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AFOEHL Bioassay Services

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December 1990

Final Report

AF Occupational and Environmental Health Laboratory (AFSC)
Human Systems Division
Brooks Air Force Base, Texas 78235-5501

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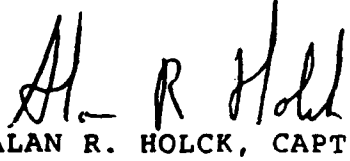

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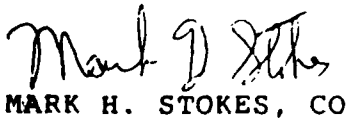

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This report introduces MAJCOM and base-level personnel to the AFOEHL bioassay program. Current services provided by the AFOEHL are discussed as well as services we hope to provide in the future.

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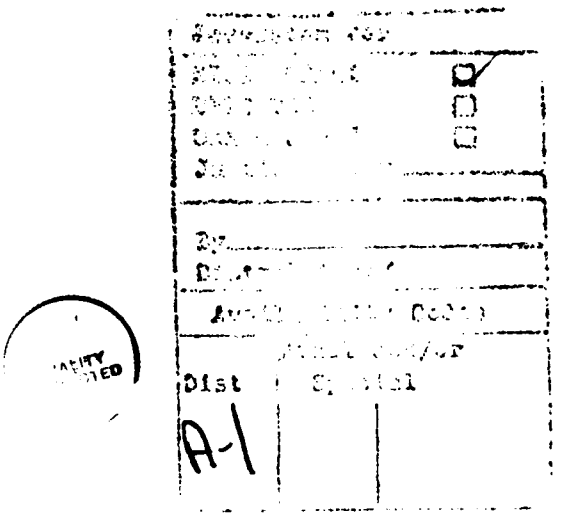

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I. INTRODUCTION

A. Purpose: To introduce MAJCOM and base-level personnel to the AFOEHL bioassay program. Current services provided by the AFOEHL are discussed as well as services we hope to provide in the future.

B. Problem: Bioassay involves the measurement of some event on life. Events classically refer to exposure to some chemical (or chemicals), but may also include the effects of temperature, noise, illumination, etc. Many Air Force (AF) installations, in order to fulfill federal, state, and local laws and regulations, require periodic bioassay of waste-water effluents. In addition, bioassay techniques can be useful to address water and/or soil quality following some sort of accidental chemical release. Personnel involved with environmental quality, environmental management, and associated areas at AF installations should be familiar with the bioassay services that the AFOEHL can provide to assist MAJCOM and base-level personnel in the performance of their mission.

C. Scope: After a brief discussion on bioassay theory, this report will focus on the type of services currently available from the AFOEHL as well as services that will be offered in the future. Current services include acute and chronic macroinvertebrate bioassays, acute and chronic fish bioassays, terrestrial bioassays using plants, and bioassay contract monitoring. Future services include terrestrial invertebrate bioassays and long-term chronic fish bioassays.

II. DISCUSSION

A. Terms Used: Before delving into a discussion on what bioassay is and the theory involved, it may be useful to define some terms which will be used in the discussion that ensues.

ACUTE: Short-term (usually <= 96 hr).

ANALYSIS OF VARIANCE (ANOVA): A method of data analysis used to differentiate between groups of ofganisms exposed to qualitatively different as well as quantitatively different stimuli.

BIOASSAY: An experimentally-based approach to determine if a living organism is impacted by some stimulus.

CERIODAPHNIA: A very small (1mm) genus of water fleas often used as a test organism for aquatic bioassay.

CHRONIC: Long-term (usually >96 hr)

CONTROL GROUP: A group of test subjects that is not exposed to the stimulus but is otherwise treated identically to the test group, thus serving as a comparison.

DAPHNIA: A genus of small (5mm) water fleas, several species of which are often used as a test organism for aquatic bioassay.

DOSE: The amount of stimulus that the test group is exposed to.

ED50 (Effective Dose, 50%): The amount of stimulus required to effect 50% of a group of test subjects when the endpoint is not death (see LD50).

ENDPOINT: The measured effect of a stimulus on a group of test subjects. Usually death, but can also be a change in growth rate, reproduction, activity, or any other measurable parameter.

LC50 (Lethal Concentration, 50%): The concentration of a stimulus in water that will kill 50% of the test organisms. (see LD50).

LD50 (Lethal Dose, 50%): The amount of a stimulus required to kill 50% of the test organisms. Similarly, the LD90 refers to the amount of a stimulus required to kill 90% of the test organisms, LD5 5%, etc.

LOEC (Lowest Observable Effect Concentration): Similar to LOEL, but refers to stimulus concentration in aquatic bioassays.

LOEL (Lowest Observable Effect Level): The lowest level of a stimulus at which an effect is observed.

NEOPLASM: A tumor; may be either malignant or benign.

NOEC (No Observable Effect Concentration): Similar to NOEL, but refers to stimulus concentration in aquatic bioassays.

NOEL (No Observable Effect Level): The level of a stimulus that produces no observed effect on a group of test organisms.

NON-PARAMETRIC ANALYSIS: A group of statistical techniques used when the data collected do not meet the assumptions required for probit analysis or ANOVA.

NPDES PERMIT: National Pollutant Discharge Elimination System permit as specified in the Clean Water Act as amended by the Water Quality Act of 1987. Issued to a pollutant discharger (i.e., AF installation) for up to a 5-year period and dictates specific allowable levels of various pollutants. May contain a bioassay requirement.

PROBIT ANALYSIS: A type of data analysis involving log-transformed linear regression. Results give a LD50/ED50 for a stimulus as well as a measure of the variability among the test organisms.

STIMULUS: The contaminant of interest. Usually refers to a chemical (or chemicals), but can also refer to noise, heat, illumination, vibration, etc.

SUBLETHAL: A dose of stimulus that does not produce an acutely fatal effect.

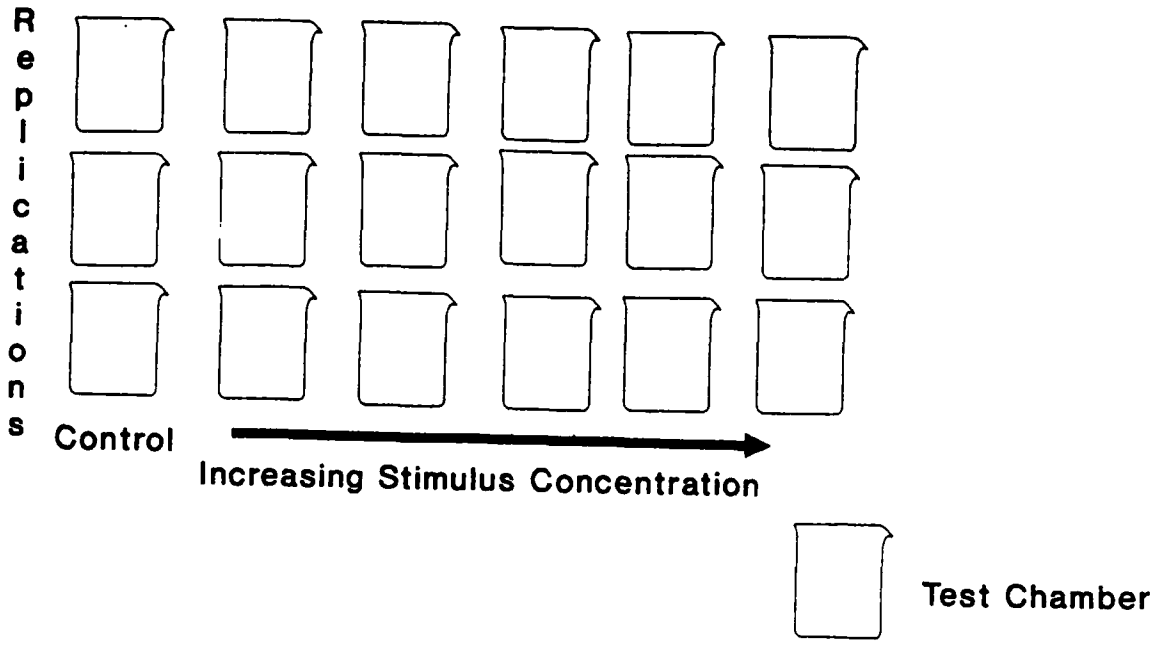
TEST GROUP(S): A group or groups of test subjects that are exposed to the stimulus.

TUMORGENIC: Capable of producing tumors.

B. Bioassay Theory:

1. The biochemistry of living organisms is incredibly complicated (Stryer 1981), but nonetheless includes certain mechanisms that are common regardless of taxonomic status. The exposure of an organism to various compounds at certain concentrations (i.e., a stimulus) will cause a change in the biochemical processes involved. This change may be so extreme as to cause death of the organism. Subacute effects may also occur, such as carcinogenesis. While great strides have been made in recent years concerning the lower limit of chemical detection in the environment, the bottom line is "What is the effect of the chemical on living organisms?" Bioassay helps us to answer that question by providing an indication of the effect of a chemical substance on the entire living organism, rather than just determining its impact on a single chemical process.

2. Bioassay is based on the theory that an organism exposed to a given stimulus will have a certain probability of responding to the stimulus. The probability will vary upon the dose of stimulus. Because the probability of an endpoint occurring on a living organism by its very nature is subject to variability, groups made up of several organisms each (typically 5 - 10) are usually exposed to the stimulus. In addition, several different levels of the stimulus are used. A control group, unexposed to the stimulus in question but otherwise treated identically to the test groups is utilized to provide background data. The total series of test groups plus the control group makes one replication. Accuracy and adequacy of data analysis is enhanced by performing several (usually 2-4) replications. The figure on the next page provides a schematic illustration of a typical bioassay setup with three replications of five stimulus concentrations plus one control concentration.



Schematic diagram of a typical bioassay.

3. Classical bioassay techniques have focused on several areas, most notably the determination of acute LD50 values for a variety of chemicals. Evidence for or against tumorgenicity may be another endpoint for chronic studies. The test organisms involved are often rats or mice. Another area that has received considerable attention in the past is the use of bioassay for water quality determination. In these aquatic bioassays, the test organisms are often macroinvertebrates or fish. The AFOEHL Bioassay Program has been very active in supporting AF installations who have both long-term (usually to meet NPDES permits) and one-time (usually for accident investigation) water quality bioassay requirements.

C. Current AFOEHL Bioassay Services:

1. Aquatic Bioassays: The AFOEHL Bioassay Program directly supports AF MAJCOMs and installations by providing several aquatic bioassay services. Often, these bioassays help installations satisfy NPDES compliance requirements. Aquatic bioassay services provided by the AFOEHL follow strict methodologies developed by the U. S. Environmental Protection Agency (Biesingeret al. 1987, Weber et al. 1989). The exact method utilized is determined by the needs of the requesting organization. Aquatic bioassays performed are as follows:

a. Fathead Minnow (Pimephales promelas) Larval Survival and Growth Test (Weber et al. 1989). This method is used to determine the effect of wastewater effluent on fathead minnow larvae over a seven-day period. Usually, five or more different effluent concentrations are used, with four

replications per concentration. The endpoints include both a differential in growth as well as in mortality between test and control groups. The test water is replaced daily. Analytical methods for the data collected include probit analysis, ANOVA, or non-parametric methods depending on the data collected.

b. Claderoceran (Ceriodaphnia dubia) Survival and Reproduction Test (Weber et al. 1989). This bioassay is used to determine the effect of wastewater effluent on the water flea Ceriodaphnia dubia over a seven-day period. A minimum of five concentrations of test water plus a control are required, with ten replications per concentration, but the total volume of water required is approximately one-half that of the fathead minnow test discussed above. Endpoints include a differential in the number of offspring as well as in mortality among the control and test groups. Data analyses performed include probit analysis and Fisher's Exact Test (non-parametric).

c. Acute Toxicity Tests (Biesinger et al. 1987, Peltier and Weber 1985). This bioassay is used to determine the acute effects of wastewater effluent on one of several different organisms over a short time period. Organisms assayed against include the water fleas Daphnia magna, D. pulex, and the fathead minnow Pimephales promelas. Time periods range from 24-96 hours. Once again, a minimum of five test group concentrations plus a control group are required. Four replications take place. Mortality is the endpoint. The LC50 is calculated using probit analysis. If little or no mortality is observed, then mean survival at each concentration is calculated.

All of the above-mentioned techniques incorporate a high level of quality control/quality assurance. As one might expect, the results are largely dependent on the test water quality. It is of the utmost importance that base-level personnel tasked with water collection follow the appropriate technique so a valid result is obtained.

2. Terrestrial Bioassay: Terrestrial bioassays are performed by the AFOEHL personnel to determine whether the soil contamination is deleterious to plant growth. Examples of contaminated soil include hazardous waste dumps and aircraft accident sites. The method used in these bioassays was developed at the AFOEHL by Lillie and Bartine (1990). Two plant species are used, the monocotyledon sorghum and the dicotyledon pinto bean. Freshly germinated seeds of both species are planted in the contaminated soil, non-contaminated soil collected from the same area as the contaminated soil and a standard potting soil. The latter two soils serve as controls. Six replications per soil are required. After a period of 14 days, the stem length is determined and the data is analyzed via analysis of variance.

3. Contract Monitoring Services: Personnel in the AFOEHL Bioassay and Ecology Function are able to develop and

monitor contracts for bioassay requirements that are not provided by the AFOEHL or exceed the lab's capacity. Several contracts are in place to cover such services. The function chief should be contacted for further details.

4. SPECIAL PROJECTS: The AFOEHL also has the ability to use bioassay techniques to address special situations or questions. For example, an AF installation recently had a potential problem with trichloroethylene (TCE) in groundwater that was being used off-site as irrigation water. A project is currently underway at the AFOEHL to determine the effect of TCE contaminated water on plant growth and to determine if TCE or its metabolites are detectable in plant tissue. Another special project involved testing a new water purification tablet against Daphnia pulex to get an idea of how it might perform against similar but pathogenic organisms that might be encountered by field personnel in contingencies.

D. Future Directions:

1. TERRESTRIAL BIOASSAY: A new technique using soil invertebrates (specifically Arthropoda: Isopoda) is being developed. This method may prove to be faster and more sensitive than the plant bioassay techniques currently employed, thus allowing a faster response to field requests. This bioassay will be portable and will allow the AFOEHL personnel to rapidly assess contamination on location at accident sites.

2. LONG-TERM CHRONIC BIOASSAY: Recently developed techniques as discussed by Gardner et al. (1990) can be used to assay the long-term effects of aquatic pollutants on test animals (typically fish). The AFOEHL is planning to utilize several of these techniques. However, at this time a timetable for their incorporation into the bioassay program is not available.

a. Fish Carcinogenicity Studies: This bioassay determines whether fish exposed to contaminated water for long periods develop any neoplasms. Evidence has been established that neoplasms in aquatic animals indicates that carcinogens are present in their environment (Dawe 1990). The technique employed involves placing 50 newly hatched fish in each of two aquaria. One aquarium is supplied with contaminated water; the other non-contaminated water. A circulation system is setup to provide five water changes per 24-hour period. After 90 days, 25 fish from each tank will be removed and sent to a contract pathology laboratory for analysis. Up to 30 distinct tissues can be examined for neoplasms. At 180 days, the remaining fish will be sacrificed and similarly analyzed. If the fish exposed to the contaminated water contain significantly more of any type of neoplasm than the clean water, this would provide evidence that the contaminant is indeed a health risk. Evidence that no real differences in number or type of neoplasms between the two

water types suggests that carcinogenicity may not be a problem.

b. Subacute Aquatic Toxicant Studies: Subacute doses of a toxicant often have negative, nonfatal, effects on the exposed organism. In fish, exposure to low doses of a toxicant can lead to a change in ventilatory rate (i.e., gill movement), depth and several other parameters. In concert, the values obtained for these parameters indicate the overall stress to which the fish are exposed. These changes can be measured electronically as discussed by van der Schalie (1986) and van der Schalie et al. (1988). These bioassays can be used not only to answer questions concerning environmental issues, but may also be employed to assay potential drinking water supplies. The results obtained from such bioassays would be of interest to personnel tasked with providing secure water sources in contingency situations. If potential water sources were constantly bioassayed with such a system, a change in the "fish stress level" could indicate an underlying change in overall water quality. This type of technique holds much promise under chemical warfare conditions to assay the quality of potentially contaminated water sources. Bioassay techniques can be used to determine if compounds that are undetectable using current techniques are present.

III. **CONCLUSIONS:** The AFOEHL/EHT Ecology and Bioassay Function offers many services to AF installations. These services range from EPA-approved NPDES monitoring to development of bioassay techniques for use in a wide range of situations where there is a question concerning the effect of some contaminant on AF personnel.

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