Bull. Environm. Contam. Toxicol. 24,543-549 (1980)

Acute Fish Toxicity of the Versene Family of Chelating Agents

T. L. Batchelder, H. C. Alexander, and W. M. McCarty
Environmental Sciences Research Laboratory, Dow Chemical U.S.A., Midland, Michigan 48640

VERSENE* family of chelating agents are readily soluble in water and have found wide use in many areas through their control of metal ions. These chelants are used in the fields of textiles, water treatment, industrial cleaners, agriculture, photography, and pulp and paper, to name only a few (STEIN 1977).

The U.S. production of specific chelating agents in 1978 (U.S. INTERNATIONAL TRADE COMMISSION 1979) was 4,267,000 lb of the pentasodium salt of diethylenetriaminepentaacetic acid (Na_5DTPA), such as VERSENEX* 80 chelating agent; 69,675,000 lb of the tetrasodium salt of ethylenediaminetetraacetic acid (Na_4EDTA), such as VERSENE 100 chelating agent; and 3,855,000 lb of the trisodium salt of N-(hydroxyethyl)-ethylenediaminetriacetic acid, such as VERSENOL* 120 chelating agent.

Both macronutrients and micronutrients (trace elements) are essential for proper plant growth. In some areas, the intensification of agricultural practices has resulted in depletion of available micronutrients. In other areas, the leveling of land for farming or improper plowing has exposed subsoils deficient in micronutrient. In order to achieve adequate agricultural production, it is necessary to add micronutrients to these soils. A chelated micronutrient is made by reacting a metallic salt with one of the chelating agents, forming a protecting glove around the metal and retarding the normal soil chemistry reactions that tie up that metal. Thus, it is more available to the plants. Chelated micronutrients are five to ten times more efficient than the nonchelated forms (THE DOW CHEMICAL COMPANY 1973).

VERSENE chelating agents are normally used only in diluted form and are relatively non-toxic to mammals, and consequently should cause no difficulty for humans in handling. Because of the high water solubility of these

---

*Trademark of The Dow Chemical Company

0007-4861/80/0024-0543 $01.40

products and the probable introduction into surface waters, especially through water treatment and agricultural uses, information on fish toxicity is needed.

METHODS

Test Compounds. Nine members of the VERSENE family of chelating agents and five VERSENE chelated micronutrients were evaluated as formulated in these toxicity tests. Test solutions were prepared in water just prior to the start of the test.

TABLE 1. Chelating Agents Tested

Product	Active Ingredient	% A.I.*
VERSENE Acid (EDTA)	Ethylenediaminetetraacetic acid (EDTA)	99.33
VERSENE 100	Tetrasodium salt of EDTA	39.00
VERSENE Powder	Tetrasodium salt of EDTA	---
VERSENE CA	Calcium chelate of disodium EDTA	97.00-102.00
VERSENE $(NH_4)_2$ EDTA	Diammonium salt of EDTA	40.09
VERSENE $(NH_4)_4$ EDTA	Tetraamonium salt of EDTA	39.00
VERSENEX 80	Pentasodium salt of diethylenetriamine pentaacetic acid	40.20
VERSENOL 120	Trisodium salt of N-(hydroxyethyl)-EDTA	41.35
VERSENE Fe-3 SPECIFIC	Sodium salt of N,N'-di(2-hydroxyethyl) glycine	41.10
VERSENE AG 7.5% Cu	Copper chelate derived from ethylenediamine-tetraacetate	45.5
VERSENOL AG 5% Fe	Iron chelate derived from N-hydroxyethylenediamine-triacetate	31.3
VERSENE AG 2.5% Mg	Magnesium chelate derived from ethylenediamine-tetraacetate	40.0
VERSENE AG 5% Mn	Manganese chelate derived from ethylenediamine-tetraacetate	35.1
VERSENE AG 1 lb Zn	Zinc chelate derived from ethylenediamine-tetraacetate	53.0

*Active Ingredient

Water. Water used in the fish laboratory comes from Whitestone Point on the west side of Saginaw Bay, Lake Huron. This raw water is chlorinated at the intake pumping station then sent by pipeline to Saginaw and Midland for further treatment. The Dow Chemical Company supply line bypasses the Midland Water Treatment Plant and enters our building where it is repressurized and sent through an activated charcoal bed to remove any free chlorine. The water is then either chilled or warmed depending on fish species being held and/or tested. Dissolved oxygen in tankage is enhanced through the use of air bubblers.

This water exhibits chemical characteristics in the following ranges: dissolved oxygen (D.O.), start of test 8.2 mg/ℓ, end of test 4.2 mg/ℓ; pH 7.6; total alkalinity, 85 mg/ℓ as $CaCO_3$; total hardness 103 mg/ℓ as $CaCO_3$; specific conductivity, 1770 μmhos/cm; and resistivity, ∿570 ohms/cm (AMERICAN PUBLIC HEALTH ASSOCIATION 1975). Since we successfully raise laboratory minnows in this water, it passes the general requirements for dilution waters stated in COMMITTEE ON METHODS FOR TOXICITY WITH AQUATIC ORGANISMS (1975).

Fish. The static water acute toxicity tests follow the methods described in the EPA publication, "Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians" (COMMITTEE ON METHODS FOR TOXICITY WITH AQUATIC ORGANISMS 1975).

The test fish, bluegill (*Lepomis macrochirus* Rafinesque), a recognized warm water test species, were received from the National Fish Hatchery at Hebron, Ohio, on September 3, 1975. The fish had an average weight of 0.74 g (range 0.62-1.28 g) and an average total length of 34 mm (range 28-38 mm); the average loading per aquarium was 0.74 g/ℓ.

The bluegill were acclimated to 22°C under laboratory conditions for at least ten days prior to use. The fish were kept in a 16-h light/8-h dark cycle. Pulverized dry Master Mix® fish pellets were fed to all specimen during the acclimation period. Feed was stopped three days prior to the bioassay to empty the digestive tract. Fish were placed into the aquaria 24 h before adding the test material.

Bioassays were conducted by placing eight liters of dechlorinated Lake Huron water in each vessel, a round glass aquarium measuring 22 cm deep with a 24.5 cm diameter, adding the fish, then aerating with plant air. If no deaths occurred in 24 h, the aerators were removed, and the test solution added with two liters of water for mixing, making a total of ten liters. Any vessel containing dead fish was cleaned, reset, and observed for another 24 h prior to compound addition. Constant temperature water troughs maintained the temperature at 22 ± 1°C. Ten fish were exposed to each concentration of the material. Between five and ten concentrations were set per product. Observations were made and recorded, and dead fish removed at the same hour for four days (96 h). Death was used for the effect criterion and was determined by lack of response when the caudal section was pinched. None of the products were neutralized prior to testing.

Three materials, VERSENE acid, VERSENE 100, and VERSENE AG 1 lb Zn, were also tested for effect in very soft and hard waters. These waters were reconstituted distilled water as per recommendations in MARKING & DAWSON (1973). Tests were conducted using the previous described procedures.

Calculations of LC Values. The LC50 is the estimated concentration of the test material at which 50% of the test organisms are dead in a specified time interval. The 96-h LC50 and its respective 95% confidence limits and slope were determined via computer with a modified version of the IBM SSP Probit Program based on Finney's method of probit analysis (FINNEY 1952).

RESULTS AND DISCUSSION

The 96-h LC50 for each of the chelant and chelated micronutrient products is tabulated in Table 2, along with the observable toxic effects and the pH's at the end of the test period.

Most inland waters containing fish have a pH between 6.7-8.6, with extremes of 6.3 and 9.0 (CALIFORNIA STATE WATER RESOURCES CONTROL BOARD 1971). The permissible pH range for any species depends on temperature, dissolved oxygen, acclimation, and various anion and cation concentrations in the water. COMMITTEE ON WATER QUALITY CRITERIA (1972) recommends a pH of "not less than 5.5 nor more than 9.5", and "no change greater than 1.5 units outside the estimated natural seasonal maximum and minimum". It would appear that pH values out of the "natural" range are stressful for fish, making them more sensitive to other components of their environment.

Two products illustrate the effect of pH in medium hard water: VERSENE acid and VERSENE powder. The acid kills 100% of the bluegill at 240 mg/ℓ in water at a pH of 3.7. Such acidity alone is lethal to bluegill (COMMITTEE ON WATER QUALITY CRITERIA 1972). If the acid is made into the sodium salt, VERSENE powder, a 100% mortality occurs at 521 mg/ℓ. The resultant pH of 8.9 is not itself toxic to bluegill. A 50% aqueous solution of this sodium salt, VERSENE 100, causes 100% mortality at 1120 mg/ℓ at a pH of 9.6. In hard water, total kill with the VERSENE acid occurs at 750 mg/ℓ and pH 3.5 (again, a pH related kill), whereas VERSENE 100 killed all bluegill at 2400 mg/ℓ and pH 9.8. A pH level of 9.5-10.0 may cause some mortality; values >10.0 are not tolerated by bluegill (COMMITTEE ON WATER QUALITY CRITERIA 1972).

Six of the VERSENE products tested would fit into category "D" of aquatic toxicity using the criteria set by the Inter-governmental Maritime Consultative Organization

TABLE 2. ACUTE TOXICITY OF SOME VERSENES TO BLUEGILL IN STATIC WATER

Product	LC50 and Other Values in mg/ℓ of Product After a 96-Hour Exposure Period				
	NAEL*	PK**	100% Kill	LC50	Slope
VERSENE Acid (EDTA)	100 (5.8)[1]	135 (4.1)[1]	240 (3.7)[1]	159 (136-204)[2]	11.1
VERSENE 100	870 (9.4)	1000 (9.5)	1120 (9.6)	1030 (980-1080)	42.9
VERSENE Powder	456 (8.1)	490 (8.6)	521 (8.9)	486 (472-500)	71.3
VERSENE CA	1000 (7.5)	2400 (7.5)	4200 (7.4)	2340 (1610-3200)	4.8
VERSENE $(NH_4)_2$ EDTA	1350 (5.0)	1800 (5.0)	5600 (4.8)	2340 (1980-2730)	6.8
VERSENE $(NH_4)_4$ EDTA	240 (7.8)	320 (7.8)	1000 (8.1)	705 (623-795)	14.2
VERSENEX 80	750	1000 (8.9)	1350 (9.1)	1115 (1005-1250)	19.5
VERSENOL 120	560 (7.9)	750 (8.3)	1000 (9.1)	808 (766-852)	39.8
VERSENE Fe-3 SPECIFIC®	1800	3200 (9.5)	5600 (9.8)	3092 (2320-3540)	10.8
VERSENE AG 7.5% Cu	320 (7.8)	420 (7.8)	750 (7.9)	555 (487-640)	10.7
VERSENOL AG 5% Fe	5600 (7.2)	7500 (6.7)	13,500 (6.4)	8100 (7220-8900)	12.2
VERSENE AG 2.5% Mg	1350 (7.6)	1800 (7.6)	4200 (7.6)	2520 (2220-2870)	11.0
VERSENE AG 5% Mn	7500 (7.5)	10,000 (7.5)	32,000 (8.0)	14,100 (11,600-16,900)	4.2
VERSENE AG 1 lb Zn	320 (7.9)	420 (8.0)	1000 (8.4)	685 (584-831)	6.7

*No (observable) adverse effect level, when compared to controls.

**Partial Kill.

[1]Values in parentheses in this column are pH measurements on the solution tested.

[2]Values in parentheses in this column are 95% confidence intervals.

(IMCO) (U.S. SENATE 1973). Category "D" is defined as "practically non-toxic", with a 96-h LC50 between 100-1000 mg/ℓ. The other eight products are <u>less toxic</u> than these, with none more toxic.

TABLE 3. ACUTE TOXICITY OF SOME VERSENES TO BLUEGILL IN STATIC WATERS OF DIFFERENT HARDNESS

LC50 and Other Values in mg/ℓ of Product After A 96-Hour Exposure Period

Water Hardness*	NAEL**	PK***	100% Kill	LC50	Slope
			VERSENE Acid (EDTA)		
Very Soft	24	32	75	41 (34-62)[1]	8.1
Medium Hard	100 (5.8)[2]	135 (4.1)[2]	240 (3.7)[2]	159 (136-204)	11.1
Very Hard	420 (4.4)	560 (4.0)	750 (3.5)	532 (473-598)	17.1
			VERSENE 100		
Very Soft	115 (7.0)	135 (7.0)	180 (7.7)	157 (147-169)	21.2
Medium Hard	870 (9.4)	1000 (9.5)	1120 (9.6)	1030 (980-1080)	4.9
Very Hard	1800 (9.5)	2100 (9.6)	2400 (9.8)	2070 (1940-2180)	33.0
			VERSENE AG 1 lb Zn		
Very Soft	560 (8.1)	750 (8.1)	1350 (8.2)	940 (869-1050)	16.2
Medium Hard	320 (7.9)	420 (8.0)	1000 (8.4)	685 (584-831)	6.7
Very Hard	320 (8.4)	420 (8.4)	750 (8.5)	513 (454-578)	15.3

*See Table 2.
**No (observable) adverse effect level.
***Partial Kill.
[1]Values in parentheses in this column are 95% confidence intervals.
[2]Values in parentheses in this column are pH measurements on the solution tested.

VERSENE acid, VERSENE 100, and VERSENE AG 1 lb Zn were tested in three hardnesses of water: very soft, medium hard, and very hard. Results are shown in Table 3. VERSENE acid and VERSENE 100 acted as expected, being most toxic in soft water. As the hardness increased, more of the chelate was converted to the chelant form by the water hardness ions, thus more chelating agent was required to exert a toxic effect. However, the chelated micronutrient, VERSENE AG 1 lb Zn, exhibited a greater toxicity at higher hardness levels. Although the conditional stability constant at pH 8 would greatly favor zinc, the observed increased toxicity at higher levels of hardness can possibly be due to the mass action effect of Ca and Mg, which allows the release of minute amounts of zinc. Zinc is known to be toxic to bluegill at 10-12 mg/L (CALIFORNIA STATE WATER RESOURCES CONTROL BOARD 1971.)

REFERENCES

AMERICAN PUBLIC HEALTH ASSOCIATION: American Water Works Association, and Water Pollution Control Federation.

Standard methods for the examination of water and wastewater, 14th Edition, New York (1975).
CALIFORNIA STATE WATER RESOURCES CONTROL BOARD: Water quality criteria, 2 ed. (1971).
COMMITTEE ON METHODS FOR TOXICITY WITH AQUATIC ORGANISMS: Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians. Ecological Research Series, EPA-660/3-75-009 (1975).
COMMITTEE ON WATER QUALITY CRITERIA: National Academy of Sciences. Water quality criteria (1972).
THE DOW CHEMICAL COMPANY: Sales brochure, VERSENE AG Chelated Micronutrients for Healthy, Vigorous Crop Growth (1973).
FINNEY, D. J.: Statistical methods in biological assay. Cambridge University Press (1952).
MARKING, L. L. and DAWSON, W. K.: Toxicity of quinalidine sulfate to fish. Invest. Fish Control No. 48. U.S. Fish Wildl. Serv., Washington D.C. (1973).
STEIN, R. C.: Personal Communication, The Dow Chemical Company (1977).
U.S. INTERNATIONAL TRADE COMMISSION: Synthetic organic chemicals, U.S. Production and Sales (1979).
U.S. SENATE: 93rd Congress, 1st Session, Series 93-52. Hearing before the Committee on Commerce on 1973 IMCO Conference on marine pollution from ships (November 1973).