ES-5
August, 1982

TECHNICAL SUPPORT DOCUMENT

FOR

ALGAL ACUTE TOXICITY TEST

OFFICE OF TOXIC SUBSTANCES
OFFICE OF PESTICIDES AND TOXIC SUBSTANCES
U.S. ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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Purpose

The purpose of this document is to provide the scientific background and rationale used in the development of Test Guideline EG-8 which uses freshwater and marine algae to evaluate the acute toxicity of chemical substances. The Document provides an account of the scientific evidence and an explanation of the logic used in the selection of the test methodology, procedures and conditions prescribed in the Test Guideline. Technical issues and practical considerations relevant to the Test Guideline are discussed. In addition, estimates of the cost of conducting the tests are provided.

II. Scientific Aspects

A. Test Procedures

1. General. A balanced growth of algae in the aquatic environment is essential, but extremes in productivity may be detrimental to other organisms. Some algae are able to inhibit or stimulate the growth of other algae, for example Selenastrum can inhibit Microcystis growth in eutrophic water (Toerien et al. 1974). Inhibition of algal growth would alter the food web and reduce the productivity of ecosystems. The toxic effect of a chemical or other inhibitor may increase the susceptibility of algae to other environmental stresses (Fisher and Wurster 1973). Stimulation of algal growth may cause an algal bloom which may have negative aesthetic effects; may adversely affect commercial sport fisheries (Lightner 1978, Lovell 1979) and recreation; may impart unpleasant taste to drinking water; may release substances deleterious to aquatic animals,

and/or may indirectly kill aquatic organisms by creating anoxic conditions (Shilo 1964, Schwimmer and Schwimmer 1967). Stimulation of algal growth, while primarily a problem in eutrophic freshwaters, has created serious ecological problems in the open ocean as well. In the spring of 1976 and extending into the fall, there was an extensive algal bloom, dominated by Ceratium tripos, located off the New Jersey coast. The bloom, together with a dearth of storm activity, anomalous surface wind conditions, and unusually warm sea surface temperatures resulted in a huge anoxic area, 100 miles long and 40 miles wide which had a severe impact on the finfish and shellfish populations in the area. The immediate effects on commercial and sport fishes, lobsters, and shellfish were not entirely known. However, an estimated 59,000 metric tons of surf clams were killed (representing twice the annual U.S. harvest), and up to 50% of other shellfish populations sampled were killed. One commercial trawler reported up to 75% of fish collected were dead. It was predicted that these mortalities would affect recruitment, population size and harvests for years to come (Sharp 1976).

Another more commonly known phenomenon is the adverse effect caused by stimulated growth of toxigenic marine algae. Frequently explosive mass development of these organisms in the form of blooms and tides occur, resulting in fish kills, contaminated shellfish, and outbreaks of paralytic shellfish poisonings in humans. (Shilo 1964, Taylor and Seliger 1979).

Even when toxigenic organisms are not present in sufficient concentrations to affect human health, red tides

may reduce the market for shellfish because of adverse publicity (Council on Environmental Quality, 1979). Furthermore, the high concentrations of phytoplankton that occur during blooms can be harmful to shellfish because the rate of water transport by molluscs is reduced and feeding ceases (Galtsoff 1964).

Algal growth was selected to measure phytotoxicity for the following reasons:

- The selection of phytoplanktonic algae for toxicity testing is based upon their importance in aquatic ecosystems. Algae were one of the first cellular life forms, dating as far back as 3.1 billion years in the fossil record (Bold and Wynne 1978) and are numerous today. Because phytoplankton are ubiquitous, it is usually the case that most marine and freshwater ecosystems are based upon the primary production of phytoplankton (Stern and Stickle 1978). Primary production is of prime significance to estuarine energetics since the primary producers are at the base of the food web. In estuaries phytoplankton are the main primary producers in the water (Vernberg 1977). Algae convert inorganic carbon to organic carbon and liberate oxygen during photosynthesis. Thus, tney are primary producers of food and energy for the lower trophic-level herbivores which in turn provide food for the upper trophic-level carnivores, generally fishes (Vance and Maki 1976). Some species fix nitrogen, required for the growth of vascular plants. Therefore, much of the

food people eat and the oxygen they breathe are the result of algal productivity.

- Inferences may be drawn from laboratory tests for inhibition or stimulation of algal growth as to the extent to which a chemical substance can interfere with primary productivity and nutrient cycling in lakes, streams, estuaries, and oceans. Further inferences may be drawn from algal bioconcentration data as to the potential of a chemical substance to bioaccumulate in food chains. However, in the natural environment there are too many factors acting to regulate algal populations which cannot be simulated in a simple laboratory test. The real value of the test guideline is to determine threshold toxicity values and to evaluate the relative toxicity of test substances to one another under rigidly controlled conditions.
- Algal testing has been well established in the literature. In 1967, the EPA began developing algal assays for evaluating the ecological effects of pollution to the environment. Initially designed for considering problems associated with eutrophication (Maloney and Miller 1975), algal assays have also been used to define the toxic effects of heavy metals (Davies 1978), pesticides (Schauberger and Wildman 1977, Walsh and Alexander 1980), oil spills (Corner 1978, Fisher and Wurster 1973, O'Brien and Dixon 1976, Vandermeulen and Ahern 1976), chemical substances (USEPA 1978 a,b,c, Harding and Phillips 1978), dyes (Little and

Chillingworth 1976), complex industrial wastes (USEPA 1978d, Walsh and Alexander 1980, Walsh et al. 1980) and natural organic components of fresh and marine water (Prakash and Rashid 1968). Over the years, extensive use of this test has sufficiently refined it to qualify as a standard method to measure water quality. Algal assays are recommended for use by the APHA (1975) USEPA (1977, 1978 a,b,c,d) and are currently under review by the American Society for Testing and Materials. Further discussion on the validity of applying algal assays in water quality assessment is found in Fitzgerald (1975); Joint Industry/Government Task Force on Eutrophication (1969); Leischman et al (1979); USEPA (1978b) Miller et al. (1978); Murray et al. (1971); Reynolds et al. (1974); and USEPA (1971, 1975a).

- The algal growth method is 1) relatively rapid, 2) inexpensive, 3) capable of being performed by persons with minimal technical training and 4) reproducible, using large numbers of organisms with sufficient replication and precision.

The test procedure involves assessment of algal growth in test chambers relative to controls by requiring a quantitative determination of algal cell numbers, and by recommending a) a qualitative appraisal of algal numbers and size by means of microscopic observation, and b) a determination of viability of growth-inhibited algae by means of mortal staining coupled with microscopic observation and/or subculturing. The test procedure is

simple because it requires only the combination of set amounts of test substance, nutrient medium and algae, and then monitoring the growth response 96 hours later. At the end of 96 hours a further assessment of growth and viability is recommended.

In the test the following procedures are required:

- Algal growth should be logarithmic at the beginning of the test and algal number should be determined.
- The number of algae should be determined at the end of the test.
- The concentration of chemical in the test solution should be determined at the beginning and end of the test and the concentration of chemical associated with the algal cells should also be determined.
- growth and bioconcentration data should be subjected to statistical analyses.

These requirements will ensure consistency and will minimize variabili y of the test results. The test also recommends testing of algicidal and/or algistatic chemical effects.

2. Range-Finding Test

It is recommended that a range-finding test be conducted prior to the definitive test in those instances where no information is available or can be elucidated on the phototoxicity of the test chemical. This approach should minimize the possibility that an inappropriate concentration series will be utilized in the definitive test and under certain circumstances may even preclude the need to conduct the definitive test. In order to minimize the cost and time

required to obtain the requisite data nominal concentrations are permitted, test duration may be shortened, replicates are not required and other test procedures and conditions are relaxed.

If test results indicate that the chemical is non-toxic or very toxic to algae and if definitive testing is not conducted, it is necessary to ascertain that the control algae have attained a logarithmic growth rate by 96 hours and that the test was conducted at the specified incubation temperature. These verifications establish that the algae tested were viable and that the test was properly conducted.

In some situations there may be enough information available on toxicity to select the appropriate concentration without a range-finding test. The range-finding test (or other available information) needs to be accurate enough to ensure that dose levels in the definitive test are spaced to result in concentrations above and below the EC-10 and EC-50 values for algal growth and mortality. If the chemical has no measurable effect at the saturation concentration (at least 1000 mg/l), it is considered relatively nontoxic to algal growth and definitive testing for effects on these processes is deemed unnecessary. In all cases, the range-finding test is conducted to reduce the expense involved with having to repeat a definitive test because of inappropriate test chemical concentrations.

3. Definitive Test

The specific requirements of the definitive test are the analytical determinations of chemical concentrations, the unbiased selection of algae for each treatment, the use of controls, the assessment of test validity, and the

recording, analysis, and presentation of data. These requirements assure that the chemical concentration - algae response relationship is accurately known, that chemical effects are not confounded by differential algal growth and that the relationships are clearly present. Reporting the occurrence of such abnormal effects as irregular cell size or shape, clumping, loss of chlorophyll, cell mortality, or other unusual effects provides qualitative data that further assist the assessment of phytotoxicity.

The purpose of the definitive test is to determine the EC-10, EC-50 and concentration-response curves for algal growth for each species tested with a minimum of testing beyond the range-finding test. The concentration range for the definitive test is based upon the results of the range-finding for that species. It is probable that each of the species tested may have a different estimated EC-50 based on the range-finding test and that more than five concentrations of a test substance in a geometric series may be needed to properly describe the dose-response relationship for either species being tested. By testing a minimum of five concentrations in a series per species the dose-response relationship will be better defined. The slope and shape of the dose-response curve can give an indication of the mode of action of the chemical and will allow estimation of the effects of lower concentrations on the algae.

The primary observations - number of algae per chemical and determination of the actual chemical concentrations employed in the definitive test, are needed to accurately describe the dose-response curve from which the EC-10 and EC-50 are calculated.

The recommended experimental design is the randomized complete block. As discussed by Hammer and Urquhart (1979), it is essential that the investigator randomly assign test containers to treatments to assure that each aliquot of algae has the same chance of receiving any of the treatments (exposure level of test chemical). To account for variation within the growth chamber and to increase the sensitivity for detecting treatment differences, small square blocks should be delineated in the growth chamber with randomization of treatment within blocks. Replication should occur over growth chambers (of the same type) as, in many cases, a within-growth chamber estimate of residual variance badly underestimates the between chamber estimate (Hammer and Urquhart 1979). This means that differences between growth chambers are often greater than differences between growth and environmental conditions within chambers.

4. Analytical Measurements

The actual chemical concentration used in the definitive test should be determined with the best available analytical precision. Analysis of stock solutions and test solutions just prior to use will minimize problems with storage (e.g., formation of degradation products, adsorption, transformation, etc.). Nominal concentrations are adequate for the purposes of the range-finding test. If definitive testing is not required because the chemical elicits an insufficient response at the 1000 mg/l level in the range-finding test, the concentration of chemical in the test solution should be determined to confirm the actual exposure level.

The pH of the test solution should be measured prior to testing to determine if it lies outside of the species optimal range. While it is recognized that algae may grow over a broad range of hydrogen-ion concentrations and typically exhibit a pH optima for logarithmic growth, this test guideline does not include pH adjustment for the following reasons: the use of acid or base may chemically alter the test substance making it more or less toxic, the amount of acid or base needed to adjust the pH may vary from one test solution concentration to the next, and the effect the test chemical has on pH may indirectly affect growth and development of the algae. Therefore, the pH of each test solution should be determined and compared to the acceptable range for growth and development of the test algae.

The data obtained in bioassays are usually expressed as standard response curves in which growth response of the test species is plotted against the concentration of the test chemical. The manner of expressing algal growth response varies considerably. For this guideline algal growth responses are expressed as direct measurements of number of algae per ml of solution. The statistical analysis (goodness-of-fit determination) facilitates accurate calculations of EC-10 and EC-50 as well as providing confidence limits for the concentration (dose)-response curve.

B. Test Conditions

1. Test Species

Both Salenastrum capricornutum and Skeletonema costatum have a number of useful characteristics as listed below,

which are necessary for an algal species to be used in bioassays (Toerien et al. 1971):

(a) broad nutrient response (grows both in oligotrophic and euthropic waters).
(b) distinct shape
(c) uniform size
(d) divide distinctly
(e) do not attach to glass or surface
(f) stay in suspension with slight agitation
(g) cells do not clump (aggregate)
(h) grow at a maximum rate in a short time in a medium simple to constitute
(i) do not excrete autotoxins
(j) cells are easy to count by both direct or indirect methods.

Selenastrum capricornutum is an excellent laboratory freshwater organism, easy to culture and count, and is both sensitive and consistent in its response to a wide range of nutrient levels (Payne and Hall 1979).

When included in multispecies toxicity screening tests, Selenastrum has been found to be a comparably sensitive species. Maki and Macek (1978) found this to be true in an environmental safety assessment for a nonphosphate detergent builder. Selenastrum was as sensitive to trinitrotoluene as the copepod, Trigriopus californicus, and was twice as sensitive as oyster larvae (Smock et al. 1976). Selenastrum was as sensitive as Daphnia and the fathead minnow to eight preparations of synfuels (Greene, personal communication). In a study of the toxicity of 56 dyes to Selenastrum and fish (fathead minnows), basic dyes do not markedly inhibit

algal growth, and "of special significance, however, is the rather startling correlation between results of algal assays and the results of fish bioassays" (Little and Chillingworth 1974). Greene (personal communication) analyzed the results of this study and found the algae appear more sensitive than fish to 35 of the dyes tested while the fish were only more sensitive to seven of the dyes tested. In a recent test conducted on 35 chemicals on the EPA priority pollutant list by EG & G Bionomics (Parrish, personal communication), there were no significant differences in the EC-0's between Selenastrum and Skeletonema, Daphnia and bluegill fish, Lepomis macrochirus. Selenastrum was significantly more sensitive than sheepshead minnow. In another 2 tests EG & G performed for Monsanto Industrial Chemical Co. (1979a,b) evaluating two phthalate esters (Santicizer 60 and 711), Selenastrum was as sensitive as Microcystis aerugenosa, Navicula pelliculosa, Skeletonema costatum and Dunaliella tertiolecta. Palmer (1969) has extensively reviewed the algal literature and has ranked the 60 most pollution tolerant genera as reported by 165 authors. In comparing two green algae often used in algal toxicity testing, Chlorella and Scenedesmus to Selenastrum, great variation is found. Of the 60 genera, Scenedesmus was the fourth most tolerant, Chlorella was the fifth most tolerant, but Selenastrum was the fifty-seventh most tolerant. This analysis is borne out by recent results obtained by Green (personal communication) in testing effluent toxicity to algae. He found that Chlorella and Scenedesmus are generally more resistant to industrial effluents and both were naturally present in 100% effluents (eight submitted by

the USEPA Industrial Environmental Laboratory, Research Triangle Park, Raleigh, North Carolina). Selenastrum only grew when the effluents were diluted to 1-10% of the original concentration (which supported Chlorella and Scenedesmus growth). This was also the case in another effluent which contained 1.7 mg/l cyanide. Both Chlorella and Scenedesmus grew in it, but Selenastrum grew only when the effluent was diluted to 1% or less. Chlorella has also recently been shown to be much less sensitive to toxics than Daphnia or fish (Kenaga and Molenaar, 1979).

While it is recognized that numerous marine algae are sensitive to toxicants (North et al. 1972); heavy metals (Davies 1978), simple organics (benzene, cresol, hexane, phenol and toluene), various inorganics (Cl, CN, Hg) and complex wastes (industrial sewage, sulfite waste liquor, detergent), and petroleum compounds (Corner 1978), Skeletonema costatum was selected for use in the toxicity test guideline. This species has been frequently reported on in the bioassay literature (US Army 1978), and is a recommended bioassay organism (APH 1975, USEPA 1977a, b, 1978, Gentile and Johnson 1974).

The testing procedure for Skeletonema has recently proven useful for the evaluation of the relative potential hazards of a compound or a complex waste by providing data for the calculation of the EC-50 or SC-20 (Walsh and Alexander 1980, Walsh et al. 1980). Skeletonema was as sensitive to the 35 priority pollutants and two phthalate esters as Selenastrum in multi-species toxicity screening tests, as in the previously described studies.

Skeletonema was found to be more sensitive (at 10ppb) to growth inhibition effects induced by PCB's than two freshwater algae (Euglena gracilis and Chlamydomonas reinhardtii) and two other marine algae (Thalassiosira pseudonana, and Dunaliella tertiolecta) (Mosser et al. 1972).

Skeletonema costatum was also more sensitive (growth inhibited) at lower concentrations of wastewater chlorination products (3-chlorobenzoic acid, 5-chlorouracil, 4-chlororesorcinol, 3-chlorophenol and Captan) than Dunaliella tertiolecta and Porphyridium sp. (Sikka and Butler 1977).

Skeletonema and Selenastrum are specified for testing toxicity of pesticides (Subpart J, Pesticide Registration Guidelines). Additional justification for selection of these test species is provided in these guidelines (see FR 45 (214): 72948-72978).

Other species may be substituted for either of these two species when appropriate. Some freshwater or marine species which are of concern or have a significant ecological role may constitute a more crucial risk population. If so, those species of particular ecological or economic value should be selected. The rationale for selection of alternative species should be discussed with the Agency and/or supported in the report of findings.

2. Facilities

a. General

The test requires a growth chamber or temperature controlled enclosure capable of maintaining a uniform temperature of 24° ± 1°C if Selenastrum is tested or

20° ± 1°C if Skeletonema is tested. Other facilities typically needed include standard laboratory glassware, culture flasks, work areas to clean and prepare equipment and to measure chemical concentrations and algal growth and proper disposal facilities. Without these facilities, the testing cannot be adequately conducted.

b. Test Containers

Sterile Erlenmeyer flasks are recommended as test and culture containers. Any flask volume may be used between 125-500 ml. However, it is imperative that flasks of the same volume be used throughout the test. Hannon and Patouillet (1979) found a marked difference (2.6x) in mercury toxicity for marine algae, Phaeodactylum tricornutum, depending on the surface : volume ratio of the culture vessel. Flasks should be stoppered with sterile plugs (such as foam rubber or cotton stoppers) which will prevent possible bacterial contamination yet allow air flow.

c. Cleaning and Sterilization

Standard good laboratory practices are recommended to remove dust, dirt, other debris, and organic and inorganic residues from the test containers and other glassware and supplies should be washed and sterilized to prevent contamination.

Algal cells are discarded at the end of a test. Algae are capable of considerable adaptation to the toxic effects of antimetabolites and antibiotics, such as streptomycin, penicillin, chloramphenicol, sulfanilimide and sodium selenate (Kumar 1964).

It is important to avoid contamination of algal cultures by bacteria. Bacteria may metabolize high molecular weight

organic compounds to produce carbon dioxide and/or cofactors that stimulate growth of Selenastrum (Tison and Lingg 1977, Sachdev and Clesceri 1978). Consequently axenic cultures of algae should be maintained by proper sterile culture techniques as well as growing and testing algae in sterile containers and nutrient medium.

d. Conditioning

Test containers are to be rinsed with appropriate test solutions prior to the beginning of the toxicity tests. This method should allow for sorption of the test substance to the test container, thereby saturating the container surface so that no further interactions of test substance will take place when new test solution is added and the test begins. Hannan and Patouillet (1979) found that up to 50% of mercury could be lost to adsorption to vessel walls in a two-day toxicity test. Therefore, with proper conditioning all the test substance in the test solution should be available to test algae and any results will reflect an accurate concentration response.

e. Nutrient Medium

The nutrient medium recommended in the test guideline, are those currently recommended by the USEPA for use in bioassays (USEPA 1977, 1978a,b,c, Walsh and Alexander 1980, Walsh et al. 1980).

Use of the nutrient media under the test conditions will ensure maximum growth rates (i.e., logarithmic) in test algae and controls. Selenastrum and Skeletonema will divide 2-3 times per day (Nielsen 1978, Lewin and Guillard 1963, USEPA 1971b). This should enhance exposure of test algae to the test substance because algal cells in this growth phase

absorb and metabolize substances at a rapid rate (Fogg 1965). Shiroyama et al. (1973) found maximum phosphorus and nitrogen uptake occurred in the first five days of growth.

Many media used for culturing algae contain a chelating agent, usually EDTA, to keep micronutrients in solution. However, a medium containing a chelating agent is less than ideal for testing toxicants because chelators can increase or decrease toxicity and can add uncertainty to the test results (Payne 1975, Fogg 1965, Prakasn and Rashid 1968, Bender 1970, Giesy 1974, Lin and Schelske 1979, Barber and Ryther 1969, Johnston 1964, Dropp 1960, 1962; Eyster 1968, Erickson et al. 1970).

3. Environmental Conditions

Selenastrum and Skeletonema will grow over a wide temperature range, from less than 5°C to 35°C (Claesson and Forsberg 1978), and between 13°C and 30°C (Fogg 1965), respectively. The temperature selected for toxicity testing using Selenastrum was 24°C because luxury uptake of ammonia nitrogen, maximum specific growth rate, and sensitivity to phenol occur at that temperature (Reynolds et al. 1974, 1975a, 1975b 1976). The test temperature 20°C selected for Skeletonema is recommended in other toxicity testing manuals (USEPA 1978a,c) and in recent publications (Walsh and Alexander 1980, (Walsh and Alexander 1980, Walsh et al. 1980).

Algae require light for photosynthesis and growth. Fitzgerald (1975) and Miller et al. (1978) have shown that light intensity will affect the rate of growth of Selenastrum. As practically all the provisional algal assay procedure (Joint Industry/Government Task Force 1969)

development work was done on Selenastrum at 400 ft-c, it was not seen as necessary to make a change (USEPA 1978b). Continuous lighting of algal cultures is required for Selenastrum in the test guideline. While this does not reflect environmental conditions, it does maximize testing for toxicity. Practically all toxicity tests using Skeletonema have recommended split day/night lighting (USEPA 1978a, 1978c, Walsh and Alexender 1980, Walsh et al. 1980). For the sake of consistency, it was not seen as necessary to make a change in the procedure.

The test guideline requires a test solution pH of 7.5 for Selenastrum because it maximizes growth. Selenastrum grows between pH 4 and 10 (Brezonik et al. 1975) and maximally between pH 7 and 9.6 (Claesson and Forsberg 1978). Maximum adenosine triphosphate (ATP) (i.e., energy production) occurs in Selenastrum cultured between pH 7.5 and 8 (Brezonik et al. 1975). The pH selected for testing with Skeletonema, 8.1, was selected because it is recommended by other toxicity testing manuals (USEPA 1978a) and in recent publications (Walsh and Alexander 1980, Walsh et al. 1980) and approximates the natural oceanic pH. The pH should be adjusted as exactly as possible to the test pH because fluctuations in pH affects toxicity.

The purposes of oscillating the cultures are to enhance exposure of algal cells to test substances and to enhance dissolution and solubilization of test substances in the test solution. Turbulence created by shaking algal cultures is important to enhance the transfer of dissolved substances between the media and the cells. Munk and Riley (1952) showed that this transfer is faster if nutrients are

continually renewed adjacent to the cell by movement of the medium.

Oscillating test containers is also analogous to wind and wave induced mixing of natural waters. This agitation and mixing serves to maximize algal exposure to the test substance.

Temperature, light intensity, pH and oscillation rate are all recorded as specified in the test guideline to ensure that the environmental conditions of the test are met.

Temperature should be recorded at least hourly to ensure that it does not exceed the specified limits. Inexpensive growth chambers are available which are equipped with adequate recording instruments or chambers may be equipped with ones at minimal cost. Severe fluctuations in temperature may affect algal growth and/or subsequent chemical uptake or metabolism.

Light intensity readings at the surface of the solutions may be made manually and ensure that all containers are receiving equal light. Light variations will affect algal growth so daily recordings are necessary to maintain uniform and constant radiation. The pH is measured at the beginning and end of the test as an indication of effects of test chemical additions and subsequent algal metabolism on the hydrogen-ion concentration. This will indicate if the test solution is outside of the algal pH optima for growth as well as show what pH variations may exist between chemical concentrations.

C. Reporting

The sponser should submit to the Agency all data developed during the test that are suggestive or predictive of phytotoxicity. If testing specifications are followed, the sponsor should report that specified procedures were followed and present the results. If alternative procedures were used instead of those recommended in the test guideline, then the protocol used should be fully described and justified.

Test temperature, chemical concentrations, test data, concentration-response curves, and statistical analyses should all be reported. The justification for this body of information is contained in this support document. If algal species other than tne two recommended were used, the rationale for the selection of the other species should be provided.

III. Economic Aspects

The Agency awarded a contract to Enviro Control, Inc. to provide an estimate of the cost for performing an acute toxicity test using freshwater algae according to the Guideline. Enviro Control supplied two estimates; a protocol estimate and a laboratory survey estimate.

The protocol estimate was $1760. This estimate was prepared by identifying the major tasks needed to do a test and estimating the hours to accomplish each task. Appropriate hourly rates were tnen applied to yield a total direct labor charge. An estimated average overhead rate of 115%, other direct costs of $400, a general and administrative rate of 10%, and a fee of 20% were then added to the direct labor charge to yield the final estimate.

Environ Control estimated that differences in salaries, equipment, overhead costs and other factors between laboratories could result in as much as 50% variation from this estimate. Consequently, they estimated that test costs could range from $878 to $2636.

The laboratory survey estimate was $1465, the mean of the estimates received from eight laboratories. The estimates ranged from $430 to #3600 and were based on the costs to perform the test according to the Guideline.

Altnough a cost analysis was not performed for a test using marine algae, the procedures used are similar to the freshwater algal test and the costs should be similar.

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