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Progress Re-evaluating Puget Sound Apparent Effects Thresholds (AETs)

Volume I:
1994 Amphipod and Echinoderm Larval AETs

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For
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LIST OF ACRONYMS

2AET	-	The second (2nd) Highest Apparent Effects Threshold
AET	-	Apparent Effects Threshold
AMPT	-	AMPhipod Toxicity bioassay
ARM	-	PSDDA Annual Review Meeting
BENA	-	BENthic Abundance
CSL	-	Cleanup Screening Level
DDD	-	DichloroDiphenylDichloroethane
DDE	-	DichloroDiphenyldichloroEthylene
DDT	-	DichloroDiphenylTrichloroethane
DO	-	Dissolved Oxygen
DNR	-	Washington State Department of Natural Resources
EC_{50}	-	Effective Concentration, 50%
ECHN	-	ECHiNoderm larval toxicity bioassay
EPA	-	U.S. Environmental Protection Agency
HAET	-	Highest Apparent Effects Threshold
HPAH	-	High molecular weight Polynuclear Aromatic Hydrocarbon compounds
LAET	-	Lowest Apparent Effects Threshold
LC_{50}	-	Lethal Concentration, 50%
LPAH	-	Low molecular weight Polynuclear Aromatic Hydrocarbon compounds
MCUL	-	Minimum CleanUp Level
MICB	-	MICrotox® luminosity Bioassay (saline extract)
ML	-	PSDDA program Maximum Level guideline concentrations
NMFS	-	National Marine Fisheries Service
NOAA	-	National Oceanic and Atmospheric and Administration
NPDES	-	National Pollutant Discharge Elimination System
OYST	-	OYSer larval Toxicity bioassay
PAH	-	Polynuclear Aromatic Hydrocarbon compounds
PCB	-	PolyChlorinated Biphenyls
PSEP	-	The Puget Sound Estuary Program
PSDDA	-	The Puget Sound Dredged Disposal Analysis agencies and program
PSWQA	-	Puget Sound Water Quality Authority
QA	-	Quality Assurance.
QA1	-	Quality Assurance, Level 1.
QA2	-	Quality Assurance, Level 2..

SEDQUAL	-	Ecology's **SED**iment **QUAL**ity values or AET database.
SIZ_{max}	-	The **max**imum allowable Sediment Impact Zone
SL	-	PSDDA program Screening Level guideline concentration.
SMARM	-	Sediment Management Annual Review Meeting
SMS	-	Washington State Sediment Management Standards.
SRM	-	Standard Reference Material
SQS	-	Sediment Quality Standard
SQV	-	Sediment Quality Value
TBT	-	TriButyl Tin
TOC	-	Total Organic Carbon.
TVS	-	Total Volatile Solids
USACE	-	U.S. Army Corps of Engineers

EXECUTIVE SUMMARY

Apparent Effects Threshold (AET) values are the concentrations of specific chemicals of concern (COC) in sediment above which a significant adverse biological effect or "Hit" always occurs. They form the basis for both the Puget Sound Dredged Disposal Analysis (PSDDA) program guidelines *(1,2)* and the criteria contained in the Sediment Management Standards (SMS) rule *(3)*.

This document is the result of the first extensive re-evaluation of sediment AETs since 1988. The re-evaluation uses extensive new synoptic data to 1) recalculate amphipod mortality AETs, 2) calculate new sediment larval AETs, and 3) determine the predictive reliability of both AET groups. A further key objective is to assess some of the potential implications of new AETs for the PSDDA and SMS programs, as well as the dredging community and other stakeholders. The scope of the re-evaluation does not include calculating AETs based on new benthic infaunal, saline extract Microtox® or 20-day juvenile polychaete *(Neanthes arenaceodentata)* endpoints.

PSDDA program staff compiled an extensive inventory of matching chemistry and bioassay, or "synoptic," samples collected up until March 1993. Many of these samples, however, were excluded from final AET calculations because they failed to meet chemical quality assurance (QA) requirements or else lacked an adequate negative control or reference area sample. Even with data exclusions, the 1994 AET re-evaluation began with a sediment quality values database that contained more than double the 1988 number of amphipod mortality samples and over 200 echinoderm larval samples.

The PSDDA agencies attempted to adhere closely to the quality assurance, biological effects interpretation, AET and reliability calculation methods used in 1988 *(4)*. This was done to avoid having to repeat certain discussions or controversies. The report does describe some method variations, though. For example, 1994 AET calculations included subsurface synoptic samples which met all general quality assurance requirements. Some of the newer sample chemistry data have yet to be fully quality assured to meet the more stringent "QA2" level requirements, as was done in 1988. Sediment larval bioassay results were interpreted using the abnormality endpoint alone and also effective mortality (abnormality plus mortality). Statistical comparisons made between test and reference samples differed slightly from those used in 1988. However, none of these was thought to have substantially affected the final 1994 AET or reliability values.

Most of the amphipod mortality AETs calculated in 1994 either remained the same or increased relative to the corresponding 1988 values, whether normalized to dry weight or organic carbon. AETs for trace metals and individual HPAHs were most affected. Dry weight-normalized arsenic, cadmium, lead, mercury and zinc AET values increased by factors ranging from 1.1 to 4.8. Six individual dry weight HPAH AETs increased by an average factor of 2.2.

The sensitivity measure of reliability for dry weight-normalized amphipod AETs declined from 58% in 1988 to 43% in 1994. However, the overall reliability of 1994 values was similar to that found in 1988, 84% and 85%, respectively.

In early 1993, the number of newer bivalve larval bioassay samples was not adequate to calculate separate bivalve larval AETs. Therefore, and on the advice of marine benthic experts, echinoderm data alone were used to calculate separate, new AET groups. These echinoderm larval AETs were based on both the abnormality endpoint and the effective mortality (abnormality + mortality) endpoint. AET values derived using the former endpoint proved more sensitive and reliable overall, so the final 1994 echinoderm larval AETs presented in this report were calculated using only abnormality.

Twenty-seven of the dry weight-normalized echinoderm abnormality AET values calculated in 1994 were lower and seven were higher than corresponding 1986 oyster values. Overall, TOC-normalized echinoderm AETs were also lower than oyster AETs. These lower echinoderm AETs may indicate a fundamentally lower tolerance of the bioassay test organisms, primarily *Dendraster excentricus*, toward chemical contaminants in Puget Sound sediments.

Reliability calculations revealed that the 1994 dry weight-normalized echinoderm abnormality AETs were far less sensitive than 1986 oyster AETs: 48% vs. 88%. However, the independent reliability calculations indicated they were substantially more efficient and had better overall reliability than the oyster AETs. Echinoderm AETs were the second most efficient of all AET types. Sensitivity and overall reliability for TOC-normalized echinoderm abnormality AETs were both reduced relative to 1986 oyster values.

Both 1994 amphipod mortality and echinoderm abnormality AETs were examined as part of a new suite of five possible AETs for Puget Sound: 1994 amphipod, 1988 benthic, 1994 echinoderm, 1986 Microtox and 1986 oyster AETs. The highest dry weight-normalized AET (HAET) values for 17 COCs were greater than reported in 1988. Most of the new HAETs were for trace metal or HPAH compounds, and were set by the recalculated 1994 amphipod AETs. Echinoderm

AETs, particularly dry weight-normalized ones, set numerous new second lowest AETs (2AET) and lowest AETs (LAET).

New HAETs set by dry weight-normalized amphipod mortality AET values have potential implications to the PSDDA program for revising ML and SL guidelines. Echinoderm AETs, which much more frequently established new 2AET or LAET values, have potential to change the SMS criteria and program.

However, proposing changes to the guidelines or the criteria used in either program will involve detailed review of the AET reliability results presented in this report, as well as other reliability calculations yet to be conducted. The values eventually adopted by either the PSDDA or SMS program will likely not compromise certain reliability measures of the current regulatory values. Finally, the practical and economic implications of any changes to PSDDA MLs and SLs, and SMS criteria, will also need to be considered prior to adopting new regulatory values.

INTRODUCTION

Management of dredged material and contaminated sediment in Washington State is based on numerical sediment quality values. Two examples of these are the PSDDA program guidelines *(1,2)* and the criteria contained in the SMS rule *(3)*. Both are derived from AET values which were last calculated in 1988 using data collected from throughout Puget Sound between March 1982 and September 1986 *(4)*.

This report represents the first extensive re-evaluation of sediment AETs since 1988. It is driven in part by the availability of additional sediment quality data, many of which are incorporated into recalculating AETs and their reliability. Results include a list of revised amphipod mortality AETs, new echinoderm larval AETs, and a comparison of these "1994" AETs to 1986 and 1988 values. These are presented and discussed in a PSDDA program context, but may also facilitate review of the SMS rule.

By re-evaluating AETs and their potential to affect the PSDDA maximum and screening levels (MLs and SLs), the report addresses an agreement to conduct an annual review of the dredging program *(2)*. It also partly fulfills Ecology's requirement to annually review the SMS rule, and the source control and cleanup criteria contained therein.

While this is a technical report, it is intended for experts and the public alike. It presents methods and results in a manner that can be repeated by future investigators, but understood by those less familiar with sediment management in Puget Sound. It offers some objective conclusions and discussion for both audiences. The report does not, however, suggest policy options or recommend specific actions relative to the use of new AET values. Regulators, in particular, should make note of this.

The report is organized into five main sections. A **Background** section provides basic information on AETs and how they are used in Puget Sound. It also outlines goals and objectives of the report. The **Methods** section details how 1994 AETs were re-calculated. **Results** contains new amphipod mortality and echinoderm larval AET values, as well as their ability predict adverse biological effects. The section which follows offers **Conclusions and Discussion**. Finally, a brief **Recommendations for Future Work** lists important next steps and suggests some possible refinements to re-evaluation methods.

In addition to the main sections, the reader will find the **Table of Contents** followed by a **List of Acronyms** (pages v-vi) and an **Executive Summary** (pages vii-ix). A **Glossary** of terms located immediately after the **References** section.

References are cited throughout the text as italicized numbers within parentheses, e.g., *(3)*.

Three separate appendices accompany this report. **Appendix A** details the methods used to conduct bioassays, interpret biological effects, calculate AETs and reliability. **Appendix B** characterizes the sediment quality values database used to calculate 1994 AETs. The final appendix, **Appendix C**, includes more complete results of AET and reliability calculations. It is provided on a single 3.5" floppy diskette as a series of spreadsheet files (Excel, version 4.0).

Finally, it is important to note that Volume I of this report does not assess the potential implications that new AETs may have on regulatory programs. That will be done under the direction of a "Regulatory Work Group" or other team of experts and will be presented in a second volume.

BACKGROUND

Apparent Effects Thresholds (AETs)

AETs are concentrations of specific chemicals of concern (COC) in sediment above which a significant adverse biological effect always occurs. They are empirically derived using synoptic sediment samples - those having undergone simultaneous testing for chemical contaminants and adverse biological effects.

Determination of a single AET value is depicted in Figure 1. All synoptic samples that do not exhibit significant adverse effects, "No Hit" samples, are ranked from highest to lowest concentration for a chemical of concern. The "No Hit" sample with the greatest concentration is identified and generally establishes the AET value. A rare exception is made when that sample is found to be chemically anomalous (see Methods and Glossary). In that case, the sample with the next highest concentration sets the AET.

There must be at least one sample exhibiting a significant adverse effect, i.e., one "Hit" sample, with a chemical concentration exceeding the AET to confirm that AET value. If no "Hit" sample has a greater concentration, then the AET is qualified as a minimum value with a "G" or ">"symbol.

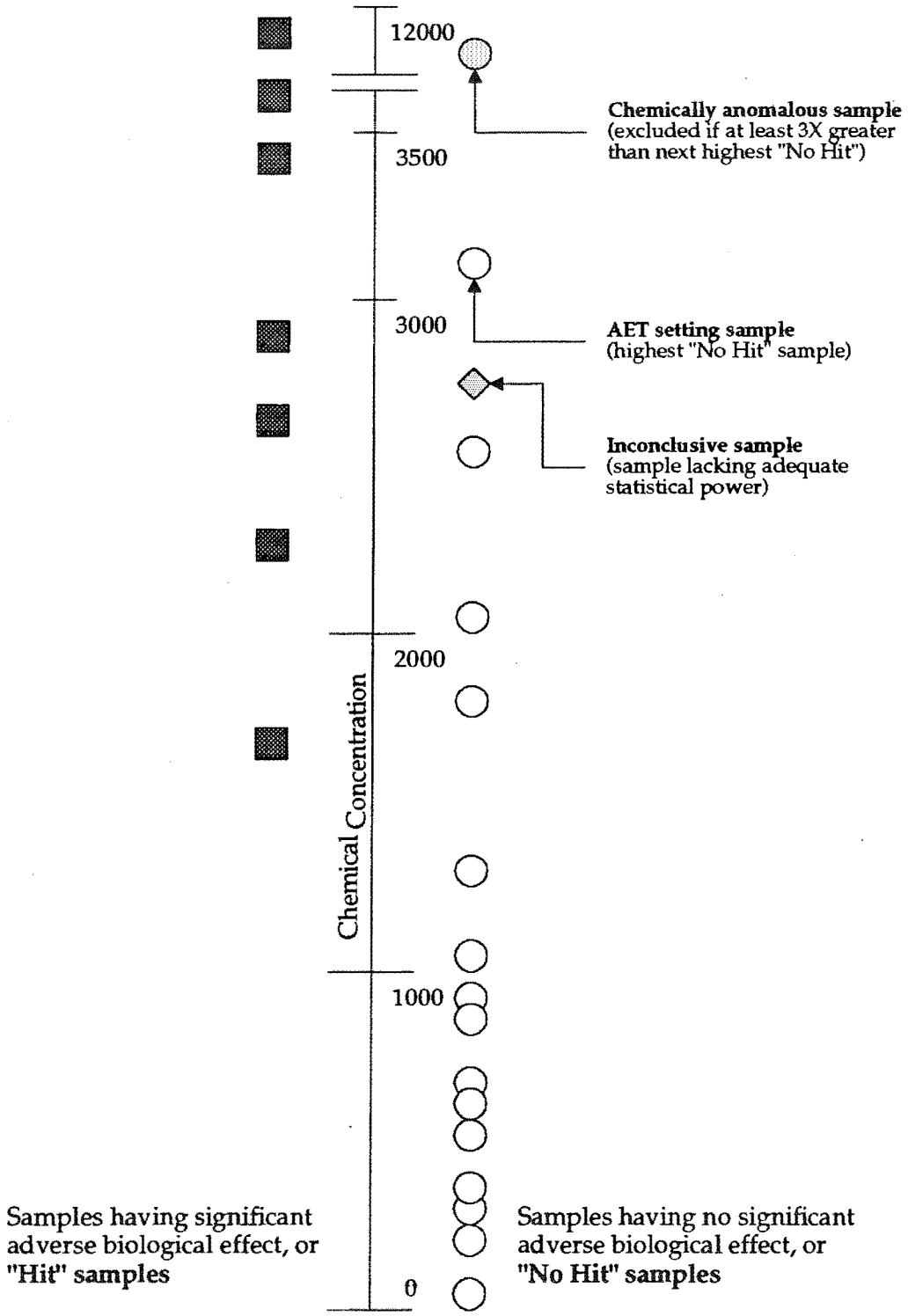
The determination of a single AET is repeated for all chemicals of concern. The resulting group of AETs is for a single biological indicator, interpretive endpoint, unit of measure and measurement basis. For example, one may calculate dry weight-normalized amphipod mortality AETs and express them in units of ppm. However, the determination process can be repeated to generate AET groups based on other biological indicators, endpoints, measurement units or bases. The 1988 AETs include values based on amphipod mortality, benthic infaunal abundance, Microtox® luminosity and oyster larval abnormality.

As stated in the Introduction, AET values are used by both the PSDDA and SMS programs to establish numerical guidelines and criteria. The relationship between the various AET and regulatory values, together with how the latter are used, is described below.

Puget Sound Dredged Disposal Analysis (PSDDA)

The PSDDA program routinely uses MLs and SLs to make decisions on the management and open water disposal of material to be dredged from Puget Sound. Current MLs and SLs are mainly based on 1988 AETs for the four biological indicators of toxicity just listed *(4)*. Those AETs incorporate extensive sediment quality data sets from Eagle Harbor, Elliott Bay and Everett Harbor

Figure 1. Determination of an Apparent Effects Threshold (AET) value.
An AET is generally set by the sample with the highest chemical concentration of a potential toxicant which does not exhibit a significant adverse biological effect ("No Hit"). The AET is qualified as a minimal value using a "G" or a ">" symbol if no "Hit" sample exceeds it. Note: Any units of measure or means of normalization may apply.



which were not used to determine the original 1986 AETs *(5)*. The highest of the four AET values, or HAET, establishes the ML for a given chemical of concern. The SL is set at one-tenth the ML, provided it is a) less than or equal to the lowest of the four AETs, or LAET, and b) greater than or equal to the average reference area concentration *(1,2)*.

In the PSDDA program, 60 different chemical ML or SL values are used to define three categories of dredged material. Material with COC concentrations exceeding an ML value is generally unsuitable for open water disposal[1] because the ML itself is an indication of several types of significant adverse biological effects. Material having chemical constituents between ML and SL concentrations may be expected to exhibit at least one type of adverse biological effect. Thus, additional biological effects testing is required to provide PSDDA agencies with the information needed for regulatory decisions. Material exhibiting sediment chemistry concentrations below all SL values is considered suitable for open water disposal because none of the adverse biological effects used to establish AETs are expected.

In order for regulatory decisions to protect biological resources, the PSDDA agencies compare high quality sediment data to ML and SL guideline values which reflect adverse biological effects.

One way PSDDA agencies ensure collection of high quality data is by recommending use of certain field and laboratory protocols *(6,7)*. These protocols encompass vessel positioning, collection of sediment samples, chemical analysis, quality assurance review, biological testing and interpretation of biological effects, etc. The PSDDA annual review process and Annual Review Meeting (ARM) facilitate adopting changes to those protocols to reflect "state-of-the-art" science, consensus-based policy making, and public input. The PSDDA process and agency responsibilities are described further in the Phase II Management Plans *(1,2, Chapter 9)*.

But regulatory decisions made by the PSDDA agencies also rely on the best available guidelines. For this reason, the PSDDA Phase II Management Plan requires an annual review of ML and SL values, too. In re-evaluating the guidelines, the agencies may consider and incorporate the following:

- synoptic sediment quality data collected during previous dredging years
- new field and laboratory experiences

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[1] Sediments which exceed two or more ML values, or one ML value by more than 100%, are considered unsuitable for placement at open water disposal sites. Sediments which exceed one ML by less than 100% must undergo biological testing.

- changes to how significant adverse biological effects are defined[2]
- results from new bioassays[3] *(10)*

Re-evaluations conducted in 1990 and 1991 *(8,9)* resulted in seven SL values being raised[4].

In preparation for this re-evaluation of MLs and SLs, PSDDA agencies obtained and reviewed additional synoptic data collected through March 1993. Preliminary AET values for amphipod mortality, bivalve and echinoderm larval abnormality, and combined larval species abnormality were presented at the 1993 and 1994 ARMs *(11,12)* and elsewhere *(13,14)*. This report incorporates comments on those presentations, as well as subsequent PSDDA agency and peer review feedback.

Sediment Management Standards (SMS)

In 1991, Ecology adopted 173-204 WAC: the Washington State Sediment Management Standards rule *(3)*. The rule addresses source control and cleanup of contaminated sediments by establishing marine chemical and biological sediment quality standards (SQS), cleanup screening level (CSL), minimum cleanup level (MCUL) and sediment impact zone (SIZ_{max}) values. In general, SQS values are based on the LAET, while CSL, MCUL and SIZ_{max} values are based on the second lowest of the four 1988 AETs (2AET).

Analogous to the PSDDA program guidelines, 47 chemical standards classify marine sediments according to levels of contamination. Sediments exceeding a CSL value are expected to show some adverse biological effects and may require cleanup[5]. Sediments exceeding a SIZ_{max} value may warrant the establishment of a sediment impact zone associated with a discharge permit. Sediments exceeding only SQS values may be expected to show at least one type of biological effect and thus require further characterization. Finally, sediments

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2 For example, PSDDA agencies adopted minor modifications to the saline extract Microtox® protocol and definition of significant adverse effects *(2, pages 5-24)*.

3 In 1992, PSDDA agencies replaced the 10-day juvenile polychaete mortality bioassay with the 20-day juvenile polychaete growth test. Both use the species *Neanthes arenaceodentata*.

4 SLs for 1,2,4-trichlorobenzene, 2-methyl-phenol, 2,4-dimethylphenol, benzoic acid, benzyl alcohol, N-nitrosodiphenylamine and pentachlorophenol were increased to 13 ppb, 20 ppb, 29 ppb, 400 ppb, 25 ppb, 28 ppb, and 100 ppb dry weight, respectively.

5 The SQS concentrations serve as cleanup goals.

with concentrations falling below the SQS are not predicted to exhibit significant adverse biological effects, and thus do not require remediation or creation of an impact zone.

Section 130(6) requires the SMS rule to be periodically reviewed and revised, as necessary *(3)*. According to Section 130(7)(a), "new or additional scientific information which is available relating surface sediment chemical quality to acute or chronic adverse effects on biological resources" must be considered during the SMS review process. As part of the process, any changes to AETs are examined for potential effect on the adopted Puget Sound marine SQS, CSL, MCUL and SIZ_{max}, values[6].

Goals and Objectives

The goal of this report is to clearly document the methods and results of the first extensive re-evaluation of AET values since the "1988 Update and Evaluation of Puget Sound AETs" *(4)*.

Specific objectives for this re-evaluation were initially determined by PSDDA agencies and grouped as mandatory, optional, or deferred *(10)*. The consensus among the agencies was that mandatory objectives had to be completed before any change to existing guidelines or criteria could be recommended. Completion of optional objectives was desirable, but was secondary to mandatory ones and dependent on agency resources. Still other objectives had to be deferred until adequate resources or technical guidance became available.

The objectives evolved throughout the project to reflect early analytical results, public comments and variable agency resources. The final objectives are summarized below.

Mandatory objectives:

- recalculate dry weight-normalized amphipod mortality AETs
- calculate new dry weight-normalized sediment larval AETs
- calculate of Total Organic Carbon or TOC-normalized AETs[7]
- determine the predictive reliability of the new 1994 AET values

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[6] Those chemical concentration criteria are listed in Section 320(2)/Table I, Section 420/Table II, and Section 520(2)/Table III, respectively).

[7] This objective was optional for the PSDDA program but considered mandatory for Ecology's review and revision of the SMS rule.

- determine the combined or "pooled" reliability of the entire new suite of Puget Sound AET values
- assess some of the potential implications of new AETs for PSDDA MLs, SLs, the program as a whole and the dredging community

Optional objectives:

- calculate echinoderm and bivalve larval AET values separately
- compare sediment larval AET values calculated using different bioassay endpoints[8]
- assess some of the potential implications of new AETs for the SMS criteria, the SMS program as a whole and stakeholders

Deferred objectives:

- recalculate of benthic infaunal AETs
- recalculate of saline extract Microtox® AETs
- calculate AETs based on the 20-day juvenile polychaete *(Neanthes arenaceodentata)* growth bioassay
- analyze patterns of sediment quality in Puget Sound, as requested by the Washington Public Ports Association *(8)*

Benthic experts recommended improvements to the interpretive endpoint for benthic infauna sample data *(15)*, but benthic AETs could not be recalculated without final reference sample performance standards. The exercise of recalculating Microtox® luminosity AETs awaited additional studies, possibly including side-by-side comparisons to the performance of other bioassays. When this re-evaluation began, the synoptic database for 20-day juvenile polychaete *(Neanthes)* growth bioassays was too limited to calculate AETs[9]. Finally, the pattern analysis mentioned was considered a lower priority than other deferred objectives.

Volume I of this report addresses all but the last of the mandatory objectives. A second volume will describe results of additional reliability analyses, the remaining mandatory objective - assessment of potential implications of new AETs - and a summary of the overall re-evaluation process. Neither volume outlines policy options or recommends actions on the use of new AETs in the PSDDA or SMS programs.

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[8] The PSDDA agencies sought to compare sediment larval AET values based on the abnormality endpoint alone to ones calculated using the combined abnormality + mortality, or "effective mortality," endpoint.

[9] Calculations of AETs result in highly variable values unless the total number of synoptic samples used approaches 50 (4, page 50).

METHODS

General Approach

The general approach used to recalculate AETs is summarized in Figure 2. First, an inventory of synoptic surveys containing bioassay data was compiled. Data for most of those surveys were then obtained and checked for completeness. Complete data sets next underwent a quality assurance (QA) review of chemical and bioassay data. Both types of data had to meet certain minimal guidelines, described later in this section. In addition, bioassay control and reference samples had to meet specific performance standards.

Bioassay test sample results were analyzed for statistically significant adverse effects, relative to one or more reference samples. This was done for all samples meeting the QA guidelines and performance standards. Samples fell into the categories illustrated in Figure 2: those exhibiting significant adverse effects ("Hit" samples), those which did not ("No Hit" samples), and those which were statistically inconclusive. The latter were excluded from calculations, as were "No Hit" samples found to be anomalous. Next, biological effects interpretations - "Hit" and "No Hit" data - were added to Ecology's sediment quality database (SEDQUAL). Finally, the new data were combined with comparable historical data, where possible, and 1994 AETs were calculated.

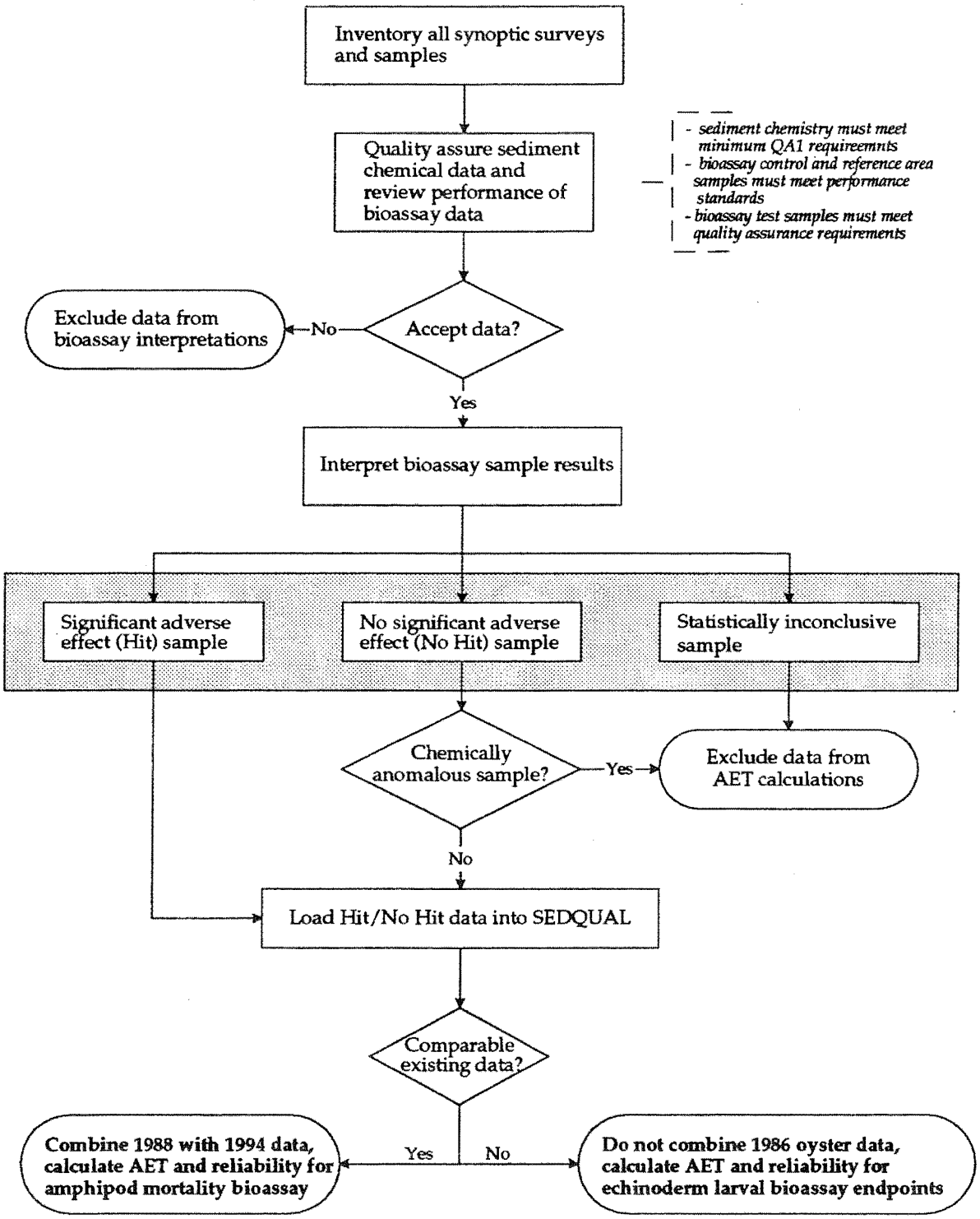
The data identification and acquisition, QA review and guidelines, bioassay performance standards, determination of biological effects, including the identification of inconclusive and anomalous samples, calculation of AETs and predictive reliability are described below.

Data Acquisition

The 1994 re-evaluation of AETs depended in part on the acquisition of high quality synoptic data not used to calculate the 1988 AETs. In order to identify and obtain such data, PSDDA program staff contacted many groups and individuals during 1992 and 1993.

PSDDA agencies asked several federal agencies to identify and submit sediment quality data, including the U.S. Army Corps of Engineers - Seattle District (USACE), U.S. Environmental Protection Agency - Region 10 (EPA), U.S. Navy, and National Oceanic and Atmospheric Administration (NOAA)/National Marine Fisheries Service (NMFS). State and regional officials from various Ecology programs, the Departments of Health and Natural Resources, the Puget Sound Water Quality Authority (PSWQA), Seattle METRO and the University of Washington were also contacted. In addition, PSDDA agencies communicated

Figure 2. General approach used to calculate 1994 Puget Sound AETs. Quality assurance review ensures use of comparable of data sets and excludes some samples from AET calculations. Bioassay interpretation yields "Hit", "No Hit" and "Inconclusive" samples (highlighted). Statistically inconclusive and anomalous samples are also excluded. The remaining biological effects data are used to calculate 1994 AET values.



with Jefferson, King, Pierce, Snohomish, Thurston, and Whatcom county staff, as well as port authorities, consulting firms and environmental laboratories.

The above groups were sent a standard letter or contacted by telephone. They were asked to identify all ambient sediment monitorings, dredging projects, compliance inspections and remedial or research investigations dating back to 1985 which involved collection of synoptic data. The responses to these inquiries were compiled into an inventory of synoptic surveys (Appendix B, Table B-1).

Chemistry: Analytical Methods and Data Quality Assurance

All chemical sediment quality data added to SEDQUAL and used in this AET re-evaluation were based on methods contained or identified in the Puget Sound Estuary Program (PSEP) Protocols and Guidelines *(6)*.

Quality assurance guidelines for sediment chemistry data depend on the ultimate use of the data. Data that PSDDA agencies use for regulatory decisions on disposal of dredged material must meet or exceed "QA1" guidelines *(6,16)*. Those guidelines verify lab and protocol performance by comparing analytical results from quality assurance samples to acceptable limits. Examples of QA samples include blanks, standard reference materials (SRM), duplicates, and matrix or surrogate spikes. Data used to develop or revise regulatory guidelines or standards must meet additional "QA2" data validation guidelines *(17)*. QA2 guidelines include checking that the continuous calibration of analytical instruments and ensuring that calculations of final results are correct.

Chemical sediment quality data used to calculate the 1988 Puget Sound AETs met the more rigorous QA2 data validation guidelines. In contrast, the 1994 AET values presented in this report were calculated using survey data reflecting different levels of QA review. While all of those data met QA1 guidelines, only a subset was known to also meet QA2 guidelines. Many survey data did not undergo full QA2 validation because of unavailable or incomplete documentation.

To reconcile this inconsistency, those surveys containing samples setting new 1994 AETs, at a minimum, will undergo an *a posteriori* QA2 review[10].

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[10] The PSDDA agencies agreed that recommendations to revise specific PSDDA ML or SL guidelines must consider results of that review.

Bioassays: Analytical Methods and Data Quality Assurance

The 1994 AET re-evaluation focused exclusively on 10-day amphipod mortality and sediment larval effects as biological indicators of sediment toxicity. All bioassays which measured those effects were conducted following the basic PSEP Protocols *(6)*. However, PSDDA survey samples may reflect slightly different bioassay protocols. That is because the PSDDA program has adopted minor modifications to the PSEP Protocols, based on the outcomes of two bioassay workshops *(18,19)* and its annual review process *(8,9,11,12)*.

One protocol modification extended the acceptable bioassay sample holding time from two weeks to eight weeks *(9)*. Thus, sediment bioassays samples from some PSDDA surveys were held longer before test initiation than 1988 AET samples. Other minor protocol modifications intended to improve test performance and interpretability included:

- measuring dissolved total ammonia and sulfides at the initiation and completion of each bioassay
- monitoring dissolved oxygen (DO) and pH daily
- aerating the overlying water if DO fell below 60% of saturation, or if high ammonia or total sulfides were present
- counting a minimum of 100 living larvae in control samples and a minimum of 20 abnormal larvae in reference and test sample replicates

The applicable sections of the PSEP Protocols (1989) are reproduced in Appendix A as Exhibit A-1. The protocol modifications made by the PSDDA agencies are summarized in Exhibit A-2.

PSDDA agencies carefully reviewed the bioassay data obtained since 1988 to ensure they were truly synoptic. Bioassay results linked to chemistry samples collected six months earlier or from a slightly different location, for example, were not used to calculate AETs. In such cases, the spatial and temporal variability observed in some Puget Sound sediments could result in high contaminant levels associated with no significant adverse biological effects, thereby setting anomalous AET values.

PSDDA agencies used both surface (0-2 cm) and subsurface (>2 cm) sediment samples for 1994 AET calculations, as long as they were synoptic and met all QA requirements. 1988 AETs were calculated using only synoptic surface sediment sample data.

Amphipod bioassays used the locally-collected marine species *Rhepoxinius abronius*. Calculations of sediment larval AETs were based on bioassays using

either bivalve (*Crassostrea gigas* or *Mytilus edulis*) or echinoderm larvae (*Dendraster excentricus* or *Strongylocentrotus droebachiensis* or *S. purpuratus*).

All new bioassay data underwent a careful, comprehensive review. PSDDA agencies examined the sample collection methods and holding times, test species, presence and performance of required control and reference samples, number of lab replicates, test duration and water quality for each bioassay batch. That information was compared to PSDDA-modified PSEP Protocols and requirements. Bioassay batches or samples were excluded from AET calculations if holding times were exceeded, or if there was no adequate negative control or reference sample.

Bioassays: Performance of Control and Reference Samples

Positive control or reference toxicant bioassay data were available for nearly all new synoptic surveys. Those data were reviewed for a dose-response relationship and an EC_{50}/LC_{50} comparable to literature values. A few bioassay batches lacking a dose-responsive positive control were used in 1994 calculations, as long as other QA or performance standards were met. None of the batches in question contained only "Hit" samples or only "No Hit" samples, indicative of unusually sensitive or insensitive test organisms. Recent investigations support use of samples which lack only a dose-responsive positive control *(20)*.

PSEP Protocols and the PSDDA program required a negative control sample to be run as part of each bioassay batch. All negative control sample data were compared to the existing performance standards presented in Table 1.

At least one reference sample per bioassay batch was also required. Reference samples had to be collected from a) recognized reference areas, such as Carr Inlet[11], b) other areas meeting the description in the PSEP Protocols *(6, page 18)*, or c) areas identified by recent reference area studies *(21,22)*.

The existing performance standards for bioassay reference samples were based on the variability observed among the 1988 amphipod reference samples and PSDDA guidelines. Before comparing new reference sample data to those performance standards, PSDDA agencies incorporated the variability observed in the reference samples from the synoptic surveys collected since 1988. The result was a new performance standard for amphipod reference samples: a standard deviation of 18% among replicate mortalities (Appendix A, Figure A-1). The comparable reference standards for sediment larval abnormality and effective

[11] Other established reference areas include Jetty Island, Samish and Sequim Bays.

mortality were standard deviations of 6% and 22%, respectively, (Appendix A, Figures A-2 and A-3).

Bioassay batches were excluded from 1994 AET calculations if their negative control or reference samples did not meet the performance standards in Table 1.

Table 1. Performance standards for bioassay control and reference samples used in 1994 re-evaluation of Puget Sound AETs.

Bioassay, Endpoint, (Test Duration)	Negative Control, Mean Value	Negative Control, Indiv. Rep.	Reference Sample, Mean Value	Reference Sample, Std. Dev.
Amphipod mortality (10-day)	≤ 10%	≤ 20%	≤20%	≤ 18% [a]
Sediment larval abnormality (48 to 96-hour)	mortality ≤ 30% abnormality ≤ 10%	none	none	≤ 6% [b]
Sediment larval effective mortality (48 to 96-hour)	mortality ≤ 30%	none	≤ 35% [c]	≤ 22% [d]

a. From the 95th percentile of standard deviations for mortality among 80 amphipod reference samples (Appendix A, Fig. A-1). The standard was 20% in 1988.
b. From the 95th percentile of standard deviations for abnormality among 62 sediment larval reference samples (Appendix A, Fig. A-2). There was no comparable standard used for 1986 oyster abnormality AETs.
c. Based on recommendations made by PSDDA in 1994 *(12)*.
d. From the 95th percentile of standard deviations for effective mortality among 62 sediment larval reference samples (Appendix A, Fig. A-3).

Determination of Adverse Biological Effects

Prior to the 1988 update of Puget Sound AETs, technical experts discussed alternative methods of determining significant adverse effects, including the

appropriate statistical tests to use. This 1994 re-evaluation of AETs did not deviate substantially from the methods used in 1988 *(4, pages 4-24 and Appendix C)*, believing that approach would preclude repeating earlier discussions and serve to maintain method consistency. The previous methods of determining significant adverse effects, and deviations from them, are highlighted in this section.

Significant Amphipod Mortality

Determining significant adverse effects for all bioassays entailed comparing a mean response among lab replicates of individual test samples to the mean response among lab replicates of one or more reference area samples[12]. Those comparisons were usually straightforward, because most surveys involved a single reference sample per batch. For some surveys or batches, however, there was more than one acceptable reference sample.

In cases of multiple reference samples, PSDDA agencies used two methods to compare a test sample to those references. When possible, the mean amphipod mortality among lab replicates of a single test sample was compared to the mean mortality among lab replicates of a single reference sample having a similar sediment grain size. If a grain size match was not possible, then the mean test sample mortality was compared to the mean mortality of all reference samples. A recent investigation supported both methods of comparison *(23)*.

The determination of significant adverse effects in the 10-day amphipod bioassay was done in five phases. Those phases are depicted in Appendix A, Figure A-4, and are described below.

Phase I — A comprehensive QA review of control and reference sample data, as discussed in the previous section

Phase II — Calculation of the mean percent mortality among lab replicates for all samples meeting the QA requirements. Test samples with a mean mortality less than 25% considered to have no significant adverse effects, and termed "No Hit" samples.

Phase III — Comparison of all test samples with a mean mortality of 25% or greater to the mean mortality of one or more reference samples. Two-tailed F-test performed to determine if the variance among

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[12] Early AET calculations used negative control samples as alternative reference samples when the latter were lacking or they did not meet QA guidelines or performance standards. It was found that doing so greatly reduced the reliability of the resulting AETs. Therefore, bioassay batches without a valid reference sample were excluded from 1994 AET calculations.

samples was homogeneous. When variances homogeneous, comparison using standard two-sample, one-tailed t-test. When variances heterogeneous (rare), comparison using Satterthwaite approximate t-test *(24)*.

Samples not statistically different from reference ($p<0.05$) and with a standard deviation among replicates of less than 15% considered "No Hit" samples. Those statistically different from reference considered "Hit" samples.

Phase IV — Estimation of statistical power for samples having a mean mortality greater than 25%, not significantly different from reference sample and with replicate variability more than 15% *(4, pages C16-22)*. Note: The addition of new amphipod bioassay test data did not change that 15% variability trigger (Appendix A, Figure A-5).

Calculation of statistical power of bioassay comparisons using a commercially available computer software program *(25)*. Samples entering this phase of determination process considered "No Hit" samples if the comparison had a power value of at least 0.6. Those having a power value less than 0.6 were labeled "inconclusive" and not used in AET calculations.

Phase V — Classification of samples as "Hit", "No Hit", or "Inconclusive." Determination of which "No Hit" samples were chemically anomalous (see Methods section, page 20).

Significant Sediment Larval Effects

Calculations of 1994 sediment larval AET values were based only on bioassay data from surveys and samples obtained since 1988 AETs were calculated. All bioassays were conducted following PSDDA-modified PSEP Protocols, and included at least five laboratory replicates. Abnormality, mortality and effective mortality test endpoint data were entered following the conventions summarized in Appendix A, Exhibit A-2. They were used to calculate a percent response and significant difference from one or more reference samples.

PSDDA agencies determined significant adverse effects in the sediment larval bioassay using two of these different test endpoints. The larval abnormality endpoint was consistent with 1988 oyster larval AETs. However, the effective mortality (abnormality plus mortality) endpoint was consistent with current regulatory program endpoints.

Sediment Larval Abnormality

Significant larval abnormality was determined as shown in Appendix A, Figure A-6. That determination was analogous to the one made for amphipod mortality, with two exceptions. First, no absolute threshold abnormality was required to define a "Hit" sample. In other words, a "Hit" based on abnormality alone was any sample with significantly greater abnormality ($p<0.05$) than an acceptable reference sample. Second, PSDDA agencies estimated the statistical power of each comparison involving high test sample variability. A standard deviation among test sample replicates exceeding 5% triggered that analysis. The 5% value was based on the 80th percentile of standard deviations among 303 sediment larval test samples (see Figure A-7). The second exception represented a departure from methods used in 1988.

Sediment Larval Effective Mortality

Adverse effects, as indicated by effective mortality in the larval bioassay were determined as illustrated in Figure A-8 (Appendix A). Three alternative threshold levels were used to define a "Hit" and subsequently calculate AETs:

- test sample effective mortality 30% greater than and significantly different from reference sample effective mortality ($p<0.05$)
- test sample effective mortality 15% greater than and significantly different from reference sample effective mortality ($p<0.05$)
- test sample effective mortality significantly greater than reference sample effective mortality ($p<0.05$)

The first of these thresholds reflected the PSDDA program's "single hit" threshold and the SMS biological standards for CSL, MCUL and SIZ_{max}. The second reflected the PSDDA program's "two hit" threshold and the SQS. The last was analogous to the abnormality determination, which lacked an absolute threshold.

For determining significant effective mortality in the sediment larval bioassay, power analysis was triggered by a standard deviation among test sample replicates of 22% (see Appendix A, Figure A-9).

Chemically Anomalous Samples

PSDDA agencies identified chemically anomalous amphipod and sediment larval samples according to the "fixed factor difference" method - the same method selected in 1988 from among several options *(4, page C-14)*. A sample was anomalous if it met the following conditions:

- it was determined to be a "No Hit" sample
- it had the greatest concentration of a given COC among all "No Hit" samples
- its concentration exceeded by a factor of three or more the concentration of the next highest ranked "No Hit" sample

Samples could be anomalous either for dry weight- or TOC-normalized chemical concentrations, or both. All samples identified as chemically anomalous were excluded from the corresponding AET calculations.

Calculating AETs and Predictive Reliability

1994 AET values were calculated using the same general methods as were used in 1988 *(4, Appendix A)*. All synoptic sediment samples which did not an exhibit significant adverse effect, "No Hit" samples, were ranked from highest to lowest concentration for a single chemical of concern. The "No Hit" sample with the greatest concentration generally established the AET. If that sample was found to be chemically anomalous, then the sample with the next highest concentration set the AET. That AET value was confirmed by one or more "Hit" samples with a higher concentration. However, if no "Hit" sample had a greater concentration, then the AET was qualified as a minimum value with a "G" or by a ">" symbol. The determination of AETs was made for all chemicals of concern. The result was a group of AETs for a single biological indicator, interpretive endpoint, unit of measure and measurement basis.

In 1994, this AET calculation process was repeated to generate several AET groups. The four principal AET groups were for dry weight- and TOC-normalized amphipod mortality and echinoderm larval abnormality, reported either in units of ppm or ppb. AET groups were also combined and evaluated as a "suite." The 1988 suite of Puget Sound AETs was extended to include 1994 echinoderm larval values.

As was done in 1988 (4), a limited list of chemicals was removed from each group of AETs so that they could not contribute in any way to the predictive reliability of the 1994 AETs. The chemicals excluded from AET groups are listed in Appendix B (Table B-10). They primarily include compounds and elements which should not be implicated in biological effects (e.g., calcium or sodium) or conventional parameters which, though they may contribute to toxicity, might not represent broadly toxic anthropogenic pollutants (e.g., ammonia).

The reliability of 1994 AET groups was assessed using the same three measures as were used in 1988: sensitivity, efficiency and overall reliability (Figure 3). Sensitivity was defined as the percentage of correctly predicted "Hit" samples -- those samples which exceed at least one AET value and exhibit

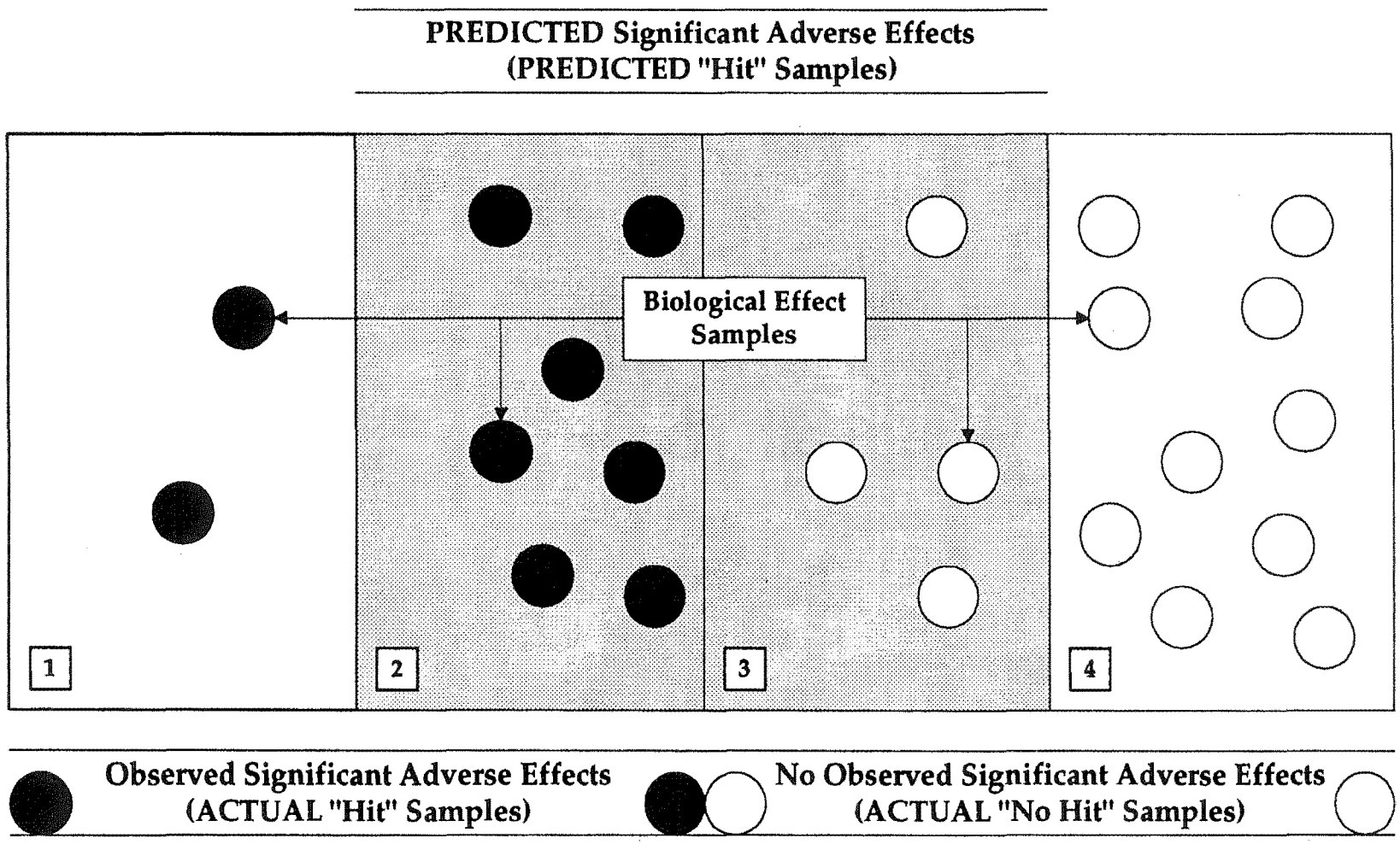
significant adverse effects. Efficiency was calculated as the percentage of all predicted "Hit" samples which actually exhibited significant adverse effects. In other words, efficiency was a measure of predictive accuracy. A highly efficient group of AETs predicted few "Hit" samples incorrectly. The last measure of reliability for a group of AETs, overall reliability, was computed as the percentage of all "Hit" and "No Hit" samples which were correctly predicted.

When the same data set used to calculate a group of AETs was compared to those AET values, efficiency was computed to be 100% by virtue of the AET definition. Any sample which exceeded the "No Hit" sample having the highest concentration for a given COC, thus setting that AET, was correctly predicted to be a "Hit" sample. Because efficiency calculated in that manner was not particularly useful, an "Independent" reliability analysis was sometimes conducted. That independent reliability analysis included a number of steps:

- temporarily withdrawing a single sample from a data set,
- computing AET values from the samples remaining,
- comparing the single sample to those AETs,
- recording whether the comparison predicted the sample which was temporarily withdrawn to be a "Hit" or a "No Hit" sample
- recording whether that prediction was correct
- repeating the process for all remaining samples (withdrawing a single sample, calculating AETs, comparing the single sample to those AETs, and so on)

Independent reliability calculations were conducted to allow meaningful comparisons of efficiency values for 1994 AET values to ones reported for corresponding 1988 AETs. Independent reliability analysis also enabled the predictive efficiency of various 1994 AET groups to be compared.

Figure 3. Predictive reliability of Apparent Effects Threshold values. Sensitivity is a measure of correctly predicted "Hit" samples. In this example, sensitivity = [(samples in box 2)/(samples in boxes 1+2)] X100 = 77%. Efficiency is a measure of accuracy of the "Hit" sample predictions [(box 2)/(boxes 2+3)] X100 = 63%. The overall reliability is the percentage of all predictions which is correct [(samples in boxes 2+4)/(all samples)] X100 = 74%.



The PSDDA agencies also calculated reliability after pooling all determinations of significant adverse effects. Any sample exhibiting at least one type of significant adverse effect was considered a "Hit" sample, even if no other adverse effects were found. The pooled reliability results showed how well a given group of AETs predicted samples exhibiting any type of significant adverse effect.

For this project, these three measures of reliability were also calculated across AET groups. For example, the sensitivity of the "LAET group" was calculated using only the lowest of the five AET values for each COC. The efficiency of the "HAET group" was similarly calculated using only the highest of five AET values.

RESULTS

This section compares the final 1988 and 1994 Puget Sound AET databases, the former being a subset of the latter. It also describes why certain synoptic survey and sample data were excluded from AET calculations. It then focuses on the results of amphipod mortality and echinoderm larval abnormality AET calculations, as well as the predictive reliability of new AET values.

Inventory of Synoptic Data for Puget Sound

The PSDDA agencies reviewed data from many dredging and non-dredging sediment surveys obtained since the "1988 Update and Evaluation of Puget Sound AET" *(4)*. Most of the data met QA requirements and were entered into SEDQUAL. At the time this report was first drafted, there were 184 surveys in SEDQUAL which contained sediment chemistry data. Some 2970 stations and 3644 samples were geographically or temporally unique. From those, PSDDA agencies compiled an inventory of synoptic surveys and samples distinct from those used to calculate 1988 AETs. That inventory is presented in Table B-1 of Appendix B.

A total of 76 surveys contained 835 samples on which amphipod bioassays were conducted. Nine surveys and 168 samples involved sediment testing using bivalve larval species. There were 35 additional surveys with 316 samples which used echinoderm larvae as bioassay test organisms[13].

Data Excluded from AET Calculations

PSDDA agencies obtained and carefully reviewed most of those surveys. A few were excluded because they were not truly synoptic or failed to meet QA1 requirements. Numerous surveys, batches and samples were also excluded from 1994 AET calculations for other reasons. They are listed in Tables B-2 through B-5 and summarized below.

Of the 824 samples in the inventory, a total of 452 amphipod bioassay samples were not used in the 1994 AET calculations. The lack of at least one matching reference sample per batch was the most common reason for excluding amphipod test samples. That was true for 31 surveys and 162 samples. Seventy-six samples from three surveys were excluded because they failed to meet QA1 requirements. PSDDA agencies also excluded bioassay batches having poor reference or negative control sample performance (81 and 65 samples, respectively).

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[13] The count of synoptic samples may include retests of some batches or samples.

The next step was to determine which among the 393 remaining samples did and which did not exhibit significant adverse effects. Three of the "No Hit" samples were found statistically inconclusive (Table B-6). The remaining 390 samples were combined with the 295 used in 1988. From that subtotal, five samples were excluded from the 1994 inventory of synoptic surveys and samples, as well as six samples from the 1988 sediment quality values database. Those 11 samples were found to be chemically anomalous (Tables B-2 and B-7). That left 674 samples on which the dry weight-normalized 1994 amphipod mortality calculations were based. Ten samples were found to be anomalous when chemical concentrations were TOC-normalized.

The PSDDA agencies inventory identified nine surveys and 168 samples on which bivalve larval bioassays were conducted. Seventy-four samples from two surveys failed to meet QA1 requirements. Four surveys contained 63 samples for which there was no negative control meeting performance standards. Only three surveys met QA guidelines and negative control performance standards: DUWO&M90, PIER53BL, and PSREF90. Those surveys contained only 31 samples, an inadequate number upon which to base AET values *(4, page 50)*.

Echinoderm larval bioassays were conducted on 35 surveys and 316 samples. Negative control sample results from four surveys and 45 samples did not meet performance standards. Matching reference samples were missing for five surveys and 30 samples. Two surveys containing 15 samples failed to meet other QA requirements. Reference samples for an additional two surveys (two samples) did not meet performance standards.

Eleven of the remaining 224 samples were found to be statistically inconclusive (Table B-4 and B6). Eight samples were excluded for being chemically anomalous (Table B-7). That left 205 samples on which 1994 dry weight-normalized echinoderm larval AETs were determined. One sample was found to be anomalous when chemical concentrations were TOC-normalized.

Neither the 1994 sediment bivalve larval nor the echinoderm larval effects data could be combined with the 1986 oyster larval effects data. The latter reflected substantially different protocols, used only two lab replicates, and determined the significance of adverse effects using a experiment-wise statistical test. In addition, the 1986 oyster AETs were based on geographically-limited data: two Commencement Bay surveys (CBMSQS, CBBLAIR).

The Puget Sound AET Database: 1988 and 1994

Sixty-two surveys, 505 stations and 590 synoptic samples were added to the AET database. Synoptic data from those samples were combined with data used in 1988 to form the basis for the analyses and results presented in this report.

Table 2 compares the final number of synoptic surveys and samples used to calculate AETs in 1994 to the number used in 1988. Amphipod mortality samples now total 674, while results from 29 surveys and 205 samples comprise the basis for echinoderm larval AETs.

Amphipod Mortality AETs

Dry Weight-Normalized Values and Samples Setting Them

Amphipod AETs for 28 PSDDA and SMS chemicals of concern remained the same as 1988 values, despite inclusion of additional synoptic data in 1994 calculations. Many AET values, however, did change. Those changes are apparent in the comparison of 1994 and 1988 amphipod AETs (Table 3) or can be discerned from the complete results presented in Appendix C (Tables C-1 through C-6).

Of the amphipod AET values which changed, all increased in magnitude except for the AET for benzyl alcohol. Those for trace metals and individual HPAHs were most affected. AETs for arsenic, cadmium, lead, mercury and zinc increased by factors ranging from 1.1 to 4.8. Values for chromium and nickel also increased, but were only estimated values, not exceeded by a "Hit" sample having a higher concentration. Six individual HPAH AETs increased by an average factor of 2.2. Values for LPAH, butyl benzyl phthalate, three substituted phenols (2-methyl phenol, 2,4-dimethyl phenol and pentachlorophenol) and p,p'-DDD increased by more modest factors of 1.1 to 1.5. In contrast, the 1994 amphipod AET for phenanthrene (21,000 ppm) was nearly triple the 1988 value, and appeared to drive the new AET for LPAH (29,000 ppm).

AET values for ethylbenzene, total xylenes and p,p'-DDT, only estimated in 1988, were confirmed by at least one 1994 "Hit" sample. Nickel and bis[2-ethylhexyl]phthalate AETs increased, but remained estimated values. Although based on limited data, amphipod AETs for hexachloroethane, trichloroethene, five pesticides, tributyl tin and other possible COCs could be calculated in 1994. Values were not reported for those chemicals in 1988.

The amphipod AET for benzyl alcohol was the only one found to decrease. It fell from 870 ppb in 1988 to 73 ppb in 1994.

Stations and samples used to calculate 1988 amphipod AETs dominated the list of those which established 1994 values (Table 3). That was because many of the 1988 AETs did not change and some of the new values were set by 1988 AET samples. The latter occurred because additional synoptic data caused a few of the 1988 AET samples to no longer be chemically anomalous. The re-introduction of those samples into 1994 calculations made it possible for them to establish new

Table 2. The Puget Sound sediment quality values (SEDQUAL) database: a comparison of synoptic surveys, stations and samples used to calculate AET values in 1988 and 1994. The number of samples with significant adverse effects ("Hit" samples) is given in parentheses. Note: SEDQUAL also contains extensive nonsynoptic data.

Biological Effect(s)	1988 Surveys/ Stations/Samples ("Hit" Samples)[a]	Added Surveys/ Stations/Samples ("Hit" Samples)[b]	1994 Surveys/ Stations/Samples ("Hit" Samples)
Amphipod mortality	9/284/287 (106)	38/329/385 (75)	47/613/674 [c] (181)
Benthic abundance	6/190/201 (108)		6/190/201 (108)
Echinoderm larval abnormality		24/176/205 (79)	24/176/205 (79)
Microtox luminosity	1/50/50 (29)		1/50/50 (29)
Oyster larval abnormality	2/56/56 (17)		2/56/56 (17)

a. Taken from "Update and Evaluation of Puget Sound AET" *(4)*
b. Synoptic surveys, stations and samples added to SEDQUAL for the 1994 re-evaluation of AETs (this report).
c. The total number of amphipod mortality samples listed here exceeds the sum because two of the 1988 samples could no longer be excluded as chemically anomalous.

AETs. For example, the EBCHEM sample SS-10 was no longer anomalous in 1994 and set four amphipod AETs.

Samples from seven newly-obtained synoptic surveys established 17 of the new 1994 amphipod AETs. Samples from the SITCUMRI survey accounted for nine of those. One sample (SITCUMRI, 300112NM5) set new AETs for three metals and three PAHs. No other newly-obtained sample set more than two.

TOC-Normalized Values and Samples Setting Them

Complete results of 1994 TOC-normalized amphipod AET calculations are presented in Appendix C (Tables 7-12). TOC-normalized values were less affected by additional synoptic data than the 1994 dry weight-normalized amphipod AETs (Table 4). Thirty-five remained the same in 1994. AETs for individual LPAHs, chlorinated organics, phthalates, phenols, miscellaneous extractables and volatile organics were particularly unaffected.

Eighteen of the recalculated TOC-normalized amphipod AETs differed from 1988 values. Trace metal and HPAH AETs accounted for more than half of those new values. The metals tended to decrease, with one remaining an estimate (chromium). TOC-normalized AETs for mercury and silver increased by 40% and 60%, respectively.

AETs for the HPAHs benzo[g,h,i]perylene, chrysene, dibenzo(a,h)anthracene and indeno(1,2,3-c,d)pyrene increased by an average of 40%. The amphipod TOC-normalized AET for phenanthrene increased, however, the increase was not extreme (690 ppm to 840 ppm) and did not affect the AET for LPAH. AETs for butyl benzyl phthalate and p,p'-DDD rose slightly.

TOC-normalized amphipod AETs for bis[2-ethylhexyl] phthalate and phenol increased from 1988 to 1994, but the new values were only estimates. In contrast, new data confirmed the AET for p,p'-DDT (16 ppm TOC) which could only be estimated in 1988. The TOC-normalized amphipod AET for benzyl alcohol decreased, as did the dry weight-normalized value. In 1994, TOC-normalized amphipod AETs could also be calculated for COCs not currently part of the PSDDA and SMS programs.

There was no lower or upper limit placed on TOC content of the samples setting the new AETs. However, anomalous "No Hit" samples were excluded based on their TOC-normalized chemistry (see Methods).

New TOC-normalized amphipod AET values were most often set by samples collected since the 1988 AETs were calculated (Table 4). Twelve samples from nine surveys caused a dozen AETs to change. Two of those samples

(SITCUMRI samples 300112NM5 and 300097NSE1A) set six new AETs. That was in contrast to seven new AETs set by eight samples from four older surveys.

Overview of Sediment Larval AETs

The PSDDA agencies calculated sediment larval abnormality AETs using both sediment bivalve and echinoderm larval bioassay data obtained since 1988. Preliminary results of those calculations were presented at the 1993 PSDDA ARM and elsewhere *(11, 18)*.

In response to those presentations, technical experts recommended calculating separate bivalve and echinoderm larval abnormality AETs[14]. However, in doing so, the PSDDA agencies found that the 1994 bivalve larval abnormality AETs would be based on synoptic data which a) was inadequate to calculate stable AETs[15], b) lacked reference samples, and c) were not geographically representative of Puget Sound. Thus, bivalve larval AET calculations were not pursued further and are not presented in this report.

In order to be consistent with the 1986 oyster AETs, echinoderm larval AETs were calculated based on the abnormality endpoint alone. The PSDDA agencies also calculated echinoderm larval AETs based on effective mortality endpoints more consistent with current regulatory definitions of significant adverse effects. Those endpoints are described in the Methods section of this report.

Early results showed echinoderm larval AETs based on the effective mortality endpoint were much less sensitive than ones based on abnormality alone (Figure C-1). In other words, AETs based on "Hit" samples having any level of significantly greater effective mortality than a reference sample correctly predicted fewer observed adverse effects than echinoderm larval abnormality AETs.

Therefore, the remainder of this section focuses exclusively on the calculation of 1994 echinoderm larval abnormality AETs. Those values are the most sensitive echinoderm larval AETs and are most consistent with the 1986 oyster abnormality AETs. Nevertheless, echinoderm larval effective mortality AET and reliability results are presented in Appendix C (Tables C-25 through C-43).

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[14] Bivalve and echinoderm larvae were expected to differ in sensitivities to chemical pollutants.

[15] More than 50 stations or samples were required for calculated AET values to be less variable and more efficient *(4, page 50)*.

Echinoderm Larval Abnormality AETs

Dry Weight-Normalized Values and Samples Setting Them

The 1994 echinoderm larval abnormality AETs were generally lower than the corresponding 1986 oyster AETs, whether dry weight-normalized (Table 5) or TOC-normalized (Table 6).

Dry weight-normalized echinoderm AETs for four trace metals decreased to levels averaging 35% of 1986 oyster AET values. Oyster abnormality AETs for antimony and silver were not reported in 1986, but were calculated using 1994 data. The chromium AET chromium was estimated to be at least 96 ppm. Only the AET for copper remained equal to that of the existing oyster AET.

Echinoderm abnormality AETs for twelve individual PAHs decreased to concentrations averaging 40% of their 1986 oyster counterparts. The summed parameters LPAH and HPAH were 23% and 46% of the original oyster AETs. Echinoderm AETs for phthalates were all lower than the corresponding oyster values. Three of those were confirmed values, while three were only estimated. Most of the phenol, miscellaneous extractable and volatile compounds were also lower.

AETs for nine chemicals of concern (mercury, nickel, silver, benzo[g,h,i] perylene, dibenzo(a,h)anthracene, indeno(1,2,3-c,d)pyrene, 2,4-dimethylphenol, pentachlorophenol, p,p'-DDT) increased, averaging 2.2 times the 1986 oyster values. The pentachlorophenol AET could only be estimated.

Confirmed echinoderm AET values for just six of the PSDDA or SMS chemicals of concern were greater than 1986 oyster AETs: mercury, silver, benzo[g,h,i]perylene, dibenzo(a,h)anthracene, indeno(1,2,3-c,d)pyrene, and 2,4-dimethylphenol.

Ten of the surveys obtained since 1988 contained 26 samples which set the echinoderm AETs (Table 5). Seven samples from the SITCUMRI survey set 25 of those values. One sample from the SITCUMRI survey (300018NSB1) set 12 AETs: five for trace metals, three for LPAHs, and four for HPAHs. Sample MET570XXS004 from the METROEBP survey set 5 AETs, including values for three HPAHs. A single PIERD_91 sample (S8) also established five AETs, mostly from the pesticide and PCBs analytical group.

TOC-Normalized Values and Samples Setting Them

TOC-normalized echinoderm larval abnormality AETs are listed in Table 6. Values for all trace metals except silver were less than the corresponding 1986

oyster AETs. 1994 echinoderm values for both LPAHs and HPAHs, however, were usually greater than the oyster analogues. Eleven of the remaining AETs could not be confirmed by a "Hit" sample with a higher concentration.

Twenty-five samples from 14 surveys established the TOC-normalized echinoderm abnormality AETs (Table 6). Twenty-six of those values were set by samples from just three surveys: METROEBP, NAVYMANC and TERMNL91. One METROEBP sample (MET570XXS004) was responsible for nine new AETs, including values for six individual HPAHs. The sample MANCHECCC002 (NAVYMANC survey) set five AETs for trace metals. A single TERMNL91 sample (TERMNL91S003) set seven TOC-normalized echinoderm AETs, including six for LPAHs.

Predictive Reliability of 1994 AETs

The three measures of reliability used to evaluate AETs - "sensitivity", "efficiency", and "overall reliability" - were described in Methods and conceptually summarized in Figure 4. Sensitivity measures the ability of chemical AETs to correctly predict samples exhibiting significant adverse biological effects ("Hit" samples). Efficiency is the fraction of all "Hit" sample predictions which are correct. AETs which are highly efficient accurately predict "Hit" samples while yielding few false positive predictions. Overall reliability is the fraction of all predictions, both "Hit" and "No Hit" samples, which are correct.

Some reliability results for the 1994 amphipod mortality and echinoderm larval abnormality AETs are summarized in Table 7. The reliability of 1988 amphipod and 1986 oyster AETs are included for comparison purposes. Those results are organized by the AET type and means of chemical normalization: dry weight or TOC. Table 7 also lists some reliability analyses which are suggested in Recommendations for Future Work or which may be recommended by technical experts.

Amphipod Mortality AETs

The sensitivity of dry weight-normalized amphipod AETs declined from 58% in 1988 to 43% in 1994. Efficiency, as indicated by independent reliability calculations, declined from 67% in 1988 to 52% in 1994. However, the overall reliability of 1994 values was similar to that found in 1988, 84% and 85%, respectively. The independent reliability calculation confirmed the fact that overall reliability was unchanged. The 1994 TOC-normalized amphipod AETs were also less sensitive than their 1988 counterparts: 36% in 1994 compared to 45% in 1988. Overall predictive reliability fell slightly, from 80% in 1988 to 78% in 1994.

Table 3. Comparison of 1994 to 1988 dry weight-normalized amphipod mortality AET values. New values are printed in bold italics. A ">" symbol indicates minimum values, not be confirmed by a "Hit" sample with a greater concentration. The ratio 1994 AET:1988 AET is given for confirmed values which changed. Stations and samples setting new 1994 AETs are also listed.

Chemical Group/ Chemical of Concern	Amphipod Mortality 1994 AET	1988 AET	1994: 1988	Survey Code and Station_ID	Sample_ID
Metals (mg/kg or ppm)					
Antimony	200	200			
Arsenic	***450***	93	4.8	EBCHEM, DR-12	DR-12
Cadmium	***14***	6.7	2.1	SITCUMRI, NM-5	300112NM5
Chromium	***>1100***	270		EBCHEM, SS-10	SS-10
Copper	1,300	1,300			
Lead	***1,200***	660	1.8	SITCUMRI, NM-5	300112NM5
Mercury	***2.3***	2.1	1.1	PSDDA1, EBZ01	EBZ01C
Nickel	***>370***	>140		EBCHEM, SS-10	SS-10
Silver	6.1	6.1			
Zinc	***3,800***	960	4.0	SITCUMRI, NM-5	300112NM5
Organic Compounds (ug/kg or ppb)					
Low molecular weight PAH					
LPAH	***29,000***	24,000	1.2	SITCUMRI, NM-5	300112NM5
2-Methylnaphthalene	1,900	1,900			
Acenaphthene	2,000	2,000			
Acenaphthylene	1,300	1,300			
Anthracene	13,000	13,000			
Fluorene	3,600	3,600			
Naphthalene	2,400	2,400			
Phenanthrene	***21,000***	6,900	3.0	SITCUMRI, NM-5	300112NM5

Table 3. Comparison of 1994 to 1988 dry weight-normalized amphipod mortality AET values. New values are printed in bold italics. A ">" symbol indicates minimum values, not be confirmed by a "Hit" sample with a greater concentration. The ratio 1994 AET:1988 AET is given for confirmed values which changed. Stations and samples setting new 1994 AETs are also listed.

Chemical Group/ Chemical of Concern	Amphipod Mortality 1994 AET	1988 AET	1994: 1988	Survey Code and Station_ID	Sample_ID
High molecular weight PAH					
HPAH	69,000	69,000			
Benz[a]anthracene	5,100	5,100			
Benzo[a]pyrene	***3,500***	3,000	1.2	EBCHEM, SS-10	SS-10
Benzo[ghi]perylene	***3,200***	1,400	2.3	EBCHEM, SS-04	SS-04
Benzofluoranthenes	***9,100***	7,800	1.2	EBCHEM, SS-10	SS-10
Chrysene	***21,000***	9,200	2.3	SITCUMRI, NM-5	300112NM5
Dibenzo(a,h)anthracene	***1,900***	540	3.5	EBCHEM, SS-04	SS-04
Fluoranthene	30,000	30,000			
Indeno(1,2,3-c,d)pyrene	***4,400***	1,800	2.4	EBCHEM, SS-04	SS-04
Pyrene	16,000	16,000			
Chlorinated organic compounds					
1,2,4-trichlorobenzene	51	51			
1,2-dichlorobenzene	>110	>110			
1,3-dichlorobenzene	>170	>170			
1,4-dichlorobenzene	120	120			
Hexachlorobenzene	130	130			
Phthalates					
Bis[2-ethylhexyl] phthalate	***>8300***	>3100		SED18903, 12	1
Butyl benzyl phthalate	***970***	900	1.1	EBCHEM, SS-04	SS-04
Di-n-butyl phthalate	1,400	1,400			
Di-n-octyl phthalate	>2100	>2100			

Table 3. Comparison of 1994 to 1988 dry weight-normalized amphipod mortality AET values. New values are printed in bold italics. A ">" symbol indicates minimum values, not be confirmed by a "Hit" sample with a greater concentration. The ratio 1994 AET:1988 AET is given for confirmed values which changed. Stations and samples setting new 1994 AETs are also listed.

Chemical Group/ Chemical of Concern	Amphipod Mortality 1994 AET	1988 AET	1994: 1988	Survey Code and Station_ID	Sample_ID
Phthalates, cont'd					
Diethylphthalate	>1200	>1200			
Dimethylphthalate	>1400	>1400			
Phenols					
2-methyl phenol	***77***	63	1.2	SITCUMRI, NC-S4	300048NCS4
2,4-dimethyl phenol	***77***	72	1.1	SITCUMRI, NC-S4	300048NCS4
4-methylphenol	3,600	3,600			
Pentachlorophenol	***400***	360	1.1	DUWO&M90, DU9003XX	DUWO&M90S003
Phenol	1,200	1,200			
Miscellaneous Extractables					
Benzyl alcohol	***73***	870	0.1	CBMSQS, HY-50	HY-50
Benzoic acid	760	760			
Dibenzofuran	1,700	1,700			
Hexachlorobutadiene	180	180			
Hexachloroethane	***140***	NA		CBMSQS, HY-24	HY-24
N-nitrosodiphenylamine	48	48			
Volatile organics					
Ethylbenzene	***50***	>50		CBMSQS, HY-17	H-17
Tetrachloroethene	>210	>210			
Xylene, Total	***160***	>160		CBMSQS, HY-17	H-17

Table 3. Comparison of 1994 to 1988 dry weight-normalized amphipod mortality AET values. New values are printed in bold italics. A ">" symbol indicates minimum values, not be confirmed by a "Hit" sample with a greater concentration. The ratio 1994 AET:1988 AET is given for confirmed values which changed. Stations and samples setting new 1994 AETs are also listed.

Chemical Group/ Chemical of Concern	Amphipod Mortality 1994 AET	1988 AET	1994: 1988	Survey Code and Station_ID	Sample_ID
Pesticides and PCBs					
Aldrin	***9.5***	NA		TERM5_91, TER50101	TER592C001
Chlordane	***2.8***	NA		TERM5_91, TER50101	TER592C001
Dieldrin	***3.5***	NA		BLGM_91A, BLGM09MC	BLGMBYXXC004
Heptachlor	***1.5***	NA		CBBLAIR, B10	B10
p,p'-DDD	***63***	43	1.5	EBCHEM, SS-04	SS-04
p,p'-DDE	***62***	15	4.1	EBCHEM, DR-10	DR-10
p,p'-DDT	***270***	>270		EBCHEM, KG-06	EBCHEM KG-06
Total DDT	***24***	NA		PIERD_91, 11	C5
Total PCBS	3,100	3,100			
Other					
TBT	***>180***	NA		PSDDA1, EBB01	EBB01C
Trichloroethene	***0.8***	NA		EBCHEM, WW-18	WW-18

Table 4. Comparison of 1994 to 1988 TOC-normalized amphipod mortality AET values for Puget Sound. New values are printed in bold italics. A ">" symbol indicates a miniumu value, not confirmed by a "Hit" samp with a greater concentration. The ratio 1994:1988 AETs is given for confirmed values which changed. Stations and samples setting new 1994 AETs are also listed.

Chemical Group/ Chemical of Concern	Amphipod Mortality AET 1994	1988	1994: 1988	Survey Code Station_ID	Sample_ID
Metals (mg/kg organic carbon; ppm)					
Antimony	***15,000***	>55,000	0.27	EBCHEM, WW-18	WW-18
				EBCHEM, WW-19	WW-19
				EBCHEM, WW-20	WW-20
Arsenic	32,000	32,000			
Cadmium	1,100	1,100			
Chromium	***>130,000***	>150,000	0.87	EBCHEM, MG-01	MG-01
Copper	100,000	100,000			
Lead	***48,000***	110,000	0.44	SITCUMRI, NM-5	300112NM5
Mercury	***300***	210	1.43	PIERD_91, 1	S1
Nickel	***20,000***	>41,000	0.49	NAVYMANC, MANC2006	MANCHEXXS007
Silver	***270***	170	1.59	SOPARK91, SPRK0302	SOPARK91C002
Zinc	***150,000***	210,000	0.71	SITCUMRI, NM-5	300112NM5
Organic Compounds (mg/kg organic carbon; ppm)					
Low molecular weight PAH					
LPAH	2,200	2,200			
2-Methylnaphthalene	>120	>120			
Acenaphthene	200	200			
Acenaphthylene	66	66			
Anthracene	1,200	1,200			
Fluorene	360	360			
Naphthalene	220	220			

Table 4. Comparison of 1994 to 1988 TOC-normalized amphipod mortality AET values for Puget Sound. New values are printed in bold italics. A ">" symbol indicates a miniumu value, not confirmed by a "Hit" samp with a greater concentration. The ratio 1994:1988 AETs is given for confirmed values which changed. Stations and samples setting new 1994 AETs are also listed.

Chemical Group/ Chemical of Concern	Amphipod Mortality AET 1994	1988	1994: 1988	Survey Code Station_ID	Sample_ID
Low molecular weight PAH, cont'd					
Phenanthrene	***840***	690	1.22	SITCUMRI, NM-5	300112NM5
High molecular weight PAH					
HPAH	5,300	5,300			
Benz[a]anthracene	270	270			
Benzo[a]pyrene	210	210			
Benzo[ghi]perylene	***100***	78	1.28	SITCUMRI, NS-E1A	300097NSE1A
Benzofluoranthenes	450	450			
Chrysene	***840***	460	1.83	SITCUMRI, NM-5	300112NM5
Dibenzo(a,h)anthracene	***50***	47	1.06	SITCUMRI, NS-E1A	300097NSE1A
Fluoranthene	3,000	3,000			
Indeno(1,2,3-c,d)pyrene	***120***	88	1.36	SED18903, 40	1
Pyrene	1,000	1,000			
Chlorinated organic compounds					
1,2,4-trichlorobenzene	1.8	1.8			
1,2-dichlorobenzene	>5.8	>5.8			
1,3-dichlorobenzene	>15	>15			
1,4-dichlorobenzene	9	9			
Hexachlorobenzene	4.5	4.5			
Phthalates					
Bis[2-ethylhexyl] phthalate	***>550***	78	7.05	SED18903, 12	1
Butyl benzyl phthalate	***49***	42	1.17	CGPIER35, PIER01MC	PIER35XXC001

Table 4. Comparison of 1994 to 1988 TOC-normalized amphipod mortality AET values for Puget Sound. New values are printed in bold italics. A ">" symbol indicates a miniumu value, not confirmed by a "Hit" samp with a greater concentration. The ratio 1994:1988 AETs is given for confirmed values which changed. Stations and samples setting new 1994 AETs are also listed.

Chemical Group/ Chemical of Concern	Amphipod Mortality AET 1994	1988	1994: 1988	Survey Code Station_ID	Sample_ID
Phthalates, cont'd					
Di-n-butyl phthalate	260	260			
Di-n-octyl phthalate	58	58			
Diethylphthalate	>110	>110			
Dimethylphthalate	53	53			
Phenols					
2-methyl phenol	3.1	3.1			
2,4-dimethyl phenol	6.5	6.5			
4-methylphenol	780	780			
Pentachlorophenol	24	24			
Phenol	***>440***	440	1.10	EVCHEM, NG-14	NG-14G
Miscellaneous Extractables					
Benzyl alcohol	***5***	73	0.07	CBMSQS, BL-21	BL-21
Benzoic acid	>170	>170			
Dibenzofuran	>170	>170			
Hexachlorobutadiene	6.2	6.2			
Hexachloroethane	***2.7***	NA		CBMSQS, HY-24	HY-24
N-nitrosodiphenylamine	>11	>11			
Volatile organics					
Ethylbenzene	>3.8	>3.8			
Tetrachloroethene	>22	>22			
Xylene, Total	>12	>12			

Table 4. Comparison of 1994 to 1988 TOC-normalized amphipod mortality AET values for Puget Sound. New values are printed in bold italics. A ">" symbol indicates a miniumu value, not confirmed by a "Hit" samp with a greater concentration. The ratio 1994:1988 AETs is given for confirmed values which changed. Stations and samples setting new 1994 AETs are also listed.

Chemical Group/ Chemical of Concern	Amphipod Mortality AET 1994	1988	1994: 1988	Survey Code Station_ID	Sample_ID
Pesticides and PCBs					
Aldrin	***0.56***	NA		TERM5_91, TER50101	TER592C001
Chlordane	***0.16***	NA		TERM5_91, TER50101	TER592C001
Dieldrin	***0.13***	NA		PSDDAM90, EB_Z01XX	EB90_Z01
Heptachlor	***>0.11***	NA		CBBLAIR, B10	B10
p,p'-DDD	***3.1***	2.2	1.41	EBCHEM, DR-10	DR-10
p,p'-DDE	***6***	1	7.04	EBCHEM, DR-10	DR-10
p,p'-DDT	***16***	>16		EBCHEM, KG-06	KG-06
Total DDT	***1.4***	NA		PIERD_91, 11	C5
Total PCBS	190	190			
Other					
TBT	***>18***	NA		PSDDA1, EBB01	EBB01C
Trichloroethene	***0.06***	NA		EBCHEM, WW-18	WW-18

Table 5. Comparison of 1994 dry weight-normalized echinoderm abnormality AETs to corresponding 1986 oyster abnormality AET values. A ">" symbol indicates a minimum value, not confirmed by a "Hit" sample with a greater concentration. The ratio 1994 echinoderm AET:oyster 1986 AET is given for values which differ. Stations and samples setting new 1994 AETs are also listed.

Chemical Group/ Chemical of Concern	Echinoderm Abnormality 1994 AET	Oyster Abnormality 1986 AET	1994: 1986	AET Set By: Survey Code and Station_ID	Sample_ID
Metals (mg/kg or ppm)					
Antimony	9.3	NA		SITCUMRI, NS-B6	300013NSB6
Arsenic	130	700	0.19	SITCUMRI, NS-B1	300018NSB1
Cadmium	2.7	9.6	0.28	SITCUMRI, NS-B1	300018NSB1
Chromium	>96	NA		OLYHARFC, OLYH26XX	OLYHFCXXS023
Copper	390	390			
Lead	430	660	0.65	SITCUMRI, NS-B1	300018NSB1
Mercury	1.4	0.59	2.37	PIERD_91, 8	S8
Nickel	110	39	2.82	BLGM_91A, BLGM0606	BLGMBYXXC006
Silver	8.4	>.56		SITCUMRI, NS-B1	300018NSB1
Zinc	460	1,600	0.29	SITCUMRI, NS-B1	300018NSB1
Organic Compounds (ug/kg or ppb)					
Low molecular weight PAH					
LPAH	1,200	5,200	0.23	SITCUMRI, NS-B1	300018NSB1
2-Methylnaphthalene	64	670	0.10	SITCUMRI, NS-B6	300013NSB6
Acenaphthene	130	500	0.26	SITCUMRI, NS-B3	300017NSB3
Acenaphthylene	71	>560		METROEBP, METR0803	MET570XXS004
Anthracene	280	960	0.29	SITCUMRI, NS-B1	300018NSB1
Fluorene	120	540	0.22	SITCUMRI, NS-B3	300017NSB3
Naphthalene	230	2,100	0.11	OLYHARFC, OLYH25MC	OLYHFCXXS012
Phenanthrene	660	1,500	0.44	SITCUMRI, NS-B1	300018NSB1

Table 5. Comparison of 1994 dry weight-normalized echinoderm abnormality AETs to corresponding 1986 oyster abnormality AET values. A ">" symbol indicates a minimum value, not confirmed by a "Hit" sample with a greater concentration. The ratio 1994 echinoderm AET:oyster 1986 AET is given for values which differ. Stations and samples setting new 1994 AETs are also listed.

Chemical Group/ Chemical of Concern	Echinoderm Abnormality 1994 AET	Oyster Abnormality 1986 AET	1994: 1986	AET Set By: Survey Code and Station_ID	Sample_ID
High molecular weight PAH					
HPAH	7,900	17,000	0.46	SITCUMRI, NS-B1	300018NSB1
Benz[a]anthracene	960	1,600	0.60	SITCUMRI, NS-B1	300018NSB1
Benzo[a]pyrene	1,100	1,600	0.69	SITCUMRI, NS-B1	300018NSB1
Benzo[ghi]perylene	920	720	1.28	METROEBP, METR0803	MET570XXS004
Benzofluoranthenes	1,800	3,600	0.50	SITCUMRI, NS-B1	300018NSB1
Chrysene	950	2,800	0.34	NAVYMANC, MANC1605	MANCHEXXS003
Dibenzo(a,h)anthracene	240	230	1.04	NAVYMANC, MANC1605	MANCHEXXS003
Fluoranthene	1,300	2,500	0.52	SITCUMRI, NS-A3	300031NSA3
				METROEBP, METR0803	MET570XXS004
Indeno(1,2,3-c,d)pyrene	760	690	1.10	METROEBP, METR0803	MET570XXS004
Pyrene	2,400	3,300	0.73	TERM5_91, TER50302	TER592C002
Chlorinated organic compounds					
1,2,4-trichlorobenzene	>4.8	64		BLAIR_91, BL915921	BLAIR91XC021
1,2-dichlorobenzene	NA	50			
1,3-dichlorobenzene	>4.4	>170		TERM5_91, TER50101	TER592C001
1,4-dichlorobenzene	NA	120			
Hexachlorobenzene	NA	230			
Phthalates					
Bis[2-ethylhexyl] phthalate	1,700	1,900	0.89	PIERD_91, 14	S14
Butyl benzyl phthalate	200	>470		OLYHARFC, OLYH07MC	OLYHFCXXS013

Table 5. Comparison of 1994 dry weight-normalized echinoderm abnormality AETs to corresponding 1986 oyster abnormality AET values. A ">" symbol indicates a minimum value, not confirmed by a "Hit" sample with a greater concentration. The ratio 1994 echinoderm AET:oyster 1986 AET is given for values which differ. Stations and samples setting new 1994 AETs are also listed.

Chemical Group/ Chemical of Concern	Echinoderm Abnormality 1994 AET	Oyster Abnormality 1986 AET	1994: 1986	AET Set By: Survey Code and Station_ID	Sample_ID
Phthalates, cont'd					
Di-n-butyl phthalate	>31	1,400		NAVYHPFC, NAVY20MC	NAVY89XXC007
Di-n-octyl phthalate	>98	>420		OLYHARFC, OLYH07MC	OLYHFCXXS013
				OLYHARFC, OLYH26XX	OLYHFCXXS023
Diethylphthalate	>62	>73		NAVYHPFC, NAVY20MC	NAVY89XXC021
Dimethylphthalate	85	160	0.53	SITCUMRI, NS-A2	300047NSA2
Phenols					
2-methyl phenol	55	63	0.87	SITCUMRI, NC-I12	300078NCI12
2,4-dimethyl phenol	55	29	1.90	SITCUMRI, NC-I12	300078NCI12
4-methylphenol	110	670	0.16	OLYHARFC, OLYH25MC	OLYHFCXXS012
Pentachlorophenol	>150	>140		PIERD_91, 14	S14
Phenol	>220	420		NAVYMANC, MANC04MC	MANCHEXXC003
Miscellaneous Extractables					
Benzyl alcohol	>12	73		PIERD_91, 2	S2
Benzoic acid	>31	650		DAYISL91, DAYI0101	DAYISL91C001
				OLYHARFC, OLHY07MC	OLYHFCXXS013
Dibenzofuran	110	540	0.20	SITCUMRI, NS-B3	300017NSB3
Hexachlorobutadiene	1.3	270	0.005	SITCUMRI, NC-I8	300084NCI8
Hexachloroethane	NA	NA			
N-nitrosodiphenylamine	>25	130		METROEBP, METR0803	MET570XXS004

Table 5. Comparison of 1994 dry weight-normalized echinoderm abnormality AETs to corresponding 1986 oyster abnormality AET values. A ">" symbol indicates a minimum value, not confirmed by a "Hit" sample with a greater concentration. The ratio 1994 echinoderm AET:oyster 1986 AET is given for values which differ. Stations and samples setting new 1994 AETs are also listed.

Chemical Group/ Chemical of Concern	Echinoderm Abnormality 1994 AET	Oyster Abnormality 1986 AET	1994: 1986	AET Set By: Survey Code and Station_ID	Sample_ID
Volatile organics					
Ethylbenzene	4.0	37	0.11	SITCUMRI, NS-B6	300013NSB6
Tetrachloroethene	>1	140		SITCUMRI, NC-I12	300078NCI12
				SITCUMRI, NC-I8	300084NCI8
Xylene, Total	21	120	0.18	SITCUMRI, NS-B6	300013NSB6
Pesticides and PCBs					
Aldrin	10	NA		TERM5_91, TER50101	TER592C001
Chlordane	>4.5	NA		PIERD_91, 2	S2
Dieldrin	1.9	NA		PIERD_91, 6	C3
Heptachlor	2	NA		BLGM_91A, BLGM6518	BLGMBYXXC018
p,p'-DDD	28	NA		PIERD_91, 8	S8
p,p'-DDE	9.3	NA		PIERD_91, 8	S8
p,p'-DDT	12	>6	2.00	PIERD_91, 11	C5
Total DDT	37	NA		PIERD_91, 8	S8
Total PCBS	450	1,100	0.41	PIERD_91, 8	S8

Table 6. Comparison of 1994 TOC-normalized echinoderm abnormality AETs to corresponding 1986 oyster abnormality AET values. A ">" symbol indicates a minimum value, not confirmed by a "Hit" sample with a greater concentration. The ratio 1994 echinoderm AET:oyster 1986 AET is given for values which differ. Stations and samples setting new 1994 AETs are also listed.

Chemical Group/ Chemical of Concern	Echinoderm Abnormality 1994 AET	Oyster Abnormality 1986 AET	1994: 1986	AET Set By: Survey Code and Station_ID	Sample_ID
Metals (mg/kg organic carbon; ppm)					
Antimony	>2,100	3,300		NAVYMANC, MANC0702	MANCHEXXC002
Arsenic	*5,800*	88,000	0.07	NAVYMANC, MANC0702	MANCHEXXC002
Cadmium	>430	1,200		NAVYMANC, MANC2207	MANCHEXXC007
Chromium	>5,400	NA		OLYHARFC, OLYH02MC	OLYHFCXXC006
Copper	30,000	49,000	0.61	NAVYMANC, MANC0702	MANCHEXXC002
Lead	22,000	66,000	0.33	BLAIR_91, BL9140MC	BLAIR91XC026
Mercury	71	210	0.34	PIERD_91, 11	C5
Nickel	>49000	NA		NAVYMANC, MANC0702	MANCHEXXC002
Silver	270	>100		SOPARK91, SPRK0302	SOPARK91C002
Zinc	60,000	>200,000		NAVYMANC, MANC0702	MANCHEXXC002
Organic Compounds (mg/kg organic carbon; ppm)					
Low molecular weight PAH					
LPAH	22	370	0.06	PIERD_91, 11	C5
2-Methylnaphthalene	>53	NA		TERMNL91, T91_03XX	TERMNL91S003
Acenaphthene	>110	16		TERMNL91, T91_03XX	TERMNL91S003
Acenaphthylene	>18	>27			
Anthracene	93	>79		TERMNL91, T91_03XX	TERMNL91S003
Fluorene	73	23	3.17	TERMNL91, T91_03XX	TERMNL91S003
Naphthalene	>190	99		TERMNL91, T91_03XX	TERMNL91S003
Phenanthrene	140	120	1.17	METROEBP, METR0803	MET570XXS004

Table 6. Comparison of 1994 TOC-normalized echinoderm abnormality AETs to corresponding 1986 oyster abnormality AET values. A ">" symbol indicates a minimum value, not confirmed by a "Hit" sample with a greater concentration. The ratio 1994 echinoderm AET:oyster 1986 AET is given for values which differ. Stations and samples setting new 1994 AETs are also listed.

Chemical Group/ Chemical of Concern	Echinoderm Abnormality 1994 AET	Oyster Abnormality 1986 AET	1994: 1986	AET Set By: Survey Code and Station_ID	Sample_ID
High molecular weight PAH					
HPAH	*150*	960	0.16	PIERD_91, 8	S8
Benz[a]anthracene	170	110	1.55	METROEBP, METR0803	MET570XXS004
Benzo[a]pyrene	230	99	2.32	METROEBP, METR0803	MET570XXS004
Benzo[ghi]perylene	>240	31		METROEBP, METR0803	MET570XXS004
Benzofluoranthenes	310	230	1.35	TERMNL91, T91_03XX	TERMNL91S003
Chrysene	220	110	2.00	METROEBP, METR0803	MET570XXS004
Dibenzo(a,h)anthracene	48	120	0.40	NAVYMANC, MANC1705	MANCHEXXS004
				NAVYMANC, MANC18XX	MANCHEXXS005
Fluoranthene	320	160	2.00	METROEBP, METR0803	MET570XXS004
Indeno(1,2,3-c,d)pyrene	>190	33		METROEBP, METR0803	MET570XXS004
Pyrene	520	>210		METROEBP, METR0803	MET570XXS004
Chlorinated organic compounds					
1,2,4-trichlorobenzene	>2.4	2.7		BLAIR_91, BL915921	BLAIR91XC021
1,2-dichlorobenzene	NA	2.3			
1,3-dichlorobenzene	>0.26	>15		TERM5_91, TER50101	TER592C001
1,4-dichlorobenzene	NA	3.1			
Hexachlorobenzene	NA	9.6			
Phthalates					
Bis[2-ethylhexyl] phthalate	130	60	2.17	NAVYMANC, MANC0101	MANCHEXXC001
Butyl benzyl phthalate	5.2	>9.2		OLYHARFC, OLYH07MC	OLYHFCXXS013
Di-n-butyl phthalate	*0.88*	260	0.00	PSDDAM92, PMONS02	PMONS02S002

Table 6. Comparison of 1994 TOC-normalized echinoderm abnormality AETs to corresponding 1986 oyster abnormality AET values. A ">" symbol indicates a minimum value, not confirmed by a "Hit" sample with a greater concentration. The ratio 1994 echinoderm AET:oyster 1986 AET is given for values which differ. Stations and samples setting new 1994 AETs are also listed.

Chemical Group/ Chemical of Concern	Echinoderm Abnormality 1994 AET	Oyster Abnormality 1986 AET	1994: 1986	AET Set By: Survey Code and Station_ID	Sample_ID
Phthalates, cont'd					
Di-n-octyl phthalate	>0.55	>57		AMPRES92, APL_01MC	AMPRES92C002
Diethylphthalate	>0.27	>5.3		NAVYHPFC, NAVY20MC	NAVY89XXC025
Dimethylphthalate	NA	>22			
Phenols					
2-methyl phenol	>2.1	3.1		OLYHARFC, OLYH17MC	OLYHFCXXC001
2,4-dimethyl phenol	NA	>1.3			
4-methylphenol	***4.7***	37	0.13	OLYHARFC, OLYH17MC	OLYHFCXXC001
Pentachlorophenol	>9.3	>11		PIERD_91, 6	C3
Phenol	***3.2***	>39		NAVYMANC, MANC18XX	MANCHEXXS005
Miscellaneous Extractables					
Benzyl alcohol	>0.71	5		PIERD_91, 2	S2
Benzoic acid	>2.0	>170		DAYISL91, DAYI0101	DAYISL91C001
Dibenzofuran	57	15	3.80	TERMNL91, T91_03XX	TERMNL91S003
Hexachlorobutadiene	NA	11			
Hexachloroethane	NA	NA			
N-nitrosodiphenylamine	>6.4	>11		METROEBP, METR0803	MET570XXS004
Volatile organics					
Ethylbenzene	NA	>3.8			
Tetrachloroethene	NA	>22			
Xylene, Total	***0.15***	>12		NAVYHPII, EVNV03MC	NAVYII90C002

Table 6. Comparison of 1994 TOC-normalized echinoderm abnormality AETs to corresponding 1986 oyster abnormality AET values. A ">" symbol indicates a minimum value, not confirmed by a "Hit" sample with a greater concentration. The ratio 1994 echinoderm AET:oyster 1986 AET is given for values which differ. Stations and samples setting new 1994 AETs are also listed.

Chemical Group/ Chemical of Concern	Echinoderm Abnormality 1994 AET	Oyster Abnormality 1986 AET	1994: 1986	AET Set By: Survey Code and Station_ID	Sample_ID
Pesticides and PCBs					
Aldrin	>0.56	NA		TERM5_91, TER50101	TER592C001
Chlordane	>0.26	NA		PIERD_91, 2	S2
Dieldrin	0.28	NA		PIERD_91, 6	C3
Heptachlor	>0.40	NA		BLGM_91A, BLGM3813	BLGMBYXXS002
p,p'-DDD	1.6	NA		PIERD_91, 16	C6
p,p'-DDE	>7.3	NA		PIERD_91, 16	C6
p,p'-DDT	>0.71	NA		PIERD_91, 11	C5
Total DDT	8.8	NA		PIERD_91, 16	C6
Total PCBS	18	>46		PIERD_91, 8	S8

Echinoderm Larval Abnormality AETs

The sensitivity of 1994 dry weight-normalized echinoderm larval abnormality AET values was 48%, much lower than the 88% value recorded for oyster larval abnormality AETs in 1986 (Table 7). Using the independent reliability calculation, the PSDDA agencies found the efficiency (54%) and overall reliability (67%) of the echinoderm AETs to exceed the values for 1986 oyster AETs (37 % and 50%, respectively). Reliability results for TOC-normalized larval AETs were: 46% sensitivity and 79% overall reliability. Corresponding values for 1986 oyster AETs were 71% and 91%.

Some Characteristics of Incorrectly Predicted Stations

The direct cause of low sensitivity was that "Hit" samples were not predicted to exhibit significant adverse effects because their chemistry did not exceed any AET value. One reason that may have occurred was that the effects observed in incorrectly predicted "Hit" samples were in response to sample handling or due to toxicity from conventional parameters, such as sulfides. A brief examination of the latter possibility is presented below.

The PSDDA agencies preliminarily examined concentrations of some sediment conventionals in "Hit" samples which were incorrectly predicted by 1994 AETs. A large fraction of those incorrectly predicted samples had "elevated" levels of bulk ammonia, percent fines, or total bulk sulfides[16] (Table 8). Percent clay and TOC were less frequently elevated. Nearly one-third of all incorrectly predicted dry weight-normalized amphipod "Hit" samples (31/104) showed elevated levels of at least two conventional parameters. One-half (24/47) of the incorrectly predicted echinoderm "Hits" fell into that category.

The PSDDA agencies considered excluding amphipod "Hit" samples having greater than 80% fines from 1994 AET calculations. They also considered another approach to reducing the number of samples in which fines content likely contributed to amphipod mortality. Modelled after DeWitt et al *(26)*, that approach was to use the relationship observed in Puget Sound between sample percent fines and amphipod mortality to correct the mean sample mortality results. That correction, performed prior to determination of significant adverse effects, could potentially have reduced the frequency of incorrectly predicted "Hit" samples.

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[16] For this initial assessment of conventional parameters associated with incorrectly predicted "Hit" samples, "elevated" bulk ammonia concentrations were operationally defined to be > 20 ppm, "elevated" fines were > 80%, and "elevated" bulk sulfides were > 50 ppm. Fine grained sediment is defined by PSEP *(6)* to be the sum of percent silt (0.063 - 0.004 mm particles) and percent clay (less than 0.004 mm).

Table 7. The predictive reliability of amphipod mortality and echinoderm larvae abnormality AETs. 1994 AETs (shaded) are compared to original 1988 amphipod and 1986 oyster AETs. "Sensitivity," "Efficiency," "Overall Reliability," "Independent AETs," and "Pooled" are defined in the Background and Glossary sections of this report. Measures of reliablity are given as percentages and fractions. Historical reliability values are from "1988 Update and Evaluation of Puget Sound AET" *(4)*.

AET Group	Database for Comparison	Total Samples	Sensitivity	Efficiency	Overall Reliability
Dry Weight Normalized					
1986 Amphipod AETs	1986 AMPT	287	56% (59/106)	69% (59/86)	74% (213/287)
1988 Amphipod AETs	1988 AMPT	287	58% (62/106)	100% (62/62)	85% (243/287)
1988 Independent AETs	1988 AMPT	287	57% (60/106)	67% (60/90)	74% (211/287)
1988 Amphipod AETs	1994 AMPT	674	48% (87/181)	38% (87/227)	65% (438/674)
1994 Amphipod AETs	1994 AMPT	674	43% (79/181)	100% (79/79)	84% (569/674)
1994 Independent AETs	1994 AMPT	674	42% (76/181)	52% (76/146)	74% (499/674)
1988 Amphipod AETs	1994 Pooled	674	39% (119/309)	47% (119/251)	52% (352/674)
1994 Amphipod AETs	1994 Pooled	674	31% (95/309)	88% (95/108)	66% (447/674)
1986 Oyster AETs	1986 OYST	56	88% (15/17)	100% (15/15)	96% (54/56)
1986 Independent AETs	1986 OYST	56	88% (15/17)	37% (15/41)	50% (28/56)
1986 OYST AETs	1994 ECHN	205	75% (59/79)	60% (59/98)	71% (146/205)
1994 Echinoderm AETs	1994 ECHN	205	48% (38/79)	100% (38/38)	80% (164/205)
1994 Independent AETs	1994 ECHN	205	46% (36/79)	54% (36/67)	67% (137/205)
1986 OYST AETs	1994 Pooled	205	67% (206/309)	58% (206/358)	NA
1994 Echinoderm AETs	1994 Pooled	205	67% (206/309)	59% (206/348)	NA

Table 7. The predictive reliability of amphipod mortality and echinoderm larvae abnormality AETs. 1994 AETs (shaded) are compared to original 1988 amphipod and 1986 oyster AETs. "Sensitivity," "Efficiency," "Overall Reliability," "Independent AETs," and "Pooled" are defined in the Background and Glossary sections of this report. Measures of reliablity are given as percentages and fractions. Historical reliability values are from "1988 Update and Evaluation of Puget Sound AET" *(4)*.

AET Group	Database for Comparison	Total Samples	Sensitivity	Efficiency	Overall Reliability
TOC Normalized					
1988 Amphipod AETs	1988 AMPT	287	45% (48/106)	100% (48/48)	80% (229/287)
1988 Amphipod AETs	1994 AMPT	671	34% (61/181)	46% (61/133)	71% (476/671)
1994 Amphipod AETs	1994 AMPT	478	36% (58/162)	100% (58/58)	78% (368/478)
1994 Independent AETs	1994 AMPT	478	NA	NA	NA
1988 Amphipod AETs	1994 Pooled	671	28% (87/309)	52% (87/168)	55% (369/671)
1994 Amphipod AETs	1994 Pooled	478	NA	NA	NA
1986 Oyster AETs	1986 OYST	56	71% (12/17)	100% (12/12)	91% (51/56)
1986 OYST AETs	1994 ECHN	205	20% (16/79)	59% (16/27)	58% (119/205)
1994 Echinoderm AETs	1994 ECHN	205	46% (36/79)	100% (36/36)	79% (162/205)
1994 Independent AETs	1994 ECHN	205	NA	NA	NA
1986 OYST AETs	1994 Pooled	205	28% (88/309)	59% (88/148)	NA
1994 Echinoderm AETs	1994 Pooled	205	NA	NA	NA

Table 7. The predictive reliability of amphipod mortality and echinoderm larvae abnormality AETs. 1994 AETs (shaded) are compared to original 1988 amphipod and 1986 oyster AETs. "Sensitivity," "Efficiency," "Overall Reliability," "Independent AETs," and "Pooled" are defined in the Background and Glossary sections of this report. Measures of reliablity are given as percentages and fractions. Historical reliability values are from "1988 Update and Evaluation of Puget Sound AET" *(4)*.

AET Group	Database for Comparison	Total Samples	Sensitivity	Efficiency	Overall Reliability
Mixed Normalization					
1988 Amphipod AET		287	55% (58/106)	100% (58/58)	83% (239/287)
1994 Amphipod AET		478	NA	NA	NA
1994 Independent AET		478	NA	NA	NA
1994 Amphipod AET, Pooled		478	NA	NA	NA
1986 Oyster AET		56	88% (15/17)	100% (15/15)	96% (54/56)
1994 Echinoderm AET		205	NA	NA	NA
1994 Independent AET		205	NA	NA	NA
1994 Echinoderm AET, Pooled		205	NA	NA	NA

Table 7. The predictive reliability of amphipod mortality and echinoderm larvae abnormality AETs. 1994 AETs (shaded) are compared to original 1988 amphipod and 1986 oyster AETs. "Sensitivity," "Efficiency," "Overall Reliability," "Independent AETs," and "Pooled" are defined in the Background and Glossary sections of this report. Measures of reliablity are given as percentages and fractions. Historical reliability values are from "1988 Update and Evaluation of Puget Sound AET" *(4)*.

AET Group	Database for Comparison	Total Samples	Sensitivity	Efficiency	Overall Reliability
Possible Additional PSDDA Reliability Analyses:					
1988 HAETs	1988		NA	NA	NA
1988 HAETs	1994		NA	NA	NA
1988 HAETs	1994 Pooled		NA	NA	NA
1994 HAETs	1994		NA	NA	NA
1994 HAETs	1994 Pooled		NA	NA	NA
Original PSDDA MLs	1988		NA	NA	NA
1994 PSDDA MLs	1994		NA	NA	NA
1994 PSDDA MLs	1994 Pooled		24% (75/309)	77% (75/98)	NA
Proposed New PSDDA MLs	1994		NA	NA	NA
1988 LAETs	1988		NA	NA	NA
1988 LAETs	1994		NA	NA	NA
1988 LAETs	1994 Pooled		NA	NA	NA
1994 LAETs	1994		NA	NA	NA
1994 LAETs	1994 Pooled		NA	NA	NA
Original PSDDA SLs	1988		NA	NA	NA
1994 PSDDA SLs	1994		NA	NA	NA
1994 PSDDA SLs	1994 Pooled		84% (260/309)	51% (260/514)	NA
1994 HAET/10	1994		NA	NA	NA
Proposed New PSDDA SL	1994		NA	NA	NA

Table 7. The predictive reliability of amphipod mortality and echinoderm larvae abnormality AETs. 1994 AETs (shaded) are compared to original 1988 amphipod and 1986 oyster AETs. "Sensitivity," "Efficiency," "Overall Reliability," "Independent AETs," and "Pooled" are defined in the Background and Glossary sections of this report. Measures of reliablity are given as percentages and fractions. Historical reliability values are from "1988 Update and Evaluation of Puget Sound AET" *(4)*.

AET Group	Database for Comparison	Total Samples	Sensitivity	Efficiency	Overall Reliability
Possible Additional SMS Reliability Analyses:					
1988 2AETs	1988		NA	NA	NA
1988 2AETs	1994		NA	NA	NA
1988 2AETs	1994 Pooled		NA	NA	NA
1994 2AETs	1994		NA	NA	NA
1994 2AETs	1994 Pooled		NA	NA	NA
1991 CSLs/MCULs	1994		NA	NA	NA
1991 CSLs/MCULs	1994 Pooled				
Proposed New CSL/MCUL	1994		NA	NA	NA
1988 LAETs	1988		NA	NA	NA
1988 LAETs	1994		NA	NA	NA
1988 LAETs	1994 Pooled				
1994 LAETs	1994		NA	NA	NA
1994 LAETs	1994 Pooled		NA	NA	NA
1991 SQSs	1994		NA	NA	NA
1991 SQSs	1994 Pooled				
Proposed New SQS			NA	NA	NA

Table 8. Number and fraction of incorrectly predicted "Hit" samples having elevated levels of bulk ammonia (> 20 ppm), percent fines (>80% silt+clay), bulk sulfides (> 50 ppm). NA = data not available.

AET Type, Normalization	High Ammonia	High Fines	High Sulfides	≥ 2 High Conventionals
Amphipod, dry weight	21/26	40/88	34/57	31/104
Amphipod, TOC	NA	NA	NA	NA
Echinoderm, dry weight	35/41	9/29	25/32	24/47
Echinoderm, TOC	NA	NA	NA	NA

However, neither of those actions was taken. There was no clear predominance of "Hit" samples among those having greater than 80% fines. And, while the PSDDA agencies have found a significant correlation between percent fines and amphipod mortality *(11)*, the correlation appeared to be of limited utility for excluding high percent fines "Hit" samples from 1994 AET calculations. So, while there was perhaps a potential case for conventional parameters influencing determinations of adverse effects, thereby lowering the sensitivity of AETs, there appeared to no objective tool by which that determination could easily be improved.

Reliability of Pooled AETs

As described in Methods (page 20), a pooled reliability analysis considers a sample exhibiting a significant adverse effect of any type - amphipod mortality, benthic abundance, echinoderm larval abnormality, Microtox luminosity, or oyster larval abnormality - to be a "Hit" sample, even if it showed no other adverse

effects. There were 309 such "Hit" samples in the 1994 AET database[17]. Using those data, PSDDA agencies calculated some pooled reliability values for the 1994 suite of five Puget Sound AETs. Those limited results appear in Table 7 and are described below.

When all biological effects samples were pooled, the most sensitive 1994 dry weight-normalized AET type was Microtox® luminosity. 1986 dry weight-normalized Microtox® AET values correctly predicted 92% of all samples showing at least one type of "Hit." The other four 1994 dry weight-normalized Puget Sound AETs, ranked from most to least sensitive, were: 1986 oyster larval abnormality (76%), 1994 echinoderm larval abnormality (67%), 1988 benthic abundance (51%), and 1994 amphipod mortality (31%). The pooled efficiency of 1994 dry weight-normalized AETs, in descending order, were 88% for amphipod mortality, 59% for echinoderm larval abnormality, 51% for oyster larval abnormality, 49% for benthic abundance, and 45% for Microtox® luminosity. The overall reliability of 1994 dry weight-normalized AETs was highest for amphipod mortality (71%), followed by echinoderm abnormality (68%), oyster abnormality (61%), benthic abundance (59%) and Microtox® luminosity (53%).

Results of pooled reliability analysis for 1994 TOC-normalized AETs revealed that the amphipod mortality AETs were the least sensitive (28%) but the most efficient (65%) and overall reliable (65%). The Microtox® AETs had the highest pooled sensitivity (79%) but were the least efficient (44%) and least reliable overall (52%). The other AET groups were intermediate in all the measures of reliability when adverse effects were pooled for synoptic samples.

PSDDA agencies also calculated the pooled reliability for the 1994 LAET, 2AET and HAET values. Results of those calculations are still being tabulated and verified. Additional reliability calculations are suggested in Table 7 and in Recommendations for Future Work. Other reliability analyses of 1994 AETs may be recommended by technical and regulatory experts reviewing this document.

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[17] This number is less than the total given in Table 2 (414) because many samples exhibit more than one significant adverse biological effect.

CONCLUSIONS AND DISCUSSION

The 1994 AET Database

The size and nature of the 1994 Puget Sound sediment quality values database changed significantly between 1988 and 1994. Newly-obtained synoptic samples nearly tripled the SEDQUAL total from 334 to 924. The total number of determinations of significant adverse effects of all types which were used to calculate AETs rose from 594 in 1988 to 1186 in 1994.

Approximately 70% of all the 1988 synoptic surveys (8/11) and samples (235/334) were conducted in or collected from primarily urban areas, with about 80% of the biological effects samples (469/594) collected from urban areas. "Hit" samples constituted nearly one-half of those (227/469), or 38% of the total (227/594) in the 1988 AET database.

By comparison, only about 55% of the newly-obtained surveys (22/41) and synoptic samples (332/590) were urban. Less than half of the urban stations (154/332) and only 26% of the total number of biological effects samples (154/590) were determined to be "Hit" samples. Two urban surveys, PIERD_91 and SITCUMRI, accounted for 44% (68/154) of all new "Hit" samples.

Just how well did the 1988 AET database represent Puget Sound in its entirety? How representative is the 1994 AET database? Those questions do not have simple answers, but the 1994 sediment quality values database is clearly less urban in character than the 1988 sediment quality values database.

Methods

PSDDA agencies' stringent QA review of chemical and bioassay data resulted in exclusion of many synoptic sample data. Over one-half of all newly-obtained amphipod mortality samples (452/833) were not used in 1994 AET calculations. Nearly all new bivalve larval bioassay samples failed to meet QA guidelines or lacked a suitable reference area sample to which test sample responses could be compared. That was also true for one-third of all echinoderm samples (112/317).

Most of the samples excluded from 1994 AET calculations lacked at least one adequate reference sample. In many cases that was intentional; the purpose of certain types of surveys did not include comparison of test to reference samples. An example of such a survey was the Puget Sound Reference Areas Study (PSREF90). Others used local "background" sites which did not adhere to the PSEP definition of reference area. Examples of those included many

Thus, it appears that the expansion of the Puget Sound AET database may depend on the careful collection and adequate performance of reference area samples. The PSDDA agencies believe that collection and performance of future reference area samples is improving. Recent surveys often collect more than one reference sample, in the event one does not meet performance standards. Concern about possible future sediment cleanup liability, and so the need to correctly characterize the biological sediment quality in a specific area, may also increase the fraction of surveys which carefully collect at least one reference sample.

While most of the synoptic sample data not used in 1994 AET calculations were excluded because they lacked a suitable reference sample, relatively few were casualties of new reference sample performance standards (see Table 1 and Figures A-1, A-2, and A-3). Similarly, few samples were excluded for being statistically inconclusive (Table B-6). For example, subjecting highly variable echinoderm larval samples to a power analysis had minimal effect on the final list of samples used to calculate 1994 AETs. Of the total inventory of synoptic echinoderm samples, only 11 samples (5%) were excluded.

Exclusion of chemically anomalous samples had a much greater effect on 1994 resulting AET values. Although defined as a "No Hit" sample that was three times higher concentration than the next highest "No Hit" sample, the average anomalous sample was more than five times higher than the next highest "No Hit" sample (Table B-7). If those anomalous samples had not been excluded, individual AET values would have been higher and generally much less sensitive. Similarly, TOC-normalized AET values would have been higher and less sensitive had the chemically anomalous TOC-normalized data been retained.

1994 Puget Sound AET Values

The synoptic data added to SEDQUAL during 1992 and 1993 were used to calculate new amphipod mortality AETs. A new, fifth type of AET was also calculated: echinoderm larval abnormality AETs.

The PSDDA agencies obtained several general types of 1994 AET results. First, many of the new AET values were unqualified, confirmed by at east one "Hit" sample having a higher chemical concentration. Some of those were newly-confirmed AETs, having only been estimated values in 1988. Others were altogether new, with no 1988 counterparts. Second, many new AETs could only be estimated, due in part to the nature of the 1994 database. With relatively few of the added synoptic samples collected from more contaminated areas, there were relatively fewer "Hit" samples to confirm new AET values. Finally, some AET values which were confirmed in 1988 could only be estimated in 1994. This occurred when at least one new "No Hit" sample having a higher chemical

concentration was added to the database and there was no confirming "Hit" sample with a higher concentration.

The majority of all new 1994 amphipod and echinoderm AETs which could be confirmed were from the chemical groups most frequently required in sampling and analysis plans, and most frequently detected: trace metals and PAHs.

Amphipod Mortality

PSDDA agencies expected that merely including more "No Hit" samples in amphipod mortality AET calculations, by virtue of the definition of AETs, would drive existing values higher. Indeed, AETs increased for six trace metals, seven individual PAHs and three substituted phenols. The largest increases were for arsenic (from 93 ppm to 450 ppm), zinc (to 3,800 ppm), dibenzo(a,h)anthracene (to 1,900 ppm) and phenanthrene (to 21,000 ppb). Estimated AET values for chromium and bis[2-ethylhexyl]phthalate increased to >1100 ppm and >8300 ppb, respectively. In addition, the amphipod AETs for ethylbenzene, total xylene and p,p'-DDT were confirmed in 1994.

1994 TOC-normalized amphipod AETs also tended to be the same or greater than 1988 values. Some of the AETs for trace metals, however, decreased. That apparently resulted when a few 1988 AET "No Hit" samples, after TOC-normalization and combination with 1994 samples, were excluded for being chemically anomalous.

As stated in the Results section, one amphipod AET which showed a particularly large decrease in 1994 was for benzyl alcohol. The dry weight-normalized value decreased from 870 ppb to 73 ppb, while the TOC-normalized values dropped from 73 ppb to just 5 ppb. The reason appeared to be that the 1988 value was set by a chemically anomalous sample (CBMSQS, HY-50) which should have been excluded from previous calculations, but was not.

Echinoderm

The PSDDA agencies could not anticipate the magnitude or reliability of a new AET type, one based on echinoderm larval abnormality. They found that the 1994 echinoderm AET values were generally lower than 1986 oyster AETs. Of the dry weight-normalized echinoderm AET values which could be confirmed, 27 were lower and seven were higher than the corresponding oyster values. Overall, TOC-normalized echinoderm AETs were also lower than oyster values, especially if estimated values were considered.

Lower echinoderm AETs might indicate a fundamentally lower tolerance of the bioassay test organisms, primarily *Dendraster excentricus*, toward chemical contaminants in Puget Sound sediments. However, without comparable echinoderm and oyster larval abnormality sample numbers, a broader geographic representation for both, and increased side-by-side testing, that conclusion would be premature.

New HAETs, 2AETs, LAETs: Potential Guidelines and Standards

Many of the 1994 amphipod and echinoderm AETs which a) increased and were confirmed, b) remained the same magnitude but were newly confirmed, or c) were for chemicals previously lacking AET values, like dieldrin, could potentially form the basis for new guidelines or standards. 1994 AET values representing new highest AET (HAET), second lowest AET (2AET) or lowest AET (LAET), could be of particular interest to regulatory programs and the regulated community, especially if there was no appreciable loss of reliability associated with those values.

For that reason, PSDDA agencies examined the new suite of five Puget Sound AETs. That was done by combining 1994 amphipod mortality and echinoderm larval abnormality AETs with the 1988 benthic abundance, 1986 Microtox luminosity and 1986 oyster larval abnormality AETs. The resulting values are detailed in Tables 9 and 10, and summarized in Table 11.

There were 17 new dry weight-normalized HAETs, all but one set by the 1994 amphipod AETs. The only new HAET value set by a 1994 echinoderm abnormality AET was for silver. HAETs increased for five trace metals (cadmium - 14 ppm, lead - 1,200 ppm, mercury - 2.3 ppm, silver - 8.4 ppm and zinc - 3,800 ppm), six PAH compounds (LPAH - 29,000 ppb, phenanthrene - 21,000 ppb, benzo[g,h,i]perylene - 3,200 ppb, chrysene - 21,000 ppb, dibenzo(a,h)anthracene - 1,900 ppb, and indeno(1,2,3-c,d)pyrene - 4,400 ppb) and six other chemicals of concern. Many of the new HAETs were for chemicals commonly found at sites proposed for dredging.

Because ML guidelines have been based on dry weight-normalized HAETs, PSDDA agencies could consider adopting new ML values for any or all of those chemicals of concern. New HAET values could also cause changes to PSDDA agencies screening level guidelines. For example, the SL for lead could be changed from 66 ppm (1/10 the current ML value of 660 ppm) to 120 ppm (1/10 the new ML of 1200 ppm).

Ten TOC-normalized HAETs also increased in 1994. However, under the current paradigm, those HAETs have no implications for regulatory programs. PSDDA program guidelines were not based on TOC-normalized AETs and SMS standards were not based on HAETs.

Table 9. The 1994 suite of dry weight-normalized Puget Sound AETs.
1994 amphipod mortality and echinoderm larvae abnormality AETs are combined with 1988 benthic abundance, 1986 Microtox luminosity and oyster abnormality AETs. A ">" symbol indicates a minimum value, not confirmed by a "Hit" sample with a higher concentration New and confirmed HAETs are in boxes, 2AETs are in bold italics, and LAETs are shaded.

Chemical Group/ Chemical of Concern	Amphipod AET (1994)	Echinoderm AET (1994)	Benthic AET (1988)	Microtox AET (1986)	Oyster AET (1986)
Metals (mg/kg or ppm)					
Antimony	200	9.3	150	NA	NA
Arsenic	450	***130***	57	700	700
Cadmium	14	2.7	***5.1***	9.6	9.6
Chromium	>1100	>96	260	NA	NA
Copper	1,300	390	530	390	390
Lead	1,200	430	***450***	530	660
Mercury	2.3	1.4	2.1	0.41	0.59
Nickel	>370	110	>140	NA	NA
Silver	6.1	8.4	>6.1	>.56	>0.56
Zinc	3,800	***460***	410	1,600	1,600
Organic Compounds (ug/kg or ppb)					
Low molecular weight PAH					
LPAH	29,000	1,200	13,000	***5,200***	5,200
2-Methylnaphthalene	1,900	64	1,400	***670***	***670***
Acenaphthene	2,000	130	730	***500***	***500***
Acenaphthylene	1,300	71	***1,300***	>560	>560
Anthracene	13,000	280	4,400	***960***	***960***
Fluorene	3,600	120	1,000	***540***	***540***
Naphthalene	2,400	230	2,700	***2,100***	***2,100***
Phenanthrene	21,000	660	5,400	***1,500***	***1,500***
High molecular weight PAH					
HPAH	69,000	7,900	69,000	***12,000***	17,000
Benz[a]anthracene	5,100	960	5,100	***1,300***	1,600
Benzo[a]pyrene	3,500	1,100	3,600	***1,600***	***1,600***
Benzo[ghi]perylene	3,200	920	2,600	670	720
Benzofluoranthenes	9,100	1,800	9,900	***3,200***	3,600
Chrysene	21,000	950	9,200	***1,400***	2,800
Dibenzo(a,h)anthracene	1,900	***240***	970	230	230
Fluoranthene	30,000	1,300	24,000	***1,700***	2,500
Indeno(1,2,3-c,d)pyrene	4,400	760	2,600	600	690
Pyrene	16,000	2,400	16,000	***2,600***	3,300

Table 9. The 1994 suite of dry weight-normalized Puget Sound AETs.
1994 amphipod mortality and echinoderm larvae abnormality AETs are combined with 1988 benthic abundance, 1986 Microtox luminosity and oyster abnormality AETs. A ">" symbol indicates a minimum value, not confirmed by a "Hit" sample with a higher concentration New and confirmed HAETs are in boxes, 2AETs are in bold italics, and LAETs are shaded.

Chemical Group/ Chemical of Concern	Amphipod AET (1994)	Echinoderm AET (1994)	Benthic AET (1988)	Microtox AET (1986)	Oyster AET (1986)
Chlorinated organic compounds					
1,2,4-trichlorobenzene	51	>4.8	NA	31	64
1,2-dichlorobenzene	>110	NA	50	35	50
1,3-dichlorobenzene	>170	>4.4	>170	>170	>170
1,4-dichlorobenzene	120	NA	110	110	120
Hexachlorobenzene	130	NA	22	70	230
Phthalates					
Bis[2-ethylhexyl] phthalate	>8,300	***1,700***	1,300	1,900	1,900
Butyl benzyl phthalate	970	***200***	900	63	>470
Di-n-butyl phthalate	1,400	>31	>5100	1,400	1,400
Di-n-octyl phthalate	>2,100	>98	6,200	NA	>420
Diethylphthalate	>1,200	>62	200	>48	>73
Dimethylphthalate	>1,400	***85***	>1400	71	160
Phenols					
2-methyl phenol	77	55	72	>72	***63***
2,4-dimethyl phenol	77	***55***	210	29	29
4-methylphenol	3,600	110	1,800	***670***	***670***
Pentachlorophenol	400	>150	690	>140	>140
Phenol	1,200	>220	1,200	1,200	420
Miscellaneous Extractables					
Benzyl alcohol	73	>12	870	57	73
Benzoic acid	760	>31	650	650	650
Dibenzofuran	1,700	110	700	***540***	***540***
Hexachlorobutadiene	180	1.3	11	120	270
Hexachloroethane	140	NA	NA	NA	NA
N-nitrosodiphenylamine	48	>25	28	40	130
Volatile organics					
Ethylbenzene	50	4.0	10	33	37
Tetrachloroethene	>210	>1	57	140	140
Xylene, Total	160	>21	40	100	120

Table 9. The 1994 suite of dry weight-normalized Puget Sound AETs.
1994 amphipod mortality and echinoderm larvae abnormality AETs are combined with 1988 benthic abundance, 1986 Microtox luminosity and oyster abnormality AETs. A ">" symbol indicates a minimum value, not confirmed by a "Hit" sample with a higher concentration New and confirmed HAETs are in boxes, 2AETs are in bold italics, and LAETs are shaded.

Chemical Group/ Chemical of Concern	Amphipod AET (1994)	Echinoderm AET (1994)	Benthic AET (1988)	Microtox AET (1986)	Oyster AET (1986)
Pesticides and PCBs					
Aldrin	9.5	9.5	NA	NA	NA
Chlordane	2.8	>4.5	NA	NA	NA
Dieldrin	3.5	1.9	NA	NA	NA
Heptachlor	1.5	2.0	NA	NA	NA
p,p'-DDD	63	***28***	16	NA	NA
p,p'-DDE	62	***9.3***	9.0	NA	NA
p,p'-DDT	>270	12	***34***	NA	>6
Total DDT	24	37	NA	NA	NA
Total PCBS	3,100	***450***	1,000	130	1,100

Table 10. The 1994 suite of TOC-normalized Puget Sound AETs.
1994 amphipod mortality and echinoderm larvae abnormality AETs are combined with 1988 benthic abundance, 1986 Microtox luminosity and oyster abnormality AETs. A ">" symbol indicates a minimum value, not confirmed by a "Hit" sample with a higher concentration. New and confirmed HAETs are in boxes, 2AETs are in bold italics and LAETs are shaded.

Chemical Group/ Chemical of Concern	Amphipod AET (1994)	Echinoderm AET (1994)	Benthic AET (1988)	Microtox AET (1986)	Oyster AET (1986)
Metals (mg/kg TOC or ppm)					
Antimony	>15,000	>2,100	5,500	3,300	3,300
Arsenic	32,000	***5,800***	4,400	88,000	88,000
Cadmium	1,100	>430	580	1,200	1,200
Chromium	>130,000	>5,400	65,000	NA	NA
Copper	100,000	***30,000***	13,000	48,000	49,000
Lead	48,000	***22,000***	18,000	66,000	66,000
Mercury	300	71	120	***77***	210
Nickel	20,000	>49,000	***31,000***	NA	NA
Silver	***270***	***270***	490	100	>100
Zinc	150,000	60,000	48,000	>200,000	>200,000
Nonionizable organic compounds (mg/kg TOC; ppm)					
Low molecular weight PAH					
LPAH	2,200	22	780	>530	***370***
2-Methylnaphthalene	>120	>53	64	NA	NA
Acenaphthene	200	>110	57	>57	16
Acenaphthylene	66	>18	66	>27	>27
Anthracene	1,200	93	***220***	>79	>79
Fluorene	360	***73***	79	>71	23
Naphthalene	220	>190	170	>170	99
Phenanthrene	840	***140***	480	>160	120
High molecular weight PAH					
HPAH	5,300	150	7,600	1,500	***960***
Benz[a]anthracene	270	***170***	650	>160	110
Benzo[a]pyrene	***210***	230	>1300	>140	99
Benzo[ghi]perylene	***100***	>240	>1200	>67	31
Benzofluoranthenes	450	***310***	1,500	>430	230
Chrysene	840	***220***	850	>200	110
Dibenzo(a,h)anthracene	50	***48***	89	33	120
Fluoranthene	3,000	***320***	1,200	>190	160
Indeno(1,2,3-c,d)pyrene	***120***	>190	900	>87	33
Pyrene	***1,000***	520	1,400	>210	>210

Table 10. The 1994 suite of TOC-normalized Puget Sound AETs.
1994 amphipod mortality and echinoderm larvae abnormality AETs are combined with 1988 benthic abundance, 1986 Microtox luminosity and oyster abnormality AETs. A ">" symbol indicates a minimum value, not confirmed by a "Hit" sample with a higher concentration. New and confirmed HAETs are in boxes, 2AETs are in bold italics and LAETs are shaded.

Chemical Group/ Chemical of Concern	Amphipod AET (1994)	Echinoderm AET (1994)	Benthic AET (1988)	Microtox AET (1986)	Oyster AET (1986)
Chlorinated organic compounds					
1,2,4-trichlorobenzene	1.8	>2.4	NA	0.81	2.7
1,2-dichlorobenzene	>5.8	NA	2.3	2.3	2.3
1,3-dichlorobenzene	>15	>0.26	>15	>15	>15
1,4-dichlorobenzene	9	NA	16	>16	3.1
Hexachlorobenzene	4.5	NA	0.38	2.3	9.6
Phthalates					
Bis[2-ethylhexyl] phthalate	>550	130	60	47	60
Butyl benzyl phthalate	49	***5.2***	64	4.9	>9.2
Di-n-butyl phthalate	260	0.88	1,700	220	260
Di-n-octyl phthalate	58	>57	4,500	NA	>57
Diethylphthalate	>110	>0.27	61	>5.3	>5.3
Dimethylphthalate	53	NA	53	>19	>22
Miscellaneous Extractables					
Dibenzofuran	>170	***57***	58	>58	15
Hexachlorobutadiene	6.2	NA	6.9	3.9	11
Hexachloroethane	2.7	NA	NA	NA	NA
N-nitrosodiphenylamine	>11	>6.4	11	>11	>11
Volatile organics					
Ethylbenzene	>3.8	NA	>3.8	>3.8	>3.8
Tetrachloroethene	>22	NA	>22	>22	>22
Xylene, Total	>12	0.15	>12	>12	>12
Pesticides and PCBs					
Aldrin	0.56	>0.56	NA	NA	NA
Chlordane	0.16	>0.26	NA	NA	NA
Dieldrin	0.13	0.28	NA	NA	NA
Heptachlor	>0.11	>0.40	NA	NA	NA
p,p'-DDD	3.1	***1.6***	1.0	NA	NA
p,p'-DDE	6.0	>7.3	0.31	NA	NA
p,p'-DDT	16	>0.71	3.7	NA	NA
Total DDT	1.4	8.8	NA	NA	NA
Total PCBS	190	***18***	65	12	>46

Table 10. The 1994 suite of TOC-normalized Puget Sound AETs.
1994 amphipod mortality and echinoderm larvae abnormality AETs are combined with 1988 benthic abundance, 1986 Microtox luminosity and oyster abnormality AETs. A ">" symbol indicates a minimum value, not confirmed by a "Hit" sample with a higher concentration. New and confirmed HAETs are in boxes, 2AETs are in bold italics and LAETs are shaded.

Chemical Group/ Chemical of Concern	Amphipod AET (1994)	Echinoderm AET (1994)	Benthic AET (1988)	Microtox AET (1986)	Oyster AET (1986)
Ionizable organic compounds (mg/kg TOC or ppm)					
Phenols					
2-methyl phenol	3.1	>2.1	10	>10	3.1
2,4-dimethyl phenol	6.5	NA	2.6	0.63	>1.3
4-methylphenol	780	4.7	250	81	37
Pentachlorophenol	24	>9.3	66	>11	>11
Phenol	>440	3.2	>140	33	>39
Miscellaneous Extractables					
Benzyl alcohol	5.0	>0.71	>73	5.0	5.0
Benzoic acid	>170	>2.0	>170	>170	>170

Table 11. Summary of the highest, second lowest and lowest AET values (HAET, 2AET, LAET, respectively) among the suite of five 1994 Puget Sound AETs. NA = data not available.

AET Rank	Dry Weight Normalized		TOC Normalized	
	Higher	Lower	Higher	Lower
New HAETs	17	0	10	0
New 2AETs	NA	<29	NA	<24
New LAETs	0	23	0	6

The number of new 2AET and LAET values was even more pronounced than the number of HAETs which changed between 1988 and 1994. This was caused primarily by the addition of a new echinoderm AET group to the suite of Puget Sound AETs. As already discussed, the 1994 echinoderm larval abnormality AETs tended to be lower than the corresponding 1986 oyster AET values.

Lower 2AET and LAET values could have potentially greater effects on the SMS program. However, this would assume, of course, the same relationship of 2AET and LAET to those criteria and that predictive reliability of 1994 values was judged adequate.

Reliability of 1994 Puget Sound AETs

There are four possible outcomes from comparisons of synoptic sediment quality data to AET values:

- correct prediction of samples exhibiting significant adverse effects (the chemistry of "Hit" samples was greater than AET values)
- correct prediction of samples with no significant adverse effects (the chemistry of "No Hit" samples was below AET values)
- incorrect prediction of samples exhibiting significant adverse effects (the chemistry of "Hit" samples was below AET values)

- incorrect prediction of samples with no significant adverse effects (the chemistry of "No Hit" samples was greater than AET values)

Comparisons yielding a high frequency of the first outcome involve sediment quality values, AETs or PSDDA SLs for example, which are highly sensitive. A high combined frequency of the first and second reflect AETs which have good overall reliability. The third and fourth outcomes translate to sediment quality values having low sensitivity and efficiency, respectively.

Reliability calculations showed the sensitivity of 1994 dry weight- and TOC- normalized amphipod AETs to be lower than in 1988. However, that result was predicted. As the 1994 amphipod AET values increased, the chemistry of fewer samples exceeded them, and fewer of the "Hit" samples were correctly predicted. The new amphipod AETs were particularly poor at predicting "Hit" samples from the EIGHTBAY, HULB90FC and PSDDA1 surveys (see Tables C-5 and C-6).

As described in the Results section, elevated levels of conventional parameters could have contributed to the adverse effects observed in those surveys. However, PSDDA agencies chose not to exclude incorrectly predicted "Hit" samples on that basis alone. If a stronger case could be made for doing so, or for correcting some "Hit" determinations based on high percent fines content, then the sensitivity of the 1994 amphipod AETs could increase approximately 10%. That would result in sensitivities similar to the 1988 dry weight- and TOC-normalized amphipod AET sensitivity values, but still lower than that of other AETs.

Another cause of low sensitivity could be that the 1994 amphipod AETs are simply too high. A weakness of AET-based sediment quality values is that a single synoptic sample sets each threshold value. For a variety of reasons, that sample may have a much higher concentration than the next highest "No Hit" sample[18]. That is also why chemically anomalous samples were excluded from 1988 amphipod and benthic AETs. It is possible, though, that a statistical test for outliers, such as the one proposed by Dixon *(27)*, would result in more exclusions. Removal of additional high "No Hit" samples from the 1994 biological effects data would, in turn, result in lower and more sensitive amphipod AETs.

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[18] Among reasons for a more contaminated sediment sample not showing significant adverse benthic or bioassay effects are: low bioavailability of contaminants or ameliorating interactive effects among chemicals of concern and other sediment constituents.

The efficiency of 1994 dry weight-normalized amphipod AETs also appeared to decline. However, the overall reliability of both the new dry weight- and TOC-normalized amphipod AETs was the same as in 1988.

Pooled reliability analysis revealed the 1994 amphipod AET to be the least sensitive of the five Puget Sound AETs, but the most efficient one. Thus, the strength of the new amphipod AET values may not be in detection of all samples having adverse biological effects. Their strength may instead lie in the accuracy with which "Hit" sample predictions are made: nearly 90%. A sample determined to be a "Hit" sample based on amphipod bioassay results could be weighted more heavily in making regulatory decisions than a "Hit" sample based on other types of biological effects.

Regarding echinoderm larval AETs, the PSDDA agencies could not predict the effect of adding a new AET type to the suite of Puget Sound AETs. Would they be more or less reliable as a group? How would they contribute to the overall reliability of the suite of Puget Sound AETs?

Results indicated the dry weight-normalized echinoderm AETs were far less sensitive than 1986 oyster AETs: 48% vs. 88%. However, the independent reliability calculations indicated they were substantially more efficient and had better overall reliability than the oyster AETs. When biological effects data were pooled, the dry weight-normalized echinoderm AETs were found to have intermediate sensitivity; Microtox and oyster were more sensitive, while benthic and amphipod AETs were less so. Echinoderm AETs were the second most efficient of all AET types.

Certain other reliability analyses may still be recommended by experts or members of a Regulatory Work Group prior to use of new AET values in regulatory programs. Reliability calculations in which single chemical substitutions are made may indicate that changing an individual guideline value or standard has no effect on reliability. For the PSDDA program, comparing the various measures of reliability for current ML and SL values to the reliability of proposed new guidelines would be helpful to technical experts and policy makers. The reliability of "mixed-normalization" AETs[19] and independent reliability calculations for TOC-normalized AETs[20] may prove useful in evaluating new AET values for the SMS program. Such calculations, along with many of those suggested in Table 7, were outside the scope of this report.

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[19] A group of AETs which has "mixed-normalization" contains dry weight-normalized trace metal and polar organic AETs, combined with TOC-normalized nonpolar organic AETs.

[20] That calculation cannot presently be calculated by SEDQUAL.

Summary

The Puget Sound sediment quality values database greatly increased in size, and perhaps in its ability to represent the whole of Puget Sound, between 1988 and 1994. However, the effort that was required to identify, obtain, quality assure, and enter significant additional synoptic data into Ecology's SEDQUAL database was substantial.

A comprehensive annual recalculation of new AETs, reliability values and their potential implications to the PSDDA and SMS regulatory programs would appear to be impractical. Given the current resource levels and priorities of the PSDDA agencies, an annual re-evaluation would require a greatly streamlined, new process. The alternative would be to plan for AET re-evaluations at less frequent intervals, perhaps every three years.

Extensive new synoptic biological sample data were analyzed for significant adverse effects, but a large number of those samples lacked an adequate reference sample to which test sample effects could be compared. Future surveys need to emphasize collection of adequate single or multiple reference area samples.

The biological effects sample data obtained since 1988 were combined with data from the 1988 sediment quality values database, and used to calculate new AET values for Puget Sound. Results showed many more of the echinoderm larval abnormality AETs represented new AET values than did the 1994 amphipod mortality AETs. The latter set 17 dry weight-normalized HAET values of potential use to the PSDDA program in revising its ML and SL guidelines. Echinoderm AETs were much more frequently either the new second lowest AET or LAET among the suite of what has become five Puget Sound AETs. That indicated that there were more potential changes to the SMS standards and program.

Changes to guidelines or standards in either program will involve review of the AET reliability results presented in this report, as well as others yet to be conducted. Values eventually adopted by either the PSDDA or SMS program will likely not compromise the reliability of current regulatory values. The practical and economic implications of any changes to PSDDA MLs an SLs, and SMS sediment quality criteria, would also need to be considered prior to adoption.

RECOMMENDATIONS FOR FUTURE WORK

The PSDDA agencies remain committed to conducting additional technical analyses needed in order to finalize the 1994 calculations of AET and reliability values presented in this report. These analyses include:

- calculating reliability of "mixed" dry weight- and TOC-normalized 1994 amphipod mortality and echinoderm larval abnormality AETs
- performing independent reliability analysis for 1994 AETs and comparing the results to 1988 reliability values, where this has not been done
- calculating additional "pooled" reliability analyses
- calculating the effect of individual new AETs on the reliability measures for AET groups[21]
- conducting other analyses to determine the quality of new 1994 AET values

Some longer-term tasks which the PSDDA agencies might consider undertaking in the future include:

- examining synoptic data only available since completing the calculations presented in this report, which might affect 1994 AET values
- consider using alternate statistical methods for comparing test sediment samples to reference sediment samples and determining significance of adverse effects
- using an established statistical test for "outliers" to detect and exclude chemically anomalous samples
- calculating AET and reliability values for subregions within Puget Sound, such as Commencement Bay, Eagle Harbor, Elliott Bay or Sinclair Inlet
- calculating new juvenile polychaete growth AETs and their reliability
- calculating new AETs based on benthic infaunal effects, and the reliability of those AETs
- performing "pooled" AET analyses which include new benthic and juvenile polychaete AETs
- examining other means of calculating empirical and probablistic effects thresholds

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[21] An example of this would be to calculate the pooled reliability of the 1988 suite of AETs, substitute a single new dry weight-normalized amphipod mortality AET (e.g., the benzo(a)pyrene value of 3500 ppb dry weight), recalculate the reliability, and compare the results.

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GLOSSARY

This glossary contains operational definitions for many of the technical terms used in this report. Definitions for terms in italics were taken in part or entirely from the 1988 "Update and Evaluation of Puget Sound AET" *(4)*.

Abnormal, Abnormality

Larvae used in sediment bioassays are abnormal if they fail to transform into a clearly defined pluteus having a pair of well-developed arms and a second pair of arms which are just budding.

Adverse Biological Effect, Adverse Effect

Any change in a biological system that results in injury or damage to an organism, population or community (e.g., death, failure to develop properly, reduced population abundance).

Amphipod

A small, shrimp-like crustacean often used in laboratory bioassays to test toxicity of sediments. Puget Sound amphipod AETs are derived from bioassays using the species *Rhepoxinius abronius*, although other species are allowed.

Anomalous

An unusual observation, unexpected result or localized phenomenon.

Anomalous Sample, Biologically or Chemically

A sample exhibiting a significant adverse biological effect, but with chemistry which does not predict one. This may be caused by interactive toxic effects among chemicals, toxic effects of unmeasured chemicals, or Type I error in the statistical test for significant adverse effects.

Or,

A sample not exhibiting a significant adverse biological effect, but with chemistry which predicts one. This may be caused toxic chemicals which are less bioavailable than in the samples used as the basis for prediction (e.g., setting the AET). It may also occur because of high biological variability, which results in inadequate statistical power to detect significant adverse effects.

Apparent Effects Threshold (AET)

The sediment concentration of various chemicals of concern above which statistically significant ($p<0.05$) adverse biological effects (relative to an appropriate reference condition) are always expected. Theoretically, an AET can be calculated for any chemical and biological indicator.

Benthic Infauna (Abundance)

Invertebrate organisms whose life cycle takes place largely within the bottom sediments of aquatic systems. Benthic AETs for Puget Sound are based on the abundance of certain major taxonomic groups of benthic organisms.

Bioassay

A laboratory test used to evaluate the toxicity of a material (commonly sediments or wastewater) by measuring behavioral, physiological, or lethal responses of organisms.

Bivalve

Any two-shelled mollusk which, as adults, usually living in association with the bottom of various waterbodies. Larval stages of bivalves are often used in laboratory bioassays to test toxicity of sediments. Puget Sound amphipod AETs are derived from bioassays using the species *Crassostrea gigas* or *Mytilus edulis*.

Contaminant

A chemical or biological substance in a form or quantity which can harm aquatic organisms, consumers of aquatic organisms or users of aquatic habitats.

Contaminated Sediment

A sediment which contains measurable levels of contaminants.

Control, Control Sample

Negative Control

A separate bioassay sample intended to distinguish between the effects on target organisms of physical or other test conditions and the effects of exposure to chemical toxicants of concern.

"Control sediment sample..." is a "...surface sediment sample which is relatively free of contamination and is physically and chemically characteristic of the area from which bioassay test animals are collected"

(PSWQA, 1991. Puget Sound Water Quality Management Plan, Element P2).

Control, Control Sample (cont'd)

Positive or Reference Toxicant Control

A series of separate bioassay samples intended to show that the test organisms are appropriately sensitive to a single toxicant over a range of environmental concentrations. See also EC_{50}.

Conventional Parameters

Routinely measured sediment variables and characteristics which may cause toxicity in laboratory tests or explain unusual field observations. Examples of conventional pollutants or "conventionals" include bulk ammonia, fine grained sediment (percent clay or fines), dissolved oxygen, pH, salinity, sulfides, temperature, total organic carbon, total volatile solids and others.

Detection Limit

The lowest concentration at which the presence of a particular chemical can be reliably established by a specified analytical protocol.

Dose Response, Dose Responsive

Any relationship in which some measure of biological toxicity increases in proportion to the increasing concentration of a known contaminant or toxicant.

Dredged Material

Sediments excavated from the bottom of a waterway or water body.

EC_{50}, Effective Concentration - 50%

The concentration of a known toxicant which causes a specified effect in 50% of test organisms.

Echinoderm

Any member of the Class Echinodermata, including sand dollars, sea stars, sea urchins, etc. Larval stages of echinoderms are often used in laboratory bioassays to test toxicity of sediments. Puget Sound echinoderm AETs are derived from bioassays using the species *Dendraster excenticus* and *Strongylocentrotus purpuratus.*

Efficiency

The proportion of all samples predicted to exhibit significant adverse biological effects which actually do exhibit them.

Endpoint

The indicator of adverse biological effects or toxicity which is evaluated upon termination of sediment bioassays. In this report, "endpoint" does not refer to the duration of the bioassay, i.e., the point in time at which a test is terminated.

Equilibrium Partitioning, Equilibrium Partitioning Coefficient (EqP)

HAET

The highest of the four types of 1988 Puget Sound AET values or the highest of the five 1994 Puget Sound AETs for a given chemical of concern. Used to set many of the PSDDA program maximum level (ML) guidelines.

Hit, Hit Sample

A significant ($p<0.05$) adverse biological effect or a sample exhibiting one.

Impacted Site, Station

A site or station having significant adverse biological effect(s).

Independent reliability (analysis or calculation)

The calculation consists of 1) temporarily withdrawing a single sample from a data set, 2) computing AET values from the samples remaining, 3) comparing the single sample to those AETs, 4) recording whether the comparison predicted the sample which was temporarily withdrawn to be a "Hit" or a "No Hit" sample, 5) recording whether or not that prediction was correct, and 6) repeating the process for all remaining samples (withdrawing a single sample, calculating AETs, comparing the single sample to those AETs, and so on). Efficiency is calculated in the usual manner, but is no longer 100% by definition. Therefore, this procedure allows a meaningful comparison of efficiency values among different groups of AET values.

LAET

The lowest of the four types of 1988 Puget Sound AET values or the lowest highest of the five 1994 Puget Sound AETs for a given chemical of concern. Used to set some of the PSDDA program screening level (SL) guidelines and most of Washington's Sediment Quality Standards (SQS).

LC_{50}, Lethal Concentration 50%

The concentration of a known toxicant which causes 50% mortality in test organisms.

Maximum Level (ML)

A sediment quality value based on the highest AET among a range of biological indicators. ML values are used in the PSDDA program to evaluate when biological testing may not be necessary to determine that a contaminated sediment is unsuitable for unconfined, open-water disposal.

Metals, Trace

Naturally occurring elements which, when present in the environment at unnatural levels, can be toxic. Some examples include cadmium, chromium, copper, lead, mercury, nickel, silver and zinc.

Microtox®

A laboratory test using light emitted by luminescent bacteria (*Photobacterium phosphoreum*) to assess the toxicity of saline or organic extracts of sediments, or of the sediments themselves.

Mortality

An indicator of adverse or toxic effects in various bioassays. It is usually calculated from a count of the total or total normal organisms surviving at the end of the test period.

Nonimpacted

Not affected adversely by human activities.

Organic Carbon

Carbon derived from living organisms.

Polychaete

Any of member of the annelid family Polychaeta. Typically a bristly marine, bottom-dwelling worm. Juvenile polychaetes, in a rapid phase of growth, are often used in laboratory bioassays to test toxicity of sediments. Puget Sound polychaete AETs, not yet calculated, will derived from bioassays using the species *Neanthes arenaceolata.*

Polychlorinated Biphenyls

A group of manmade organic chemicals, including 209 different but closely related compounds (congeners) made of carbon, hydrogen and chlorine. If released to the environment, they persist for long periods of time and can enter food chains.

Pooled reliability

The reliability of an AET or other sediment quality values group in which a sample is considered a "Hit" if it exhibits *any* significant adverse biological effect.

Power Analysis

A mathematical technique to determine the capacity of a statistical test to detect true differences between treatments (e.g., between amphipod mortality measured in a test sediment and that measured in a reference sediment).

Quality Assurance

The general process by which the precision and accuracy of (environmental) data are assessed.

QA1

Quality Assurance, Level 1. The level of quality assurance which includes independent evaluation of the results from various quality control samples. Those samples include laboratory blanks and duplicates, matrix (sediment) and/or surrogate spikes, and standard reference materials. Sediment management programs use data meeting QA1 requirements to make regulatory decisions.

QA2

Quality Assurance, Level 2. The level of quality assurance which includes independent evaluation of instrument calibration records, review of actual instrument outputs (paper or tape), and recalculation of final test sample results by for 10% of all samples. Sediment management programs use data meeting QA2 requirements to calculate or revise regulatory guidelines or standards.

Reference Area

" ... an area believed to be free from chemical contamination ... "

Reference Sample, Reference

A "reference sediment sample ... " is a " ... surface sediment sample which serves as a laboratory indicator of a test animal's tolerance to important natural physical and chemical characteristics of the sediment ... " *(18)*

"...represent the non-anthropogenically affected background surface sediment quality of the sediment sample."

'Where physical/chemical characteristics of control sediments differ from test sediments, reference sediment samples must be run.'

reference samples must be "...collected from an area believed to be free from chemical contamination..."

Reference Sample, Reference (continued)

reference area "sediments should be relatively clean."

Reliability, or Predictive Reliability

The ability of a given AET or other sediment quality values group to correctly predict significant adverse biological effects, or the lack thereof. Three measures of reliability are sensitivity, efficiency and overall reliability.

Sample

A volume of sediment collected from one station using any of a variety of devices. A single sediment sample can be collected from multiple lowerings and retrievals of that device, if all the sediment is homogenized before being placed in sample containers. If there is no homogenization from lowering and retrieval, then field replicates are collected.

Screening Level (SL) Concentration or Guideline

A sediment quality value which is based on the highest of the suite of Puget Sound AETs, but modified with a 1/10 safety factor (HAET/10). SLs are used in the PSDDA program to evaluate when biological testing should be conducted to determine the suitability of sediment for unconfined, open-water disposal.

Sediment

Material suspended in or settling to the bottom of a water body, such as sand and mud which make up much of the shorelines and bottom of the Puget Sound. Sediment comes from natural sources (e.g., soil erosion) and anthropogenic sources (e.g., construction practices). Certain contaminants tend to collect on or adhere to sediment particles. Puget Sound sediments may contain elevated levels of some contaminants relative to reference areas located away from major contaminant sources.

Sediment Quality Value

Any of ... A "benchmark" number for an environmentally acceptable concentration of a given chemical in sediments. A chemical concentration that is expected to be below the level that would consistently lead to adverse biological effects for a wide variety of species, habitats, and sediment types.

SEDQUAL

Sediment quality database and menu-driven program developed for the Puget Sound Estuary Program and used by Ecology to calculate sediment

quality values, evaluate the suitability of dredged material for disposal in open water, assess the need for sediment cleanup, etc. Also, the "AET database."

Sensitivity

The proportion of all sediment samples exhibiting a significant adverse biological effect which are correctly predicted by a sediment quality value, e.g., AET.

Station

Any physical location, unique in space and time, at which sediment (or other) samples are collected. Station locations are usually stored as degrees, minutes and decimal seconds of latitude and longitude, along with water depth.

Statistically Significant

A quantitative determination of the statistical degree to which multiple measurements of the same variable can be shown to be different, given the variability of the measurement.

Survey

Any field investigation involving collection of (marine sediment) samples for a single identified purpose, whether that be related to dredging, monitoring, reconnaissance, remediation or scientific study. Surveys may be completed in a single day or may be conducted over many weeks or months.

Synoptic Data

Chemistry and bioassay data representing subsamples taken from the same homogenized sediment sample, collected on the same day, as part of the same sampling effort.

Synoptic Survey

Any survey which collects synoptic data.

Toxicant

Any man-made or naturally-occurring chemical which causes acute, chronic or other adverse effects in living organisms.

APPENDIX A

METHODS

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AMPHIPOD SEDIMENT BIOASSAY

USE AND LIMITATIONS

The amphipod (*Rhepoxynius abronius* or *Eohaustorius estuarius*) sediment bioassay is used to characterize the toxicity of marine or estuarine sediments. This bioassay may be used alone as a screening tool in broad-scale sediment surveys, in combination with sediment chemistry and *in situ* biological indices, and in laboratory experiments addressing a variety of sediment and water quality manipulations. Mortality is the primary endpoint in the amphipod bioassay. Sublethal endpoints, such as emergence of amphipods from the sediment during the exposure period and failure to rebury in sediment at the end of the exposure period, may also be used to assess sediment toxicity. Total effective mortality, a combined endpoint representing the sum of percent mortality and percent nonreburial, has also been used in this bioassay. The basis for the combined endpoint is the assumption that individuals that fail to rebury in sediments at the end of the exposure period would die in nature as a result of predation.

The *R. abronius* bioassay is appropriate for sediments with interstitial water salinity of ≥25 ppt. The *E. estuarius* bioassay is appropriate for sediments with interstitial water salinity of 2-28 ppt. In addition, the following constraints apply:

- For the *R. abronius* bioassay, an interstitial water salinity of ≥25 ppt is necessary to ensure that there are no salinity effects. In general, adjustment of interstitial water salinity should not be attempted because of potential effects of adjustment on toxicological properties of the sediment. However, for dredged material that will be disposed of in the marine environment (where *in situ* sediments have interstitial water salinities >25 ppt), salinity adjustment may be desirable. If interstitial water salinities of dredged material are between 15 and 24 ppt, they may be adjusted upward for use in the *R. abronius* bioassay. For other testing purposes, use of the *E. estuarius* bioassay is preferred for sediments with salinities <25 ppt.
- Grain size may have an effect on the animals at extremes of fine and coarse material (DeWitt et al. 1988). If the clay content of the test sediments exceeds 50 percent or the gravel content exceeds 35 percent, controls for the effects of particle size distribution (i.e., a reference area sediment similar in grain size to the test sediment) are recommended for interpretation of toxicity test results (Swartz et al. 1985).
- As in all bioassays using natural populations, there is a possibility that relative sensitivity of the amphipods will vary with season or other factors. Accordingly, a positive control is recommended. This should comprise a 96-hour LC_{50} measurement with a reference toxicant (e.g., cadmium chloride) conducted in the absence of sediment. The salinity and temperature of the dilution water should be equivalent to the values specified for sediment testing (i.e., 28 ppt and 15°C, respectively).

- Identification of *R. abronius* and *E. estuarius* must be confirmed by a qualified taxonomist prior to initiation of the bioassay, and representative specimens should be preserved and archived for future reference.
- Predators generally are not a problem in the bioassay, but potential problems can be avoided by observation and predator removal (if necessary).

These guidelines have been adapted from Swartz et al. (1985) for *R. abronius* and DeWitt et al. (1989) for *E. estuarius*. A new standard guide for conducting 10-day static sediment toxicity tests for marine and estuarine amphipods has been developed by ASTM (1990).

FIELD PROCEDURES

Collection

Test Animals—Both species of amphipods can be collected using benthic grabs (e.g., van Veen, Smith-McIntyre) or small dredges. *E. estuarius* can also be collected by shovel at low tide. If a dredge is used, a short haul (10 meters) will minimize potential damage to the animals during collection. *R. abronius* inhabits fine sands from the low intertidal zone to a maximum depth of 60 meters. *E. estuarius* is found in intertidal estuarine sediments from +0.5 meters to +2.0 meters above mean lower low water. Approximately one-third more animals than are required for the bioassay are collected. Surface and bottom seawater salinity and temperature are measured at the collection site. Sediment temperature is recorded from the first and last dredge sample. It is recommended that bioassays be conducted within 10 days of amphipod collection.

Sediment—Control, reference, and test sediments should be stored in glass jars that have PTFE-lined lids and have been cleaned according to the procedures described in the section entitled *General QA/QC Guidelines*. Each jar should be filled completely to exclude air. A minimum sediment sample size of 0.25 liters for each bioassay beaker is recommended for each kind of sediment. Because five replicate tests are conducted for each field sample, a minimum sediment sample size of 1.25 liters is recommended for each station.

Processing

Test Animals—Contents of the dredge or grab sampler are gently washed into a container using seawater of similar temperature and salinity to that at the collection depth. Samples that show evidence of contamination (e.g., oil sheen) are rejected. Amphipods will typically bury in the sediment and if necessary can be held in the containers for several hours (at the temperature of the collection depth) prior to sieving. It is preferable to minimize the delay between collection and sieving. To avoid handling stress, each dredge sample is placed in a separate container. Amphipods are maintained and transported in clean coolers, should be held in sediment during transport to the laboratory, and should be kept at or below the collection site temperature. During a long transport, aeration may be required.

Sediment—Control, reference, and test sediments should be stored at 4°C in the dark. Holding time should not exceed 14 days.

LABORATORY PROCEDURES

The laboratory procedures are those described by Swartz et al. (1985) and DeWitt et al. (1989) with the following changes incorporated:

- The salinity of the overlying water is adjusted to 28 ppt for *R. abronius*
- A 1.0-mm screen is used to sieve out amphipods prior to initiation of testing
- Holding time for amphipods is standardized to between 2 and 10 days
- Sediment holding time prior to testing is set at a maximum of 14 days
- Additional details are provided concerning maintenance and transportation of amphipods, confirmation of taxonomic identifications, and freeing of amphipods trapped by water surface tension during testing
- A specific procedure for adjustment of interstitial water salinity for testing dredged material is included.

Test Animals

Sieving—A 1.0-mm sieve is used to remove *R. abronius* and *E. estuarius* from sediment. Mature amphipods (3.0–5.0 mm total length) are used in the sediment bioassay. Gentle sieving is essential to reduce handling stress. The sieve is placed in a large tub filled with seawater at ambient salinity and temperature for the collection site sediments. The entire contents of each holding container, including water, are washed through the sieve using seawater pumped at low pressure through a fan spray nozzle. The sieve can be shaken gently, but the bottom of the screen must be beneath the water surface at all times. Material retained on the screen is washed into buckets for sorting. Large pieces of detritus and obvious predators are discarded. If there is a delay of more than 1 hour before sorting begins, the buckets should contain enough sieved sediment to allow the amphipods to bury. The buckets must be kept at or below collection site temperature. Aeration may be necessary.

Sorting—An aliquot of detritus or sediment containing amphipods is placed in a sorting tray. Healthy, active animals are removed with a bulb pipette (5-mm opening) and placed in 10-cm-diameter finger bowls filled with 28 ppt seawater and a 2-cm-deep layer of 0.5-mm sieved collection site sediment. Twenty amphipods are held in each bowl and enough bowls are prepared to provide at least one-third more specimens than are required for the bioassay. Seawater temperature during sorting of amphipods must not exceed 18°C. Filled finger bowls are submerged in holding tanks supplied with flowing water or aeration where temperature and salinity approximate bioassay conditions. If temperature and salinity adjustments are necessary, they should be made gradually. Healthy amphipods will remain in the finger bowl sediment and can be retrieved easily when the bioassay is set up. Amphipods should be acclimated to laboratory conditions for a minimum of 2 days and a maximum of 10 days before testing.

The identification of amphipods as *R. abronius* or *E. estuarius* must be confirmed by a qualified taxonomist. In addition, representative specimens from each bioassay series should be preserved and archived for future reference.

Control Sediment

R. abronius and *E. estuarius* typically inhabit well-sorted, fine sand. Suggestions for sieving and settling may have to be adjusted for other sediment types.

Approximately 0.25 liters of control sediment should be collected for each bioassay beaker. This sediment is sieved twice: first, to remove the test species and other macrobenthos and second, to adjust interstitial water salinity. The entire contents of one or more sediment samples, including water and suspended particulate matter, are sieved through a 0.5-mm screen without allowing overflow from the container. After the first sieving, the sediment is allowed to settle for at least 4 hours (preferably 12-16 hours). Overlying water is then decanted and the sediment resieved through a 0.5-mm screen into water of the bioassay salinity (28 ppt for *R. abronius*, ambient salinity for *E. estuarius*). Again, the sediment is allowed to settle for at least 4 hours (preferably 12-16 hours), overlying water is decanted, and the control sediment is held at 4°C until the bioassay chambers are prepared.

Test and Reference Area Sediment

Approximately 0.25 liters of test sediment should be collected for each bioassay beaker. Test sediments should not be wet-sieved, but if large predators or other large organisms are present, they can be removed using forceps or by pressing the sediment through a 2.0-mm screen. The natural geochemical properties of test sediment collected from the field must be within the tolerance limits of the test species. *R. abronius* may be adversely affected by salinity stress if the interstitial water salinity is below 25 ppt. For estuarine dredged material designated for disposal in the marine environment, interstitial water salinities below 25 ppt may require adjustment upwards. Both *R. abronius* and *E. estuarius* are tolerant of a range of sediment grain size. However, controls for the effects of particle size distribution are recommended if the silt and clay content exceeds 50 percent or the gravel content exceeds 35 percent.

The *R. abronius* test requires a minimum water column salinity regime of 28 ppt. When the interstitial (i.e., pore water) salinity is below 25 ppt, it must be raised if this test is to be used. Adjustment of interstitial water salinity is appropriate only for dredged material destined for marine disposal. The following procedure is recommended in such cases. The interstitial salinity of the sediments is determined (e.g., by refractometer using interstitial water collected by centrifugation) and the sediments are placed in the bioassay chambers with overlying water of a salinity calculated to raise interstitial salinities to a minimum of 25 ppt. The sediments are then carefully and slowly stirred by hand with a clean glass rod for 1 minute, and allowed to settle for at least 4 hours (preferably 12-16 hours). The majority (approximately 75 percent) of the overlying water is then carefully decanted and the interstitial salinity in each chamber confirmed prior to bioassay initiation. The decant water can be retained, salinity adjusted if necessary, and used as the overlying water in the bioassay. Alternatively, fresh seawater with the appropriate salinity can be used as the overlying water. Sediments are slowly mixed with a glass rod after adding the decant water. When the bioassays are terminated, the interstitial salinities are reconfirmed (e.g., by refractometer using interstitial water collected by centrifugation).

Bioassay Seawater

Seawater used in the bioassay is maintained at a salinity of 28 ± 1 ppt for the *R. abronius* test and at ambient interstitial salinity for the sediment collection site for the *E. estuarius* test. Temperature of seawater

used in bioassays of either species is maintained at 15 ± 1°C. The bioassay seawater must be uncontaminated, which may necessitate collection of seawater at the amphipod collection site. Natural and reconstituted seawater should be held at ≤15°C for no longer than 2 days before inoculation. The quantity of seawater required is dependent on sieving and holding needs and on the number of bioassay chambers.

Bioassay seawater is passed through a filter with 0.45-µm pore diameter. If necessary, salinity is reduced by addition of deionized distilled water or raised by addition of clean oceanic water or reagent grade chemicals (ASTM 1989). Seawater is prepared within 2 days of the bioassay and stored in covered, clean containers at the bioassay temperature.

Facilities and Equipment

The bioassay chamber is a standard 1-liter glass beaker (10-cm internal diameter) covered with an 11.4-cm diameter glass watchglass. The beakers are placed in a shallow water bath or temperature-controlled room with overhead aeration source. Aeration to each beaker is provided through a 1-mL glass pipette that extends between the beaker spout and watchglass to a depth not closer than 2 cm from the sediment surface. Air is bubbled into the beakers at a rate that does not disturb the sediment. The bioassay temperature is maintained by either the water bath or room temperature control.

All laboratory glassware is cleaned according to the procedures specified in the section entitled *General QA/QC Guidelines*. Large plastic containers and plastic sieves used for preparation and storage of sediment and seawater are preconditioned initially by soaking for 24 hours in seawater and rinsed after each use with clean seawater. They are used only for bioassays and stored in a clean room. Sieves and containers used to collect and store amphipods, seawater, and control sediment are kept separate from those used for test sediment.

Bioassay Procedure

The day before the bioassay is initiated, approximately 175 mL of test sediment are placed in the bottom of the 1-liter bioassay chamber to create a 2-cm-deep layer. Five replicate tests are conducted for each field sample. Beakers are filled to 750 mL with seawater at 28 ppt for the *R. abronius* test or ambient interstitial salinity for the *E. estuarius* test, covered with a watchglass, and placed in a 15°C water bath. Constant illumination is provided by overhead lights. Water in the beakers is aerated without disturbing the sediment surface. The system is allowed to equilibrate overnight before the amphipods are added. When the test is initiated, 20 amphipods are placed in each beaker and the seawater level is brought up to 950 mL. The bioassay is terminated after 10 days of exposure.

The primary endpoint is mortality after 10 days exposure to test or control sediment. The secondary endpoints that also can be measured are daily emergence of amphipods from sediment and failure to rebury in sediment at the end of the exposure period.

Initiation—The day before the bioassay is initiated, each test sediment sample is homogenized and an aliquot sufficient to make a 2-cm-deep layer is added to a bioassay beaker. For replicate bioassay samples, the weight of sediment necessary to make a 2-cm-deep layer (approximately 175 mL) in the first beaker is added to

the other replicates. The same procedure applies to control sediment. Treatments are randomly assigned to prenumbered bioassay beakers.

The sediment aliquot in the beaker can be settled by smoothing with a spoon, and bubbles can be removed by tapping the beaker against the palm of the hand. A disk (attached by a string for removal) is placed on the sediment surface. This minimizes sediment disruption as bioassay seawater is added up to the 750-mL mark on the beakers. This disk is removed and rinsed in bioassay water between beakers and changed between treatments. The beakers are covered with watchglasses, put into the 15°C water bath or temperature-controlled room, and aerated. The beakers are allowed to equilibrate overnight to bioassay conditions. Normal room lighting is maintained continuously during the bioassay. If the experimental design requires monitoring of sediment chemistry [e.g., metals, total volatile solids, oxidation potential (Eh)], additional beakers must be set up for this purpose. Monitoring the quality of seawater overlying the sediment can be accomplished in the bioassay beakers without disturbing the sediment. Temperature is recorded from a thermometer maintained in a separate beaker containing control sediment and bioassay water but no amphipods.

On the day the bioassay is initiated, amphipods are distributed among all beakers so that each receives 20 individuals. It is usually not logistically possible to distribute amphipods to all beakers at the same time, so it is necessary to select a portion of the beakers (as many as 15) to be processed together. The exact number of beakers to receive amphipods at one time is dependent on the size and design of the experiment. At least one replicate from each treatment, including control and reference area sediment, is processed at a time if possible. Otherwise, selection is random.

Amphipods are removed from the holding sediment using a 1.0-mm sieve, and then transferred to sorting trays. About one-third more fingerbowls are removed from the holding tank at one time than are required for the number of beakers. This allows selection of active, apparently healthy animals for the bioassay. Amphipods are removed from the sorting tray and sequentially distributed among clean 10-cm fingerbowls each containing 150 mL of bioassay seawater without sediment. The number of amphipods distributed to each fingerbowl is recounted by transferring them to a separate fingerbowl.

Amphipods are added to the bioassay beakers by placing a black plastic disk on the seawater surface and gently pouring the entire contents of the fingerbowl into the beaker. The fingerbowl is washed with bioassay water to remove adhering amphipods. The seawater level is brought up to 950 mL with bioassay water, and the disk is removed and rinsed between samples. Amphipods are allowed to bury in the sediment and any that are floating on the seawater surface are pushed down with the edge of the beaker cover or a clean glass rod. After 15 minutes, amphipods that have not buried are removed and replaced. Normally, less than 1 percent of the animals will fail to bury in 1 hour.

Monitoring—If samples for chemical analysis are desired, seawater and sediment samples can be taken from beakers at the initiation of the bioassay. A small quantity of seawater can be taken from beakers at the initiation of the bioassay, but chemistry beakers have to be sacrificed to obtain sediment samples. This is accomplished by siphoning the overlying seawater without disturbing the sediment surface and then taking appropriate sediment aliquots for chemical analyses. It is not necessary to add amphipods to chemistry beakers that are sacrificed at the initiation of the bioassay, but amphipods are added to those sacrificed later. Certain

sediment and water quality variables (e.g., dissolved oxygen, pH, Eh) can be monitored by inserting analytical probes into the chemistry beakers.

During the course of the bioassay, certain observations are made daily. Temperature in the beaker set up for this purpose is monitored. Lighting and aeration systems are checked. Each beaker is carefully examined but not disturbed except for the temporary removal of the aeration pipette and watchglass. Notes are made on sediment appearance and unusual conditions. The number of amphipods that have emerged from the sediment, either floating on the water surface or lying on top of the sediment, is recorded. Amphipods that have emerged are not removed, even if they are dead. These data are used to document the temporal pattern of emergence. Amphipods trapped by surface tension at the water surface are gently pushed down with a clean instrument (e.g., pipette, glass rod, beaker cover).

Termination—The bioassay is terminated after 10 days of exposure. After daily observations are recorded, the contents of the bioassay beakers are sieved through a 0.5-mm screen. Material retained on the screen is placed in clean bioassay water in a sorting tray. The numbers of live and dead amphipods are recorded. The sum of these numbers may not always equal 20 because of death and subsequent decomposition of amphipods. An amphipod is counted as alive if there is any sign of life (e.g., pleopod twitch observed under magnification, response to gentle prodding with a clean instrument).

If the reburial endpoint is to be evaluated, amphipods that survive the test are transferred to dishes containing a 2-cm layer of negative-control sediment and observed under constant illumination. The numbers of individuals able to bury after an exposure period of 1 hour is then recorded.

Experimental Design

Logistics—A typical sediment bioassay involves about 50–60 bioassay beakers. Collection and preparation of animals, sediment, and seawater requires at least four people for 2 days. Three or four people are required on the days experiments are initiated and terminated. One person can monitor the experiment in progress.

Controls—Five replicates of the amphipod collection-site control sediment are included in all bioassays. These beakers comprise a negative (clean) control that allows comparisons among experiments and among laboratories of the validity of the procedures used in individual investigations. In the negative control, mean mortality should be ≤10 percent and individual replicate mortality should be ≤20 percent for the test to be considered valid. Experiments in which contaminants are added to sediment may require additional solvent control replicates to determine effects of solvent addition.

A positive (contaminated) control is also required for all testing. This involves determining 96-hour LC_{50} values for *R. abronius* exposed to a reference toxicant in clean, filtered seawater without sediment (following standard bioassay procedures and under the same general test conditions as the sediment bioassays). Such data are necessary to determine the relative sensitivity of the animals (e.g., seasonal difference in sensitivity) for each test series to ensure comparability of the data. The commonly used reference toxicant is reagent-grade cadmium chloride. Swartz et al. (1986) determined a 96-hour LC_{50} of 1.61 mg/L for cadmium chloride. Acute lethality results for a reference toxicant must be reported along with the sediment bioassay results. Bioassays to establish

an LC_{50} involve four or five logarithmic concentration series and a control. At least one treatment should give a partial response below the LC_{50} and one above the LC_{50}. Statistical procedures for the LC_{50} estimate are given in APHA (1985) and ASTM 1989).

Reference Area Sediment—The design of field surveys typically includes a reference sediment involving five replicate laboratory tests of samples from an area believed to be free from sediment contamination. This provides a site-specific basis for comparison of potentially toxic and nontoxic conditions while controlling for the effects of exposing amphipods to non-native sediments. The grain size composition (as measured by percent silt plus clay and percent gravel) of the reference area sediment should be as similar as possible to that of the test sediment. Organic carbon content of reference area sediment should also be matched with the test sediment as closely as possible. However, it should be recognized that matching of organic carbon content may not be warranted in cases where pollution (e.g., from pulp mills, sewage outfalls, combined sewer overflows) is responsible for high organic content of test sediments.

DeWitt et al. (1988) found that sediments having a high percentage of fine-grained material could increase the mortality rate of *Rhepoxynius abronius* in the absence of apparent chemical contamination. DeWitt et al. (1988) developed a regression model to predict the relationship between amphipod mortality and sediment grain size. In their approach, test results (i.e., mean mortality values) that lie outside the 95-percent prediction limit developed from reference area data are considered indicative of chemical toxicity. A similar model can be applied to the *E. estuarius* bioassay (DeWitt et al. 1989).

Response Criteria—Endpoints examined in the amphipod bioassay include mortality, emergence from sediment, and failure to rebury in sediment at the end of the exposure period. Data on emergence and reburial are used to monitor sublethal behavioral responses of the amphipods during (i.e., emergence) and after (i.e., reburial) the 10-day exposure. Mortality after 10 days of exposure is the primary criterion of toxicity. An estimate of total effective mortality may also be calculated by summing percent mortality and percent failure to rebury. Each of these response criteria must be monitored in a "blind" fashion; that is, the observer must have no knowledge of the treatment of the sediment in the beakers. This is accomplished through randomization of beaker numbers.

DATA REPORTING REQUIREMENTS

The following data should be reported by all laboratories performing this bioassay:

- Water quality measurements during testing [i.e., dissolved oxygen, temperature, salinity, pH, sulfides (optional), and ammonia (optional)]
- Daily emergence for each beaker and the 10-day mean and standard deviation for each treatment

- Failure to rebury (optional) for each beaker and the mean and standard deviation for each treatment
- 10-day mortality and total effective mortality (optional) in each beaker and the mean and standard deviation for each treatment
- Interstitial water salinity for control, reference, and test sediments
- 96-hour LC_{50} values with reference toxicants (results for metallic compounds should be reported in terms of the metal ion rather than as the weight of the whole salt)
- Any problems that may have influenced data quality.

Exhibit A-1
Laboratory Sediment Bioassays
Amphipod Bioassay
Revised July 1991

BIVALVE LARVAE SEDIMENT BIOASSAY

USE AND LIMITATIONS

The bivalve larvae bioassay technique is described in *Standard Methods* (APHA 1985) and by ASTM (1989) as a rapid and reliable indicator of environmental quality. Pacific oysters (*Crassostrea gigas*) and blue mussels (*Mytilus edulis*) are recommended for testing. During the first 48 hours of embryonic development, fertilized oyster and mussel eggs normally develop into free-swimming, fully shelled larvae (prodissoconch I). Failure of the eggs to survive or the proportion of larvae developing in an abnormal manner have been used as the primary indicators of toxicity. A combined mortality and abnormality endpoint may also be calculated from the number of normal surviving larvae.

This sediment bioassay can be used to characterize the toxicity of marine sediments. It may be used alone as a screening tool in broad-scale sediment surveys, in combination with sediment chemistry and *in situ* biological indices, and in laboratory experiments addressing a variety of sediment and water quality manipulations.

The two species recommended for testing may show different levels of sensitivity to various contaminants. Therefore, the quantitative results for corresponding endpoints may not be strictly comparable between the two species. Nevertheless, results of statistical comparisons of test sediments with reference area sediments based on the two bivalve species as well as the related echinoderm embryo bioassay may be considered interchangeable for some purposes (e.g., regulatory decision-making).

The bivalve larvae bioassay probably can be used in sediments that have interstitial salinities less than 1 ppt, as the sediments are mixed and equilibrated with seawater prior to testing. However, because further testing is required to determine the validity of using this technique with such low salinity sediments, this bioassay is not recommended for sediments that have an interstitial salinity of less than 10 ppt. In addition, the following caveats apply:

- Bivalve larvae such as those of *C. gigas* normally are not associated with the types of sediments that generally are tested using this method. Hence, this bioassay is primarily an indicator of the relative toxicity among different samples because its direct ecological significance with respect to *in situ* sediments has not yet been established.
- Spawning of *C. gigas* occurs naturally in the Puget Sound area in summer. The natural spawning period for *M. edulis* is late spring to early summer. Both of these bivalves can be induced to spawn at other times of the year, but may show decreased viability of gametes. Gamete viability may also vary depending on the brood stock used. Accordingly, a positive control is recommended. This should comprise 48-hour LC_{50} and EC_{50} measurements with a reference toxicant in seawater only.

- Relative sensitivity of the three endpoints (percent mortality, percent abnormality, and percent combined mortality/abnormality) to toxic chemicals, natural chemical factors (e.g., total organic carbon, paralytic shellfish poison) and physical factors (e.g., suspended sediment) has not been evaluated.
- High mortalities in the seawater control and/or reference sediment tests may be occasionally observed. The cause of such mortality is unknown, but may be related to natural factors that reduce embryo quality.
- It is possible that abnormalities induced during testing may be underestimated due to poor recovery of living abnormal larvae from the sediments. Accordingly, it is recommended that a few sediment samples from each set of bioassays conducted with this technique be examined to determine whether living larvae are present in the sediment. The results should be quantified and reported.

FIELD PROCEDURES

Collection

Both test and reference area sediment should be collected in glass jars that have PTFE-lined lids and have been cleaned according to the procedures described in the section titled *General QA/QC Guidelines*. Each jar should be filled completely with sediment to exclude air. A minimum sediment sample size of 20 grams for each bioassay chamber is recommended for both kinds of sediment. Because five replicate tests are conducted for each field sample, and additional sediment is used for water quality monitoring, a minimum sediment sample size of 200 grams is recommended for each station.

Processing

Both test sediment and reference area sediment should be stored at 4°C in the dark. Holding time should not exceed 14 days.

LABORATORY PROCEDURES

The following procedures apply equally to larvae of both *C. gigas* and *M. edulis*, and are as described by Chapman and Morgan (1983) with the following changes incorporated:

- The salinity of test water is adjusted to 28 ppt
- Exposure time can range from 48 to 60 hours and depends on larval development in the negative controls
- Replication is increased from two to five to allow adequate statistical comparisons
- Larvae of *M. edulis* are included in the bioassay protocol
- Sediment holding time prior to testing is set at a maximum of 14 days

- Seawater holding time prior to testing is set at a maximum of 2 days for field-collected and reconstituted seawater
- Sediment resuspension in the test chambers is adequately accomplished by vigorous shaking for 10 seconds; there is no need to rotate the chambers for 3 hours at 10 rpm
- Twenty grams of sediment is suspended in 1 liter of seawater rather than 15 grams in 750 mL
- Sediments are allowed to settle in the bioassay chamber for 4 hours prior to inoculation with embryos
- pH is not adjusted before the bioassay starts and is only monitored
- Aeration is specified for test chambers in which dissolved oxygen concentrations decline below 60 percent of saturation
- A positive control (reference toxicant) is recommended
- Additional seawater controls are added for monitoring the stage of larval development
- Minimum sample sizes of 100 larvae in each replicate control chamber and 20 larvae in each replicate test chamber are recommended for abnormality measurements
- Additional details provided by ASTM (1989) for conditioning and spawning adults are included.

Bioassay Species

The species selected for testing depends on the availability of brood stock and spawning success during recent bioassays or pilot tests. For a given test or series of related tests, adult bivalves (brood stock) should be obtained from the same source: either commercial rearing facilities (oysters) or a chemically uncontaminated area (mussels). If brood stock is obtained from a commercial source, the original collection area should preferably be identified. Brood stock should be sexually mature individuals with normal, well-developed shells. Within 24 hours of collection or purchase, adults should be transported to the test laboratory and placed into flowing seawater similar in character to that from which they were taken. Rough handling, extended periods of desiccation, and abrupt changes in temperature, salinity, or other water quality variables must be avoided as these may induce premature spawning or render the stock useless for later controlled spawning or both. Upon receipt, adults should be cleaned of fouling organisms and detritus and placed in flowing seawater for conditioning.

Adult bivalves are held at recommended conditioning temperatures to stimulate final maturation of the gametes. The desired conditioning temperature (20 ± 1°C for oysters and 16 ± 1°C for mussels) and salinity (28 ± 1 ppt) should be attained gradually at increments not exceeding 2°C/day and 5 ppt/day. Conditioning may extend from a few days to several weeks depending on the physiological and gametogenic status of the adults. The length of the conditioning period is determined empirically by periodic sacrificial examination and spawning of representative individuals. Adults should be spawned or discarded within 2-3 weeks after attaining acceptable maturity because gamete quality will deteriorate rapidly with excessive conditioning. Adults should be provided with an adequate supply of natural or cultured phytoplankton. Natural seawater flow should be

about 28 liters/hour per individual adult. ASTM (1989) describes procedures for maintaining holding tanks. Procedures for inducing spawning in bivalves, enhancing the quality of gametes, and preparing embryos are described in ASTM (1989).

Bioassay Sediment

The bivalve larvae bioassay is conducted with reference area sediment in addition to seawater controls. Reference area sediment typically consists of material collected from an area documented to be free of chemical contamination and nontoxic to bivalve larvae.

Bioassay Seawater

Seawater used in the bioassay is maintained at a salinity of 28 ± 1 ppt and temperature of 20 ± 1°C for oysters and 16 ± 1°C for mussels. Seawater should be collected from uncontaminated areas (e.g., deep or offshore waters) to avoid contamination and should be held at ≤20°C for no longer than 2 days before inoculation. Reconstituted seawater (ASTM 1989) should be held at ≤20°C for no longer than 2 days before use. The bioassay seawater must be uncontaminated and of acceptably low toxicity. The biological criterion of acceptability is that the larvae, spawned by adults in the dilution water, must not incur more than 10-percent abnormal development or 30-percent mortality during 48 hours of exposure to the bioassay seawater .

Bioassay seawater is passed through an ultraviolet sterilizer or a filter with 0.45-µm pore diameter. If necessary, salinity of the bioassay water is reduced by addition of deionized distilled water or raised by addition of clean oceanic water, sea salt, or reagent grade chemicals (ASTM 1989). Artificial seawater is prepared within 2 days of use and is stored in clean, covered containers at the requisite temperature.

Facilities and Equipment

All laboratory glassware is cleaned according to the procedures specified in the section entitled *General QA/QC Guidelines*. The bioassay chamber is a 1-liter glass bottle with a screw-top lid. Bioassays are conducted at 20 ± 1°C for oysters and 16 ± 1°C for mussels, with the bottles in shallow water baths, incubators, or temperature-controlled rooms.

If adults are to be conditioned for spawning out of season, a continuous supply of temperature-controlled, aerated seawater is needed. Laboratory facilities should be well-ventilated and free of organic vapors. Holding and conditioning chambers preferably should not be in a room in which toxicity tests are conducted, stock or test solutions are prepared, or equipment is cleaned. Air used for aeration should be free of organic vapors, oil, and water. Raw seawater can be used for holding and conditioning, but feeding the adults a natural or cultivated alga is necessary to deter starvation. The flow rates used for adult conditioning must be high enough (typically >28 liters/hour/individual) to prevent water quality degradation and provide as much food as possible to the adults.

Tanks and trays are necessary for holding the adults, and a water bath, incubator, or temperature-controlled room is necessary during the bioassay. Adult holding and conditioning tanks should be cleaned several times each week to prevent accumulation of organic matter and bacteria. Dead specimens should be removed immediately and the tanks cleaned. The tanks should be cleaned with detergent and rinsed with clean seawater,

and if microbial growth is present, rinsed with 200 mg/L of hypochlorite and then seawater. With enriched waters and elevated conditioning temperatures, more frequent cleaning may be required.

Bioassay Procedure

Initiation—Adult bivalves, conditioned as necessary in the laboratory, are induced to spawn with selected thermal and biological (i.e., sperm) stimulation. Selected densities of the resulting embryos are exposed to the test or reference area sediments for 48 hours, during which the embryos normally will develop into prodissoconch I larvae. A slightly longer exposure period may be used if necessary to achieve adequate development of larvae in seawater controls. Exposure time should not exceed 60 hours for an acceptable test. Data from tests with longer exposures (>48 hours) may not be comparable to those from tests conducted using the standard 48-hour exposure. Toxicity test endpoints are based on abnormal shell development and larval death.

The bivalves are spawned by rapidly raising the water temperature to 5-10°C above the conditioning temperature. Individuals are additionally stimulated to spawn by the addition of sperm from a sacrificed or naturally spawned male.

Spawning is conducted by placing the bivalves in individual, clean Pyrex™ dishes containing filtered, ultraviolet (UV)-treated seawater. Fertilization is accomplished within 1 hour of spawning by combining eggs and sperm (i.e., at a concentration of 10^5–10^7 sperm/mL) in a 1-liter Nalgene beaker. The fertilized eggs are then washed through a 0.25-mm Nitex screen to remove excess gonadal material and suspended in 2 liters of filtered, UV-treated seawater at incubating temperature. The embryos are kept suspended by frequent agitation using a perforated plunger, and used in the bioassay within 2 hours of fertilization. When microscopic examination of fertilized eggs reveals the formation of polar bodies, egg density is determined from triplicate counts of the number of eggs in 1.0-mL samples of a 1:99 dilution of homogeneous egg suspension.

Sediment bioassays are conducted in clean, 1-liter glass bottles. Five replicate tests are conducted for each field sample. An additional bioassay chamber is prepared for water quality monitoring. Twenty grams (wet weight) of the appropriate sediment is added to each bottle and volume is brought up to 1 liter with filtered or UV-treated seawater (28 ± 1 ppt salinity) to make a final concentration in all containers of 20 grams (wet weight) of sediment per liter of seawater. The reference area sediment chambers each contain 20 grams of clean sediment. In addition, negative and positive controls for determination of LC_{50} and EC_{50} are prepared consisting of clean seawater without sediment.

The sediments are suspended by vigorous shaking for 10 seconds and the suspended sediments are allowed to settle for 4 hours prior to addition of larvae. No additional agitation is provided. The seawater controls are treated similarly except for the lack of sediments.

Within 2 hours of fertilization, each container is inoculated with 20,000–40,000 developing embryos to give a concentration of about 20–40/mL. The containers are covered and incubated for 48 hours (or longer if required) at 20 ± 1°C for oysters and 16 ± 1°C for mussels under a 14-hour light:10-hour dark photoperiod. Test chambers generally are not aerated during the bioassay. However, if the dissolved oxygen concentration in any test chamber declines below 60 percent of saturation, the water in that chamber should be aerated gently for

the remainder of the test. A random numbering method should be used to distribute the chambers in the water bath (or incubator or cold room).

The mean embryo concentration at 0 hours should be determined by collecting five replicate 10-mL samples from control cultures and preserving them in 5-percent buffered formalin. This method of determining the initial embryo concentration is one of three methods recommended by ASTM (1989) for larval bioassays of water and elutriates. The other two methods include 1) direct subsampling of each test chamber after inoculation and 2) direct subsampling of the stock solution. According to ASTM (1989), the preferred method is direct subsampling of test chambers after inoculation. This method provides the best estimate of embryo densities within each chamber and the variability of densities among chambers. However, this method cannot be easily used for the present bioassay because the sediment present in each test chamber prevents the contents of each chamber from being homogenized adequately for representative subsampling. Because the method recommended for the present bioassay does not rely on direct assessments of embryo densities in the test chambers, the resulting density estimates have an unquantified error component associated with them. This error reduces the reliability of larval mortality estimates and may thereby influence the results of statistical analyses. However, it does not affect larval abnormality estimates because they are based on known numbers of survivors.

Monitoring—Temperature, pH, salinity, and dissolved oxygen are measured daily in the replicates prepared specifically for monitoring water quality. Measurements are taken just prior to introduction of the embryos to the test beakers, then at the same time each day until the conclusion of the bioassay. Measurements of conventional water quality variables (e.g., sulfides, ammonia) should be made at the start and termination of the bioassay if it is suspected these variables may interfere with the results for the test sediment.

Termination—The bioassay is terminated when greater than 95 percent of the embryos in the duplicate seawater control have reached the prodissoconch I stage (approximately 48–60 hours). Once this stage has been achieved in the control beakers, final water quality measurements are recorded and the test is terminated. The bioassay is terminated in the following manner. The water and larvae overlying the settled sediment in each container are carefully poured into a clean 1-liter beaker. This water is then stirred, and 10-mL aliquots of the well-mixed sample are removed by pipette and placed in 10-mL screw-cap vials. The contents of each vial are preserved in 5-percent buffered formalin.

Preserved samples (equal in volume to those containing 300–500 larvae in controls) are examined in Sedgewick-Rafter cells. Normal and abnormal larvae are enumerated to determine percent survival and percent abnormality. A minimum sample size of 20 living larvae in each of the five replicate bioassay chambers for test sediment and reference area sediment and 100 larvae in each replicate chamber for the seawater control should be scored for abnormalities. Percent survival for each replicate bioassay chamber is based on the number of larvae surviving relative to the mean number of survivors in the seawater controls. Larvae that fail to transform to the fully shelled, straight-hinged, D-shaped prodissoconch I stage are considered abnormal. Percent abnormal for each replicate bioassay chamber is based on the number of survivors that are abnormal. Definitions of normal development specified at the PSDDA larval bioassay workshop should be followed. These definitions include the following:

- An uninterrupted shell must be formed around the margin; any indication that the shell cannot close (e.g., chips or knobs) constitutes an abnormality. Classification of open shells or shells

seen in other than side view should be made on best professional judgment. Empty shells that are complete count as normal, because they developed successfully to the shelled stage, no matter what happened thereafter.

- Larvae classified as normal must have a straight hinge by termination of experiment. If larvae have not reached D or prodissoconch I stage by the end of the exposure time (set by the duplicate sacrificial control vessel) they are considered abnormal.

Photomicrographs or drawings that illustrate the proper interpretation of "normal" and "abnormal" development are available from U.S. Army Corps of Engineers, Seattle District, or EPA Region 10.

Controls

Five replicates of the seawater control are included in all bioassays. These comprise negative (clean) controls that allow comparison among experiments and among laboratories of the validity of the procedures used in individual investigations. At least 70 percent of the larvae must survive the 48-hour exposure with seawater alone, and of these at least 90 percent must show no abnormalities. Experiments in which contaminants are added to sediment may require control replicates to determine effects of solvent addition.

A positive (toxic) control is also required. This involves determining 48-hour (or longer if required) LC_{50} and EC_{50} values for bivalve larvae exposed to reference toxicants in clean, filtered or UV-treated seawater without sediment [following standard ASTM (1989) bioassay procedures and under the same general test conditions as the sediment bioassays]. Such data are necessary to determine the relative sensitivity of the larvae. Two commonly used reference toxicants are reagent-grade cadmium chloride and sodium dodecyl sulfate. Either of these reference toxicants may be used, but the results must be reported along with the sediment bioassay results. Bioassays to establish an LC_{50} or an EC_{50} involve four or five logarithmic concentration series and a control. At least one treatment should give a partial response below the LC_{50} and EC_{50} and one above the LC_{50} and EC_{50}. Statistical procedures for the LC_{50} and EC_{50} estimates are given in APHA (1985) and ASTM (1989).

Reference Area Sediment

The design of field surveys may include a reference sediment from an area known to be free from chemical contamination. This provides a basis for comparison of potentially toxic and nontoxic conditions.

DATA REPORTING REQUIREMENTS

The following data should be reported by all laboratories performing this bioassay:

- Source, qualitative condition, and holding time of brood stock
- All water quality measurements [e.g., dissolved oxygen, temperature, salinity, pH, sulfides (optional), ammonia (optional)]
- Individual replicate and mean and standard deviation data for larval percent mortality after 48-hour exposure
- Individual replicate and mean and standard deviation data for larval percent abnormality after 48-hour exposure
- Individual replicate and mean and standard deviation data for larval percent combined mortality and abnormality after 48-hour exposure (optional)
- 48-hour LC_{50} and EC_{50} values for reference toxicants (with results for metallic compounds reported in terms of the metal ion, not as weight of the whole salt)
- Data on larval presence in the sediment
- Any problems that may have influenced data quality.

ECHINODERM EMBRYO SEDIMENT BIOASSAY

USE AND LIMITATIONS

The echinoderm embryo bioassay is described by Dinnel and Stober (1985) as a rapid and sensitive technique for assessing the toxicity of marine sediments. Purple sea urchins (*Strongylocentrotus purpuratus*), green sea urchins, (*S. droebachiensis*), and sand dollars (*Dendraster excentricus*) are the recommended species for testing. During the first 48–96 hours of embryonic development, fertilized echinoderm eggs normally develop into the pluteus stage. Failure of the eggs to survive and the proportion of larvae developing in an abnormal manner are used as indicators of toxicity. A combined mortality and abnormality endpoint may also be calculated from the number of normal surviving larvae.

The echinoderm embryo bioassay can be used to characterize the toxicity of marine sediments. It may be used alone as a screening tool in broad-scale sediment surveys, in combination with sediment chemistry and *in situ* biological indices, and in laboratory experiments addressing a variety of sediment and water quality manipulations.

The three species recommended for testing may show different levels of sensitivity to various contaminants. Therefore, the quantitative results for corresponding endpoints may not be strictly comparable between the three species. Nevertheless, results of statistical comparisons of test sediments with reference area sediments based on the three echinoderm species, as well as the related bivalve larvae bioassay, may be considered interchangeable for some purposes (e.g., regulatory decision-making).

The echinoderm bioassay probably can be used in sediments that have interstitial salinities less than 1 ppt, as the sediments are mixed and equilibrated with seawater prior to testing. However, because further testing is required to determine the validity of using this technique with such low salinity sediments, this bioassay is not recommended for sediments that have an interstitial salinity of less than 10 ppt. In addition the following caveats apply:

- Echinoderm larvae normally reside in the water column and are not intimately associated with sediments. Hence, this bioassay is primarily an indicator of the relative toxicity among different samples because its direct ecological significance with respect to *in situ* sediments has not yet been established.
- Spawning of *Strongylocentrotus* spp. occurs naturally in the Puget Sound region from December to April. The natural spawning period for *D. excentricus* is from April to October. Echinoderms can be induced to spawn at other times of the year, but may show decreased viability of gametes. Gamete viability may also vary depending on the brood stock used.

Accordingly, a positive control is recommended. This should comprise 48-hour LC_{50} and EC_{50} measurements with a reference toxicant in seawater only.

- Relative sensitivity of the three endpoints (percent mortality, percent abnormality, and percent combined mortality/abnormality) to toxic chemicals, natural chemical factors (e.g., total organic carbon, paralytic shellfish poison) and physical factors (e.g., suspended sediment) has not been evaluated.
- High mortalities in the seawater control and/or reference sediment tests may be observed occasionally. The cause of such mortality is unknown, but may be related to natural factors that reduce embryo quality.
- It is possible that abnormalities induced during testing may be underestimated due to poor recovery of living larvae from the sediments. Accordingly, it is recommended that a few sediment samples from each set of bioassays conducted with this technique be examined to determine whether living larvae are present in the sediment. The results should be quantified and reported.

FIELD PROCEDURES

Collection

Test Animals—All recommended echinoderm species can be collected off the coast of Washington. Purple sea urchins can be found in the intertidal zone and are usually ripe from December through March in Washington waters. Green sea urchins occur in the shallow subtidal zone and are usually ripe from January through April. Sea urchins should be collected with care to avoid injury from the sharp spines. Sand dollars are the preferred test species during the summer months as they are in spawning condition from about April through October. Sand dollars can be collected by hand on many Puget Sound beaches during low tide. All animals should be collected from uncontaminated areas.

Sediment—Both reference area and test sediment should be collected in glass jars that have PTFE-lined lids and have been cleaned according to the procedures described in the section entitled *General QA/QC Guidelines*. Each jar should be filled completely with sediment to exclude air. A minimum sediment sample size of 20 grams for each bioassay chamber is recommended for both kinds of sediment. Because five replicate tests are conducted for each field sample and additional sediment is used for water quality monitoring, a minimum sediment sample size of 200 grams is recommended for each station.

Processing

Sediment—Both control and test sediment should be stored at 4°C in the dark. Holding time should not exceed 14 days.

LABORATORY PROCEDURES

The following procedures are synthesized primarily from Dinnel and Stober (1985) and Dinnel and Kocan (1988). The following changes were incorporated:

- The salinity of test water is adjusted to 28 ppt
- Seawater temperature during the bioassay is maintained at 15 ± 1°C for all test species
- Exposure time can range from 48 to 96 hours and depends on larval development in the negative controls
- Replication is increased from three to five to allow adequate statistical comparisons
- Sediment holding time prior to testing is set at a maximum of 14 days
- Seawater holding time prior to testing is set at a maximum of 2 days for field-collected and reconstituted seawater
- Sediment is included in each test chamber
- Sediment resuspension in each test chamber is adequately accomplished by vigorous shaking for 10 seconds
- Sediment is allowed to settle in test chambers for 4 hours before addition of fertilized eggs
- pH is not adjusted before the bioassay starts and is only monitored
- Aeration is specified for test chambers in which dissolved oxygen concentrations decline below 60 percent of saturation
- A positive control (reference toxicant) is recommended
- Additional seawater controls are added for monitoring the stage of embryo development
- Two endpoints are measured: mortality and abnormality (a combined mortality/abnormality endpoint can also be calculated)
- Minimum sample sizes of 100 larvae in each replicate control chamber and 20 larvae in each replicate test chamber are recommended for abnormality measurements.

Bioassay Species

The species selected for testing depends on the availability of brood stock and spawning success during recent bioassays or pilot tests. For a given test or series of related tests, adult echinoderms (brood stock) should be obtained from the same source: either commercial harvesters or a chemically uncontaminated area. If brood stock is obtained from a commercial source, the original collection area should be identified. Within 24 hours of collection or purchase, adults should be transported to the test laboratory and placed into flowing seawater similar in character to that from which they were taken. Because epidemic spawning can occur when echinoderms are transported in seawater, test animals can be transported in ice chests containing only kelp or other moist material. If animals are transported in seawater, the seawater should be kept cool (4°C) and, if necessary, aerated. Rough handling, extended periods of desiccation, and abrupt changes in temperature, salinity, or other water quality variables must be avoided as these induce premature spawning or render the

stock useless for later controlled spawning or both. Upon receipt, adults should be cleaned of detritus and placed in flowing seawater. Sand dollars are best held on a bed of sand in flowing or well-aerated seawater.

Bioassay Sediment

The echinoderm embryo bioassay is conducted with reference area sediment in addition to seawater controls. Reference area sediment typically consists of material collected from an area documented to be free of chemical contamination and nontoxic to echinoderm embryos.

Bioassay Seawater

Seawater used in the bioassay is maintained at a salinity of 28 ± 1 ppt. Water temperature should be maintained at 15 ± 1°C for all test species. Seawater should be collected from uncontaminated areas (e.g., deep or offshore waters) to avoid contamination and should be held at ≤15°C for no longer than 2 days before inoculation. Reconstituted seawater (ASTM 1989) should be held at ≤15°C for no longer than 2 days before use. The bioassay seawater must be uncontaminated and of acceptable low toxicity. The biological criterion of acceptability is that the larvae, spawned by adults in the dilution water, must not incur more than 10-percent abnormal development or 30-percent mortality during 48 hours of exposure to the bioassay seawater.

Bioassay seawater is passed through an ultraviolet sterilizer or a filter with a 0.45-µm pore diameter. If necessary, salinity is reduced by addition of deionized distilled water or raised by addition of clean oceanic water, sea salt, or reagent-grade chemicals (ASTM 1989).

Facilities and Equipment

All laboratory glassware is cleaned according to the procedures specified in the section entitled *General QA/QC Guidelines*. The bioassay chamber is a standard 1-liter glass beaker (10-cm internal diameter) covered with an 11.4-cm-diameter watchglass. The beakers are maintained at 15 ± 1°C in a shallow water bath, incubator, or temperature-controlled room with an overhead aeration source. General recommendations of ASTM (1989) should be followed for materials used for test equipment, cleaning procedures, and good laboratory practices.

If adults are to be conditioned for spawning out of season, a continuous supply of temperature-controlled, aerated seawater is needed. Laboratory facilities should be well ventilated and free of organic vapors. Holding and conditioning chambers preferably should not be in a room in which toxicity tests are conducted, stock or test solutions are prepared, or equipment is cleaned. Air used for aeration should be free of organic vapors, oil, and water. Raw seawater can be used for holding and conditioning, but feeding the adults a natural or cultivated alga is necessary to deter starvation. The flow rates used for adult conditioning must be high enough (typically >28 liters/hour/individual) to prevent water quality degradation and provide as much food as possible to the adults.

Tanks and trays are necessary for holding adults. These are placed in a water bath, incubator, or temperature-controlled room to maintain proper temperature. Adult holding tanks should be cleaned several times each week to prevent accumulation of organic matter and bacteria. Dead specimens should be removed immediately and the tanks cleaned. The tanks should be cleaned with detergent and rinsed with clean seawater, and if

microbial contamination is present, rinsed with 200 mg/L of hypochlorite and then seawater. With enriched waters and elevated conditioning temperatures, more frequent cleaning may be required.

Bioassay Procedure

Initiation—Adult echinoderms, conditioned as necessary in the laboratory, are induced to spawn with chemical stimulation. Selected densities of the resulting embryos are exposed to the test or reference area sediments for 48 hours, during which the embryos normally will develop into the four-armed pluteus stage. A slightly longer exposure period may be used if necessary to achieve adequate development of embryos in seawater controls. Exposure time should not exceed 96 hours for an acceptable test. Data from tests with longer exposures (>48 hours) may not be comparable to those from tests conducted using the standard 48-hour exposure. Toxicity test endpoints are based on abnormal shell development and larval death.

Adult sea urchins are spawned by injecting 1 mL of 0.5-molar potassium chloride (KCl) through the peristomal membrane into the coelomic cavity. Sand dollars are injected with 0.5 mL of 0.5-molar KCl through the oral opening, with the syringe held at an angle. Animals are rinsed with clean seawater and inverted over individual 150–250 mL beakers filled with seawater for about 30 minutes until spawning is completed. As many as 12 females may need to be spawned to ensure an adequate quantity of eggs. The spawning beakers should be placed in a water bath or temperature-controlled room to maintain temperature at acclimation levels.

Eggs from females discharging relatively small numbers of eggs (e.g., <100,000) are discarded. The retained eggs are examined microscopically for viability and ripeness. Ripe, viable eggs are normally round, uniform in size, free of excessive debris, and appear slightly granular. Immature eggs contain a large, clear spot (the germinal vesicle) in the cytoplasm; overripe eggs are usually less circular, have inconsistent granularity of the cytoplasm, and are often associated with increased debris. If the proportion of underripe or overripe eggs in a beaker exceeds 10 percent, the eggs are discarded. Eggs that are accepted are pooled together into a 1-liter beaker and washed three times by repeatedly decanting the water above the eggs and adding 500–1,000 mL new seawater (allow the eggs to settle to the bottom of the beaker between washes). Small subsamples of eggs are counted using a dissecting microscope to determine the number of eggs per milliliter.

The solutions of sperm from males producing thick, viscous discharges are combined to provide a stock solution. Sperm density is determined by immobilizing the sperm (i.e., by heat shock or exposure to 10-percent glacial acetic acid) and counting on a hemocytometer. Fertilization should be initiated within 1 hour of spawning by adding sperm to the beaker containing the eggs, at a sperm:egg ratio of ≤2,000:1. A perforated plastic plunger is used to gently mix the contents of the beaker. Care should be taken that excessive amounts of sperm are not used. Fertilization is monitored by examining successive 1-mL aliquots microscopically and determining the percentage of eggs with a raised fertilization membrane. When greater than 90 percent of the eggs show membrane formation (about 10-15 minutes), the developing eggs are counted and the density is adjusted to 20,000–30,000 per mL, either by diluting with seawater to decrease density or decanting excess surficial water to increase density.

Test beakers should be prepared prior to spawning of the adult echinoderms to allow enough time for sediments to settle in the test beakers before inoculation with the fertilized eggs. Five replicate tests are conducted for each field sample. An additional bioassay chamber is prepared for water quality monitoring. Twenty grams of reference or test sediment is added to each beaker. Filtered or UV-treated seawater (28 ppt salinity) is added to each beaker up to 1 liter to make a final concentration in all containers of 20 grams (wet weight) of

sediment per liter of seawater. Each reference area sediment chamber also contains 20 grams/liter of clean sediment. In addition, two control series are prepared consisting of clean seawater without sediment (one series is used as a duplicate, sacrificial control to monitor embryo development).

The sediments are suspended by vigorous shaking for 10 seconds and then allowed to settle for 4 hours prior to addition of the embryos. No additional agitation is provided. The seawater control beakers are treated similarly.

Within 2 hours of fertilization, a 1-mL aliquot of the solution of embryos (about 25,000 embryos) is added to each bioassay chamber using an automatic pipette. The containers are covered with a watchglass and incubated for 48 hours (or longer if required) at 15 ± 1°C under a 14-hour light:10-hour dark photoperiod. Test chambers generally are not aerated during the bioassay. However, if the dissolved oxygen concentration in any test chamber declines below 60 percent of saturation, the water in that chamber should be aerated gently for the remainder of the test. A random numbering method should be used to distribute the chambers in the water bath (or incubator or cold room).

The mean embryo concentration at 0 hours should be determined by collecting five replicate 10-mL samples from thoroughly mixed control cultures and preserving them in 5-percent buffered formalin. This method of determining the initial embryo concentration is one of three methods recommended by ASTM (1989) for larval bioassays of water and elutriates. The other two methods include 1) direct subsampling of each test chamber after inoculation and 2) direct subsampling of the stock solution. According to ASTM (1989), the preferred method is direct subsampling of test chambers after inoculation. This method provides the best estimate of embryo densities within each chamber and the variability of densities among chambers. However, this method cannot be easily used for the present bioassay because the sediment present in each test chamber prevents the contents of each chamber from being homogenized adequately for representative subsampling. Because the method recommended for the present bioassay does not rely on direct assessments of embryo densities in the test chambers, the resulting density estimates have an unquantified error component associated with them. This error reduces the reliability of larval mortality estimates. However, it does not affect larval abnormality estimates because they are based on known numbers of survivors.

Monitoring—Temperature, pH, salinity, and dissolved oxygen are measured daily in the replicates prepared specifically for monitoring water quality. Measurements are taken just prior to introduction of the embryos to the test beakers, then at the same time each day until the conclusion of the bioassay. Measurements of conventional water quality variables (e.g., sulfides, ammonia) should be made at the start and termination of the bioassay if it is suspected these variables may interfere with the results for the test sediment.

Termination—The bioassay is terminated when greater than 95 percent of the embryos in the duplicate seawater control have reached the four-armed pluteus stage (approximately 48–96 hours). Once this stage has been achieved in the control beakers, final water quality measurements are recorded and the test is terminated.

The bioassay is terminated in the following manner. The water and larvae overlying the settled sediment in each beaker are carefully poured into a clean 1-liter beaker. The water is then mixed thoroughly using a perforated plunger and 10-mL aliquots of the sample are removed by pipette and placed in 10-mL screw-cap vials. The contents of each vial are preserved in 5-percent buffered formalin.

Preserved samples (equal in volume to those containing 300–500 larvae in controls) are examined in Sedgewick-Rafter cells. Normal and abnormal larvae are enumerated to determine percent survival and percent abnormality. Percent survival for each replicate bioassay chamber is based on the number of larvae surviving in each test container relative to the initial number. Percent mortality is then calculated, including correction for mortality in the seawater control. Larvae that fail to transform into clearly defined pluteus with two well-developed arms and the second pair of arms budding are considered abnormal. A minimum sample size of 20 living larvae in each of the five replicate bioassay chambers for test sediment and reference area sediment and 100 larvae in each replicate chamber for the seawater control should be scored for abnormalities. Aliquot counts should be completed even after the minimum sample size is achieved. Percent survival for each replicate bioassay chamber is based on the number of larvae surviving relative to the mean number of survivors in the seawater controls. Embryos that fail to transform to the four-armed pluteus stage are considered abnormal. Percent abnormal for each replicate bioassay chamber is based on the number of survivors that are abnormal.

Controls

Five replicates of the seawater control are included in all bioassays. These comprise negative (clean) controls that allow comparison among experiments and among laboratories of the validity of the procedures used in individual investigations. At least 70 percent of the larvae must survive the 48-hour exposure with seawater alone, and of these at least 90 percent must show no abnormalities. Experiments in which contaminants are added to sediment may require control replicates to determine effects of solvent addition.

A positive (toxic) control is also required. This involves determining 48-hour (or longer) LC_{50} and EC_{50} values for echinoderm larvae exposed to reference toxicants in clean, filtered or UV-treated seawater without sediment [following standard ASTM (1989) bioassay procedures and under the same general test conditions as the sediment bioassays]. Such data are necessary to determine the relative sensitivity of the larvae. Two commonly used reference toxicants are reagent-grade cadmium chloride and sodium dodecyl sulfate. Either of these reference toxicants may be used, but the results must be reported along with the sediment bioassay results. Bioassays to establish an LC_{50} or an EC_{50} involve four or five logarithmic concentration series and a control. At least one treatment should give a partial response below the LC_{50} and EC_{50} and one above the LC_{50} and EC_{50}. Statistical procedures for LC_{50} and EC_{50} estimates are given in APHA (1985) and ASTM (1989).

Reference Area Sediment

The design of field surveys may include a reference sediment from an area known to be free from chemical contamination. This provides a basis for comparison of potentially toxic and nontoxic conditions.

DATA REPORTING REQUIREMENTS

The following data should be reported by all laboratories performing this assay:

- Source, qualitative condition, and holding time of brood stock
- All water quality measurements [e.g., dissolved oxygen, temperature, salinity, pH, sulfides (optional), and ammonia (optional)]

- Individual replicate and mean and standard deviation data for larval percent mortality at termination of bioassay
- Individual replicate and mean and standard deviation data for larval percent abnormality at termination of bioassay (optional)
- Individual replicate and mean and standard deviation data for larval percent combined mortality plus abnormalities at termination of bioassay
- LC_{50} and EC_{50} values for reference toxicants (with results for metallic compounds reported in terms of the metal ion, not as weight of the whole salt)
- Data on larval presence in the sediment
- Any problems that may have influenced data quality.

Exhibit A-2
Modifications to Biossay Protocols

Bioassay Protocols:

PSDDA Modifications to the Amphipod and Sediment Larval Tests

The following outline lists changes to the amphipod and sediment larval bioassay protocols which the PSDDA agencies have adopted or recommended since the PSEP Protocols (*6*) were published.

I. 10-day amphipod mortality bioassay modifications.

   A. Bioassay laboratories should avoid sexually dimorphic male amphipods (*19*).
   B. Reference sediments should be collected subtidally and be grain size matched to test sediment samples using a wet sieving technique (*8,19*).
   C. Laboratories should analyze reference sediment samples for bulk ammonia and total sulfides for 10-day amphipod mortality bioassay (*8*).
   D. Laboratories should collect and report amphipod reburial data (*9*).
   E. The holding time for bioassay test samples was extended to a maximum of eight weeks (*9*).
   F. Under certain circumstances, *Ampelisca abdita* or *Eohaustorius estuarius* may be substituted for *Rhepoxinius abronius* (*11*).

II. Sediment larval bioassay modifications.

   A. Echinoderm larval bioassays should be conducted at 15°C for a minimum of 48 hours. Guidelines for initiating and terminating the test were recommended (*19*)
   B. Alternate reference areas may be used, if the sediment chemistry of those areas is adequately characterized (*8*).
   C. Laboratories should analyze reference sediment samples for bulk ammonia and total sulfides for echinoderm larval bioassay (*8*).
   D. PSDDA adopted several changes to the test temperature, duration, endpoint and termination of the echinoderm larval bioassay protocol (*9*).

E. The holding time for bioassay test samples was extended to a maximum of eight weeks (*9*).

F. Seawater control and reference sample performance standards were revised. The seawater control abnormality standard was eliminated. The performance standard for seawater control samples was changed to $\leq$ 30% effective mortality (abnormality plus mortality). Reference samples should not exceed 30% effective mortality when seawater control-normalized (*12*).

G. In order to increase statistical power, certain modifications were made to interpretive guidelines for the sediment larval bioassay (*12*).

Exhibit A-3
Sediment Larvae Bioassay
Data Entry and Response Calculation

Sediment Larval Bioassays: Data entry and Response Calculation.

Sediment larval bioassay laboratory results were entered in the sediment quality values database (SEDQUAL) using the same standard conventions as were used prior to calculating 1988 AETs *(PTI Environmental Services, Inc., personal communication)*. Total and normal larvae surviving, both at test initiation and conclusion, were entered as described below. The percent abnormality, mortality and effective mortality (abnormality + mortality) endpoints for echinoderm and bivalve larval bioassays were then calculated from those data.

I. Data Entry

A. Abnormality Endpoint.

1. Sea water (negative) control sample.
   a. Initial Value = the total number of survivors in each replicate (normal + abnormal) at the end of the sea water control exposure.
   b. Final Value = the total number of normal survivors in each replicate at the end of the sea water control exposure.

2. Positive control sample.
   a. Initial Value = the total number of survivors in each replicate (normal + abnormal) at the end of the positive control exposure.
   b. Final Value = the total number of normal survivors in each replicate at the end of the positive control exposure.

3. Reference area and test sediment samples.
   a. Initial Value = the total number of survivors in each replicate (normal + abnormal) at the end of the test/reference sediment sample exposure.
   b. Final Value = the total number of normal survivors in each replicate at the end of the test/reference sample exposure.

I. **Data Entry** (continued).

B. **Mortality Endpoint.**

1. Sea water (negative) control sample.
   a. Initial Value = the stocking density = the average number of larvae introduced to individual beakers or containers at the beginning of the sea water control exposure.
   b. Final Value = the total number of survivors (normal + abnormal) in each replicate at the end of the sea water control exposure.

2. Positive control sample.
   a. Initial Value = the average total number of survivors (average of all replicates) at the end of the sea water control exposure.
   b. Final Value = the total number of survivors in each replicate (normal + abnormal) at the end of the positive control exposure.

3. Test/reference area sediment samples.
   a. Initial Value = the average total number of survivors (average of all replicates) at the end of the sea water control exposure.
   b. Final Value = the total number of survivors in each replicate (normal + abnormal) at the end of the test/reference sample exposure.

I. **Data Entry** (continued).

C. **Effective Mortality Endpoint (Abnormality + Mortality).**

1. Sea water (negative) control sample.
   a. Initial Value = the stocking density = the average number of larvae introduced to individual beakers or containers at the beginning of the sea water control exposure.
   b. Final Value = the total number of normal survivors in each replicate at the end of the sea water control exposure.

2. Positive control sample.
   a. Initial Value = the average total number of normal survivors (average of all replicates) at the end of the sea water control exposure.
   b. Final Value = total number of normal survivors in each replicate at end of positive control exposure.

3. Test/reference sediment samples.
   a. Initial Value = the average total number of normal survivors (average of all replicates) at the end of the sea water control exposure.
   b. Final Value = the total number of normal survivors in each replicate at the end of the test/reference sediment sample exposure.

Exhibit A-3
Sediment Larvae Bioassay
Data Entry and Response Calculation

II. Calculation of percent abnormality, mortality and effective mortality.

Percent larvae abnormality, mortality and effective mortality were calculated using the appropriate Initial and Final Values, described above, as:

$$\frac{[\text{Initial (test sample) Value} - \text{Final (test sample) Value}]}{\text{Initial (test sample) Value}} \times 100$$

Figure A-1. Reference sample amphipod mortality: frequency distribution of standard deviations. Arrow indicates 95th percentile value.

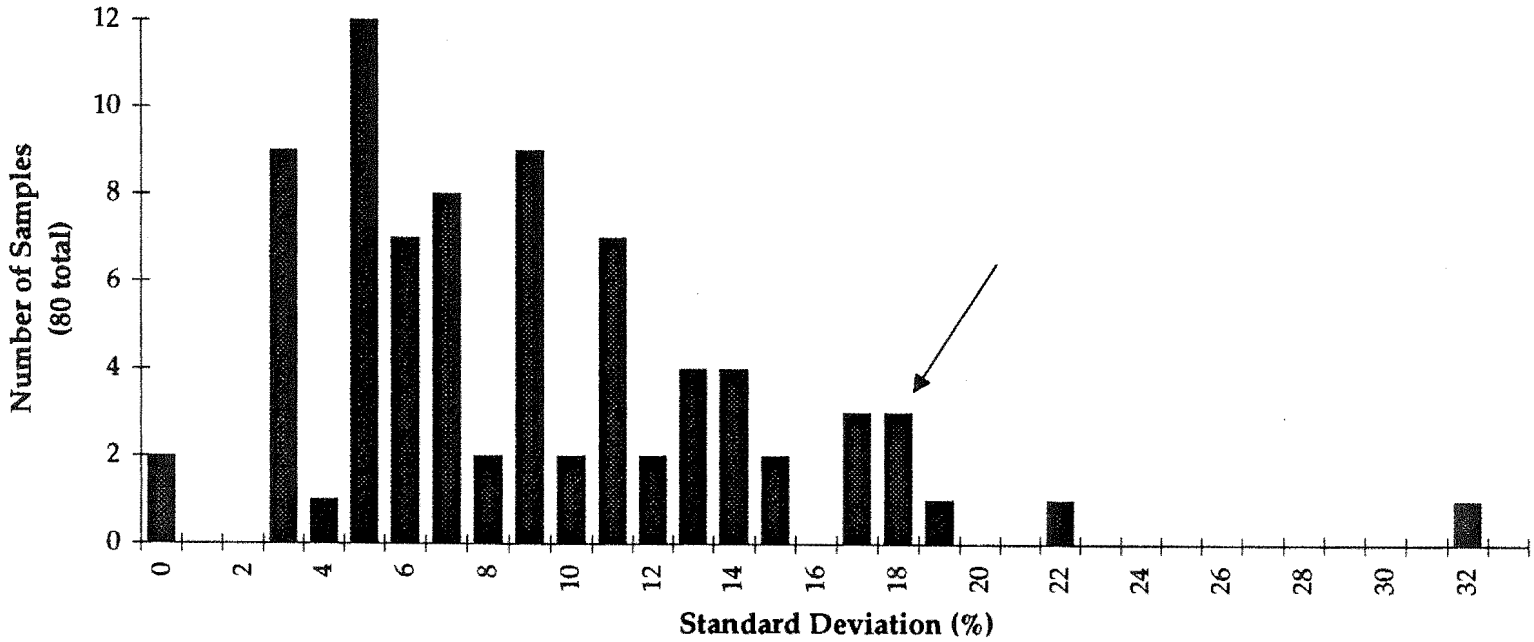


Figure A-2. Reference sample larval abnormality: frequency distribution of standard deviations. Arrow indicates 95th percentile value.

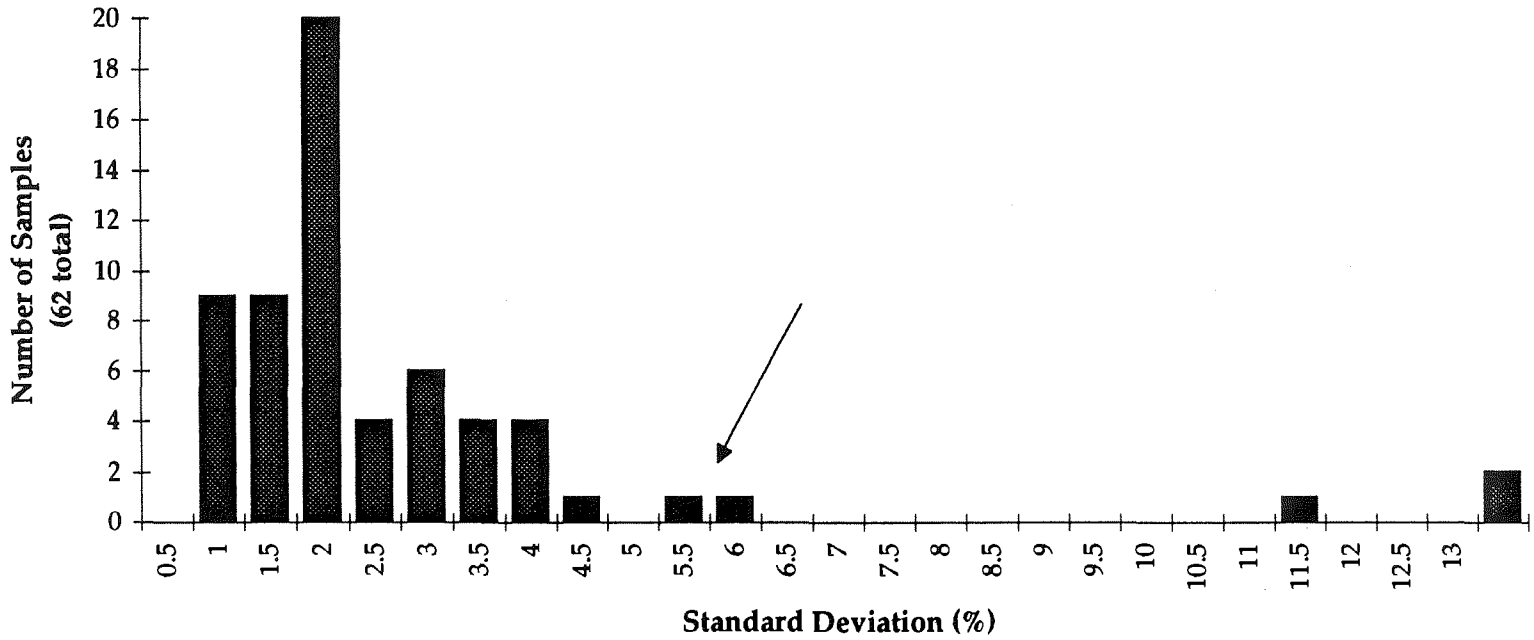


Figure A-3. Reference larval effective mortality: frequency distribution of standard deviations. Arrow indicates 95th percentile value.

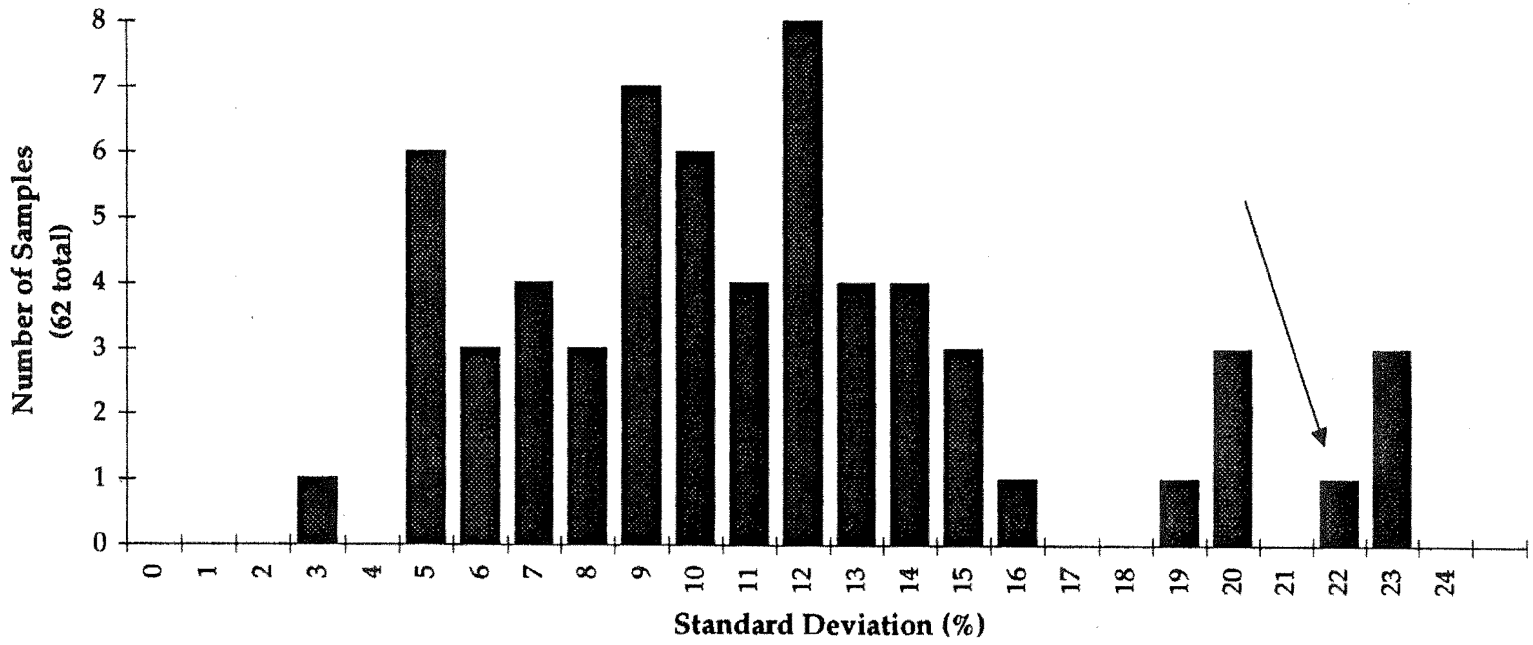


Figure A-4. Phased determination of significant adverse effects in amphipod mortality bioassay samples. A test sample is considered to be a "Hit" if it exhibits greater than or equal to 25% mortality and is significantly different ($p<0.05$) from the corresponding reference sample(s).

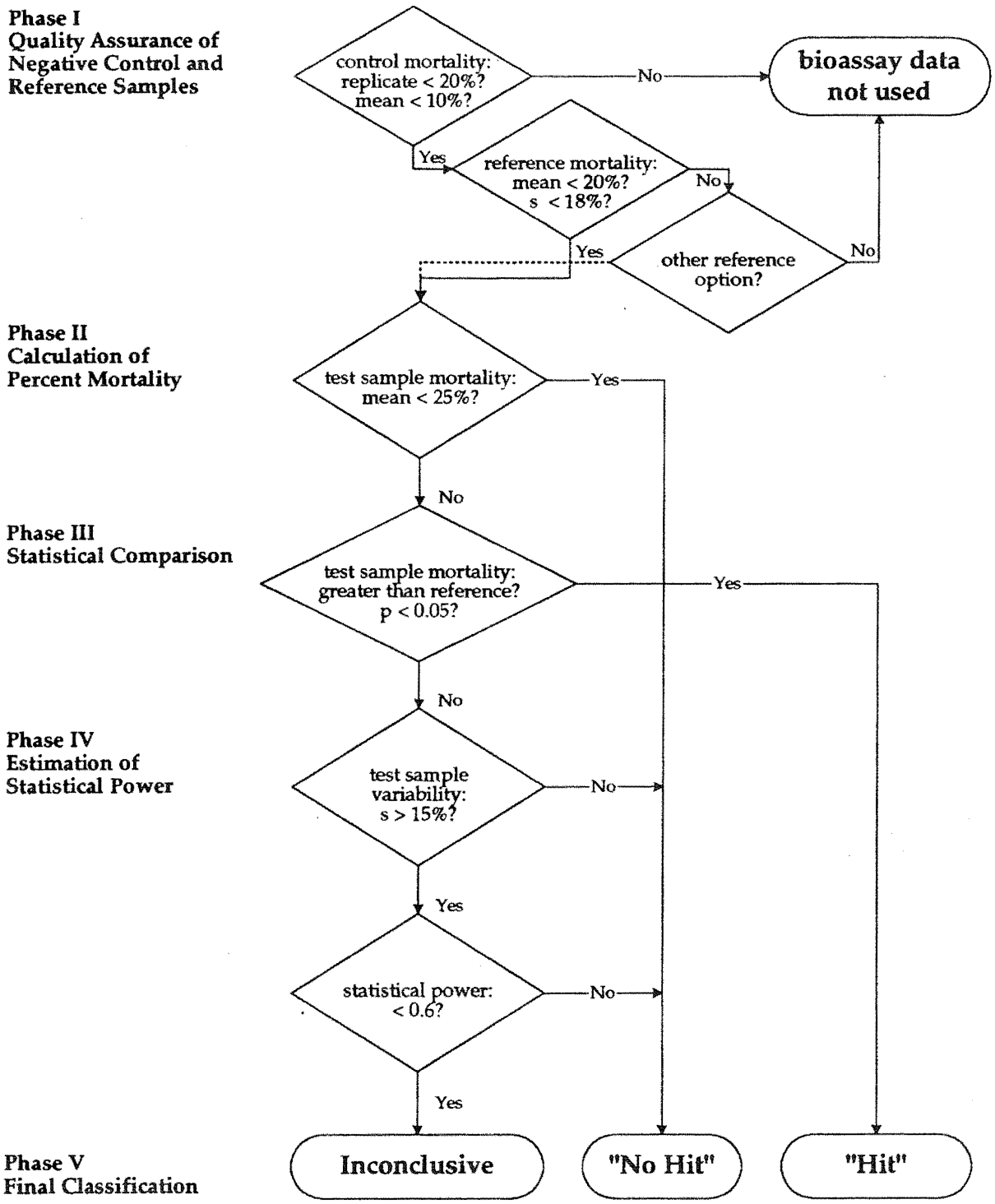


Figure A-5. Test sample amphipod mortality: frequency distribution of standard deviations. Arrow indicates 80th percentile value.

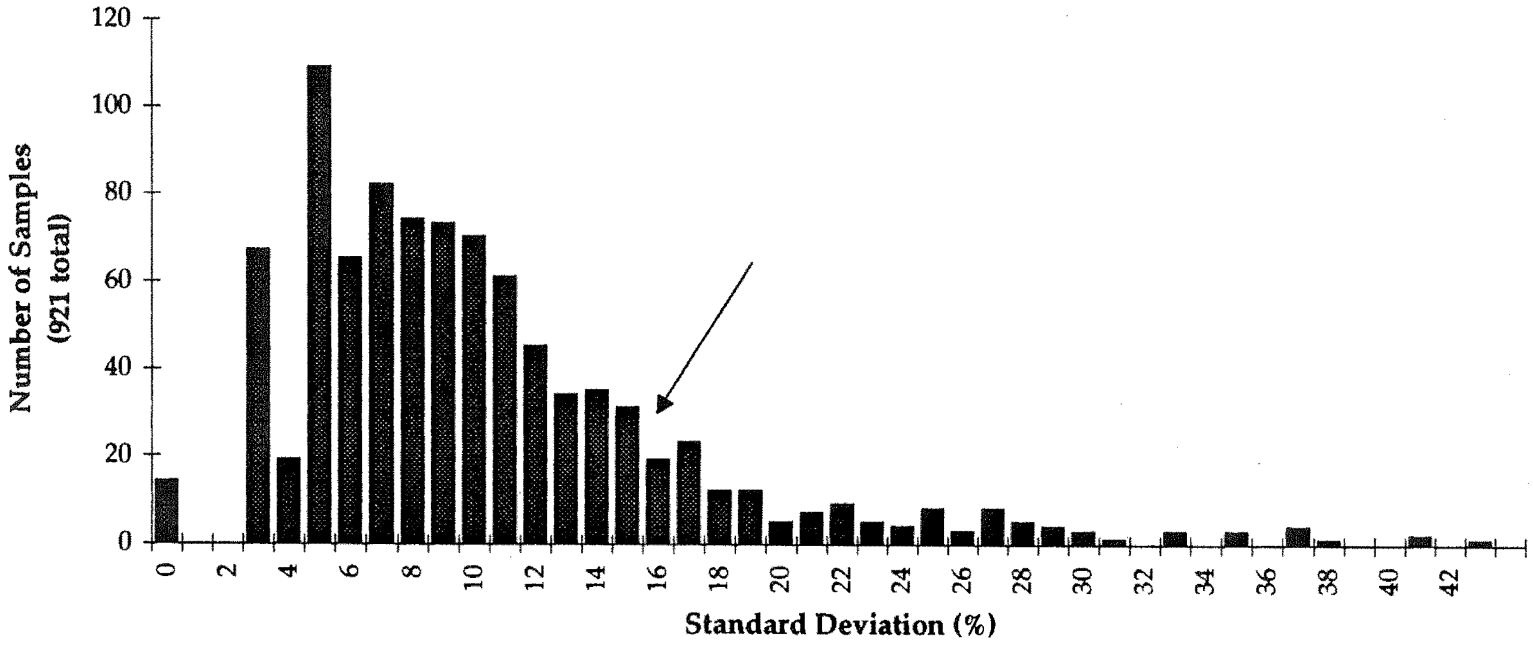


Figure A-6. Phased evaluation procedure for determining significant adverse effects in echinoderm larvae abnormality bioassays. A test sample is considered to be a "Hit" if it exhibits significantly greater abnormality ($p<0.05$) than the corresponding reference sample(s).

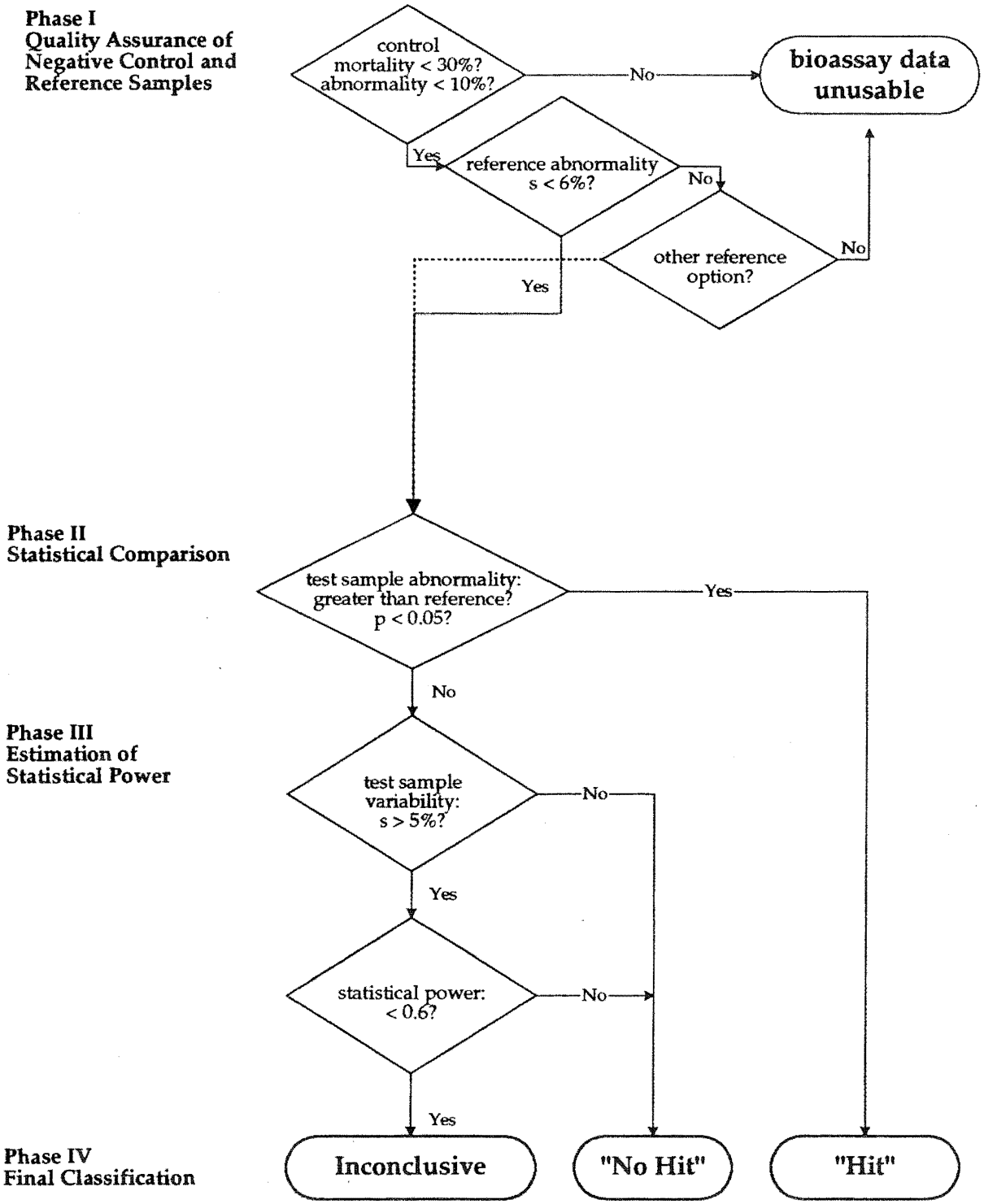


Figure A-7. Test sample larval abnormality: frequency distribution of standard deviations. Arrow indicates 80th percentile value.

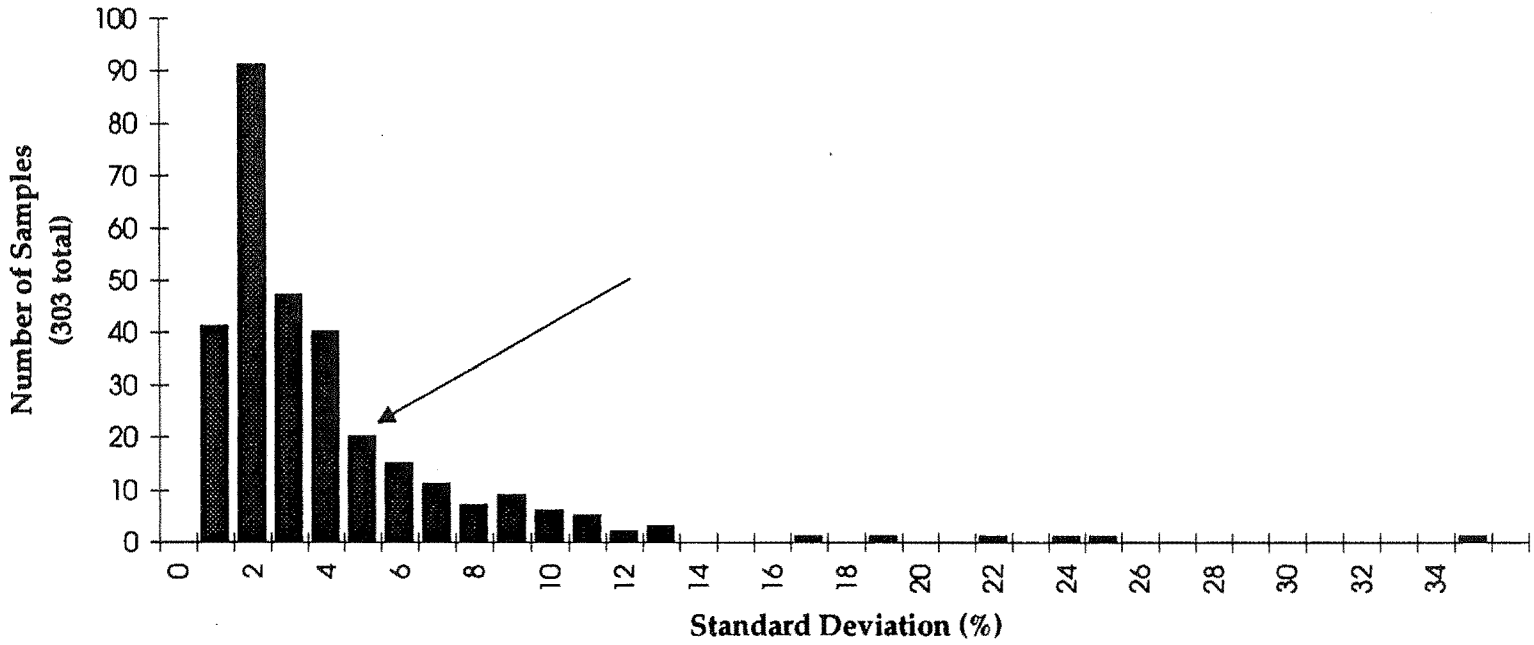


Figure A-8. Phased determination of significant adverse effects in echinoderm larval effective mortality bioassays. Three separate definitions of a "Hit" sample are explored in this investigation: if the effective mortality (abnormality plus mortality) of test samples is a) significantly greater than that of the corresponding reference sample ($p<0.05$), b) 15% > the reference ($p<0.05$), and c) 30% > the reference ($p<0.05$).

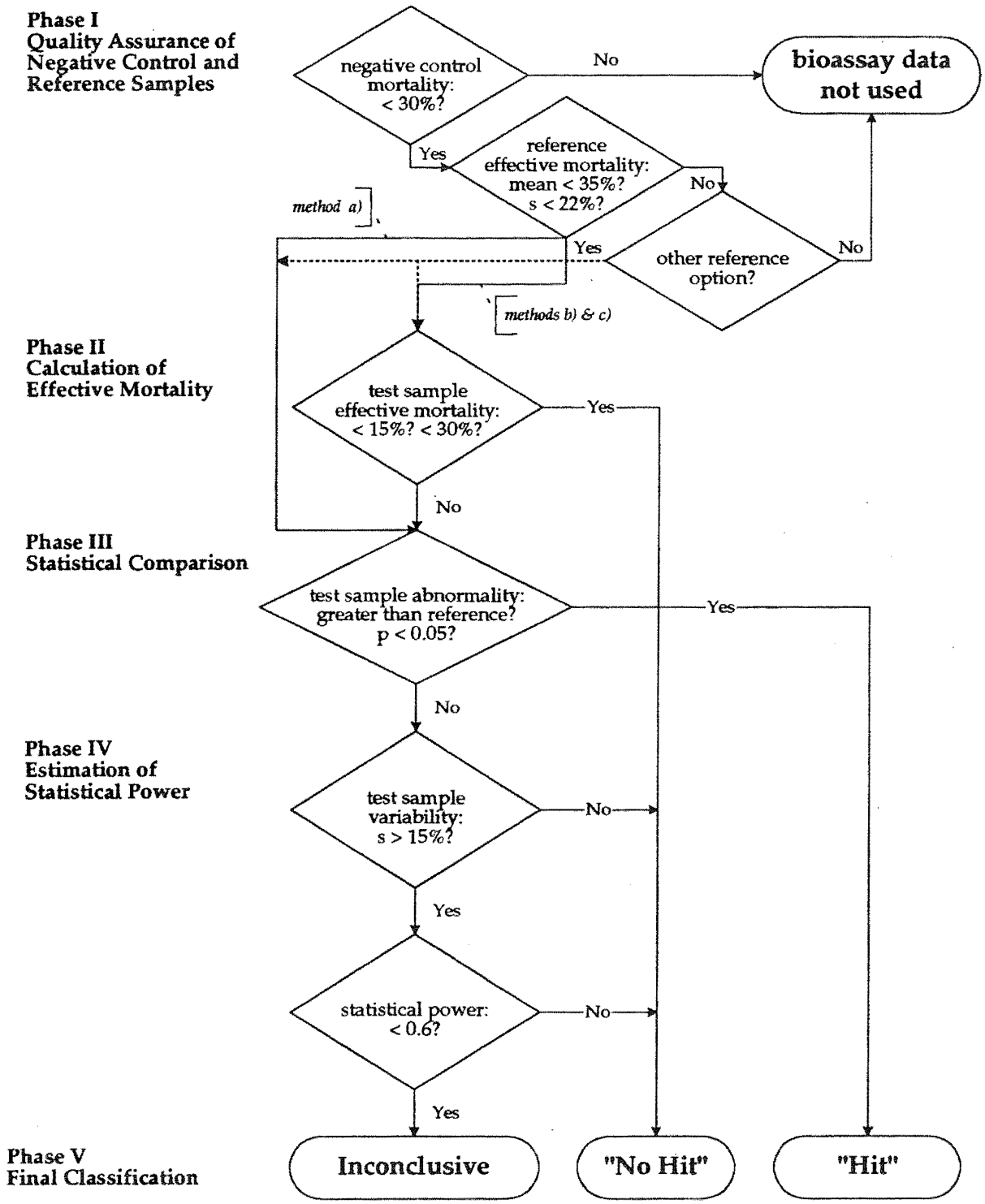
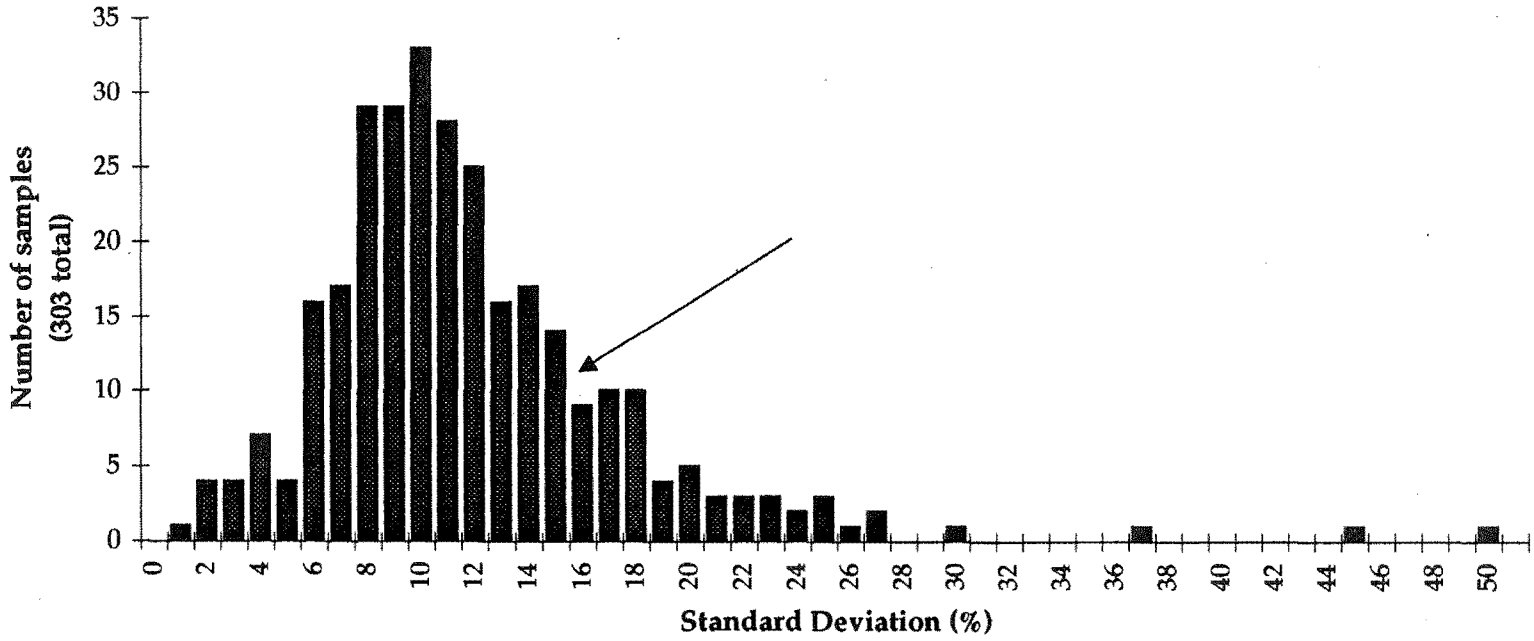


Figure A-9. Test sample larval effective mortality: frequency distribution of standard deviations. Arrow indicates 80th percentile value.



APPENDIX B:

The 1994 Puget Sound Sediment Quality Database (SEDQUAL)

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Table B-1. Inventory of synoptic surveys and bioassay samples for potential use in calculating 1994 AETs. Surveys are ordered alphabetically by Survey Code. Those used to derive 1988 amphipod, 1988 benthic, 1986 Micr and 1986 oyster AET and reliability values are highlighted. A = amphipod mortality (Rhepoxinius abronius), B= b infaunal abundance, E = echinoderm larval abnormality (Dendraster excentricus, Stongylocentrotus purpuratus, S. droebachiensis,), M = Microtox luminosity, and O = oyster/bivalve larval abnormality (Crassostrea gigas or Mytilus edulis). Sample numbers are totalled at the end.

Survey Code	Survey Name	A	B	E	M	O
1TREE_89	LOTT outfall dredging sediment characterization	3				
ALKI	METRO survey of Alki Point sewage treatment outfall		**11**			
AMPRES92	American President's Line maintenance dredging	2		2		
ANCHOR90	Anchor Cove condominium marina project	1		1		
ARCOCPC2	ARCO Cherry Point refinery, Class II inspection	2		2		
BCWTACC2	Boise Cascade's W. Tacoma mill, Class II inspection	2				
BLAIR_91	Port of Tacoma, Blair Waterway project	27		27		
BLGM_91A	Maintenance/other dredging of Bellingham Bay.	37		20 ?		
BPFERNC2	BP Oil Refinery, Class II inspection	3				
BREMWTC2	Bremerton treatment plant, Class II inspection	3				
CASCADRI	Cascade Pole Remedial Investigation	1		1		
CBBLAIR	Commencmnt Bay RI Blair Waterway dredging	**6**	**6**			**6**
CBMSQS	Commencement Bay RI, main sediment quality survey	**52**	**48**		**50**	**50**
CGPIER35	U.S. Coast Guard Pier 35 dredging project	3		3		
CHEVMD90	Chevron USA Edmonds dock maintenance dredging	3		3		
CNKTSPC2	1988 Central Kitsap treatment plant, Class II inspection	3				
COLUMBIA	Columbia Cement proposed maintenance dredging	1				
DAYISL91	Day Island Yacht Club (Tacoma) sediment characterizat	1		1		
DUWO&M89	Duwamish River maintenance dredging project, 1989	30		30		
DUWO&M90	Duwamish River maintenance dredging, Phase 1	4				4
DUWO&M91	Duwamish River maintenance dredging, Phase 2	14		14		
DUWRIV1	PSDDA Duwamish River I data set	**9**				
DUWRIV2	PSDDA Duwamish River II data set	**31**				
DUYACHT1	Duwamish Yacht Club marina maintenance dredging	5		5		

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Survey Code	Survey Name	A	B	E	M	O
EBCHEM	1985 Elliott Bay sediment quality survey	**110**	**82**			
EDMDWTC2	Edmonds treatment plant, Class II inspection	3				
EHCHEM	Eagle Harbor sediment chemistry survey	**10**	**10**			
EHRITM10	Eagle Harbor RI, Technical Memo. #10	6				6
EIGHTBAY	1985 Puget Sound Eight-Bay survey	**48**				
EVCHEM	1985 Everett Harbor chemistry & biological data	**29**	**19**			
EVERETT1	A subset of NAVYHP85 (Navy Homeport EIS)	**6**				
EVMAR_88	Everett Marina maintenance dredging	5				5
EVRT10TH	Everett Harbor, 10th St. boat ramp expansion	1		1		
EVRT12TH	Everett Harbor, 12th St. barge channel dredging	6		6		
EVWEYCII	Everett Weyerhaueser, Class II inspection	3				
GAPAC_C2	Georgia Pacific (Bellingham), Class II inspection	3				
HULB90FC	Hulbert Mill proposed 12th St. marina	8		8		
HURLEN89	Hurlen Construction Co. maintenance dredging	1				
INTALCC2	1988 Intalco, Class II inspection	4				
LONEST89	Lonestar NW, maintenance dredging in Duwamish Riv	1				
LONEST92	Lonestar Northwest, west terminal dredging	1		1		
LOTTO041	Olympia treatment plant (LOTT) outfall sampling, DY8	3				
LOTTO043	Olympia treatment plant (LOTT) outfall sampling, DY9	1		1		
METAMB88	1988 METRO NPDES & ambient subtidal monitoring	8				
METAMB90	1990METRO NPDES & ambient subtidal monitoring	8				
METAMB92	1992 METRO NPDES & ambient subtidal monitoring	8				
METROEBP	METRO's West Point emergency bypass outfall samplir	7		7		
MORTON89	Morton Marine wharf construction & draft increase	1				

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Survey Code	Survey Name	A	B	E	M	O
MORTON92	Morton Marine maintenance dredging	1		1		
NAVYHP85	U.S. Navy Homeport sediment characterization, 1985	6				
NAVYHPFC	U.S. Navy Homeport (full sediment characterization)	31		31		
NAVYHPII	U.S. Navy Homeport Element II full characterization	4		4		
NAVYMANC	U.S. Navy Manchester fuel pier replacement	15		15		
OLYHARFC	Olympia Harbor planning, full characterization	13		13		
OLYTERC2	Olympus Terrace treatment plant, Class II inspection	3		3		
PENNWLC2	Pennwalt, Class II inspection	3				
PIER53BL	Pier 53-55 sediment capping project, baseline	6				6
PIERD_91	U.S. Navy Bremerton Pier D	28		28		
POSTPTC2	Post Point (Bellingham) treatment plant, Class II inspec	3				
PSDDA1	PSDDA Phase 1 baseline survey	14				14
PSDDA2	PSDDA Phase 2 baseline survey	6				6
PSDDAM90	1990 PSDDA post-disposal site monitoring	10		10		
PSDDAM91	1991 PSDDA site monitoring/Port Gardner PGB09	1		1		
PSDDAM92	1992 PSDDA full monitoring, Elliott Bay	3		3		
PSREF90	Puget Sound reference areas survey	21		21		21
PTORCHC2	Port Orchard treatment plant, Class II inspection	3				
PTWNPCC2	Port Townsend Paper Company, Class II inspection	3				
PTWNPENR	Port Townsend pen-reared salmon study	2				
SEATLSC2	Seattle Steel, Class II inspection	2				
SED18804	Puget Sound non-urban reconnaisance survey	24				
SED18903	Puget Sound Ambient Monitoring - 1989	50				
SED19003	Puget Sound Ambient Monitoring - 1990	82				

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Survey Code	Survey Name	A	B	E	M	O
SED19103	Puget Sound Ambient Monitoring - 1991	61				
SHELLCII	Shell Oil refinery (Anacortes), Class II inspection	1				
SITCUMRI	Port of Tacoma RI/NRDA (Sitcum/Milwaukee/Blair)	79		38 ?		38
SNDREF92	Sound Refining NPDES sediment monitoring	3		3		
SOPARK91	South Park Marina maintenance dredging, 1991	2		2		
SOTERM86	Port of Everett, Hewitt Ave./South Terminal characteri:	17				
SSRECON	South Puget Sound Reconnaissance	17				
TACCENC2	Tacoma Central treatment plant, Class II inspection	3				
TERM5_91	Terminal 5 (Elliott Bay, West Waterway) maint. dredgir	3		3		
TERMNL91	Terminal 91, west side apron construction	3		3		
TEXACOC2	Texaco Inc. refinery (Anacortes), Class II inspection	3				
TPPS3AB	METRO's Toxicant Priority Pollutant Survey, 198?		29			
TXNPDS92	Texaco Inc. refinery (Anacortes), NPDES sediment stud	3				
USOILVLF	U.S. Oil refinery (Blair Waterway) maintenance dredgin	13		4 ?		
WYCKOFRI	Wyckoff RI (Eagle Harb.), effluent study	68				68
1988 INVENTORY		301	205	0	50	56
NEW INVENTORY		833	0	316	0	168
1994 INVENTORY		1134	205	316	50	224

Table B-2. Samples excluded from 1994 amphipod mortality AET calculations. Sample data were excluded because they were not synoptic, failed to meet chemica quality assurance (QA1) or various bioassay QA requirements, were statistically in clusive or were chemically anomalous. Exclusions are ordered by survey code.

Surveys/Batches/Samples Excluded			Criteria for Exclusion:						
Survey Code	Batch	Number of Samples	Not Synopyic	Failed Chemical QA	Insufficient Lab Replicates	Negative Control Sample Failure	No Reference or Reference Failur	Statistically Inconclusive	Chemically Anomalous
1TREEISL	1	3					X		
ARCOCPC2	1	2					X		
BCWTACC2	1	2					X		
BLAIR_91	1	27					X		
BLAIR_91	?	(5)						X	
BLGM_91A	1	18					X		
BLGM_91A	2	10					X		
BLGM_91A	4	3					X		
BPFERNC2	1	3					X		
BREMWTC2	1	3					X		
CASCADRI	1	1					X		
CBMSQS	1	2							X
CNKTSPC2	1	3					X		
COLUMBIA	1	1				X			
DUWO&M89	1	18				X			
DUWO&M91	1	13				X			
DUWO&M91	1	(1)						X	
EBCHEM	1	4						X	
EBCHEM	1	6							X
EDMDWTC2	1	3					X		
EHRITM10	1	6		X					
EVRT12TH	1	6				X			
EVWEYCII	1	3					X		
GAPAC_C2	1	3					X		
INTALCC2	1	4					X		
METAMB88	1	8					X		
METAMB90	1	8					X		
METAMB92	1	8					X		
METROEBP	1	7					X		
OLYHARFC	1	1						X	
OLYHARFC	2	1					X		

Table B-2. Samples excluded from 1994 amphipod mortality AET calculations. Sample data were excluded because they were not synoptic, failed to meet chemica quality assurance (QA1) or various bioassay QA requirements, were statistically in clusive or were chemically anomalous. Exclusions are ordered by survey code.

Surveys/Batches/Samples Excluded			Criteria for Exclusion:						
Survey Code	Batch	Number of Samples	Not Synopyic	Failed Chemical QA	Insufficient Lab Replicates	Negative Control Sample Failure	No Reference or Reference Failur	Statistically Inconclusive	Chemically Anomalous
OLYTERC2	1	3					X		
PENNWLC2	1	3					X		
PIER53BL	1	6					X		
POSTPTC2	1	3					X		
PSDDA1	1	1						X	
PSDDA1	1	1							X
PSREF90	1	21					X		
PTORCHC2	1	3					X		
PTWNPCC2	1	3					X		
PTWNPENR	1	3					X		
SEATTLSTL	1	2		X					
SED18804	1	12				X			
SED18804	2	12					X		
SED18903	1	2					X		
SED18903	1	1						X	
SED19003	1	24					X		
SED19003	2	20					X		
SED19003	3	21					X		
SED19003	4	17					X		
SED19103	2	1			X				
SHELLCII	1	1					X		
SITCUMRI	2	4							X
SNDREF92	1	3					X		
SOTERM86	1	17					X		
SSRECON	1	17					X		
TACCENC2	1	3					X		
TEXACOC2	1	3					X		
WYCKOFF	1	68		X					
TOTAL	72	452							

Table B-3. Data excluded from 1994 bivalve abnormality AET calculations. Sample data were excluded from 1994 AET calculations because they were not synoptic, failed to meet minimum chemical quality assurance (QA1) requirements, failed to meet various bioassay QA requirements, were statistically inconclusive or were considered chemically anomalous. Exclusions are ordered by survey code.

Surveys/Batches/Samples Excluded			Criteria for Exclusion:						
Survey Code	Batch	Number of Samples	Not Synopyic	Failed Chemical QA	Insufficient Lab Replicates	Negative Control Sample Failure	No Reference or Reference Failur	Statistically Inconclusive	Chemically Anomalous
CBBLAIR	1	6			X				
CBMSQS	1	50			X				
DUWO&M90	1	4							X
EHRITM10	1	6		X					
PIER53BL	1	6					X		
PSDDA1	1	14					X		
PSDDA1	1	6					X		
PSREF90	1	21					X		
SITCUMRI	1	38				X			X
WYCKOFF	1	68		X					
TOTAL	10	219							

Table B-4. Data excluded from 1994 echinoderm abnormality AET calculations. Sample data were excluded from 1994 AET calculations because they were not synoptic, failed to meet minimum chemical quality assurance (QA1) requirements, failed to meet various bioassay QA requirements, were statistically inconclusive or were considered chemically anomalous. Exclusions are ordered by survey code.

Surveys/Batches/Samples Excluded			Criteria for Exclusion:						
Survey Code	Batch	Number of Samples	Not Synopyic	Failed Chemical QA	Insufficient Lab Replicates	Negative Control Sample Failure	No Reference or Reference Failur	Statistically Inconclusive	Chemically Anomalous
ANCHOR90	1	1					X		
ARCOCPC2	1	2					X		
BLAIR_91	1	1						X	
BLGM_91A	1	7					X		
BLGM_91A	2	2							X
BLGM_91A	3	1						X	
CASCADRI	1	1					X		
CGPIER35	1	1							X
CHEVMD90	1	1						X	
DUWO&M89	1	?					X		
DUWO&M89	2	11					X		
DUWO&M89	3	1					X		
DUWO&M91	1	13				X			
DUWO&M91	2	12							
DUWO&M91	2	2	X						
DUYACHT1	1	5					X		
EVRT10TH		1							X
HULB90FC	1	8				X			
LONEST92	1	1						X	
NAVYHPII	2	4				X	X		
NAVYMANC	1	1			X				
OLYHARFC	1	2						X	
OLYTERC2	1	3					X		
PIER_D91	1	4						X	
PIER_D91	1	2							X
PSDDAM90	1	1						X	
PSREF90	1	21					X		
SITCUMRI	1	1							X

Table B-4. Data excluded from 1994 echinoderm abnormality AET calculations. Sample data were excluded from 1994 AET calculations because they were not synoptic, failed to meet minimum chemical quality assurance (QA1) requirements, failed to meet various bioassay QA requirements, were statistically inconclusive or were considered chemically anomalous. Exclusions are ordered by survey code.

Surveys/Batches/Samples Excluded			Criteria for Exclusion:						
Survey Code	Batch	Number of Samples	Not Synopyic	Failed Chemical QA	Insufficient Lab Replicates	Negative Control Sample Failure	No Reference or Reference Failur	Statistically Inconclusive	Chemically Anomalous
SNDREF92	1	3					X		
TERMNL91	1	1							X
TOTAL	38	114							

Table B-5. Exclusions from 1994 echinoderm effective mortality AET calculatior
Sample data were excluded from 1994 AET calculations because they were not synoptic, failed to meet minimum chemical quality assurance (QA1) requirements, failed to meet various bioassay QA requirements, were statistically inconclusive or were considered chemically anomalous. Exclusions are ordered by survey code.

Surveys/Batches/Samples Excluded			Criteria for Exclusion:						
Survey Code	Batch	Number of Samples	Not Synopyic	Failed Chemical QA	Insufficient Lab Replicates	Negative Control Sample Failure	No Reference or Reference Failur	Statistically Inconclusive	Chemically Anomalous
AMPRES92									
ANCHOR90									
ARCOCPC2									
BLAIR_91									
BLGM_91A									
CASCADRI									
DAYISL91									
DUWO&M89									
DUWO&M89									
DUWO&M89									
DUWO&M91									
DUYACHT1									
EVRT10TH									
EVRT12TH									
HULB90FC									
LONEST92									
LOTTO043									
METROEBP									
MORTON92									
NAVYHPFC									
NAVYHPII									
NAVYMANC									
OLYHARFC									
OLYTERC2									
PIERD_91									
POSTPTC2									
PSDDAM90									
PSDDAM91									

Table B-5. Exclusions from 1994 echinoderm effective mortality AET calculatior
Sample data were excluded from 1994 AET calculations because they were not synoptic, failed to meet minimum chemical quality assurance (QA1) requirements, failed to meet various bioassay QA requirements, were statistically inconclusive or were considered chemically anomalous. Exclusions are ordered by survey code.

Surveys/Batches/Samples Excluded			Criteria for Exclusion:						
Survey Code	Batch	Number of Samples	Not Synopyic	Failed Chemical QA	Insufficient Lab Replicates	Negative Control Sample Failure	No Reference or Reference Failur	Statistically Inconclusive	Chemically Anomalous
PSDDAM92									
PSREF90									
SITCUMRI									
SNDREF92									
SOPARK91									
TERM_91									
TREMNL91									
USOILVLF									
TOTAL									

Table B-6. List of 1994 synoptic samples for which the determination of significant adverse biological effects was statistically inconclusive.
Samples in italics were determined to be inconclusive for 1988 AET calculatio:

Survey Code	Station_ID	Sample_ID	AET Test Type	Test Endpoint
EBCHEM	*DR-05*	*DR-05*	*AMPT*	*Mortality*
EBCHEM	*DR-08*	*DR-08*	*AMPT*	*Mortality*
EBCHEM	*EW-03*	*EW-03*	*AMPT*	*Mortality*
EBCHEM	*NS-04*	*NS-04*	*AMPT*	*Mortality*
EVCHEM	*EW-14*	*EW-14G*	*AMPT*	*Mortality*
EVCHEM	*SD-02*	*SD-02G*	*AMPT*	*Mortality*
EVCHEM	*SR-07*	*SR-07G*	*AMPT*	*Mortality*
BLAIR_91	BL9106MC	BLAIR91XC003	AMPT	Mortality
BLAIR_91	BL9109MC	BLAIR91XC004	AMPT	Mortality
BLAIR_91	BL9112MC	BLAIR91XC005	AMPT	Mortality
BLAIR_91	BL9136MC	BLAIR91XC013	AMPT	Mortality
BLAIR_91	BL9141MC	BLAIR91XC015	AMPT	Mortality
DUWO&M91	DU9111XX	DUWO&M91S003	AMPT	Mortality
OLYHARFC	OLYH06MC	OLYHFCXXC020	AMPT	Mortality
PSDDA1	CBB02	CBB02C	AMPT	Mortality
SED18903	20	1	AMPT	Mortality
BLAIR_91	BL912409	BLAIR91XC009	ECHN	Abnormality
BLGM_91A	BLGM3312	BLGMBYXXC012	ECHN	Abnormality
CHEVMD90	CHEV02XX	CHEV90XXS001	ECHN	Abnormality
LONEST92	LSNW0101	LONEST92C001	ECHN	Abnormality
OLYHARFC	OLYH09MC	OLYHFCXXC005	ECHN	Abnormality
OLYHARFC	OLYH21MC	OLYHFCXXC019	ECHN	Abnormality
PIERD_91	3	C2	ECHN	Abnormality
PIERD_91	3	S3	ECHN	Abnormality
PIERD_91	4	S4	ECHN	Abnormality
PIERD_91	15	S15	ECHN	Abnormality
PSDDAM90	EB_BO3BX	EB90_B03B	ECHN	Abnormality

Table B-7. Summary of chemically anomalous samples excluded from 1994 dry weight-normalized AET calculations. Data are listed alphabetically first by AET type, then by chemical of concern. AMPT = 10-day amphipod mortality AETs. ECHN = 96-hour echinoderm larvae abnormality AETs. The survey, station and "No Hit" sample having the highest concentration for a given chemical is listed, together with its ratio to the next highest "No Hit" sample.

AET Type	Survey Code	Station_ID	"No Hit" Sample_ID	Chemical of Concern	Sample Concentration(s)	Ratio(s) to Next Highest "No Hit" Sample Conc.
AMPT	CBMSQS	CI-16	CI-16	1,2-Dichlorobenzene	350	3.2
AMPT	SITCUMRI	NC-S6	300042NCS6	2-Methylphenol, 2,4-Dimethylphenol	355	4.6
AMPT	SITCUMRI	NC-S5	300043NCS5	2-Methylphenol, 2,4-Dimethylphenol	370	4.8
AMPT	EBCHEM	NH-10	NH-10	Aldrin	90	9.5
AMPT	EBCHEM	WW-14	WW-14	Antimony	1370	6.9
AMPT	EBCHEM	EW-12	EW-12	Benzyl alcohol	870	11.9
AMPT	CBMSQS	HY-12	HY-12	Di-n-butyl phthalate	5100	3.2
AMPT	EBCHEM	NS-06	NS-06	Di-n-octyl phthalate	9900	4.7
AMPT	EBCHEM	NH-10	NH-10	Dieldrin	51	14.6
AMPT	PSDDA1	EBS02	EBS02C	Mercury	7.3	3.2
AMPT	CBMSQS	CI-16	CI-16	N-Nitroso diphenylamine	220	4.6
AMPT	SITCUMRI	NS-D6	300095NSD6	Silver	24.5	4.0
AMPT	SITCUMRI	NS-D4	300098NSD4	Silver	33.4	5.5
ECHN	TERMNL91	T91_03XX	TERMNL91S003	2-methylnaphthalene	790	4.9
ECHN	BLGM_91A	BLGM3813	BLGMBYXXS002	Antimony	110	3.1
ECHN	CGPIER35	PIER01MC	PIER35XXC001	Antimony	36	3.9
ECHN	SITCUMRI	NS-E3	300105NSE3	Antimony	36	3.9
ECHN	EVRT10TH	EV100101	EV10ST92C001	Benzoic acid	240	7.7
ECHN	CGPIER35	PIER01MC	PIER35XXC001	Butylbenzylphthalate	760	4.8
ECHN	PIERD_91	16	C6	Dieldrin	44	3.7
ECHN	PIERD_91	18	C7	Total DDT	420	6.3
ECHN	PIERD_91	11	C5	Total PCBs	920	3.2
ECHN	PIERD_91	16	C6	Total PCBs	910	3.1

Table B-8. Final list of synoptic surveys and samples used to calculate 1994 Puget Sound AETs.
Surveys and sample numbers in bold italics were used to calculate 1988 AETs.

Survey Code	Survey Description	Type and Number of Biological Effect Samples: A	B	E mort	E abnm	M	O abnm
ALKI	***METRO - Alki Point outfall sediments, 1982***		***11***				
AMPRES92	American President's Line maint. dredge, 1992	2			2		
ANCHOR90	Anchor Cove condominium marina proj., 1990	1					
BLAIR_91	Port of Tacoma, Blair Waterway proj., 1991				26		
BLGM_91A	maint./other dredge of Bellingham Bay, 1991	6			17		
CBBLAIR	***Commencmnt Bay RI Blair Waterway dredge***	***6***	***6***				***6***
CBMSQS	***Commencement Bay RI, main sediment quality survey***	***48***	***48***			***50***	***50***
CGPIER35	U.S. Coast Guard dredge and construction	3			2		
CHEVMD90	Chevron USA Edmonds dock maint. dredge	3			2		
DAYISL91	Day Island Yacht Club (Tacoma) sediment characterizatic	1			1		
DUWO&M89	Duwamish R. maint. dredge proj., 1989	12					
DUWO&M90	Duwamish R. maint. dredge 1990, Phase 1	4					
DUWO&M91	Duwamish R. maint. dredge 1990, Phase 2 ,	1					
DUWRIV1	***PSDDA Duwamish R. I data set***	***9***					
DUWRIV2	***PSDDA Duwamish R. II data set***	***31***					
DUYACHT1	Duwamish Yacht Club marina maint. dredge	5					
EBCHEM	***1985 Elliott Bay sediment quality survey***	***100***	***80***				
EHCHEM	***Eagle Harbor sediment chemistry survey***	***10***	***10***				
EIGHTBAY	***1985 Puget Sound Eight-Bay survey***	***48***					
EVCHEM	***1985 Everett Harbor chemistry & biological data***	***29***	***19***				
EVERETT1	***A subset of NAVYHP85 (Navy Homeport EIS)***	***6***					
EVMAR_88	Everett Marina maint. dredge, 1988	5					
EVRT10TH	Everett Harbor, 10th St. boat ramp expansion	1					
EVRT12TH	Everett Harbor, 12th St. barge channel dredge				6		
HULB90FC	Hulbert Mill proposed 12th St. marina dredge	8			4		
HURLEN89	Hurlen Construction Co. maint. dredge	1					
LONEST89	Lonestar NW, maint. dredge in Duwamish R., 1989	1					
LONEST92	Lonestar NW, west terminal dredge, 1992	1					
LOTTO041	Olympia treatment plant (LOTT) outfall sampling, DY89	3					
LOTTO043	Olympia treatment plant (LOTT) outfall sampling, DY91	1			1		
METROEBP	METRO West Point emergency bypass outfall sampling				4		

Table B-8. Final list of synoptic surveys and samples used to calculate 1994 Puget Sound AETs.
Surveys and sample numbers in bold italics were used to calculate 1988 AETs.

Survey Code	Survey Description	Type and Number of Biological Effect Samples: A	B	E mort	E abnm	M	O abnm
MORTON89	Morton Marine wharf construction & draft increase	1					
MORTON92	Morton Marine maint. dredge, 1992	1			1		
NAVYHPFC	U.S. Navy Homeport (full characterization)	31			30		
NAVYHPII	U.S. Navy Homeport Element II (full characterization)	4			4		
NAVYMANC	U.S. Navy Manchester fuel pier replacement	15			11		
OLYHARFC	Olympia Harbor planning (full characterization)	11			9		
PIERD_91	U.S. Navy Bremerton Pier D	28			24		
PSDDA1	PSDDA Phase 1 baseline survey	13					
PSDDA2	PSDDA Phase 2 baseline survey	6					
PSDDAM90	1990 PSDDA post-disposal site monitoring	10			9		
PSDDAM91	1991 PSDDA site monitoring/Port Gardner PGB09	1			1		
PSDDAM92	1992 PSDDA full monitoring, Elliott Bay	3			3		
SED18903	Puget Sound Ambient Monitoring - 1989	48					
SED19103	Puget Sound Ambient Monitoring - 1991	57					
SITCUMRI	Port of Tacoma RI/NRDA (Sitcum/Milwaukee/Blair)	79			36		
SOPARK91	South Park Marina maint. dredge, 1991	2			1		
TERM5_91	Terminal 5 (Elliott Bay, West Waterway) maint. dredge	3			3		
TERMNL91	Terminal 91, west side apron construction	3			3		
TPPS3AB	***METRO Toxic Priority Pollutant Survey, 198?***		***27***				
TXNPDS92	Texaco Inc. refinery (Anacortes), NPDES sediment studie	3					
USOILVLF	U.S. Oil refinery (Blair Waterway) maint. dredge	9			5		
1988 Subtotal		***287***	***201***		***0***	***50***	***56***
1994 Subtotal		387	0		205	0	0
Grand Total		674	201		205	50	56

Table B-9. Final biological effects file used for 1994 AET calculations.
Results are ordered alphabetically by Survey Code and Sample ID. A = amphipod bioassay, B = benthic infaunal abundance, ECHN abnorm. = echinoderm larval abnormality, ECHN mort = echinoderm larval effective mortality, M = Microtox luminosity (saline extract), and O = oyster/bivalve larval abnormality.

			Bioeffect Type:		ECHN	ECHN		
Survey Code	Station ID	Sample ID	A	B	abnorm.	Eff. Mort.	M	O
1TREE_89	TR890101	TR89FDL4C001	NO HIT					
1TREE_89	TR890702	TR89FDL5C002	HIT					
1TREE_89	TR892305	TR89DVR8C005	NO HIT					
ALKI	AP-01	AP-01		NO HIT				
ALKI	AP-02	AP-02		NO HIT				
ALKI	AP-03	AP-03		NO HIT				
ALKI	AP-04	AP-04		HIT				
ALKI	AP-05	AP-05		NO HIT				
ALKI	AP-06	AP-06		NO HIT				
ALKI	AP-07	AP-07		NO HIT				
ALKI	PW-01	PW-01		NO HIT				
ALKI	PW-02	PW-02		NO HIT				
ALKI	PW-03	PW-03		NO HIT				
ALKI	PW-04	PW-04		NO HIT				
AMPRES92	APL_01MC	AMPRES92C001	NO HIT		HIT			
AMPRES92	APL_01MC	AMPRES92C002	NO HIT		NO HIT			
ANCHOR90	ANCH0101	ANCH90XXC001	NO HIT		HIT			
ARCOCPC2	CP9101XX	CP91EDGES001	HIT					
ARCOCPC2	CP9102XX	CP91BKGDS002	NO HIT					
BCWTACC2	BC8901XX	BC89MIDPS001	NO HIT					
BCWTACC2	BC8902XX	BC89OUTZS002	NO HIT					
BLAIR_91	BL910101	BLAIR91XC001	HIT		HIT			
BLAIR_91	BL910402	BLAIR91XC002	NO HIT		NO HIT			
BLAIR_91	BL9106MC	BLAIR91XC003			NO HIT			
BLAIR_91	BL9109MC	BLAIR91XC004			HIT			
BLAIR_91	BL9112MC	BLAIR91XC005			HIT			
BLAIR_91	BL911606	BLAIR91XC006	NO HIT		NO HIT			
BLAIR_91	BL911907	BLAIR91XC007	NO HIT		NO HIT			

Table B-9. Final biological effects file used for 1994 AET calculations.
Results are ordered alphabetically by Survey Code and Sample ID. A = amphipod bioassay, B = benthic infaunal abundance, ECHN abnorm. = echinoderm larval abnormality, ECHN mort = echinoderm larval effective mortality, M = Microtox luminosity (saline extract), and O = oyster/bivalve larval abnormality.

			Bioeffect Type:		ECHN	ECHN		
Survey Code	Station ID	Sample ID	A	B	abnorm.	Eff. Mort.	M	O
BLAIR_91	BL912208	BLAIR91XC008	NO HIT		HIT			
BLAIR_91	BL912409	BLAIR91XC009	HIT					
BLAIR_91	BL9127MC	BLAIR91XC010	NO HIT		HIT			
BLAIR_91	BL913011	BLAIR91XC011	NO HIT		HIT			
BLAIR_91	BL913412	BLAIR91XC012	NO HIT		HIT			
BLAIR_91	BL9136MC	BLAIR91XC013			NO HIT			
BLAIR_91	BL9138MC	BLAIR91XC014	HIT		HIT			
BLAIR_91	BL9141MC	BLAIR91XC015			HIT			
BLAIR_91	BL914516	BLAIR91XC016	HIT		HIT			
BLAIR_91	BL914817	BLAIR91XC017	NO HIT		HIT			
BLAIR_91	BL915118	BLAIR91XC018	NO HIT		HIT			
BLAIR_91	BL915419	BLAIR91XC019	NO HIT		HIT			
BLAIR_91	BL915720	BLAIR91XC020	NO HIT		HIT			
BLAIR_91	BL915921	BLAIR91XC021	NO HIT		HIT			
BLAIR_91	BL916322	BLAIR91XC022	NO HIT		NO HIT			
BLAIR_91	BL9105MC	BLAIR91XC023	NO HIT		NO HIT			
BLAIR_91	BL9110MC	BLAIR91XC024	NO HIT		NO HIT			
BLAIR_91	BL9123MC	BLAIR91XC025	NO HIT		NO HIT			
BLAIR_91	BL9140MC	BLAIR91XC026	NO HIT		NO HIT			
BLAIR_91	BL9127MC	BLAIR91XC027	NO HIT		NO HIT			
BLGM_91A	BLGM09MC	BLGMBYXXC004	NO HIT		NO HIT			
BLGM_91A	BLGM0606	BLGMBYXXC006	NO HIT		NO HIT			
BLGM_91A	BLGM1608	BLGMBYXXC008	NO HIT		HIT			
BLGM_91A	BLGM3312	BLGMBYXXC012	NO HIT					
BLGM_91A	BLGM3713	BLGMBYXXC013	HIT		NO HIT			
BLGM_91A	BLGM4714	BLGMBYXXC014	NO HIT		NO HIT			
BLGM_91A	BLGM6518	BLGMBYXXC018	HIT		HIT			
BLGM_91A	BLGM3713	BLGMBYXXS001	HIT		HIT			

Table B-9. Final biological effects file used for 1994 AET calculations.
Results are ordered alphabetically by Survey Code and Sample ID. A = amphipod bioassay, B = benthic infaunal abundance, ECHN abnorm. = echinoderm larval abnormality, ECHN mort = echinoderm larval effective mortality, M = Microtox luminosity (saline extract), and O = oyster/bivalve larval abnormality.

			Bioeffect Type:		ECHN	ECHN		
Survey Code	Station ID	Sample ID	A	B	abnorm.	Eff. Mort.	M	O
BLGM_91A	BLGM3813	BLGMBYXXS002	HIT		NO HIT			
BLGM_91A	BLGM39XX	BLGMBYXXS003	HIT		HIT			
BLGM_91A	BLGM40XX	BLGMBYXXS004	HIT		NO HIT			
BLGM_91A	BLGM41XX	BLGMBYXXS005	HIT		HIT			
BLGM_91A	BLGM42XX	BLGMBYXXS006	NO HIT		NO HIT			
BLGM_91A	BLGM43XX	BLGMBYXXS007	NO HIT		HIT			
BLGM_91A	BLGM45XX	BLGMBYXXS009	HIT		NO HIT			
BLGM_91A	BLGM46XX	BLGMBYXXS010	HIT		HIT			
BLGM_91A	BLGM4714	BLGMBYXXS011	HIT		HIT			
BLGM_91A	BLGM4814	BLGMBYXXS012	HIT		HIT			
BLGM_91A	BLGM5015	BLGMBYXXS014			HIT			
BLGM_91A	BLGM57XX	BLGMBYXXS024			HIT			
BPFERNC2	BP8901XX	BPATOUTFS001	NO HIT					
BPFERNC2	BP8903XX	BPCNTROLS003	NO HIT					
BPFERNC2	BP8902XX	BPNROUTFS002	NO HIT					
BREMWTC2	BM8801XX	BM88EASTS001	NO HIT					
BREMWTC2	BM8802XX	BM88WESTS002	NO HIT					
BREMWTC2	BM8803XX	BM88CTRLS003	NO HIT					
CASCADRI	CASC2D3	E	NO HIT		NO HIT			
CBBLAIR	B03	B03	NO HIT	NO HIT				NO HIT
CBBLAIR	B04	B04	NO HIT	NO HIT				NO HIT
CBBLAIR	B09	B09	NO HIT	NO HIT				NO HIT
CBBLAIR	B10	B10	NO HIT	NO HIT				HIT
CBBLAIR	B12	B12	NO HIT	NO HIT				NO HIT
CBBLAIR	B15	B15	HIT	NO HIT				NO HIT
CBMSQS	BL-11	BL-11	NO HIT	NO HIT			NO HIT	NO HIT
CBMSQS	BL-13	BL-13	NO HIT	NO HIT			HIT	NO HIT
CBMSQS	BL-21	BL-21	NO HIT	NO HIT			NO HIT	NO HIT

Table B-9. Final biological effects file used for 1994 AET calculations.
Results are ordered alphabetically by Survey Code and Sample ID. A = amphipod bioassay, B = benthic infaunal abundance, ECHN abnorm. = echinoderm larval abnormality, ECHN mort = echinoderm larval effective mortality, M = Microtox luminosity (saline extract), and O = oyster/bivalve larval abnormality.

			Bioeffect Type:		ECHN	ECHN		
Survey Code	Station ID	Sample ID	A	B	abnorm.	Eff. Mort.	M	O
CBMSQS	BL-25	BL-25	HIT	NO HIT			NO HIT	NO HIT
CBMSQS	BL-28	BL-28	NO HIT	NO HIT			NO HIT	NO HIT
CBMSQS	BL-31	BL-31	NO HIT	NO HIT			HIT	NO HIT
CBMSQS	CI-11	CI-11	HIT	HIT			HIT	HIT
CBMSQS	CI-13	CI-13	NO HIT	HIT			HIT	HIT
CBMSQS	CI-16	CI-16		HIT			HIT	HIT
CBMSQS	CI-17	CI-17	NO HIT	HIT			HIT	NO HIT
CBMSQS	CI-20	CI-20	HIT	NO HIT			NO HIT	HIT
CBMSQS	CI-22	CI-22	NO HIT	NO HIT			NO HIT	NO HIT
CBMSQS	CR-11	CR-11	NO HIT	NO HIT			NO HIT	NO HIT
CBMSQS	CR-12	CR-12	NO HIT	NO HIT			NO HIT	NO HIT
CBMSQS	CR-13	CR-13	NO HIT	NO HIT			NO HIT	NO HIT
CBMSQS	CR-14	CR-14	NO HIT	NO HIT			NO HIT	NO HIT
CBMSQS	HY-12	HY-12	NO HIT	NO HIT			HIT	HIT
CBMSQS	HY-14	HY-14	NO HIT	HIT			HIT	NO HIT
CBMSQS	HY-17	HY-17	NO HIT	HIT			HIT	HIT
CBMSQS	HY-22	HY-22	HIT	HIT			HIT	HIT
CBMSQS	HY-23	HY-23	HIT	HIT			HIT	HIT
CBMSQS	HY-24	HY-24	NO HIT	HIT			HIT	NO HIT
CBMSQS	HY-28	HY-28	NO HIT	HIT			NO HIT	NO HIT
CBMSQS	HY-32	HY-32	NO HIT	HIT			NO HIT	NO HIT
CBMSQS	HY-37	HY-37	NO HIT	HIT			HIT	NO HIT
CBMSQS	HY-42	HY-42	HIT	HIT			HIT	NO HIT
CBMSQS	HY-43	HY-43	NO HIT	HIT			HIT	NO HIT
CBMSQS	HY-44	HY-44	NO HIT	NO HIT			NO HIT	NO HIT
CBMSQS	HY-47	HY-47	HIT	HIT			HIT	HIT
CBMSQS	HY-50	HY-50	NO HIT	NO HIT			HIT	NO HIT
CBMSQS	MD-12	MD-12	NO HIT	HIT			NO HIT	NO HIT

Table B-9. Final biological effects file used for 1994 AET calculations.
Results are ordered alphabetically by Survey Code and Sample ID. A = amphipod bioassay, B = benthic infaunal abundance, ECHN abnorm. = echinoderm larval abnormality, ECHN mort = echinoderm larval effective mortality, M = Microtox luminosity (saline extract), and O = oyster/bivalve larval abnormality.

			Bioeffect Type:		ECHN	ECHN		
Survey Code	Station ID	Sample ID	A	B	abnorm.	Eff. Mort.	M	O
CBMSQS	MI-11	MI-11	HIT	NO HIT			NO HIT	NO HIT
CBMSQS	MI-13	MI-13	NO HIT	NO HIT			HIT	NO HIT
CBMSQS	MI-15	MI-15	HIT	NO HIT			HIT	NO HIT
CBMSQS	RS-12	RS-12	NO HIT	NO HIT			NO HIT	NO HIT
CBMSQS	RS-13	RS-13	HIT	NO HIT			NO HIT	HIT
CBMSQS	RS-14	RS-14	NO HIT	NO HIT			NO HIT	NO HIT
CBMSQS	RS-18	RS-18	HIT	HIT			HIT	HIT
CBMSQS	RS-19	RS-19	HIT	HIT			HIT	HIT
CBMSQS	RS-20	RS-20	NO HIT	HIT			HIT	NO HIT
CBMSQS	RS-22	RS-22	NO HIT	NO HIT			NO HIT	NO HIT
CBMSQS	RS-24	RS-24	HIT	NO HIT			NO HIT	NO HIT
CBMSQS	SI-11	SI-11	NO HIT	HIT			HIT	NO HIT
CBMSQS	SI-12	SI-12	HIT	HIT			HIT	NO HIT
CBMSQS	SI-15	SI-15	HIT	NO HIT			NO HIT	NO HIT
CBMSQS	SP-11	SP-11	NO HIT	HIT			HIT	NO HIT
CBMSQS	SP-12	SP-12	NO HIT	NO HIT			HIT	HIT
CBMSQS	SP-14	SP-14	HIT	HIT			HIT	HIT
CBMSQS	SP-15	SP-15	HIT	HIT			HIT	HIT
CBMSQS	SP-16	SP-16	HIT	HIT			HIT	HIT
CGPIER35	PIER01MC	PIER35XXC001	NO HIT		NO HIT			
CGPIER35	PIER01MC	PIER35XXC002	HIT		HIT			
CGPIER35	PIER02MC	PIER35XXS001	NO HIT		HIT			
CHEVMD90	CHEV01MC	CHEV90XXC001	NO HIT		NO HIT			
CHEVMD90	CHEV0302	CHEV90XXC002	NO HIT		HIT			
CHEVMD90	CHEV02XX	CHEV90XXS001	NO HIT					
CNKTSPC2	CK8801XX	CK88OUTFS001	NO HIT					
CNKTSPC2	CK8802XX	CK88OUTFS002	NO HIT					
CNKTSPC2	CK8803XX	CK88BKGDS003	NO HIT					

Table B-9. Final biological effects file used for 1994 AET calculations.
Results are ordered alphabetically by Survey Code and Sample ID. A = amphipod bioassay, B = benthic infaunal abundance, ECHN abnorm. = echinoderm larval abnormality, ECHN mort = echinoderm larval effective mortality, M = Microtox luminosity (saline extract), and O = oyster/bivalve larval abnormality.

			Bioeffect Type:					
Survey Code	Station ID	Sample ID	A	B	ECHN abnorm.	ECHN Eff. Mort.	M	O
DAYISL91	DAYI0101	DAYISL91C001	NO HIT		NO HIT			
DUWO&M89	27	C8	NO HIT		NO HIT			
DUWO&M89	DU891111	DUWR89XXC011	NO HIT					
DUWO&M89	DU891312	DUWR89XXC012	NO HIT					
DUWO&M89	DU8906XX	DUWR89XXS006	NO HIT					
DUWO&M89	DU8908XX	DUWR89XXS008	NO HIT					
DUWO&M89	DU8909XX	DUWR89XXS009	NO HIT					
DUWO&M89	DU8910XX	DUWR89XXS010	NO HIT					
DUWO&M89	DU8915XX	DUWR89XXS013	NO HIT					
DUWO&M89	DU8916XX	DUWR89XXS014	HIT					
DUWO&M89	DU8917XX	DUWR89XXS015	HIT					
DUWO&M89	DU8918XX	DUWR89XXS016	HIT					
DUWO&M89	DU8919XX	DUWR89XXS017	NO HIT					
DUWO&M90	DU9001XX	DUWO&M90S001	HIT		HIT			HIT
DUWO&M90	DU9003XX	DUWO&M90S003	NO HIT		HIT			HIT
DUWO&M90	DU9004XX	DUWO&M90S004	HIT		HIT			HIT
DUWO&M90	DU9005XX	DUWO&M90S005	NO HIT		HIT			HIT
DUWO&M91	DU9111XX	DUWO&M91S003	NO HIT					
DUWRIV1	DR-01	DR-01	NO HIT					
DUWRIV1	DR-02	DR-02	NO HIT					
DUWRIV1	DR-03	DR-03	NO HIT					
DUWRIV1	DR-04	DR-04	NO HIT					
DUWRIV1	DR-05	DR-05	NO HIT					
DUWRIV1	DR-06	DR-06	NO HIT					
DUWRIV1	DR-07	DR-07	HIT					
DUWRIV1	DR-08	DR-08	HIT					
DUWRIV1	SQ-09	SQ-09	NO HIT					
DUWRIV2	DR-10	CA1	HIT					

Table B-9. Final biological effects file used for 1994 AET calculations.
Results are ordered alphabetically by Survey Code and Sample ID. A = amphipod bioassay, B = benthic infaunal abundance, ECHN abnorm. = echinoderm larval abnormality, ECHN mort = echinoderm larval effective mortality, M = Microtox luminosity (saline extract), and O = oyster/bivalve larval abnormality.

			Bioeffect Type:					
Survey Code	Station ID	Sample ID	A	B	ECHN abnorm.	ECHN Eff. Mort.	M	O
DUWRIV2	DR-11	CA2	HIT					
DUWRIV2	DR-12	CA3	NO HIT					
DUWRIV2	DR-13	CB1	NO HIT					
DUWRIV2	DR-14	CB2	NO HIT					
DUWRIV2	DR-15	CB3	NO HIT					
DUWRIV2	DR-16	CB4	HIT					
DUWRIV2	DR-17	CB5	NO HIT					
DUWRIV2	DR-18	CC1	NO HIT					
DUWRIV2	DR-19	CC2	NO HIT					
DUWRIV2	DR-20	CC3	NO HIT					
DUWRIV2	DR-21	CC4	NO HIT					
DUWRIV2	DR-22	CC5	NO HIT					
DUWRIV2	DR-23	CD1	NO HIT					
DUWRIV2	DR-24	CD2	NO HIT					
DUWRIV2	DR-25	CE1	HIT					
DUWRIV2	DR-26	CE2	HIT					
DUWRIV2	DR-27	CE3	HIT					
DUWRIV2	DR-28	CF1	NO HIT					
DUWRIV2	DR-29	CF2	HIT					
DUWRIV2	DR-30	CF3	NO HIT					
DUWRIV2	DR-31	CF4	NO HIT					
DUWRIV2	DR-32	CF5	NO HIT					
DUWRIV2	DR-33	CG1	NO HIT					
DUWRIV2	DR-34	CG2	NO HIT					
DUWRIV2	DR-35	CG3	NO HIT					
DUWRIV2	DR-36	CG4	NO HIT					
DUWRIV2	DR-37	CG5	NO HIT					
DUWRIV2	DR-38	CH1	NO HIT					

Table B-9. Final biological effects file used for 1994 AET calculations.
Results are ordered alphabetically by Survey Code and Sample ID. A = amphipod bioassay, B = benthic infaunal abundance, ECHN abnorm. = echinoderm larval abnormality, ECHN mort = echinoderm larval effective mortality, M = Microtox luminosity (saline extract), and O = oyster/bivalve larval abnormality.

			Bioeffect Type:		ECHN	ECHN		
Survey Code	Station ID	Sample ID	A	B	abnorm.	Eff. Mort.	M	O
DUWRIV2	DR-39	CW/A1	NO HIT					
DUWRIV2	SQ-21	SEQUIM	NO HIT					
DUYACHT1	DUWY01XX	DUWYATXXS001	HIT					
DUYACHT1	DUWY02XX	DUWYATXXS002	HIT					
DUYACHT1	DUWY03XX	DUWYATXXS003	NO HIT					
DUYACHT1	DUWY04XX	DUWYATXXS004	HIT					
DUYACHT1	DUWY05XX	DUWYATXXS005	HIT					
EBCHEM	AB-01	AB-01	HIT					
EBCHEM	AB-02	AB-02	NO HIT	NO HIT				
EBCHEM	AB-03	AB-03	NO HIT	HIT				
EBCHEM	AB-04	AB-04	NO HIT	NO HIT				
EBCHEM	DR-01	DR-01	NO HIT					
EBCHEM	DR-02	DR-02	HIT					
EBCHEM	DR-03	DR-03	NO HIT					
EBCHEM	DR-04	DR-04	NO HIT					
EBCHEM	DR-06	DR-06	NO HIT					
EBCHEM	DR-07	DR-07	NO HIT					
EBCHEM	DR-09	DR-09	NO HIT					
EBCHEM	DR-10	DR-10	NO HIT					
EBCHEM	DR-11	DR-11	NO HIT					
EBCHEM	DR-12	DR-12	NO HIT					
EBCHEM	DR-13	DR-13	HIT					
EBCHEM	DR-14	DR-14	HIT					
EBCHEM	DR-15	DR-15	HIT					
EBCHEM	DR-16	DR-16	HIT					
EBCHEM	DR-17	DR-17	NO HIT					
EBCHEM	DR-25	DR-25	HIT					
EBCHEM	EW-01	EW-01	NO HIT					

Table B-9. Final biological effects file used for 1994 AET calculations.
Results are ordered alphabetically by Survey Code and Sample ID. A = amphipod bioassay, B = benthic infaunal abundance, ECHN abnorm. = echinoderm larval abnormality, ECHN mort = echinoderm larval effective mortality, M = Microtox luminosity (saline extract), and O = oyster/bivalve larval abnormality.

Survey Code	Station ID	Sample ID	Bioeffect Type: A	B	ECHN abnorm.	ECHN Eff. Mort.	M	O
EBCHEM	EW-02	EW-02	HIT	HIT				
EBCHEM	EW-03	EW-03		NO HIT				
EBCHEM	EW-04	EW-04	HIT	HIT				
EBCHEM	EW-05	EW-05	HIT	HIT				
EBCHEM	EW-05	EW-05	HIT	HIT				
EBCHEM	EW-06	EW-06	HIT	HIT				
EBCHEM	EW-07	EW-07	HIT	HIT				
EBCHEM	EW-08	EW-08	HIT	HIT				
EBCHEM	EW-09	EW-09	HIT	HIT				
EBCHEM	EW-10	EW-10	HIT	NO HIT				
EBCHEM	EW-11	EW-11	HIT	HIT				
EBCHEM	EW-12	EW-12	NO HIT	NO HIT				
EBCHEM	EW-13	EW-13	NO HIT	NO HIT				
EBCHEM	EW-14	EW-14	NO HIT	NO HIT				
EBCHEM	EW-15	EW-15	NO HIT	NO HIT				
EBCHEM	EW-16	EW-16	HIT	NO HIT				
EBCHEM	KG-01	KG-01	NO HIT	HIT				
EBCHEM	KG-02	KG-02	HIT					
EBCHEM	KG-03	KG-03	HIT	NO HIT				
EBCHEM	KG-04	KG-04	NO HIT					
EBCHEM	KG-05	KG-05	HIT	HIT				
EBCHEM	KG-06	KG-06	NO HIT	HIT				
EBCHEM	KG-07	KG-07	NO HIT	HIT				
EBCHEM	KG-08	KG-08	NO HIT	HIT				
EBCHEM	KG-09	KG-09	HIT	NO HIT				
EBCHEM	KG-10	KG-10	HIT					
EBCHEM	KG-11	KG-11	HIT	HIT				
EBCHEM	MG-01	MG-01	NO HIT	HIT				

Table B-9. Final biological effects file used for 1994 AET calculations.
Results are ordered alphabetically by Survey Code and Sample ID. A = amphipod bioassay, B = benthic infaunal abundance, ECHN abnorm. = echinoderm larval abnormality, ECHN mort = echinoderm larval effective mortality, M = Microtox luminosity (saline extract), and O = oyster/bivalve larval abnormality.

			Bioeffect Type:		ECHN	ECHN		
Survey Code	Station ID	Sample ID	A	B	abnorm.	Eff. Mort.	M	O
EBCHEM	MG-02	MG-02	NO HIT	HIT				
EBCHEM	MG-03	MG-03	NO HIT	HIT				
EBCHEM	MG-04	MG-04	NO HIT	HIT				
EBCHEM	NH-01	NH-01	NO HIT	HIT				
EBCHEM	NH-02	NH-02	HIT	HIT				
EBCHEM	NH-03	NH-03	HIT	HIT				
EBCHEM	NH-04	NH-04	HIT	HIT				
EBCHEM	NH-05	NH-05	HIT	HIT				
EBCHEM	NH-06	NH-06	HIT	HIT				
EBCHEM	NH-07	NH-07	NO HIT					
EBCHEM	NH-08	NH-08	HIT	HIT				
EBCHEM	NH-09	NH-09	HIT	NO HIT				
EBCHEM	NH-10	NH-10	NO HIT					
EBCHEM	NH-11	NH-11	HIT	HIT				
EBCHEM	NS-01	NS-01	HIT					
EBCHEM	NS-02	NS-02	NO HIT	NO HIT				
EBCHEM	NS-03	NS-03	NO HIT	NO HIT				
EBCHEM	NS-05	NS-05	NO HIT	NO HIT				
EBCHEM	NS-06	NS-06		HIT				
EBCHEM	NS-07	NS-07	HIT	NO HIT				
EBCHEM	NS-08	NS-08	HIT	HIT				
EBCHEM	PS-01	PS-01	NO HIT	NO HIT				
EBCHEM	PS-01	PS-01	NO HIT	NO HIT				
EBCHEM	PS-02	PS-02	NO HIT	NO HIT				
EBCHEM	PS-03	PS-03	NO HIT	NO HIT				
EBCHEM	PS-04	PS-04	NO HIT	NO HIT				
EBCHEM	SS-01	SS-01	NO HIT	NO HIT				
EBCHEM	SS-03	SS-03	HIT	HIT				

Table B-9. Final biological effects file used for 1994 AET calculations.
Results are ordered alphabetically by Survey Code and Sample ID. A = amphipod bioassay, B = benthic infaunal abundance, ECHN abnorm. = echinoderm larval abnormality, ECHN mort = echinoderm larval effective mortality, M = Microtox luminosity (saline extract), and O = oyster/bivalve larval abnormality.

Survey Code	Station ID	Sample ID	Bioeffect Type: A	B	ECHN abnorm.	ECHN Eff. Mort.	M	O
EBCHEM	SS-04	SS-04	NO HIT	HIT				
EBCHEM	SS-05	SS-05	NO HIT	NO HIT				
EBCHEM	SS-05	SS-05	NO HIT	NO HIT				
EBCHEM	SS-06	SS-06	HIT	HIT				
EBCHEM	SS-07	SS-07	HIT	NO HIT				
EBCHEM	SS-08	SS-08	HIT	HIT				
EBCHEM	SS-09	SS-09	HIT	HIT				
EBCHEM	SS-11	SS-11	NO HIT	NO HIT				
EBCHEM	SS-12	SS-12	NO HIT	NO HIT				
EBCHEM	WW-01	WW-01	NO HIT	HIT				
EBCHEM	WW-02	WW-02	HIT					
EBCHEM	WW-03	WW-03	NO HIT	HIT				
EBCHEM	WW-04	WW-04	NO HIT	HIT				
EBCHEM	WW-05	WW-05	NO HIT	HIT				
EBCHEM	WW-06	WW-06	NO HIT	HIT				
EBCHEM	WW-06	WW-06	NO HIT	HIT				
EBCHEM	WW-08	WW-08	HIT	HIT				
EBCHEM	WW-09	WW-09	HIT	HIT				
EBCHEM	WW-10	WW-10	NO HIT	HIT				
EBCHEM	WW-11	WW-11	HIT	HIT				
EBCHEM	WW-12	WW-12	HIT	HIT				
EBCHEM	WW-13	WW-13	NO HIT	NO HIT				
EBCHEM	WW-14	WW-14		HIT				
EBCHEM	WW-15	WW-15	NO HIT					
EBCHEM	WW-16	WW-16	NO HIT	HIT				
EBCHEM	WW-17	WW-17	NO HIT	HIT				
EBCHEM	WW-18	WW-18	NO HIT	HIT				
EBCHEM	WW-19	WW-19	NO HIT	HIT				

Table B-9. Final biological effects file used for 1994 AET calculations.
Results are ordered alphabetically by Survey Code and Sample ID. A = amphipod bioassay, B = benthic infaunal abundance, ECHN abnorm. = echinoderm larval abnormality, ECHN mort = echinoderm larval effective mortality, M = Microtox luminosity (saline extract), and O = oyster/bivalve larval abnormality.

Survey Code	Station ID	Sample ID	Bioeffect Type: A	B	ECHN abnorm.	ECHN Eff. Mort.	M	O
EBCHEM	WW-20	WW-20	NO HIT	HIT				
EDMDWTC2	ED8901XX	OUTRIDGAS001	NO HIT					
EDMDWTC2	ED8902XX	SWOUTFLBS002	NO HIT					
EDMDWTC2	ED8903XX	ED89BKGDS003	NO HIT					
EHCHEM	BH-01	B1	NO HIT	NO HIT				
EHCHEM	BH-02	B1	NO HIT	NO HIT				
EHCHEM	EH-01	B1	NO HIT	NO HIT				
EHCHEM	EH-02	B1	NO HIT	HIT				
EHCHEM	EH-05	V1	NO HIT	HIT				
EHCHEM	EH-08	V1	HIT	HIT				
EHCHEM	EH-15	V1	NO HIT	HIT				
EHCHEM	EH-16	V1	NO HIT	HIT				
EHCHEM	EH-03	V6	NO HIT	HIT				
EHCHEM	EH-06	V6	NO HIT	HIT				
EIGHTBAY	BH-03	BH-03	NO HIT					
EIGHTBAY	BH-04	BH-04	NO HIT					
EIGHTBAY	BH-05	BH-05	HIT					
EIGHTBAY	BH-07	BH-07	NO HIT					
EIGHTBAY	BH-11	BH-11	NO HIT					
EIGHTBAY	BH-12	BH-12	NO HIT					
EIGHTBAY	BH-23	BH-23	HIT					
EIGHTBAY	BH-24	BH-24	NO HIT					
EIGHTBAY	CS-01	CS-01	HIT					
EIGHTBAY	CS-11	CS-11	HIT					
EIGHTBAY	CS-15	CS-15	HIT					
EIGHTBAY	CS-17	CS-17	HIT					
EIGHTBAY	DB-01	DB-01	NO HIT					
EIGHTBAY	DB-05	DB-05	NO HIT					

Table B-9. Final biological effects file used for 1994 AET calculations.
Results are ordered alphabetically by Survey Code and Sample ID. A = amphipod bioassay, B = benthic infaunal abundance, ECHN abnorm. = echinoderm larval abnormality, ECHN mort = echinoderm larval effective mortality, M = Microtox luminosity (saline extract), and O = oyster/bivalve larval abnormality.

			Bioeffect Type:		ECHN	ECHN		
Survey Code	Station ID	Sample ID	A	B	abnorm.	Eff. Mort.	M	O
EIGHTBAY	DB-07	DB-07	HIT					
EIGHTBAY	DB-15	DB-15	HIT					
EIGHTBAY	EL-09	EL-09	NO HIT					
EIGHTBAY	EL-10	EL-10	NO HIT					
EIGHTBAY	EL-12	EL-12	NO HIT					
EIGHTBAY	EL-17	EL-17	NO HIT					
EIGHTBAY	EL-20	EL-20	NO HIT					
EIGHTBAY	EL-22	EL-22	NO HIT					
EIGHTBAY	EL-23	EL-23	NO HIT					
EIGHTBAY	EL-24	EL-24	NO HIT					
EIGHTBAY	EV-01	EV-01	HIT					
EIGHTBAY	EV-02	EV-02	HIT					
EIGHTBAY	EV-03	EV-03	HIT					
EIGHTBAY	EV-04	EV-04	HIT					
EIGHTBAY	EV-05	EV-05	HIT					
EIGHTBAY	EV-06	EV-06	NO HIT					
EIGHTBAY	EV-07	EV-07	NO HIT					
EIGHTBAY	EV-11	EV-11	HIT					
EIGHTBAY	SC-06	SC-06	HIT					
EIGHTBAY	SC-07	SC-07	NO HIT					
EIGHTBAY	SC-08	SC-08	HIT					
EIGHTBAY	SC-14	SC-14	HIT					
EIGHTBAY	SC-17	SC-17	HIT					
EIGHTBAY	SC-18	SC-18	HIT					
EIGHTBAY	SC-19	SC-19	NO HIT					
EIGHTBAY	SC-20	SC-20	HIT					
EIGHTBAY	SM-01	SM-01	HIT					
EIGHTBAY	SM-03	SM-03	HIT					

Table B-9. Final biological effects file used for 1994 AET calculations.
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			Bioeffect Type:		ECHN	ECHN		
Survey Code	Station ID	Sample ID	A	B	abnorm.	Eff. Mort.	M	O
EIGHTBAY	SM-07	SM-07	HIT					
EIGHTBAY	SM-20	SM-20	HIT					
EIGHTBAY	SQ-14	SQ-14	NO HIT					
EIGHTBAY	SQ-17	SQ-17	NO HIT					
EIGHTBAY	SQ-18	SQ-18	NO HIT					
EIGHTBAY	SQ-20	SQ-20	NO HIT					
EVCHEM	ES-01	ES-01G	NO HIT					
EVCHEM	ES-02	ES-02G	NO HIT					
EVCHEM	ES-03	ES-03G	NO HIT					
EVCHEM	EW-01	EW-01G	HIT	HIT				
EVCHEM	EW-04	EW-04G	HIT	HIT				
EVCHEM	EW-07	EW-07G	HIT	HIT				
EVCHEM	EW-10	EW-10G	HIT	HIT				
EVCHEM	EW-12	EW-12G	NO HIT	HIT				
EVCHEM	EW-14	EW-14G		HIT				
EVCHEM	NG-01	NG-01G	NO HIT	NO HIT				
EVCHEM	NG-02	NG-02G	NO HIT	HIT				
EVCHEM	NG-03	NG-03G	NO HIT	HIT				
EVCHEM	NG-04	NG-04G	HIT	HIT				
EVCHEM	NG-06	NG-06G	HIT	NO HIT				
EVCHEM	NG-10	NG-10G	NO HIT	NO HIT				
EVCHEM	NG-12	NG-12G	NO HIT					
EVCHEM	NG-13	NG-13G	NO HIT					
EVCHEM	NG-14	NG-14G	NO HIT					
EVCHEM	NG-15	NG-15G	NO HIT					
EVCHEM	OG-03	OG-03G	HIT					
EVCHEM	PS-02	PS-02G	NO HIT	NO HIT				
EVCHEM	PS-03	PS-03G	NO HIT	NO HIT				

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			Bioeffect Type:		ECHN	ECHN		
Survey Code	Station ID	Sample ID	A	B	abnorm.	Eff. Mort.	M	O
EVCHEM	PS-04	PS-04G	NO HIT	NO HIT				
EVCHEM	SD-01	SD-01G	NO HIT	HIT				
EVCHEM	SD-02	SD-02G		NO HIT				
EVCHEM	SR-01	SR-01G	NO HIT					
EVCHEM	SR-02	SR-02G	NO HIT					
EVCHEM	SR-04	SR-04G	NO HIT					
EVCHEM	SR-07	SR-07G		HIT				
EVCHEM	SR-08	SR-08G	NO HIT	HIT				
EVCHEM	SS-01	SS-01G	NO HIT					
EVCHEM	SS-03	SS-03G	NO HIT					
EVERETT1	EV-20	EV-20	NO HIT					
EVERETT1	EV-21	EV-21	NO HIT					
EVERETT1	EV-22	EV-22	NO HIT					
EVERETT1	EV-23	EV-23	NO HIT					
EVERETT1	EV-24	EV-24	HIT					
EVERETT1	EV-25	EV-25	NO HIT					
EVMAR_88	EVMR0601	EVRTMRXXC001	NO HIT					NO HIT
EVMAR_88	EVMR0302	EVRTMRXXC002	HIT					NO HIT
EVMAR_88	EVMR0203	EVRTMRXXC003	NO HIT					NO HIT
EVMAR_88	EVMR0405	EVRTMRXXC005	NO HIT					NO HIT
EVMAR_88	EVMR0506	EVRTMRXXC006	NO HIT					NO HIT
EVRT10TH	EV100101	EV10ST92C001	NO HIT		NO HIT			
EVRT12TH	EV1201MC	EV12TH92C001			HIT			
EVRT12TH	EV1202MC	EV12TH92C002			HIT			
EVRT12TH	EV1204MC	EV12TH92C004			HIT			
EVRT12TH	EV1205MC	EV12TH92C005			HIT			
EVRT12TH	EV1206MC	EV12TH92C006			HIT			
EVRT12TH	EV1207MC	EV12TH92C007			HIT			

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			Bioeffect Type:					
Survey Code	Station ID	Sample ID	A	B	ECHN abnorm.	ECHN Eff. Mort.	M	O
EVWEYCII	WE8801XX	FIELDCNTS001	NO HIT					
EVWEYCII	WE8802XX	OUTFALLXS002	NO HIT					
EVWEYCII	WE8803XX	BELOWOUTS003	NO HIT					
GAPAC_C2	GAPA02XX	GA_PACXXS002	NO HIT					
GAPAC_C2	GAPA03XX	GA_PACXXS003	NO HIT					
GAPAC_C2	GAPA04XX	GA_PACXXS004	HIT					
HULB90FC	HULB0101	HULB90FCC001	HIT		NO HIT			
HULB90FC	HULB0402	HULB90FCC002	HIT		NO HIT			
HULB90FC	HULB07MC	HULB90FCC003	HIT		NO HIT			
HULB90FC	HULB10MC	HULB90FCC004	HIT		NO HIT			
HULB90FC	HULB0305	HULB90FCC005	NO HIT		HIT			
HULB90FC	HULB0402	HULB90FCC006	HIT		NO HIT			
HULB90FC	HULB07MC	HULB90FCC007	HIT		NO HIT			
HULB90FC	HULB10MC	HULB90FCC008	HIT		NO HIT			
HURLEN89	HURL0101	HURLEN89C001	NO HIT					
INTALCC2	INC201XX	INTALCC2S001	HIT					
INTALCC2	INC202XX	INTALCC2S002	NO HIT					
INTALCC2	INC203XX	INTALCC2S003	NO HIT					
INTALCC2	INC204XX	INTALCC2S004	NO HIT					
LONEST89	LONE0101	LONE89XXC001	NO HIT					
LONEST92	LSNW0101	LONEST92C001	NO HIT					
LOTTO041	1	S1	NO HIT					
LOTTO041	1	S2	NO HIT					
LOTTO041	2	S3	NO HIT					
LOTTO043	1	C1	NO HIT		NO HIT			
METAMB88	JSTU01	METAMB88S614	NO HIT					
METAMB88	JSWW01	METAMB88S615	NO HIT					
METAMB88	KSGX02	METAMB88S617	NO HIT					

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			Bioeffect Type:					
Survey Code	Station ID	Sample ID	A	B	ECHN abnorm.	ECHN Eff. Mort.	M	O
METAMB88	KSIV01	METAMB88S618	NO HIT					
METAMB88	KSRK02	METAMB88S619	NO HIT					
METAMB88	KSTK06	METAMB88S620	NO HIT					
METAMB88	LSKQ04	METAMB88S621	NO HIT					
METAMB88	LSLR03	METAMB88S622	NO HIT					
METAMB90	JSTU01	METAMB90S680	NO HIT					
METAMB90	JSWW01	METAMB90S686	NO HIT					
METAMB90	KSGX02	METAMB90S693	NO HIT					
METAMB90	KSIV01	METAMB90S699	NO HIT					
METAMB90	KSRK02	METAMB90S705	NO HIT					
METAMB90	KSTK06	METAMB90S711	NO HIT					
METAMB90	LSKQ04	METAMB90S717	NO HIT					
METAMB90	LSLR03	METAMB90S723	NO HIT					
METAMB92	JSTU01	METAMB92S770	NO HIT					
METAMB92	JSWW01	METAMB92S776	NO HIT					
METAMB92	KSGX02	METAMB92S783	NO HIT					
METAMB92	KSIV01	METAMB92S789	NO HIT					
METAMB92	KSRK02	METAMB92S795	NO HIT					
METAMB92	KSTK06	METAMB92S801	NO HIT					
METAMB92	LSKQ04	METAMB92S807	NO HIT					
METAMB92	LSLR03	METAMB92S813	NO HIT					
METROEBP	METR0101	MET154XXC001	NO HIT		NO HIT			
METROEBP	METR0402	MET407XXC002	NO HIT		HIT			
METROEBP	METR0703	MET571XXC003	NO HIT		NO HIT			
METROEBP	METR0201	MET153XXS001	NO HIT		HIT			
METROEBP	METR0301	MET155XXS002	NO HIT		HIT			
METROEBP	METR0602	MET406XXS003	NO HIT		NO HIT			
METROEBP	METR0803	MET570XXS004	NO HIT		NO HIT			

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Survey Code	Station ID	Sample ID	Bioeffect Type: A	B	ECHN abnorm.	ECHN Eff. Mort.	M	O
MORTON89	MORT0101	MORTONXXC001	NO HIT					
MORTON92	MORT0101	MORT92XXC001	NO HIT		NO HIT			
NAVYHPFC	NAVY01MC	NAVY89XXC001	NO HIT		NO HIT			
NAVYHPFC	NAVY0402	NAVY89XXC002	NO HIT		NO HIT			
NAVYHPFC	NAVY07MC	NAVY89XXC003	NO HIT		NO HIT			
NAVYHPFC	NAVY12MC	NAVY89XXC005	NO HIT		NO HIT			
NAVYHPFC	NAVY16MC	NAVY89XXC006	NO HIT		NO HIT			
NAVYHPFC	NAVY20MC	NAVY89XXC007	NO HIT		NO HIT			
NAVYHPFC	NAVY21MC	NAVY89XXC008	NO HIT		NO HIT			
NAVYHPFC	NAVY28MC	NAVY89XXC009	NO HIT		NO HIT			
NAVYHPFC	NAVY01MC	NAVY89XXC010	NO HIT		NO HIT			
NAVYHPFC	NAVY05MC	NAVY89XXC011	NO HIT		NO HIT			
NAVYHPFC	NAVY09MC	NAVY89XXC012	NO HIT		NO HIT			
NAVYHPFC	NAVY21MC	NAVY89XXC013	NO HIT		NO HIT			
NAVYHPFC	NAVY28MC	NAVY89XXC014	NO HIT		NO HIT			
NAVYHPFC	NAVY01MC	NAVY89XXC015	NO HIT		HIT			
NAVYHPFC	NAVY07MC	NAVY89XXC016	NO HIT		NO HIT			
NAVYHPFC	NAVY20MC	NAVY89XXC017	NO HIT		HIT			
NAVYHPFC	NAVY25MC	NAVY89XXC018	NO HIT		HIT			
NAVYHPFC	NAVY01MC	NAVY89XXC019	NO HIT		HIT			
NAVYHPFC	NAVY05MC	NAVY89XXC020	NO HIT		HIT			
NAVYHPFC	NAVY20MC	NAVY89XXC021	NO HIT		HIT			
NAVYHPFC	NAVY25MC	NAVY89XXC022	NO HIT		HIT			
NAVYHPFC	NAVY01MC	NAVY89XXC023	NO HIT		HIT			
NAVYHPFC	NAVY05MC	NAVY89XXC024	NO HIT		HIT			
NAVYHPFC	NAVY20MC	NAVY89XXC025	NO HIT		HIT			
NAVYHPFC	NAVY01MC	NAVY89XXC026	NO HIT		HIT			
NAVYHPFC	NAVY20MC	NAVY89XXC027	NO HIT		HIT			

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			Bioeffect Type:					
Survey Code	Station ID	Sample ID	A	B	ECHN abnorm.	ECHN Eff. Mort.	M	O
NAVYHPFC	NAVY01MC	NAVY89XXC028	HIT		HIT			
NAVYHPFC	NAVY20MC	NAVY89XXC029	HIT		HIT			
NAVYHPFC	NAVY01MC	NAVY89XXC030	NO HIT		HIT			
NAVYHPFC	NAVY20MC	NAVY89XXC031	HIT		HIT			
NAVYHPFC	NAVY11MC	NAVY89XXS004	NO HIT		NO HIT			
NAVYHPII	EVNV03MC	NAVYII90C002	NO HIT		HIT			
NAVYHPII	EVNV07MC	NAVYII90C003	NO HIT		HIT			
NAVYHPII	EVNV05MC	NAVYII90C005	HIT		HIT			
NAVYHPII	EVNV05MC	NAVYII90C007	NO HIT		HIT			
NAVYMANC	MANC0101	MANCHEXXC001	NO HIT		HIT			
NAVYMANC	MANC0702	MANCHEXXC002	NO HIT		HIT			
NAVYMANC	MANC04MC	MANCHEXXC003	NO HIT		NO HIT			
NAVYMANC	MANC1204	MANCHEXXC004	NO HIT		NO HIT			
NAVYMANC	MANC1505	MANCHEXXC005	NO HIT		HIT			
NAVYMANC	MANC1906	MANCHEXXC006	NO HIT					
NAVYMANC	MANC2207	MANCHEXXC007	NO HIT		NO HIT			
NAVYMANC	MANC1204	MANCHEXXS001	NO HIT		HIT			
NAVYMANC	MANC1505	MANCHEXXS002	NO HIT		HIT			
NAVYMANC	MANC1605	MANCHEXXS003	NO HIT		NO HIT			
NAVYMANC	MANC1705	MANCHEXXS004	NO HIT		NO HIT			
NAVYMANC	MANC18XX	MANCHEXXS005	HIT		HIT			
NAVYMANC	MANC1906	MANCHEXXS006	NO HIT		HIT			
NAVYMANC	MANC2006	MANCHEXXS007	NO HIT		NO HIT			
NAVYMANC	MANC2106	MANCHEXXS008	NO HIT		NO HIT			
OLYHARFC	OLYH17MC	OLYHFCXXC001	NO HIT		NO HIT			
OLYHARFC	OLYH19MC	OLYHFCXXC002	NO HIT		NO HIT			
OLYHARFC	OLYH09MC	OLYHFCXXC005	HIT					
OLYHARFC	OLYH02MC	OLYHFCXXC006	NO HIT		NO HIT			

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			Bioeffect Type:					
Survey Code	Station ID	Sample ID	A	B	ECHN abnorm.	ECHN Eff. Mort.	M	O
OLYHARFC	OLYH08MC	OLYHFCXXC007	NO HIT		NO HIT			
OLYHARFC	OLYH07MC	OLYHFCXXC017	NO HIT					
OLYHARFC	OLYH21MC	OLYHFCXXC019	NO HIT					
OLYHARFC	OLYH06MC	OLYHFCXXC020	HIT		NO HIT			
OLYHARFC	OLYH25MC	OLYHFCXXS012			NO HIT			
OLYHARFC	OLYH07MC	OLYHFCXXS013	NO HIT		NO HIT			
OLYHARFC	OLYH24MC	OLYHFCXXS014	NO HIT		NO HIT			
OLYHARFC	OLYH25MC	OLYHFCXXS015	NO HIT		NO HIT			
OLYHARFC	OLYH26XX	OLYHFCXXS023	HIT		NO HIT			
OLYTERC2	OT9201XX	NORTHOUTS001	NO HIT		NO HIT			
OLYTERC2	OT9202XX	SOUTHOUTS002	NO HIT		HIT			
OLYTERC2	OT9203XX	OT92BKGDS003			HIT			
PENNWLC2	PN8801XX	EASTOUTFS001	NO HIT					
PENNWLC2	PN8802XX	WESTOUTFS002	NO HIT					
PENNWLC2	PN8803XX	MIDCHANLS003	NO HIT					
PIER53BL	P53_92S1	PIER53BLS294	NO HIT					HIT
PIER53BL	P53_92S2	PIER53BLS295	NO HIT					HIT
PIER53BL	P53_92T2	PIER53BLS296	NO HIT					HIT
PIER53BL	P5392S11	PIER53BLS297	NO HIT					HIT
PIER53BL	P5392ST1	PIER53BLS298	NO HIT					HIT
PIER53BL	P53_92S9	PIER53BLS299	NO HIT					HIT
PIERD_91	1	C1	HIT		HIT			
PIERD_91	3	C2	HIT					
PIERD_91	6	C3	HIT		HIT			
PIERD_91	8	C4	HIT		HIT			
PIERD_91	11	C5	NO HIT		NO HIT			
PIERD_91	16	C6	HIT		HIT			
PIERD_91	18	C7	HIT		NO HIT			

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			Bioeffect Type:		ECHN	ECHN		
Survey Code	Station ID	Sample ID	A	B	abnorm.	Eff. Mort.	M	O
PIERD_91	21	C8	HIT		NO HIT			
PIERD_91	18	C9	HIT		HIT			
PIERD_91	20	C10	HIT		HIT			
PIERD_91	22	C11	HIT		HIT			
PIERD_91	1	S1	NO HIT		HIT			
PIERD_91	2	S2	HIT		HIT			
PIERD_91	3	S3	HIT					
PIERD_91	4	S4	HIT					
PIERD_91	5	S5	HIT		HIT			
PIERD_91	6	S6	HIT		HIT			
PIERD_91	7	S7	HIT		HIT			
PIERD_91	8	S8	HIT		NO HIT			
PIERD_91	9	S9	HIT		HIT			
PIERD_91	10	S10	HIT		HIT			
PIERD_91	11	S11	HIT		HIT			
PIERD_91	12	S12	HIT		HIT			
PIERD_91	13	S13	HIT		HIT			
PIERD_91	14	S14	HIT		NO HIT			
PIERD_91	15	S15	NO HIT					
PIERD_91	16	S16	HIT		HIT			
PIERD_91	17	S17	HIT		HIT			
POSTPTC2	POST01XX	POSTPTXXS001	HIT					
POSTPTC2	POST03XX	POSTPTXXS003	HIT					
PSDDA1	CBB01	CBB01C	HIT					
PSDDA1	CBB03	CBB03C	HIT					
PSDDA1	CBS01	CBS01C	NO HIT					
PSDDA1	CBS08	CBS08C	NO HIT					
PSDDA1	CBZ01	CBZ01C	HIT					

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			Bioeffect Type:		ECHN	ECHN		
Survey Code	Station ID	Sample ID	A	B	abnorm.	Eff. Mort.	M	O
PSDDA1	EBB01	EBB01C	NO HIT					
PSDDA1	EBB02	EBB02C	NO HIT					
PSDDA1	EBS01	EBS01C	NO HIT					
PSDDA1	EBS02	EBS02C	NO HIT					
PSDDA1	EBZ01	EBZ01C	NO HIT					
PSDDA1	PGB01	PGB01C	HIT					
PSDDA1	PGB02	PGB02C	HIT					
PSDDA1	PGZ01	PGZ01C	HIT					
PSDDA2	AKB02	AKB02C	NO HIT					
PSDDA2	AKZ01	AKZ01C	NO HIT					
PSDDA2	BBB01	BBB01C	NO HIT					
PSDDA2	BBB02	BBB02C	NO HIT					
PSDDA2	BBB04	BBB04C	NO HIT					
PSDDA2	BBZ01	BBZ01C	NO HIT					
PSDDAM90	EB_B01XX	EB90_B01	NO HIT		NO HIT			
PSDDAM90	EB_B02XX	EB90_B02	NO HIT		NO HIT			
PSDDAM90	EB_B03BX	EB90_B03B	HIT					
PSDDAM90	EB_B04AX	EB90_B04	NO HIT		NO HIT			
PSDDAM90	EB_Z01XX	EB90_Z01	NO HIT		HIT			
PSDDAM90	PG_B01XX	PG90_B01	NO HIT		NO HIT			
PSDDAM90	PG_B02XX	PG90_B02	HIT		HIT			
PSDDAM90	PG_S04XX	PG90_S04	NO HIT		HIT			
PSDDAM90	PG_S08XX	PG90_S08	NO HIT		HIT			
PSDDAM90	PG_Z06XX	PG90_Z06	NO HIT		HIT			
PSDDAM91	P91PG1XX	P91PGB09S001	HIT		HIT			
PSDDAM92	PMONZ01	PMONZ01S001	NO HIT		HIT			
PSDDAM92	PMONS02	PMONS02S002	NO HIT		HIT			
PSDDAM92	PMONS04	PMONS04S003	HIT		HIT			

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			Bioeffect Type:					
Survey Code	Station ID	Sample ID	A	B	ECHN abnorm.	ECHN Eff. Mort.	M	O
PSREF90	CR02	SD0002	NO HIT		NO HIT			NO HIT
PSREF90	CR20	SD0003	NO HIT		HIT			NO HIT
PSREF90	CR21	SD0004	NO HIT		NO HIT			HIT
PSREF90	CR22	SD0005	NO HIT		NO HIT			HIT
PSREF90	CR23	SD0006	NO HIT		NO HIT			NO HIT
PSREF90	CR24	SD0007	NO HIT		HIT			HIT
PSREF90	CR25	SD0008	NO HIT		NO HIT			NO HIT
PSREF90	SM30	SD0010	NO HIT		HIT			HIT
PSREF90	SM31	SD0011	NO HIT		NO HIT			HIT
PSREF90	SM32	SD0012	NO HIT		HIT			HIT
PSREF90	SM33	SD0013	NO HIT		NO HIT			HIT
PSREF90	SM34	SD0014	NO HIT		NO HIT			HIT
PSREF90	SM35	SD0015	NO HIT		NO HIT			HIT
PSREF90	SM36	SD0016	NO HIT		NO HIT			HIT
PSREF90	HH01	SD0017	NO HIT		HIT			HIT
PSREF90	HH02	SD0018	NO HIT		NO HIT			HIT
PSREF90	HM03	SD0019	NO HIT		NO HIT			HIT
PSREF90	HM04	SD0020	NO HIT		NO HIT			HIT
PSREF90	HM05	SD0021	NO HIT		HIT			HIT
PSREF90	HM06	SD0024	NO HIT		HIT			HIT
PSREF90	HM07	SD0025	NO HIT		HIT			HIT
PTORCHC2	PO8901XX	POATOUTFS001	NO HIT					
PTORCHC2	PO8902XX	PONROUTFS002	NO HIT					
PTORCHC2	PO8903XX	POCNTROLS003	NO HIT					
PTWNPCC2	PTC203XX	PTPCC287K003	NO HIT					
PTWNPCC2	PTC201XX	PTPCC287S001	NO HIT					
PTWNPCC2	PTC202XX	PTPCC287S002	NO HIT					
PTWNPENR	PT8702XX	PTWGLENCS002	NO HIT					

Table B-9. Final biological effects file used for 1994 AET calculations.
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Survey Code	Station ID	Sample ID	Bioeffect Type: A	B	ECHN abnorm.	ECHN Eff. Mort.	M	O
PTWNPENR	PT8703XX	PTEGLENCS003	NO HIT					
PTWNPENR	PT8705XX	PTPTWLSNS005	NO HIT					
SED18804	OH-1	OH-1	NO HIT					
SED18804	OH-2	OH-2	NO HIT					
SED18804	OH-3	OH-3	NO HIT					
SED18804	OH-4	OH-4	NO HIT					
SED18804	OH-5	OH-5	NO HIT					
SED18804	OH-6	OH-6	NO HIT					
SED18804	PA-1	PA-1	NO HIT					
SED18804	PA-2	PA-2	NO HIT					
SED18804	PA-3	PA-3	NO HIT					
SED18804	PA-4	PA-4A	NO HIT					
SED18804	PA-5	PA-5	NO HIT					
SED18804	PA-6	PA-6	NO HIT					
SED18903	1	1	NO HIT					
SED18903	2	1	NO HIT					
SED18903	3	1	NO HIT					
SED18903	4	1	NO HIT					
SED18903	5	1	NO HIT					
SED18903	6	1	NO HIT					
SED18903	7	1	NO HIT					
SED18903	8	1	NO HIT					
SED18903	9	1	NO HIT					
SED18903	10	1	NO HIT					
SED18903	11	1	NO HIT					
SED18903	12	1	NO HIT					
SED18903	13	1	NO HIT					
SED18903	14	1	NO HIT					

Table B-9. Final biological effects file used for 1994 AET calculations.
Results are ordered alphabetically by Survey Code and Sample ID. A = amphipod bioassay, B = benthic infaunal abundance, ECHN abnorm. = echinoderm larval abnormality, ECHN mort = echinoderm larval effective mortality, M = Microtox luminosity (saline extract), and O = oyster/bivalve larval abnormality.

Survey Code	Station ID	Sample ID	Bioeffect Type: A	B	ECHN abnorm.	ECHN Eff. Mort.	M	O
SED18903	15	1	NO HIT					
SED18903	16	1	NO HIT					
SED18903	17	1	NO HIT					
SED18903	18	1	NO HIT					
SED18903	19	1	NO HIT					
SED18903	20	1	NO HIT					
SED18903	21	1	NO HIT					
SED18903	22	1	NO HIT					
SED18903	23	1	NO HIT					
SED18903	24	1	HIT					
SED18903	25	1	NO HIT					
SED18903	26	1	NO HIT					
SED18903	27	1	NO HIT					
SED18903	28	1	NO HIT					
SED18903	29	1	NO HIT					
SED18903	30	1	NO HIT					
SED18903	31	1	NO HIT					
SED18903	32	1	NO HIT					
SED18903	33	1	NO HIT					
SED18903	34	1	NO HIT					
SED18903	35	1	HIT					
SED18903	36	1	NO HIT					
SED18903	37	1	NO HIT					
SED18903	38	1	NO HIT					
SED18903	39	1	NO HIT					
SED18903	40	1	NO HIT					
SED18903	41	1	NO HIT					
SED18903	42	1	NO HIT					

Table B-9. Final biological effects file used for 1994 AET calculations.
Results are ordered alphabetically by Survey Code and Sample ID. A = amphipod bioassay, B = benthic infaunal abundance, ECHN abnorm. = echinoderm larval abnormality, ECHN mort = echinoderm larval effective mortality, M = Microtox luminosity (saline extract), and O = oyster/bivalve larval abnormality.

			Bioeffect Type:					
Survey Code	Station ID	Sample ID	A	B	ECHN abnorm.	ECHN Eff. Mort.	M	O
SED18903	43	1	NO HIT					
SED18903	44	1	NO HIT					
SED18903	45	1	NO HIT					
SED18903	46	1	NO HIT					
SED18903	47	1	NO HIT					
SED18903	48	1	NO HIT					
SED18903	49	1	NO HIT					
SED18903	50	1	NO HIT					
SED19003	1	1	HIT					
SED19003	3	3	NO HIT					
SED19003	4	4	HIT					
SED19003	5	5	NO HIT					
SED19003	5	51	NO HIT					
SED19003	5	52	HIT					
SED19003	5	53	HIT					
SED19003	8	8	NO HIT					
SED19003	12	12	HIT					
SED19003	14	14	HIT					
SED19003	15	15	NO HIT					
SED19003	17	17	HIT					
SED19003	18	18	HIT					
SED19003	19	19	HIT					
SED19003	20	20	HIT					
SED19003	21	21	NO HIT					
SED19003	22	22	NO HIT					
SED19003	26	26	NO HIT					
SED19003	29	29	NO HIT					
SED19003	30	30	NO HIT					

Table B-9. Final biological effects file used for 1994 AET calculations.
Results are ordered alphabetically by Survey Code and Sample ID. A = amphipod bioassay, B = benthic infaunal abundance, ECHN abnorm. = echinoderm larval abnormality, ECHN mort = echinoderm larval effective mortality, M = Microtox luminosity (saline extract), and O = oyster/bivalve larval abnormality.

Survey Code	Station ID	Sample ID	Bioeffect Type: A	B	ECHN abnorm.	ECHN Eff. Mort.	M	O
SED19003	32	32	NO HIT					
SED19003	32	57	NO HIT					
SED19003	32	58	NO HIT					
SED19003	32	59	NO HIT					
SED19003	33	33	NO HIT					
SED19003	34	34	HIT					
SED19003	35	35	HIT					
SED19003	35	72	HIT					
SED19003	35	73	HIT					
SED19003	35	74	HIT					
SED19003	38	38	HIT					
SED19003	38	60	NO HIT					
SED19003	38	61	HIT					
SED19003	38	62	HIT					
SED19003	39	39	NO HIT					
SED19003	40	40	NO HIT					
SED19003	41	41	NO HIT					
SED19003	43	43	NO HIT					
SED19003	44	44	NO HIT					
SED19003	44	63	NO HIT					
SED19003	44	64	NO HIT					
SED19003	44	65	NO HIT					
SED19003	45	45	NO HIT					
SED19003	47	47	NO HIT					
SED19003	48	48	HIT					
SED19003	49	49	HIT					
SED19003	69	69	NO HIT					
SED19003	70	70	NO HIT					

Table B-9. Final biological effects file used for 1994 AET calculations.
Results are ordered alphabetically by Survey Code and Sample ID. A = amphipod bioassay, B = benthic infaunal abundance, ECHN abnorm. = echinoderm larval abnormality, ECHN mort = echinoderm larval effective mortality, M = Microtox luminosity (saline extract), and O = oyster/bivalve larval abnormality.

Survey Code	Station ID	Sample ID	Bioeffect Type: A	B	ECHN abnorm.	ECHN Eff. Mort.	M	O
SED19003	71	71	NO HIT					
SED19003	101R	101R	HIT					
SED19003	102R	102R	HIT					
SED19003	103R	103R	NO HIT					
SED19003	104R	104R	HIT					
SED19003	105R	105R	HIT					
SED19003	106R	106R	HIT					
SED19003	108R	108R	NO HIT					
SED19003	109R	109R	HIT					
SED19003	110R	110R	HIT					
SED19003	111R	111R	NO HIT					
SED19003	112R	112R	NO HIT					
SED19003	113R	113R	NO HIT					
SED19003	114R	114R	HIT					
SED19003	115R	115R	HIT					
SED19003	116R	116R	NO HIT					
SED19003	46R	46R	NO HIT					
SED19103	1	1	NO HIT					
SED19103	3	3	NO HIT					
SED19103	4	4	NO HIT					
SED19103	5	5	NO HIT					
SED19103	5	51	NO HIT					
SED19103	5	52	NO HIT					
SED19103	5	53	NO HIT					
SED19103	8	8	NO HIT					
SED19103	12	12	NO HIT					
SED19103	14	14	NO HIT					
SED19103	15	15	NO HIT					

Table B-9. Final biological effects file used for 1994 AET calculations.
Results are ordered alphabetically by Survey Code and Sample ID. A = amphipod bioassay, B = benthic infaunal abundance, ECHN abnorm. = echinoderm larval abnormality, ECHN mort = echinoderm larval effective mortality, M = Microtox luminosity (saline extract), and O = oyster/bivalve larval abnormality.

Survey Code	Station ID	Sample ID	Bioeffect Type: A	B	ECHN abnorm.	ECHN Eff. Mort.	M	O
SED19103	17	17	NO HIT					
SED19103	18	18	HIT					
SED19103	19	19	NO HIT					
SED19103	20	20	NO HIT					
SED19103	21	21	NO HIT					
SED19103	22	22	NO HIT					
SED19103	26	26	NO HIT					
SED19103	29	29	NO HIT					
SED19103	30	30	NO HIT					
SED19103	32	32	NO HIT					
SED19103	32	57	NO HIT					
SED19103	32	58	NO HIT					
SED19103	32	59	NO HIT					
SED19103	33	33	NO HIT					
SED19103	34	34	NO HIT					
SED19103	35	35	NO HIT					
SED19103	35	72	NO HIT					
SED19103	35	73	NO HIT					
SED19103	35	74	NO HIT					
SED19103	38	38	NO HIT					
SED19103	38	60	NO HIT					
SED19103	38	61	NO HIT					
SED19103	38	62	NO HIT					
SED19103	39	39	NO HIT					
SED19103	40	40	NO HIT					
SED19103	41	41	NO HIT					
SED19103	43	43	NO HIT					
SED19103	44	44	NO HIT					

Table B-9. Final biological effects file used for 1994 AET calculations.
Results are ordered alphabetically by Survey Code and Sample ID. A = amphipod bioassay, B = benthic infaunal abundance, ECHN abnorm. = echinoderm larval abnormality, ECHN mort = echinoderm larval effective mortality, M = Microtox luminosity (saline extract), and O = oyster/bivalve larval abnormality.

Survey Code	Station ID	Sample ID	Bioeffect Type: A	B	ECHN abnorm.	ECHN Eff. Mort.	M	O
SED19103	44	63	NO HIT					
SED19103	44	64	NO HIT					
SED19103	44	65	NO HIT					
SED19103	45	45	NO HIT					
SED19103	47	47	NO HIT					
SED19103	48	48	NO HIT					
SED19103	49	49	NO HIT					
SED19103	69	69	NO HIT					
SED19103	70	70	NO HIT					
SED19103	71	71	NO HIT					
SED19103	R09	9	NO HIT					
SED19103	R10	10	NO HIT					
SED19103	R11	11	NO HIT					
SED19103	R13	13	NO HIT					
SED19103	R201	201	NO HIT					
SED19103	R202	202	NO HIT					
SED19103	R203	203	NO HIT					
SED19103	R204	204	NO HIT					
SED19103	R206	206	NO HIT					
SED19103	R207	207	HIT					
SED19103	R208	208	NO HIT					
SED19103	R209	209	NO HIT					
SHELLCII	SH8901XX	SH89OUTFS001	NO HIT					
SITCUMRI	NB-1	300001NB1	HIT					
SITCUMRI	NB-2	300055NB2	NO HIT					
SITCUMRI	NB-A1	300056NBA1	NO HIT					
SITCUMRI	NB-A2	300057NBA2	HIT					
SITCUMRI	NB-A3	300027NBA3	NO HIT					

Table B-9. Final biological effects file used for 1994 AET calculations.
Results are ordered alphabetically by Survey Code and Sample ID. A = amphipod bioassay, B = benthic infaunal abundance, ECHN abnorm. = echinoderm larval abnormality, ECHN mort = echinoderm larval effective mortality, M = Microtox luminosity (saline extract), and O = oyster/bivalve larval abnormality.

Survey Code	Station ID	Sample ID	Bioeffect Type: A	B	ECHN abnorm.	ECHN Eff. Mort.	M	O
SITCUMRI	NB-A4	300003NBA4	NO HIT					
SITCUMRI	NB-A5	300023NBA5	NO HIT					
SITCUMRI	NB-A6	300032NBA6	NO HIT					
SITCUMRI	NB-A7	300034NBA7	NO HIT					
SITCUMRI	NB-B1	300029NBB1	NO HIT					
SITCUMRI	NB-B2	300030NBB2	NO HIT					
SITCUMRI	NB-B3	300054NBB3	NO HIT					
SITCUMRI	NB-B4	300041NBB4	NO HIT					
SITCUMRI	NB-B5	300053NBB5	NO HIT					
SITCUMRI	NB-B6	300052NBB6	NO HIT					
SITCUMRI	NB-C1	300087NBC1	NO HIT					
SITCUMRI	NB-C2	300104NBC2	NO HIT					
SITCUMRI	NB-C3	300103NBC3	NO HIT					
SITCUMRI	NB-C4	300102NBC4	NO HIT					
SITCUMRI	NB-C5	300101NBC5	NO HIT					
SITCUMRI	NB-C6	300100NBC6	NO HIT					
SITCUMRI	NC-I1	300092NCI1	NO HIT					
SITCUMRI	NC-I10	300080NCI10	NO HIT		NO HIT			
SITCUMRI	NC-I11	300079NCI11	NO HIT		HIT			
SITCUMRI	NC-I12	300078NCI12	NO HIT		NO HIT			
SITCUMRI	NC-I2	300091NCI2	NO HIT					
SITCUMRI	NC-I3	300090NCI3	NO HIT					
SITCUMRI	NC-I4	300089NCI4	NO HIT					
SITCUMRI	NC-I5	300088NCI5	NO HIT					
SITCUMRI	NC-I6	300086NCI6	NO HIT					
SITCUMRI	NC-I7	300085NCI7	HIT		HIT			
SITCUMRI	NC-I8	300084NCI8	NO HIT		NO HIT			
SITCUMRI	NC-I9	300081NCI9	NO HIT		NO HIT			

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Survey Code	Station ID	Sample ID	Bioeffect Type: A	B	ECHN abnorm.	ECHN Eff. Mort.	M	O
SITCUMRI	NC-S1	300051NCS1	NO HIT					
SITCUMRI	NC-S2	300050NCS2	NO HIT					
SITCUMRI	NC-S3	300049NCS3	NO HIT					
SITCUMRI	NC-S4	300048NCS4	NO HIT					
SITCUMRI	NC-S5	300043NCS5	NO HIT					
SITCUMRI	NC-S6	300042NCS6	NO HIT					
SITCUMRI	NC-S7	300033NCS7	NO HIT					
SITCUMRI	NM-1	300113NM1	NO HIT					
SITCUMRI	NM-2	300115NM2	NO HIT					
SITCUMRI	NM-3	300020NM3	NO HIT					
SITCUMRI	NM-4	300114NM4	NO HIT					
SITCUMRI	NM-5	300112NM5	NO HIT					
SITCUMRI	NM-6	300111NM6	NO HIT					
SITCUMRI	NM-7	300109NM7	NO HIT					
SITCUMRI	NS-1	300004NS1	NO HIT		NO HIT			
SITCUMRI	NS-2	300001NS2	NO HIT		HIT			
SITCUMRI	NS-3	300108NS3	NO HIT		NO HIT			
SITCUMRI	NS-A1	300016NSA1	NO HIT		HIT			
SITCUMRI	NS-A2	300047NSA2	NO HIT		NO HIT			
SITCUMRI	NS-A3	300031NSA3	NO HIT		NO HIT			
SITCUMRI	NS-A4	300012NSA4	NO HIT		NO HIT			
SITCUMRI	NS-A5	300014NSA5	NO HIT		HIT			
SITCUMRI	NS-A6	300015NSA6	NO HIT		HIT			
SITCUMRI	NS-B1	300018NSB1	HIT		NO HIT			
SITCUMRI	NS-B2	300046NSB2	NO HIT		HIT			
SITCUMRI	NS-B3	300017NSB3	NO HIT		HIT			
SITCUMRI	NS-B4	300009NSB4	NO HIT		HIT			
SITCUMRI	NS-B5	300010NSB5	HIT		NO HIT			

Table B-9. Final biological effects file used for 1994 AET calculations.
Results are ordered alphabetically by Survey Code and Sample ID. A = amphipod bioassay, B = benthic infaunal abundance, ECHN abnorm. = echinoderm larval abnormality, ECHN mort = echinoderm larval effective mortality, M = Microtox luminosity (saline extract), and O = oyster/bivalve larval abnormality.

Survey Code	Station ID	Sample ID	Bioeffect Type: A	B	ECHN abnorm.	ECHN Eff. Mort.	M	O
SITCUMRI	NS-B6	300013NSB6	NO HIT		NO HIT			
SITCUMRI	NS-C1	300005NSC1	NO HIT		NO HIT			
SITCUMRI	NS-C2	300008NSC2	NO HIT		HIT			
SITCUMRI	NS-C3	300019NSC3	HIT		HIT			
SITCUMRI	NS-C4	300006NSC4	NO HIT		HIT			
SITCUMRI	NS-C5	300044NSC5	NO HIT		NO HIT			
SITCUMRI	NS-C6	300011NSC6	HIT		NO HIT			
SITCUMRI	NS-D1A	300082NSD1A	NO HIT		HIT			
SITCUMRI	NS-D2	300083NSD2	HIT		HIT			
SITCUMRI	NS-D3	300093NSD3	NO HIT		HIT			
SITCUMRI	NS-D4	300098NSD4	NO HIT		HIT			
SITCUMRI	NS-D5	300099NSD5	HIT		HIT			
SITCUMRI	NS-D6	300095NSD6	HIT		HIT			
SITCUMRI	NS-E1A	300097NSE1A	NO HIT		HIT			
SITCUMRI	NS-E2	300096NSE2	NO HIT		HIT			
SITCUMRI	NS-E3	300105NSE3	NO HIT		NO HIT			
SITCUMRI	NS-E4	300106NS34	NO HIT		HIT			
SITCUMRI	NS-E5	300094NSE5	NO HIT		HIT			
SNDREF92	SD920101	SD92OUTFC001	NO HIT		HIT			
SNDREF92	SD920602	SD92HYLBC002	NO HIT		NO HIT			
SNDREF92	SD921103	SD92UPSTC003	NO HIT		NO HIT			
SOPARK91	SPRK0101	SOPARK91C001	NO HIT		NO HIT			
SOPARK91	SPRK0302	SOPARK91C002	NO HIT		NO HIT			
SOTERM86	ST86H101	ST86HA1SC001	NO HIT					
SOTERM86	ST86H302	ST86HA3SC002	NO HIT					
SOTERM86	ST86H503	ST86HA5SC003	NO HIT					
SOTERM86	ST86H704	ST86HA7SC004	HIT					
SOTERM86	ST86H905	ST86HA9SC005	NO HIT					

Table B-9. Final biological effects file used for 1994 AET calculations.
Results are ordered alphabetically by Survey Code and Sample ID. A = amphipod bioassay, B = benthic infaunal abundance, ECHN abnorm. = echinoderm larval abnormality, ECHN mort = echinoderm larval effective mortality, M = Microtox luminosity (saline extract), and O = oyster/bivalve larval abnormality.

			Bioeffect Type:					
Survey Code	Station ID	Sample ID	A	B	ECHN abnorm.	ECHN Eff. Mort.	M	O
SOTERM86	ST86W106	ST86W1S_C006	NO HIT					
SOTERM86	ST86W407	ST86W4S_C007	HIT					
SOTERM86	ST86W708	ST86W7S_C008	NO HIT					
SOTERM86	ST86H302	ST86HA3B_S01	NO HIT					
SOTERM86	ST86H905	ST86HA9B_S04	NO HIT					
SOTERM86	ST861005	ST86HA10BS05	NO HIT					
SOTERM86	ST86W206	ST86W2B__S06	NO HIT					
SOTERM86	ST86W306	ST86W3B__S07	NO HIT					
SOTERM86	ST86W507	ST86W5B__S08	NO HIT					
SOTERM86	ST86W607	ST86W6B__S09	NO HIT					
SOTERM86	ST86W10X	ST86W10S_S10	NO HIT					
SOTERM86	ST86W10X	ST86W10B_S11	NO HIT					
SSRECON	BUD-9	C00001	NO HIT					
SSRECON	BUD-10	C00002	NO HIT					
SSRECON	BUD-8	C00003	NO HIT					
SSRECON	BUD-4	C00004	NO HIT					
SSRECON	BUD-7	C00005	NO HIT					
SSRECON	BUD-5	C00006	NO HIT					
SSRECON	BUD-2	C00007	NO HIT					
SSRECON	BUD-3	C00008	NO HIT					
SSRECON	BUD-6	C00009	NO HIT					
SSRECON	BUD-1	C00010	NO HIT					
SSRECON	BUD-11	C00011	NO HIT					
SSRECON	BUD-12	C00012	NO HIT					
SSRECON	ELD-16	C00013	NO HIT					
SSRECON	HEND-17	C00014	NO HIT					
SSRECON	CR-23	C00019	NO HIT					
SSRECON	WOLL-24	C00021	NO HIT					

Table B-9. Final biological effects file used for 1994 AET calculations.
Results are ordered alphabetically by Survey Code and Sample ID. A = amphipod bioassay, B = benthic infaunal abundance, ECHN abnorm. = echinoderm larval abnormality, ECHN mort = echinoderm larval effective mortality, M = Microtox luminosity (saline extract), and O = oyster/bivalve larval abnormality.

			Bioeffect Type:		ECHN	ECHN		
Survey Code	Station ID	Sample ID	A	B	abnorm.	Eff. Mort.	M	O
SSRECON	CORM-20	C00024	NO HIT					
TACCENC2	TC8901XX	TACCN89AS001	NO HIT					
TACCENC2	TC8902XX	TACCN89BS002	NO HIT					
TACCENC2	TC8903XX	TACCN89CS003	NO HIT					
TERM5_91	TER50101	TER592C001	NO HIT		NO HIT			
TERM5_91	TER50302	TER592C002	NO HIT		NO HIT			
TERM5_91	TER50503	TER592C003	NO HIT		NO HIT			
TERMNL91	T91_01XX	TERMNL91S001	HIT		HIT			
TERMNL91	T91_02XX	TERMNL91S002	NO HIT		HIT			
TERMNL91	T91_03XX	TERMNL91S003	HIT		NO HIT			
TEXACOC2	TX8801XX	TX88DOCKS001	NO HIT					
TEXACOC2	TX8802XX	TX88DISCS002	NO HIT					
TEXACOC2	TX8803XX	TX88CTRLS003	NO HIT					
TPPS3AB	EB-33	1779		HIT				
TPPS3AB	EB-33	2080		HIT				
TPPS3AB	EB-35	1775		HIT				
TPPS3AB	EB-35	2079		HIT				
TPPS3AB	EB-36	1776		HIT				
TPPS3AB	EB-36	2072		HIT				
TPPS3AB	EB-38	1778		HIT				
TPPS3AB	EB-38	2074		HIT				
TPPS3AB	WP-01	2088		NO HIT				
TPPS3AB	WP-02	2089		NO HIT				
TPPS3AB	WP-03	2090		HIT				
TPPS3AB	WP-04	2091		NO HIT				
TPPS3AB	WP-05	2092		NO HIT				
TPPS3AB	WP-06	2084		NO HIT				
TPPS3AB	WP-07	2093		NO HIT				

Table B-9. Final biological effects file used for 1994 AET calculations.
Results are ordered alphabetically by Survey Code and Sample ID. A = amphipod bioassay, B = benthic infaunal abundance, ECHN abnorm. = echinoderm larval abnormality, ECHN mort = echinoderm larval effective mortality, M = Microtox luminosity (saline extract), and O = oyster/bivalve larval abnormality.

			Bioeffect Type:		ECHN	ECHN		
Survey Code	Station ID	Sample ID	A	B	abnorm.	Eff. Mort.	M	O
TPPS3AB	WP-08	2083		HIT				
TPPS3AB	WP-09	2082		NO HIT				
TPPS3AB	WP-10	2076		NO HIT				
TPPS3AB	WP-11	1789						
TPPS3AB	WP-12	1786		NO HIT				
TPPS3AB	WP-12	2069		NO HIT				
TPPS3AB	WP-13	1784		NO HIT				
TPPS3AB	WP-13	2070		NO HIT				
TPPS3AB	WP-14	1785		NO HIT				
TPPS3AB	WP-14	2085						
TPPS3AB	WP-15	1817		NO HIT				
TPPS3AB	WP-15	2094		NO HIT				
TPPS3AB	WP-16	1816		HIT				
TPPS3AB	WP-16	2086		NO HIT				
TXNPDS92	TX920101	TX92OUTFC001	NO HIT					
TXNPDS92	TX920602	TX92DILZC002	NO HIT					
TXNPDS92	TX921103	TX92AMBSC003	NO HIT					
USOILVLF	USOL0101	USOILVLFC001	NO HIT					
USOILVLF	USOL0101	USOILVLFS001	NO HIT		HIT			
USOILVLF	USOL0101	USOILVLFS002	NO HIT					
USOILVLF	USOL0201	USOILVLFS003	NO HIT		HIT			
USOILVLF	USOL0201	USOILVLFS004	NO HIT					
USOILVLF	USOL03XX	USOILVLFS005	NO HIT		HIT			
USOILVLF	USOL0401	USOILVLFS006	HIT					
USOILVLF	USOL0501	USOILVLFS007	NO HIT		HIT			
USOILVLF	USOL0501	USOILVLFS008	NO HIT					

Table B-10. Chemicals eliminated from AET groups prior to database comparisons and calculations of predictive reliability. Chemicals appear in bold italics were eliminated from 1988 AET comparisons.

Chemical Name
Acid volatile sulfides
Ammonia-nitrogen
Ammonium (NH4+)
Biochemical oxygen demand
Biochemical oxygen demand-5 day test
Calcium
Carbon Dioxide
Carbon Monoxide
Chemical oxygen demand
Conductivity
Dissolved oxygen
Dissolved oxygen-% Saturation
Dry weight of sediment or tissue
Iron
Manganese
Oil And Grease
PCB-1242
PCB-1248
PCB-1254
PCB-1260
Percent clay
Percent gravel
Percent moisture
Percent rocks
Percent sand
Percent silt
Percent sulfur
pH
Phi Class -1.00+ To 0.00
Phi Class 0.00+ To 1.00
Phi Class 1.00+ To 2.00
Phi Class 2.00+ To 3.00
Phi Class 3.00+ To 4.00
Phosphorus
Potassium
Salinity
Silica
Silicate
Sodium
Specific Gravity
Sulfate
Sulfates

Table B-10. Chemicals eliminated from AET groups prior to database comparisons and calculations of predictive reliability. Chemicals appear in bold italics were eliminated from 1988 AET comparisons.

Chemical Name
Total alkalinity
Total carbon
Total nitrogen (no2+no3)
Total nitrogen (no3+no3+nh4)
Total NO2-n (nitrite Nitrogen)
Total NO3-n (nitrate Nitrogen)
Total phosphate
Total Solids
Total Suspended Solids
Total volatile solids
Viscosity
Wet weight of sediment or tissue