

Continental Shelf Associates, Inc.

TOXICITY OF BALLAST-WATER EFFLUENT TO REPRESENTATIVE MARINE ANIMALS AND PLANTS

-- Derivation of Water Quality
Criteria for Effluent --



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Criteria for Effluent --

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SUMMARY

Effluent discharged from Alyeska's Ballast Water Treatment Facility was evaluated during the period of September 1989 through December 1991 for acute and chronic toxicity to, respectively, 11 and 7 representative species of marine animals. Chronic toxicity of effluent to 2 representative species of plants was also assessed. The most sensitive of acutely evaluated animal species (which included two indigenous species; i.e., coonstripe shrimp [Pandalus hypsinotus] and pink salmon [Oncorhynchus gorbuscha]) was the mysid shrimp (Mysidopsis bahia), which was characterized by a mean (geometric mean) 96-hr median lethal concentration (96-hr LC50) of approximately 51.4% effluent. The most sensitive of chronically assessed animal species (which also included pink salmon) was the inland silverside (Mendia beryllina), which generated a mean Maximum Acceptable Toxicant Concentration (MATC) of about 15.6% effluent. The two evaluated species of plants, a diatom (Thalassiosira pseudonana) and a red alga (Champia parvula), were not deleteriously affected at the highest concentration of effluent tested (50.0% effluent).

Water quality criteria were derived for effluent by interpreting the above-referenced toxicological data in the context of the rigorous protocols promulgated by the U. S. Environmental Protection Agency for the derivation of numerical national water quality criteria for discharged substances. The acute criterion -- the Criteria Maximum Concentration (CMC) -- was determined to be 21% effluent; whereas, the chronic criterion -- the Criteria Continuous Concentration (CCC) -- is 16% effluent. The CCC was derived by the "application factor approach," in which a Final Application Factor (FAF) of 0.39 was developed from paired acute and chronic toxicological data. This FAF is the inverse of a Final Acute:Chronic Ratio (FACR) of 2.56 and is substantially lower than the 0.01 AF identified in the State of Alaska's present water quality standards.

The CMC (21% effluent) and CCC (16% effluent) must be achieved at the boundaries of the mixing zone (MZ) established in the vicinity of the effluent outfall. Dilution factors approximating just 4.8 (CMC) and 6.2 (CCC) are required to disperse effluent within the MZ to environmentally protective levels. In addition, lethality to aquatic organisms passing through the MZ can be prevented if the CMC is achieved

within a short distance of the effluent outfall (which is characteristic of high-velocity discharge structures) or, more quantitatively, if drifting organisms are not exposed to 1-hr mean concentrations of effluent exceeding the CMC.

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LIST OF MAJOR TERMS AND ASSOCIATED ACRONYMS
AND/OR DEFINITIONS

<u>Major Term</u>	<u>Associated Acronym and/or Definition</u>
<u>General Terms and Acronyms/Definitions</u>	
Chronic value	CV (CV = MATC)
Median effective concentration	EC50
Median lethal concentration	LC50
Lowest-observed-effect concentration	LOEC
Maximum acceptable toxicant concentration	MATC (MATC = CV)
Mixing zone	MZ
No-observed-effect concentration	NOEC
National Pollution Discharge Elimination System Permit	NPDES Permit
Quality assurance/quality control	QA/QC

Terms and Acronyms/Definitions
Directly Employed in Derivation of
Acute Water Quality Criterion
(Criteria Maximum Concentration) for Effluent

Criteria maximum concentration	CMC (CMC = 0.5 FAV)
Final acute value	FAV (FAV = 2 CMC)
Genus mean acute value	GMAV (GMAV = geometric mean of all SMAVs for genus)
Species mean acute value	SMAV (SMAV = geometric mean of all LC50s for species)

Major Term

Associated Acronym
and/or Definition

Terms and Acronyms/Definitions
Directly Employed in Derivation of
Chronic Water Quality Criterion
(Criteria Maximum Concentration) for Effluent

Acute:chronic ratio	ACR (ACR = 1/AF)
Application factor	AF (AF = 1/ACR)
Chronic value	CV (CV = MATC)
Criteria continuous concentration	CCC (CCC = lowest of FCV, FPV, and FRV)
Final acute:chronic ratio	FACR (FACR = 1/FAF)
Final application factor	FAF (FAF = 1/FACR)
Final chronic value	FCV (FCV = FAV/FACR)
Final plant value	FPV
Final residue value	FRV (not applicable to ballast- water effluent)
Species mean acute:chronic ratio	SMACR (SMACR = 1/SMAF)
Species mean application factor	SMAF (SMAF = 1/SMACR)

1. INTRODUCTION

The objectives of this report are two-fold. The first objective is to present in an easily assimilable format the results of numerous aquatic toxicity tests conducted during the last several years with effluent discharged from Alyeska's Ballast Water Treatment Facility (BWTF). The second objective is to employ these toxicological results to derive water quality criteria for the effluent. This latter objective is predicated upon development of a Final Application Factor (FAF) or Final Acute:Chronic Ratio (FACR) for the effluent. (The FAF and FACR bear an inverse relationship to each other.)

The aquatic toxicity tests presented in this report were performed during the period of September 1989 through December 1991 by four different aquatic toxicology laboratories. These laboratories collectively tested 11 representative species of marine animals for acute sensitivity to effluent, as well as 7 representative species of marine animals and 2 representative species of marine plants for chronic sensitivity to effluent. Many of these species were tested repetitively over the above-referenced 28-month period. Several of the species were evaluated in response to conditions included in the U.S. Environmental Protection Agency's (EPA, 1989) National Pollution Discharge Elimination System (NPDES) Permit for effluent (Part I.B.9.e-f of permit, acute survival tests; and Part I.B.9.a-d of permit; sublethal tests). The toxicological data base presented in this report consists of a total of 58 separate acute or chronic (in most cases, "short-term" chronic) toxicity tests. Results of additional toxicity tests performed with effluent are not presented in the report because of unacceptable responses of control organisms (EPA; 1985,1989).

Water quality criteria for effluent were derived by employing the protocols promulgated by the EPA for the derivation of numerical national water quality criteria for discharged substances (e.g., EPA, 1986 [the "Gold Book"]; Appendix A of this report). These protocols are substantially more rigorous than the procedures normally recommended by the EPA (1991) for deriving water quality criteria for individual "whole" effluents. However, both sets of protocols are based, in part, upon the development of a FAF (or FACR) to "convert" acute toxicological data (e.g., median lethal concentrations [LC50s]) into chronic water quality criteria. The State of Alaska's

present water quality standards (State of Alaska, undated) mandates exclusive use of an arbitrary AF of 0.01 for protection of aquatic life and wildlife from a variety of discharged substances (i.e., toxics and other deleterious organic and inorganic substances, total aromatic hydrocarbons, and total hydrocarbons). Contemporary toxicological guidelines for deriving water quality criteria for discharged substances (e.g., EPA; 1986, 1991) recommend the use of empirically derived (measured) AFs, as compared to assumed factors (such as the 0.01 factor). In the absence of empirically derived AFs for whole effluents, an assumed factor of 0.10 is recommended for use (EPA, 1991).

The State of Alaska's water quality standards may be updated as a consequence of its 1989-1991 "Triennial Review" to reflect the above-referenced contemporary toxicological guidelines. For example, the Alaska Department of Environmental Conservation (ADEC, 1991) has noted that "the 0.01 factor is scientifically outdated, and provides unjustifiably low criteria values." The water quality criteria derived in this report are responsive to the ADEC's conclusion that such criteria should be based upon scientifically justified procedures.

2. PROCEDURES

Aquatic toxicity of ballast-water effluent was documented by evaluating sensitivity of representative species of marine animals and plants to effluent (Table 1). Eleven (11) representative marine animals were evaluated for acute sensitivity to effluent and 7 representative marine animals were assessed for chronic sensitivity to effluent. Two (2) representative marine plants were also evaluated for chronic sensitivity to effluent. The evaluated species represent the various taxonomic categories recommended by the EPA (1986; Appendix A of this report) for deriving numerical national water quality criteria for discharged substances. Five (5) species of marine animals -- sheepshead minnows, inland silversides, pink salmon, mysid shrimp, and purple sea urchins -- were assessed for both acute and chronic sensitivity to effluent in order to develop Application Factors (AFs) and inversely related Acute:Chronic Ratios (ACRs) for effluent.

Aquatic toxicity testing was performed by four aquatic toxicology laboratories: Continental Shelf Associates (1990), ENSR (1990a-1990l), Battelle (1990a-1990b; 1991a-1991d; 1992), and T.H.E. Laboratories (1991). Acute toxicity tests, 72- or 96-hr in duration, were conducted according to procedures recommended by the EPA (1985). Most organisms were exposed to test media under static-renewal conditions. However, purple sea urchins (embryos and larvae) were tested by static, non-renewal techniques. Also, pink salmon and coonstripe shrimp (juveniles/adults) were sometimes tested by both static-renewal and dynamic (flow-through) techniques.

Short-term chronic toxicity tests were performed with early-life stages of sheepshead minnows and inland silversides over a 7-day period by static-renewal methods recommended by the EPA (1988). Similar 7-day, static-renewal tests were performed with mysid shrimp (partial-life-cycle tests). Bay mussels (larvae), sand dollars (gametes), purple sea urchins (gametes), diatoms, and red algae were tested over shorter exposure periods by static, non-renewal techniques. Pink salmon were evaluated in a 28-day partial-life-cycle test by dynamic techniques.

Toxicological data presented in this report conform with standards recommended for data employed in the derivation of numerical national water quality criteria for

discharged substances (EPA, 1986) and/or, in the case of some chronic data (i.e., data generated by short-term chronic toxicity tests), more contemporary toxicological standards (EPA; 1988, 1991). In particular, toxicological values reported as "greater than" (>) values are included in the report (EPA, 1986). This EPA-recommended practice mandates that calculations employed to develop the Final Acute Value (FAV) for effluent, as well as the Final Application Factor (FAF) and Final Acute:Chronic Ratio (FACR) for effluent, must be based upon absolute values.

Water quality criteria for effluent -- i.e., the Criteria Maximum Concentration (CMC) and Criteria Continuous Concentration (CCC) -- were derived in accordance with protocols promulgated for the derivation of numerical national water quality criteria for discharged substances (EPA, 1986). The CMC was derived from a FAV (CMC = 0.5 FAV) that was not based upon a Final Acute Equation since there was no indication that acute toxicity of effluent is related to specific ambient water quality characteristics. The CCC is defined by the EPA (1986) as being equal to the lowest of the Final Chronic Value (FCV), Final Plant Value (FPV), and Final Residue Value (FRV). In the case of ballast-water effluent, the FCV was less than the FPV (as will be indicated later in this report) and a FRV was not applicable. (In the latter case, Maximum Permissible Tissue Concentrations have not been promulgated for chemical constituents that are characteristic of effluent.) Consequently, the CCC for effluent was equivalent to the FCV, which, in turn, was derived by the "application factor approach," in which the FAV is divided by the FACR. Chronic toxicity of effluent has not been demonstrated to be dependent upon specific ambient water quality characteristics. Therefore, derivation of the FCV was not predicated upon a Final Chronic Equation.

Table 1. Representative marine animals and plants evaluated for sensitivity to ballast-water effluent

Taxonomic category ^a represented by marine animal or plant	Marine animal or plant evaluated for sensitivity to ballast-water effluent
<u>Marine Animals Evaluated for Acute Sensitivity to Effluent^b</u>	
1. Family in phylum Chordata	Sheepshead minnow (<u>Cyprinodon variegatus</u>) -- Phylum: Chordata; Family: Cyprinodontidae ^c Inland silverside (<u>Menidia beryllina</u>) -- Phylum: Chordata; Family: Atherinidae ^c
2. Second family in phylum Chordata	Rainbow trout (<u>Oncorhynchus mykiss</u>) -- Phylum: Chordata; Family: Salmonidae Pink salmon (<u>Oncorhynchus gorbuscha</u>) -- Phylum: Chordata; Family: Salmonidae ^{c, d}
3. Either Mysidae or Penaeidae family (not chordates)	Mysid shrimp (<u>Mysidopsis bahia</u>) -- Phylum: Arthropoda; Family: Mysidae ^c
4. Second family in phylum other than Chordata (can include family not evaluated for above-referenced Category 3)	Coonstripe shrimp (<u>Pandulus hypsinotus</u>) -- Phylum: Arthropoda; Family: Pandalidae ^d
5. Third family in phylum other than Chordata	Amphipod (<u>Rheopoxynius abronius</u>) -- Phylum: Arthropoda; Family: Phoxocephalidae Amphipod (<u>Corophium spinicorne</u>) -- Phylum: Arthropoda; Family: Oedicerotidae
6. Fourth family in phylum other than Chordata	Polychaete worm (<u>Neanthes arenaceodentata</u>) -- Phylum: Annelida; Family: Nereidae
7. Family in phylum other than Chordata and Arthropoda	Blue mussel (<u>Mytilus edulis</u>) -- Phylum: Mollusca; Family: Mytilidae
8. Any family not referenced above	Purple sea urchin (<u>Strongylocentrotus purpuratus</u>) -- Phylum: Echinodermata; Family: Strongylocentrotidae ^c

Table 1. __ Representative marine animals and plants evaluated for sensitivity to ballast-water effluent -- cont.

Taxonomic category ^a represented by marine animal or plant	Marine animal or plant evaluated for sensitivity to ballast-water effluent
<u>Marine Animals Evaluated for Chronic Sensitivity to Effluent^e</u>	
1. Family in Superclass Pisces (a fish species)	<p>Sheepshead minnow (<u>C. variegatus</u>) -- Phylum: Chordata; Family: Cyprinodontidae^c</p> <p>Inland silverside (<u>M. beryllina</u>) -- Phylum: Chordata; Family: Atherinidae^c</p> <p>Pink salmon (<u>O. gorbuscha</u>) -- Phylum: Chordata; Family: Salmonidae^c</p>
2. Family of invertebrates (an invertebrate species)	<p>Bay mussel (<u>M. spp.</u>) -- Phylum: Mollusca; Family: Mytilidae^f</p> <p>Sand dollar (<u>Dendraster excentricus</u>) -- Phylum: Echinodermata; Family: Dendrasteridae^f</p>
3. Acutely sensitive saltwater species in any family not referenced above	<p>Mysid shrimp (<u>M. bahia</u>) -- Phylum: Arthropoda; Family: Mysidae^c</p> <p>Purple sea urchin (<u>S. purpuratus</u>) -- Phylum: Echinodermata; Family: Strongylocentrotidae^{c, f}</p>
<u>Marine Plants Evaluated for Chronic Sensitivity to Effluent^g</u>	
1. Phylum of saltwater algae or vascular plants	<p>Diatom (<u>Thalassiosira pseudonana</u>) -- Phylum: Chrysophyta</p>
2. Second phylum of saltwater algae or vascular plants	<p>Red alga (<u>Champia parvula</u>) -- Phylum: Rhodophyta</p>

Table 1. __ Representative marine animals and plants evaluated for sensitivity to ballast-water effluent -- cont.

a The taxonomic categories identified in this column are recommended (EPA, 1986; Appendix A of report) for evaluation during derivation of numerical national water quality criteria for discharged substances.

b Eight different families of animals distributed over eight different taxonomic categories are recommended (EPA, 1986; Appendix A of report) for evaluation of acute sensitivity of marine animals to discharged substances. In the case of ballast-water effluent, more than one family in a single category was sometimes evaluated.

c Acute and chronic sensitivity of five families (species) of marine animals (sheepshead minnow, inland silverside, pink salmon, mysid shrimp, and purple sea urchin) was evaluated, thus permitting the development of application factors (AFs) and acute:chronic ratios (ACRs) for these animals.

d Pink salmon and coonstrip shrimp were evaluated as a condition (Part I. B. 9.e-f; acute survival tests) of the National Pollution Discharge Elimination System (NPDES) Permit for ballast-water effluent (EPA, 1989).

e Three different families of animals distributed over three different taxonomic categories are recommended (EPA, 1986; Appendix A of report) for evaluation of chronic sensitivity of marine animals to discharged substances. In the case of ballast-water effluent, two or three families in each category were evaluated.

f Bay mussels, sand dollars, and purple sea urchins were evaluated as a condition (Part I. B.9. a-d; sublethal tests) of the National Pollution Discharge Elimination System (NPDES) Permit for ballast-water effluent (EPA, 1989).

g One or two different phyla of plants are recommended (EPA, 1986; Appendix A of report) for evaluation of sensitivity of marine plants to discharged substances. Evaluation of the second phylum is recommended only if plants are among the aquatic organisms most sensitive to a discharged substance.

3. RESULTS

Toxicity of ballast-water effluent and derivation of water quality criteria for effluent are addressed separately in this part of the report.

3.1 Toxicity of Ballast-Water Effluent

Toxicity of this salt-water effluent to representative marine animals and representative marine plants was evaluated.

3.1.1 Toxicity to Representative Marine Animals

Acute and chronic toxicity of effluent to animals was assessed.

(a) Acute Toxicity

Effluent was relatively non-toxic to the 11 representative species of marine animals that were evaluated under acute exposure periods (Table 2). In the case of 4 species -- the amphipod Rheopoxynius abronius, polychaete worms, blue mussels, and sheepshead minnows -- 96-hr LC50s were greater than 100% effluent. Juvenile/adult coonstripe shrimp were also characterized by 96-hr LC50s greater than 100% effluent. Two additional species -- inland silversides and pink salmon -- generally exhibited 96-hr LC50s that exceeded 100% effluent (or, in the case of inland silversides, values approximating 100% effluent). The 96-hr LC50 of 71.0% effluent for rainbow trout was generated by fish that had been acclimated to freshwater. The species most acutely sensitive to effluent -- mysid shrimp -- was characterized by a mean (geometric mean) 96-hr LC50 of > 51.4% effluent.

Although larval coonstripe shrimp were characterized by a mean 96-hr LC50 (65.2% effluent) that was lower than the mean value for juveniles/adults (>100% effluent), that difference is basically less than a 2-fold difference and, therefore, is considered to be inconsequential (EPA, 1986). Similarly, no species that was repetitively evaluated for acute sensitivity to effluent was characterized by 96-hr LC50s that differed by more than a factor of 10 (EPA, 1986).

Table 2. ___ Acute toxicity of ballast-water effluent to representative marine animals^{a,b}

Representative marine animal	Acute toxicity of ballast-water effluent (% effluent) ^{c, d}		Date of acute toxicity test	Reference
	96-hr median lethal concentration (96-hr LC50)	95% confidence interval of 96-hr LC50		
1. Mysid shrimp (<i>Mysidopsis bahia</i>)-- juveniles	>100.0	--	Sept. 26-30, 1989	Continental Shelf Associates, 1990
	42.7	34.9 - 51.2	March 1-5, 1990	Battelle, 1990a
	56.2	49.5 - 63.9	May 22-26, 1990	Battelle, 1990b
	>100.0	--	July 18-22, 1990	ENSR, 1990j
	81.0	--	July 19-23, 1990	Battelle, 1990c
	43.5	36.0 - 52.6	Oct. 19-23, 1990	Battelle, 1990d
	36.6	29.8 - 45.0	April 22-26, 1991	Battelle, 1991b
	45.8	36.3 - 57.9	May 13-17, 1991	Battelle, 1991c
	21.8	16.2 - 29.2	Sept. 26-30, 1991	Battelle, 1991d
	41.6	31.2 - 55.4	Dec. 9-13, 1991	Battelle, 1992
Geometric mean:	>51.4	--	--	--
2. Coonstripe shrimp (<i>Pandalus hypsinotus</i>)	--	--	--	--
• Larvae	56.1	50.7 - 62.2	April 22-26, 1991	Battelle, 1991b
	75.8	61.9 - 92.9	May 13-17, 1991	Battelle, 1991c
Geometric mean (larvae):	65.2	--	--	--
• Juveniles/adults	>100.0 ^e	--	May 22-26, 1990	ENSR, 1990a
	>100.0 ^f	--	June 9-13, 1990	ENSR, 1990g
Geometric mean (juveniles/adults):	>100.0	--	--	--
Geometric mean (species):	>80.7	--	--	--
3. Amphipod (<i>Rheopoxynius abronius</i>) -- adults	>100.0	--	Dec. 10-14, 1990	Battelle, 1991a
Geometric mean:	>100.0	--	--	--

Table 2. ___ Acute toxicity of ballast-water effluent -- cont.

Representative marine animal	Acute toxicity of ballast-water effluent (% effluent) ^{c, d}		Date of acute toxicity test	Reference
	96-hr median lethal concentration (96-hr LC50)	95% confidence interval of 96-hr LC50		
4. Amphipod (<u>Corophium spinicorne</u>) -- adults	65.2	53.5 - 79.8	Dec. 10-14, 1990	Battelle, 1991a
Geometric mean:	65.2	--	--	--
5. Polychaete worm (<u>Neanthes arenaceodentata</u>) -- juveniles	>100.0	--	Dec. 10-14, 1990	Battelle, 1991a
Geometric mean:	>100.0	--	--	--
6. Blue mussel (<u>Mytilus edulis</u>) -- juveniles	>100.0	--	Sept. 26-30, 1989	Continental Shelf Associates, 1990
Geometric mean:	>100.0	--	--	--
7. Purple sea urchin (<u>Strongylocentrotus purpuratus</u>) -- embryos/larvae	>100.0 ^{g, h} 27.4 ^{g, i}	-- 25.5 - 29.5	May 22-25, 1990 July 19-22, 1990	Battelle, 1990b Battelle, 1990c
Geometric mean:	>52.3	--	--	--
8. Sheepshead minnow (<u>Cyprinodon variegatus</u>) -- juveniles	>100.0	--	Sept. 26-30, 1989	Continental Shelf Associates, 1990
	>100.0 ^j	--	Dec. 10-14, 1990	Battelle, 1991a
Geometric mean:	>100.0	--	--	--

Table 2. ___ Acute toxicity of ballast-water effluent -- cont.

Representative marine animal	Acute toxicity of ballast-water effluent (% effluent) ^{c, d}		Date of acute toxicity test	Reference
	96-hr median lethal concentration (96-hr LC50)	95% confidence interval of 96-hr LC50		
9. Inland silverside (<i>Menidia beryllina</i>) -- larvae/juveniles	>98.0	--	March 6-10, 1990 (Trial 2)	Battelle, 1990a
	>97.9	--	May 22-26, 1990 (Trial D)	Battelle, 1990b
	>100.0	--	July 18-22, 1990	ENSR, 1990k
	>96.2	--	July 19-23, 1990	Battelle, 1990c
	>100.0	--	Oct. 19-23, 1990	Battelle, 1990d
	28.8	20.8 - 40.0	Dec. 10-14, 1990	Battelle, 1991a
	>97.3	--	April 22-26, 1991	Battelle, 1991b
	65.7	47.5 - 90.8	May 13-17, 1991	Battelle, 1991c
	>97.9	--	Sept. 23-27, 1991	Battelle, 1991d
	>98.5	--	Dec. 9-13, 1991	Battelle, 1992
Geometric mean:	>83.5	--	--	--
10. Rainbow trout (<i>Oncorhynchus mykiss</i>) -- juveniles ^k	71.0	50.0 - 100.0	Sept. 29-Oct. 3, 1989	Continental Shelf Associates, 1990
	Geometric mean:	71.0	--	--
11. Pink salmon (<i>Oncorhynchus gorbuscha</i>) ^l -- fry/smolts	>100.0 ^m	--	May 22-26, 1990	ENSR, 1990c
	>100.0 ⁿ	--	June 2-6, 1990	ENSR, 1990e
	79.5	51.0 - >100.0	April 22-26, 1991	Battelle, 1991b
	>100.0	--	May 13-17, 1991	Battelle, 1991c
	Geometric mean:	>94.4	--	--

^a Marine animals evaluated for acute sensitivity to ballast-water effluent are representative of the eight taxonomic categories that are recommended (EPA, 1986; Appendix A of report) for evaluation during derivation of numerical national water quality criteria for discharged substances. Coonstripe shrimp larvae and pink salmon fry were evaluated as a condition (Part I.B.9.e-f; acute survival tests) of the National Pollution Discharge Elimination System (NPDES) Permit for effluent (EPA, 1989).

Table 2. ___ Acute toxicity of ballast-water effluent -- cont.

b Acute toxicity of ballast-water effluent to representative marine animals was usually determined in 96-hr static-renewal toxicity tests. However, in the case of purple sea urchins, static, non-renewal techniques, in which organisms were exposed to effluent for 72 hr, were employed. Results of a sea urchin test conducted with effluent in March 1990 (Battelle, 1990a) are not presented in this table because of unacceptably low survival of control organisms.

c Acute values are always expressed in terms of % effluent as contrasted to % effluent and % brine employed to adjust salinity of effluent to 30 ppt, a convention employed to describe toxicity in some Battelle reports (Battelle; 1990a, 1990b, 1991b [inland silversides]).

d The 96-hr median lethal concentration (96-hr LC50) is the theoretical concentration of ballast-water effluent that killed 50% of test animals exposed to effluent for 96 hr.

e An identical 96-hr LC50 (i.e., >100.0% ballast-water effluent) was generated in a parallel dynamic (flow-through) toxicity test conducted during May 22-26, 1990, with effluent and coonstripe shrimp juveniles/adults (ENSR, 1990b).

f An identical 96-hr LC50 (i.e., >100.0% ballast-water effluent) was generated in a parallel dynamic (flow-through) toxicity test conducted during June 9-13, 1990, with effluent and coonstripe shrimp juveniles/adults (ENSR, 1990h).

g This LC50 for ballast-water effluent and purple sea urchins is a 72-hr value.

h A 72-hr median effective concentration (72-hr EC50) also was derived for ballast-water effluent and purple sea urchins (Battelle, 1990b). That value, which reflected the presence of abnormal pluteus larvae as well as dead larvae, was 34.2% effluent (95% confidence interval: 30.7 - 38.2% effluent).

i A 72-hr median effective concentration (72-hr EC50) also was derived for ballast-water effluent and purple sea urchins (Battelle, 1990c). That value, which reflected the presence of abnormal pluteus larvae as well as dead larvae, was 26.8% effluent (95% confidence interval: 25.0 - 28.7% effluent).

j A 96-hr EC50 also was derived for ballast-water effluent and sheepshead minnows (Battelle, 1991a). That value, which reflected the presence of moribund fish as well as dead fish, was 100.0% effluent.

k Rainbow trout evaluated for toxicity were acclimated to freshwater.

l Pink salmon evaluated for toxicity were acclimated to saltwater.

m An identical 96-hr LC50 (i.e., >100.0% ballast-water effluent) was generated in a parallel dynamic (flow-through) toxicity test conducted during May 22-26, 1990, with effluent and pink salmon (ENSR, 1990d).

n An identical 96-hr LC50 (i.e., >100.0% ballast-water effluent) was generated in a parallel dynamic (flow-through) toxicity test conducted during June 2-6, 1990, with effluent and pink salmon (ENSR, 1990f).

Coonstripe shrimp (juveniles/adults) and pink salmon were sometimes evaluated for acute sensitivity to effluent in both static-renewal and dynamic (flow-through) toxicity tests. No difference in toxicity was associated with use of the two testing techniques (96-hr LC50s were always >100% effluent).

(b) Chronic Toxicity

Mysid shrimp exposed to effluent were characterized by a mean (geometric mean) Maximum Acceptable Toxicant Concentration (MATC) of 26.4% effluent (Table 3). Higher ("less toxic") mean MATCs were generated by bay mussels (29.3% effluent), pink salmon (35.4% effluent), and sheephead minnows (>50.0% effluent); whereas lower MATCs were associated with sand dollars (<21.9% effluent), purple sea urchins (<18.3% effluent), and inland silversides (<15.6% effluent).

Inland silversides were determined to be the species most chronically sensitive to effluent partly because of two extremely low MATC values (<6.1 and <6.2% effluent) generated early in the testing program (winter and fall of 1990). These low values did not reoccur in later testing (winters of 1990 and 1991). Sand dollars also generated a low and seemingly aberrant MATC value of <6.2% effluent (in the spring of 1990). Tests with sand dollars and purple sea urchins, in which success of fertilization of eggs by sperm was evaluated, were often of unacceptable quality (Table 3; Footnote b). This poor quality was related to fertilization success of control eggs and sperm, which frequently was outside of the recommended (EPA, 1988) limits of 70 to 90%.

3.1.2 Toxicity to Representative Marine Plants
(Chronic Toxicity)

Effluent was relatively non-toxic to the two representative species of marine plants that were evaluated for chronic sensitivity to effluent (Table 4). In both cases, the chronic value (96-hr EC50 or MATC) was greater than 50.0% effluent, which was the highest concentration of effluent tested.

Table 3. ___ Chronic toxicity of ballast-water effluent to representative marine animals^{a,b}

Representative marine animal	Chronic toxicity of ballast-water effluent		Date of toxicity test	Reference
	Chronic value (MATC) -- % effluent ^{c,d}	Biological endpoint(s) measured		
1. Mysid shrimp (<i>Mysidopsis bahia</i>)	35.4	Survival and growth of juveniles (7 days old)	Sept. 26-Oct. 3, 1989	Continental Shelf Associates, 1990
	34.3	Survival, growth, and reproduction of juveniles (7 days old) -- glass testing containers	March 1-8, 1990	Battelle, 1990a
	34.5	Survival, growth, and reproduction of juveniles (7 days old) -- plastic testing containers	March 1-8, 1990	Battelle, 1990a
	17.4	Survival and growth of juveniles (7 days old)	May 22-29, 1990	Battelle, 1990b
	17.6	Survival and growth of juveniles (7 days old)	Oct. 19-26, 1990	Battelle, 1990d
Geometric mean:	26.4	--	--	--
2. Bay mussel (<i>Mytilus</i> spp.)	34.5	Development of larvae to normal "D-shaped" forms	July, 1990	Battelle, 1990c
	24.8	Development of larvae to normal "D-shaped" forms	Sept. 23-25, 1991	Battelle, 1991d
	Geometric mean:	29.3	--	--
3. Purple sea urchin (<i>Strongylocentrotus purpuratus</i>)	12.0 ^e	Fertilization success	March 3 and 8, 1990 (glass containers)	Battelle, 1990a
	<20.1 ^e	Fertilization success	May 26 and 31, 1990	Battelle, 1990b
	25.3	Fertilization success	May 14, 1991	Battelle, 1991c
	Geometric mean:	<18.3	--	--

Table 3. ___ Chronic toxicity of ballast-water effluent -- cont.

Representative marine animal	Chronic toxicity of ballast-water effluent		Date of toxicity test	Reference
	Chronic value (MATC) -- % effluent ^{c,d}	Biological endpoint(s) measured		
4. Sand dollar (<i>Dendraster excentricus</i>)	<6.2	Fertilization success	June 4, 1990	Battelle, 1990b
	67.9	Fertilization success	July 25, 1990	Battelle, 1990c
	24.8	Fertilization success	Sept. 23, 1991	Battelle, 1991d
	Geometric mean:	<21.9	--	--
5. Sheepshead minnow (<i>Cyprinodon variegatus</i>)	>50.0	Survival and growth of larvae (<24 hr old)	Sept. 26-Oct.3, 1989	Continental Shelf Associates, 1990
	Geometric mean:	>50.0	--	--
6. Inland silverside (<i>Menidia beryllina</i>)	<6.1 (8.7 based on comparison to brine control)	Survival and growth of larvae (10 days post hatch)	March 1-8, 1990 (Trial 2)	Battelle, 1990a
	<6.2	Survival and growth of larvae (11 days post hatch)	Oct. 19-26, 1990	Battelle, 1990d
	25.3	Survival and growth of larvae (11 days post hatch)	Dec. 10-17, 1990	Battelle, 1991a
	62.3	Survival and growth of larvae (11 days post hatch)	Dec. 9-16, 1991	Battelle, 1992
	Geometric mean:	<15.6	--	--
7. Pink salmon (<i>Oncorhynchus gorbuscha</i>)	35.4 ^f	Survival and growth of smolts (203 days post hatch)	May 22-June 19, 1990	ENSR, 1990i
	Geometric mean:	35.4	--	--

^a Marine animals evaluated for chronic sensitivity to ballast-water effluent are representative of the three taxonomic categories that are recommended (EPA, 1986; Appendix A of report) for evaluation during derivation of numerical national water quality criteria for discharged substances. Bay mussels, purple sea urchins, and sand dollars were evaluated as a condition (Part I.B.9.a-d; sublethal tests) of the National Pollution Discharge Elimination System (NPDES) Permit for effluent (EPA, 1989).

Table 3. ___ Chronic toxicity of ballast-water effluent -- cont.

b Chronic toxicity of ballast-water effluent to representative marine animals was usually determined in 7-day static-renewal partial-life-cycle (mysid shrimp) or early-life-stage (sheepshead minnows and inland silversides) toxicity tests. However, in the case of purple sea urchins and sand dollars, static, non-renewal techniques (in which gametes were exposed to effluent for up to 1.3 hr) were employed. Static, non-renewal techniques were also utilized for bay mussel larvae (which were exposed to effluent for 48 hr). The pink salmon test was a partial-life-cycle test (28 days in duration) conducted under dynamic (flow-through) conditions. Results of some tests conducted with effluent and mysid shrimp (Battelle, 1990c), purple sea urchins (Battelle; 1990a, 1990b, 1990c, 1991b, 1991c, 1992), sand dollars (Battelle; 1990b, 1990c, 1990d, 1991d), sheepshead minnows (Battelle, 1991a), and inland silversides (ENSR, 1990; Battelle, 1990b) are not presented in this table because of unacceptably low survival (mysid shrimp, sheepshead minnows, and inland silversides evaluated by Battelle), low weight (inland silversides evaluated by ENSR), or poor fertilization success (purple sea urchins and sand dollars) of control organisms. Results of another test with inland silversides (Battelle, 1990c) are not presented in this table because of extreme temperature fluctuations during the test.

c The chronic value (or Maximum Acceptable Toxicant Concentration, MATC) for ballast-water effluent is the geometric mean of the lower chronic limit (or No-Observed-Effect Concentration, NOEC) and upper chronic limit (or Lowest-Observed-Effect Concentration, LOEC) for effluent. The NOEC is the highest tested concentration of effluent that did not cause a statistically significant ($P = 0.05$) deleterious effect in animals exposed to effluent as compared to animals exposed to control medium. The LOEC is the lowest tested concentration of effluent that caused such a statistically significant effect.

d Chronic values are always expressed in terms of % effluent as contrasted to % effluent and % brine employed to adjust salinity of effluent to 30 ppt, a convention employed to describe toxicity in some Battelle reports (Battelle; 1990a [inland silversides], 1990d [mysid shrimp]).

e This chronic value for ballast-water effluent and purple sea urchins is the geometric mean of chronic values derived in two tests performed during the indicated testing period.

f This chronic value for ballast-water effluent and pink salmon (35.4% effluent) reflects corrections to originally reported data (ENSR, 1990i). The corrections pertain to NOEL (6.25 to 25% effluent), LOEC (12.5 to 50% effluent), and chronic value (8.8 to 35.4% effluent).

Table 4. ___ Chronic toxicity of ballast-water effluent to representative marine plants^{a,b}

Representative marine plant	Chronic toxicity of ballast-water effluent (% effluent)		Date of toxicity test	Reference
	96-hr median effective concentration (96-hr EC50) ^c	Maximum Acceptable Toxicant Concentration (MATC) ^d		
1. Diatom (<i>Thalassiosira pseudonana</i>)	>50.0 (cell production)	--	Nov. 27-Dec. 1, 1991	T.H.E. Laboratories, 1991
2. Red alga (<i>Champia parvula</i>)	--	>50.0 (cystocarp production)	Nov. 27-Dec. 6, 1991	T.H.E. Laboratories, 1991

Final Plant Value (FPV) = >50.0% effluent				

^a Marine plants evaluated for chronic sensitivity to ballast-water effluent are representative of the two taxonomic categories (phyla) that are recommended (EPA, 1986; Appendix A of report) for evaluation during derivation of numerical national water quality criteria for discharged substances.

^b Chronic toxicity of ballast-water effluent to diatoms was determined in a static, non-renewal toxicity test. Chronic toxicity of effluent to red alga was evaluated by exposing algae to static test media for 2 days, followed by static exposure to control (recovery) media for 7 days. Results of a number of tests conducted with effluent and red algae (Battelle; 1990b, 1990c, 1990d, 1991a, 1992) are not presented in this table because of unacceptably low cystocarp production by control organisms.

^c The 96-hr median effective concentration (96-hr EC50) is the theoretical concentration of ballast-water effluent that caused a 50% reduction (from control values) in number of plant cells. The 96-hr EC50 is considered to be a measurement of chronic toxicity in single-celled organisms, which have a relatively short life span (EPA, 1986).

^d The Maximum Acceptable Toxicant Concentration (MATC) for ballast-water effluent is the geometric mean of the lower chronic limit (or No-Observed-Effect Concentration, NOEC) and upper chronic limit (or Lowest-Observed-Effect Concentration, LOEC) for effluent. The NOEC is the highest tested concentration of effluent that did not cause a statistically significant ($P = 0.05$) deleterious effect in plants exposed to effluent as compared to plants exposed to control medium. The LOEC is the lowest tested concentration of effluent that caused such a statistically significant effect.

3.2 Derivation of Water Quality Criteria for Ballast-Water Effluent

A Criteria Maximum Concentration (CMC) and Criteria Continuous Concentration (CCC) were derived for effluent.

3.2.1 Criteria Maximum Concentration

The Final Acute Value (FAV) derived for effluent is 41.42% effluent (Table 5). The appropriateness of this FAV is supported by several factors (EPA, 1986). First, Species Mean Acute Values (SMAVs) for commercially or recreationally important species -- in particular, coonstripe shrimp (>80.7% effluent) and pink salmon (>94.4% effluent) -- are greater than the FAV (41.42% effluent). Second, the range of SMAVs for the genus Oncorhynchus (the only genus for which more than one species was evaluated) is less than a factor of 10 (the range approximates a factor of 1.3). Third, the range of Genus Mean Acute Values (GMAVs) for the four most sensitive genera is less than a factor of 10 (the range approximates a factor of 1.6). Finally, the FAV is "reasonable" in comparison to all SMAVs and GMAVs.

Since the FAV for effluent is 41.42% effluent, the CMC for effluent is 21% effluent (CMC = 0.5 FAV).

3.2.2 Criteria Continuous Concentration (Application Factor Approach)

The Final Application Factor (FAF) developed for effluent is 0.39 (Table 6). The FAF was developed as the grand mean (geometric mean) of all five Species Mean Application Factors (SMAFs) because these SMAFs differ by less than a factor of 10 (the maximum difference approximates a factor of 2.7) and no major trend is apparent between SMAFs and associated mean acute values (EPA, 1986). Since the FAF for effluent is 0.39, the Final Acute:Chronic Ratio (FACR) for effluent is 2.56 (FACR = 1/FAF).

The Final Chronic Value (FCV) for effluent is 16% effluent (FCV = FAV/FACR = 41.42% effluent/2.56 = 16% effluent), which is slightly less than the CMC for effluent (21% effluent). The reasonableness of this FCV is further evidenced by a number of factors (EPA, 1986). First, chronic values (MATCs) are available for acutely sensitive species -- i.e., mysid shrimp and purple sea urchins. Second,

Table 5. ___ Calculation of Final Acute Value (FAV) for ballast-water effluent^a

Ranking of representative marine animals for acute sensitivity to effluent (from most sensitive to least sensitive)	Statistics associated with Genus Mean Acute Value (GMAV)							
	Representative marine animal	Species Mean Acute Value (SMAV) -- % effluent ^b	GMAV (%effluent) ^c	Ranking (R) of GMAV (from lowest to highest)	Cumulative probability (P) of GMAV ^{d,e}	P ^{1/2} of GMAV ^d	Natural logarithm (1n) of GMAV (%effluent) ^{d,f}	1n of GMAV ² (%effluent) ^{d,f}
1.	Mysid shrimp (<i>Mysidopsis bahia</i>)	>51.4	>51.4	1	0.0909	0.3015	3.9396	15.5204
2.	Purple sea urchin (<i>Strongylocentrotus purpuratus</i>)	>52.3	>52.3	2	0.1818	0.4264	3.9570	15.6578
3.	Amphipod (<i>Corophium spinicorne</i>)	65.2	65.2	3	0.2727	0.5222	4.1775	17.4515
4.	Rainbow trout (<i>Oncorhynchus mykiss</i>)	71.0	>81.9	5	--	--	--	--
5.	Coonstripe shrimp (<i>Pandalus hypsinotus</i>)	>80.7	>80.7	4	0.3636	0.6030	4.3907	19.2782
6.	Inland silverside (<i>Menidia beryllina</i>)	>83.5	>83.5	6	--	--	--	--
7.	Pink salmon (<i>Oncorhynchus gorbuscha</i>)	>94.4	>81.9	5	--	--	--	--
8.	Amphipod (<i>Rheopoxynius abronius</i>)	>100.0	>100.0	7	--	--	--	--
9.	Polychaete worm (<i>Neanthes arenaceodentata</i>)	>100.0	>100.0	8	--	--	--	--

Table 5. Calculation of Final Acute Value (FAV) for ballast-water effluent -- cont.

Ranking of representative marine animals for acute sensitivity to effluent (from most sensitive to least sensitive)	Representative marine animal	Species Mean Acute Value (SMAV) -- % effluent ^b	GMAV (%effluent) ^c	Ranking (R) of GMAV (from lowest to highest)	Statistics associated with Genus Mean Acute Value (GMAV)				
					Cumulative probability (P) of GMAV ^{d,e}	P ^{1/2} of GMAV ^d	Natural logarithm (1n) of GMAV (%effluent) ^{d,f}	1n of GMAV ² (%effluent) ^{d,f}	
10.	Blue mussel (<i>Mytilus edulis</i>)	>100.0	>100.0	9	--	--	--	--	
11.	Sheepshead minnow (<i>Cyprinodon variegatus</i>)	>100.0	>100.0	10	--	--	--	--	
-----					N = 10	Σ = 0.9090	Σ = 1.8531	Σ = 16.4648	Σ = 67.9079
					$s^2 = \frac{\sum 1n \text{ GMAV}^2 - \frac{(\sum 1n \text{ GMAV})^2}{4}}{\sum P - \frac{(\sum P^{1/2})^2}{4}} = \frac{67.9079 - \frac{16.4648^2}{4}}{0.9090 - \frac{1.8531^2}{4}} = \frac{0.1355}{0.0505} = 2.6832$				
					$s = \sqrt{s^2} = 1.6380$				
					$L = \frac{\sum 1n \text{ GMAV} - (s)(\sum P^{1/2})}{4} = \frac{16.4648 - (1.6380)(1.8531)}{4} = 3.3574$				
					$A = (s)(0.05^{1/2}) + L = (1.6380)(0.05^{1/2}) + 3.3574 = 3.7237$				
					$\text{FAV} = eA = e3.7237 = 41.42\% \text{ effluent}$				

Table 5. ___ Calculation of Final Acute Value (FAV) for ballast-water effluent -- cont.

- a The Final Acute Value (FAV) pertains only to marine animals (i.e., not to marine plants).
- b Species Mean Acute Values (SMAVs) are initially presented in Table 2 as 96-hr LC50s.
- c Genus Mean Acute Values (GMAVs) are identical to SMAVs except in the case of the genus Oncorhynchus, which is represented by two species (rainbow trout and pink salmon). In this case, the GMAV is the geometric mean of the two SMAVs.
- d This statistic is calculated for only those four GMAVs characterized by P values nearest to 0.05.
- e Cumulative probabilities (Ps) of GMAVs are mathematically defined as $P = R/N + 1$.
- f The calculation of this statistic is based upon the absolute values of GMAVs (EPA, 1986).

Table 6. ___ Application factors (AFs) and acute:chronic ratios (ACRs) for ballast-water effluent and representative marine animals

Representative marine animal	Corresponding acute and chronic values (% effluent) ^a		Application factor (AF) ^d	Acute:chronic ratio (ACR) ^e	Date of corresponding toxicity tests ^a	Reference
	Acute value (96-hr LC50) ^b	Chronic value (MATC) ^c				
1. Mysid shrimp (<i>Mysidopsis bahia</i>)	>100.0	35.4	<0.35	>2.86	Sept. 26-Oct. 3, 1989	Continental Shelf Associates, 1990
	42.7	34.3	0.80	1.25	March 1-8, 1990	Battelle, 1990a
	56.2	17.4	0.31	3.23	May 22-29, 1990	Battelle, 1990b
	43.5	17.6	0.40	2.50	Oct. 19-26, 1990	Battelle, 1990d
	Geometric mean:	>56.8	24.7	<0.43	>2.33	--
2. Purple sea urchin (<i>Strongylocentrotus purpuratus</i>)	34.2 ^f	<20.1	<0.59	>1.69	May 22-31, 1990	Battelle, 1990b
	Geometric mean:	34.2	<20.1	<0.59	>1.69	--
3. Sheepshead minnow (<i>Cyprinodon variegatus</i>)	>100.0	>50.0	0.50	2.00	Sept. 26-Oct. 3, 1989	Continental Shelf Associates, 1990
	Geometric mean:	>100.0	>50.0	0.50	2.00	--
4. Inland silverside (<i>Menidia beryllina</i>)	>98.0	<6.1	<0.06	>16.67	March 1-10, 1990	Battelle, 1990a
	>100.0	<6.2	<0.06	>16.67	Oct. 19-26, 1990	Battelle, 1990d
	28.8	25.3	0.88	1.14	Dec. 10-17, 1990	Battelle, 1991a
	>98.5	62.3	<0.63	>1.59	Dec. 9-16, 1991	Battelle, 1992
	Geometric mean:	>72.6	<15.6	<0.21	>4.76	--

Table 6. ___ Application factors (AFs) and acute:chronic ratios (ACRs) for ballast-water effluent -- cont.

Representative marine animal	Corresponding acute and chronic values (% effluent) ^a		Application factor (AF) ^d	Acute:chronic ratio (ACR) ^e	Date of corresponding toxicity tests ^a	Reference
	Acute value (96-hr LC50) ^b	Chronic value (MATC) ^c				
5. Pink salmon (<i>Oncorhynchus gorbuscha</i>)	>100.0	35.4	<0.35	>2.86	May 22- June 19, 1990	ENSR; 1990c, 1990e, 1990i
Geometric mean:	>100.0	35.4	<0.35	>2.86	--	--

Final Application Factor (FAF): 0.399 Final Acute:Chronic Ratio (FACR): 2.56 ^h						

a Corresponding (paired) acute and chronic values were derived during the same general study (i.e., a study conducted by the same aquatic toxicology laboratory on the same approximate date with the same dilution water).

b Acute values (96-hr LC50s) are abstracted from Table 2.

c Chronic values (MATCs) are abstracted from Table 3.

d The application factor (AF) is the quotient of the chronic value divided by the acute value.

e The acute:chronic ratio (ACR) is the inverse of the application factor.

f This acute value (34.2% effluent) is a 72-hr EC50.

g The Final Application Factor (FAF) is estimated (EPA, 1986) as the absolute value of the geometric mean of the five Species Mean Application Factors (SMAFs).

h The Final Acute:Chronic Ratio (FACR) is the inverse of the Final Application Factor (FAF).

the chronic value for a commercially and recreationally important species -- pink salmon (35.4% effluent) -- is greater than the Final Chronic Value (16% effluent) . Last, the Final Chronic Value (FCV) is compatible with all acute and chronic toxicological data presented in this report.

In the case of ballast-water effluent, the Criteria Continuous Concentration (CCC) is equivalent to the FCV (16% effluent). This is because the FCV is less than the Final Plant Value (>50.0% effluent; Table 4) and a Final Residue Value is not applicable to effluent. Consequently, the CCC for effluent is 16% effluent.

4. DISCUSSION

The Criteria Maximum Concentration (CMC) and Criteria Continuous Concentration (CCC) for any discharged substance consist of three components: 1) a "magnitude" element; 2) a "duration" element; and 3) a "frequency" element (EPA, 1991). In the case of ballast-water effluent, the previously discussed values of 21% effluent (for CMC) and 16% effluent (for CCC) pertain to the magnitude (concentrations) of effluent that are protective of approximately 95% of resident aquatic species represented by the animals employed in the toxicological testing program (EPA, 1986). These concentrations are compared to environmental levels of effluent averaged over 1 hr (for CMC) and 4 days (for CCC), which represent the durational aspect of the water quality criteria. For both the CMC and CCC, environmental levels of effluent cannot exceed protective concentrations of effluent more than on an average of once every 3 years, which is the frequency element of the water quality criteria.

In the case of states that allow mixing zones (such as Alaska), CMCs and CCCs must be achieved at the boundaries of the zones (EPA, 1991). For ballast-water effluent, dilution factors of 4.8 (CMC) and 6.2 (CCC) are required to disperse effluent within the mixing zone (MZ) to environmentally protective levels ($100\% \text{ effluent} / 4.8 = \text{CMC of } 21\% \text{ effluent}$; $100\% \text{ effluent} / 6.2 = \text{CCC of } 16\% \text{ effluent}$). Additional environmental safeguards must prevail in the MZ proper. The EPA (1991) has recommended that a MZ: 1) not impair the "overall" biological integrity of the water body; 2) not endanger critical resource areas, including fish-harvesting areas; 3) not restrict passage of free-swimming organisms (e.g., fishes) into tributaries; 4) provide for a continuous corridor of passage that meets water quality criteria for free-swimming organisms and drifting organisms (e.g., plankton); and 5) prevent lethality to organisms "passing through" the MZ. (Prevention of deleterious impacts to benthos in the MZ is not a criterion for establishment of a MZ.) The EPA (1991) has concluded that lethality to organisms passing through the MZ can be prevented if the CMC is achieved within a "very short" distance of the effluent outfall (which is characteristic of high-velocity discharge structures) or, more quantitatively, if drifting organisms are not exposed to 1-hr mean concentrations of effluent exceeding the CMC. It is beyond the scope of this report to definitively address the environmental fate of discharged effluent. However, it

appears that hydrodynamic conditions within the MZ for ballast-water effluent conform with EPA guidelines for preventing lethality of organisms drifting through the zone.

5. CONCLUSIONS

The acute and chronic toxicological data presented in this report, interpreted in the context of rigorous EPA-recommended protocols for the derivation of water quality criteria for discharged substances, indicate that effluent discharged from Alyeska's Ballast Water Treatment Facility does not represent an aquatic hazard to resident marine organisms if an approximately 6-fold dilution of effluent by receiving water is achieved at the boundaries of the mixing zone (MZ) for effluent. In addition, the appropriate Final Application Factor (FAF) for effluent is 0.39 (Final Acute:Chronic Ratio = 2.56), which differs substantially from the 0.01 AF identified in the State of Alaska's present water quality standards (State of Alaska, undated). This FAF of 0.39 cannot be considered unusually high considering that AFs developed for oil-refinery effluents and mysid shrimp have ranged from 0.54 to 0.67 (EPA, 1991).

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CSA APPENDICES

APPENDIX A

PROTOCOLS FOR DERIVATION OF NUMERICAL NATIONAL WATER QUALITY CRITERIA FOR DISCHARGED SUBSTANCES (UNITED STATES ENVIRONMENTAL PROTECTION AGENCY)

Derivation of numerical national water quality criteria for the protection of aquatic organisms and their uses is a complex process that uses information from many areas of aquatic toxicology. After a decision is made that a national criterion is needed for a particular material, all available information concerning toxicity to, and bioaccumulation by, aquatic organisms is collected, reviewed for acceptability, and sorted. If enough acceptable data on acute toxicity to aquatic animals are available, they are used to estimate the highest 1-hour average concentration that should not result in unacceptable effects on aquatic organisms and their uses. If justified, this concentration is made a function of a water quality characteristic such as pH, salinity, or hardness. Similarly, data on the chronic toxicity of the material to aquatic animals are used to estimate the highest 4-day average concentration that should not cause unacceptable toxicity during a long-term exposure. If appropriate, this concentration is also related to a water quality characteristic.

Data on toxicity to aquatic plants are examined to determine whether plants are likely to be unacceptably affected by concentrations that should not cause unacceptable effects on animals. Data on bioaccumulation by aquatic organisms are used to determine if residues might subject edible species to restrictions by the U.S. Food and Drug Administration or if such residues might harm some wildlife consumers of aquatic life. All other available data are examined for adverse effects that might

be biologically important.

If a thorough review of the pertinent information indicates that enough acceptable data are available, numerical national water quality criteria are derived for fresh water or saltwater or both to protect aquatic organisms and their uses from unacceptable effects due to exposures to high concentrations for short periods of time, lower concentrations for longer periods of time, and combinations of the two.

I. Collection of Data

- A. Collect all available data on the material concerning (a) toxicity to, and bioaccumulation by, aquatic animals and plants, (b) FDA action levels [12], and (c) chronic feeding studies and long-term field studies with wildlife species that regularly consume aquatic organisms.
- B. All data that are used should be available in typed, dated, and signed hard copy (publication, manuscript, letter, memorandum, etc.) with enough supporting information to indicate that acceptable test procedures were used and that the results are probably reliable. In some cases it may be appropriate to obtain additional written information from the investigator, if possible. Information that is confidential or privileged or otherwise not available for distribution should not be used.
- C. Questionable data, whether published or

unpublished, should not be used. For example, data should usually be rejected if they are from tests that did not contain a control treatment, tests in which too many organisms in the control treatment died or showed signs of stress or disease, and tests in which distilled or deionized water was used as the dilution water without addition of appropriate salts.

- D. Data on technical grade materials may be used if appropriate, but data on formulated mixtures and emulsifiable concentrates of the material of concern should not be used.
- E. For some highly volatile, hydrolyzable, or degradable materials it is probably appropriate to use only results of flow-through tests in which the concentrations of test material in the test solutions were measured often enough using acceptable analytical methods.
- F. Data should be rejected if they were obtained using:
 1. Brine shrimp, because they usually occur naturally only in water with salinity greater than 35 g/kg.
 2. Species that do not have reproducing wild populations in North America (See Appendix 1).
 3. Organisms that were previously exposed to substantial concentrations of the test material or other contaminants.

- G. Questionable data, data on formulated mixtures and emulsifiable concentrates, and data obtained with nonresident species or previously exposed organisms may be used to provide auxiliary information but should not be used in the derivation of criteria.

II. Required Data

- A. Certain data should be available to help ensure that each of the four major kinds of possible adverse effects receives adequate consideration. Results of acute and chronic toxicity tests with representative species of aquatic animals are necessary so that data available for tested species can be considered a useful indication of the sensitivities of appropriate untested species. Fewer data concerning toxicity to aquatic plants are required because procedures for conducting tests with plants and interpreting the results of such tests are not as well developed. Data concerning bioaccumulation by aquatic organisms are required only if relevant data are available concerning the significance of residues in aquatic organisms.
- B. To derive a criterion for freshwater aquatic organisms and their uses, the following should be available:
1. Results of acceptable acute tests (see Section

- IV) with at least one species of freshwater animal 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 in the class Osteichthyes, preferably a commercially or recreationally important warmwater species (e.g., bluegill, channel catfish, etc.)
 - c. a third family in the phylum Chordata (may be in the class Osteichthyes or may be an amphibian, etc.)
 - d. a planktonic crustacean (e.g., cladoceran, copepod, etc.)
 - e. a benthic crustacean (e.g., ostracod, isopod, amphipod, crayfish, etc.)
 - f. an insect (e.g., mayfly, dragonfly, damselfly, stonefly, caddisfly, mosquito, midge, etc.)
 - g. a family in a phylum other than Arthropoda or Chordata (e.g., Rotifera, Annelida, Mollusca, etc.)
 - h. a family in any order of insect or any phylum not already represented.
2. Acute-chronic ratios (see Section VI) with species of aquatic animals in at least three different families provided that of the three species:

- a. at least one is a fish
 - b. at least one is an invertebrate
 - c. at least one is an acutely sensitive freshwater species (the other two may be saltwater species).
3. Results of at least one acceptable test with a freshwater alga or vascular plant (see Section VIII). 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 also be available.
 4. At least one acceptable bioconcentration factor determined with an appropriate freshwater species, if a maximum permissible tissue concentration is available (see Section IX).
- c. To derive a criterion for saltwater aquatic organisms and their uses, the following should be available:
1. Results of acceptable acute tests (see Section IV) with at least one species of saltwater animal in at least eight different families such that all of the following are included:
 - a. two families in the phylum Chordata
 - b. a family in a phylum other than Arthropoda or Chordata

- c. either the Mysidae or Penaeidae family
 - d. three other families not in the phylum Chordata (may include Mysidae or Penaeidae, whichever was not used above)
 - e. any other family.
2. Acute-chronic ratios (see section VI) with species of aquatic animals in at least three different families provided that of the three species:
 - a. at least one is a fish
 - b. at least one is an invertebrate
 - c. at least one is an acutely sensitive saltwater species (the other one may be a freshwater species).
 3. Results of at least one acceptable test with a saltwater alga or vascular plant (see Section VIII. If plants are among the aquatic organisms most sensitive to the material, results of a test with a plant in another phylum (division) should also be available.
 4. At least one acceptable bioconcentration factor determined with an appropriate saltwater species, if a maximum permissible tissue concentration is available (see Section IX).
- D. If all the required data are available, a numerical criterion can usually be derived, except in special cases. For example, derivation of a criterion

might not be possible if the available acute-chronic ratios vary by more than a factor of 10 with no apparent pattern. Also, if a criterion is to be related to a water quality characteristic T (see Sections V and VII), 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 might be possible to derive a criterion if the available data clearly indicate that the Final Residue Value should be much lower than either the Final Chronic Value or the Final Plant Value.

- E. Confidence in a criterion usually increases as the amount of available pertinent data increases. Thus, additional data are usually desirable.

III. Final Acute Value

- A. Appropriate measures of the acute (short-term) toxicity of the material to a variety of species of aquatic animals are used to calculate the Final Acute Value. The Final Acute Value is an estimate of the concentration of the material corresponding to a cumulative probability of 0.05 in the acute toxicity values for the genera with which acceptable acute tests have been conducted on the material. However, in some cases, if the Species

Mean Acute Value of a commercially or recreationally important species is lower than the calculated Final Acute Value, then that Species Mean Acute Value replaces the calculated Final Acute Value in order to provide protection for that important species.

- B. Acute toxicity tests should have been conducted using acceptable procedures [13].
- C. Except for tests with saltwater annelids and mysids, results of acute tests during which the test organisms were fed should not be used, unless data indicate that the food did not affect the toxicity of the test material.
- D. Results of acute tests conducted in unusual dilution water, e.g., dilution water in which total organic carbon or particulate matter exceeded 5 mg/L, should not be used, unless a relationship is developed between acute toxicity and organic carbon or particulate matter or unless data show that organic carbon, particulate matter, etc., do not affect toxicity.
- E. Acute values should be based on endpoints which reflect the total severe acute adverse impact of the test material on the organisms used in the test. Therefore, only the following kinds of data on acute toxicity to aquatic animals should be used:

1. Tests with daphnids and other cladocerans should be started with organisms less than 24 hours old and tests with midges should be stressed with second- or third-instar larvae. The result should be the 48-hr EC50 based on percentage of organisms immobilized plus percentage of organisms killed. If such an EC50 is not available from a test, the 48-hr LC50 should be used in place of the desired 48-hr EC50. An EC50 or LC50 of longer than 48 hours can be used as long as the animals were not fed and the control animals were acceptable at the end of the test.
2. The result of a test with embryos and larvae of barnacles, bivalve molluscs (clams, mussels, oysters, and scallops), sea urchins, lobsters, crabs, shrimp, and abalones should be the 96-hr EC50 based on the percentage of organisms with incompletely developed shells plus the percentage of organisms killed. If such an EC50 is not available from a test, the lower of the 96-hr EC50 based on the percentage of organisms with incompletely developed shells and the 96-hr LC50 should be used in place of the desired 96-hr EC50. If the duration of the test was between 48 and 96 hours, the EC50 or LC50 at the end of the test should be used.

3. The acute values from tests with all other freshwater and saltwater animal species and older life stages of barnacles, bivalve molluscs, sea urchins, lobsters, crabs, shrimps, and abalones should be the 96-hr EC50 based on the percentage of organisms exhibiting loss of equilibrium plus the percentage of organisms immobilized plus the percentage of organisms killed. If such an EC50 is not available from a test, the 96-hr LC50 should be used in place of the desired 96-hr EC50.
 4. Tests with single-celled organisms are not considered acute tests, even if the duration was 96 hours or less.
 5. If the tests were conducted properly, acute values reported as "greater than" values and those which are above the solubility of the test material should be used, because rejection of such acute values would unnecessarily lower the Final Acute Value by eliminating acute values for resistant species.
- F. If the acute toxicity of the material to aquatic animals apparently has been shown to be related to a water quality characteristic such as hardness or particulate matter for freshwater animals or

salinity or particulate matter for saltwater animals, a Final Acute Equation should be derived based on that water quality characteristic. Go to Section V.

- G. If the available data indicate that one or more life stages are at least a factor of 2 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 Species Mean Acute Value (SMAV) because a species can only be considered protected from acute toxicity if all life stages are protected.
- H. The agreement of the data within and between species should be considered. Acute values that appear to be questionable in comparison with other acute and chronic data for the same species and for other species in the same genus probably should not be used in calculation of a Species Mean Acute Value. For example, if the acute values available for a species or genus differ by more than a factor of 10, some or all of the values probably should not be used in calculations.
- I. For each species for which at least one acute value is available, the Species Mean Acute Value should be calculated as the geometric mean of the results of all flow-through tests in which the concentrations of test material were measured. For a species for which no such result is available,

the Species Mean Acute Value should be calculated as 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 concentrations of test material (nominal concentrations are acceptable for most test materials if measured concentrations are not available).

NOTE: Data reported by original investigators should not be rounded off. Results of all intermediate calculations should be rounded [14] to four significant digits.

NOTE: The geometric mean of N numbers is the N^{th} root of the product of the N numbers. Alternatively, the geometric mean can be calculated by adding the logarithms of the N numbers, dividing the sum by N , and taking the antilog of the quotient. The geometric mean of two numbers is the square root of the product of the two numbers, and the geometric mean of one number is that number. Either natural (base e) or common (base 10) logarithms can be used to calculate geometric means as long as they are used consistently within each set of data, i.e., the antilog used must match the logarithm used.

NOTE: Geometric means, rather than arithmetic means, are used here because the distributions of sensitivities of individual organisms in toxicity tests on most materials and the distributions of sensitivities of species within a genus are more likely to be lognormal than normal. Similarly, geometric means are used for acute-chronic ratios and bioconcentration factors because quotients are likely to be closer to lognormal than normal distributions. In addition, division of the geometric mean of a set of numerators by the geometric mean of the set of corresponding denominators will result in the geometric mean of the set of corresponding quotients.

- J. For each genus for which one or more Species Mean Acute Values are available, the Genus Mean Acute Value should be calculated as the geometric mean of the Species Mean Acute Values available for the genus.
- K. Order the Genus Mean Acute Value from high to low.
- L. Assign ranks, R , to the Genus Mean Acute Value from "1" for the lowest to "N" for the highest. If two or more Genus Mean Acute Values are identical, arbitrarily assign them successive ranks.
- M. Calculate the cumulative probability, P , for each Genus Mean Acute Value as $R/(N+1)$.

N. Select the four Genus Mean Acute Value which have cumulative probabilities closest to 0.05 (if there are less than 59 Genus Mean Acute Value, these will always be the four lowest Genus Mean Acute Values).

O. Using the selected Genus Mean Acute Values and F_s , calculate:

$$S_2 = \frac{E(\ln \text{GMAV})_2 - ((E \ln \text{GMAV}))_2/4}{(P) - ((E / ^p))_2/4}$$

$$L = (E(\ln \text{GMAV}) - S(E(/^p)))/4$$

$$A = S(/^{0.05}) + L$$

$$\text{FAV} = e^A$$

(See [11] for development of the calculation procedure and Appendix 2 for example calculation and computer program.)

NOTE: Natural logarithms (logarithms to base e, denoted as ln) are used herein merely because they are easier to use on some hand calculators and computers than common (base 10) logarithms. Consistent use of either will produce the same result.

P. If for a commercially or recreationally important species the geometric mean of the acute values from flow-through tests in which the concentrations of test material were measured is lower than the calculated Final Acute Value, then that geometric mean should be used as the Final Acute Value instead of the calculated Final Acute

Value.

Q. Go to Section VI.

IV. Final Acute Equation

- A. When enough data are available to show that acute toxicity to two or more species is similarly related to a water quality characteristic, the relationship should be taken into account as described in Sections B-G below or using analysis of covariance [15,16]. The two methods are equivalent and produce identical results. The manual method described below provides an understanding of this application of covariance analysis, but computerized versions of covariance analysis are much more convenient for analyzing large data tests. If two or more factors affect toxicity, multiple regression analysis should be used.
- B. 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 the acute toxicity values on the corresponding values of the water quality characteristic to obtain the slope and its 95 percent confidence limits for each species.

NOTE: Because the best documented relationship fitting these data is that between hardness and acute toxicity of metals in fresh water and a log-log

relationship, geometric means and natural logarithms of both toxicity and water quality are used in the rest of this section. For relationships based on other water quality characteristics such as pH, temperature, or salinity, no transformation or a different transformation might fit the data better, and appropriate changes will be necessary throughout this section.

- C. Decide whether the data for each species are useful, taking into account the range and number of the tested values of the water quality characteristic and the degree of agreement within and between species. For example, a slope based on six data points might be of limited value if it is based only on data for a very narrow range of values of the water quality characteristic. A slope based on only two data points, however, might be useful if it is consistent with other information and if the two points cover a broad enough range of the water quality characteristic. In addition, acute values that appear to be questionable in comparison with other acute and chronic data available for the same species and for other species in the same genus probably should not be used. For example, if after adjustment for the water quality characteristic, the acute values available for a species or genus.

differ by more than a factor of 10, probably some or all of the values should be rejected. If useful slopes are not available for at least one fish and one invertebrate or if the available slopes are too dissimilar or if too few data are available to adequately define the relationship between acute toxicity and the water quality characteristic, return to Section IV.G, using the results of tests conducted under conditions and in waters similar to those commonly used for toxicity tests with the species.

- D. Individually for each species calculate the geometric mean of the available acute values and then divide each of the acute values for species by the mean for the species. This normalizes the values so that the geometric mean of the normalized values for each species individually 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 acute 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 Section B.

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 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 center of the graph.
- H. For each species calculate the geometric mean, W , of the acute toxicity values and the geometric mean, X , of the values of the water quality characteristic. (These were calculated in steps D and E.)
- I. For each species calculate the logarithm, Y , of the Species Mean Acute Value at a selected value, Z , of the water quality characteristic using the equation:
- $$Y = \ln W - V(\ln X - \ln Z).$$
- J. For each species calculate the SMAV at Z using the equation: $SMAV = e^Y$.

NOTE: Alternatively, the Species Mean Acute Values at Z can be obtained by skipping step H using the

equations in steps I and J to adjust each acute value individually to Z, and then calculating the geometric mean of the adjusted values for each species individually. This alternative procedure allows an examination of the range of the adjusted acute values for each species.

- K. Obtain the Final Acute Value at Z by using the procedure described in Section IV.J-0.
- L. If the Species Mean Acute Value at Z of a commercially or recreationally important species is lower than the calculated Final Acute Value at Z, then that Species Mean Acute Value should be used as the Final Acute Value at Z instead of the calculated Final Acute Value.
- M. The Final Acute Equation is written as: Final Acute Value = $e^{(V[\ln(\text{water quality characteristic})] + \ln A - V[\ln Z])}$, where V = pooled acute slope and A = Final Acute Value at Z. Because V, A, and Z are known, the Final Acute Value can be calculated for any selected value of the water quality characteristic.

V. Final Chronic Value

- A. Depending on the data that are available concerning chronic toxicity to aquatic animals, the Final Chronic Value might be calculated in the same manner as the Final Acute Value or by dividing the Final Acute Value by the Final Acute-

Chronic Ratio. In some cases it may not be possible to calculate a Final Chronic Value.

NOTE: As the name implies, the acute-chronic ratio is a way of relating acute and chronic toxicities. The acute-chronic ratio is basically the inverse of the application factor, but this new name is better because it is more descriptive and should help prevent confusion between "application factors" and "safety factors." Acute-chronic ratios and application factors are ways of relating the acute and chronic toxicities of a material to aquatic organisms. Safety factors are used to provide an extra margin of safety beyond the known or estimated sensitivities of aquatic organisms. Another advantage of the acute-chronic ratio is that it will usually be greater than 1; this should avoid the 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.

- B. Chronic values should be based on results of flow-through (except renewal is acceptable for daphnids) chronic tests in which the concentrations of test material in the test solutions were properly measured at appropriate times during the test.
- C. Results of chronic tests in which survival,

growth, or reproduction in the control treatment was unacceptably low should not be used. The limits of acceptability will depend on the species.

D. Results of chronic tests conducted in unusual dilution water, e.g., dilution water in which total organic carbon or particulate matter exceeded 5 mg/L, should not be used, unless a relationship is developed between chronic toxicity and organic carbon or particulate matter or unless data show that organic carbon, particulate matter, etc., do not affect toxicity.

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

1. Life-cycle toxicity tests consisting of exposures of each of two or more groups of individuals of a species to a different concentration of the test material throughout a life cycle. To ensure that all life stages and life processes are exposed, tests with fish should begin with embryos or newly hatched young less than 48 hours old, continue through maturation and reproduction, and should end not less than 24 days (90 days for salmonids) after the hatching of the next generation. Tests with daphnids should begin

with young less than 24 hours old and last for not less than 21 days. Tests with mysids should begin with young less than 24 hours old and continue until 7 days past the median time of first brood release in the controls. 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. For mysids, data should be obtained and analyzed on survival, growth, and young per female.

2. Partial life-cycle toxicity tests consisting of exposures of each of two or more groups of individuals of a species of fish to a concentration of the test material through most portions of 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 immature juveniles at least 2 months prior to active gonad development, continue through maturation and reproduction,

and end not less than 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.

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

NOTE: Results of an early life-stage test are used as predictions 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 are possibly not good predictions of the results of comparable life-cycle or partial life-cycle tests.

F. A chronic value may be obtained by calculating the geometric mean of the 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 adverse effect on any of the specified biological measurements, and (c) below which no tested concentration caused 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 adverse effect on one or more of the specified biological measurements, and (c) above which all tested concentrations also caused such an effect.

NOTE: Because various authors have used a variety of terms and definitions to interpret and report results of chronic tests, reported results should be reviewed carefully. The amount of effect that is considered unacceptable is often based on a statistical hypothesis test, but might also be defined in terms of a specified percent reduction from the controls. A small percent reduction (e.g., 3 percent) might be considered acceptable even if it is statistically significantly different from the control, whereas a large

percent reduction (e.g., 30 percent) might be considered unacceptable even if it is not statistically significant.

- G. If the chronic toxicity of the material to aquatic animals apparently has been shown to be related to a water quality characteristic such as hardness or particulate matter for freshwater animals or salinity or particulate matter for saltwater animals, a Final Chronic Equation should be derived based on that water quality characteristic. Go to Section VII.
- H. If chronic values are available for species in eight families as described in Sections III.B.1 or III.C.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 available for the species, and appropriate Genus Mean Chronic Values should be calculated. The Final Chronic Value should then be obtained using the procedure described in Section IV.J-0. Then go to Section VI.M.
- I. 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 the results of all acceptable flow-through (except static is acceptable for daphnids) acute tests in

the same dilution water and in which the concentrations were measured. For fish, the acute test(s) should have been conducted with juveniles. The 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, but in a different study, may be used. If no such acute tests are available, results of acute tests conducted in the same dilution water in a different laboratory may be used. If no such acute tests are available, an acute-chronic ratio should not be calculated.

- J. For each species, calculate the species mean acute-chronic ratio as the geometric mean of all acute-chronic ratios available for that species.
- K. For some materials the acute-chronic ratio seems to be the same for all species, but for other materials the ratio seems to increase or decrease as the Species Mean Acute Value (SMAV) increases. Thus the Final Acute-Chronic Ratio can be obtained in four ways, depending on the data available:
 - 1. If the Species Mean Acute-Chronic ratio seems to increase or decrease as the Species Mean Acute Value increases, the Final Acute-Chronic Ratio should be calculated as the geometric mean of the acute-chronic ratios for species

whose Species Mean Acute Values are close to the Final Acute Value.

2. If no major trend is apparent and the acute-chronic ratios for a number of species are within a factor of 10, the Final Acute-Chronic Ratio should be calculated as the geometric mean of all the Species Mean Acute-Chronic Ratios available for both freshwater and saltwater species.
3. For acute tests conducted on metals and possibly other substances with embryos and larvae of barnacles, bivalve molluscs, sea urchins, lobsters, crabs, shrimp, and abalones (see Section IV.E.2), it is probably appropriate to assume that the acute-chronic ratio is 2. Chronic tests are very difficult to conduct with most such species, but it is likely that the sensitivities of embryos and larvae would determine the results of life-cycle tests. Thus, if the lowest available Species Mean Acute Values were determined with embryos and larvae of such species, the Final Acute-Chronic Ratio should probably be assumed to be 2, so that the Final Chronic Value is equal to the Criterion Maximum Concentration (see Section XI.B)

4. If the most appropriate Species Mean Acute-Chronic Ratios are less than 2.0, and especially if they are less than 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 assumed to be 2, so that the Final Chronic Value is equal to the Criterion Maximum Concentration (see Section XI.B).

If the available Species Mean Acute-Chronic Ratios do not fit one of these cases, a Final Acute-Chronic Ratio probably cannot be obtained, and a Final Chronic Value probably cannot be calculated.

- L. Calculate the Final Chronic Value by dividing the Final Acute Value by the Final Acute-Chronic Ratio. If there was a Final Acute Equation rather than a Final Acute Value, see also Section VII.A.
- M. If the Species Mean Chronic Value of a commercially or recreationally important species is lower than the calculated Final Chronic Value, then that Species Mean Chronic Value should be used as the Final Chronic Value instead of the calculated Final Chronic Value.
- N. Go to Section VIII.

VI. Final Chronic Equation

- A. A Final Chronic Equation can be derived in two ways. The procedure described here in Section A will result in the chronic slope being the same as the acute slope. The procedure described in Sections B-N usually will result in the chronic slope being different from the acute slope.
1. If acute-chronic ratios are available for enough species at enough values of the water quality characteristic to indicate that the acute-chronic ratio is probably the same for all species and is probably independent of the water quality characteristic, calculate the Final Acute-Chronic Ratio as the geometric mean of the available Species Mean Acute-Chronic Ratios.
 2. Calculate the Final Chronic Value at the selected value Z of the water quality characteristic by dividing the Final Acute Value at Z (see Section V.M) by the Final Acute-Chronic Ratio.
 3. Use V = pooled acute slope (see section V.M) as L = pooled chronic slope.
 4. Go to Section VII.M.
- B. 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 taken into account as described in Sections B-G or using analysis of covariance [15,16]. The two methods are equivalent and produce identical results. The manual method described below provides an understanding of this application of covariance analysis, but computerized versions of covariance analysis are much more convenient for analyzing large data sets. If two or more factors affect toxicity, multiple regression analysis should be used.

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 the chronic toxicity values on the corresponding values of the water quality characteristic to obtain the slope and its 95 percent confidence limits for each species.

NOTE: Because the best documented relationship fitting these data is that between hardness and acute toxicity of metals in freshwater and a log-log relationship, geometric means and natural logarithms of both toxicity and water quality are used in the rest of this section. For relationships based on other water quality characteristics such as pH, temperature, or

salinity, no transformation or a different transformation might fit the data better, and appropriate changes will be necessary throughout this section. It is probably preferable, but not necessary, to use the same transformation that was used with the acute values in Section V.

- D. Decide whether the data for each species are useful, taking into account the range and number of the tested values of the water quality characteristic and the degree of agreement within and between species. For example, a slope based on six data points might be of limited value if it is based only on data for a very narrow range of values of the water quality characteristic. A slope based on only two data points, however, might be useful if it is consistent with other information and if the two points cover a broad enough range of the water quality characteristic. In addition, chronic values that appear to be questionable in comparison with other acute and chronic data available for the same species and for other species in the same genus probably should not be used. For example, if after adjustment for the water quality characteristic, the chronic values available for a species or genus differ by more than a factor of 10, probably some or all of the values should be rejected. If

a useful chronic slope is not available for at least one species or if the available slopes are too dissimilar or if too few data are available to adequately define the relationship between chronic toxicity and the water quality characteristic, it might be appropriate to assume that the chronic slope is the same as the acute slope, which is equivalent to assuming that the acute-chronic ratio is independent of the water quality characteristic. Alternatively, return to Section VI.H, using the results of tests conducted under conditions and in waters similar to those commonly used for toxicity tests with the species.

- E. Individually 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 individually and for any combination of species is 1.0.
- F. Similarly normalize the values of the water quality characteristic for each species individually.
- G. 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 the 95 percent confidence

limits will be identical to those obtained in Section B. 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.

- H. 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.
- I. For each species calculate the geometric mean, M , of the toxicity values and the geometric mean, P , of the values of the water quality characteristic. (These were calculated in steps E and F.)

J. For each species calculate the logarithm, Q , of the Species Mean Chronic Value at a selected value, Z , of the water quality characteristic using the equation: $Q = \ln M - L(\ln P - \ln Z)$.

NOTE: Although it is not necessary, it will usually be best to use the same value of the water quality characteristic here as was used in section V.I.

K. For each species calculate a Species Mean Chronic Value at Z using the equation: $SMCV = e^Q$.

NOTE: Alternatively, the Species Mean Chronic Values at Z can be obtained by skipping step J, using the equations in steps J and K to adjust each acute value individually to Z , and then calculating the geometric means of the adjusted values for each species individually. This alternative procedure allows an examination of the range of the adjusted chronic values for each species.

L. Obtain the Final Chronic Value at Z by using the procedure described in Section IV.J-0.

M. If the Species Mean Chronic Value at Z of a commercially or recreationally important species is lower than the calculated Final Chronic Value at Z , then that Species Mean Chronic Value should be used as the Final Chronic Value at Z instead of the calculated Final Chronic Value.

N. The Final Chronic Equation is written as: Final Chronic Value = $e^{(L[\ln(\text{water quality characteristic})] + \ln S - L[\ln Z])}$, where L = pooled chronic slope and S = Final Chronic Value at Z. Because L, S and Z are known, the Final Chronic Value can be calculated for any selected value of the water quality characteristic.

VII. Final Plant Value

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

B. A plant value is the result of a 96-hr test conducted with an alga or a chronic test conducted with an aquatic vascular plant.

NOTE: A test of the toxicity of a metal to a plant usually should not be used if the medium contained an excessive amount of a complexing agent, such as EDTA, that might affect the toxicity of the metal. Concentrations of EDTA above about 200 ug/L should probably be considered excessive.

- C. The Final Plant Value should be obtained by selecting the lowest result from a test with an important aquatic plant species in which the concentrations of test material were measured and the endpoint was biologically important.

VIII. Final Residue Value

- A. The Final Residue Value is intended to (a) prevent concentrations in commercially or recreationally important aquatic species from affecting marketability because of exceedence of applicable FDA action levels and (b) protect wildlife, including fishes and birds, that consume aquatic organisms from demonstrated unacceptable effects. The Final Residue Value is the lowest of the 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) an FDA action level [12] for fish oil or for the edible portion of fish or shellfish, or (b) a maximum acceptable dietary intake 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 X because no Final Residue Value can be derived.

B. Bioconcentration Factors (BCFs) and bioaccumulation factors (BAFs) are quotients of the concentration of a material in one or more tissues of an aquatic organism divided by the average concentration in the solution in which the organism had been living. A BCF is intended to account only for net uptake directly from water, and thus almost has to be measured in a laboratory test. Some uptake during the bioconcentration test might not be directly from water if the food sorbs some of the test material before it is eaten by the test organisms. A BAF is intended to account for net uptake from both food and water in a real-world situation. A BAF almost has to be measured in a field situation in which predators accumulate the material directly from water and by consuming prey that itself could have accumulated the material from both food and water. The BCF and BAF are probably similar for a material with a low BCF, but the BAF is probably higher than the BCF for materials with high BCFs. Although BCFs are not too difficult to determine, very few BAFs have been measured acceptably because it is necessary to make enough measurements of the concentration of the material in water to show that it was reasonably constant for a long enough period of time over the range of territory inhabited by the

organisms. Because so few acceptable BAFs are available, only BCFs will be discussed further. However, if an acceptable BAF is available for a material, it should be used instead of any available BCFs.

- C. If a maximum permissible tissue concentration is available for a substance (e.g., parent material, parent material plus metabolites, etc.), the tissue concentration used in the calculation of the BCF should be for the same substance. Otherwise the tissue concentration used in the calculation of the BCF 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.
- D. 1. A BCF should be used only if the test was flow-through, the BCF was calculated based on measured concentrations of the test material in tissue and in the test solution, and the exposure continued at least until either apparent steady-state or 28 days was reached. Steady-state is reached when the BCF does not change significantly over a period of time, such as 2 days or 16 percent of the length of the exposure, whichever is longer. The BCF used from a test should be the highest of (a) the apparent steady-state BCF, if apparent steady-state was reached, (b) the highest BCF

- obtained, if apparent steady-state was not reached, and (c) the projected steady-state BCF, if calculated.
2. Whenever a BCF is determined for a lipophilic material, the percent lipids should also be determined in the tissue(s) for which the BCF was calculated.
 3. A BCF obtained from an exposure that adversely affected the test organisms may be used only if it is similar to a BCF obtained with unaffected organisms of the same species at lower concentrations that did not cause adverse effects.
 4. Because maximum permissible tissue concentrations are almost never 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 BCF by 0.1 for plankton and by 0.2 for individual species of fishes and invertebrates [17].
 5. If more than one acceptable BCF is available for a species, the geometric mean of the available values should be used, except that if the BCFs are from different lengths of exposure and the BCF increases with length of

exposure, the BCF for the longest exposure should be used.

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

1. For each available maximum acceptable dietary intake derived from a chronic 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 the tested wildlife species.

2. For an FDA action level for fish or shellfish, the appropriate BCF is the highest geometric mean species BCF for the edible portion (muscle for decapods, muscle with or without skin for fishes, adductor muscle for scallops, and total soft tissue for other bivalve molluscs) of a consumed species. The highest species BCF is used because FDA action levels are applied on a species-by-species basis.

F. For lipophilic materials, it might be possible to calculate additional residue values. Because the steady-state BCF for a lipophilic material seems to be proportional to percent lipids from one tissue to another and from one species to another

[18-20], extrapolations can be made from tested tissues or species to untested tissues or species on the basis of percent lipids.

1. For each BCF for which the percent lipids is known for the same tissue for which the BCF was measured, normalize the BCF to a 1 percent lipid basis by dividing the BCF by the percent lipids. This adjustment to a 1 percent lipid basis is intended to make all the measured BCFs for a material comparable regardless of the species or tissue with which the BCF was measured.
2. Calculate the geometric mean normalized BCF. Data for both saltwater and freshwater species should be used to determine the mean normalized BCF, unless the data show that the normalized BCFs are probably not similar.
3. Calculate all possible residue values by dividing the available maximum permissible tissue concentrations by the mean normalized BCF and by the percent lipids values appropriate to the maximum permissible tissue concentrations, i.e.,

$$\text{Residue value} = \frac{\text{(maximum permissible tissue concentration)}}{\text{(mean normalized BCF) (appropriate percent lipids)}}$$

$$\text{Residue value} = \frac{\text{(maximum permissible tissue concentration)}}{\text{(mean normalized BCF) (appropriate percent lipids)}}$$

a. For an FDA action level for fish oil, the

appropriate percent lipids value is 100.

- b. For an FDA action level for fish, the appropriate percent lipids value is 11 for freshwater criteria and 10 for saltwater criteria because FDA action levels are applied on a species-by-species basis to commonly consumed species. The highest lipid contents in the edible portions of important consumed species are about 11 percent for both the freshwater chinook salmon and lake trout and about 10 percent for the saltwater Atlantic herring [21].
 - c. For a maximum acceptable dietary intake derived from a chronic feeding study or a long-term field study with wildlife, the appropriate percent lipids is that of an aquatic species or group of aquatic species which constitute a major portion of the diet of the wildlife species.
- G. The Final Residue Value is obtained by selecting the lowest of the available residue values.

NOTE: In some cases the Final Residue Value will not be low enough. For example, a residue value calculated from an FDA action level will probably result in an average concentration in the edible portion of a fatty species that is at the action level. Some individual organisms, and possibly some species, will have residue concentrations higher than the mean value but no mechanism has been devised to provide appropriate additional protection. Also, some chronic feeding studies and long-term field studies with wildlife identify concentrations that cause adverse effects but do not identify concentrations which do not cause adverse effects; again, no mechanism has been devised to provide appropriate additional protection. These are some of the species and uses that are not protected at all times in all places.

X. Other Data

Pertinent information that could not be used in earlier sections might 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 adverse effect that has been shown to be biologically important. Especially important are data for species for which no

other data are available. Data from behavioral, biochemical, physiological, microcosm, and field studies might also be available. Data might be available from tests conducted in unusual dilution water (see IV.D and VI.D), from chronic tests in which the concentrations were not measured (see VI.B), from tests with previously exposed organisms (see II.F), and from tests on formulated mixtures or emulsifiable concentrates (see II.D). Such data might affect a criterion if the data were obtained with an important species, the test concentrations were measured, and the endpoint was biologically important.

XI. Criterion

- A. A criterion consists of two concentrations: the Criterion Maximum Concentration and the Criterion Continuous Concentration.
- B. The Criterion Maximum Concentration (CMC) is equal to one-half the Final Acute Value.
- C. The Criterion Continuous Concentration (CCC) is equal to the lowest of the Final Chronic Value, the Final Plant Value, and the Final Residue Value, unless other data (see Section X) show that a lower value should be used. If toxicity is related to a water quality characteristic, the Criterion Continuous Concentration is obtained from the Final Chronic Equation, the Final Plant Value, and the Final Residue Value by selecting

the one, or the combination, that results in the lowest concentrations in the usual range of the water quality characteristic, unless other data (see Section X) show that a lower value should be used.

D. Round [14] both the Criterion Maximum Concentration and the Criterion Continuous Concentration to two significant digits.

E. 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 4-day average concentration of (2) does not exceed (3) ug/L more than once every 3 years on the average and if the 1-hour average concentration does not exceed (4) ug/L more than once every 3 years on the average.

where (1) = insert "freshwater" or "saltwater"

(2) = insert name of material

(3) = insert the Criterion Continuous
Concentration

(4) = insert the Criterion Maximum
Concentration.

XII. Final Review

A. The derivation of the criterion should be carefully reviewed by rechecking each step of the Guidelines. Items that should be especially checked are:

1. If unpublished data are used, are they well documented?
2. Are all required data available?
3. Is the range of acute values for any species greater than a factor of 10?
4. Is the range of Species Mean Acute Values for any genus greater than a factor of 10?
5. Is there more than a factor of 10 difference between the four lowest Genus Mean Acute Values?
6. Are any of the four lowest Genus Mean Acute Values questionable?
7. Is the Final Acute Value reasonable in comparison with the Species Mean Acute Values and Genus Mean Acute Values?
8. For any commercially or recreationally important species, is the geometric mean of the acute values from flow-through tests in which the concentrations of test material were measured lower than the Final Acute Value?
9. Are any of the chronic values questionable?

10. Are chronic values available for acutely sensitive species?
 11. Is the range of acute-chronic ratios greater than a factor of 10?
 12. Is the Final Chronic Value reasonable in comparison with the available acute and chronic data?
 13. Is the measured or predicted chronic value for any commercially or recreationally important species below the Final Chronic Value?
 14. Are any of the other data important?
 15. Do any data look like they might be outliers?
 16. Are there any deviations from the Guidelines?
Are they acceptable?
- B. On the basis of all available pertinent laboratory and field information, determine if the criterion is consistent with sound scientific evidence. If it is not, another criterion, either higher or lower, should be derived using appropriate modifications of these Guidelines.