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AMBIENT AQUATIC LIFE WATER QUALITY CRITERIA FOR 2,4-DIMETHYLPHENOL

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U.S. ENVIRONMENTAL PROTECTION AGENCY

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NOTICES

This document has been reviewed by the Environmental Research Laboratories, Duluth, MN and Narragansett, RI, Office of Research and Development and the Health and Ecological Criteria Division, Office of Science and Technology, U.S. Environmental Protection Agency, and approved for publication.

This document is available to the public through the National Technical Information Service (NTIS), 5285 Port Royal Road, Springfield, VA 22161.

FOREWORD

Section 304(a) (1) of the Clean Water Act of 1977 (P.L. 95-217) requires the Administrator of the Environmental Protection Agency to publish water quality criteria that accurately reflect the latest scientific knowledge on the kind and extent of all identifiable effects on health and welfare that might be expected from the presence of pollutants in any body of water, including ground water. This document is a revision of proposed criteria based upon consideration of comments received from other federal agencies, state agencies, special interest groups, and individual scientists. Criteria contained in this document replace any previously published EPA aquatic life criteria for the same pollutant(s).

The term "water quality criteria" is used in two sections of the Clean Water Act, section 304(a)(1) and section 303(c)(2). The term has a different program impact in each section. In section 304, the term represents a non-regulatory, scientific assessment of ecological effects. Criteria presented in this document are such scientific assessments. If water quality criteria associated with specific stream uses are adopted by a state as water quality standards under section 303, they represent maximum acceptable pollutant concentrations in ambient waters within that state that are enforced through issuance of discharge limitations in NPDES permits. Water quality criteria adopted in state water quality standards could have the same numerical values as criteria developed under section 304. However, in many situations states might want to modify water quality criteria developed under section 304 to reflect local environmental conditions and human exposure patterns. Alternatively, states may use different data and assumptions than EPA in deriving numeric criteria that are scientifically defensible and protective of designated uses. It is not until their adoption as part of state water quality standards that criteria become regulatory. Guidelines to assist the states and Indian tribes in modifying the criteria presented in this document are contained in the Water Quality Standards Handbook (December 1983). This handbook and additional guidance on the development of water quality standards and other water-related programs of this Agency have been developed by the Office of Water.

This document, if finalized, would be guidance only. It would not establish or affect legal rights or obligations. It would not establish a binding norm and would not be finally determinative of the issues addressed. Agency decisions in any particular situation will be made by applying the Clean Water Act and EPA regulations on the basis of specific facts presented and scientific information then available.

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Introduction

2,4-Dimethylphenol (2,4-DMP) is a naturally occurring, substituted phenol derived from the cresol fraction of petroleum or coal tars by fractional distillation and extraction with aqueous alkaline solutions (Gruse and Stevens 1942; Lowry 1963; Rudolfs 1953; U.S. EPA 1976). 2,4-DMP, also known as 1-hydroxy-2,4-dimethylbenzene, m-xylenol, 2,4-xylenol or m-4-xylenol, has the empirical formula $C_8H_{10}O$ (Weast 1972). 2,4-DMP is used commercially as an important chemical feedstock or constituent for the manufacture of a wide range of commercial products for industry and agriculture. It is also used in the manufacture of phenolic antioxidants, disinfectants, solvents, pharmaceuticals, insecticides, fungicides, plasticizers, rubber chemicals, polyphenylene oxide, wetting agents, and dyestuffs; and is an additive or constituent of lubricants, gasolines, and cresylic acid. No direct commercial application for 2,4-DMP appears to exist at present.

Five other positional isomers of dimethylphenol or xylenol exist and include 2,3-, 2,5-, 2,6-, 3,4-, and 3,5-dimethylphenol. Since these isomers result from the different positioning of the two methyl groups on the phenol ring, they are referred to as positional isomers. As would be expected, there are variations in their physical, chemical, and biological properties.

2,4-DMP has a molecular weight of 122.17 and in its normal state exists as a colorless, crystalline solid (Bennet 1974; Weast 1972). It has a melting point of 27 to 28°C, a boiling point of 210°C (760 mm Hg), a vapor pressure of 1 mm Hg at 52.8°C, and a density of 0.9650 at 20°C (Bennet 1974; Jordan 1954; Weast 1972). 2,4-DMP is slightly soluble in water and, as a weak acid (pK_a of 10.6), is also soluble in alkaline solutions (Sober 1970). 2,4-DMP readily dissolves in organic solvents such as alcohol and ether (Weast 1972).

A large number of products utilize 2,4-DMP as a feedstock or constituent. Hence, disposal of chemical and industrial process wastes and distribution from normal product applications represent feasible routes for entry of 2,4-DMP into the environment. Examples of the latter route include pesticide applications, asphalt and roadway runoff, and the washing of dyed materials (U.S. EPA 1975).

Information regarding the concentration, persistence, fate and effects of 2,4-DMP in the environment is limited. However, its presence in petroleum distillate fractions and coal tars, together with its use as a chemical feedstock or constituent for the manufacture of many products, clearly indicate the potential for both point and nonpoint source water contamination. 2,4-DMP has been detected in the effluent from coal gasification plants and in finished drinking water (Shackelford and Keith 1976). The concentration of 2,4-DMP in sediments collected near the Los Angeles County Sanitation District's sewage outfall located off of Palos Verdes, California, was 40 μg/kg (Schwartz et al. 1985). It was below detection limits at six other stations located further away from the outfall (Schwartz et al. 1985).

It is inferred that 2,4-dimethylphenol will undergo some photolysis in well-aerated surface waters in spite of its apparent persistence (Callahan et al. 1979). Richards and Shieh (1986) rank it as a persistent, volatile and accumulative chemical. Callahan et al. (1979), on the other hand, indicate that there should be little tendency for it to volatilize from water. The complete biodegradation of 2,4-DMP has been reported to occur in approximately two months, although the conditions were not stated (Rodd 1952).

2,4-DMP can be oxidized to form pseudoquinone (Rodd 1952). However, the conditions required for this reaction generally are not found in the environment. 2,4-DMP reacts with aqueous alkaline solutions to form the corresponding salt. Such salts are readily soluble in water, provided that an alkaline pH is maintained. The free position on the aromatic ring, ortho to the hydroxyl group, may be alkylated (Kirk and Othmer 1964) or halogenated (Rodd 1952). However, such reactions have not been reported to occur under normal environmental conditions.

2,4-DMP causes a detectable odor in water when present at relatively low concentrations (Buikema et al. 1979). Hoak (1957) reported an odor threshold of 55.5 μg/L.

All concentrations reported herein are expressed as 2,4-DMP. The criteria presented herein supersede previous aquatic life water quality criteria for 2,4-DMP (U.S. EPA 1980) because these new criteria were derived using improved procedures and additional information. Whenever adequately justified, a national criterion may be replaced by a site-specific criterion (U.S. EPA 1983a) that may include not only site-specific concentrations (U.S. EPA 1983b) but also site-specific frequencies of allowed excursions (U.S. EPA 1985).

A comprehension of the "Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses" (Stephan et al. 1985), hereafter referred to as the Guidelines, and the response to public comment (U.S. EPA 1985) is necessary to evaluate the following text, tables, and calculations.

The latest comprehensive literature search for information for this document was conducted in September, 1992. Some more recent information is included.

Acute Toxicity to Aquatic Animals

The data that are available according to the Guidelines concerning the acute toxicity of 2,4-DMP are presented in Table 1. Freshwater Species Mean Acute Values were calculated according to the Guidelines as geometric means of the available acute values. Of the 12 freshwater genera for which mean acute values are available, the most sensitive genus, Ceriodaphnia, is about 20 times more sensitive than the most resistant, Lumbriculus. Both the most sensitive and most resistant genera are invertebrates. Fish were intermediate in sensitivity with a range in Genus Mean Acute Values from 6,300 μg/L to 19,300 μg/L. The freshwater Final Acute Value for 2,4-DMP was calculated to be 2,670 μg/L using the procedure described in the Guidelines and the Genus Mean Acute Values in Table 3. The Final Acute Value is lower than the lowest available freshwater Species Mean Acute Value.

The acute toxicity of 2,4-DMP to resident North American saltwater animals has been determined with six species of invertebrates and three species of fish (Table 1). The acute toxicity of 2,4-DMP differs by a factor of 42 for saltwater animals, with acute values based on 96-hr LC50s ranging from 1,320 μg/L for juvenile inland silversides, Menidia beryllina, to 55,900 μg/L for adult archiannelid worms, Dinophilus gyrociliatus (Thursby and Berry 1987a). Mortality increased with increased duration during 96-hr tests with six of seven species for which daily survival data are available. The saltwater Final Acute Value, based on nine Genus Mean Acute Values, is 548.8 μg/L. The Final Acute Value is lower than the lowest available saltwater Species Mean Acute Value.

Chronic Toxicity to Aquatic Animals

The data that are available according to the Guidelines concerning the chronic toxicity of 2,4-DMP are presented in Table 2. The freshwater cladoceran, Ceriodaphnia dubia, was tested in a 7-day life-cycle chronic exposure (Spehar 1987). Mean 2,4-DMP concentrations were 210, 470, 810, 1,870 and 3,410 μg/L. Survival was slightly reduced, but not significantly at these concentrations. However, young production was significantly lower ($p \leq 0.05$) than controls at the two highest concentrations, with reductions of about 64 and 90 percent in young produced at 1,870 and 3,410 μg/L, respectively. The chronic limits for this test were between 810 and 1,870 μg/L, which results in a chronic value of 1,230 μg/L (Table 2). Division of the companion acute value for Ceriodaphnia of 3,340 μg/L by the chronic value results in an acute-chronic ratio of 2.715.

Fathead minnows (Pimephales promelas) were exposed to 2,4-DMP in a 32-day early life-stage test at concentrations of 900, 1,360, 1,970, 3,110, and 5,130 μg/L (Holcombe et al. 1982). The percentage of normal appearing larvae at hatch was similar for each exposure as in the control. Survival of juvenile fish was reduced at the highest exposure and weight was reduced (≥15.6%) at the two highest exposures. The control fish at the end of the study averaged 72.6 mg in wet weight which is low for fathead minnows of this age in a toxicity test. Based upon growth, the chronic limits were 1,970 and

3,110 μg/L. The chronic value is 2,475 μg/L. Division of the companion acute value of 17,000 μg/L by this value results in an acute-chronic ratio of 6.869.

LeBlanc (1984) published an early life-stage study in which fathead minnows were exposed to 750, 1,500, 3,200, 7,400, and 15,000 μg/L 2,4-DMP. No fish survived at 15,000 μg/L, and only 12 percent survived at 7,400 μg/L. Length and weight were significantly less than controls at 7,400 and 3,200 μg/L. Based upon growth, the chronic limits were 1,500 and 3,200 μg/L. The chronic value is 2,200 μg/L. No corresponding acute value is available to determine an acute-chronic ratio.

Fathead minnows were exposed to 2,4-DMP in a third 32-day life-stage test at concentrations of 398, 605, 966, 1,573, 2,580, and 4,052 μg/L (Russom 1993). The study was conducted in the same laboratory as the Holcombe et al. (1982) study, but ten years later. Significant negative effects were observed for percentage of normal appearing larvae at hatch, and in survival at the end of the study at the highest exposure concentration of 4,052 μg/L. Significant differences in growth (wet weight and total length) from control fish were observed at concentrations ≥605 μg/L. Wet weight and total length were reduced 10.4% and 4.8%, respectively, at the exposure concentration of 605 μg/L. The mean wet weight of the control fish was 144 mg. Based upon growth, the chronic limits were 398 and 605 μg/L. The chronic value is 491 μg/L. A corresponding acute value for this test was not measured; therefore, an acute chronic ratio cannot be calculated.

The chronic toxicity of 2,4-DMP has been determined in an early life-stage toxicity test with the saltwater inland silverside, Menidia beryllina (Thursby and Berry 1987b). Ninety percent of the embryos exposed to 722 μg/L died prior to hatch; all hatched fish died. Survival of fish hatched in 296 μg/L was significantly reduced; 39 percent survived the 28-day test in contrast to 72 percent of the controls. No significant effects on survival or growth were detected in early life-stages of inland silversides exposed to 131 μg/L. The chronic limits determined from this test were 131 and 296 μg/L. The chronic value is 196.9 μg/L (Table 2). Division of this value by the companion acute value of 1,320 μg/L results in an acute-chronic ratio of 6.704.

The Final Acute-Chronic Ratio of 5.000 is the geometric mean of the acute-chronic ratio of 2.715 for the freshwater cladoceran, Ceriodaphnia dubia; 6.869 for the freshwater fathead minnow, Pimephales promelas; and 6.704 for the saltwater inland silverside, Menidia beryllina. Division of the Final Acute Value of 2,670 μg/L for freshwater species by the ratio of 5.000 results in a Final Chronic Value of 534.0 μg/L for freshwater aquatic life. The value of 534.0 μg/L is a factor of 2.3 less than the chronic value for the life-cycle test with Ceriodaphnia dubia and is slightly greater than the lowest chronic value of 491 μg/L reported for the fathead minnow.

Division of the Final Acute Value of 548.8 μg/L for saltwater species by the ratio of 5.000 results in a Final Chronic Value of 109.8 μg/L for saltwater aquatic life. The value of 109.8 μg/L is a factor of 1.8 less than the chronic value of 196.9 μg/L determined from the early life-stage test with inland silversides.

Toxicity to Aquatic Plants

Results of a test with one species of freshwater algae and 2,4-DMP is shown in Table 4. A four-day exposure with the alga, Scenedesmus quadricauda, indicated that 2,4-DMP concentrations of 40,000 μg/L and above inhibited growth (Bringman and Kuhn 1959a,b). No acceptable saltwater plant data with 2,4-DMP were found in the literature. A Final Plant Value, as defined in the Guidelines, cannot be calculated for 2,4-DMP.

Bioaccumulation

A study to determine the bioconcentration of 2,4-DMP with one freshwater species is shown in Table 5. ^{14}C radiolabelled 2,4-DMP bioconcentrated 150-fold in the whole body of the bluegill, Lepomis macrochirus (Barrows et al. 1980; Veith et al. 1980) (Table 5). A BCF determined on the basis of radiolabelling may contain some radiolabelled metabolites; therefore, the BCF of 150 may be greater than that for parent 2,4-DMP. 2,4-DMP has a measured partition coefficient (log n-octanol/water) of 2.42, and the BCF of 150 appears to be a reasonable estimate when compared to other chemicals (Veith et al. 1980).

No U.S. FDA action level or other maximum acceptable concentration in tissue, as defined in the Guidelines, is available for 2,4-DMP. Therefore, no Final Residue Value can be calculated.

Other Data

The incipient inhibition concentration for the bacterium, *Escherichia coli*, was in excess of 100,000 μg/L (Bringman and Kuhn 1959a) (Table 6). Exposure of the alga, *Chlorella pyrenoidosa*, to 100,000 μg/L for 72 hr resulted in a 52 percent reduction of chlorophyll a (Huang and Gloyna 1967,1968). A 28-hr exposure of the protozoan, *Microregma heterostoma*, produced an incipient inhibition concentration of 70,000 μg/L (Bringman and Kuhn 1959b), while a 60-hr EC50 of 130,510 μg/L (based on cell number) was obtained with the protozoan, *Tetrahymena pyriformis* (Schultz and Riggin 1985).

Spehar (1987) and Norberg-King (1987) reported 48-hr LC50s ranging from 3,100 μg/L to 6,300 μg/L for *Ceriodaphnia dubia* in eight separate tests in which daphnids were fed. Bringman and Kuhn (1959a,b) reported immobilization of *Daphnia magna* at 24,000 μg/L.

Rainbow trout, *Oncorhynchus mykiss*, were exposed to acutely lethal concentrations of 2,4-dimethylphenol to determine the symptomology of poisoning (Bradbury et al. 1989). At an exposure concentration of 9,040 μg/L, the trout had a mean survival time of 6 hr. They exhibited a significant increase from pre-exposure measurements in cough frequency, and significant decreases in gill oxygen uptake efficiency, total blood carbon dioxide (arterial) and hematocrit. These responses were consistent with a toxic mode of action referred to as type II (polar) narcosis. The mean LC50 for fathead minnows after 8 days was 13,500 μg/L (Phipps et al. 1981).

The number of sporophytes was reduced in brown kelp, *Laminaria* saccharina, exposed for two days to 12,000 µg/L of 2,4-DMP in two tests which began with either five- or seven-day-old plants (Thursby and Steele 1987; Table 6). Reproduction of kelp in 7,200 µg/L was not reduced.

Unused Data

A screening study by Applegate et al. (1957) was not used because not enough fish were tested per concentration. High control mortalities occurred in some tests reported by Thursby and Berry (1987a,b), and these results were not included in the data tables. 2,4-DMP toxicity was reported in cell cultures only by Babich and Borenfreund (1987). Methods were not adequately described in some studies (e.g., Curtis et al. 1982; Grushko et al. 1975). Data were not used when 2,4-DMP was a component in a mixture (e.g., Giddings and Franco 1985; Swartz et al. 1985) or effluent (Horning et al. 1984; Pickering 1983). Studies were not used if the exposure duration was not specified (e.g., Blum and Speece 1991).

Reports of 2,4-DMP toxicity were not used when the data had been compiled from other sources (e.g., Alexander et al. 1983; Enslein 1987; Hall and Kier 1984a,b; Kenaga 1982; Sabljic 1987; Schultz et al. 1986; Veith and Broderius 1987). Similarly, reviews on bioconcentration (Davies and Dobbs 1984) and taste or odor (Persson 1984) were not used.

Summary

The acute toxicity of 2,4-DMP has been determined for 12 species of freshwater animals. Acute values ranged between 3,340 µg/L and 67,600 µg/L. Of the eight invertebrate and four fish species tested, two cladocerans, Ceriodaphnia dubia and Daphnia magna, were the most sensitive. Acute values for freshwater fish ranged from 6,300 µg/L to 19,300 µg/L. The bluegill, Lepomis macrochirus, was the most sensitive freshwater species.

The chronic value for Ceriodaphnia dubia was 1,230 µg/L. In three tests with the fathead minnow, Pimephales promelas, chronic values of 2,475, 2,200 and 491 µg/L were obtained. Acute-chronic ratios were 2.715 and 6.869 for

Ceriodaphnia and Pimephales, respectively.

The acute toxicity of 2,4-DMP has been determined for nine species of saltwater animals. Acute values ranged from 1,320 µg/L for juvenile inland silversides, Menidia beryllina, to 55,900 µg/L for archiannelid worms, Dinophilus gyrociliatus. Of the six invertebrate and three fish species tested, no taxonomic group appeared particularly sensitive.

Chronic toxicity data for saltwater organisms are available from an early life-stage test with the inland silverside, Menidia beryllina. Survival of hatched fish was reduced in 296 µg/L of 2,4-DMP. No effects on survival or growth were observed at 131 µg/L. The acute-chronic ratio for this species is 6.704.

Limited plant data indicate that concentrations of 40,000 µg/L or more result in reduced growth of freshwater algae. No acceptable saltwater plant data were found in the literature.

One test showed that the BCF for 2,4-DMP was 150 based on data for the bluegill, Lepomis macrochirus. No acceptable saltwater BCFs were found in the literature.

The freshwater Final Acute Value and Final Chronic Value for 2,4-DMP are 2,670 and 534 µg/L, respectively. The value of 534 µg/L is slightly greater than the lowest chronic value of 491 µg/L reported for the fathead minnow, indicating that this species might not be adequately protected if ambient water concentrations exceed this concentration for long periods of time. The saltwater Final Acute Value and Chronic Value are 548.8 and 109.8 µg/L, respectively. Chronic adverse effects to the only saltwater species exposed to 2,4-DMP occurred at concentrations that are higher than the Final Chronic Value which should be protective of saltwater species.

National Criteria

The procedures described in the "Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses" indicate that, except possibly where a locally important species is very sensitive, freshwater aquatic organisms and their uses should not be affected unacceptably if the four-day average concentration of 2,4-DMP does not exceed 530 $\mu g/L$ more than once every three years on the average and if the one-hour average concentration does not exceed 1,300 $\mu g/L$ more than once every three years on the average.

The procedures described in the "Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses" indicate that, except possibly where a locally important species is very sensitive, saltwater aquatic organisms and their uses should not be affected unacceptably if the four-day average concentration of 2,4-DMP does not exceed 110 $\mu g/L$ more than once every three years on the average and if the one-hour average concentration does not exceed 270 $\mu g/L$ more than once every three years on the average.

Implementation

As discussed in the Water Quality Standards Regulation (U.S. EPA 1983a) and the Foreword to this document, a water quality criterion for aquatic life has regulatory impact only when it has been adopted in a state water quality standard. Such a standard specifies a criterion for a pollutant that is consistent with a particular designated use. With the concurrence of the U.S. EPA, states designate one or more uses for each body of water or segment thereof and adopt criteria that are consistent with the use(s) (U.S. EPA 1983b, 1987). Water quality criteria adopted in state water quality standards could have the same numerical values as criteria developed under Section 304, of the Clean Water Act. However, in many situations states might want to adjust water quality criteria developed under Section 304 to reflect local environmental conditions and human exposure patterns. Alternatively, states may use different data and assumptions than EPA in deriving numeric criteria that are scientifically defensible and protective of designated uses. State water quality standards include both numeric and narrative criteria. A state may adopt a numeric criterion within its water quality standards and apply it either state-wide to all waters designated for the use the criterion is designed to protect or to a specific site. A state may use an indicator parameter or the national criterion, supplemented with other relevant information, to interpret its narrative criteria within its water quality standards when developing NPDES effluent limitations under 40 CFR 122.44(d)(1)(vi).2

Site-specific criteria may include not only site-specific criterion concentrations (U.S. EPA 1983b), but also site-specific, and possibly pollutant-specific, durations of averaging periods and frequencies of allowed excursions (U.S. EPA 1991). The averaging periods of "one hour" and "four days" were selected by the U.S. EPA on the basis of data concerning how rapidly some aquatic species react to increases in the concentrations of some aquatic pollutants, and "three years" is the Agency's best scientific judgment of the average amount of time aquatic ecosystems should be provided between excursions (Stephan et al. 1985; U.S. EPA 1991). However, various species and ecosystems react and recover at greatly differing rates. Therefore, if adequate justification is provided, site-specific and/or pollutant-specific concentrations, durations, and frequencies may be higher or lower than those given in national water quality criteria for aquatic life.

Use of criteria, which have been adopted in state water quality standards, for developing water quality-based permit limits and for designing waste treatment facilities requires selection of an appropriate wasteload allocation model. Although dynamic models are preferred for the application of these criteria (U.S. EPA 1991), limited data or other considerations might require the use of a steady-state model (U.S. EPA 1986).

Guidance on mixing zones and the design of monitoring programs is also available (U.S. EPA 1987, 1991).

Table 1. Acute Toxicity of 2,4-Dimethylphenol to Aquatic Animals

Species	Method[a]	Chemical[b]	pH	LC50 or EC50 (µg/L)	Species Mean Acute Value (µg/L)	Reference
			FRESHWATER SPECIES			
Coelenterate, Hydra oligactis	F, M	-	7.0-7.8	62,500	62,500	Sabourin 1987
Annelid, Lumbriculus variegatus	F, M	-	7.0-7.8	67,600	67,600	Sabourin 1987
Snail (adult), Aplexa hypnorum	F, M	99%	7.7	41,600	41,600	Holcombe and Phipps 1987
Cladoceran (<24 hr), Ceriodaphnia dubia	S, M	97%	7.6	3,340	3,340	Spehar 1987
Cladoceran (<24 hr), Daphnia magna	S, U	>80%	7.0	2,100	-	LeBlanc 1980
Cladoceran (<24 hr), Daphnia magna	S, U	-	7.7	2,370	-	Randall and Knopp 1980
Cladoceran (<24 hr), Daphnia magna	F, M	97%	7.7	4,800	4,800	Holcombe and Phipps 1987
Glass shrimp (adult), Palaemonetes kadiakensis	F, M	97%	7.7	16,000	16,000	Holcombe and Phipps 1987
Crayfish (adult), Orconectes immunis	F, M	97%	7.7	36,300	36,300	Holcombe and Phipps 1987
Midge (3rd & 4th instar), Tanytarsus dissimilis	F, M	97%	7.7	33,400	33,400	Holcombe and Phipps 1987
Rainbow trout (juvenile), Oncorhynchus mykiss	F, M	97%	7.7	9,200	9,200	Holcombe and Phipps 1987

Table 1. (continued)

Species	Method[a]	Chemical[b]	pH	LC50 or EC50 (µg/L)	Species Mean Acute Value (µg/L)	Reference
Fathead minnow (30-35 day), Pimephales promelas	F, M	-	7.6-9.1	17,000	-	Phipps et al. 1981
Fathead minnow (juvenile), Pimephales promelas	F, M	97%	7.7	18,100	17,359	Holcombe and Phipps 1987
Channel catfish, Ictalurus punctatus	F, M	-	7.0-7.8	19,300	19,300	Sabourin 1987
Bluegill (juvenile), Lepomis macrochirus	S, U	>80%	6.7-7.4	7,800	-	Buccafusco et al. 1981
Bluegill (juvenile), Lepomis macrochirus	F, M	97%	7.7	6,300	6,300	Holcombe and Phipps 1987
			SALTWATER SPECIES			
Archiannelid worm (adult), Dinophilus gyrociliatus	R, U	100%	7.7-7.9	55,900	55,900	Thursby and Berry 1987a
Eastern oyster (embryo), Crassostrea virginica	S, U	100%	7.7-8.0	8,500	8,500	Thursby and Berry 1987a
Mysid (juvenile), Mysidopsis bahia	R, U	100%	8.2-8.3	1,600	1,600	Thursby and Berry 1987a
Amphipod (sub-adult), Ampelisca abdita	R, U	100%	8.0-8.1	10,500	10,500	Redmond and Scott 1987a
Grass shrimp (larva), Palaemonetes pugio	R, U	100%	7.9-8.0	4,800	4,800	Thursby and Berry 1987a

Table 1. (continued)

Species	Method[a]	Chemical[b]	pH	LC50 or EC50 (μg/L)	Species Mean Acute Value (μg/L)	Reference
SALTWATER SPECIES						
Sea urchin (embryo-larva), Arbacia punctulata	S, U	100%	7.9-8.0	21,400	21,400	Thursby and Berry 1987a
Sheepshead minnow (juvenile), Cyprinodon variegatus	R, U	100%	8.0-8.1	11,800	11,800	Thursby and Berry 1987a
Inland silverside (juvenile), Menidia beryllina	F, M	100%	7.5	1,320	1,320	Thursby and Berry 1987a
Winter flounder (larva), Pseudopleuronectes americanus	S, U	100%	8.0-8.4	>40,000	>40,000	Thursby and Berry 1987a

[a] S = static; R = renewal; F = flow-through; M = measured; U = unmeasured.

[b] Purity of the test chemical.

Table 2. Chronic Toxicity of 2,4-Dimethylphenol to Aquatic Animals

Species	Test[a]	Chemical[b]	pH	Chronic Limits (μg/L)[c]	Chronic Value (μg/L)	Reference
FRESHWATER SPECIES						
Cladoceran, *Ceriodaphnia dubia*	LC	97%	7.8	810-1,870	1,230	Spehar 1987
Fathead minnow, *Pimephales promelas*	ELS	Reagent Grade	7.2-7.9	1,970-3,110	2,475	Holcombe et al. 1982
Fathead minnow, *Pimephales promelas*	ELS	-	-	1,500-3,200	2,200	LeBlanc 1984
Fathead minnow, *Pimephales promelas*	ELS	97%	7.4	398-605	491	Russom 1993
SALTWATER SPECIES						
Inland silverside, *Menidia beryllina*	ELS	100%	7.1-7.5	131-296	196.9	Thursby and Berry 1987b

[a] LC = life-cycle or partial life-cycle; ELS = early life-stage.
[b] Purity of the test chemical.
[c] Results are based on measured concentrations of 2,4-dimethylphenol.

Table 2. (continued)

Acute-Chronic Ratio

Species	pH	Acute Value (µg/L)	Chronic Value (µg/L)	Ratio
Cladoceran, *Ceriodaphnia dubia*	7.6-7.8	3,340	1,230	2.715
Fathead minnow, *Pimephales promelas*	7.2-9.1	17,000	2,475	6.869
Inland silverside, *Menidia beryllina*	-	1,320	196.9	6.704

Table 3. Ranked Genus Mean Acute Values with Species Mean Acute-Chronic Ratios

Rank[a]	Genus Mean Acute Value ($\mu g/L$)	Species	Species Mean Acute Value ($\mu g/L$)[b]	Species Mean Acute-Chronic Ratio[c]
		FRESHWATER SPECIES		
12	67,600	Annelid, *Lumbriculus variegatus*	67,600	-
11	62,500	Coelenterate, *Hydra oligactis*	62,500	-
10	41,600	Snail, *Aplexa hypnorum*	41,600	-
9	36,300	Crayfish, *Orconectes immunis*	36,300	-
8	33,400	Midge, *Tanytarsus dissimilis*	33,400	-
7	19,300	Channel catfish, *Ictalurus punctatus*	19,300	-
6	17,359	Fathead minnow, *Pimephales promelas*	17,359	6.869
5	16,000	Glass shrimp, *Palaemonetes kadiakensis*	16,000	-
4	9,200	Rainbow trout, *Oncorhynchus mykiss*	9,200	-
3	6,300	Bluegill, *Lepomis macrochirus*	6,300	-
2	4,800	Cladoceran, *Daphnia magna*	4,800	-

Table 3. (continued)

Rank[a]	Genus Mean Acute Value (µg/L)	Species	Species Mean Acute Value (µg/L)[b]	Species Mean Acute-Chronic Ratio[c]
1	3,340	Cladoceran, *Ceriodaphnia dubia*	3,340	2.715
		SALTWATER SPECIES		
9	55,900	Archiannelid worm, *Dinophilus gyrociliatus*	55,900	-
8	>40,000	Winter flounder, *Pseudopleuronectes americanus*	>40,000	-
7	21,400	Sea urchin, *Arbacia punctulata*	21,400	-
6	11,800	Sheepshead minnow, *Cyprinodon variegatus*	11,800	-
5	10,500	Amphipod, *Ampelisca abdita*	10,500	-
4	8,500	Eastern oyster, *Crassostrea virginica*	8,500	-
3	4,800	Grass shrimp, *Palaemonetes pugio*	4,800	-
2	1,600	Mysid, *Mysidopsis bahia*	1,600	-

Table 3. (continued)

Rank[a]	Genus Mean Acute Value (µg/L)	Species	Species Mean Acute Value (µg/L)[b]	Species Mean Acute-Chronic Ratio[c]
1	1,320	Inland silverside, Menidia beryllina	1,320	6.704

[a] Ranked from most resistant to most sensitive based on Genus Mean Acute Value.
[b] From Table 1.
[c] From Table 2.

Fresh water

Final Acute Value = 2,670 µg/L

Criterion Maximum Concentration = (2,670 µg/L) / 2 = 1,335 µg/L

Final Acute-Chronic Ratio = 5.000 (see text)

Final Chronic Value = (2,670 µg/L) / 5.000 = 534 µg/L

Salt water

Final Acute Value = 548.8 µg/L

Criterion Maximum Concentration = (548.8 µg/L) / 2 = 274.4 µg/L

Final Acute-Chronic Ratio = 5.000 (see text)

Final Chronic Value = (548.8 µg/L) / 5.000 = 109.8 µg/L

Table 4. Toxicity of 2,4-Dimethylphenol to Aquatic Plants

Species	Chemical[a]	pH	Duration (days)	Effect	Concentration (µg/L)	Reference
FRESHWATER SPECIES						
Alga, *Scenedesmus quadricauda*	-	7.5-7.8	4	Incipient inhibition	40,000	Bringman and Kuhn 1959a,b
SALTWATER SPECIES						
(No acceptable plant data for saltwater species)						

[a] Purity of test chemical.

Table 5. Bioaccumulation of 2,4-Dimethylphenol by Aquatic Organisms

Species	pH	Concentration in Water (μg/L)[a]	Duration (days)	Tissue	Percent Lipids	BCF or BAF[b]	Normalized BCF or BAF[c]	Reference
FRESHWATER SPECIES								
Bluegill, *Lepomis macrochirus*	7.1	10.2	28	Whole body	-	150	-	Barrows et al. 1980; Veith et al. 1980
SALTWATER SPECIES								
(No acceptable bioaccumulation data for saltwater species)								

[a] Measured concentration of 2,4-dimethylphenol.
[b] Bioconcentration factors (BCFs) and bioaccumulation factors (BAFs) are based on measured concentrations of radiolabel in water and in tissue.
[c] When possible, the factors were normalized to 1% lipids by dividing the BCFs and BAFs by the percent lipids.

Table 6. Other Data on Effects of 2,4-Dimethylphenol on Aquatic Organisms

Species	Chemical[a]	pH	Duration	Effect	Concentration (µg/L)	Reference
FRESHWATER SPECIES						
Bacteria, Escherichia coli	-	7.5	6-48 hr	Incipient inhibition	>100,000	Bringman and Kuhn 1959a
Alga, Chlorella pyrenoidosa	-	7.0	72 hr	52% reduction in Chlorophyll a	100,000	Huang and Gloyna 1967,1968
Protozoa, Tetrahymena pyriformis	-	-	60 hr	EC50 (cell number)	130,510	Schultz and Riggin 1985
Protozoa, Microregma heterostoma	-	7.5-7.8	28 hr	Incipient inhibition	70,000	Bringman and Kuhn 1959b
Cladoceran (<24 hr), Ceriodaphnia dubia	97%	7.5-7.9	48 hr	LC50 (fed)	3,400	Norberg-King 1987
Cladoceran (<24 hr), Ceriodaphnia dubia	97%	7.4-7.8	48 hr	LC50 (fed)	3,100	Norberg-King 1987
Cladoceran (<24 hr), Ceriodaphnia dubia	97%	7.5-7.8	48 hr	LC50 (fed)	6,300	Norberg-King 1987
Cladoceran (<24 hr), Ceriodaphnia dubia	97%	7.5-7.8	48 hr	LC50 (fed)	5,400	Norberg-King 1987
Cladoceran (<24 hr), Ceriodaphnia dubia	97%	7.7-8.0	48 hr	LC50 (fed)	5,400	Norberg-King 1987
Cladoceran (<24 hr), Ceriodaphnia dubia	97%	7.7-8.0	48 hr	LC50 (fed)	5,400	Norberg-King 1987
Cladoceran (<24 hr), Ceriodaphnia dubia	97%	7.6-8.0	48 hr	LC50 (fed)	5,400	Norberg-King 1987

Table 6. (continued)

Species	Chemical[a]	pH	Duration	Effect	Concentration (µg/L)	Reference
			FRESHWATER SPECIES			
Cladoceran (<24 hr), Ceriodaphnia dubia	97%	7.8	48 hr	LC50 (fed)	3,540	Spehar 1987
Cladoceran (<24 hr), Daphnia magna	-	7.5-7.8	48 hr	Immobilization	24,000 1959a,b	Bringman and Kuhn
Rainbow trout (600-1,000g), Oncorhynchus mykiss	97%	7.7	3.5 to 17.3 hr	Mean survival time of 6.0 hr	9,040	Bradbury et al. 1989
Rainbow trout (600-1,000g), Oncorhynchus mykiss	97%	7.7	3.5 to 17.3 hr	Significant increase in cough frequency and decreases in gill oxygen uptake efficiency, total blood carbon dioxide (arterial), and hematocrit	9,040	Bradbury et al. 1989
Fathead minnow, Pimephales promelas	-	7.6-9.1	8 day	LC50	14,000	Phipps et al. 1981
Fathead minnow, Pimephales promelas	-	7.6-9.1	8 day	LC50	13,000	Phipps et al. 1981
			SALTWATER SPECIES			
Brown kelp (5-day old plants), Laminaria saccharina	100%	-	2 day exposure, 5-7 day recovery period	Sporophytes reduced; no effect at 7,200 µg/L	12,000	Thursby and Steel 1987
Brown kelp (7-8 day old plants), Laminaria saccharina	100%	-	2 day exposure, 5-7 day recovery period	Sporophytes reduced; no effect at 7,200 µg/L	12,000	Thursby and Steel 1987

[a] Purity of test chemical.

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AMBIENT AQUATIC LIFE WATER QUALITY CRITERIA FOR

ANILINE

(CAS Registry Number 62-53-3)

SEPTEMBER 1993

U.S. ENVIRONMENTAL PROTECTION AGENCY

OFFICE OF WATER
OFFICE OF SCIENCE AND TECHNOLOGY
HEALTH AND ECOLOGICAL CRITERIA DIVISION
WASHINGTON, D.C.

OFFICE OF RESEARCH AND DEVELOPMENT
ENVIRONMENTAL RESEARCH LABORATORIES
DULUTH, MINNESOTA
NARRAGANSETT, RHODE ISLAND

NOTICES

This document has been reviewed by the Environmental Research Laboratories, Duluth, MN and Narragansett, RI, Office of Research and Development and the Health and Ecological Criteria Division, Office of Science and Technology, U.S. Environmental Protection Agency, and approved for publication.

This document is available to the public through the National Technical Information Service (NTIS), 5285 Port Royal Road, Springfield, VA 22161.

FOREWORD

Section 304(a) (1) of the Clean Water Act of 1977 (P.L. 95-217) requires the Administrator of the Environmental Protection Agency to publish water quality criteria that accurately reflect the latest scientific knowledge on the kind and extent of all identifiable effects on health and welfare that might be expected from the presence of pollutants in any body of water, including ground water. This document is a revision of proposed criteria based upon consideration of comments received from other federal agencies, state agencies, special interest groups, and individual scientists. Criteria contained in this document replace any previously published EPA aquatic life criteria for the same pollutant(s).

The term "water quality criteria" is used in two sections of the Clean Water Act, section 304(a)(1) and section 303(c)(2). The term has a different program impact in each section. In section 304, the term represents a non-regulatory, scientific assessment of ecological effects. Criteria presented in this document are such scientific assessments. If water quality criteria associated with specific stream uses are adopted by a state as water quality standards under section 303, they represent maximum acceptable pollutant concentrations in ambient waters within that state that are enforced through issuance of discharge limitations in NPDES permits. Water quality criteria adopted in state water quality standards could have the same numerical values as criteria developed under section 304. However, in many situations states might want to modify water quality criteria developed under section 304 to reflect local environmental conditions and human exposure patterns. Alternatively, states may use different data and assumptions than EPA in deriving numeric criteria that are scientifically defensible and protective of designated uses. It is not until their adoption as part of state water quality standards that criteria become regulatory. Guidelines to assist the states and Indian tribes in modifying the criteria presented in this document are contained in the Water Quality Standards Handbook (December 1983). This handbook and additional guidance on the development of water quality standards and other water-related programs of this Agency have been developed by the Office of Water.

This document, if finalized, would be guidance only. It would not establish or affect legal rights or obligations. It would not establish a binding norm and would not be finally determinative of the issues addressed. Agency decisions in any particular situation will be made by applying the Clean Water Act and EPA regulations on the basis of specific facts presented and scientific information then available.

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CONTENTS

TABLES

Introduction

Aniline (aminobenzene, benzenamine, phenylamine) is the simplest of the aromatic amines ($C_6H_5NH_2$). It occurs naturally in coal-tars (Shelford 1917) and is manufactured by the catalytic reduction of nitrobenzene, amination of chlorobenzene and ammonolysis of phenol.

The major users of aniline are the polymer, rubber, agricultural and dye industries. Demand for aniline by the dye industry was high prior to the 1970's but decreased markedly in the United States thereafter because of the increased use of synthetic fabrics. Aniline is used today primarily by the polymer industry to manufacture products such as polyurethanes. The rubber industry uses large amounts of aniline to manufacture antioxidants, antidegradants and vulcanization accelerators. The pharmaceutical industry uses aniline in the manufacture of sulfa drugs and other products. Important agricultural uses for aniline derivatives include herbicides, fungicides, insecticides, repellents and defoliants. Aniline has also been used as an antiknock compound in gasolines (Kirk-Othmer 1982).

Aniline is soluble in water up to 34,000,000 µg/L (Verschueren 1977). The $\log_{10}$ of the octanol-water partition coefficient for aniline is 0.90 (Chiou 1985a). Through direct disposal, such as industrial discharges and non-point sources associated with agricultural uses, it enters the aquatic environment. It is removed from the aquatic environment by several mechanisms. The major pathway of removal from water is by microbial decomposition (Lyons et al. 1984, 1985). Several minor pathways have been identified including evaporation, binding to humic substances and autoxidation.

Additions to the aniline molecule of certain functional groups have been found to increase toxicity (Brooke et al. 1984; Geiger et al. 1986, 1987). Tests with the fathead minnow (*Pimephales promelas*) have demonstrated that substitutions with halogens, (chlorine, fluorine, and bromine) increased toxicity. The addition of alkyl groups also increased toxicity; the toxicity increases in proportion to the increase in chain length. Twenty-four substitutions were tested and all except *para* additions of methyl and nitro groups increased the toxicity to the fathead minnow.

All concentrations reported herein are expressed as aniline. Results of such intermediate calculations as recalculated LC50's and Species Mean Acute Values are given to four significant figures to prevent round-off error in subsequent calculations, not to reflect the precision of the value. Whenever adequately justified, a national criterion may be replaced by a site-specific criterion (U.S. EPA 1983a) that may include not only site-specific concentrations (U.S. EPA 1983b) but also site-specific frequencies of allowed excursion (U.S. EPA 1985).

A comprehension of the "Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses" (Stephan et al. 1985), hereinafter referred to as the Guidelines, and the response to public comment (U.S. EPA 1985), is necessary to understand the following text, tables, and calculations. The latest comprehensive literature search for information for this document was conducted in September 1992; some more recent information is included.

Acute toxicity to Aquatic Animals

The data that are available according to the Guidelines concerning the acute toxicity of aniline are presented in Table 1. Cladocera were the most sensitive group of the 19 species tested. Several species of larval midges and embryos and larvae of the clawed toad, Xenopus laevis, were the most resistant to aniline in acute exposures. Fish tended to be in the mid-range of sensitivity for aquatic organisms.

Forty-eight-hour EC50s for the cladocerans Ceriodaphnia dubia and Daphnia magna were 44 µg/L and 530 µg/L, respectively. Several independent exposures conducted with both species showed consistency among the tests (Table 1). However, there appears to be a large increase in tolerance of aniline between cladocerans and other aquatic species. The 96-hr LC50 for the next most sensitive species, a planarian, Dugesia tigrina, was 31,600 µg/L.

Ninety-six-hour LC50s for fish ranged from 10,600 to 187,000 µg/L. The rainbow trout (<u>Oncorhyncus mykiss</u>) was the most sensitive species of fish tested, with 96-hr LC50s ranging from 10,600 to 41,000 µg/L. The bluegill (<u>Lepomis macrochirus</u>) was slightly more tolerant of aniline with a 96-hr LC50 of 49,000 µg/L. Fathead minnows, <u>Pimephales promelas</u>, and goldfish, <u>Carassius auratus</u>, were the most tolerant of aniline of the fish species tested. Ninety-six-hour LC50s for tests with fathead minnows ranged from 32,000 to 134,000 µg/L. A 96-hr LC50 for the goldfish was 187,000 µg/L.

Franco et al. (1984) exposed four species of midge larvae to aniline and found them to be the most tolerant of aniline of all species tested. The midge, <u>Clinotanypus pinguis</u>, was the most tolerant of the four species tested; a 48-hr LC50 of 477,900 µg/L was calculated for this species. LC50s for other midge species tested by Franco et al. (1984), ranged downward to 272,100 µg/L. Holcombe et al. (1987) tested another species of midge (<u>Tanytarsus dissimilis</u>) and reported a 48-hr LC50 >219,000 µg/L.

The African clawed frog, <u>Xenopus laevis</u>, was relatively tolerant of aniline. In a series of three tests, Davis et al. (1981) found that embryos of African clawed frogs were more tolerant than the larvae. The 96-hr LC50s for embryos and tailbud embryos were 550,000 and 940,000 µg/L, respectively, compared to 150,000 µg/L for the larvae.

Genus Mean Acute Values (GMAVs) are ranked from most sensitive to most resistant for the nineteen freshwater genera tested (Table 3). The freshwater Final Acute Value (FAV) of 56.97 µg/L was calculated using the GMAVs for the four most sensitive genera, <u>Ceriodaphnia</u>, <u>Daphnia</u>, <u>Dugesia</u>, and <u>Oncorhynchus</u> which differ from one another within a factor of 251. The Final Acute Value is 2.2 times less than the acute value for the most sensitive freshwater species.

The acute toxicity of aniline to resident North American saltwater animals has been determined with five species of invertebrates and three species of fish (Thursby and Berry 1987a, 1987b; Redmond and Scott 1987; Table 1). Grass shrimp, tested as larvae, was the most sensitive species based on an acute value of 610 µg/L. Crustaceans comprised the three most sensitive species tested; acute values ranged from 610 to 16,600 μg/L. Acute values for three fishes, a mollusc and an echinoderm ranged from 17,400 to >333,000 μg/L. Mortalities in acute tests with mysids, grass shrimp, sheepshead minnows and inland silversides increased during 96-hr tests. GMAVs are ranked from the most sensitive to the most resistant (Table 3) for the eight saltwater genera tested. The Final Acute Value for saltwater species is 153.4 μg/L which is four times less than the acute value for the most sensitive saltwater species tested.

Chronic Toxicity to Aquatic Animals

The data that are available according to the Guidelines concerning the chronic toxicity of aniline are presented in Table 2. Four chronic toxicity tests exposing freshwater organisms to aniline have been reported. The cladoceran, Ceriodaphnia dubia, was exposed to initial concentrations ranging from 1.07 to 26.5 μg/L for seven days with daily renewed exposures (Spehar 1987). Survival was not significantly affected at any exposure concentration; however, effects on young production were observed at 12.7 μg/L, but not at 8.1 μg/L. The chronic value, based upon reproductive impairment, is 10.1 μg/L. This number may be under-protective since it is based upon initial measured concentrations of aniline and did not take into consideration that the study showed nearly 100% loss of aniline from solution in 24 hr. A companion acute test was conducted with the chronic study and resulted in a 48-hr EC50 of 44 μg/L. Division of this value by the chronic value generates an acute-chronic ratio of 4.356 for Ceriodaphnia dubia.

Daphnia magna were exposed to aniline for 21 days in a renewal test (Gersich and Milazzo 1988). Mean concentrations for the exposures ranged from 12.7 to 168.6 μg/L for the five concentrations tested. Mean total young/surviving adult and mean brood size/surviving adult were not significantly different from the control organisms at 24.6 μg/L but were significantly different at 46.7 μg/L. Based upon these two reproduction endpoints, the chronic value is 33.9 μg/L. The companion acute value (48-hr

EC50) used to compute an acute-chronic ratio was 170 µg/L (Gersich and Mayes, 1986). Division of this value by the chronic value of 33.9 µg/L results in an acute-chronic ratio of 5.015.

A 90-day early life-stage test was conducted with rainbow trout (Spehar 1987). The test was started with newly fertilized embryos. After 56 days (swim-up stage), wet weight was significantly reduced at concentrations of 4,000 µg/L and above. After 90 days of exposure, an effect was not seen at 4,000 µg/L but weight was reduced at 7,800 µg/L. Survival was reduced at only the highest exposure concentration (15,900 µg/L). The chronic value for rainbow trout is 5,600 µg/L, based upon growth. Spehar (1987) also conducted a 96-hr acute test which resulted in an acute value of 30,000 µg/L. Division of the acute value by the chronic value generates an acute-chronic ratio of 5.357.

The fathead minnow was exposed to aniline concentrations that ranged from 316 to 2,110 µg/L in 32-day exposures (Russom 1993). Percentage normal fry at hatch and survival at the end of the test did not differ significantly from the control fish at any aniline concentrations. Growth (weight and length) was significantly ($p<0.05$) reduced at aniline concentrations of 735 µg/L and greater, but not at 422 µg/L. Wet weight was reduced by 13.3% and total length by 6.4% compared to control fish wet weight and total length at 735 µg/L. The chronic value for this test, based upon growth, is 557 µg/L. The companion acute test resulted in a 96-hr LC50 of 112,000 µg/L (Geiger et al. 1990). Division of this value by the chronic value results in an acute-chronic ratio of 201.1.

The only chronic toxicity test with aniline and saltwater species was conducted with the mysid, Mysidopsis bahia (Thursby and Berry 1987b). Ninety-five percent of the mysids exposed during a life-cycle test to 2,400 µg/L died and no young were produced by the survivors. Reproduction of mysids in 1,100 µg/L was reduced 94 percent relative to controls. No significant effects were detected on survival, growth, or reproduction in mysids exposed to ≤540 µg/L for 28 days. The chronic value for this species is 770.7 µg/L, based upon reproductive impairment. A comparison acute test was conducted with the chronic test which resulted in an acute value of 1,930 µg/L. Division of this value by the chronic value results in an acute-chronic ratio of 2.504.

The Final Acute-Chronic Ratio of 4.137 is the geometric mean of the acute-chronic ratios of 4.356 for the freshwater cladoceran, Ceriodaphnia dubia, 5.015 for the freshwater cladoceran, Daphnia magna, 5.357 for the rainbow trout, Oncorhynchus mykiss, and 2.504 for the saltwater mysid, Mysidopsis bahia (Table 2). The acute-chronic ratio of 201.1 for the fathead minnow was not used in this calculation because, as described in the Guidelines, this species is not acutely sensitive to aniline and its Species Mean Acute Value is not close to the Final Acute Value (Table 3). Division of the freshwater Final Acute Value of 56.97 µg/L by 4.137 results in a freshwater Final Chronic Value of 13.77 µg/L. Division of the saltwater Final Acute Value of 153.4 µg/L by 4.137 results in a saltwater Final Chronic Value of 37.08 µg/L. The freshwater Final Chronic Value is approximately 1.4 times greater than the lowest freshwater chronic value of 10.1 µg/L for Ceriodaphnia dubia. The saltwater Final Chronic Value is a factor of 21 times less than the only saltwater chronic value of 770.7 µg/L.

Toxicity to Aquatic Plants

Results of tests with two species of freshwater green alga exposed to aniline are shown in Table 4. Sensitivity to aniline differed between the two species. Four-day exposures with aniline and Selenastrum capricornutum showed that the EC50s ranged from 1,000 µg/L (Adams et al. 1986) to 19,000 µg/L (Calamari et al. 1980, 1982) with reduced growth as the effect. Slooff (1982) determined an EC50 of 20,000 µg/L for an unidentified species of Selenastrum with reduced biomass as the effect. The studies by Adams et al. (1986) were conducted both with and without a carrier solvent (acetone). The lowest 96-hr EC50s were obtained from exposures using acetone. However, this relationship was reversed when the exposure duration was increased to five and six days

(Table 4). The green alga, Chlorella vulgaris, is considerably more tolerant to aniline than Selenastrum. In 14-day exposures, growth of C. vulgaris was reduced 58% by 306,000 μg/L and 16% by 184,000 μg/L (Ammann and Terry 1985). The study also demonstrated that aniline had significant effects upon respiration and photosynthesis of the species. There are no acceptable plant data for saltwater species for aniline. A Final Plant Value, as defined in the Guidelines, cannot be obtained for aniline.

Bioaccumulation

Studies to determine the bioconcentration of aniline with three species of organisms have been reported (Table 5). In all these studies, steady-state bioconcentrations were not demonstrated. Daphnia magna bioconcentrated aniline five times in a 24-hr exposure (Dauble et al. 1984, 1986), a green alga 91 times in a 24- to 25-hr exposure (Hardy et al. 1985) and rainbow trout 507 times in a 72-hr exposure (Dauble et al. 1984). Because tests were not of sufficient duration according to the Guidelines, and no U.S. FDA action level or other maximum acceptable concentration in tissue is available for aniline, no Final Residue Value can be calculated.

Other Data

Other data available concerning aniline toxicity are presented in Table 5. Effects on two species of bacteria were seen at aniline concentrations ranging from 30,000 to 130,000 μg/L.

Three genera of algae were exposed to aniline. One species of bluegreen algae, Microcystis aeruginosa, (Bringmann and Kuhn 1976, 1978a,b), showed more sensitivity to aniline than other species. Inhibition of cell replication of this species was observed after an 8-day exposure to 160 μg/L. Fitzgerald et al. (1952) reported a 24-hr LC50 of 20,000 μg/L with the same species. A 66% reduction of photosynthesis by the green algae, Selenastrum capricornutum, was reported by Giddings (1979) after a 4-hr exposure to 100,000 μg/L of aniline.

Several species of protozoans were exposed to aniline. A 28-hr aniline exposure with Microreqma heterostoma showed that food ingestion was reduced at 20,000 µg/L (Bringmann and Kuhn 1959a). Other species of protozoa were tested and showed less sensitivity to aniline (Table 5).

The hydrazoan, Hydra oligactis, showed sensitivity to aniline in a 48-hr test. The LC50 for this species of 406 µg/L was determined by Slooff (1983) in a static, unmeasured test using river water. Other organisms such as planarians (Dugesia lugubris), tubificid worms (Tubificidae), and snails (Lymnea stagnalis) were also tested and had much higher 48-hr LC50s of 155,000, 450,000 and 800,000 µg/L, respectively.

Cladocera appeared to be the group most sensitive to aniline. Spehar (1987) reported a 48-hr LC50 of 132 µg/L for Ceriodaphnia dubia in an exposure in which the organisms were fed their culturing ration. In the same study, a LC50 of 44 µg/L was determined for unfed Ceriodaphnia dubia. The difference in results could have been due to the complexation of aniline by the food and/or increased hardiness of the fed organisms. Daphnia magna was affected (acoustic reaction and mortality) at aniline concentrations ranging from 400 to 2,000 µg/L (Bringmann and Kuhn 1959a,b, 1960; Lakhnova 1975) for 48-hr exposures. Calamari et al. (1980, 1982) found this species to be more resistant to aniline with a reported 24-hr EC50 of 23,000 µg/L.

Insects showed varying sensitivities to aniline. Puzikova and Markin (1975) exposed the midge, Chironomus dorsalis, to aniline through its complete life cycle and reported 100% survival at 3,000 µg/L and 5% survival at 7,800 µg/L. Slooff (1983) exposed mayfly and mosquito larvae to aniline for 48 hr and reported LC50s of 220,000 and 155,000 µg/L, respectively.

The toxicity values for rainbow trout in Table 5 are in general agreement with those used in Table 1. Rainbow trout were exposed to aniline by several workers using different exposure durations. Shumway and Palensky (1973) found 100% mortality of rainbow trout at 100,000 µg/L in a 48-hr exposure and 100% survival at 10,000 µg/L. Lysak and Marcinek (1972) also reported 100% mortality for a 24-hr exposure at 21,000 µg/L and observed no mortality at 20,000 µg/L. Abram and Sims (1982) determined the 7-day LC50 to be 8,200 μg/L in two separate tests using rainbow trout.

Several tests were run with aniline in dilution waters of different water quality. Water hardness appeared to have little, if any, impact on aniline toxicity (Birge et al. 1979a,b). Young channel catfish, Ictalurus punctatus, were exposed to aniline in waters with a four-fold difference in hardness (53.3 and 197.5 mg/L as $CaCO_3$). The resulting LC50s indicated only a slight decrease in toxicity with increasing hardness. In a similar test they also exposed goldfish and largemouth bass, Micropterus salmoides, and reported the opposite effect on toxicity. pH does not appear to affect toxicity of aniline with aquatic organisms (Table 5).

The African clawed frog demonstrated varied effects over a broad range of concentrations of aniline. Davis et al. (1981) and Dumpert (1987) observed that aniline concentrations of 50 and 70 μg/L resulted in reduced epidermal pigmentation or failure of larvae to develop normal pigmentation. In a 12-week exposure, Dumpert (1987) showed that 1,000 μg/L of aniline slowed metamorphosis and reduced growth. At an exposure concentration of 10,000 μg/L for 96-hr, 6% of the frog larvae developed abnormalities (Dumont et al. 1979; Davis et al. 1981). Frog embryos had 50% teratogeny in 120- and 96-hr exposures at 91,000 and 370,000 μg/L, respectively (Table 5). One hundred percent mortality of immature frogs occurred during a 12-day exposure to 90,000 μg/L (Dumpert 1987) and 50% mortality during a 48-hr exposure to 560,000 μg/L (Slooff 1982; Slooff and Baerselman 1980).

Concentrations of the free amino acids aspartate, glutamate and alanine in the sea anemone, Bunodosoma cavernata, increased after seven days of exposure to aniline at 500,000 μg/L (Kasschau et al. 1980; Table 5). The lethal threshold (geometric mean of the highest concentration with no mortality and the next higher concentration) was 29,400 μg/L for sand shrimp, Crangon septemspinosa, and >55,000 for soft-shelled clams, Mya arenaria (McLeese et al. 1979).

Unused Data

Some data on the effects of aniline on aquatic organisms were not used because the studies were conducted with species that are not resident in North America or Hawaii (Freitag et al. 1984; Hattori et al. 1984; Inel and Atalay 1981; Juhnke and Ludemann 1978; Lallier 1971; Slooff and Baerselman 1980; Tonogai et al. 1982; Yoshioka et al. 1986a). Chiou (1985b); Hermens et al. (1985); Hodson (1985); Koch (1986); Newsome et al. (1984); Persson (1984); Schultz and Moulton (1984); Slooff et al. (1983); Vighi and Calamari (1987) compiled data from other sources. Results were not used where the test procedures or test material were not adequately described (Buzzell et al. 1968; Canton and Adema 1978; Carlson and Caple 1977; Clayberg 1917; Demay and Menzies 1982; Kuhn and Canton 1979; Kwasniewska and Kaiser 1984; Pawlaczyk-Szpilowa et al. 1972; Sayk and Schmidt 1986; Shelford 1917; Wellens 1982). Data were not used when aniline was part of a mixture (Giddings and Franco 1985; Lee et al. 1985; Winters et al. 1977) or when the organisms were exposed to aniline in food (Lee et al. 1985; Loeb and Kelly 1963).

Babich and Borenfreund (1988), Batterton et al. (1978), Bols et al. (1985); Buhler and Rasmusson (1968), Carter et al. (1984), Elmamlouk et al. (1974), Elmamlouk and Gessner (1976), Fabacher (1982), Lindstrom-Seppa et al. (1983), Maemura and Omura (1983), Pedersen et al. (1976), Sakai et al. (1983), and Schwen and Mannering (1982) exposed only enzymes, excised or homogenized tissue, or cell cultures. Anderson (1944), and Bringmann and Kuhn (1982) cultured organisms in one water and conducted tests in another. Batterton et al. (1978) conducted a study in which organisms were not tested in water but were tested on agar in the "algal lawn" test.

Results of one laboratory test were not used because the test was conducted in distilled or deionized water without addition of appropriate salts (Mukai 1977). Results of laboratory bioconcentration tests were not used when the test was not flow-through or renewal (Freitag et al. 1985; Geyer et al. 1981; Geyer et al. 1984) and BCFs obtained from microcosm or model ecosystem studies were not used where the concentration of aniline in water decreased with time (Lu and Metcalf 1975; Yount and Shannon 1987). Douglas et al. (1986) had insufficient mortalities to calculate an LC50 and Sollmann (1949) conducted studies without control exposures.

<u>Summary</u>

Data on the acute toxicity of aniline are available for nineteen species of freshwater animals. Cladocera were the most acutely sensitive group tested. Mean 48-hr EC50s ranged from 125.8 μg/L for <u>Ceriodaphnia dubia</u> to 250 μg/L for <u>Daphnia magna</u>. The planarian, <u>Dugesia tigrina</u>, was the fourth most sensitive species to aniline with a 96-hr LC50 of 31,600 μg/L.

Freshwater fish 96-hr LC50s ranged from 10,600 to 187,000 μg/L. Rainbow trout, <u>Oncorhynchus mykiss</u>, were the most sensitive fish tested, with species mean acute values of 26,130 μg/L. The bluegill, <u>Lepomis macrochirus</u>, was nearly as sensitive to aniline as rainbow trout, with a 96-hr LC50 of 49,000 μg/L reported for this species. The fathead minnow, <u>Pimephales promelas</u>, and goldfish, <u>Carassius auratus</u>, were the most tolerant fish species exposed to aniline, with species mean acute values of 106,000 μg/L and 187,000 μg/L, respectively.

The most tolerant freshwater species tested with aniline was a midge, <u>Clinotanypus pinguis</u>, with a 48-hr LC50 of 477,000 μg/L. Developmental stages of an amphibian, <u>Xenopus laevis</u>, had differing sensitivities to aniline. The embryos were the most tolerant with a 96-hr LC50 of 550,000 μg/L and the larvae had a 96-hr LC50 of 150,000 μg/L.

Data on the acute toxicity of aniline are available for eight species of saltwater animals. Species Mean Acute Values ranged from >333,000 μg/L for larval winter flounder, <u>Pseudopleuronectes americanus</u>, to 610 μg/L for larval grass shrimp, <u>Palaemonetes pugio</u>. Arthropods appear particularly sensitive to aniline. There are no data to support the derivation of a salinity- or temperature-dependent Final Acute Equation.

Chronic tests have been conducted with four species of freshwater organisms. A chronic value of 10.1 μg/L for the cladoceran, <u>Ceriodaphnia</u>

dubia, was based upon reproductive impairment. A chronic value of 33.9 μg/L for another cladoceran, Daphnia magna, was also based on reproductive impairment. Rainbow trout were exposed for 90 days to aniline and the results showed that survival was reduced at 15,900 μg/L and growth (wet weight) at 7,800 μg/L. The chronic value for trout of 5,600 μg/L was based upon growth. The fathead minnow was exposed for 32 days in an early life-stage test. The chronic value of 557 μg/L was also based upon growth.

One saltwater chronic value was found. A chronic value of 770.7 μg/L for the mysid, Mysidopsis bahia, was based upon reproductive impairment.

Effects due to aniline have been demonstrated with two freshwater plant species. The green alga, Selenastrum capricornutum, had EC50s ranging from 1,000 to 19,000 μg/L in 4-day exposures. Another green alga, Chlorella vulgaris, was considerably more resistant to aniline, showing a growth reduction of 58% by 306,000 μg/L in a 14-day exposure. No acceptable saltwater plant data have been found. Final Plant Values, as defined in the Guidelines, could not be obtained for aniline.

No suitable data have been found for determining the bioconcentration of aniline in freshwater or saltwater organisms.

Acute-chronic ratio data that are acceptable for deriving numerical water quality criteria are available for three species of freshwater animals and one species of saltwater animal. The acute-chronic ratios range from 2.504 to 5.357 with a geometric mean of 4.137.

The freshwater Final Acute Value for aniline is 56.97 μg/L and the Final Chronic Value is 13.77 μg/L. The Freshwater Final Chronic Value is 1.4 times greater than the lowest chronic value observed for one species of Cladocera indicating that sensitive species of this group may not be adequately protected if ambient water concentrations exceed this value. The saltwater Final Acute Value for aniline is 153.4 μg/L and the Final Chronic Value is 37.08 μg/L. Chronic adverse effects to the only saltwater species exposed to aniline occurred at concentrations that are higher than the saltwater Final Chronic Value which should be protective of saltwater organisms.

National Criteria

The procedures described in the "Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses" indicate that, except for certain sensitive species of Cladocera, freshwater organisms and their uses should not be affected unacceptably if the four-day average concentration of aniline does not exceed 14 µg/L more than once every three years on the average and if the one-hour average concentration does not exceed 28 µg/L more than once every three years on the average.

The procedures described in the "Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses" indicate that, except possibly where a locally important species is very sensitive, saltwater organisms and their uses should not be affected unacceptably if the four-day average concentration of aniline does not exceed 37 µg/L more than once every three years on the average and if the one-hour average concentration does not exceed 77 µg/L more than once every three years on the average.

Implementation

As discussed in the Water Quality Standards Regulation (U.S. EPA 1983a) and the Foreword to this document, a water quality criterion for aquatic life has regulatory impact only after it has been adopted in a state water quality standard. Such a standard specifies a criterion for a pollutant that is consistent with a particular designated use. With the concurrence of the U.S. EPA, states designate one or more uses for each body of water or segment thereof and adopt criteria that are consistent with the use(s) (U.S. EPA 1983b, 1987). Water quality criteria adopted in state water quality standards could have the same numerical values as criteria developed under Section 304, of the Clean Water Act. However, in many situations states might want to adjust water quality criteria developed under Section 304 to reflect local environmental conditions and human exposure patterns. Alternatively, states may use different data and assumptions than EPA in deriving numeric criteria that are scientifically defensible and protective of designated uses. State water quality standards include both numeric and narrative criteria. A state may adopt a numeric criterion within its water quality standards and apply it either state-wide to all waters designated for the use the criterion is designed to protect or to a specific site. A state may use an indicator parameter or the national criterion, supplemented with other relevant information, to interpret its narrative criteria within its water quality standards when developing NPDES effluent limitations under 40 CFR 122.44(d)(1)(vi).2

Site-specific criteria may include not only site-specific criterion concentrations (U.S. EPA 1983b), but also site-specific, and possibly pollutant-specific, durations of averaging periods and frequencies of allowed excursions (U.S. EPA 1991). The averaging periods of "one hour" and "four days" were selected by the U.S. EPA on the basis of data concerning how rapidly some aquatic species react to increases in the concentrations of some pollutants, and "three years" is the Agency's best scientific judgment of the average amount of time aquatic ecosystems should be provided between excursions (Stephan et al. 1985; U.S. EPA 1991). However, various species and ecosystems react and recover at greatly differing rates. Therefore, if adequate justification is provided, site-specific and/or pollutant-specific concentrations, durations and frequencies may be higher or lower than those given in national water quality criteria for aquatic life.

Use of criteria, which have been adopted in state water quality standards, for developing water quality-based permit limits and for designing waste treatment facilities requires selection of an appropriate wasteload allocation model. Although dynamic models are preferred for the application of these criteria (U.S. EPA 1991), limited data or other considerations might require the use of a steady-state model (U.S. EPA 1986).

Guidance on mixing zones and the design of monitoring programs is available (U.S. EPA 1987, 1991).

Table 1. Acute Toxicity of Aniline to Aquatic Animals

Species	Method[a]	Chemical[b]	pH	LC50 or EC50 (µg/L)	Species Mean Acute Value µg/L	Reference
			FRESHWATER SPECIES			
Planarian, *Dugesia tigrina*	S,U	Reagent Grade	6.5-8.5	31,600	31,600	Ewell et al. 1986
Annelid, *Lumbriculus variegatus*	S,U	Reagent Grade	6.5-8.5	>100,000	>100,000	Ewell et al. 1986
Snail (adult), *Aplexa hypnorum*	F,M	-	7.4	>219,000	>219,000	Holcombe et al. 1987
Snail, *Helisoma trivolvis*	S,U	Reagent Grade	6.5-8.5	100,000	100,000	Ewell et al. 1986
Cladoceran (<24-hr), *Ceriodaphnia dubia*	S,U	99.5%	7.4-7.9	119	-	Norberg-King 1987
Cladoceran (<24-hr), *Ceriodaphnia dubia*	S,U	99.5%	7.4-7.7	193	-	Norberg-King 1987
Cladoceran (<24-hr), *Ceriodaphnia dubia*	S,U	99.5%	7.4-7.9	146	-	Norberg-King 1987
Cladoceran (<24-hr), *Ceriodaphnia dubia*	S,U	99.5%	7.4-7.7	184	-	Norberg-King 1987
Cladoceran (<24-hr), *Ceriodaphnia dubia*	S,U	99.5%	7.5-8.0	146	-	Norberg-King 1987
Cladoceran (<24-hr), *Ceriodaphnia dubia*	S,M	99.5%	7.8	44	125.8	Spehar 1987
Cladoceran (<24-hr), *Daphnia magna*	S,M	-	-	150	-	Biesinger 1987
Cladoceran (<24-hr), *Daphnia magna*	S,M	-	-	530	-	Biesinger 1987
Cladoceran (juvenile), *Daphnia magna*	S,U	Reagent Grade	6.5-8.5	210	-	Ewell et al. 1986
Cladoceran (<24-hr), *Daphnia magna*	S,U	>99%	7.7-7.9	170	-	Gersich and Mayes 1986

Table 1. (continued)

Species	Method[a]	Chemical[b]	pH	LC50 or EC50 (µg/L)	Species Mean Acute Value µg/L	Reference
Cladoceran (<24-hr), Daphnia magna	F,M	-	7.4	250	250.0	Holcombe et al. 1987
Isopod, Asellus intermedius	S,U	Reagent Grade	6.5-8.5	>100,000	>100,000	Ewell et al. 1986
Amphipod, Gammarus fasciatus	S,U	Reagent Grade	6.5-8.5	>100,000	>100,000	Franco et al. 1986
Midge (larva), Chironomus tentans	S,U	Reagent Grade	7.8	399,900	399,900	Franco et al. 1984
Midge (larva), Clinotanypus pinguis	S,U	Reagent Grade	7.8	477,900	477,900	Franco et al. 1984
Midge (larva), Einfeldia natchitocheae	S,U	Reagent Grade	7.8	427,900	427,900	Franco et al. 1984
Midge (larva), Tanypus neopunctipennis	S,U	Reagent Grade	7.8	272,100	272,100	Franco et al. 1984
Midge (3rd-4th instar), Tanytarsus dissimilis	F,M	-	7.4	>219,000	>219,000	Holcombe et al. 1987
Rainbow trout (juvenile), Oncorhynchus mykiss	F,M	-	7.1-7.7	10,600	-	Abram and Sims 1982
Rainbow trout, Oncorhynchus mykiss	S,M	Analytical Grade	-	41,000	-	Calamari et al. 1980, 1982
Rainbow trout, Oncorhynchus mykiss	S,M	Analytical Grade	-	20,000	-	Calamari et al. 1980, 1982
Rainbow trout, Oncorhynchus mykiss	F,M	-	7.6-8.2	36,220	-	Hodson et al. 1984
Rainbow trout (juvenile), Oncorhynchus mykiss	F,M	-	7.4	40,500	-	Holcombe et al. 1987
Rainbow trout, Oncorhynchus mykiss	F,M	99.5%	7.8	30,000	26,130	Spehar 1987

Table 1. (continued)

Species	Method[a]	Chemical[b]	pH	LC50 or EC50 (μg/L)	Species Mean Acute Value μg/L	Reference
Fathead minnow (juvenile), *Pimephales promelas*	F,M	99%	7.6	134,000	-	Brooke et al. 1984
Fathead minnow (juvenile), *Pimephales promelas*	S,U	Reagent Grade	6.5-8.5	32,000	-	Ewell et al. 1986
Fathead minnow (juvenile), *Pimephales promelas*	F,M	-	7.4	77,900	-	Holcombe et al. 1987; Geiger et al. 1990
Fathead minnow (juvenile), *Pimephales promelas*	F,M	99%	7.5	114,000	106,000	Geiger et al. 1990
Goldfish (juvenile), *Carassius auratus*	F,M	-	7.4	187,000	187,000	Holcombe et al. 1987
Bluegill (juvenile) *Lepomis macrochirus*	F,M	-	7.4	49,000	49,000	Holcombe et al. 1987
White sucker (juvenile), *Catastomus commersoni*	F,M	-	7.4	78,400	78,400	Holcombe et al. 1987
African clawed frog (embryo), *Xenopus laevis*	S,U	-	-	550,000[c]	-	Davis et al. 1981
African clawed frog (tailbud embryo), *Xenopus laevis*	S,U	-	-	940,000[c]	-	Davis et al. 1981
African clawed frog (larva), *Xenopus laevis*	S,U	-	-	150,000	150,000	Davis et al. 1981
			SALTWATER SPECIES			
Eastern oyster (embryos), *Crassostrea virginica*	S,U	100%	7.9-8.0	>30,000	>30,000	Thursby and Berry 1987a
Mysid (juvenile), *Mysidopsis bahia*	R,U	100%	7.4-7.5	1,090	-	Thursby and Berry 1987a

Table 1. (continued)

Species	Method[a]	Chemical[b]	pH	LC50 or EC50 (µg/L)	Species Mean Acute Value µg/L	Reference
Mysid (juvenile), Mysidopsis bahia	F,M	100%	7.5-7.6	1,930	1,930	Thursby and Berry 1987b
Amphipod (juvenile), Ampelisca abdita	R,U	100%	7.5-7.6	16,600	16,600	Redmond and Scott 1987
Grass shrimp (larva), Palaemonetes pugio	R,U	100%	7.9-8.0	610	610	Thursby and Berry 1987a
Sea urchin (embryo-larva), Arbacia punctulata	S,U	100%	7.6-7.7	>200,000	>200,000	Thursby and Berry 1987a
Sheepshead minnow (juvenile), Cyprinodon variegatus	R,U	100%	7.8-8.2	120,000	120,000	Thursby and Berry 1987a
Inland silverside (juvenile), Menidia beryllina	R,U	100%	8.0-8.2	17,400	17,400	Thursby and Berry 1987a
Winter flounder (larva), Pseudopleuronectes americanus	S,U	100%	7.9-8.1	>330,000	>330,000	Thursby and Berry 1987a

[a] S = Static; R = Renewal; F = Flow-through; M = Measured; U = Unmeasured.
[b] Purity of the test chemical.
[c] Results from less sensitive life stages are not used in the calculation of the Species Mean Acute Value.

Table 2. Chronic Toxicity of Aniline to Aquatic Animals

Species	Test[a]	Chemical[b]	pH	Chronic Limits (µg/L)[c]	Chronic Value (µg/L)	Reference
			FRESHWATER SPECIES			
Cladoceran, *Ceriodaphnia dubia*	LC	99.5%	7.8	8.1-12.7	10.14	Spehar 1987'
Cladoceran, *Daphnia magna*	LC	99%	7.8-8.1	24.6-46.7	33.89	Gersich and Milazzo 1988
Rainbow trout, *Oncorhynchus mykiss*	ELS	99.5%	7.8	4,000-7,800	5,600	Spehar 1987
Fathead minnow, *Pimephales promelas*	ELS	99.5%	7.93	422-735	557	Russom 1993
			SALTWATER SPECIES			
Mysid, *Mysidopsis bahia*	LC	100%	7.4-7.6	540-1,100	770.7	Thursby and Berry 1987b

[a] LC = life-cycle or partial life-cycle; ELS = early life-stage.
[b] Purity of the test chemical.
[c] Results are based on measured concentrations of aniline.

Table 2. (continued)

Species	pH	Acute-Chronic Ratio Acute Value (µg/L)	Chronic Value (µg/L)	Ratio
Rainbow trout, *Oncorhynchus mykiss*	7.8	30,000	5,600	5.357
Cladoceran, *Daphnia magna*	7.7-8.1	170	33.9	5.015
Cladoceran, *Ceriodaphnia dubia*	7.8	44	10.1	4.356
		SALTWATER SPECIES		
Mysid, *Mysidopsis bahia*	7.4-7.6	1,930	770.7	2.504

Table 3. Ranked Genus Mean Acute Values with Species Mean Acute-Chronic Ratios

Rank[a]	Genus Mean Acute Value (µg/L)	Species	Species Mean Acute Value (µg/L)[b]	Species Mean Acute-Chronic Ratio[c]
		FRESHWATER SPECIES		
19	477,900	Midge, *Clinotanypus pinguis*	477,900	-
18	427,900	Midge, *Einfeldia natchitocheae*	427,900	-
17	399,900	Midge, *Chironomus tentans*	399,900	-
16	272,100	Midge, *Tanypus neopunctipennis*	272,100	-
15	>219,000	Midge, *Tanytarsus dissimilis*	>219,000	-
14	>219,000	Snail, *Aplexa hypnorum*	>219,000	-
13	187,000	Goldfish, *Carassius auratus*	187,000	-
12	150,00	African clawed frog, *Xenopus laevis*	150,000	-
11	106,000	Fathead minnow, *Pimephales promelas*	106,000	-
10	>100,000	Annelid, *Lumbriculus variegatus*	>100,000	-
9	>100,000	Amphipod, *Gammarus fasciatus*	>100,000	-
8	>100,000	Isopod, *Asellus intermedius*	>100,000	-
7	100,000	Snail, *Helisoma trivolvis*	100,000	-
6	78,400	White sucker, *Catastomus commersoni*	78,400	-

Table 3. (continued)

Rank[a]	Genus Mean Acute Value (µg/L)	Species	Species Mean Acute Value (µg/L)[b]	Species Mean Acute-Chronic Ratio[c]
5	49,000	Bluegill, *Lepomis macrochirus*	49,000	-
4	31,600	Planarian, *Dugesia tigrina*	31,600	-
3	26,130	Rainbow trout, *Oncorhynchus mykiss*	26,130	5.357
2	250	Cladoceran, *Daphnia magna*	250.0	5.015
1	125.8	Cladoceran, *Ceriodaphnia dubia*	125.8	4.356
		SALTWATER SPECIES		
8	>333,000	Winter flounder, *Pseudopleuronectes americanus*	>333,000	-
7	>200,000	Sea urchin, *Arbacia punctulata*	>200,000	-
6	120,000	Sheepshead minnow, *Cyprinodon variegauts*	120,000	-
5	>30,000	Eastern oyster, *Crassostrea virginica*	>30,000	-
4	17,400	Inland silverside, *Menidia beryllina*	17,400	-
3	16,600	Amphipod, *Ampelisca abdita*	16,600	-

Table 3. (continued)

Rank[a]	Genus Mean Acute Value (µg/L)	Species	Species Mean Acute Value (µg/L)[b]	Species Mean Acute-Chronic Ratio[c]
2	1,930	Mysid, *Mysidopsis bahia*	1,930	2.504
1	610	Grass shrimp, *Palaemonetes pugio*	610	-

[a] Ranked from most resistant to most sensitive based on Genus Mean Acute Value.
[b] From Table 1.
[c] From Table 2.

Fresh water

Final Acute Value = 56.97 µg/L

Criterion Maximum Concentration = 56.97 µg/L / 2 = 28.49 µg/L

Final Acute-Chronic Ratio = 4.137 (see text)

Final Chronic Value = (56.97 µg/L) / 4.137 = 13.77 µg/L

Salt water

Final Acute Value = 153.4 µg/L

Criterion Maximum Concentration = (153.4 µg/L) / 2 = 76.7 µg/L

Final Acute-Chronic Ratio = 4.137 (see text)

Final Chronic Value = (153.4 µg/L) / 4.137 = 37.08 µg/L

Table 4. Toxicity of Aniline to Aquatic Plants

Species	Chemical[a]	pH	Duration	Effect	Result (µg/L)	Reference
			FRESHWATER SPECIES			
Green algae, *Selenastrum capricornutum*	Analytical Grade	-	4 days	EC50 (growth)	19,000	Clamari et al. 1980, 1982
Green algae, *Selenastrum capricornutum*	-	-	7 days	No effect (cell number)	<5,000	Adams et al. 1986
Green algae, *Selenastrum capricornutum*	-	-	7 days	No effect (growth rate)	10,000	Adams et al. 1986
Green algae, *Selenastrum capricornutum*	-	-	4 days	Incipient effect (growth)	3,000	Adams et al. 1986
Green algae, *Selenastrum capricornutum*	-	-	4 days	Incipient effect (growth)	1,000[b]	Adams et al. 1986
Green algae, *Selenastrum capricornutum*	-	-	5 days	Incipient effect (growth)	3,000	Adams et al. 1986
Green algae, *Selenastrum capricornutum*	-	-	5 days	Incipient effect (growth)	5,000[b]	Adams et al. 1986
Green algae, *Selenastrum capricornutum*	-	-	6 days	Incipient effect (growth)	3,000	Adams et al. 1986
Green algae, *Selenastrum capricornutum*	-	-	6 days	Incipient effect (growth)	5,000[b]	Adams et al. 1986
Green algae, *Selenastrum* sp.	-	-	4 days	EC50 (biomass)	20,000	Sloof 1982
Green alga, *Chlorella vulgaris*	-	-	14 days	16% reduction in growth	184,000	Ammann and Terry 1985

Table 4. (continued)

Species	Chemical[a]	pH	Duration	Effect	Result (µg/L)	Reference
Green alga, *Chlorella vulgaris*	-	-	14 days	58% reduction in growth	306,000	Ammann and Terry 1985
Green alga, *Chlorella vulgaris*	-	-	14 days	66% reduction in growth	613,200	Ammann and Terry 1985
Green alga, *Chlorella vulgaris*	-	-	14 days	75% reduction in growth	817,000	Ammann and Terry 1985
			SALTWATER SPECIES			
			No acceptable toxicity data for saltwater plants			

[a] Purity of the test chemical.
[b] Acetone carrier used.

Table 5. Other Data on the Effects of Aniline on Aquatic Organisms

Species	Chemical*	pH	Duration	Effect	Concentration (µg/L)	Reference
			FRESHWATER SPECIES			
Bacterium, *Pseudomonas putida*	-	7.0	16 hr	Incipient inhibition	130,000	Bringmann 1973; Bringmann and Kuhn 1976, 1977b, 1980b
Bacterium, *Spirillum volutans*	-	6.8	1 hr	Inhibition of motility	30,000	Bowdre and Krieg 1974
Blue-green alga, *Microcystis aeruginosa*	-	-	24 hr	50% mortality	20,000	Fitzgerald et al. 1952
Blue-green alga, *Microcystis aeruginosa*	-	-	8 days	Incipient inhibition	160	Bringmann and Kuhn 1976, 1978a,b
Green algae, *Scenedesmus quadricauda*	-	7.5	4 days	Incipient inhibition	10,000	Bringmann and Kuhn 1959a,b
Green algae, *Scenedesmus quadricauda*	-	-	8 days	Incipient inhibition	8,300	Bringmann and Kuhn 1977b, 1978a,b, 1980b
Green alga, *Scenedesmus quadricauda*	-	-	24-25 hr	BCF = 91	-	Hardy et al. 1985
Green algae, *Selenastrum capricornutum*	Reagent Grade	-	4 hr	66% reduction in photosynthesis	100,000	Giddings 1979
Protozoan, *Chilomonas paramaecium*	-	-	48 hr	Incipient inhibition	250,000	Bringmann et al. 1980; Bringmann and Kuhn 1981
Protozoan, *Entosiphon sulcatum*	-	6.9	72 hr	Incipient inhibition	24,000	Bringmann 1978; Bringmann and Kuhn 1980b, 1981

Table 5. (continued)

Species	Chemical[a]	pH	Duration	Effect	Concentration (µg/L)	Reference
Protozoan, Microregma heterostoma	-	7.5-7.8	28 hr	Incipient inhibition	20,000	Bringmann and Kuhn 1959a
Protozoan, Tetrahymena pyriformis	-	6.3	72 hr	EC50 (growth)	154,270	Schultz and Allison 1979
Protozoan, Uronema parduczi	-	6.9	20 hr	Incipient inhibition	91,000	Bringmann and Kuhn 1980a, 1981
Hydrozoan, Hydra oligactis	>98%	-	48 hr	LC50	406,000	Slooff 1983
Planarian, Dugesia lugubris	>98%	-	48 hr	LC50	155,000	Slooff 1983
Tubificid worm, Tubificidae	>98%	-	48 hr	LC50	450,000	Slooff 1983
Snail, Lymnaea stagnalis	>98%	-	48 hr	LC50	800,000	Slooff 1982, 1983
Cladoceran, Ceriodaphnia dubia	99.5%	7.8	48 hr	EC50 (fed)	132	Spehar 1987
Cladoceran, Daphnia magna	-	7.5	48 hr	EC50 (acoustic reaction)	400	Bringmann and Kuhn 1959a,b 1960
Cladoceran, Daphnia magna	-	7.6-7.7	24 hr	EC50 (immobility)	500	Bringmann and Kuhn 1977a
Cladoceran, Daphnia magna	Pure Analytical Grade	7.4	24 hr	EC50	23,000	Clamari et al. 1980, 1982
Cladoceran, Daphnia magna	-	-	24 hr	BCF = 5.0	-	Dauble et al. 1984, 1986
Cladoceran, Daphnia magna	-	-	10 hr	LT50	10,000	Lakhnova 1975
Cladoceran, Daphnia magna	-	-	12 hr	LT50	8,000	Lakhnova 1975

Table 5. (continued)

Species	Chemical[a]	pH	Duration	Effect	Concentration (µg/L)	Reference
Cladoceran, Daphnia magna	-	-	1.0 day	LT50	6,000	Lakhnova 1975
Cladoceran, Daphnia magna	-	-	1.5 days	LT50	4,000	Lakhnova 1975
Cladoceran, Daphnia magna	-	-	2.0 days	LT50	2,000	Lakhnova 1975
Cladoceran, Daphnia magna	-	-	3.5 days	LT50	1,000	Lakhnova 1975
Cladoceran, Daphnia magna	99%	-	14 days	MATC	29.9	Gersich and Milazzo 1990
Cladoceran, Daphnia magna	99%	-	14 days	MATC	14.9	Gersich and Milazzo 1990
Cladoceran (adult), Moina macrocopa	Analytical Grade	-	3 hr	LC50	1,000,000	Yoshioka et al. 1986b
Midge, Chironomus dorsalis	-	-	20-21 days	95% Mortality	7,800	Puzikova and Markin 1975
Midge, Chironomus dorsalis	-	-	20-21 days	30% Mortality	7,000	Puzikova and Markin 1975
Midge, Chironomus dorsalis	-	-	20-21 days	0% Mortality	3,000	Puzikova and Markin 1975
Mayfly (larva), Cloeon dipterum	>98%	-	48 hr	LC50	220,000	Slooff 1983
Mosquito (3rd instar), Aedes aegypti	>98%	-	48 hr	LC50	155,000	Slooff 1982
Rainbow trout (juvenile), Oncorhynchus mykiss	-	7.4	7 days	LC50	8,200	Abram and Sims 1982

Table 5. (continued)

Species	Chemical[a]	pH	Duration	Effect	Concentration (µg/L)	Reference
Rainbow trout (juvenile), Oncorhynchus mykiss	-	7.4	7 days	LC50	8,200	Abram and Sims 1982
Rainbow trout (juvenile), Oncorhynchus mykiss	-	7.4	72 hr	BCF = 507	-	Dauble et al. 1984
Rainbow trout (2 yr), Oncorhynchus mykiss	-	-	24 hr	No mortality	10,000-20,000	Lysak and Marcinek 1972
Rainbow trout (2 yr), Oncorhynchus mykiss	-	-	24 hr	LC100	21,000	Lysak and Marcinek 1972
Rainbow trout, Oncorhynchus mykiss	-	7.0-8.0	48 hr	No impairment of flavor	10,000	Shumway and Palensky 1973
Rainbow trout, Oncorhynchus mykiss	-	7.0-8.0	48 hr	100% mortality	100,000	Shumway and Palensky 1973
Guppy, Poecilia reticulata	99%	-	14 days	LC50	125,629	Hermens et al. 1984
Fathead minnow (3-4 wk), Pimephales promelas	>98%	-	48 hr	LC50	65,000	Slooff 1982
Channel catfish (embryo, larva), Ictalurus punctatus	-	7.7	To hatch (4.5 days)	LC50	5,600 (5,500)[b]	Birge et al. 1979b
Channel catfish (embryo, larva), Ictalurus punctatus	-	7.7	8.5 days (4 days post-hatch)	LC50	5,000 (5,000)[b]	Birge et al. 1979b

Table 5. (continued)

Species	Chemical[a]	pH	Duration	Effect	Concentration (µg/L)	Reference
Channel catfish (embryo, larva), Ictalurus punctatus	-	7.7	To hatch (4.5 days)	LC50	7,400 (6,300)[b]	Birge et al. 1979b
Channel catfish (embryo, larva), Ictalurus punctatus	-	7.7	8.5 days (4 days post-hatch)	LC50	7,000 (6,200)[b]	Birge et al. 1979b
Goldfish (embryo, larva), Carassius auratus	-	7.7	To hatch (3.5 days)	LC50	10,200 (9,300)[b]	Birge et al. 1979b
Goldfish (embryo, larva), Carassius auratus	-	7.7	7.5 days (4 days post-hatch)	LC50	5,600 (5,500)[b]	Birge et al. 1979b
Goldfish (embryo, larva), Carassius auratus	-	7.7	11.5 days (4 days post-hatch)	LC50	5,500	Birge et al. 1979b
Goldfish (embryo, larva), Carassius auratus	-	7.7	To hatch (3.5 days)	LC50	10,000 (7,600)[b]	Birge et al. 1979b
Goldfish (embryo, larva), Carassius auratus	-	7.7	7.5 days (4 days post-hatch)	LC50	4,800 (4,600)[b]	Birge et al. 1979b
Goldfish (embryo, larva), Carassius auratus	-	7.7	11.5 days (8 days post-hatch)	LC50	4,700	Birge et al. 1979b
Largemouth bass (embryo, larva), Micropterus salmoides	-	7.7	To hatch (2.5-3.5 days)	LC50	47,300 (32,700)[b]	Birge et al. 1979b
Largemouth bass (embryo, larva), Micropterus salmoides	-	7.7	6.5-7.5 days (4 days post-hatch)	LC50	10,500 (7,100)[b]	Birge et al. 1979b

Table 5. (continued)

Species	Chemical[a]	pH	Duration	Effect	Concentration (µg/L)	Reference
Largemouth bass (embryo, larva), Micropterus salmoides	-	7.7	10.5-11.5 days (8 days post-hatch)	LC50	5,200	Birge et al. 1979b
Largemouth bass (embryo, larva), Micropterus salmoides	-	7.7	To hatch (2.5-3.5 days)	LC50	43,200 (29,900)[b]	Birge et al. 1979b
Largemouth bass (embryo, larva), Micropterus salmoides	-	7.7	6.5-7.5 days (4 day post-hatch)	LC50	8,400 (7,100)[b]	Birge et al. 1979b
Largemouth bass (embryo, larva), Micropterus salmoides	-	7.7	10.5-11.5 days (8 days post-hatch)	LC50	4,400	Birge et al. 1979b
African clawed frog (embryo), Xenopus laevis	-	-	96 hr	EC50 (teratogeny)	370,000	Davis et al. 1981
African clawed frog (embryo), Xenopus laevis	-	-	120 hr	EC50 (teratogeny)	91,000	Davis et al. 1981
African clawed frog (larva), Xenopus laevis	-	-	96 hr	6% abnormalities	10,000	Dumont et al. 1979; Davis et al. 1981
African clawed frog (tadpole), Xenopus laevis	-	-	12 days	100% mortality	90,000	Dumpert 1987
African clawed frog (embryo), Xenopus laevis	-	-	12 weeks	Slowed metamorphosis, reduced growth	1,000	Dumpert 1987
African clawed frog (3-4 wk), Xenopus laevis	98%	-	LC50	560,000		Slooff 1982; Slooff and Baerselman 1980

Table 5. (continued)

Species	Chemical[a]	pH	Duration	Effect	Concentration (µg/L)	Reference
			SALTWATER SPECIES			
Sea anemone, *Bunodosoma cavernata*	-	-	7 days	Significant increase in concentration of free aspartate, glutamate, alanine	500,000	Kasschau et al. 1980
Sand shrimp (adult), *Crangon septemspinosa*	-	-	96 hr	Lethal threshold	29,400	McLeese et al. 1979

[a] Purity of the test chemical.
[b] Data in parenthesis are from Birge et al. 1979a.

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