxicity. The surfactant in the Daconate formulations probably contributed to toxicity, either by making the arsonate more available to the organism or by being toxic itself. Other inert ingredients or chemicals may have had effects also; however, in light of the great differences in toxicities among the four formulations produced by the same manufacturer (SDS Biotech) with presumably the same inert ingredients, surfactants seemed the most likely cause of those differences.

Table 2. 96-h LC50s (and 95% confidence intervals) for MSMA and its formulations to channel catfish and fathead minnows at 12°C in soft, reconstituted water[a]

Formulation	Channel catfish	Fathead minnows
Arsonate Liquid® (Technical MSMA) (51.0% a.i.)	2,390 (2,004–2,851)	1,210 (977–1,499)
Bueno 6® (48.3% a.i.)	2,460 (2,051–2,950)	1,290 (902–1,845)
Daconate® (35.19% a.i.)	385 (337–440)	448 (377–532)
Daconate 6® (48.3% a.i.)	675 (618–738)	550 (488–620)

[a]All concentrations (mg/L) based on the active ingredient (a.i.) of the formulations.

Mixture toxicity

The additive toxicity indices and 95% confidence intervals for the mixture of technical dinoseb and technical MSMA were 0.51 (0.14–1.16) for channel catfish and 5.78 (2.92–9.92) for fathead minnows. In both cases, the additive indices and 95% confidence intervals were above zero and demonstrated varying degrees of synergism or greater than additive toxicity. The synergism may be explained through the modes of action of chemicals similar to dinoseb and MSMA.

Arsonates are uncouplers of glycolysis and also affect the Krebs cycle [10]. They act by competing with phosphate molecules to form acyl arsonates. For example, at the 1,3-diphosphoglycerate step of glycolysis, arsonate binds with glyceraldehyde-3-phosphate to form 1-arsono-3-phosphoglycerate. Glycolysis proceeds normally, but the ATPs usually produced in the next step are lost because of the attachment of arsonate instead of phosphate. Thus, the energy as ATP typically obtained from glycolysis is not produced [10].

Dinitrophenol (DNP) acts as an uncoupler of oxidative phosphorylation and as a metabolic stimulant [10]. DNP uncouples oxidative phosphorylation by transporting protons across the inner mitochondrial membranes. However, normal elec-

tron flow from NADH to O_2 at the end of the electron transport chain is not interrupted. DNP dissipates the proton gradient across the inner mitochondrial membrane that normally provides the energy for the reaction of ADP and PI to form ATP in oxidative phosphorylation. Thus the ATPs normally obtained from oxidative phosphorylation are not produced, but other metabolism is maintained because NADH is still oxidized. Metabolic stimulation results from the loss of respiratory control due to an increase in the ADP:ATP ratio. The body interprets the ADP increase as a decrease in ATP; therefore, metabolism and O_2 consumption are increased to compensate for the ATP loss, thus raising the metabolic rate. This increase in metabolic rate and drop in ATP production provide the basis for the toxicity of the compound [10].

MSMA and dinoseb would be synergistic (as shown in this study) if their modes of action were similar to those for inorganic arsonate and DNP. This would be accomplished by the arsonate acting on substrate-level phosphorylation and the DNP eliminating ATP production from oxidative phosphorylation, thus effectively halting most ATP-producing reactions in the cell. Metabolic stimulation by DNP would further contribute to the effect by increasing the rate at which the cell would consume its energy reserves. This action would affect nerve tissue most severely because it requires glucose as the primary energy source to satisfy its high energy requirements. Uncoupling of the glycolytic and oxidative phosphorylation metabolic pathways would cause nerve tissue to lose function and cause problems in respiration, circulation, movement and other bodily functions. Spastic behavior such as labored respiration, twitching, erratic swimming and defecation were seen throughout the mixture experiments, which lends support to this hypothesis. Further research would be needed for verification.

The degree of difference in the additive index of the mixture for channel catfish (0.51) and for fathead minnows (5.78) was quite large. Assuming that the synergistic action of these chemicals was the same at the cellular level for both fish species, a similar degree of synergism would be expected for each. Because this was not found, other physiological processes must contribute to or be absent from these species to account for the difference in susceptibility. However, it was beyond the scope of this study to investigate any mechanisms that might explain the difference in the degree of synergism between the two species.

SUMMARY AND CONCLUSIONS

The 96-h LC50s for technical dinoseb at 12°C in soft, reconstituted water were 0.058 mg/L for channel catfish and 0.088 mg/L for fathead minnows. Formulations of dinoseb had little effect on the toxicity to either species.

The 96-h LC50s for technical MSMA were 2,390 mg/L for channel catfish and 1,210 mg/L for fathead minnows at 12°C in soft water. Formulations of MSMA significantly affected toxicity and made the compounds from one to six times more toxic than the technical material. This may be due to additional toxicity from the surfactant or from the surfactant's making the arsonate more available to the organism.

Toxicity of the mixture of technical MSMA and technical dinoseb was greater than additive to both channel catfish and fathead minnows. It is hypothesized that the synergism is due to the uncoupling of glycolysis by arsonate combined with the uncoupling of oxidative phosphorylation by DNP, causing a drop in ATP production by the cell. This action is thought to affect nerve tissue, causing a disruption of body functions and ultimately the death of the organism. Further research, however, is needed to verify this hypothesis.

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REFERENCES

1. *Farm Chemicals Handbook*. 1985. Meister Publishing Co., Willoughby, OH.
2. *Herbicide Handbook of the Weed Science Society of America*. 1983. Weed Science Society of America, Champaign, IL.
3. **Peltier, W.H.** and **C.I. Weber**, eds. 1985. Methods for measuring the acute toxicity of effluents to freshwater and marine organisms. EPA 600/4-85/013. U.S. Environmental Protection Agency, Cincinnati, OH.
4. **Litchfield, J.T.** and **F.A. Wilcoxon.** 1949. A simplified method of evaluating dose effect experiments. *J. Pharmacol. Exp. Ther.* **96**:99–113.
5. **Marking, L.L.** and **V.K. Dawson.** 1975. Method for assessment of toxicity or efficiency of mixtures of chemicals. *Investigations in Fish Control* **67**:1–7.
6. **American Public Health Association, American Water Works Association** and **Water Pollution Con-**

trol Federation. 1985. *Standard Methods for the Examination of Water and Wastewater*, 16th ed. American Public Health Association, Washington, DC.

7. **Woodward, D.F.** 1976. Toxicity of the herbicides dinoseb and picloram to cutthroat (*Salmo clarki*) and lake trout (*Salvelinus namaycush*). *J. Fish. Res. Board Can.* **33**:1671–1676.
8. **Call, D.J., L.T. Brooke, R.J. Kent, S.H. Poirer, M.L. Knuth, P.J. Shubat** and **E.J. Slick.** 1984. Toxicity, uptake, and elimination of the herbicides alachlor and dinoseb in freshwater fish. *J. Environ. Qual.* **13**:493–498.
9. **Johnson, W.W.** and **M.T. Finley.** 1980. Handbook of acute toxicity of chemicals to fish and aquatic invertebrates. U.S. Department of the Interior Fish and Wildlife Service Resource Publication 137. Washington, DC.
10. **Stryer, L.** 1981. *Biochemistry*. W.H. Freeman and Co., San Francisco, CA.