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Water Quality Criteria for Nitrocellulose

FINAL REPORT

Michael G. Ryon

JUNE 1986

SUPPORTED BY

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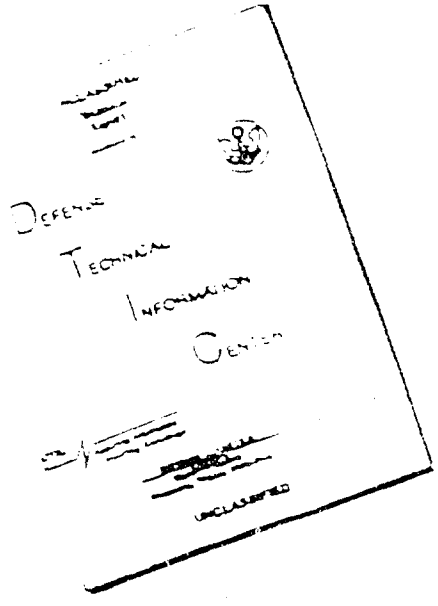

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Available data on macroinvertebrates, fish, and algae indicate nitrocellulose is not very toxic for most aquatic species. In most evaluations the EC_{50} and LC_{50} values were >1,000 mg/L. Only for the effect on chlorophyll a content of Selenastrum capricornutum was a lower value estimated, an EC_{50} of 579 mg/L.

Available data on human health effects and mammalian toxicity suggest nitrocellulose is virtually nontoxic. The LD_{50} values were in excess of 5,000 mg/kg. Chronic toxicity studies in mice demonstrated only physical effects (fiber impaction) in the digestive tract (presumably because of the small size of the mouse digestive tract). Genotoxicity and developmental toxicity studies did not demonstrate any other significant toxic effects. Carcinogenicity data generated by an epidemiology study of occupational exposure during production of nitrocellulose suggest some association between nitrocellulose and rectal/digestive tract cancers; this should be researched further. Metabolism data in rats indicate no absorption from the GI tract.

However, nitrocellulose does appear to produce significant abiotic environmental effects. Because of its fibrous nature, nitrocellulose would blanket benthic habitats (limiting available oxygen) and fill in interstitial spaces used as cover for benthic organisms. This habitat alteration is compounded by the resistance of nitrocellulose to environmental degradation. In contrast to its low toxicity, habitat alteration becomes a significant aspect of regulatory control. Keywords: munitions product

Using available data and USEPA guidelines, an attempt was made to calculate criteria to protect aquatic life and its uses and to protect human health. However, the data did not satisfy all USEPA guideline requirements and valid criteria could not be generated. The general conclusion was that nitrocellulose would best be regulated based on its physical characteristics (e.g., levels of suspended or dissolved solids) and research to provide the required data for calculation of water quality criteria by the USEPA methodology would not be necessary. The general water criteria for total suspended solids (TSS) is:

> "Settleable and suspended solids should not reduce the depth of the compensation point for photosynthetic activity by more than 10 percent from the seasonally established norm for aquatic life." (USEPA 1976).

Actual levels of TSS that might be applicable for regulation of nitrocellulose include (1) 10 ppm in wastewater discharges (Barkley and Rosenblatt 1978), (2) a maximum of 250 mg/kg product and a 30-day average of 84 mg/kg product for point sources of wastewater discharged from explosives manufacturing (USEPA 1984), and (3) a maximum value of 260 mg/kg product and a 30-day average of 88 mg/kg product for point sources of wastewater discharged from an LAP facility (USEPA 1984).

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WATER QUALITY CRITERIA FOR NITROCELLULOSE

FINAL REPORT

Michael G. Ryon

Chemical Effects Information Task Group
Information Research and Analysis
Biology Division

JUNE 1986

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EXECUTIVE SUMMARY

Based on available data for four species of macroinvertebrates, four species of fish, and three species of algae, nitrocellulose does not appear to be very toxic for most aquatic species. In the majority of evaluations the EC_{50} and LC_{50} values were >1,000 mg/L. Only for the effect on the chlorophyll a content of Selenastrum capricornutum was a lower value estimated, an EC_{50} of 579 mg/L (Bentley et al. 1977).

The available data on the human health effects and mammalian toxicity of nitrocellulose generally suggest that it is virtually nontoxic. The acute toxicity data indicated LD_{50} values in excess of 5,000 mg/kg. Chronic toxicity studies in mice demonstrated that it is harmful only due to its physical effects (fiber impaction) in the digestive tract (presumably because of the small size of the mouse digestive tract). Genotoxicity and developmental toxicity studies did not demonstrate any significant toxic effects not related to these fiber effects. The data on carcinogenicity generated by an epidemiology study of occupational exposure during production of nitrocellulose does suggest some association between nitrocellulose and rectal or digestive tract cancers and this possibility should be evaluated with further research. Data on the metabolism of nitrocellulose by rats indicate that it is not absorbed into the gastrointestinal tract and further supports the general conclusion that nitrocellulose does not represent a significant human health risk.

However, nitrocellulose does appear to produce significant abiotic environmental effects when released from Army Ammunition Plants. Because of its fibrous nature, nitrocellulose would tend to blanket benthic habitats (perhaps depriving organisms of oxygen) and fill in interstitial spaces used as cover for benthic invertebrates and substrates for periphyton. This potential for habitat alteration is further compounded by the apparent resistance of released nitrocellulose to environmental degradation. When contrasted to the low toxicity of the compound, this habitat alteration becomes a significant aspect of regulatory control.

Using the data reviewed above and the USEPA guidelines, an attempt was made to calculate criteria to protect aquatic life and its uses and to protect human health. However, the data did not satisfy all of the USEPA guideline requirements and appropriate criteria could not be generated. The general conclusion was that nitrocellulose would best be regulated based on its physical characteristics (e.g., levels of suspended or dissolved solids) and research to provide the required data for calculation of water quality criteria by the USEPA methodology would not be neces-

sary. The general water criteria for total suspended solids (TSS) is:

> ''Settleable and suspended solids should not reduce the depth of the compensation point for photosynthetic activity by more than 10 percent from the seasonally established norm for aquatic life.'' (USEPA 1976).

In actual levels, several figures might be applicable for regulation of nitrocellulose. A value of 10 ppm was given for total suspended solids in wastewater discharges (Barkley and Rosenblatt 1978). For point source categories of wastewater discharged from an explosives manufacturing site, a maximum value of 0.25 kg/1,000 kg of product and an average of daily values for 30 days of 0.084 kg/1,000 kg of product were given for TSS (USEPA 1984). For point source categories of wastewater discharged from a Load, Assemble, and Pack facility, the maximum value for TSS was 0.26 kg/1,000 kg of product and the 30-day average value for TSS was 0.088 kg/1,000 kg of product (USEPA 1984).

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1. INTRODUCTION

Cellulose nitrate, commonly known as nitrocellulose, is a munitions compound manufactured or handled at several U.S. Army facilities, including Radford Army Ammunition Plant (AAP), Badger AAP, Indiana AAP, Lake City AAP, Sunflower AAP, and Alabama AAP. Although current production is limited to Radford, the other sites contain sediment deposits in settling ponds and represent a potential for future aquatic pollution, particularly if production is renewed (Barkley and Rosenblatt 1978). The objective of this report is to review the available data on the aquatic and human health effects of nitrocellulose and using the latest USEPA guidelines, generate water quality criteria values. Appendix A is a summary of the USEPA guidelines for generating water quality criteria for the protection of aquatic life and its uses (Stephan et al. 1985). Appendix B is a summary version of the USEPA guidelines for generating water quality criteria for the protection of human health (USEPA 1980).

1.1 PHYSICAL AND CHEMICAL PROPERTIES

Nitrocellulose (CAS No. 9004-70-0) is a cellulose ester of nitric acid (Figure 1) and is usually a fibrous, solid polymer. Nitrocellulose is nonvolatile, has a low solubility, and has a variable degree of nitration. Typically, nitrocellulose has three nitrate ester groups per glucose ring (cellulose trinitrate) with a theoretical nitrogen content of 14.15 percent, but due to incomplete nitration, it can have a nitrogen content as low as 6.76 percent (Barkley and Rosenblatt 1978; Urbanski 1983). The glucose rings are linked together to form chains of beta, 1→4-linked glucoside units in which the hydroxyl groups of the glucose subunits have reacted to form nitrate esters (Sullivan et al. 1978). Table 1 summarizes the available information on synonyms and the chemical and physical properties of nitrocellulose. The virtual insolubility of nitrocellulose in aqueous solutions is perhaps the most important of its properties for this analysis. In aquatic systems, nitrocellulose is not found in solution in high concentrations in water tending to settle onto the sediments.

Under actual manufacturing conditions, nitrocellulose wastes (fines) are produced with a range of particle sizes. Helton (1976) examined a sample of nitrocellulose taken from the poacher pits at Radford AAP and found that 66 percent (by weight) of the particles were greater than 88 microns in size (based on ability to pass through sieves and membrane filters), 23 percent were between 88 and 44 microns, 11 percent were between 44 and 5 microns, and less than 0.3 percent were smaller than 5 microns. Another report classifying sizes of nitrocellulose particles from boiling tub

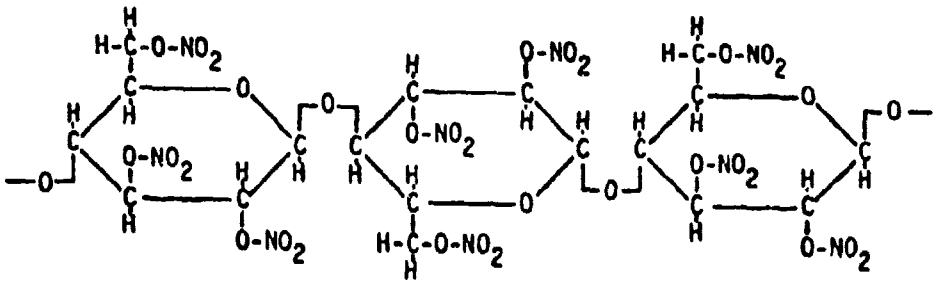

Figure 1. Chemical Structure of a Section of the Nitrated Glucose Chain Forming Nitrocellulose (From Sullivan et al. 1978).

TABLE 1. SYNONYMS AND PROPERTIES OF NITROCELLULOSE

Property	Value	Reference
CAS No.	9004-70-0	Sax 1984
Synonyms	C 2018, CA 80-15, Celloidin, Cellulose nitrate (9CI), Cellulose tetranitrate, Collodion, Collodion cotton, Collodion wool, Colloxylin, Coriat EM finish F, E 1440, Flexible collodion, FM-NTS, Guncotton, HX 3/5, Kodak LR 115, LR 115, Nitrocellulose E950, Nitrocotton, Nitron, Nixon N/C, NTs 62, NTs 218, NTs 222, NTs 539, NTs 542, Parlodion, Pyralin, Pyroxylin, Pyroxylin plastic (DOT), RF 10, R.S. Nitrocellulose, Soluble gun cotton, Synpor, Tsapolak 964, Xyloidin	Tatken and Lewis 1983
Molecular formula	$[C_6H_7O_2(ONO_2)_3]_n$	Hawley 1981
Molecular weight	459.28-594.28[a]	ILO 1983
Physical state	White pulpy, cotton-like, amorphous solid (when dry) or colorless liquid to semisolid (solution) depending on degree of nitration	Hawley 1981; ILO 1983
Melting point	Ignites at 169-170°C	ILO 1983
Flash point	12.8°C	ILO 1983
Solubility	High nitrogen form is insoluble in mixtures of ethanol and ether, but soluble in acetone; low-nitrogen form (pyroxylin) is soluble in both	ITII 1975
	Soluble in 25 parts of a mixture of 1 volume alcohol and 3 volumes ether; also soluble in methanol, acetone, glacial acetic acid, and amyl acetate	Windholz et al. 1983
Specific gravity	1.66	Hawley 1981

a. Exact weight depends on chain length and degree of nitration (Sullivan et al. 1978).

pit water, found that 97 percent were smaller than 20 microns and 30 percent were smaller than 1 micron (Rosenblatt et al. 1973).

1.2 MANUFACTURING AND ANALYTICAL TECHNIQUES

Nitrocellulose is manufactured by treating cellulose, either wood pulp or cotton linters (short-fibered cotton hairs), with mixtures of nitric and sulfuric acids (Lindner 1980). To obtain different products or levels of nitration, variations are used in the strength of acids, temperature and time of reaction, acid/cellulose ratio, and types of acids (Hawley 1981). The cellulose trinitrate form can only be manufactured using nitric anhydride or a mixture of nitric and phosphoric acids (Lindner 1980). The excess acids are removed by a series of washing, digesting, and boiling procedures (ILO 1983). When made using the conventional techniques, nitrocellulose retains the fibrous structure of the cellulose, which helps it provide mechanical strength as well as readily available energy for gun and rocket propellants (Lindner 1980). Manufacturing techniques are available for both batch and continuous production (Lindner 1980).

Analytical techniques for nitrocellulose have concentrated on determining the degree of nitration of the cellulose moiety or the content of nitrocellulose in a propellant mixture (Barkley and Rosenblatt 1978). A variety of analytical methods have been developed based on titration methods involving ferrous and/or titanous ions, but these are generally time-consuming, low in sensitivity, and do not have potential for automation (Barkley and Rosenblatt 1978). Another approach involves hydroxide ion attack on nitrocellulose in an acetone solution and measurement of the released nitrite ions (Barkley and Rosenblatt 1978). One automated technique using the Technicon AutoAnalyzer, is based on colorimetric analysis of the nitrocellulose solution and includes a dialysis step to reduce interference from nitrite and nitrate ions (Barkley and Rosenblatt 1978).

2. ENVIRONMENTAL EFFECTS AND FATE

2.1 ABIOTIC ENVIRONMENTAL EFFECTS

Release of nitrocellulose into the aquatic environment may have an impact on the quality of that habitat by affecting physical or chemical parameters of the area. For many chemicals this consideration would be minor, overshadowed by toxic effects, but nitrocellulose appears to have a very low toxicity and therefore these considerations

carry more importance in this case. Data on these effects are limited primarily to environmental reports on Army Ammunition Plants (AAPs).

Cooper et al. (1975) evaluated the aquatic impact from nitrocellulose production at Badger AAP and from the packing of nitrocellulose into ammunition at Lake City AAP. At Badger, nitrocellulose was found in all but one of the water samples from associated streams and ponds with levels of <1 to 10 ppm (Table 2). The highest level was taken below the outfall of an industrial waste treatment plant. A level of 7 ppm was found in the last of three settling ponds, indicating some transport of nitrocellulose. The <1 ppm value was taken from the large Wisconsin River next to BAAP and indicates either settling or dilution effects. These sampling areas were associated with water quality characteristics that differed from control stations, particularly for NO_2/NO_3 levels and dissolved and total solids. Sediment levels were not measured but the authors felt that nitrocellulose levels would be higher in the sediments. At Lake City AAP, the values for nitrocellulose in water and sediment samples were <1 to 4.6 ppm and were associated with sites having elevated dissolved and total solids levels. Cooper et al. did mention that the nitrocellulose values represented more than just nitrocellulose, probably being more a measure of total organic nitrogen.

Stilwell et al. (1976) reported the final data from the aquatic survey of Badger AAP, initiated by Cooper et al. (1975), evaluating the effects on water quality of wastewater discharge from nitrocellulose manufacturing. Despite the fact that the nitrocellulose production area had not been immediately operating prior to sampling (production ceased in May 1975 and sampling occurred in June 1975), detectable levels of nitrocellulose were found in the water (<1 to 12.1 ppm) and sediments (17.8 to 296.0 ppm) of streams and ponds receiving the waste discharges. Water quality of these aquatic systems was affected, including elevated concentrations of NO_3/NO_2, sulfate and chloride (considered higher than desirable for protection of aquatic life), and higher dissolved solid levels (twice the normal background). Sediment analysis indicated elevated levels of volatile solids, chemical oxygen demand, and nutrients (total Kjeldahl nitrogen and phosphate) associated with the nitrocellulose wastewater discharge. However, the authors did conclude that many of the negative biological effects associated with these areas could not be based on nitrocellulose and those that could (e.g., benthic macroinvertebrate distributions) may be more a reflection of habitat alteration rather than toxic effects of nitrocellulose alone.

Two other AAPs were evaluated for the effects of nitrocellulose on habitat quality, but no harmful effects or definite associations were detected. These surveys included

TABLE 2. WASTEWATER CHARACTERISTICS FROM ASSOCIATED AQUATIC SYSTEMS AT BADGER ARMY AMMUNITION PLANT (AAP)[a]

Pollutant/ Characteristic (mg/L[b])	Sampling Sites						
	ITP[c]	SL-1[d]	RP-2[e]	SL-3[f]	GBT-1[g]	WRT-3[h]	OC[i]
Nitrocellulose	10	3	6	5	7	<1	<1
Conductivity	370	960	360	710	800	220	100
Hardness	116	460	-	436	356	96	64
Suspended solids	24	24	-	12	<10	<10	44
Dissolved solids	230	874	-	810	566	68	26
Total solids	254	898	-	822	566	68	70
COD[j]	12	21	-	17	34	30	13
TOC[k]	11	12	-	10	13	12	8
NO_2	0.4	4.9	-	3.6	6.6	<0.1	<0.1
NO_3	4.8	125	-	125	25.1	0.1	2.2

a. Adapted from Cooper et al. 1975.

b. Only concentrations given in mg/L.

c. ITP = industrial waste treatment plant outfall.

d. SL-1 = head of settling lake below ITP outfall.

e. RP-2 = rocket pond effluent below nitrocellulose and nitroglycerin production areas.

f. SL-2 = base of third settling lake below ITP outfall and RP-2 outfall.

g. GBT-1 = transect across Greuber's Bay below ITP outfall and RP-2 outfall.

h. WRT-3 = partial transect in Wisconsin River below Badger AAP.

i. OC = Otter Creek, reference site not associated with Badger AAP.

j. COD = chemical oxygen demand.

k. TOC = total organic carbon.

Radford AAP (Huff et al. 1975; Weitzel et al. 1976; Heffinger 1984) and Indiana AAP (Wiese 1981).

A characterization of the wastewater effluent from the nitrocellulose production facility of Radford AAP was reported by Luh and Szachta (1978), which indicated high levels of suspended and dissolved solids due to nitrocellulose (Table 3). Another characterization study of nitrocellulose wastewater at Radford AAP reported that suspended solids ranged from 80 to 900 ppm in one of the waste lines and that more than 2 tons per day of nitrocellulose fines were overflowing the boiler-line settling pits (Epstein et al. 1978).

Barkley and Rosenblatt (1978) have proposed the conclusion that nitrocellulose is toxicologically inert and should be controlled based on a criterion for regulating total suspended solids in wastewater discharges. This conclusion is also supported by the Sullivan et al. (1978) report aimed at generating water quality criteria. The authors state that nitrocellulose fines released from AAPs could physically alter the habitat permanently and in particular affect the benthic community structure. Ellis et al. (1980) concluded that the only appropriate water quality criterion would be based on physical parameters, such as total suspended solids or clarity.

2.2 ENVIRONMENTAL FATE

Information on the fate of nitrocellulose in the environment was limited to comments made by various authors concerning the potential biodegradation and long-term persistence of nitrocellulose. Bluhm (1976) states in an introduction to a study of degradation products from the alkaline hydrolysis of nitrocellulose that direct biodegradation of nitrocellulose is not feasible. In order for some biodegradation to occur, nitrocellulose must undergo alkaline hydrolysis. This view is also supported by Wendt and Kaplan (1976) who discuss the early studies of nitrocellulose indicating that direct decomposition of nitrocellulose by microorganisms is not possible (Siu 1951; Urbanski 1965, both as reported in Wendt and Kaplan 1976). Barkley and Rosenblatt (1978) state that nitrocellulose is essentially inert and long lasting under most environmental conditions. Sullivan et al. (1978) comment that nitrocellulose particles (fines) released from AAPs, tend to accumulate in the sediments of aquatic systems due to their particulate character, low solubility, and specific gravity. Once in the sediments, it is extremely stable and may persist for an indefinite length of time. Thus, the general consensus is that nitrocellulose will remain in the environment essentially unchanged and any harmful effects will be restricted to its habitat alteration effects.

TABLE 3. WASTEWATER CHARACTERISTICS FROM NITROCELLULOSE PRODUCTION AREA[a] AT RADFORD ARMY AMMUNITION PLANT[b]

Pollutant/ Characteristic	Value[c]
Chemical oxygen demand	2,915 mg/L
Total organic carbon	875 mg/L
Nitrates	565 mg/L
Sulfates	16 mg/L
Color	1,050
Alkalinity	225 mg/L
Nitroglycerin	0 mg/L
Lead	0 mg/L
Suspended solids	1,800 mg/L[d]
Dissolved solids	2,716 mg/L
pH	7.0

a. Alcohol rectification area, includes nitroglycerin and nitrocellulose production lines.

b. Adapted from Luh and Szachta 1978.

c. Average values, based on flow rate of 65,000 gal/day.

d. Solely nitrocellulose.

2.3 SUMMARY

Nitrocellulose appears to produce significant abiotic environmental effects when released from AAPs. Because of its fibrous nature, nitrocellulose would tend to blanket benthic habitats (perhaps depriving organisms of oxygen) and fill in interstitial spaces used as cover for invertebrates and periphyton. This potential for habitat alteration is further compounded by the apparent lack of environmental degradation. When contrasted to the low toxicity of the compound, this habitat alteration becomes a significant aspect of regulatory control.

3. AQUATIC TOXICOLOGY

3.1 ACUTE TOXICITY TO ANIMALS

Data available on the acute toxicity of nitrocellulose to aquatic animals are limited to some EC_{50} (median effective concentration) based on immobilization and LC_{50} (median lethal concentration) values generated by Bentley et al. (1977). The tests were performed on several aquatic macroinvertebrate and fish species using a nitrocellulose slurry taken from poacher pit fines at Radford AAP (nitrocellulose was assayed as 11.8 percent active ingredient). The macroinvertebrates (Daphnia magna, Gammarus fasciatus, Asellus militaris, and Chironomus tentans) were exposed in a 250-mL beaker to 200 mL of test solution with 15 animals per concentration (3 replicates, 5 animals per replicate). No efforts were made to keep the nitrocellulose suspended in the water. The fish species (Lepomis macrochirus, Salmo gairdneri, Ictalurus punctatus, and Pimephales promelas) were exposed in 19.6-L tanks containing 15 L of test solution with 30 animals of each species per concentration (3 replicates, 10 animals per replicate). In testing the fish species, the nitrocellulose was kept in suspension by means of a mechanical stirrer. Tests were static exposures and test methods were based on the USEPA publication, Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians (USEPA 1975a). Based on range-finding tests, concentrations of 0, 560, 750, and 1,000 mg/L were used for all tests; the highest concentration represents a level, three orders of magnitude higher than that expected in the receiving waters associated with Radford AAP. The results from these tests were negative, with LC_{50} and EC_{50} values >1,000 mg/L (Table 4).

Bentley et al. (1977) also tested various life stages of P. promelas for toxic effects from nitrocellulose and the impact of several water quality variables on toxicity to L. macrochirus. The water conditions tested included a range of temperatures (15°C, 20°C, and 25°C), various water

TABLE 4. ACUTE TESTS FOR MORTALITY[a] OR IMMOBILIZATION[b] OF AQUATIC SPECIES FOLLOWING EXPOSURE TO NITROCELLULOSE[c]

Test Species	Test Method	Test Duration	LC_{50} (mg/L)	EC_{50} (mg/L)	Genus Mean Acute Value (mg/L)
Arthropoda					
Crustacea					
Daphnidae					
Daphnia magna[d]	Se	48 hr	NA[f]	>1,000	>1,000
Gammaridae					
Gammarus fasciatus[g]	S	48 hr	NA	>1,000	>1,000
Asellidae					
Asellus militaris[g]	S	48 hr	NA	>1,000	>1,000
Insecta					
Chironomidae					
Chironomus tentans[h]	S	48 hr	NA	>1,000	>1,000
Osteichthyes					
Centrarchidae					
Lepomis macrochirus[i]	S	96 hr	>1,000	NA[f]	>1,000
Salmonidae					
Salmo gairdneri[j]	S	96 hr	>1,000	NA	>1,000
Ictaluridae					
Ictalurus punctatus[k]	S	96 hr	>1,000	NA	>1,000
Cyprinidae					
Pimephales promelas[l]	S	96 hr	>1,000	NA	>1,000

a. Mortality tests were designed to give LC_{50} values.

b. Immobilization tests were designed to give EC_{50} values.

c. Adapted from Bentley et al. 1977.

d. Test animals were 0-24 hours old at start of test.

e. S = static.

f. NA = not applicable; LC_{50} was only determined for fish species and EC_{50} was only determined for macroinvertebrates.

g. Test animals were juveniles at start of test.

h. Test animals were second or third instars at start of test.

i. Test animals had a mean weight of 1.0±0.3 g and a standard length of 35±6 mm at start of test.

j. Test animals had a mean weight of 0.9±0.3 g and a standard length of 43±4 mm at start of test.

k. Test animals had a mean weight of 1.3±0.5 g and a standard length of 57±11 mm at start of test.

l. Test animals had a mean weight of 1.0±0.4 g and a standard length of 43±8 mm at start of test.

hardness values (35, 100, and 250 mg/L $CaCO_3$), and several pH levels (6.0, 7.0, and 8.0). The various life stages (embryo, 1-hr old fry, 7-day-old fry, 30-day-old fry, and 60-day-old fry) were exposed in static toxicity tests over a 144-hr period for the embryos and 96-hr periods for the remaining stages. Three replicates of 10 animals per replicate were used for each test. As in prior acute tests with P. promelas and L. macrochirus, no toxic effects were found.

Based on these experiments, nitrocellulose does not appear to be acutely toxic to aquatic animals at the concentrations tested up to 1,000 mg/L.

3.2 CHRONIC TOXICITY TO ANIMALS

No real chronic toxicity tests with aquatic organisms were located. The only aquatic study of longer duration than an acute study was an evaluation of the effects of exposure of two generations of Chironomus tentans to nitrocellulose in sediment (Bentley et al. 1977). Although aquatic exposure would normally be conducted by mixing in water, C. tentans is a burrowing species of midge and would experience contact with nitrocellulose in sediments. Also nitrocellulose released from Army Ammunition Plants would settle on the sediments and thus this test is a valid aquatic exposure situation. Nitrocellulose in water was added to dry sediment and the resulting sediment concentrations were placed in aquaria with a water volume of 1.75 L each and allowed to settle. One hundred animals were placed in each test concentration and monitored for 10 to 15 days of exposure. At this point daily records were kept of emergence, adult survival, and egg production. When adult mortality exceeded emergence, the tank was cleaned of fungus, dead organisms, exuviae, and remaining larvae and the second generation was initiated in the same manner as the first. No significant effects were noted following exposure of the first generation to initial nitrocellulose concentrations of up to 540 ± 112 mg nitrocellulose/kg (dry weight) of sediment (Table 5). By the end of the exposure period for the first generation (28 days), the two highest levels had decreased from 540 and 220 mg/kg to $<223 \pm 133$ and <140 mg/kg dry weight, respectively. Exposure of the second generation to levels as high as 540 mg/kg also failed to produce any significant effect on emergence or survival. However, any conclusions from this study must consider shortcomings in the study protocol. Since the nitrocellulose was mixed with the sediment, the amount of test material that C. tentans would come into contact with or ingest would be hard to determine, thus the exposure concentrations might not be as high as indicated.

TABLE 5. MEAN[a] RELATIVE PERCENT[b] EMERGENCE AND SURVIVAL OF FIRST GENERATION ADULT Chironomus tentans EXPOSED TO NITROCELLULOSE IN SEDIMENT FOR 28 DAYS[c]

Concentration[d] (mg/kg)	Emergence (%)	Survival (%)
Control	100 (61)[e]	100 (52)
25[f]	200 (47)	87 (73)
50[f]	116 (71)	100 (13)
100[f]	216 (33)	61 (100)
220[g] (29)[e]	211 (39)	87 (50)
540[g] (21)[e]	122 (45)	95 (34)

a. Each value represents the mean of four replicates per treatment, N = 100/replicate.

b. Control values represent unity against which all other measurements are compared.

c. Adapted from Bentley et al. 1977.

d. Concentration, mg nitrocellulose per kg sediment (dry weight).

e. Coeffecient of variation.

f. Nominal concentrations, the minimum detectable limit of analytical method is 140 mg/kg, mean measured.

g. Concentrations at initiation of exposure.

3.3 TOXICITY TO PLANTS

Two studies of the effects of nitrocellulose on aquatic plants were found in the literature, both in the report by Bentley et al. (1977). The assays were performed according to methods described in Algal Assay Procedure: Bottle Assay (USEPA 1971). The algal species, Selenastrum capricornutum (green algae), Microcystis aeruginosa (blue-green algae), and Navicula pelliculosa (diatom), were observed for a decrease in the number of cells per mL over a 96-hr period following exposure to nitrocellulose. At concentrations of 100 to 1,000 mg/L, concentration-dependent decreases in cells per mL were noted, particularly for S. capricornutum (Table 6). The effects on this species were considered significant by the authors who stated that the EC_{50} values for the other species would be >1,000 mg/L. In addition to evaluating changes in the numbers of cells, Bentley et al. (1977) also determined the effect of nitrocellulose on the chlorophyll a content of the three previous algal species plus another blue-green species, Anabeana flos-aquae (Table 7). Again the EC_{50} values for species besides S. capricornutum were >1,000 mg/L. The authors calculated a 96-hr EC_{50} value for S. capricornutum of 579 mg/L with a 95 percent confidence interval range of 138 to 2,400 mg/L.

Discharges of wastewater containing nitrocellulose from Badger AAP were linked to harmful effects on the periphyton and algal communities of associated aquatic systems (Cooper et al. 1975). Species diversity was lower in streams receiving nitrocellulose wastewater than in reference streams for the periphyton community. Also the standing crop for periphyton was lowered by wastewater from nitrocellulose areas. For algal communities, the effects included lowered numbers of species, lowered numbers of individuals, and lowered species diversity. These effects were associated with nitrocellulose levels of <1 to 10 mg/L in water, although associated sediments may have contained higher levels. However, since Badger AAP also produces nitroglycerin and rocket paste, the effects on periphyton and algal communities cannot be directly and exclusively linked to nitrocellulose. Also, no mechanisms were identified to determine whether toxic effects or habitat alteration effects of nitrocellulose were the primary factors.

3.4 BIOACCUMULATION

No data were found to support the bioaccumulation (uptake and storage) or bioconcentration of nitrocellulose by aquatic species. Cooper et al. (1975) tested the tissues of carp (Cyprinus carpio) and largemouth bass (Micropterus salmoides) exposed in local streams to wastewater from Badger AAP for bioaccumulation of nitrocellulose. By taking tissue samples from adult fish that were native to streams

TABLE 6. DECREASE IN NUMBER OF CELLS/mL OF ALGAL SPECIES FOLLOWING EXPOSURE TO NITROCELLULOSE[a]

Test Species	Concentration (mg/L)	Percent Decrease[b] 24-hr	48-hr	96-hr
Selenastrum capricornutum	100	0	0	5
	135	0	0	14
	240	0	0	19
	420	2	19	31
	750	11	18	37
	1,000	12	23	42
Microcystis aeruginosa	10	0	0	0
	32	0	0	0
	100	0	0	14
	320	0	0	23
	1,000	0	9	32
Navicula pelliculosa	10	0	0	0
	32	0	3	0
	100	0	0	9
	320	0	3	16
	1,000	0	11	23

a. Adapted from Bentley et al. 1977.

b. Percent decrease as compared to controls; control represented unity against which all other measurements are compared to determine percent decrease.

TABLE 7. PERCENT DECREASE[a] IN CHLOROPHYLL a CONCENTRATION IN ALGAL SPECIES 96-HR AFTER EXPOSURE TO NITROCELLULOSE[b]

Test Species	Concentration (mg/L)								
	10	32	100	135	240	320	420	750	1,000
Selenastrum capricornutum	NT[c]	NT	19	17	24	NT	40	58	66
Microcystis aeruginosa	0	0	9	NT	NT	27	NT	NT	30
Anabeana flos-aquae	1	0	3	NT	NT	7	NT	NT	10
Navicula pelliculosa	0	0	0	NT	NT	21	NT	NT	33

a. Percent decrease compared to controls; control represented unity against which all other measurements are compared.

b. Adapted from Bentley et al. 1977.

c. NT = not tested at this concentration.

known to contain nitrocellulose and using infrared analysis, Cooper et al. found no evidence for the presence of nitrocellulose (detection limit 10 ppm).

3.5 SUMMARY

Based on available data for four species of macroinvertebrates, four species of fish, and three species of algae, nitrocellulose does not appear to be very toxic for most aquatic species. In the majority of evaluations the EC_{50} and LC_{50} values were >1,000 mg/L. Only for the effect on the chlorophyll a content of Selenastrum capricornutum was a lower value estimated, an EC_{50} of 579 mg/L (Bentley et al. 1977).

4. HUMAN HEALTH EFFECTS AND MAMMALIAN TOXICOLOGY

4.1 PHARMACOKINETICS

4.1.1 Animal Studies

Information on the uptake, metabolism, and elimination of nitrocellulose in laboratory mammals was limited to one report. Ellis et al. (1976) reported the results of a metabolism study in which two adult (607 and 715 g BW) male Charles River rats were given nitrocellulose (12.9 percent nitrogen by weight) made with ^{14}C-labeled cotton. The nitrocellulose was cut and ground to a size small enough to pass through an 18-gauge needle and then put into either an aqueous suspension or a methyl cellulose/Tween 80 suspension. After fasting, the rats were dosed by gavage with the suspensions of nitrocellulose at a rate of 1 mL/100 g/day (about 20,000 dpm/mL for total radioactive doses of 485,600 and 572,000 dpm) for four days and kept in a Rotu-Delmar metabolism chamber. Expired CO_2, feces, and urine were collected separately for analysis. Twenty-four hours after the last dose, the rats were sacrificed and various organs examined for radioactivity (Table 8). No detectable radioactivity was found in any tissues, body fluids, or in the expired CO_2. Radioactivity was only found in the gastrointestinal tract and feces and the authors concluded that the nitrocellulose molecule was not absorbed by the rat.

4.1.2 Human Studies

Data from human studies were not found.

TABLE 8. DISTRIBUTION AND EXCRETION OF RADIOACTIVITY AFTER ORAL ADMINISTRATION OF ^{14}C-NITROCELLULOSE[a]

Tissue or Substance Samples	Total Radioactivity Recovered (dpm)	
	Rat No. 1[b]	Rat No. 2[c]
Stomach[d]	169,575	6,867
Small intestine[d]	4,979	0
Cecum[d]	60,735	0
Large intestine[d]	3,222	0
Feces	168,579	488,720
Expired air	0	0
Blood	0	0
Urine	0	0
Liver	0	0
Spleen	0	0
Kidneys	0	0
Lungs	0	0
Muscle	0	0

a. Adapted from Ellis et al. 1976.

b. Rat No. 1 received the ^{14}C-nitrocellulose as an aqueous suspension with a total radioactive dose of approximately 485,600 dpms (disintegrations per minute).

c. Rat No. 2 received the ^{14}C-nitrocellulose as a suspension in 0.2 percent methylcellulose - 0.4 percent Tween 80 with a total radioactive dose of approximately 572,000 dpms.

d. Plus internal contents.

4.2 ACUTE TOXICITY

4.2.1 Animal Studies

The acute toxicity of nitrocellulose in rats and mice was determined by Lee et al. (1975). After fasting 16 hr, male and female Charles River rats and albino Swiss mice were given, by gastric intubation, two doses 30 min apart (due to the large volume required for the highest dose), of a 5 percent (dry weight) nitrocellulose suspension in water. No toxic effects were noted in the animals receiving the highest dose of 5,000 mg/kg, even after 14 days of observation. Two of 10 male mice died at this dose, but no gross lesions were observed and the authors projected an LD_{50} of >5,000 mg/kg. Lee et al. also tested the primary skin and eye irritancy potential of nitrocellulose using rabbits. New Zealand rabbits were exposed to a 33 percent solution of nitrocellulose and the intact and abraided skin and eyes were evaluated at 24 and 72 hr. These slightly modified Draize tests were also negative.

4.2.2 Human Studies

One report of human acute response from exposure to a mixture of nitrocellulose and nitromethane described massive intravascular hemolysis followed by secondary neuropathy with anuria (Kaiffer et al. 1972). The authors did not specify whether these effects were due to exposure to nitrocellulose alone, nitromethane alone, or the mixture.

4.3 SUBCHRONIC AND CHRONIC TOXICITY

4.3.1 Animal Studies

Toxic effects of nitrocellulose ingestion were evaluated in a 13-week subchronic study using beagle dogs, Charles River rats, and albino Swiss mice (Ellis et al. 1976). Dietary levels of 0, 1, 3, or 10 percent (dry weight) were given to groups of two male and two female dogs, and to groups of eight male and eight female rats and mice. The animals were monitored for effects on food consumption, body weight, blood analysis, organ weights, and gross and microscopic pathology. Control groups consisted of a group receiving just animal chow mixture and a group receiving 10 percent cotton linters in the diet as a cotton control. The 10 percent cotton linter control group was included to determine any effects from the processing of nonnutritive bulk by the gastrointestinal system. Estimated food consumption was elevated in the mice and rat treatment groups due to the amount of nonnutritive bulk in their diets. At the 10 percent nitrocellulose and cotton linter levels, apparent food consumption was further increased because of the separation of nitrocellulose and control

fibers from the feed diets by the test animals. No adverse effects were found in animals given 1 or 3 percent nitrocellulose in their diets or in dogs receiving 10 percent nitrocellulose or 10 percent cotton linter diets. Rats and mice receiving the highest dose and those controls receiving the cotton failed to gain as much weight as controls receiving just the normal diet, but did not show any lesions or blood changes. Some mice in these two exposure groups died apparently due to fiber impaction in the lower intestinal tract.

These subchronic studies of nitrocellulose were followed up by the same laboratory with a two-year study using the same dietary levels and species (Ellis et al. 1980). The exposure groups consisted of six dogs of each sex, 32 rats of each sex, and 58 mice of each sex at every dose level. Based on the average weight gain and apparent food consumption during the two years, calculations were made to roughly determine equivalent mg/kg/day dose rates for the three species (Table 9). Because of the separation of fibers from the diet mix at the 10 percent levels, Ellis et al. made attempts to determine the amount of fiber not ingested by the animals. At the 10 percent nitrocellulose levels, they calculated that only 90 percent of diet mixture was ingested; however, at the 10 percent cotton control level, the percentage was extremely variable (0 to 100 percent) and the food consumption estimate was not reliable. The same types of observations were made in these studies as in the subchronic studies, with an interim sacrifice and necropsy of some animals at each dose level after one year of exposure. Most effects observed in the nitrocellulose treatment groups were also seen in the cotton fiber control group and were considered "fiber" effects by the authors. The "fiber" effects included intestinal impaction, increased food consumption, and dermal irritation and were a result of the physical form of the nitrocellulose and cotton fibers. Dogs and rats showed a proportional increase in food consumption related to dose level, and rats at the 10 percent levels had somewhat lower body weights than controls due to decreased body fat levels. Mice showed some additional effects, including a transient irritation of the extremities and deaths from intestinal fiber impaction (usually during first week of study). There was also a cluster of mouse deaths at the 10 percent nitrocellulose and cotton control groups after nine months with no apparent cause. These deaths occurred three times more frequently in the 10 percent nitrocellulose group than in the 10 percent cotton linter group and Ellis et al. could not rule out a nitrocellulose compound effect as the cause. This was the only effect noticed more frequently in the nitrocellulose group than in the cotton group. Ellis et al. concluded that nitrocellulose in the diet acts as nonnutritive bulk and has no adverse effects at the 10 percent level in dogs (approximately 5,135 mg/kg/day for males and 5,737 mg/kg/day for

TABLE 9. BODY WEIGHTS, FOOD CONSUMPTION, AND CALCULATED DOSE RATES FOR RATS, MICE, AND DOGS REPEATEDLY EXPOSED TO NITROCELLULOSE

Species (Sex)	Test[a] Duration	NC Dose[b] (%)	Body Weight[c] (g)	Food Intake[d] (g/day)	Calculated Dose[e] (mg/kg/day)
Rat	13 wk	0	415.3	26.0	0
(male)	13 wk	10Cf	336.8	67.3	19,982[f]
	13 wk	1	415.0	26.9	648.2
	13 wk	3	400.0	31.7	2,378
	13 wk	10	349.5	58.1	16,624
Rat	13 wk	0	253.3	17.5	0
(female)	13 wk	10Cf	246.8	52.2	21,151
	13 wk	1	239.0	20.1	841.0
	13 wk	3	264.0	22.9	2,602
	13 wk	10	250.5	46.5	18,563
Mouse	13 wk	0	32.2	4.9	0
(male)	13 wk	10Cf	27.9	13.6	48,746
	13 wk	1	29.9	4.9	1,638.8
	13 wk	3	31.2	5.4	5,192
	13 wk	10	25.8	19.2	74,419
Mouse	13 wk	0	25.7	4.2	0
(female)	13 wk	10Cf	23.3	12.2	52,361
	13 wk	1	27.3	4.7	1,721.6
	13 wk	3	23.3	5.6	7,210
	13 wk	10	21.4	9.7	45,327
Dog	13 wk	0	9,650	720	0
(male)	13 wk	10Cf	13,100	677	5,168
	13 wk	1	12,025	637	529.7
	13 wk	3	10,725	709	1,983
	13 wk	10	11,350	701	6,176
Dog	13 wk	0	7,950	554	0
(female)	13 wk	10Cf	7,800	641	8,218
	13 wk	1	10,725	652	607.9
	13 wk	3	6,900	639	2,778
	13 wk	10	9,275	730	7,871
Rat	24 mo	0	710§	27.2	0
(male)	24 mo	10Cf	565§	54.1	9,575
	24 mo	1	733§	28.2	384.7
	24 mo	3	716§	30.1	1,261
	24 mo	10	549§	48.3	8,798
Rat	24 mo	0	416§	18.6	0
(female)	24 mo	10Cf	357§	45.9	12,857
	24 mo	1	428§	19.2	448.6
	24 mo	3	465§	21.6	1,394
	24 mo	10	375§	38.9	10,373

TABLE 9. (Cont.)

Species (Sex)	Test[a] Duration	NC Dose[b] (%)	Body Weight[c] (g)	Food Intake[d] (g/day)	Calculated Dose[e] (mg/kg/day)
Mouse	24 mo	0	44	5.9	0
(male)	24 mo	10C[f]	38.7[g]	15.8	40,827[h]
	24 mo	1	42.6[g]	6.5	1,526
	24 mo	3	NA[i]	6.7	ND[j]
	24 mo	10	38.3[g]	13.8	36,031[k]
Mouse	24 mo	0	37	6.2	0
(female)	24 mo	10C[f]	34.2	12.8	37,427[h]
	24 mo	1	34.8	6.4	1,839
	24 mo	3	34.7	6.5	5,620
	24 mo	10	33.7	14.9	44,214[k]
Dog	24 mo	0	11,767[g]	422	0
(male)	24 mo	10C[f]	11,567[g]	514	4,444
	24 mo	1	NA	463	ND
	24 mo	3	NA	456	ND
	24 mo	10	11,333[g]	582	5,135
Dog	24 mo	0	9,200[g]	411	0
(female)	24 mo	10C[f]	9,200[g]	492	5,348
	24 mo	1	NA	428	ND
	24 mo	3	NA	429	ND
	24 mo	10	9,133[g]	524	5,737

a. Data on 13-wk tests from Ellis et al. 1976 and data on 24 mo test from Ellis et al. 1980.

b. Dose of nitrocellulose (NC) given as a percentage of diet.

c. Average body weight in grams.

d. Average food consumption in grams per animal per day.

e. Calculated dose in mg/kg/day using food intake, body weight, and percent dose.

f. 10C = Cotton control group with 10 percent cotton fibers in diet.

g. Values taken from graph based on 3 to 6 points.

h. Values are unreliable due to variable amounts of fiber removed from diet mix by animals.

i. NA = data not available.

j. ND = not determined.

k. Values are high due to separation of fiber from diet by animals; for use in criteria calculations, number given will be reduced by 10 percent.

females) and in rats (approximately 8,798 mg/kg/day for males and 10,373 mg/kg/day for females) and at the 3 percent level in mice (approximately 5,620 mg/kg/day for females).

Based on the subchronic and chronic studies performed by Ellis and his co-workers, the long-term effects of nitrocellulose seem to be minimal and correspond well with the lack of effects in acute studies. The no-observed-adverse-effect levels were approximately 5,135 to 5,737 mg/kg/day (for dogs) and approximately 8,798 to 10,373 mg/kg/day (for rats) and the only observed-adverse-effect level was a frank-effect-level (FEL) of approximately 32,428 to 39,793 mg/kg/day (based on 90 percent of 36,031 to 44,214 mg/kg/day levels for skin irritation and death in mice). These values are probably artificially high because calculations of food consumption and ingested nitrocellulose did not account for separation of the nitrocellulose fibers from the diet mixture by the animals.

4.3.2 Human Studies

Data from human studies were not found.

4.4 GENOTOXICITY

The genotoxic effects of nitrocellulose were studied in several short-term assays, including the Ames test. Ellis et al. (1978) exposed Salmonella typhimurium strains TA-98, TA-100, TA-1535, TA-1537, and TA-1538 to nitrocellulose concentrations of 0, 100, 1,000 and 5,000 g/plate and the number of revertant colonies per plate were counted. None of the tested concentrations, with or without S-9 activation, increased significantly the number of revertant colonies over controls. Therefore, nitrocellulose was considered to be nongenotoxic by the authors.

In a later study from the same laboratory, Ellis et al. (1980) evaluated the cytogenetic effects in rats fed nitrocellulose in the diet for 24 months. Cell cultures were prepared using blood lymphocytes, bone marrow cells, and kidney cells taken from Charles River rats exposed to 0, 1, 3, or 10 percent nitrocellulose diets. From these cultures, cell ploidy was estimated by examining 200 cells, and chromosomes were counted and observed for morphological aberrations by looking at 50 metaphase cells. No significant chromosomal effects were noted in this evaluation, thus supporting the authors earlier conclusion that nitrocellulose is not genotoxic.

4.5 DEVELOPMENTAL/REPRODUCTIVE TOXICITY

4.5.1 Animal Studies

Data on the developmental toxicity of nitrocellulose are limited primarily to a three generation reproductive study using Charles River rats (Ellis et al. 1980). Nitrocellulose was fed in the diet at levels of 0, 1, 3, and 10 percent, and a control of 10 percent cotton linters was used. Ten males and 20 females from each dose group were mated after being on test diets for 6 months and were used as the parental group (F_0). The first offspring (F_{1a}) were discarded after weaning and the F_0 rats were mated again to provide the second generation rats (F_{1b}). These rats were mated and their offspring (F_{2a} and F_{2b}) treated in the same manner as the F_0 rats. The F_{2b} rats were mated again to form the third generation (F_{3a} and F_{3b}). The fertility of the males and females appeared not to be affected by the nitrocellulose, with mating ratio, pregnancy ratio, and ratios of fertile to mated males or females not significantly different from controls. For the reproductive parameters, the results were also generally not significant (Table 10) with only random changes in litter size, live-born index, birth weight, viability index, or ratio of males to total offspring. Significant reductions did occur in the lactation index and in the weight of pups at weaning in the 10 percent nitrocellulose and 10 percent cotton linter groups. This suggests that the nitrocellulose does not directly affect reproduction, but the effects of the fibers can cause some adverse consequences on related processes, such as nutrition.

4.5.2 Human Studies

Two reports in the Bulgarian literature were found that related exposure to organic solvents and nitrocellulose lacquers in the work environment to occurrence of obstetric and gynecologic diseases or problems (Panova 1967, 1968). No specific details were given in the abstract regarding the degree of involvement of nitrocellulose in these occupational exposures.

4.6 CARCINOGENICITY

4.6.1 Animal Studies

The chronic two-year studies in dogs, rats, and mice performed by Ellis et al. (1980) and described in Section 4.3 also included tissue examinations for carcinogenic effects; no lesions or tumors were found in these studies.

TABLE 10. REPRODUCTIVE PERFORMANCE OF FEMALE RATS GIVEN NITROCELLULOSE IN A THREE-GENERATION STUDY[a]

Dose (% Diet)	Litter Number	Litter Size	Live-born Index (%)	Weight at Birth (g)	Viability Index (%)[b]	Lactation Index (%)[c]	Weight at Weaning (g)	Sex Ratio Males:Total
0	F1a	9.3±1.1(7)[d]	95±4	6.3±0.3	98±2	86±14	47±4(6)	27:49
0	F1b	13.2±1.1(4)	96±2	6.3±0.3	96±8	100	44±1	21:48
0	F2a	14.4±0.6(14)	100	6.7±0.2	99±1	98±1	42±1	96:194
0	F2b	11.0±1.0(14)	98±1	7.3±0.4	99±1	100	50±2	73:148
0	F3a	14.1±0.7(15)	99±1	6.7±0.1	98±1	96±2	42±2	97:196
0	F3b	14.6±0.7(14)	98±1	6.6±0.1	98±1	97±1	38±2	92:190
1	F1a	7.8±2.0(8)	86±12	7.5±0.7	82±14(7)	100(6)	53±5(6)	30:57
1	F1b	14.2±1.2(6)	100	7.2±0.6	91±9	99±1	49±4	34:76
1	F2a	11.6±0.9(16)	100	6.9±0.3	101±1	94±3	52±3	69:175
1	F2b	10.0±1.0(16)	93±4	7.0±0.2	99±1	98±2	53±2	77:149
1	F3a	13.1±0.5(17)	99±1	6.6±0.1	98±2	97±3	46±1	102:209
1	F3b	12.3±1.2(13)	83±10	5.6±0.7	92±8(12)	93±5(11)	39±2	72:140
3	F1a	7.8±2.0(8)	86±12	7.5±0.7	82±14(7)	100(6)	53±5(6)	30:57
3	F1b	10.2±1.6(6)	94±6	7.2±0.2	96±4	96±2	56±5	21:52
3	F2a	12.4±0.9(16)	100	7.1±0.4	95±2	89±4	50±2	85:169
3	F2b	11.4±1.0(13)	94±3	6.6±0.2	96±2	90±4	48±3	67:121
3	F3a	13.1±0.5(17)	98±1	6.5±0.1	100	98±2	45±2	97:212
3	F3b	12.9±0.8(16)	99±1	6.4±0.1	99±1	91±3	44±2	106:183
10	F1a	9.5±1.7(17)	98±2	7.2±0.6	97±2	82±8	41±8(15)	55:116
10	F1b	10.2±1.2(10)	100	7.9±0.4[e]	99±1	73±10	26±3(9)[e]	39:76
10	F2a	12.5±0.5(18)	10[illegible]	6.9±0.1	98±1	51±9[e]	23±3[e]	44:103
10	F2b	10.1±1.2(17)	100	7.2±0.4	100±10	56±9[e]	34±4[e]	50:102
10	F3a	12.4±0.6(18)	99±1	6.7±0.2	94±6	98±1(17)	42±2	100:205
10	F3b	13.8±0.6(15)	97±1	6.2±0.4	91±7	98±1(14)	35±2	81:176
10[f]	F1a	6.6±1.1(13)	92±5	6.3±0.3	88±8	66±12(12)	27±3(9)	22:48
10[f]	F1b	10.7±0.6(12)	95±4	6.8±0.2	98±1	70±11	24±3(10)[e]	42:79
10[f]	F2a	14.1±0.6(19)	100	6.7±0.2	98±1	66±7[e]	24±2[e]	78:166
10[f]	F2b	12.7±0.9(17)	99±1	6.5±0.2	100	80±8	23±3[e]	69:158
10[f]	F3a	11.9±0.7(16)	93±5	6.6±0.1	99±1	99±1	43±3	90:179
10[f]	F3b	13.8±0.7(16)	99±1	6.6±0.1	99±1	90±2	37±3	104:193

a. Adapted from Ellis et al. (1980).

b. Viability index is given in percentage of live-born pups surviving to 4 days.

c. Lactation index is given in percentage of young alive at day 4 surviving to weaning.

d. Mean ± Standard Error and, in parentheses, the number of litters included in the mean.

e. Significantly different from the mean value of the respective control litter.

f. Fed 10 percent cotton linters.

4.6.2 Human Studies

The only report on carcinogenic effects of nitrocellulose describes an epidemiological study of a plastics plant that produces cellulose nitrate, cellulose acetate, polyvinyl butyral, formaldehyde, polystyrene, vinyl chloride products, and melamines. Occupational exposure categories for this plant included styrene polymerization, polystyrene processing, vinyl chloride polymerization, polyvinyl chloride processing, vinyl acetate copolymerization, cellulose acetate sheet production, cellulose nitrate processing, cellulose acetate processing, resin production/processing, polyvinyl butyral sheet production, polyvinyl butyral processing, alcohol recovery, and plating operations. A review of the mortality among workers, based on job and work location, was performed using a matched case-control study nested within a retrospective cohort design (Marsh 1983). A general comparison of mortality among 2,490 male wage earners with chemical exposure (routes not specified) and who worked at least one year in the plant between 1949 and 1966 did indicate a slight increase in digestive cancer rates and a significant increase ($P < 0.05$) in genitourinary cancer rates above the local cancer rates. When job type and work location variables were analyzed, a possible association between rectal cancer and cellulose nitrate production was indicated, although not at statistically significant levels. The odds ratios for all digestive system cancers combined showed an increase with length of exposure to cellulose nitrate production, ranging from a ratio of 1.07 for one month to 1.91 for five years to 2.85 for ten years. In terms of specific cancers, a statistically significant odds ratio of 8.90 ($P < 0.5$) for rectal cancer and cellulose nitrate production was found (based on four exposure cases). Although these results do generate some concern about the carcinogenicity of nitrocellulose, Marsh cautions that these data merely suggest an association, and many confounding factors, such as chemicals other than nitrocellulose in the production area, could be responsible for the observed ratios.

4.7 SUMMARY

The available data on the human health effects and mammalian toxicity of nitrocellulose exposure in water generally indicate that it is virtually nontoxic. The acute toxicity data indicated LD_{50} values in excess of 5,000 mg/kg. Subchronic and chronic toxicity studies in laboratory animals indicated that it is harmful only due to its physical effects in the digestive tract (fiber impaction). Genotoxicity and developmental toxicity studies did not demonstrate any significant toxic effects unrelated to these fiber effects. Carcinogenicity data,

generated by an epidemiology study of occupational exposure during production of nitrocellulose, does suggest some association between nitrocellulose production and rectal or digestive tract cancers, and this possibility should be evaluated with further research. Data on the metabolism of nitrocellulose by rats indicate that it is not absorbed into the gastrointestinal tract and further support the general conclusion that nitrocellulose in water does not represent a significant human health risk.

5. CRITERION FORMULATION

5.1 EXISTING GUIDELINES AND STANDARDS

A few references were found that suggested guidelines or values for safe exposure levels for nitrocellulose. The USEPA (1975b) published an effluent guideline standard for cellulose nitrate production designed to limit the allowable pollutants or pollutant characteristics. They recommended maximum daily values for cellulose nitrate of: 9.4 lb/1,000 lb product for biological oxygen demand, 47 lb/1,000 lb product for chemical oxygen demand, and 2.5 lb/1,000 lb product for total suspended solids. These values were chosen to account for the application of the best available technology economically achievable. Helton (1976) reported a value for suspended solids concentration of <25 ppm proposed by the Ammunition Procurement and Supply Agency for nitrocellulose fines. Martynova et al. (1978), in a discussion of effects from occupational exposure to the synthetic nitrocellulose polymer nitron, recommended a maximum permissible concentration of 5 mg/m^3 for dust in air. No federal or state water quality standards or guidelines were reported for nitrocellulose in a review by Cogley et al. (1979).

5.2 OCCUPATIONAL EXPOSURE

Reports of occupational exposure to nitrocellulose are limited and deal principally with exposure to mixtures containing nitrocellulose and various other chemicals.

Two articles by Panova (1967, 1968) examined the exposure of women polishers and upholsterers to nitrocellulose and organic solvents. The author found that exposure disrupted the menstrual cycle, but did not attribute the effects to nitrocellulose alone.

Allergic contact dermatitis resulting from contact with nail lacquers containing nitrocellulose, plasticizer, solvent, coloring agent, and adhesives (sulfonamide and formaldehyde resins) was reported by Kim et al. (1977). The face and neck were usually affected, but, again, a direct link to nitrocellulose was not established.

Martynova et al. (1978) reported the effects of exposure to dusts of synthetic polymers including nitron (a synonym of nitrocellulose). In the abstract, the authors comment that exposure produced weak fibrogenic and general toxic effects, although no specific link to nitron was mentioned.

Engibaryan and Frangulyan (1983) examined the allergic diseases occurring with exposure to hexamethyleneidamine, styrene, varnish, titanium oxide, and nitrocellulose and found that most of the 310 workers examined developed atonic forms of bronchial asthma, allergic rhinitis, or dermatitis. Again a specific connection between nitrocellulose and the observed effects was not made.

An epidemiological study evaluating the potential for increased mortality due to certain digestive and genitourinary cancers following exposure to chemicals, including nitrocellulose, in a plastics producing plant was reported by Marsh (1983). An association between nitrocellulose production and rectal cancer was indicated, but Marsh cautions that the significance of this finding should not be overly emphasized due to the low numbers and confounding factors involved in the analysis.

5.3 PREVIOUSLY CALCULATED CRITERIA

Previous attempts were made to calculate a water quality criterion value for nitrocellulose based on the then current USEPA guidelines (Sullivan et al. 1978). The authors looked at the available information (essentially the same aquatic data contained in this report) and concluded that the toxicity of nitrocellulose to aquatic species is very low to nil. Therefore, they felt that nitrocellulose should be regulated based on physical factors alone, and recommended that the USEPA criteria for solids and turbidity were sufficient for control:

> ''Settleable and suspended solids should not reduce the depth of the compensation point for photosynthetic activity by more than 10 percent from the seasonally established norm for aquatic life.'' (USEPA 1976).

In actual levels, several figures might be applicable for regulation of nitrocellulose. A value of 10 ppm was given for total suspended solids (TSS) in wastewater discharges (Barkley and Rosenblatt 1978). For point source categories of wastewater discharged from an explosives manufacturing site, a maximum value of 0.25 kg/1,000 kg of product and an average of daily values for 30 days of 0.084 kg/1,000 kg of product were given for TSS (USEPA 1984). For point source categories of wastewater discharged from a Load, Assemble, and Pack facility, the maximum value for

TSS was 0.26 kg/1,000 kg of product and the 30-day average value for TSS was 0.088 kg/1,000 kg of product (USEPA 1984).

Another attempt to calculate nitrocellulose water quality criteria using EPA guidelines for aquatic and human health was made by the U.S. Army Medical Bioengineering Research and Development Laboratory (USAMBRDL) in an interim report to the Surgeon General's Office (U.S. Army 1982, 1983). This report concluded that recommendation of criteria was not justified because of the insolubility of nitrocellulose in water and the general lack of toxicity to mammalian and aquatic species following exposure.

The conclusions of the USAMBRDL assessment were generally supported in an earlier paper by Pearson and Glennon (1979) in which they discuss the U.S. Army's approach to hazard assessment. They listed the following requirements that would lead to not generating a water quality criterion for a compound: relatively nontoxic (e.g., LC_{50} values of >1,000 mg/L); toxicity is not increased by environmental parameters or any aging effects; a low potential for bioconcentration (e.g, a BCF of <100); and low potential for adverse environmental effects, based on results from mammalian and environmental fate/chemistry studies. These requirements are met by nitrocellulose, indicating that criteria probably do not need to be calculated.

A water quality criterion designed to protect aquatic life was estimated by Bentley et al. (1977). They based their estimate on a 96-hr EC_{50} value for Selenastrum capricornutum (579 mg/L) multiplied by an application factor of 0.1. Bentley et al. felt the resulting value, 50 mg/L, "should provide reasonable protection of aquatic life."

5.4 AQUATIC CRITERIA

The aquatic criteria consist of two values, a criterion maximum concentration and a criterion continuous concentration (Stephan et al. 1985). The criterion maximum concentration is calculated by dividing the Final Acute Value (FAV) by two. The criterion continuous concentration is equal to the lowest of the Final Chronic Value (FCV), the Final Plant Value, or the Final Residue Value.

Data available for calculating aquatic criteria for nitrocellulose do not meet all the requirements specified by the USEPA guidelines (Stephan et al. 1985), thus strictly speaking one should not calculate aquatic criteria. However, because most of the data indicate the same level of toxicity, an attempt was made to generate water quality criteria using the formula provided in the USEPA guidelines (Stephan et al. 1985). Despite not testing enough genera

and not using flow-through tests, the calculated FAV, 1,000 mg/L, would be expected to be conservatively accurate because all of the acute values were >1,000 mg/L. Not enough data were available for calculating a FCV, with the only nonacute test failing to demonstrate adverse effects at concentrations up to 540 mg nitrocellulose/kg sediment (dry weight) (Table 5). The Final Residue Value also could not be calculated due to a lack of data. The plant value, an EC_{50} based on chlorophyll a decrease and taken from the lowest value of all tests, was 579 mg/L.

Due to the lack of data on chronic effects and final residue values, meaningful criteria cannot be calculated. Thus the available data could only indicate the relative toxic potential of nitrocellulose.

5.5 HUMAN HEALTH CRITERIA

Data on nitrocellulose have not shown any significant carcinogenic pattern. Also, reliable evidence from human exposures (either threshold or nonthreshold effects) was not available for calculating a human health criterion. Therefore, the chronic and subchronic animal data generated by Ellis et al. (1980) were used to generate the following criterion. Because the only observed-adverse-effect-level occurred in the 2-yr mouse study, the criteria calculations were based on that species. The frank-effect-level was determined to be at concentrations of approximately 32,428 mg/kg/day (males) and 39,793 mg/kg/day (females) for an unexplained cluster of deaths judged significant by the authors. Within this mouse study, the highest no-observed-adverse-effect-level (NOAEL) was approximately 5,620 mg/kg/day for females (no mg/kg/day estimate was available for males at this diet level). Applying an uncertainty factor of 100 to these NOAEL data, as recommended by the USEPA (1980) guidelines, and multiplying by 70 kg gives a human ADI (acceptable daily intake) of 3,934 mg/day. Inserting this ADI into the human health criterion equation gives a human health criterion of 1,967 mg/kg/day.

$$C = \frac{ADI - (DT + IN)}{[2L + (0.0065\ kg \times BCF)]},$$

$$C = \frac{3{,}934 - (0 + 0)}{[2L + (0.0065\ kg \times 0)]},$$

where,

C = criterion,
DT = nonfish dietary intake,
IN = inhalation intake,
2L = daily water intake in liters,
0.0065 kg = daily dietary fish intake, and
BCF = bioconcentration factor,

Of course these daily intake values are based on approximations of the amount of nitrocellulose eaten by the mice in their dietary exposures and may represent higher levels than actually ingested. Based on physical/chemical properties of nitrocellulose and occupational exposure data, the non-fish dietary and inhalation intakes were assumed to be zero. Also, based on the limited data of Ellis et al. (1976), the bioconcentration factor was assumed to be zero. These values suggest that nitrocellulose is not a major concern for human health following exposure to aquatic concentrations of nitrocellulose associated with the manufacturing or processing in Army Ammunition Plants.

5.6 RESEARCH RECOMMENDATIONS

As mentioned previously, the data on nitrocellulose are incomplete as far as calculating water quality criteria are concerned. The following research recommendations are intended to produce data needed to meet the USEPA guideline requirements (USEPA 1980; Stephan et al. 1985) for generating water quality criteria.

1. Acute aquatic tests using species from a genus in a phylum other than Arthropoda or Chordata (e.g., Annelida or Mollusca).

2. Chronic tests in three different aquatic families where at least one is a fish, one is an invertebrate, and one is a sensitive freshwater species.

3. Bioconcentration data for an appropriate aquatic species or on the significance of residues in aquatic species.

4. More precise data from chronic dietary exposure studies in laboratory mammals, particularly regarding doses actually ingested.

5. Possible effects of nitrocellulose fibers on respiratory function in animals that use gills, e.g., fish mortality due to mucous buildup on the gills from physical irritation by fibers.

6. Further evaluation of the suggested link between occupational nitrocellulose exposure and rectal cancer.

7. Examine the effect of nitrocellulose fibers on macrophyte communities, e.g, decrease photosynthesis due to fiber buildup.

The available data on nitrocellulose indicate that its toxicity is so low that extensive testing would probably not reveal additional significant information. Only one area that lacks significant data should be evaluated by further research: chronic toxicity to aquatic animals. A study (e.g., an early life stage test) evaluating the impact of nitrocellulose in the water column (maintained in suspension) on an appropriate fish species should be conducted. If the results from this test indicate that the impact of nitrocellulose is minimal, then the recommendation of this review is to cease activity aimed at producing water quality criteria and to regulate nitrocellulose based on its physical effects on aquatic habitats. Wastewater discharges from AAPs should be monitored for color, suspended solids, and dissolved solids, which are characteristics of nitrocellulose that will have the most significant impact on the aquatic systems associated with manufacturing and production at AAPs. This recommendation is further supported by the conclusions of Sullivan et al. (1978), USEPA (1976), and USAMBRDL (U. S. Army 1982, 1983).

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7. GLOSSARY

AAP	Army Ammunition Plant
ADI	Acceptable daily intake
BCF	Bioconcentration factor (R)
BOD	Biological oxygen demand
COD	Chemical oxygen demand
dpm	Disintegrations per minute
EC_{50}	Effective concentration based on immobilization
FEL	Frank effect level
fines	Waste particles of nitrocellulose
gpm	Gallons per minute
LAP	Load, assemble, and pack
LC_{50}	Lethal concentration
NOAEL	No observed adverse effect level
NOEL	No observed effect level
NPDES	National Pollution Discharge Elimination System
ppm	Parts per million
TNT	α-Trinitrotoluene (unless mentioned otherwise)
USAMBRDL	U.S. Army Medical Bioengineering Research and Development Laboratory
USATHAMA	U.S. Army Toxic and Hazardous Materials Agency

APPENDIX A:
SUMMARY OF USEPA METHODOLOGY FOR DERIVING NUMERICAL WATER QUALITY CRITERIA FOR THE PROTECTION OF AQUATIC ORGANISMS AND THEIR USES

The following summary is a condensed version of the 1985 final US Environmental Protection Agency (USEPA) guidelines for calculating a water quality criteria to protect aquatic life and is slanted towards the specific regulatory needs of the US Army (e.g., discussion of saltwater aspects of the criteria calculation are not included). The guidelines are the most recent document outlining the required procedures and were written by the following researchers from the USEPA's regional research laboratories: C.E. Stephan, D.I. Mount, D.J. Hansen, J.H. Gentile, G.A. Chapman, and W.A. Brungs. For greater detail on individual points consult Stephan et al. (1985).

1. INTRODUCTION

The Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses describe an objective, internally consistent, and appropriate way of estimating national criteria. Because aquatic life can tolerate some stress and occasional adverse effects, protection of all species all of the time was not deemed necessary. If acceptable data are available for a large number of appropriate taxa from a variety of taxonomic and functional groups, a reasonable level of protection should be provided if all except a small fraction are protected, unless a commercially, recreationally, or socially important species was very sensitive. The small fraction is set at 0.05 because other fractions resulted in criteria that seemed too high or too low in comparison with the sets of data from which they were calculated. Use of 0.05 to calculate a Final Acute Value does not imply that this percentage of adversely affected taxa should be used to decide in a field situation whether a criterion is appropriate.

To be acceptable to the public and useful in field situations, protection of aquatic organisms and their uses should be defined as prevention of unacceptable long-term and short-term effects on (1) commercially, recreationally, and socially important species and (2) (a) fish and benthic invertebrate assemblages in rivers and streams and (b) fish, benthic invertebrate, and zooplankton assemblages in lakes, reservoirs, estuaries, and oceans. These national guidelines have been developed on the theory that effects which occur on a species in appropriate laboratory tests will generally occur on the same species in comparable field situations.

Numerical aquatic life criteria derived using these national guidelines are expressed as two numbers, so that the criteria can more accurately reflect toxicological and practical realities. The combination of a maximum concentration and a continuous concentration is designed to provide adequate protection of aquatic life and its uses from acute and chronic toxicity to animals, toxicity to plants, and

bioaccumulation by aquatic organisms without being as restrictive as a one-number criterion would have to be in order to provide the same degree of protection.

Criteria produced by these guidelines should be useful for developing water quality standards, mixing zone standards, and effluent standards. Development of such standards may have to consider additional factors such as social, legal, economic, and additional biological data. It may be desirable to derive site-specific criteria from these national criteria to reflect local conditions (USEPA 1982). The two factors that may cause the most difference between the national and site-specific criteria are the species that will be exposed and the characteristics of the water.

Criteria should provide reasonable and adequate protection with only a small possibility of considerable overprotection or underprotection. It is not enough that a criterion be the best estimate obtainable using available data; it is equally important that a criterion be derived only if adequate appropriate data are available to provide reasonable confidence that it is a good estimate. Thus, these guidelines require that certain data be available if a criterion is to be derived. If all the required data are not available, usually a criterion should not be derived; however, availability of all required data does not ensure that a criterion can be derived. The amount of guidance in these national guidelines is significant, but much of it is necessarily qualitative rather than quantitative; much judgement will be required to derive a water quality criterion for aquatic life. All necessary decisions should be based on a thorough knowledge of aquatic toxicology and an understanding of these guidelines and should be consistent with the spirit of these guidelines - which is to make best use of all available data to derive the most appropriate criterion.

2. DEFINITION OF MATERIAL OF CONCERN

1. Each separate chemical that does not ionize significantly in most natural bodies of water should be considered a separate material, except possibly for structurally similar organic compounds that only exist in large quantities as commercial mixtures of the various compounds and apparently have similar biological, chemical, physical, and toxicological properties.

2. For chemicals that do ionize significantly, all forms that would be in chemical equilibrium should usually be considered one material. Each different oxidation state of a metal and each different nonionizable covalently bonded organometallic compound should usually be considered a separate material.

3. Definition of the material should include an operational analytical component. It is also necessary to reference or

describe analytical methods that the term is intended to denote. Primary requirements of the operational analytical component is that it be appropriate for use on samples of receiving water, that it be compatible with toxicity and bioaccumulation data without making extrapolations that are too hypothetical, and that it rarely result in underprotection of aquatic life and its uses.

NOTE: Analytical chemistry of the material may have to be considered when defining the material or when judging acceptability of some toxicity tests, but a criterion should not be based on sensitivity of an analytical method. When aquatic organisms are more sensitive than analytical techniques, the proper solution is to develop better analytical methods, not to underprotect aquatic life.

3. COLLECTION OF DATA

1. Collect all available data on the material concerning (a) toxicity to, and bioaccumulation by, aquatic animals and plants; (b) FDA action levels (FDA Guidelines Manual); and (c) chronic feeding studies and long-term field studies with wildlife that regularly consume aquatic organisms.

2. All data used should be available in typed, dated and signed hardcopy with enough supporting information to indicate that acceptable test procedures were used and the results should be reliable.

3. Questionable data, whether published or not, should not be used.

4. Data on technical grade materials may be used if appropriate, but data on formulated mixtures and emulsifiable concentrates of the test material should not be used.

5. For some highly volatile, hydrolyzable, or degradable materials it may be appropriate to only use results of flow-through tests in which concentration of test material in test solutions were measured using acceptable analytical methods.

6. Do not use data obtained using brine shrimp, species that do not have reproducing wild populations in North America, or organisms that were previously exposed to significant concentrations of the test material or other contaminants.

4. REQUIRED DATA

1. Results of acceptable acute tests (see Section 5) with freshwater animals in at least eight different families such that all of the following are included:

   a. the family Salmonidae in the class Osteichthyes;

   b. a second family (preferably an important warmwater species) in the class Osteichthyes (e.g., bluegill, fathead minnow, or channel catfish);

   c. a third family in the phylum Chordata (e.g, fish or amphibian);

   d. a planktonic crustacean (e.g, cladoceran or copepod);

   e. a benthic crustacean (e.g, ostracod, isopod, or amphipod);

   f. an insect (e.g., mayfly, midge, stonefly);

   g. a family in a phylum other than Arthropoda or Chordata (e.g, Annelida or Mollusca); and

   h. a family in any order of insect or any phylum not represented.

2. Acute-chronic ratios (see Section 7) for species of aquatic animals in at least three different families provided that of the three species at least (a) one is a fish, (b) one is an invertebrate, and (c) one is a sensitive freshwater species.

3. Results of at least one acceptable test with a freshwater alga or a chronic test with a freshwater vascular plant (see Section 9). If plants are among the aquatic organisms that are most sensitive to the material, results of a test with a plant in another phylum (division) should be available.

4. At least one acceptable bioconcentration factor determined with an appropriate aquatic species, if a maximum permissible tissue concentration is available (see Section 10).

If all required data are available, a numerical criterion can usually be derived, except in special cases. For example, if a criterion is to be related to a water quality characteristic (see Sections 6 and 8), more data will be necessary. Similarly if all required data are not available a numerical criterion should not be derived except in special cases. For example, even if not enough acute and chronic data are available, it

may be possible to derive a criterion if the data clearly indicate that the Final Residue Value would be much lower than either the Final Chronic Value or the Final Plant Value. Confidence in a criterion usually increases as the amount of data increases. Thus, additional data are usually desirable.

5. FINAL ACUTE VALUE

1. The Final Acute Value (FAV) is an estimate of the concentration of material corresponding to a cumulative probability of 0.05 in the acute toxicity values for the genera with which acute tests have been conducted on the material. However, in some cases, if the Species Mean Acute Value (SMAV) of an important species is lower than the calculated FAV, then that SMAV replaces the FAV to protect that important species.

2. Acute toxicity tests should have been conducted using acceptable procedures (e.g., ASTM Standard E 724 or 729).

3. Generally, results of acute tests in which food was added to the test solution should not be used, unless data indicate that food did not affect test results.

4. Results of acute tests conducted in unusual dilution water, e.g., dilution water containing high levels of total organic carbon or particulate matter (higher than 5 mg/L) should not be used, unless a relationship is developed between toxicity and organic carbon or unless data show that organic carbon or particulate matter, etc. do not affect toxicity.

5. Acute values should be based on endpoints which reflect the total adverse impact of the test material on the organisms used in the tests. Therefore, only the following kinds of data on acute toxicity to freshwater aquatic animals should be used:

    a. Tests with daphnids and other cladocerans should be started with organisms <24 hr old and tests with midges should be started with second- or third-instar larvae. The result should be the 48-hr EC_{50} based on percentage of organisms immobilized plus percentage of organisms killed. If such an EC_{50} is not available from a test, the 48-hr LC_{50} should be used in place of the desired 48-hr EC_{50}. An EC_{50} or LC_{50} of longer than 48 hr can be used provided animals were not fed and control animals were acceptable at the end of the test.

b. The result of tests with all other aquatic animal species should be the 96-hr EC_{50} value based on percentage of organisms exhibiting loss of equilibrium plus percentage of organisms immobilized plus percentage of organisms killed. If such an EC_{50} value is not available from a test, the 96-hr LC_{50} should be used in place of the desired EC_{50}.

c. Tests with single-cell organisms are not considered acute tests, even if the duration was $\leq$96 hr.

d. If the tests were conducted properly, acute values reported as greater than values and those acute values which are above solubility of the test material are acceptable.

6. If the acute toxicity of the material to aquatic animals has been shown to be related to a water quality characteristic (e.g., total organic carbon) for freshwater species, a Final Acute Equation should be derived based on that characteristic.

7. If the data indicate a that one or more life stages are at least a factor of 2 times more resistant than one or more other life stages of the same species, the data for the more resistant life stages should not be used in the calculation of the SMAV because a species can only be considered protected from acute toxicity if all life stages are protected.

8. Consider the agreement of the data within and between species. Questionable results in comparison to other acute and chronic data for the species and other species in the same genus probably should not be used.

9. For each species for which at least one acute value is available, the SMAV should be calculated as the geometric mean of all flow-through test results in which the concentration of test material were measured. For a species for which no such result is available, calculate the geometric mean of all available acute values, i.e., results of flow-through tests in which the concentrations were not measured and results of static and renewal tests based on initial total concentrations of test material.

   NOTE: Data reported by original investigators should not be rounded off and at least four significant digits should be retained in intermediate calculations.

10. For each genus for which one or more SMAV is available, calculate the Genus Mean Acute Value (GMAV) as the geometric mean of the SMAVs.

11. Order the GMAVs from high to low and assign ranks (R) to the GMAVs from "1" for the lowest to "N" for the highest. If two or more GMAVs are identical, arbitrarily assign them successive ranks.

12. Calculate the cumulative probability (P) for each GMAV as R/(N+1).

13. Select the four GMAVs which have cumulative probabilities closest to 0.05 (if there are <59 GMAVs, these will always be the four lowest GMAVs).

14. Using the selected GMAVs and Ps, calculate

$$S^2 = \frac{\Sigma((\ln GMAV)^2) - ((\Sigma(\ln GMAV))^2/4)}{\Sigma(P) - ((\Sigma(\sqrt{P}))^2/4}$$

$$L = (\Sigma(\ln GMAV) - S(\Sigma(\sqrt{P})))/4$$

$$A = S(\sqrt{0.05}) + L$$

$$FAV = e^A$$

15. If for an important species, such as a recreationally or commercially important species, the geometric mean of acute values from flow-through tests in which concentrations of test material were measured is lower than the FAV, then that geometric mean should be used as the FAV.

16. Go to Section 7.

6. FINAL ACUTE EQUATION

1. When enough data show that acute toxicity to two or more species is similarly related to a water quality characteristic, the relationship should be considered as described below or using analysis of covariance (Dixon and Brown 1979, Neter and Wasserman 1974). If two or more factors affect toxicity, multiple regression analyses should be used.

2. For each species for which comparable acute toxicity values are available at two or more different values of the water quality characteristic, perform a least squares regression of acute toxicity values on values of the water quality characteristic.

3. Decide whether the data for each species is useful, considering the range and number of tested values of the water quality characteristic and degree of agreement within and between species. In addition, questionable results, in comparison with other acute and chronic data for the species and other species in the same genus, probably should not be used.

4. Individually for each species calculate the geometric mean of the acute values and then divide each of the acute values for a species by the mean for the species. This normalizes the acute values so that the geometric mean of the normalized values for each species individually and for any combination of species is 1.0

5. Similarly normalize the values of the water quality characteristic for each species individually.

6. Individually for each species perform a least squares regression of the normalized acute toxicity values on the corresponding normalized values of of the water quality characteristic. The resulting slopes and 95 percent confidence limits will be identical to those obtained in 2. above. Now, however, if the data are actually plotted, the line of best fit for each individual species will go through the point 1,1 in the center of the graph.

7. Treat all the normalized data as if they were all for the same species and perform a least squares regression of all the normalized acute values on the corresponding normalized values of the water quality characteristic to obtain the pooled acute slope (V) and its 95 percent confidence limits. If all the normalized data are actually plotted, the line of best fit will go through the point 1,1 in the cent of the graph.

8. For each species calculate the geometric mean (W) of the acute toxicity values and the geometric mean (X) of the related values of the water quality characteristic (calculated in 4. and 5. above).

9. For each species calculate the logarithmic intercept (Y) of the SMAV at a selected value (Z) of the water quality characteristic using the equation: $Y = \ln W - V(\ln X - \ln Z)$.

10. For each species calculate the SMAV using: $SMAV = e^{Y}$.

11. Obtain the F[illegible] by using the procedure described in Section 5. (No[illegible].

12. If the SMAV for an important species is lower than the FAV at Z, then that SMAV should be used as the FAV at Z.

13. The Final Acute Equation is written as: FAV = $e^{(V[\ln(\text{water quality characteristic}) + \ln A - V[\ln Z])}$, where V = pooled acute slope and A = FAV at Z. Because V, A, and Z are known, the FAV can be calculated for any selected value of the water quality characteristic.

7. FINAL CHRONIC VALUE

1. Depending on available data, the Final Chronic Value (FCV) might be calculated in the same manner as the FAV or by dividing the FAV by the Final Acute-Chronic Ratio.

   NOTE: Acute-chronic ratios and application factors are ways of relating acute and chronic toxicities of a material to aquatic organisms. Safety factors are used to provide an extra margin of safety beyond known or estimated sensitivities of aquatic organisms. Another advantage of the acute-chronic ratio is that it should usually be greater than one; this should avoid confusion as to whether a large application factor is one that is close to unity or one that has a denominator that is much greater than the numerator.

2. Chronic values should be based on results of flow-through (except renewal is acceptable for daphnids) chronic tests in which concentrations of test material were properly measured at appropriate times during testing.

3. Results of chronic tests in which survival, growth, or reproduction in controls was unacceptably low should not be used. Limits of acceptability will depend on the species.

4. Results of chronic tests conducted in unusual dilution water should not be used, unless a relationship is developed between toxicity and the unusual characteristic or unless data show the characteristic does not affect toxicity.

5. Chronic values should be based on endpoints and exposure durations appropriate to the species. Therefore, only results of the following kinds of chronic toxicity tests should be used:

a. Life-cycle toxicity tests consisting of exposures of two or more groups of a species to a different concentration of test material throughout a life cycle. Tests with fish should begin with embryos or newly hatched young <48 hr old, continue through maturation and reproduction, and should end not <24 days (90 days for salmonids) after the hatching of the next generation. Tests with daphnids should begin with young <24 hr old and last for not <21 days. For fish, data should be obtained and analyzed on survival and growth of adults and young, maturation of males and females, eggs spawned per female, embryo viability (salmonids only), and hatchability. For daphnids, data should be obtained and analyzed on survival and young per female.

b. Partial life-cycle toxicity tests consisting of exposures of two or more groups of a species to a different concentration of test material throughout a life cycle. Partial life-cycle tests are allowed with fish species that require more than a year to reach sexual maturity, so that all major life stages can be exposed to the test material in less than 15 months. Exposure to the test material should begin with juveniles at least 2 months prior to active gonadal development, continue through maturation and reproduction, and should end not <24 days (90 days for salmonids) after the hatching of the next generation. Data should be obtained and analyzed on survival and growth of adults and young, maturation of males and females, eggs spawned per female, embryo viability (salmonids only), and hatchability.

c. Early life-stage toxicity tests consisting of 28- to 32-day (60 days posthatch for salmonids) exposures of early life stages of a species of fish from shortly after fertilization through embryonic, larval, and early juvenile development. Data should be obtained on growth and survival.

NOTE: Results of an early life-stage test are used as predictors of results of life-cycle and partial life-cycle tests with the same species. Therefore, when results of a life-cycle or partial life-cycle test are available, results of an early life-stage test with the same species should not be used. Also, results of early life-stage tests in which the incidence of mortalities or abnormalities increased substantially near the end of the test should not be used because results of such tests may be poor estimates of results of a comparable life-cycle or partial life-cycle test.

6. A chronic value may be obtained by calculating the geometric mean of lower and upper chronic limits from a chronic test or by analyzing chronic data using regression analysis. A lower chronic limit is the highest tested concentration (a) in an acceptable chronic test, (b) which did not cause an unacceptable amount of an adverse effect on any specified biological measurements, and (c) below which no tested concentration caused such an unacceptable effect. An upper chronic limit is the lowest tested concentration (a) in an acceptable chronic test, (b) which did cause an unacceptable amount of an adverse effect on one or more of specified biological measurements, and (c) above which all tested concentrations caused such an effect.

7. If chronic toxicity of material to aquatic animals appears to be related to a water quality characteristic, a Final Chronic Equation should be derived based on that water quality characteristic. Go to Section 8.

8. If chronic values are available for species in eight families as described in Section 4 (No. 1), a Species Mean Chronic Value (SMCV) should be calculated for each species for which at least one chronic value is available by calculating the geometric mean of all chronic values for the species and appropriate Genus Mean Chronic Values should be calculated. The FCV should then be obtained using procedures described in Section 5 (No. 10-14). Then go to Section 7 (No. 13).

9. For each chronic value for which at least one corresponding appropriate acute value is available, calculate an acute-chronic ratio, using for the numerator the geometric mean of results of all acceptable flow-through (except static is acceptable for daphnids) acute tests in the same dilution water and in which concentrations were measured. For fish, the acute test(s) should have been conducted with juveniles. Acute test(s) should have been part of the same study as the chronic test. If acute tests were not conducted as part of the same study, acute tests conducted in the same laboratory and dilution water may be used. If acute tests were not conducted as part of the same study, acute tests conducted in the same dilution water but a different laboratory may be used. If such acute tests are not available, an acute-chronic ratio should not be calculated.

10. For each species, calculate the species mean acute-chronic ratio as the geometric mean of all acute-chronic ratios for that species.

11. For some materials the acute-chronic ratio is about the same for all species, but for other materials the ratio increases or decreases as the SMAV increases. Thus, the Final Acute-Chronic Ratio can be obtained in three ways, depending on the data.

    a. If the species mean acute-chronic ratio increases or decreases as the SMAV increases, the final Acute-Chronic Ratio should be calculated as the geometric mean of all species whose SMAVs are close to the FAV.

    b. If no major trend is apparent and the acute-chronic ratios for a number of species are within a factor of ten, the Final Acute-Chronic Ratio should be calculated as the geometric mean of all species mean acute-chronic ratios for both freshwater and saltwater species.

    c. If the most appropriate species mean acute-chronic ratios are <2.0, and especially if they are <1.0, acclimation has probably occurred during the chronic test. Because continuous exposure and acclimation cannot be assured to provide adequate protection in field situations, the Final Acute-Chronic Ratio should be set at 2.0 so that the FCV is equal to the Criterion Maximum Concentration.

    If the acute-chronic ratios do not fit one of these cases, a Final Acute-Chronic Ratio probably cannot be obtained, and a FCV probably cannot be calculated.

12. Calculate the FCV by dividing the FAV by the Final Acute-Chronic Ratio.

13. If the SMAV of an important species is lower than the calculated FCV, then that SMCV should be used as the FCV.

14. Go to Section 9.

8. FINAL CHRONIC EQUATION

1. A Final Chronic Equation can be derived in two ways. The procedure described in this section will result in the chronic slope being the same as the acute slope.

    a. If acute-chronic ratios for enough species at enough values of the water quality characteristics indicate that the acute-chronic ratio is probably the same for all species and independent of the water quality

characteristic, calculate the Final Acute-Chronic Ratio as the geometric mean of the species mean acute-chronic ratios.

b. Calculate the FCV at the selected value Z of the water quality characteristic by dividing the FAV at Z by the Final Acute-Chronic Ratio.

c. Use V = pooled acute slope as L = pooled chronic slope.

d. Go to Section 8, No. 2, item m.

2. The procedure described in this section will usually result in the chronic slope being different from the acute slope.

a. When enough data are available to show that chronic toxicity to at least one species is related to a water quality characteristic, the relationship should be considered as described below or using analysis of covariance (Dixon and Brown 1979, Neter and Wasserman 1974). If two or more factors affect toxicity, multiple regression analyses should be used.

b. For each species for which comparable chronic toxicity values are available at two or more different values of the water quality characteristic, perform a least squares regression of chronic toxicity values on values of the water quality characteristic.

c. Decide whether data for each species is useful, taking into account range and number of tested values of the water quality characteristic and degree of agreement within and between species. In addition, questionable results, in comparison with other acute and chronic data for the species and other species in the same genus, probably should not be used. If a useful chronic slope is not available for at least one species or if the slopes are too dissimilar or if data are inadequate to define the relationship between chronic toxicity and water quality characteristic, return to Section 7 (No. 8), using results of tests conducted under conditions and in water similar to those commonly used for toxicity tests with the species.

d. For each species calculate the geometric mean of the available chronic values and then divide each chronic value for a species by the mean for the species.

This normalizes the chronic values so that the geometric mean of the normalized values for each species and for any combination of species is 1.0.

e. Similarly normalize the values of the water quality characteristic for each species individually.

f. Individually for each species perform a least squares regression of the normalized chronic toxicity values on the corresponding normalized values of the water quality characteristic. The resulting slopes and 95 percent confidence limits will be identical to those obtained in 1. above. Now, however, if the data are actually plotted, the line of best fit for each individual species will go through the point 1,1 in the center of the graph.

g. Treat all the normalized data as if they were all for the same species and perform a least squares regression of all the normalized chronic values on the corresponding normalized values of the water quality characteristic to obtain the pooled chronic slope (L) and its 95 percent confidence limits. If all the normalized data are actually plotted, the line of best fit will go through the point 1,1 in the center of the graph.

h. For each species calculate the geometric mean (M) of toxicity values and the geometric mean (P) of related values of the water quality characteristic.

i. For each species calculate the logarithm (Q) of the SMCVs at a selected value (Z) of the water quality characteristic using the equation: $Q = \ln M - L(\ln P - \ln Z)$.

j. For each species calculate a SMCV at Z as the antilog of Q ($SMCV = e^{Q}$).

k. Obtain the FCV at Z by using the procedure described in Section 5 (No. 10-14).

l. If the SMCV at Z of an important species is lower than the calculated FCV at Z, then that SMCV should be used as the FCV at Z.

m. The Final Chronic Equation is written as: $FCV = e^{(L[\ln(\text{water quality characteristic})] + \ln S - L[\ln Z])}$, where L = mean chronic slope and S = FCV at Z.

9. FINAL PLANT VALUE

1. Appropriate measures of toxicity of the material to aquatic plants are used to compare relative sensitivities of aquatic plants and animals. Although procedures for conducting and interpreting results of toxicity tests with plants are not well developed, results of such tests usually indicate that criteria which adequately protect aquatic animals and their uses also protect aquatic plants and their uses.

2. A plant value is the result of any test conducted with an alga or an aquatic vascular plant.

3. Obtain the Final Plant Value by selecting the lowest result obtained in a test on an important aquatic plant species in which concentrations of test material were measured and the endpoint is biologically important.

10. FINAL RESIDUE VALUE

1. The Final Residue Value (FRV) is intended to (a) prevent concentrations in commercially or recreationally important aquatic species from exceeding applicable FDA action levels and (b) protect wildlife, including fish and birds, that consume aquatic organisms from demonstrated unacceptable effects. The FRV is the lowest of residue values that are obtained by dividing maximum permissible tissue concentrations by appropriate bioconcentration or bioaccumulation factors. A maximum permissible tissue concentration is either (a) a FDA action level (FDA administrative guidelines) for fish oil or for the edible portion of fish or shellfish or (b) a maximum acceptable dietary intake (ADI) based on observations on survival, growth, or reproduction in a chronic wildlife feeding study or a long-term wildlife field study. If no maximum permissible tissue concentration is available, go to Section 11., because a Final Residue Value cannot be derived.

2. Bioconcentration Factors (BCFs) and Bioaccumulation Factors (BAFs) are the quotients of the concentration of a material in one or more tissues of an aquatic organism divided by the average concentration in the solution to which the organism has been exposed. A BCF is intended to account only for net uptake directly from water, and thus almost has to be measured in a laboratory test. A BAF is intended to account for net uptake from both food and water in a real-world situation, and almost has to be measured in a field situation in which predators accumulate the material directly from

water and by consuming prey. Because so few acceptable BAFs are available, only BCFs will be discussed further, but an acceptable BAF can be used in place of a BCF.

3. If a maximum permissible tissue concentration is available for a substance (e.g, parent material or parent material plus metabolite), the tissue concentration used in BCF calculations should be for the same substance. Otherwise the tissue concentration used in the BCF calculation should be that of the material and its metabolites which are structurally similar and are not much more soluble in water than the parent material.

   a. A BCF should be used only if the test was flow-through, the BCF was calculated based on measured concentrations of test material in tissue and in the test solution, and exposure continued at least until either apparent steady-state (BCF does not change significantly over a period of time, such as two days or 16 percent of exposure duration, whichever is longer) or 28 days was reached. The BCF used from a test should be the highest of (a) the apparent steady-state BCF, if apparent steady-state was reached; (b) highest BCF obtained, if apparent steady-state was not reached; and (c) projected steady-state BCF, if calculated.

   b. Whenever a BCF is determined for a lipophilic material, percentage of lipids should also be determined in the tissue(s) for which the BCF is calculated.

   c. A BCF obtained from an exposure that adversely effected the test organisms may be used only if it is similar to that obtained with unaffected individuals at lower concentrations that did cause effects.

   d. Because maximum permissible tissue concentrations are rarely based on dry weights, a BCF calculated using dry tissue weights must be converted to a wet tissue weight basis. If no conversion factor is reported with the BCF, multiply the dry weight by 0.1 for plankton and by 0.2 for species of fishes and invertebrates.

   e. If more than one acceptable BCF is available for a species, the geometric mean of values should be used, unless the BCFs are from different exposure durations, then the BCF for the longest exposure should be used.

4. If enough pertinent data exist, several residue values can be calculated by dividing maximum permissible tissue concentrations by appropriate BCFs:

   a. For each available maximum ADI derived from a feeding study or a long-term field study with wildlife, including birds and aquatic organisms, the appropriate BCF is based on the whole body of aquatic species which constitute or represent a major portion of the diet of tested wildlife species.

   b. For an FDA action level for fish or shellfish, the appropriate BCF is the highest geometric mean species BCF for the edible portion of a consumed species. The highest species BCF is used because FDA action levels are applied on a species-by-species basis.

5. For lipophilic materials, it may be possible to calculate additional residue values. Because the steady-state BCF for a lipophilic material seems to be proportional to percentage of lipids from one tissue to another and from one species to another (Hamelink et al. 1971, Lundsford and Blem 1982, Schnoor 1982), extrapolations can be made from tested tissues or species to untested tissues or species on the basis of percentage of lipids.

   a. For each BCF for which percentage of lipids is known for the same tissue for which the BCF was measured, normalize the BCF to a one percent lipid basis by dividing the BCF by percentage of lipids. This adjustment makes all the measured BCFs comparable regardless of species or tissue.

   b. Calculate the geometric mean normalized BCF.

   c. Calculate all possible residue values by dividing available maximum permissible tissue concentrations by the mean normalized BCF and by the percentage of lipids values appropriate to the maximum permissible tissue concentration.

      - For an FDA action level for fish oil, the appropriate percentage of lipids value is 100.

      - For an FDA action level for fish, the appropriate percentage of lipids value is 11 for freshwater criteria, based on the highest levels for important consumed species (Sidwell 1981).

- For a maximum ADI derived from a chronic feeding study or long-term field study with wildlife, the appropriate percentage of lipids is that of an aquatic species or group of aquatic species which constitute a major portion of the diet of the wildlife species.

6. The FRV is obtained by selecting the lowest of available residue values.

11. OTHER DATA

Pertinent information that could not be used in earlier sections may be available concerning adverse effects on aquatic organisms and their uses. The most important of these are data on cumulative and delayed toxicity, flavor impairment, reduction in survival, growth, or reproduction, or any other biologically important adverse effect. Especially important are data for species for which no other data are available.

12. CRITERION

1. A criterion consists of two concentrations: the Criterion Maximum Concentration and the Criterion Continuous Concentration.

2. The Criterion Maximum Concentration (CMC) is equal to one-half of the FAV.

3. The Criterion Continuous Concentration (CCC) is equal to the lower of the FCV, the Final Plant Value, and the FRV unless other data show a lower value should be used. If toxicity is related to a water quality characteristic, the CCC is obtained from the Final Chronic Equation, the Final Plant Value, and the FRV by selecting the value or concentration that results in the lowest concentrations in the usual range of the water quality characteristic, unless other data (see Section 11) show that a lower value should be used.

4. Round both the CCC and CMC to two significant figures.

5. The criterion is stated as:

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) (1) aquatic organisms and their uses should not be affected unacceptably if the four-day average concentration of (2) does not exceed (3) µg/L more than once every three years on the average and if the one-hour average concentration does not exceed (4) µg/L more than once every three years on the average.

Where

(1) = insert freshwater or saltwater,

(2) = name of material,

(3) = insert the Criterion Continuous Concentration, and

(4) = insert the Criterion Maximum Concentration.

13. REFERENCES

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APPENDIX B:
SUMMARY OF USEPA METHODOLOGY FOR DETERMINING WATER QUALITY CRITERIA FOR THE PROTECTION OF HUMAN HEALTH

The following summary is a condensed version of the 1980 final US Environmental Protection Agency (USEPA) guidelines for calculating a water quality criteria to protect human health and is slanted towards the specific regulatory needs of the US Army. The guidelines are the most recent document outlining the required procedures and were published in the Federal Register (USEPA 1980). For greater detail on individual points consult that reference.

1. INTRODUCTION

The EPA's water quality criteria for the protection of human health are based on one or more of the following properties of a chemical pollutant:

a) Carcinogenicity, b) Toxicity, and c) Organoleptic (taste and odor) effects.

The meanings and practical uses of the criteria values are distinctly different depending on the properties on which they are based. Criteria based solely on organoleptic effects do not necessarily represent approximations of acceptable risk levels for human health. In all other cases the criteria values represent either estimations of the maximum allowable ambient water concentrations of a pollutant which would prevent adverse health effects or, for suspect and proven carcinogens, estimations of the increased cancer risk associated with incremental changes in the ambient water concentration of the substance. Social and economic costs and benefits are not considered in determining water quality criteria. In establishing water quality standards, the choice of the criterion to be used depends on the designated water use. In the case of a multiple-use water body, the criterion protecting the most sensitive use is applied.

2. DATA NEEDED FOR HUMAN HEALTH CRITERIA

Criteria documentation requires information on: (1) exposure levels, (2) pharmacokinetics, and (3) range of toxic effects of a given water pollutant.

2.1 EXPOSURE DATA

For an accurate assessment of total exposure to a chemical, consideration must be given to all possible exposure routes including ingestion of contaminated water and edible aquatic and nonaquatic organisms, as well as exposure through inhalation and dermal contact. For water quality criteria the most important exposure routes to be considered are ingestion of water and consumption of fish and shellfish.

Generally, exposure through inhalation, dermal contact, and non-aquatic diet is either unknown or so low as to be insignificant; however, when such data are available, they must be included in the criteria evaluation.

The EPA guidelines for developing water quality criteria are based on the following assumptions which are designed to be protective of a healthy adult male who is subject to average exposure conditions:

1. The exposed individual is a 70-kg male person (International Commission on Radiological Protection 1977).

2. The average daily consumption of freshwater and estuarine fish and shellfish products is equal to 6.5 grams.

3. The average daily ingestion of water is equal to 2 liters (Drinking Water and Health, National Research Council 1977).

Because fish and shellfish consumption is an important exposure factor, information on bioconcentration of the pollutant in edible portions of ingested species is necessary to calculate the overall exposure level. The bioconcentration factor (BCF) is equal to the quotient of the concentration of a substance in all or part of an organism divided by the concentration in ambient water to which the organism has been exposed. The BCF is a function of lipid solubility of the substance and relative amount of lipids in edible portions of fish or shellfish. To determine the weighted average BCF, three different procedures can be used depending upon lipid solubility and availability of bioconcentration data:

(1) For lipid soluble compounds, the average BCF is calculated from the weighted average percent lipids in ingested fish and shellfish in the average American diet. The latter factor has been estimated to be 3 percent (Stephan 1980, as cited in USEPA 1980)

Because steady-state BCFs for lipid soluble compounds are proportional to percent lipids, the BCF for the average American diet can be calculated as follows:

$$BCF_{avg} = BCF_{sp} \times \frac{3.0\%}{PL_{sp}},$$

where BCF_{sp} is the bioconcentration factor for an aquatic species and PL_{sp} is the percent lipids in the edible portions of that species.

(2) Where an appropriate bioconcentration factor is not available, the BCF can be estimated from the octanol/water partition coefficient (P) of a substance as follows:

$$\log BCF = (0.85 \log P) - 0.70$$

for aquatic organisms containing about 7.6 percent lipids (Veith et al. 1980, as cited in USEPA 1980). An adjustment for percent

lipids in the average diet (3 percent versus 7.6 percent) is made to derive the weighted average bioconcentration factor.

(3) For nonlipid-soluble compounds, the available BCFs for edible portions of consumed freshwater and estuarine fish and shellfish are weighted according to consumption factors to determine the weighted BCF representative of the average diet.

2.2 PHARMACOKINETIC DATA

Pharmacokinetic data, encompassing information on absorption, distribution, metabolism, and excretion, are needed for determining the biochemical fate of a substance in human and animal systems. Information on absorption and excretion in animals, together with a knowledge of ambient concentrations in water, food, and air, are useful in estimating body burdens in humans. Pharmacokinetic data are also essential for estimating equivalent oral doses based on data from inhalation or other routes of exposure.

2.3 BIOLOGICAL EFFECTS DATA

Effects data which are evaluated for water quality criteria include acute, subchronic, and chronic toxicity; synergistic and antagonistic effects; and genotoxicity, teratogenicity, and carcinogenicity. The data are derived primarily from animal studies, but clinical case histories and epidemiological studies may also provide useful information. According to the EPA (USEPA 1980), several factors inherent in human epidemiological studies often preclude their use in generating water quality criteria (see NAS 1977). However, epidemiological data can be useful in testing the validity of animal-to-man extrapolations.

From an assessment of all the available data, a biological endpoint, i.e., carcinogenicity, toxicity, or organoleptic effects is selected for criteria formulation.

3. HUMAN HEALTH CRTITERIA FOR CARCINOGENIC SUBSTANCES

If sufficient data exist to conclude that a specific substance is a potential human carcinogen (carcinogenic in animal studies, with supportive genotoxicity data, and possibly also supportive epidemiological data) then the position of the EPA is that the water quality criterion for that substance (recommended ambient water concentration for maximum protection of human health) is zero. This is because the EPA believes that no method exists for establishing a threshold level for carcinogenic effects, and, consequently, there is no scientific basis for establishing a "safe" level. To better define the carcinogenic risk associated with a particular water pollutant, the EPA has developed a methodology for determining ambient water concentrations of the substance which would correspond to incremental lifetime cancer risks of 10^{-7} to

10^{-5} (one additional case of cancer in populations ranging from ten million to 100,000, respectively). These risk estimates, however, do not represent an EPA judgment as to an "acceptable" risk level.

3.1 METHODOLOGY FOR DETERMINING CARCINOGENICITY (NONTHRESHOLD) CRITERIA

The ambient water concentration of a substance corresponding to a specific carcinogenic risk can be calculated as follows:

$$C = \frac{70 \times PR}{q_1^* (2 + 0.0065BCF)}$$

where,
C = ambient water concentration;
PR = the probable risk (e.g., 10^{-5}; equivalent to one case in 100,000);
BCF = the bioconcentration factor; and
q_1^* = a coefficient (defined below) (USEPA 1980).

By rearranging the terms in this equation, it can be seen that the ambient water concentration is one of several factors which define the overall exposure level:

$$PR = q_1^* \times \frac{C\,(2 + 0.0065\ BCF)}{70}$$

or

$$PR = q_1^* \times \frac{2C + (0.0065\ BCF \times C)}{70},$$

where, 2C is the daily exposure resulting from drinking 2 liters of water per day and (0.0065 x BCF x C) is the average daily exposure resulting from the consumption of 6.5 mg of fish and shellfish per day. Because the exposure is calculated for a 70-kg man, it is normalized to a per kilogram basis by the factor of 1/70. In this particular case, exposure resulting from inhalation, dermal contact, and nonaquatic diet is considered to be negligible.

In simplified terms the equation can be rewritten

$$PR = q_1^* X,$$

where X is the total average daily exposure in mg/kg/day or

$$q_1^* = \frac{PR}{X},$$

showing that the coefficient q_1^* is the ratio of risk to dose; an indication of the carcinogenic potency of the compound.

The USEPA guidelines state that for the purpose of developing water quality criteria, the assumption is made that at low dose levels there

is a linear relationship between dose and risk (at high doses, however, there may be a rapid increase in risk with dose resulting in a sharply curved dose/response curve). At low doses then, the ratio of risk to dose does not change appreciably and q_1^* is a constant. At high doses the carcinogenic potency can be derived directly from experimental data, but for risk levels of 10^{-7} to 10^{-5}, which correspond to very low doses, the q_1^* value must be derived by extrapolation from epidemiological data or from high dose, short-term animal bioassays.

3.2 CARCINOGENIC POTENCY CALCULATED FROM HUMAN DATA

In human epidemiological studies, carcinogenic effect is expressed in terms of the relative risk [RR(X)] of a cohort of individuals at exposure X compared to the risk in the control group [PR(control)] (e.g., if the cancer risk in group A is five times greater than that of the control group, then RR(X) = 5). In such cases the "excess" relative cancer risk is expressed as RR(X) - 1, and the actual numeric, or proportional excess risk level [PR(X)] can be calculated:

$$PR(X) = [RR(X) - 1] \times PR(control).$$

Using the standard risk/dose equation:

$$PR(X) = b \times X$$

and substituting for PR(X):

$$[RR(X) - 1] \times PR(control) = b \times X$$

or

$$b = \frac{[RR(X) - 1] \times PR(control)}{X},$$

where b is equal to the carcinogenic potency or q_1^*.

3.3 CARCINOGENIC POTENCY CALCULATED FROM ANIMAL DATA

In the case of animal studies where different species, strains, and sexes may have been tested at different doses, routes of exposure, and exposure durations, any data sets used in calculating the health criteria must conform to certain standards:

1. The tumor incidence must be statistically significantly higher than the control for at least one test dose level and/or the tumor incidence rate must show a statistically significant trend with respect to dose level.

2. The data set giving the highest estimate of carcinogenic lifetime risk (q_1^*) should be selected unless the sample size is quite small and another data set with a similar dose-response relationship and larger sample size is available.

3. If two or more data sets are comparable in size and identical with respect to species, strain, sex, and tumor site, then the geometric mean of q_1^* from all data sets is used in the risk assessment.

4. If in the same study tumors occur at a significant frequency at more than one site, the cancer incidence is based on the number of animals having tumors at any one of those sites.

In order to make different data sets comparable, the EPA guidelines call for the following standardized procedures:

1. To establish equivalent doses between species, the exposures are normalized in terms of dose per day (m) per unit of body surface area. Because the surface area is proportional to the 2/3 power of the body weight (W), the daily exposure (X) can be expressed as:

$$X = \frac{m}{W^{2/3}}.$$

2. If the dose (s) is given as mg per kg of body weight:

$$S = \frac{m}{W},$$

then

$$m = s \times W$$

and the equivalent daily exposure (X) would be

$$X = \frac{(s \times w)}{W^{2/3}}$$

or

$$X = s \times W^{1/3}.$$

3. The dose must also be normalized to a lifetime average exposure. For an carcinogenic assay in which the average dose per day (in mg) is m, and the length of exposure is l_e, and the total length of the experiment is L_e, then the lifetime average exposure (X_m) is

$$X_m = \frac{l_e \times m}{L_e \times W^{2/3}}.$$

4. If the duration of the experiment (L_e) is less than the natural life span (L) of the test animal, the value of q_1^* is increased by a factor of $(L/L_e)^3$ to adjust for an age-specific increase in the cancer rate.

5. If the exposure is expressed as the dietary concentration of a substance (in ppm), then the dose per day (m) is

$$m = ppm \times F \times r,$$

where F is the weight of the food eaten per day in kg, and r is the absorption fraction (which is generally assumed to be equal to 1). The weight of the food eaten per day can be expressed as a function of body weight

$$F = fW,$$

where f is a species-specific, empirically derived coefficient which adjusts for differences in F due to difference in the caloric content of each species diet (f is equal to 0.028 for a 70-kg man; 0.05 for a 0.35-kg rat; and 0.13 for a 0.03-kg mouse).

Substituting (ppm x F) for m and fW for F, the daily exposure (dose/surface area/day or $m/W^{2/3}$) can be expressed as

$$X = \frac{ppm \times F}{W^{2/3}} = \frac{ppm \times f \times W}{W^{2/3}} = ppm \times f \times W^{1/3}.$$

6. When exposure is via inhalation, calculation can be considered for two cases: (1) the substance is a water soluble gas or aerosol, and is absorbed proportionally to to the amount of air breathed in and (2) the substance is not very water soluble and absorption, after equilibrium is reached between the air and the body compartments, will be proportional to the metabolic rate which is proportional to rate of oxygen consumption; which, in turn, is a function of total body surface area.

3.4 EXTRAPOLATION FROM HIGH TO LOW DOSES

Once experimental data have been standardized in terms of exposure levels, they are incorporated into a mathematical model which allows for calculation of excess risk levels and carcinogenic potency at low doses by extrapolation from high dose situations. There are a number of mathematical models which can be used for this procedure (see Krewski et al. 1983 for review). The EPA has selected a "linearized multi-stage" extrapolation model for use in deriving water quality criteria (USEPA 1980). This model is derived from a standard "general product" time-to-response (tumor) model (Krewski et al. 1983):

$$P(t;d) = 1 - \exp[-g(d)H(t)],$$

where P(t;d) is the probable response for dose d and

time t; g(d) is the polynomial function defining the effect of dose level, and H(t) the effect of time:

$$g(d) = \sum_{i=0}^{a} \alpha_i d^i,$$

$$H(t) = \sum_{i=1}^{b} \beta_i t^i,$$

(with α and $\beta \geq 0$, and $\sum \beta_i = 1$).

This time-to-response model can be converted to a quantal response model by incorporation of the time factor into each α as a multiplicative constant (Crump 1980):

$$P(d/t) = 1 - \exp[- \sum_{i=0}^{a} \alpha d^i],$$

or as given in the EPA guidelines (USEPA 1980):

$$P(d) = 1 - \exp[-(q_o + q_1 d + q_2 d^2 + \ldots + q_k d^k)],$$

where P(d) is the lifetime risk (probability) of cancer at dose d.

For a given dose the excess cancer risk A(d) above the background rate P(o) is given by the equation:

$$A(d) = \frac{P(d) - P(o)}{1 - P(o)},$$

where

$$A(d) = 1 - \exp[-q_1 d + q_2 d^2 + \ldots + q_k d^k)],$$

Point estimates of the coefficients $q_1 \ldots q_k$ and consequently the extra risk function A(d) at any given dose are calculated by using the statistical method of maximum likelihood. Whenever q_1 is not equal to 0, at low doses the extra risk function A(d) has approximately the form:

$$A(d) = q_1 \times d.$$

Consequently, $q_1 \times d$ represents a 95 percent upper confidence limit on the excess risk, and R/q_1 represents a 95 percent lower confidence limit on the dose producing an excess risk of R. Thus A(d) and R will be a function of the maximum possible value of q_1 which can be determined from the 95 percent upper confidence limits on q_1. This is accomplished by using the computer program GLOBAL 79 developed by Crump and Watson (1979). In this procedure q_1^*, the 95 percent upper confidence limit, is calculated by increasing q_1 to a value which, when incorporated into the log-likelihood function, results in a maximum value satisfying the equation:

$$2(L_o - L_1) = 2.70554,$$

where L_o is the maximum value of the log-likelihood function.

Whenever the multistage model does not fit the data sufficiently, data at the highest dose are deleted and the model is refitted to the data. To determine whether the fit is acceptable, the chi-square statistic is used:

$$\chi^2 = \sum_{i=1}^{h} \frac{(X_i - N_i P_i)^2}{N_i P_i \times (1 - P_i)},$$

where N_i is the number of animals in the ith dose group, X_i is the number of animals in the ith dose group with a tumor response, P_i is the probability of a response in the ith dose group estimated by fitting the multistage model to the data, and h is the number of remaining groups.

The fit is determined to be unacceptable whenever chi-square (X^2) is larger than the cumulative 99 percent point of the chi-square distribution with f degrees of freedom, where f equals the number of dose groups minus the number of nonzero multistage coefficients.

4. HEALTH CRITERIA FOR NONCARCINOGENIC TOXIC SUBSTANCES

Water quality criteria that are based on noncarcinogenic human health effects can be derived from several sources of data. In all cases it is assumed that the magnitude of a toxic effect decreases as the exposure level decreases until a threshold point is reached at, and below which, the toxic effect will not occur regardless of the length of the exposure period. Water quality criteria (C) establish the concentration of a substance in ambient water which, when considered in relation to other sources of exposure [i.e., average daily consumption of nonaquatic organisms (DT) and daily inhalation (IN)], place the Acceptable Daily Intake (ADI) of the substance at a level below the toxicity threshold, thereby preventing adverse health effects:

$$C = \frac{ADI - (DT + IN)}{[2L + (0.0065\ kg \times BCF)]}$$

where 2L is the amount of water ingested per day, 0.0065 kg is the amount of fish and shellfish consumed per day, and BCF is the weighted average bioconcentration factor.

In terms of scientific validity, an accurate estimate of the ADI is the major factor in deriving a satisfactory water quality criteria.

The threshold exposure level, and thus the ADI, can be derived from either or both animal and human toxicity data.

4.1 NONCARCINOGENIC HEALTH CRITERIA BASED ON ANIMAL TOXICITY DATA (ORAL)

For criteria derivation, toxicity is defined as any adverse effects which result in functional impairment and/or pathological lesions which may affect the performance of the whole organism, or which reduce an organism's ability to respond to an additional challenge (USEPA 1980).

A bioassay yielding information as to the highest chronic (90 days or more) exposure tolerated by the test animal without adverse effects (No-Observed-Adverse-Effect-Level or NOAEL) is equivalent to the toxicity threshold and can be used directly for criteria derivation. In addition to the NOAEL, other data points which can be obtained from toxicity testing are

(1) NOEL = No-Observed-Effect-Level,
(2) LOEL = Lowest-Observed-Effect-Level,
(3) LOAEL = Lowest-Observed-Adverse-Effect-Level,
(4) FEL = Frank-Effect-Level.

According to the EPA guidelines, only certain of these data points can be used for criteria derivation:

1. A single FEL value, without information on the other response levels, should not be used for criteria derivation because there is no way of knowing how far above the threshold it occurs.

2. A single NOEL value is also unsuitable because there is no way of determining how far below the threshold it occurs. If only multiple NOELs are available, the highest value should be used.

3. If a LOEL value alone is available, a judgement must be made as to whether the value actually corresponds to a NOAEL or an LOAEL.

4. If an LOAEL value is used for criteria derivation, it must be adjusted by a factor of 1 to 10 to make it approximately equivalent to the NOAEL and thus the toxicity threshold.

5. If for reasonably closely spaced doses only a NOEL and a LOAEL value of equal quality are available, the NOEL is used for criteria derivation.

The most reliable estimate of the toxicity threshold would be one obtained from a bioassay in which an NOEL, NOAEL, LOAEL, and clearly defined FEL were observed in relatively closely spaced doses.

Regardless of which of the above data points is used to estimate the toxicity threshold, a judgement must be made as to whether the experimental data are of satisfactory quality and quantity to allow for a valid extrapolation for human exposure situations. Depending on whether the data are considered to be adequate or inadequate, the

toxicity threshold is adjusted by a "safety factor" or "uncertainty factor" (NAS 1977). The "uncertainty factor" may range from 10 to 1000 according to the following general guidelines:

1. Uncertainty factor 10. Valid experimental results from studies on prolonged ingestion by man, with no indication of carcinogenicity.

2. Uncertainty factor 100. Data on chronic exposures in humans not available. Valid results of long-term feeding studies on experimental animals, or in the absence of human studies, valid animal studies on one or more species. No indication of carcinogenicity.

3. Uncertainty factor 1000. No long-term or acute exposure data for humans. Scanty results on experimental animals with no indication of carcinogenicity.

Uncertainty factors which fall between the categories described above should be selected on the basis of a logarithmic scale (e.g., 33 being halfway between 10 and 100).

The phrase "no indication of carcinogenicity" means that carcinogenicity data from animal experimental studies or human epidemiology are not available. Data from short-term carcinogenicity screening tests may be reported, but they are not used in criteria derivation or for ruling out the uncertainty factor approach.

4.2 CRITERIA BASED ON INHALATION EXPOSURES

In the absence of oral toxicity data, water quality criteria for a substance can be derived from threshold limit values (TLVs) established by the American Conference of Governmental and Industrial Hygienists (ACGIH), the Occupational Safety and Health Administration (OSHA), or the National Institute for Occupational Safety and Health (NIOSH), or from laboratory studies evaluating the inhalation toxicity of the substance in experimental animals. TLVs represent 8-hr time-weighted averages of concentrations in air designed to protect workers from various adverse health effects during a normal working career. To the extent that TLVs are based on sound toxicological evaluations and have been protective in the work situation, they provide helpful information for deriving water quality criteria. However, each TLV must be examined to decide if the data it is based on can be used for calculating a water quality criteria (using the uncertainty factor approach). Also the history of each TLV should be examined to assess the extent to which it has resulted in worker safety. With each TLV, the types of effects against which it is designed to protect are examined in terms of its relevance to exposure from water. It must be shown that the chemical is not a localized irritant and there is no significant effect at the portal of entry, regardless of the exposure route.

The most important factor in using inhalation data is in determining equivalent dose/response relationships for oral exposures. Estimates of equivalent doses can be based upon (1) available pharmacokinetic data for oral and inhalation routes, (2) measurements of absorption efficiency from ingested or inhaled chemicals, or (3) comparative excretion data when associated metabolic pathways are equivalent to those following oral ingestion or inhalation. The use of pharmacokinetic models is the preferred method for converting from inhalation to equivalent oral doses.

In the absence of pharmacokinetic data, TLVs and absorption efficiency measurements can be used to calculate an ADI value by means of the Stokinger and Woodward (1958) model:

$$ADI = TLV \times BR \times DE \times d \times A_A/(A_O \times SF),$$

where,

BR = daily air intake (assume 10 m^3),
DE = duration of exposure in hours per day,
d = 5 days/7 days,
A_A = efficiency of absorption from air,
A_O = efficiency of absorption from oral exposure, and
SF = safety factor.

For deriving an ADI from animal inhalation toxicity data, the equation is:

$$ADI = C_A \times DE \times d \times AA \times BR \times 70\ kg/(BWA \times AO \times SF),$$

where,

C_A = concentration in air (mg/m^3),
D_E = duration of exposure (hr/day),
d = number of days exposed/number of days observed,
A_A = efficiency of absorption from air,
BR = volume of air breathed (m^3/day),
70 kg = standard human body weight,
BWA = body weight of experimental animals (kg),
AO = efficiency of absorption from oral exposure, and
SF = safety factor.

The safety factors used in the above equations are intended to account for species variability. Consequently, the mg/surface area/day conversion factor is not used in this methodology.

5. ORGANOLEPTIC CRITERIA

Organoleptic criteria define concentrations of substances which impart undesirable taste and/or odor to water. Organoleptic criteria are based on aesthetic qualities alone and not on toxicological data, and therefore have no direct relationship to potential adverse human health effects. However, sufficiently intense organoleptic effects may,

under some circumstances, result in depressed fluid intake which, in turn, might aggravate a variety of functional diseases (i.e., kidney and circulatory diseases).

For comparison purposes, both organoleptic criteria and human health effects criteria can be derived for a given water pollutant; however, it should be explicitly stated in the criteria document that the organoleptic criteria have no demonstrated relationship to potential adverse human health effects.

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