



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  
WASHINGTON, D.C. 20460

July 31, 1989

EPA-SAB-EETFC-89-027

The Honorable William Reilly  
Administrator  
U.S. Environmental Protection Agency  
401 M. Street, S.W.  
Washington, D.C. 20460

Dear Mr. Reilly:

The Sediment Criteria Subcommittee of the Science Advisory Board has completed its review of the Apparent Effects Threshold (AET) Approach to setting sediment criteria. This approach was developed by EPA's Region 10, Office of Puget Sound. The review was conducted at the request of Region 10's Administrator, Mr. Robie Russell, and was conducted on October 27 and 28 in Seattle, Washington.

The AET approach is designed to identify adverse effects due to chemical contamination in sediments by determining specific chemical concentrations above which adverse effects will always occur. The method has major strengths in its ability to determine biological effects and assess interactive chemical effects. The method is considered by the Subcommittee to contain sufficient scientific merit that, with appropriate validation, it could be used to estimate sediment quality at specific sites.

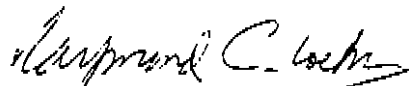
In the Subcommittee's opinion, the AET approach should not be used to develop general, broadly applicable sediment quality criteria. Some major limitations drive this opinion, including the site specific nature of the approach, its inability to describe cause and effect relationships, its lack of independent validation, and its inability to describe differences in bioavailability of chemicals on different sediments.

The Subcommittee recognizes the Apparent Effects Threshold Approach as a credible step towards development of a technically defensible and publicly acceptable tool for managing contaminated sediments. The approach provides a constructive beginning towards assessing the impact of mixtures of chemicals as they occur in actual situations. Such innovative empirical approaches that assess actual contamination and concomitant effects are encouraged and applauded by the Subcommittee. However, the Subcommittee also recommends that neither the AET or any other existing methodology be used as a stand-alone decision tool to provide absolute pass/fail criteria to dictate regulatory action.

The Subcommittee has several suggestions for strengthening the AET approach. These include utilizing replicate sediment samples for assessments, devising criteria for selection of reference sites, including considerations of physical factors, and developing measures of variance. In addition, the use of both carbon normalization and benthic infaunal assays are strongly supported by the Subcommittee.

The Subcommittee appreciates the opportunity to conduct this scientific review. We request that the Agency formally respond to the scientific advice transmitted in the attached report.

Sincerely



Dr. Raymond Loehr, Chairman  
Executive Committee  
Science Advisory Board



Dr. Kenneth Dickson, Chairman  
Environmental Effects,  
Transport and Fate  
Committee



Dr. Robert Huggett, Chairman  
Sediment Criteria Subcommittee

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Robie Russell  
Chris Zarba  
Catherine Krueger  
Donald Barnes

United States

Environmental Protection

Agency

Office of the Administrator

Science Advisory Board

Washington, D.C. 20460

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# **Report of the Sediment Criteria Subcommittee**

## **Evaluation of the Apparent Effects Threshold (AET) Approach for Assessing Sediment Quality**



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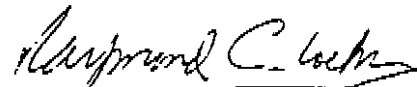
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The Subcommittee recognizes the Apparent Effects Threshold Approach as a credible step towards development of a technically defensible and publicly acceptable tool for managing contaminated sediments. The approach provides a constructive beginning towards assessing the impact of mixtures of chemicals as they occur in actual situations. Such innovative empirical approaches that assess actual contamination and concomitant effects are encouraged and applauded by the Subcommittee. However, from a scientific standpoint, the Subcommittee recommends that multiple approaches be used to estimate sediment quality, develop criteria, and guide regulatory action, since the AET approach alone provides insufficient certainty for broad-scale decision making.

The Subcommittee has several suggestions for strengthening the AET approach. These include utilizing replicate sediment samples for assessments, devising criteria for selection of reference sites, including considerations of physical factors, and developing measures of variance. In addition, the use of both carbon normalization and benthic infaunal assays are strongly supported by the Subcommittee.

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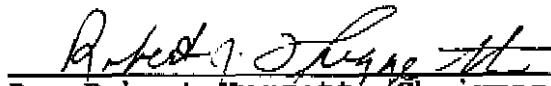
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## **ABSTRACT**

This report presents the conclusions and recommendations of the U.S. Environmental Protection Agency's Science Advisory Board summarizing a review of EPA's Apparent Effects Threshold Approach for setting sediment quality criteria. The AET approach integrates data from bulk sediment chemistry, sediment bioassays and infaunal species measurements to provide estimates of sediment chemical concentrations above which adverse environmental effects will occur. An objective of the AET methodology is to identify adverse effects due to chemicals occurring in mixtures in sediments by determining specific chemical concentrations above which adverse effects will always be found. The method has major strengths in its ability to determine biological effects and assess interactive chemical effects. The method is considered by the Subcommittee to contain sufficient scientific merit that, with appropriate validation of the AET values, it could be used to establish sediment quality values for use at specific sites. In the Subcommittee's opinion, the AET approach should not be used to develop general, broadly applicable sediment quality criteria. Some major limitations drive this opinion, including the site specific nature of the approach, its inability to describe cause and effect relationships, its lack of independent validation, and its inability to describe differences in bioavailability of chemicals on different sediments. The Subcommittee has several suggestions for strengthening the AET approach including: building in replicate sediment samples to assessments, devising criteria for selection of reference sites, including considerations of physical factors, and developing measures of variance.

**Key Words:** Sediment, AET; Apparent Effects Threshold

# U.S. ENVIRONMENTAL PROTECTION AGENCY

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### Appendix A - Briefing Report to the EPA Science Advisory Board: The Apparent Effects Threshold Approach

## 1.0 EXECUTIVE SUMMARY

The Apparent Effects Threshold (AET) approach for deriving sediment quality values integrates data from bulk sediment chemistry, sediment bioassays and infaunal species measurements to provide estimates of sediment chemical concentrations. Concentrations above these estimates are thought to result in adverse environmental effects. An objective of the AET methodology is to identify adverse effects due to chemicals occurring in mixtures in sediments by determining specific chemical concentrations above which adverse effects will always be found.

The major strengths of the approach are a) the fact that both infaunal analyses and laboratory bioassays are incorporated to determine biological effects and b) the fact that the approach has the ability to incorporate interactive chemical effects, such as synergism and antagonism under the specific conditions encountered in the environment where the AET is applied. The method is considered by the Subcommittee to contain sufficient scientific merit that, with appropriate validation of the AET values, it could be used to establish sediment quality values for use at specific sites.

The major limitations of the AET method are a) its site-specific nature b) its inability to describe cause and effect relationships for specific chemicals c) its lack of independent validation of the AET values and 4) its inability to describe differences in bioavailability of chemicals on different sediments. These factors restrict the applicability of the specific AET values to the locality and conditions under which they were developed. In the Subcommittee's opinion, the AET approach should not be used to develop general, broadly applicable sediment quality criteria.

The validity of the AET estimates can be improved by formalizing criteria, designating reference sites, and developing measures of uncertainty for specific AET values. The Subcommittee recommends that AET values be derived based on more than one sediment bioassay, and that benthic infaunal analyses be included in AET development. In addition, the accuracy of AET values can be assessed by comparison with endpoints and through other experimental approaches that permit examination of the major convergences and divergences in the values.

## **2.0 INTRODUCTION**

### **2.1 Request for Science Advisory Board Review**

At the request of the Regional Administrator of EPA's Region 10, the Science Advisory Board (SAB) agreed to conduct a scientific review of the Apparent Effects Threshold (AET) approach. The SAB's Environmental Effects, Transport and Fate Committee authorized the formation of a Subcommittee to perform a series of tasks related to the technical aspects of sediment quality assessment and criteria development, with the approval of the Board's Executive Committee.

#### **2.1.1 Charge to the Subcommittee**

This review is the first to be completed in a series of SAB reviews related to sediment quality criteria. Preliminary reviews focus on approaches to examining non-ionic organic contaminants. Specifically, the charge accepted by the Subcommittee is to:

a) Evaluate individually the scientific and technical foundations for the various methodologies available to the Agency to estimate sediment toxicity and biological impact of contaminated sediments in situ.

b) Evaluate the feasibility of utilizing each methodology to determine extent of contamination and risk posed to the environment and human health.

c) Identify research directions that would provide information to strengthen each methodology.

d) Conduct a technical review of documents provided by the Agency that compare and contrast the scientific and technical strengths and weaknesses of the methodologies available to the Agency to estimate sediment toxicity and biological impact of contaminated sediments in situ.

### **2.2 Subcommittee Review Procedures**

The Sediment Criteria Subcommittee met on August 8 and 9, 1988, in Denver, Colorado, to assess the Agency's activities regarding contaminated sediment and to explore avenues for providing oversight. A second meeting was held on October 27 and 28, 1988, in Seattle, Washington. Informative and well-prepared briefings were provided on the objectives, historical perspective and technical components of the AET. The briefings were supported by extensive documentation provided to Subcommittee members prior to the meeting. Both the briefings and the documentation were considered by the Subcommittee to be very well done and relevant to the issues under review. This preparation and support was provided by Region 10 staff and associated contractors (PTI

Environmental Service, Inc.). The Environmental Protection Agency is divided into 10 Regional Offices, and Region 10 serves the states of Alaska, Washington, Oregon, and Idaho from its base in Seattle, Washington.

The contamination present in Puget Sound was characterized. Biological assays used in AET development were described. Additional briefings highlighted the statistical procedures utilized in the method, and procedures used to treat uncertainties in both biological and chemical data. Comments were heard from interested members of industry and the public, and intended application of the method was described by the Washington State Department of Ecology.

### **2.3 Expected Future Activities**

The Sediment Criteria Subcommittee plans to review and report on the technical appropriateness of the Equilibrium Partitioning (EP) method in the near future. In addition, bioassay procedures that are used as an integral part of the AET, EP and other approaches will be subjected to review for scientific adequacy. Other methods, including methods for assessing metal contaminants, are expected to be developed and existing approaches refined, and as this occurs, they will be transmitted to the Subcommittee for review. The Contaminated Sediment Technical Committee, established by EPA's Office of Water, is in the process of preparing a manual which describes currently available methods that may be applied to establishing sediment criteria. The Subcommittee will comment on this manual in the future.

During the course of these critical evaluation processes, it is likely that areas for additional or future research will be targeted. To facilitate the incorporation of these recommendations into EPA research planning, the Subcommittee may conduct a review of the Office of Research and Development's proposed Sediment Initiative. The time sequence of these proposed events is contingent on their completion by Agency staff.

### **3.0 EVALUATION OF THE APPARENT EFFECTS THRESHOLD APPROACH**

The AET approach provides useful information for developing the weight of evidence needed to make decision regarding contaminants in sediment. The AET is a statistically-based empirical approach which attempts to establish quantitative relationships between sediment contaminants and resulting biological effects. It has been developed using synoptic data on chemical contaminants and biological effects (as assessed by benthic infaunal analyses and sediment bioassays) at suspected contaminated sites and reference sites. The AET represents a credible step towards development of a technically defensible and publicly acceptable tool for managing contaminated sediments.

The use of quantified data for the derivation of relationships between exposure and effects is the only scientifically justifiable basis for describing such relationships quantitatively. Thus, the technical acceptability of the development of sediment quality values depends upon the fidelity with which measures of exposures and effects on the environment can reflect "true" environmental conditions, and upon the characteristics of the model that seeks to summarize these complex relationships in the form of a descriptor of sediment quality.

The AET approach provides a constructive beginning toward assessing the impact of mixtures of chemicals as they occur in actual situations, as opposed to solely assessing the influence of single chemicals under laboratory conditions. Its major strength is its ability to assess impacts of contaminated sediments on aquatic life. Innovative empirical approaches such as the AET that assess actual contamination and concomitant effects are encouraged and applauded by the Subcommittee.

#### **3.1 Quality of Data for AET Determination**

The Subcommittee is concerned that several limitations of the data sets used to develop the AET values have influenced the interpretation of relationships between sediment contamination levels and resulting biological effects. This concern stems in part from the lack of true field replication of the chemistry and bioassay data used in the development of the AET. The preponderance of the chemical and bioassay data is based on single samples from each site. While a sample may be divided into sub-samples and chemical analyses and/or bioassays performed on sub-samples (thus measuring the precision of the respective methods), there is no estimate of the within-site variability of chemical concentrations and sediment toxicity (as measured by sediment bioassays). Although in some cases (e.g., assessments of the suitability of material for dredging) within-site variability is less important than in other cases (e.g., determination of the extent and significance of problem areas), some estimate of this variability is considered necessary.

On the other hand, the data base used to develop an AET does contain an estimate of infaunal variability at each site, since replicate field samples are independently analyzed. The appropriate level of infaunal community structure analysis must be carefully evaluated and applied. Since there is no estimate of the variance for any of the chemical concentrations, an undefined uncertainty exists around the AET. The Subcommittee recommends that future application of the AET include replicate sediment samples for determination of chemical contaminants and for assessment of toxicity. By so doing, the uncertainties can be better defined and a stronger relationship between contaminant levels and biological effects established. In addition, with field replication data available on chemical contaminants, it may be feasible to statistically estimate the variance of the chemical data used in the development of the AET, a feature not presently incorporated in this or most other field approaches.

### **3.2 Determining Spatial Extent of Contamination.**

One of the proposed uses of AET is to quantitatively define the spatial extent of biological impacts associated with contaminated sediments. Because the method depends on a statistical comparison of test sites to reference sites, it is critical that valid reference sites be determined. A crucial factor in determining the location of the reference sites is that they be physically similar to the test sites. Although several sediment characteristics were considered in the selection of reference sites (e.g. season, water depth, grain size, or organic carbon content) and decisions were made using best professional judgment, it does not appear that a formalized set of decision criteria have been established. If inappropriate reference sites are used, an assessment of whether or not a test site is impacted is difficult. The Subcommittee recommends that criteria for selecting reference sites be formalized. The selection/rejection criteria need to be clearly defined and the rationale for their choice explained.

Furthermore, it is important that the AET approach clearly recognize and incorporate systematic temporal changes that may impact both the materials present in the sediment and the effects they may have. In reference areas there will be temporal as well as spatial changes in the composition and functioning of the resident faunal community. In impacted areas there will also be temporal changes in the concentrations of chemicals as a result of physical (burial, re-suspension, etc.), chemical (degradation, sorption, etc.), and biological (biodegradation) processes. Biologically, the community that is present at any site exists in response to the matrix of chemical, biological and physical factors that are present. Therefore, the presence of pollutants represents a selective influence that may lead over time to a community adapted to the conditions present, which may be different than the reference area but not necessarily "impacted" in a functional sense.

In its development, the AET methodology has not clearly addressed the influences of temporal factors on the distribution of benthic biota and the bioavailability of sedimentary contaminants. It would be useful to conduct repeated sampling over some time period at a few stations to establish whether changes occur that would significantly alter the generated AET value. Some knowledge of how temporal changes impact the AET is needed to characterize the uncertainty that may be added by this variable.

### 3.3 Field Applicability and Site Specificity

The AET values produced from the Puget Sound data appear to work well in Puget Sound. The Subcommittee recognizes the merits of the AET for identifying potential problem areas and potential problem chemicals. Since AET are currently being proposed for use as part of a process that involves site-specific biological testing in Puget Sound, as opposed to broader, more generic application, this application seems to be consistent with the Subcommittee's recommendations.

However, application of Puget Sound AET to another location or different physical setting must be done with extreme caution because the effects of physical, chemical and biological factors on AET are not well understood. For example, differences in current and wave conditions from one site to another may lead to very different bottom sediment compositions, even within the same body of water. The density, size distribution, salinity and degree of flocculation of sediments may be quite different at different sites. Moreover, the AET's inability to address the causality of biological response by a single chemical, although not unique to the AET approach, must be taken into consideration. Even though one can argue that an AET may be generically protective, there is presently no evidence that AETs developed in one place are protective in another environment.

High levels of chemical contamination may result in toxicity to laboratory species, but may cause no alteration of the real world benthos. This situation may indicate a potential problem that is not yet realized due to such factors as adaptation, hormesis (i.e., stimulation of growth and/or fecundity in the presence of low levels of contamination or stress-induced vigor), etc. Such information is valuable and could be misinterpreted by the AET as presently applied. The Subcommittee recommends that multiple approaches be used to estimate sediment quality, determine criteria and guide regulatory actions since the AET approach alone provides insufficient certainty for broad-scale decision making.

### 3.4 Cause-and-Effect Relationships

The AET approach conceptually allows for generation of sediment quality values for virtually any chemical. However, the

method does not provide a way of isolating biological effects that are caused by one specific chemical when the same chemical is present in a mixture of known and unknown chemicals sorbed to sediment. Therefore the scientific defensibility of chemical-specific AET values is unknown. A logical conclusion is that the AET method is not capable of demonstrating specific cause and effect relationships for any one specific chemical. Furthermore, the report provided to the Board ("Briefing Report to the EPA Science Advisory Board", page 15, see excerpt in Appendix A) states that "... it cannot distinguish and quantify the contributions of interactive effects, unmeasured chemicals or matrix effects in environmental samples."

The AET method can generate values for a wide variety of chemicals. However, to assess the general applicability of AETs, the implied relationship between the chemical sediment concentration and biological effect must be validated. The following points are presented to support this contention:

1. The existing data set does not contain verification data from experiments with spiked sediments or from toxicity identification evaluations which would assess the accuracy of the AETs. The work of Swartz with fluoranthene and amphipods is an example of the type of study that could be done to assess AET accuracy (Dr. Richard Swartz, EPA/ORD, Newport, Oregon; personal communication). Additionally, toxicity fractionation-identification for specific chemicals from elutriate or liquid-phase sediments at selected sites could be used.

2. The AET values for phthalates do not reflect the physical-chemical properties of this group of chemicals. It would be expected that the AET values would increase for the more water soluble, less toxic phthalates such as dimethyl and diethyl phthalate. This is not the case. This may reflect an inadequate data set for the phthalate class, which would improve with additional sampling, but it also indicates a fundamental lack of consistency with the existing literature on this class of compounds. Related comments can be made for some other chemicals. It is recommended that the data set be reviewed for all the AETs in relation to their physical-chemical properties (e.g.,  $K_{oc}$ , Log P and water solubility) and their existing toxicological properties (e.g., acute versus chronic or genetic effects) for the purpose of establishing the sensitivity of AET values to parameters that are known to affect toxicity. These types of data can be used as supportive evidence of cause-and-effect relationships.

3. Several of the AETs are below existing acute and chronic toxicity values for aquatic organisms based on aqueous exposure. In water, most chemicals are assumed to be bioavailable, with some exceptions. Bioavailability is generally considered to be reduced when a chemical is sorbed to sediment. Therefore it does not seem plausible that AET values for protecting aquatic life from chemicals sorbed to sediments can be lower than chronic water exposure values. It is recommended that the AET values be

compared to existing toxicity values for aquatic organisms for the purpose of identifying those AET values which are inconsistent with the existing body of toxicological data.

4. The sediment bioassay and field infaunal biological effects measurements do not show a strong dose-response relationship above the AET values. A basic premise of toxicology is that effects intensify with increasing dose. Inability to demonstrate this relationship argues against a causal relationship between exposure and effect for these chemicals near the AET concentration. It therefore diminishes the weight of evidence of the accuracy of the AET values as they relate to specific chemicals and indicates that the toxicity may be due to more than one chemical or that bioavailability or population resistance is changing from station to station. Both factors detract from the method's ability to relate observed effects to a specific chemical or chemical concentration. It is recommended that additional research be conducted to evaluate AETs relative to the applicable, available data for dose-response relationships. Additionally, most of the existing AETs have only a limited number of stations that fall above the AET value. This suggests that additional sampling and analysis could be beneficial to the AET method if the samples were collected from sites with high chemical concentrations.

5. Several of the AET values are for chemicals which do not show acute toxicity in laboratory aqueous toxicity tests. This is generally due to the limited solubility of the test chemical. Since the AET values are derived primarily from acute toxicity tests, one would expect a priori that these same chemicals would not be acutely toxic when sorbed to sediments. Since the bioavailability of chemicals sorbed to sediments is usually less than that in the aqueous phase, the trend is for chemicals to become less toxic on sediments, not more toxic. These data suggest that the effects are due, in part, to the presence of other chemicals, the physical properties of the sediment, or some other unknown factor(s). Inconsistency with known toxicological principles raises questions about the accuracy of AET values and, therefore, the scientific validity of the method.

The AET has clear utility for assessing contaminated sediments. Points such as those raised above, once addressed through additional research or comparison with existing literature, can go a long way towards improving confidence in the method and in demonstrating its consistency with established scientific principles.

### 3.5 Endpoint Considerations

The ability of the AET to effectively assess impacts at contaminated sites is related to the choice of the biological effect endpoint(s) used. The application of the AET to Puget Sound utilizes data on benthic infaunal assessments, amphipod sediment bioassays, oyster larvae bioassays and Microtox<sup>(R)</sup> bio-

assays. The AET can theoretically incorporate any biological effect(s) endpoint. In the generation of the AET, a battery of biological effects endpoints should be used. In the selection of biological effects endpoints one should consider:

1. The range of organismal responses to the contaminated sediments.
2. The methods used for assessment of acute and chronic toxicity.

The AET incorporates the first consideration by requiring multiple biological effects endpoints. In the Puget Sound data base, the latter concern was also addressed by including benthic infaunal assessments. However, if infaunal data are not available, the AET fails to consider any estimate of chronic toxicity. This conclusion is based on recognizing that the infaunal analyses are integrated measurements of chronic and acute toxicities while the bioassays chosen and available to date reflect acute toxicities only.

It is the conclusion of the Subcommittee that properly designed and applied infaunal analyses are extremely valuable to the development and validation of the AET and that they should be included in future activities. Research should be initiated to develop improved toxicity assay methods that can be used to assess long-term impacts of sediment toxicity.

The AET approach factors in both in situ biological effects through the benthic infaunal analysis and laboratory toxicity tests through bioassays. The former measures alteration, but is prone to all the problems inherent in field data. These include natural variability from biotic (predation, competition) as well as abiotic factors (grain-size, salinity). As the method is presently being used, few ecological endpoints are being measured. A 50% reduction in major taxa is a gross change indicative of major adverse effects. The use of major taxa rather than species changes may be correct, but it is heavily weighted to serious acute effects. It may incorporate chronic and more subtle changes, but these may be masked by the overriding lethal effects, even though chronic effects can also result in lethality.

Regarding bioassays, both acute and chronic tests should be used. The later is defined as a test that encompasses at least one full life-cycle of an organism. The more tests the better, given the value of a preponderance of evidence approach, but it is recognized that there are always going to be limitations to resources. It is recommended that not only different organisms be used but also that different exposure routes and life-stages be used. It is important that those using AET clearly and precisely specify what each biological parameter measured is assumed to mean. For instance, it is very difficult from the data presented to determine what Microtox<sup>[R]</sup> is supposed to indicate. If the test is intended to indicate impacts on the procaryotic level of the community, then its use in sediment is

probably inappropriate since it is based on photoluminescence by aerobic organisms that are not normally inhabitants of sediments, which are usually anaerobic. If it is used as an empirically derived indicator of toxicity, then the choice is more reasonable. The need for clearly defining the assumptions is most important for the potential users of both the AET value and the methodology.

The biological indicators used in Puget Sound for the development of AET values are some of the best tools presently available, but this is a rapidly evolving field. At present there is a need for better sediment bioassays, in particular chronic bioassays. We are now using first generation (water bioassays adapted for sediment, usually through the use of elutriates) and second generation bioassays (acute bioassays specifically adapted for use with whole sediments). The developers of the AET approach in Puget Sound are to be complimented for incorporating a range of biological evaluation tests in their approach. The fact that there may be other methods or even better ones does not detract from the effort. As stated above, there is a definite need to incorporate more than one assay and the concordance of the results provides reassuring evidence for effects.

### **3.6 Complex Mixtures**

In some circumstances, the AET method has the potential to assess the impact of complex chemical mixtures on indigenous benthic infauna. This is because organisms utilized in the bioassays and organisms enumerated in the environment are exposed to the array of chemicals incorporated in the sediment of question. If the composition of the complex mixture responsible for the measured biological effects remains relatively constant in sediments throughout the area of interest (i.e., that portion of a water body to which an AET value is to be applied), then the predictive potential of the AET is increased. It is also necessary that the chemicals measured be those responsible for the biological effects or else that they act as surrogates for those that are the causative agents. In some situations, such as impacts from PAH, these criteria may be met, but in situations where the complex mixture changes in composition rather than concentration or where the chemicals measured do not vary proportionally with the concentrations of the substances responsible for the effects, AETs will be faulty predictors of biological impacts of complex mixtures.

Cause/effect relationships, as pointed out in section 3.4 and in the briefing document provided to the SAB, are not well defined by the AET approach. This is because there is no certainty that the chemical substances measured are, in fact, those responsible for the noted biological effect. A chemical(s) which appears to be the causative agent may only co-exist and co-vary with the toxicants rather than be involved in the toxicity.

In such cases, one may regulate surrogates rather than the toxicants.

Even with these limitations, there are definite advantages associated with this or similar multivariate methodologies over univariate methodologies which consider only single chemicals. These advantages are that additive, synergistic or antagonistic effects can be taken into account, as long as the composition of the contaminants remains constant.

### **3.7 Physical Factors**

Attention should be given to the effects of physical factors on biological response(s) to chemicals since many areas of concern are in environments that are highly dynamic. It should be noted, however, that many other areas are relatively placid and physical factors are less important. The currents, turbulent mixing, and dispersion of sediments generally vary significantly with time and space according to the tides, winds, waves, and freshwater discharges. All of these factors can have significant impacts on biological response(s) to chemicals. For example, variation in currents, salinity, turbulence level, sediment characteristics (mineralogical composition, size distribution), and dissolved oxygen levels can all affect the biota.

The Puget Sound study concentrated on the chemical and biological data and used little or no physical data (currents, salinity, turbulence, and sediment characteristics) in the development of AET. Until the effect of physical factors on AET is adequately studied, the present AET values could contain significant errors and the AET cannot be applied generically with confidence.

A comprehensive study should be conducted to determine the effect of physical factors on biological response to chemicals. It is recommended that hydrodynamic and sedimentary information (via monitoring or modeling effort) be collected and used to guide the selection of AET sampling stations and reference sites.

Such effort will not only provide a scientifically more defensible basis for site selection, but may also lead to a reduction in the number of required sampling sites.

### **3.8 Carbon Normalization**

A large amount of data exists in the literature, from both laboratory and field studies, demonstrating the utility of carbon normalization for relating the bioavailability of non-ionic organic chemicals sorbed to sediments. These data indicate that the free form of the chemical which is available for organism uptake, whether by ingestion or by transport across respiratory and external membranes, can best be approximated by carbon normalization of the measured sediment chemical concentrations. This also tends to reduce variability in the data.

The information presented on the AET approach indicates that no improvement in the sensitivity of the method was achieved by carbon normalization of the data for the individual stations with subsequent recalculation of the AET values. Several reasons may exist for this lack of change in sensitivity.

a) Most of the sediment chemical concentration data have organic carbon values in the range of 1-3%. This is not a wide range, and therefore one would not expect major changes in the normalized values.

b) Chemical concentrations on sediments do not always correlate well with organic carbon in areas impacted by massive discharges of chemicals above their water solubilities, i.e., due to spills. In such situations it is possible to find high concentrations of chemicals on sediments which are very low in carbon.

The Subcommittee recommends that carbon normalization be used to develop the proposed AET values. The use of this approach is consistent with theory and it provides an AET which is based on mass of chemical per mass of carbon. In practice, converting a carbon-normalized AET for a specific chemical into non-normalized concentrations results in a range of (AET) values depending upon the value of carbon that is used to make the conversion. For example, the carbon-normalized Microtox<sup>[R]</sup> AET for hexachlorobenzene is listed as 2.3 mg/Kg C (USEPA, Briefing Report to the EPA Science Advisory Board, page 35, Table 4, September, 1988). To interpret environmental sediment chemical concentrations in relation to this normalized AET (2.3 mg/Kg C), the non-normalized sediment chemical concentration would have to be divided by its respective sediment total organic carbon (TOC) content. This means that a non-normalized sediment concentration, which is what is usually determined analytically, would exceed the normalized AET value (2.3 mg/Kg C) if it contained 0.02 mg/Kg hexachlorobenzene and a TOC of 1.0% (0.20 mg/Kg/1% TOC = 20 mg/Kg C). However, if the same sediment sample had a TOC of 10%, it would not exceed the carbon normalized AET (0.20 mg/Kg/10% = 2.0 mg/Kg C).

Carbon normalization does not assume that all sediments are equal. The same sediment chemical concentration detected in a range of different sediments may or may not exceed the AET depending on the TOC of the sediment. The use of TOC-normalized values in the above manner should eliminate the criticism that the AET approach is insensitive to differences in sediment types and differences in bioavailability.

### 3.9 Uncertainties in the AET

The AET approach relies on the use of a single AET value for each chemical and each biological indicator/endpoint. The choice of such single value(s) is necessarily somewhat subjective. Any

uncertainty and variability of the actual data are not adequately incorporated into single-valued AET.

Uncertainties in the data used for the AET process are based in part upon the variabilities inherent in sampling, naturally occurring patchiness in the distribution of organisms, and similar patchiness in the spatial distribution of chemicals. It may be possible to display these kinds of uncertainties through the use of appropriate sampling strategies.

Uncertainties that may be due to inadvertent biases or due to uncontrolled variables are much more difficult to uncover. The inclusion of such uncertainty may lead to artificially elevated AET values. This factor is of concern since AET values (by definition) can only be elevated as more data is generated. Examples of factors that may give rise to biased relationships between the exposure and response variables are:

- a. Measurement of total metals, rather than of indicators of bio-available metals.
- b. Failure to consider the strength of sorption of contaminants to the sediment matrix.
- c. Estimation of effects based on responses of higher taxa; changes in lower taxa would be expected to be more sensitive.
- d. Bioassays conducted with homogenized sediments or with supernatants derived from agitated sediments as opposed to undisturbed sediments.
- e. Use of acute bioassays and consequent lack of consideration for chronic and genotoxic response.

Environmental significant parameters that have not been measured may influence uncertainties and biases in unpredictable ways. The authors of the AET methodology clearly were aware of some of the major categories of important variables that have not been measured. Among such neglected variables that may influence effects are:

- a. Interactions between chemicals in the production of effects.
- b. Matrix effects that influence bioavailability.
- c. Physical parameters that influence the distribution of chemicals and benthic organisms.

The AET method correctly assumes that, as the exposure variable is increased, the response or effects variable will eventually become resolvable. In the method, the exposure variables are maintained throughout as individual chemical concentration measurements. Although the list of chemicals tested for is rela-

tively small, the list contains those substances that are of current regulatory concern, and it can be increased to a broader list of chemicals in the future.

The effects variables are treated in a more simplistic fashion. Indicators of effects are limited to an in situ assessment of four major benthic taxa, which should provide information on chronic and interactive effects. In addition, the sediment samples are subjected to one to three short-term laboratory bioassays. The observed deviations from "reference" conditions are then converted into quantal or affected/non-affected categories. This process collapses the continuous data of the effects variable into two categories, losing most of the information related to the variability of effects in the process. A further problem associated with the categorization into affected vs. non-affected (non-impacted) conditions is based on the selection of "reference" conditions. The exact categorization criteria are not rigorous or self-evident, but are based upon best professional judgment. Use of the term "inconclusive" rather than non-affected or non-impacted may better indicate the subjective nature of this determination.

The categorization process into affected/non-affected has the advantage of providing a starting point for the development of single-value delimiters for the AET methodology. Such single-value delimiters have obvious attractiveness as administrative tools for setting criteria, standards, or other limits. However, these values are dependent on the available data, their central tendency, and more importantly on the range and variability of the available data. This makes it very important to investigate the variabilities associated with the development of the exposure and effect functions, to examine the relationships critically, and to incorporate indicators or measures of the degree of variability as part of any apparent effects threshold.

If no categorization step is undertaken initially, and if the effects function is expressed in terms of the exposure function, then the relationship and its associated variability can be described directly, leaving the need for professional judgment as the final step. It should also be possible to substitute a formalized decision process for a portion of the professional judgment required in the last step. Another alternative would be to display the variability inherent in the process as outlined above as an adjunct to the development of the AET along lines similar to those suggested in the present methodology. The Subcommittee recommends that a measure of variance for AET values be developed in addition to the single-valued statistical mean AET.

#### **4.0 Summary of Subcommittee Recommendations.**

- a. The Subcommittee recommends that future application of the AET include replicate sediment samples for determination of chemical contaminants and for assessment of toxicity.
- b. The Subcommittee recommends that criteria for selecting reference sites be formalized. The selection/rejection criteria need to be clearly defined and the rationale for their choice explained.
- c. Some knowledge of how temporal changes impact the AET is needed to characterize the uncertainty that may be added by this variable.
- d. The Subcommittee recommends that multiple approaches be used to estimate sediment quality, determine criteria and guide regulatory actions since the AET approach alone provides insufficient certainty for broad-scale decision making.
- e. It is recommended that the data set be reviewed for all the AETs in relation to their physical-chemical properties (e.g.  $K_{oc}$ , Log P and water solubility) and their existing toxicological properties (e.g., acute versus chronic or genetic effects) for the purpose of establishing the sensitivity of AET values to parameters that are known to affect toxicity.
- f. It is recommended that the AET values be compared to existing toxicity values for aquatic organisms for the purpose of identifying those AET values which are inconsistent with the existing body of toxicological data.
- g. It is recommended that additional research be conducted to evaluate AETs relative to the applicable, available data for dose-response relationships.
- h. It is the conclusion of the Subcommittee that properly designed and applied infaunal analyses are extremely valuable to the development and validation of the AET and that they should be included in future activities. Research should be initiated to develop improved toxicity assay methods that can be used to assess long-term impacts of sediment toxicity.
- i. It is recommended that not only different organisms be used but also that different exposure routes and life-stages be used.
- j. A comprehensive study should be conducted to determine the effect of physical factors on biological response to chemicals. It is recommended that hydrodynamic and sedimentary information (via monitoring or modeling effort) be collected and used to guide the selection of AET sampling stations and reference sites.
- k. The Subcommittee recommends that carbon normalization be used to develop the proposed AET values.

1. The Subcommittee recommends that a measure of variance for AET values be developed in addition to the single-valued statistical mean AET.

**APPENDIX A:**

**Briefing Report to the EPA Science Advisory Board:**  
**The Apparent Effects Threshold Approach**

**BRIEFING REPORT TO THE  
EPA SCIENCE ADVISORY BOARD:**

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**THE APPARENT EFFECTS THRESHOLD APPROACH**

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PTI Contract No. C714-01

September 1988

## 2. THE CONCEPT OF AET

An AET is defined as the sediment concentration of a given chemical above which statistically significant ( $P \leq 0.05$ ) biological effects (e.g., depressions in the abundances of indigenous benthic infauna) are always expected. If any chemical exceeds its AET for a particular biological indicator, an adverse biological effect is predicted for that indicator. If all chemical concentrations are below their AET for a particular biological indicator, then no adverse effect is predicted.

In this section, AET generation is described and the AET concept is discussed as it relates to the interpretation of chemical and biological data in field-collected sediments. AET generation is a conceptually simple process and incorporates the complexity of biological-chemical interrelationships in the environment without relying upon *a priori* assumptions as to the mechanistic nature of these interrelationships. The concept of the AET is presented in this section with little reference to specific chemicals, specific biological tests, or specific chemical normalizations, because the approach is not inherently limited to specific subsets of these variables. The specific use of the AET concept to generate AET values from Puget Sound data is described in Section 3.

### DESCRIPTION OF THE AET APPROACH

The focus of the AET approach is to identify concentrations of contaminants that are associated exclusively with sediments exhibiting statistically significant biological effects relative to reference sediments. The calculation of AET for each chemical and biological indicator is straightforward:

1. **Collect "matched" chemical and biological effects data**--Conduct chemical and biological effects testing on subsamples of the same field sample (to avoid unaccountable losses of benthic organisms, benthic infaunal and chemical analyses are conducted on separate samples collected concurrently)
2. **Identify "impacted" and "nonimpacted" stations**--Statistically test the significance of adverse biological effects relative to suitable reference conditions for each sediment sample and biological indicator; suitable reference conditions are established by sediments containing very low or undetectable concentrations of any toxic chemicals
3. **Identify AET using only "nonimpacted" stations**--For each chemical, the AET can be identified for a given biological indicator as the highest detected concentration among sediment samples that do not exhibit statistically significant effects (if the chemical is undetected in all nonimpacted samples, no AET can be established for that chemical and biological indicator)
4. **Check for preliminary AET**--Verify that statistically significant biological effects are observed at a chemical concentration higher than the AET;

otherwise the AET is only a preliminary minimum estimate (or may not exist).

5. Repeat Steps 1-4 for each biological indicator.

A pictorial representation of the AET approach for two example chemicals is presented in Figure 2 based on results for a toxicity bioassay. Two subpopulations of all sediments analyzed for chemistry and subjected to a bioassay are represented by bars in the figure and include:

- Sediments that did not exhibit statistically significant ( $P > 0.05$ ) toxicity relative to reference conditions ("nonimpacted" stations)
- Sediments that exhibited statistically significant ( $P \leq 0.05$ ) toxicity in bioassays relative to reference conditions ("impacted" stations).

The horizontal axis in each figure represents sedimentary concentrations of chemicals (lead or 4-methylphenol) on a log scale. Dry weight normalized data are presented in Figure 2, although the AET approach is not limited to any particular normalization. For the toxicity bioassay under consideration, the AET for lead is the highest lead concentration corresponding to sediments that did not exhibit significant toxicity (the top bar for lead in Figure 2). Above this lead AET, significant toxicity was always observed in the data set. The AET for 4-methylphenol was determined analogously.

## INTERPRETATION OF AET

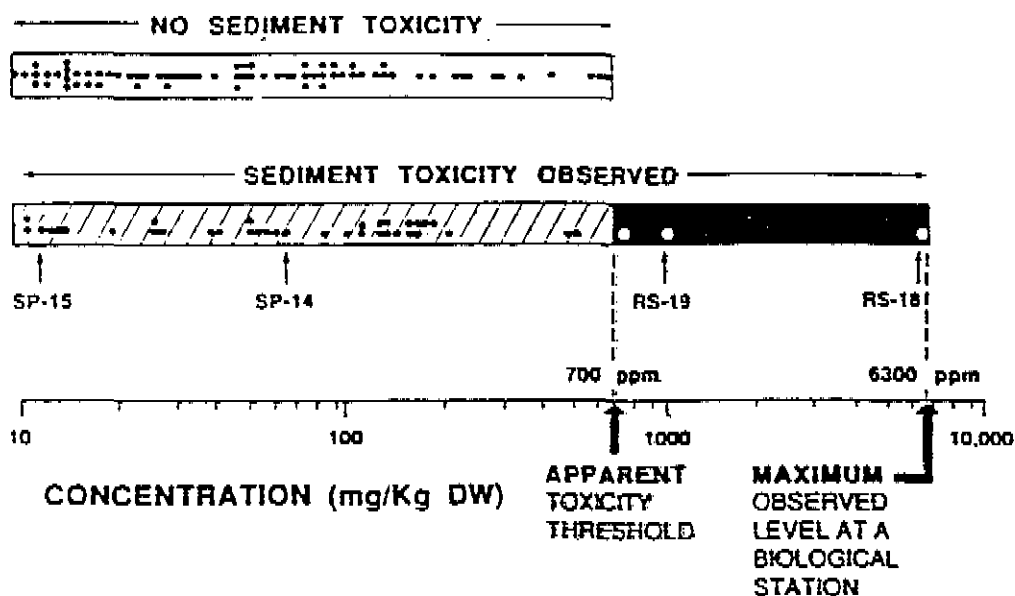
An AET corresponds to the sediment concentration of a chemical above which all samples for a particular biological indicator were observed to have adverse effects. Thus, the AET is based on noncontradictory evidence of biological effects. Data are treated in this manner to reduce the weight given to samples in which factors other than the contaminant examined (e.g., other contaminants, environmental variables) may be responsible for the biological effect.

### Relationships Among Chemical-Specific AET

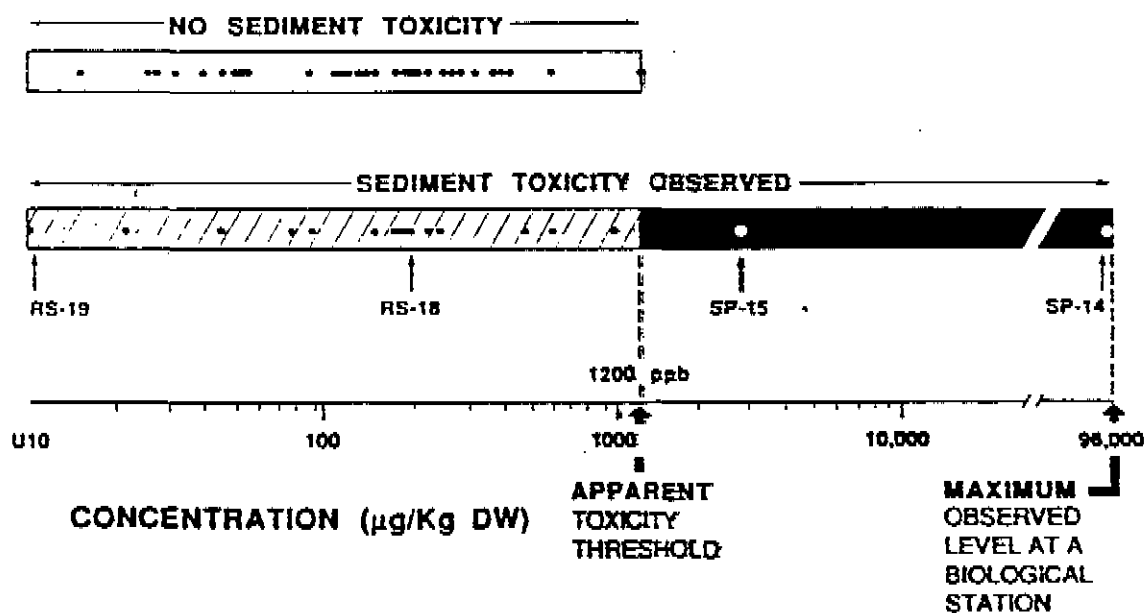
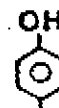
Using Figure 2 as an example, sediment from Station SP-14 exhibited severe toxicity, potentially related to a greatly elevated level of 4-methylphenol (7,400 times reference levels). The same sediment from Station SP-14 contained a relatively low concentration of lead that was well below the AET for lead (Figure 2). Despite the toxic effects associated with the sample, sediments from many other stations with higher lead concentrations than SP-14 exhibited no statistically significant biological effects. These results were interpreted to suggest that the effects at Station SP-14 were potentially associated with 4-methylphenol (or a substance with a similar environmental distribution) but were less likely to be associated with lead.

A converse argument can be made for lead and 4-methylphenol in sediments from Station RS-18. In this manner, the AET approach helps to identify measured chemicals that are potentially associated with observed effects at each biologically impacted site and eliminates from consideration chemicals that are far less likely to be associated with effects (i.e., the latter chemicals have been observed at higher concentrations at

# LEAD



# 4-METHYL PHENOL



U - undetected at detection limit shown

Figure 2. The AET approach applied to sediments tested for lead and 4-methyl phenol concentrations and toxicity response during bioassays.

other sites without associated biological effects). Based on the results for lead and 4-methylphenol, effects at 4 of the 28 impacted sites shown in the figures may be associated with elevated concentrations of 4-methylphenol, and effects at 7 other sites may be associated with elevated concentrations of lead (or similarly distributed contaminants).

These results illustrate that the occurrence of biologically impacted stations at concentrations below the AET of a single chemical does not imply that AET in general are not protective against biological effects, only that single chemicals may not account for all stations with biological effects. By developing AET for multiple chemicals, a high percentage of all stations with biological effects are accounted for with the AET approach (reliability results are presented in Section 3 of this briefing document).

AET can be expected to be more predictive when developed from a large, diverse database with wide ranges of chemical concentrations and a wide diversity of measured chemicals. Data sets that have large concentration gaps between stations and/or do not cover a wide range of concentrations must be scrutinized carefully (e.g., to discern whether chemical concentrations in the data set exceed reference concentrations) before generation of AET is appropriate.

#### **Dose-Response Relationships and AET**

The AET concept is consistent with empirical observations in the laboratory of dose-response relationships between increasing concentrations of individual toxic chemicals and increasing biological effects. A simple hypothetical example of such single-chemical relationships is shown for chemicals X and Y in Figure 3. In the example, data are shown for laboratory exposures of a test organism to sediment containing only increasing concentrations of chemical X, and independently, for exposures to sediment containing only increasing concentrations of chemical Y. The magnitude of toxic response in the example differs for the two chemicals and occurs over two different concentration ranges. It is assumed that at some level of response, for example >25 percent, the two different responses can be distinguished from reference conditions (i.e., responses resulting from exposure to sediments containing very low or undetectable concentrations of any toxic chemicals).

These single-chemical relationships cannot be proven in the field because organisms are exposed to complex mixtures of chemicals in environmental samples. In addition, unrelated discharges from different sources can result in uncorrelated distributions of chemicals in environmental samples. To demonstrate the potential effects of these distributions, response data are shown in Figure 4 for a random association of chemicals X and Y using the same concentration data as in Figure 3. The data have been plotted according to increasing concentrations of chemical X, and the same dose-response relationship observed independently for the two chemicals in the laboratory has been assumed. The contributions of chemicals X and Y to the toxic response shown for these simple mixtures is intended only for illustration purposes to enable direct comparison to the relationships shown in Figure 3, but are analogous to an additive toxic response. Other interactive effects are not considered in this example.

In Figure 4, a significant response relative to reference conditions would result whenever elevated concentrations of either chemical X or chemical Y occurred in a sample. Because of the random association of Y with X in these samples, the significant responses would appear to occur randomly over the lower concentration range of

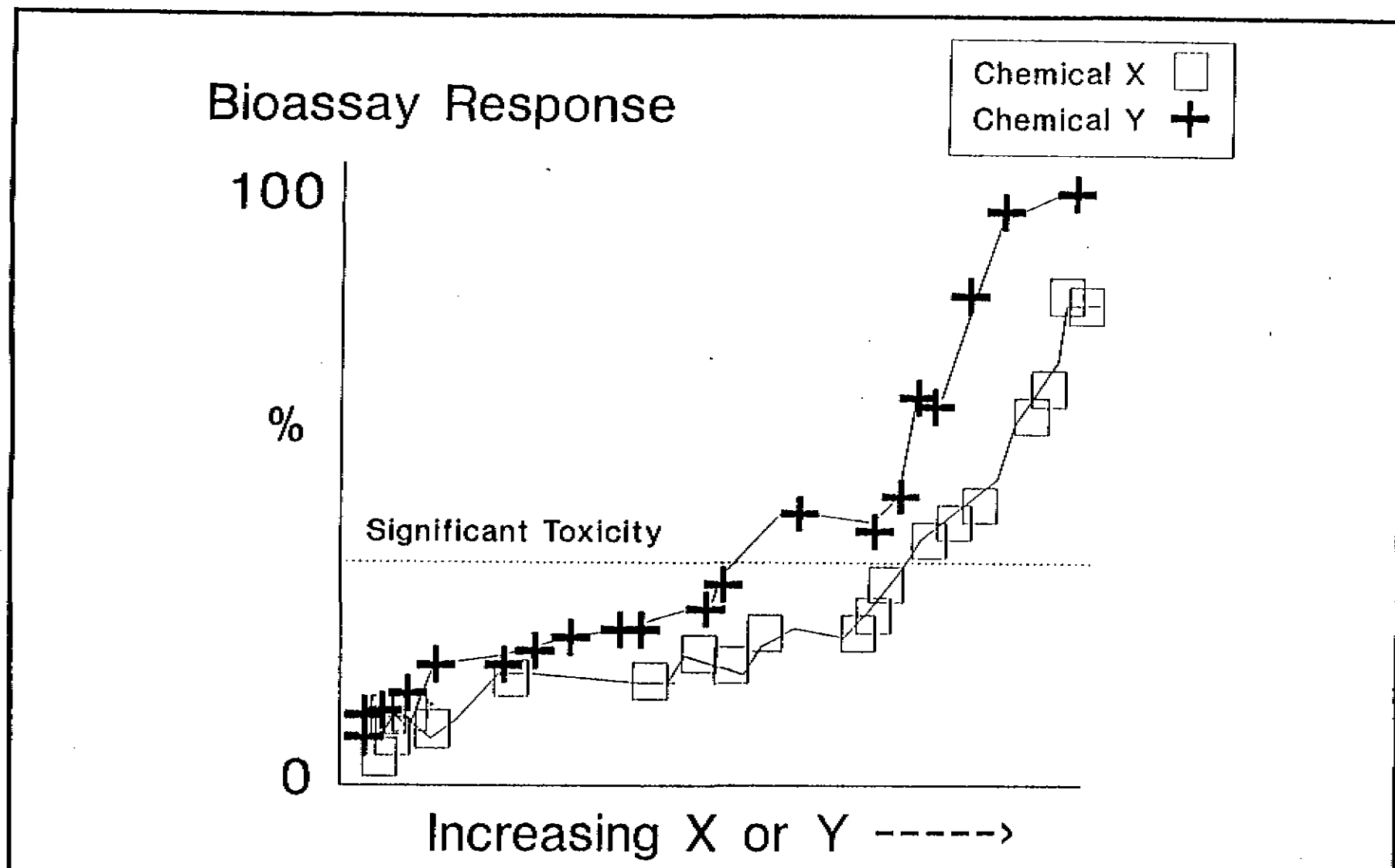


Figure 3. Hypothetical example of dose-response relationship resulting from laboratory exposure to single chemicals X and Y.

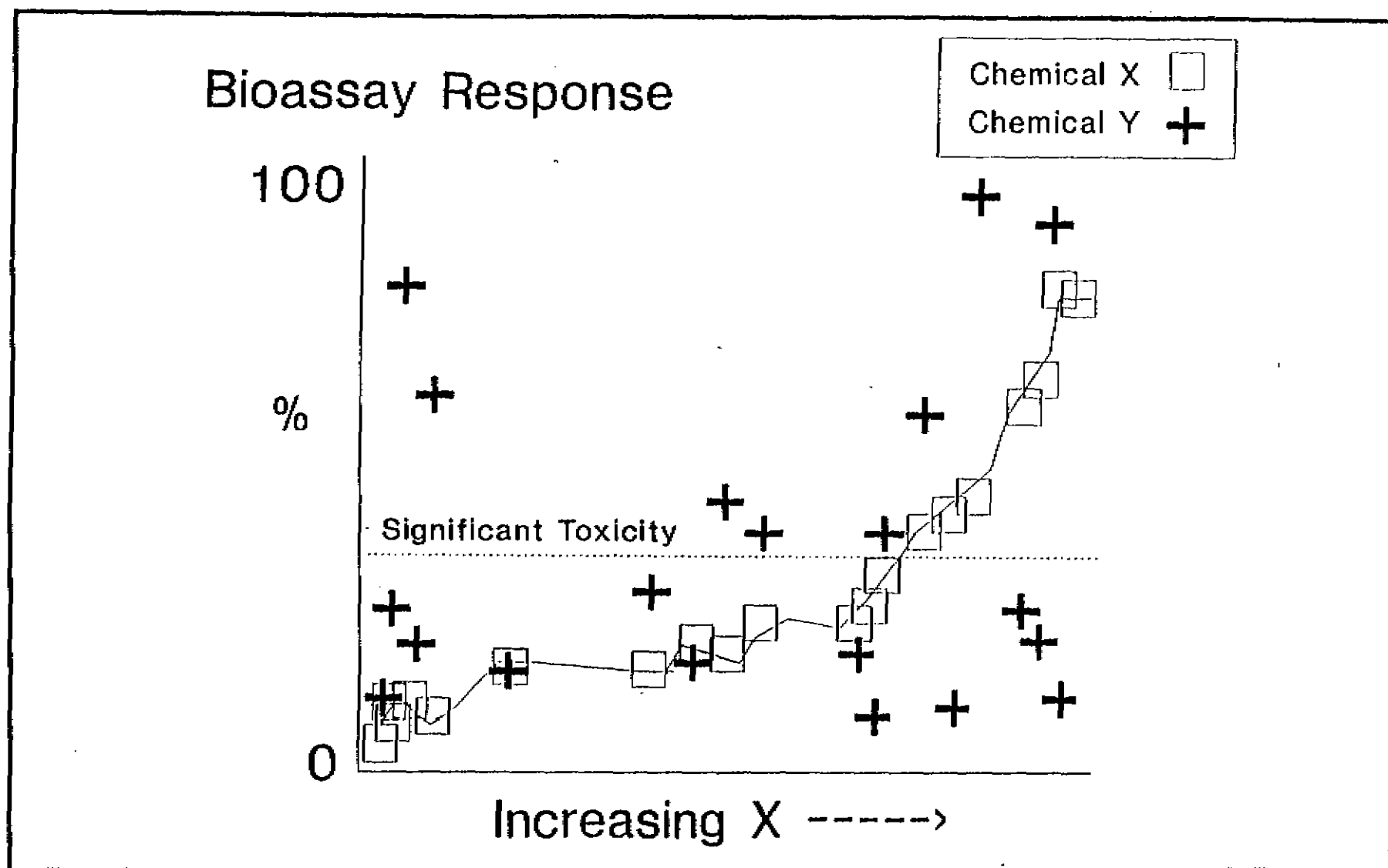


Figure 4. Hypothetical example of toxic response resulting from exposure to environmental samples of sediment contaminated with chemicals X and Y.

chemical X. The classification of the responses shown in Figure 4 into significant and nonsignificant groups (i.e., >25 percent response for either chemical) results in generation of Figure 5.

Figure 5 represents the appearance of the environmental results when ranked according to concentration of chemical X using these data. Below the AET for chemical X, significant toxicity is produced by elevated concentrations of chemical Y, which is randomly associated with the distribution of chemical X. Above the AET for chemical X, significant toxicity is always produced by elevated concentrations of chemical X, although in some samples, elevated concentrations of chemical Y also contribute to the overall toxicity. The AET for chemical X corresponds conceptually, in this simple example, to the concentration in Figure 3 at which a significant difference in response was observed in the laboratory for chemical X.

In environmental samples that contain complex mixtures of chemicals, a monotonic dose-response relationship such as in this simple two-chemical example may not always apply. For example, a consistently increasing biological response may not always occur at increasing concentrations of a chemical above its AET. Such observations could indicate that the AET is coincidental (i.e., that the observed toxicity in some or all samples above the AET is unrelated to the presence of that chemical), or that changing environmental factors in samples exceeding an AET obscure a monotonic dose-response relationship. Such factors are discussed in the following section.

#### **Influence of Environmental Factors on AET Interpretation**

Although the AET concept is simple, the generation of AET values based on environmental data incorporates many complex biological-chemical interrelationships. For example, the AET approach incorporates the net effects of the following factors that may be important in field-collected sediments:

- Interactive effects of chemicals (e.g., synergism, antagonism, and additivity)
- Unmeasured chemicals and other unmeasured, potentially adverse variables
- Matrix effects and bioavailability [i.e., phase associations between contaminants and sediments that affect bioavailability of the contaminants, such as the incorporation of polycyclic aromatic hydrocarbons (PAH) in soot particles].

The AET approach cannot distinguish and quantify the contributions of interactive effects, unmeasured chemicals, or matrix effects in environmental samples, but AET values may be influenced by these factors. To the extent that the samples used to generate AET are representative of samples for which AET are used to predict effects, the above environmental factors may not detract from the predictive reliability of AET. Alternatively, the infrequent occurrence of the above environmental factors in a data set used to generate AET could detract from the predictive reliability of those AET values. If confounding environmental factors render the AET approach unreliable, this should be evident from validation tests in which biological effects are predicted in environmental samples. Tests of AET values generated from Puget Sound data (see Section 3) indicate that the approach is relatively reliable in predicting biological effects despite the potential uncertainties of confounding environmental factors.

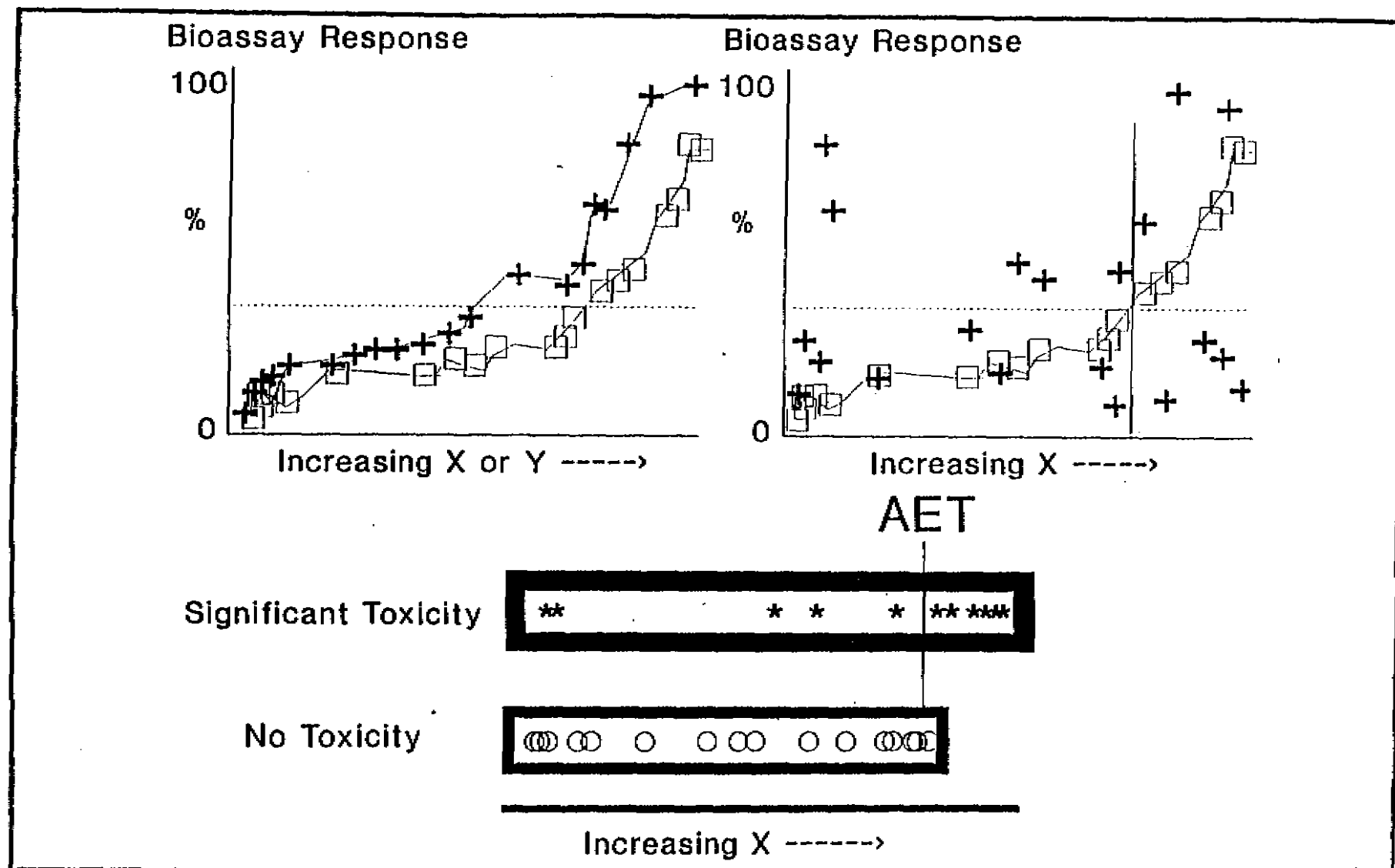


Figure 5. Hypothetical example of AET calculation for chemical X based on classification of significant and nonsignificant responses for environmental samples contaminated with both chemicals X and Y. [Previous Figures 3 and 4 are also shown for comparison; dashed line indicates level of significant toxicity]

Although the above environmental factors can influence the generation of field-based sediment quality values such as AET, they also may influence the application of all sediment quality value approaches for the prediction of adverse biological effects. For example, sediment quality values based on laboratory sediment bioassays spiked with single chemicals would not be susceptible to the effects of the environmental factors listed above. However, in applying such values to field-collected samples, predictions of biological effects could be less successful to the extent that interactive effects, unmeasured chemicals, and matrix effects occur in the environment.

The nature of the relationships between AET values and confounding environmental factors is discussed in the remainder of this section.

**Interactive Effects and AET**--AET uncertainty is increased by the possibility of interactive effects; the increase in uncertainty is expected to be less pronounced when large data sets collected from diverse areas are used to generate AET. Additivity and synergism can produce a comparatively low AET for a given chemical by causing impacts at concentrations that would not cause impacts in the absence of these interactive effects. This would effectively reduce the pool of nonimpacted stations used to generate AET. This effect should be reduced if a diverse database is used such that chemicals occur over a wide range of concentrations at stations where additivity and synergism are not operative. For chemicals that covary regularly in the environment (e.g., fluoranthene and pyrene), even a large, diverse database will not reduce the effects of additivity and/or synergism on AET generation. The resulting AET values for such chemicals may be reliable in predicting biological effects in environmental samples although not representative of the toxicities of the chemicals acting independently.

Antagonism will produce comparatively high AET values if (and only if) the AET is established at a station where antagonism occurs. A large, diverse database could not rectify this elevation of AET if the station at which antagonism occurred was the nonimpacted station with the highest concentration (i.e., the station setting an AET). An AET set by a station at which antagonism occurred would not be representative of the toxicity of the chemical acting independently. Hence, if antagonism did not occur widely, such antagonistic effects would cause the AET to be less sensitive in predicting adverse effects in the environment.

Empirical approaches such as the AET do not provide a means for characterizing interactive effects. Only laboratory-spiked sediment bioassays offer a systematic and reliable method for identifying and quantifying additivity, synergism, and antagonism. A great deal of research effort would be required to test the range of chemicals potentially occurring in the environment (both individually and in combination), a sufficiently wide range of organisms, and a wide range of sediment matrices to establish criteria. In addition, the applicability of bioassays conducted with laboratory-spiked sediments to environmentally-contaminated sediments requires further testing.

**Unmeasured Chemicals and AET**--Another source of uncertainty for AET and other field-based approaches is the possibility of effects being caused by unmeasured, covarying chemicals. Such chemicals would not be expected to substantially decrease the ability of AET to predict biologically impacted stations (excluding interactive effects discussed above). If an unmeasured chemical (or group of chemicals) varies consistently in the

environment with a measured chemical, then the AET established for the measured contaminant will indirectly apply to, or result in the management of, the unmeasured contaminant. In such cases, a measured contaminant would act as a surrogate for an unmeasured contaminant (or group of unmeasured contaminants). Because all potential contaminants cannot be measured routinely, management strategies must rely to some extent on "surrogate" chemicals.

If an unmeasured toxic chemical (or group of chemicals) does not always covary with a measured chemical (e.g., if a certain industry releases an unusual mixture of contaminants), the effect should be mitigated if a sufficiently large and diverse data set is used to establish AET. Use of a large data set comprising samples from a variety of areas with wide-ranging chemical concentrations would decrease the likelihood that an unrealistically low AET would be set. Because AET are set by the highest concentration of a given chemical in samples without observed biological effects, AET will not be affected by less contaminated samples in which unmeasured contaminants cause biological effects.

If an unmeasured toxic chemical does not covary with any of the measured chemicals, it is unlikely that the AET (or any other chemical-specific approach) could predict impacts at stations where the chemical is inducing toxic effects. The frequency of occurrence of stations with biological effects but no chemicals exceeding AET is the subject of validation tests (see Section 3 of this briefing document).

**Matrix Effects and Bioavailability**--Geochemical associations of contaminants with sediments that reduce bioavailability of those contaminants would affect AET analogously to antagonistic effects (i.e., they would increase AET relative to sediments in which this factor was not operative). Sediment matrices observed in Puget Sound that may reduce bioavailability of certain contaminants include slag material (containing high concentrations of various metals and metalloids, such as copper and arsenic) and coal or soot (which may contain high concentrations of largely unavailable PAH, as opposed to oil or creosote, in which PAH would be expected to be far more bioavailable; e.g., Farrington and Teal 1982). Many kinds of matrices may occur in the environment and a large proportion may be difficult to classify based upon appearance or routinely measured sediment variables. Hence, the use of matrix-specific data sets to generate AET, although desirable, would be difficult to implement.

To address this concern from a technical perspective (i.e., representativeness of data used in AET generation), the AET database could be screened for sediment with chemical concentrations that are anomalously high relative to those in other nonimpacted sediments from different geographic areas. From a management perspective, this guideline would generate more protective (sensitive) sediment quality standards that may also be less efficient in only identifying problem sediments. These sediments would be considered nonrepresentative and not used in AET generation unless and until additional data could substantiate that they are representative. Such data treatment methods are discussed in the following section.