EVALUATION OF PROCEDURES FOR PREPARING ENVIRONMENTAL AND WASTE SAMPLES FOR MUTAGENICITY TESTING: Environmental Waters and Wastewaters

by

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ABSTRACT

To complement the standardized protocol of the EPA for conducting the Ames assay, consensus protocol for the preparation of environmental samples for testing are currently under evaluation. The first of these is for environmental water and wastewater.

The Salmonella/mammalian-enzyme assay developed by Dr. B.N. Ames and his colleagues has been widely accepted as the most frequently-used and reliable short-term mutagenicity assay available. It is one of the most cost-effective screening tools for evaluating the distribution of mutagenic pollutants in the environment. The application of the Ames assay for mutagenicity measurement can also be valuable for prioritizing environmental samples for chemical analysis, for facilitating the detection of potentially hazardous environmental contaminants, and eventually, for assessing risks of human and environmental exposure.

However, most complex environmental samples can not be tested directly in the Ames assay due to the presence of very low concentrations of mutagens or the existence of toxic components which mask mutagenic effects. Protocols are needed for preparation of environmental and waste samples for mutagenicity testing. Under the sponsorship of the EPA, a project to develop guidelines for sample preparation of various environmental media including wastewater, drinking water, soils and sediments, solid wastes, air, and nonaqueous liquid wastes has been initiated. Standardization procedures are crucial for ensuring comparability of test results for scientific evaluation and for potential enforcement and litigation actions arising from surveillance of hazardous waste sites.

The first protocol evaluated is for environmental water and wastewater. Samples used in the evaluation were selected from a range of generic wastewater types and include effluents from industrial and municipal wastewater treatment plants, contaminated groundwater, surface runoff from a hazardous waste landfill, the aqueous fraction from a RCRA solid waste extraction procedure, and brackish surface water receiving industrial effluents and areal runoff. The aqueous wastes were processed by a liquid-liquid extraction scheme similar to the EPA method 625. A 3 L sample was typically used for the extraction. The sample was adjusted to

pH 11, extracted with dichloromethane, then adjusted to pH 2 and reextracted with dichloromethane. The dichloromethane fractions were either combined or were kept separated, then concentrated to dryness. The residues of the solvent extracts were tested in the Ames assay using strains TA98 and TA100 in the absence and the presence of 2%, 10%, or 30% rat liver activation systems (S-9). All the field samples were found to be mutagenic, and mutagen levels ranged from approximately 200 to 4000 revertants per liter of water. TA98 was the more sensitive strain although mutagenic activity was also detected in TA100 for some samples.

ASTM Type I water, which served as field and travel blanks as well as method blank, was extracted, and the residue was tested in the Ames assay. It was established as the method background by using several statistical analyses. Control charts for precision and accuracy of the background were constructed for routine quality control checks on data acceptability. The average water blank values in TA98 were 35 (without S-9), 49 (2% S-9), 51 (10% S-9), and 39 (30% S-9) revertants/plate. The detection limit was set as twice that of the background according to the Ames two-fold rule.

Replicate or triplicate measurements of mutagenicity were performed on the wastewater samples. The results of several samples with relatively stable activities were evaluated for precision of the experimental process. Standard deviations representing the precision of these measurements varied from 2% to 33%. The accuracy of the methodology was evaluated indirectly through recovery studies. The extraction efficiency based on the recovery of spiked mutagens was determined with both chemical and mutagenicity analyses. The recovery for benzo(a)pyrene, a neutral compound, was 90% to greater than 100% in a municipal wastewater sample. For 4-nitrobenzoic acid, an acidic compound, the recovery was 40% to 60%. The recovery of 2-aminoanthracene, a basic compound, was approximately 25% to 35%.

The study established the validity of the consensus protocol for the preparation of a variety of generic types of wastewater samples for Ames testing. Emphasis was on minimum requirements for routine screening of wastewater samples, consistent with the intended purpose of the sample preparation protocol for eventual use in hazardous materials monitoring.

This report covers a period from March 1, 1985, to December 31, 1985, and work (including draft report preparation) was completed as of March 31, 1986.

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TABLES

ABBREVIATIONS

ACS:	American Chemical Society
AIHL:	Air and Industrial Hygiene Laboratory, CDHS
ASTM:	American Society for Testing and Materials
2AA:	2-Aminoanthracene
B(a)P:	Benzo(a)pyrene
CDHS:	California State Department of Health Services
CDHF:	California Public Health Foundation
CFR:	Code of Federal Regulations
CWQCB:	California State Water Quality Control Board
DMSO:	Dimethylsulfoxide
EP:	Extraction Procedure (40 CFR 261.24)
EPA:	Environmental Protection Agency
EMIC:	Environmental Mutagen, Carcinogen, and Teratogen Information Center, Oak Ridge, TN
EMSL:	Environmental Monitoring System Laboratory, EPA, Las Vegas, NV
FIFRA:	The Federal Insecticide, Fungicide and Rodenticide Act
HML:	Hazardous Materials Laboratory, CDHS
HPLC:	High Pressure Liquid Chromatography
K-D:	Kuderna-Danish Concentrator
4NBA:	4-Nitrobenzoic Acid
NBS:	National Bureau of Standards
2NF:	2-Nitrofluorene
4NQO:	4-Nitroquinoline-N-Oxide
QA:	Quality Assurance
QC:	Quality Control
RCRA:	The Resource Conservation and Recovery Act
SAIC:	Science Applications International Corporation, La Jolla, CA
SD:	Standard Deviation
SPRM:	Spiked Reference Mutagens
SR:	Spontaneous Mutation Revertants
TCLP:	Toxicity Characteristics Leachate Procedure
TSCA:	The Toxic Substances Control Act
TSCD:	The Toxic Substances Control Division, CDHS

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SECTION 1

INTRODUCTION

There is increasing evidence that environmental mutagens are a cause of cancer and of genetic birth defects and that they may also contribute to aging and heart disease (Ames, 1979). Of particular concern are toxic and hazardous chemical wastes which are produced in quantities of over 250 million metric tons a year in the United States. The U.S. Environmental Protection Agency (EPA) has found that the mismanagement of these wastes causes environmental and public health damage such as contamination of groundwater and surface waters, pollution of air and soils, and poisoning and chronic illness of humans and animals via the food chains or direct contact (USEPA, 1979a). The chemical complexity of hazardous wastes and their residues precludes complete chemical analysis of toxic components. Furthermore, toxicological synergism and antagonism between chemical waste components is likely. These interactions make risk assessment of hazardous wastes on a chemical-by-chemical basis extremely difficult. A critical need is to develop short-term biological methods to assist in assessing the potential hazards of chemicals in complex waste samples. To this end, EPA laboratories and program offices with responsibility for toxic and hazardous substances management have expressed an immediate need to apply the Ames and other mutagenicity tests in the analysis of complex waste samples. Short-term bioassays, such as the Ames test, are now used by many public and private laboratories in screening of complex mixtures for mutagenic activity (Hollstein and McCann, 1979). Results of these tests are often widely circulated and interpreted. It is thus critical that testing procedures be standardized and that the quality of the data be assured so that valid interlaboratory and intralaboratory comparisons of results obtained by different laboratories can be obtained.

This report describes progress in developing guidelines for preparing environmental waste samples for mutagenicity (Ames) testing. Procedures for the preparation and the mutagenicity testing of environmental waters and wastewater are described. This work was carried out under a cooperative agreement (CR810022-02-0) between the Environmental Monitoring Systems Laboratory (EMSL), EPA, Las Vegas, Nevada,

and the Air and Industrial Hygiene Laboratory (AIHL), California State Department of Health Services (CDHS), administered through the California Public Health Foundation (CPHF) during the period from March 1, 1985 to December 31, 1985.

GOALS AND TASKS

The ultimate objective of this work is to develop a quality assurance (QA) program for biological testing of complex environmental samples using the Ames Salmonella mutagenicity assay. Mutagenicity testing can be used to determine the mutagenic potential of complex environmental pollution mixtures. In preparing mixtures for Ames testing, it is critical to evaluate differences in capability and efficiency of sample preparation procedures toward organic compounds that might not be detected by chemical analyses alone. Such knowledge will increase the value of the Ames test to EPA hazardous waste monitoring programs and to other programs assessing environmental and human health risks.

The Salmonella/mammalian-enzyme mutagenicity assay developed by Ames and his colleagues (Ames et al., 1975; Maron and Ames, 1983) has been extensively studied and widely accepted as the most reliable and efficient short-term mutagenicity assay available (Brusick and Young, 1981; Hollstein and McCann, 1979, Sexton et al., 1981). In a collaborative study supported by the EPA, a standard Ames test protocol has been established and validated (Adams et al., 1984; Williams and Preston, 1983, Williams, 1985). The utility of this procedure as a means of providing routine detection of potentially mutagenic substances in the environment has also been recommended (Sugimura and Nagao, 1982).

However, the interim procedures of the EPA for conducting the Ames test (Williams and Preston, 1983) do not address the sample preparation of environmental wastes and substances. Further, there are no generally accepted or standard methods specifically designed for preparing these types of samples for biological testing. The EPA requires standardized procedures for the preparation of environmental samples for mutagenicity testing to ensure comparability of test results for scientific evaluation and for potential enforcement and litigation actions arising from surveillance of hazardous waste sites.

The purpose of this phase of the project is to validate guidelines for preparing environmental water and wastewater samples for the Ames test. The guidelines are based on a consensus protocol for wastewater sample preparation developed under the auspices of the Quality Assurance Division, EMSL-Las Vegas, EPA (ICAIR, 1985). At present, preliminary protocols for the following six media are available: air particulate matter, drinking water, environmental waters and wastewater, nonaqueous liquid wastes, soils and sediments, and solid wastes. However, these protocols have not been validated, and no guidance for QA exists in these protocols. Therefore the main tasks of this project are protocol validation and QA development. In this report, guidelines for preparing wastewater are developed. Guidelines to the other five media will be developed in future phases of the work.

The goal of the sample preparation protocol is to provide samples which accurately and reliably reflect the mutagenic potential of the original complex material. These procedures should also yield adequate products that can be appropriately tested in the Ames assay and to other biological assay systems. In addition, since the protocols are intended for routine use in the examination of a large number of samples, the procedures need to be simple and cost-effective. The extent of sample preparation processing required varies for different media. For example, nonaqueous liquid waste samples in many cases can be tested directly for mutagenic activity without any sample preparation. However, extraction and concentration are required for the other five media: air particulate matter, drinking water, wastewater, soils and sediments, and solid wastes (ICAIR, 1985). In general, further fractionation of an extract is required if cytotoxicity of the extract prevents assessment of its mutagenic potential. By using standardized and validated procedures for sample preparation and for mutagenicity testing, it will be possible to increase the accuracy and the precision of the results and to permit more meaningful interlaboratory comparisons.

Another essential aspect of the validation of the wastewater preparation protocol includes the development of a comprehensive QA program incorporating the protocol. This protocol may also be used for preparing wastewater samples for other bioassay systems and for chemical analyses. Once validated, these standardized testing procedures can be applied to assist in the wastewater monitoring effort of the EPA.

APPROACH

The development of a comprehensive QA program for mutagenicity testing of environmental mixtures using the Ames test involves three phases. In the first phase, a standard Ames test protocol is established and is validated through inter-laboratory testing. This is now completed (Adams et al., 1984; Williams and Preston, 1983). In the second phase, standard protocols for the preparation of complex environmental mixtures for Ames testing are developed and validated. In the third phase, the validated protocols for sample preparation and mutagenicity testing of complex mixtures are applied to actual waste samples by laboratories in support of EPA hazardous waste and other programs. In this stepwise fashion, mutagenicity testing procedures can be standardized and can be applied to the monitoring of hazardous waste materials by EPA.

This Cooperative Agreement is concerned with phase two, the evaluation of sample preparation protocols. Specifically, the consensus sample preparation protocol for wastewater is validated during this reporting period. Although the application phase is beyond the scope of the current EPA-CDHS/CPHF Cooperative Agreement, actual waste samples are used in developing the standardized sample preparation protocol. This provides preliminary indications as to the efficacy of using the Ames assay and sample preparation protocols as a screening mechanism for potentially hazardous environmental water and wastewater samples.

The following four-step approach is used in the protocol evaluation and validation:

- Establish the method background with field blanks and with reagent blanks. Several quality control (QC) procedures to avoid potential problems such as chemical contamination, i.e., artifact mutagen formation and negative interference, are developed.

- Establish the accuracy of the method through the use of spikes (analyte addition) or surrogates (chemical homologues) or both. To assess accuracy for the sample collection process, travel blanks are carried along with the field samples during collection and transport.

- Establish the precision of the method through testing of replicate aliquots of the same sample in a batch. The precision is affected by the homogeneity and the stability of the sample as well as the extraction and the analytical techniques of the individual analyst.

- Establish the best available protocols through method comparison for recovery efficiency and for cost-effectiveness with an emphasis on the minimum requirements for the purpose of routine screening.

Two major tasks, the validation of the wastewater protocol and the development of a QA program, have been completed during the period of time from March 1, 1985, to December 31, 1985. The progress on technical design, procedure documentation, experimental method and results, statistical analyses, and precision and accuracy establishment are presented in this report.

SECTION 2

CONCLUSIONS

Based on the data gathered so far, it is concluded that the EPA-consensus liquid-liquid extraction protocol (Appendix A), as modified herein, can provide extracts suitable for the Ames testing from the six types of wastewater samples (Table 1). As long as the sample material is available and the sample stream is not constantly changing over time, the liquid-liquid extraction can be cost-effective. Standard deviation (SD), used for precision evaluation, was 9% for the industrial effluent (No. AIHL-85-0406), 20% for the municipal effluent (No. AIHL-85-0403), 2% for the brackish receiving water (No. AIHL-85-044A), and 33% for the bay water (No. AIHL-85-0404). The recovery of spiked reference mutagens (SPRM's) ranged from 25% to 35% for 2-aminoanthracene (2AA), 40% to 60% for 4-nitrobenzoic acid (4NBA), to 90% to 105% for benzo(a)pyrene (B(a)P). The method background was established using the water blank value which was 35 (without S-9), 49 (2% S-9), 51 (10% S-9), and 39 (30% S-9) revertants/plate in TA98.

Three major problems occurred during the interference by extraction process: (1) precipitation at pH 11, (2) formation of emulsions, and (3) interference by residual water. Modifications to improve the extraction procedure are: (1) extracting the

TABLE 1. MUTAGENICITY OF WASTEWATER SAMPLES PREPARED BY THE LIQUID-LIQUID EXTRACTION METHOD IN STRAIN TA98

Sample (No. AIHL-85-)		S-9 Condition	Mutagenic Activity ± SD[1] (Revertants/L)
Stringfellow Groundwater (on-site well, OW-2)	(0402)	2%	3600[2]
NBS Reference Sludge - TCLP Leachate	(0401)	2%	2300[3]
Industrial Treatment Plant Effluent	(0406)	30%	1700 ± 160[4]
Landfill Surface Runoff	(0405)	without	610[2]
Municipal Treatment Plant Effluent	(0403)	10%	420 ± 80
Brackish Receiving Wastewater	(044A)	2%	240 ± 6
Brackish S.F. Bay Water	(0404)	2%	230 ± 70
Stringfellow Groundwater (upgradient well, UGB-8)	(042A)	2%	170[5]

1. Standard deviation (SD) representing precision measurements was obtained from results or replicate or triplicate experiments.

2. No replicate result was obtained as a result of possible sample degradation.

3. Only one acceptable result was produced, see Section 6.1.1.6, EPA/NBS Reference Sludge - TCLP Leachate, for details.

4. Data produced approximately four months after sample collection. See Section 6.1.1.4, Industrial Wastewater Treatment Plant Effluent, for details.

5. Only one experiment was performed for this sample, see Section 6.1.1.5, Contaminated Groundwater from the Stringfellow Hazardous Waste Disposal Facility, for details.

samples which precipitate at pH 11, at pH 2 first; (2) eliminating the emulsion with glass wool, and (3) absorbing the residual water with a sodium sulfate column.

In addition to the sample preparation procedures, a sequential Ames mutagenicity testing strategy developed in this project proved to be efficient in cost and effort. A screening experiment is performed at first, and only the optimum condition is repeated. Only the two most sensitive strains, TA98 and TA100, for environmental complex mixtures are used. Four conditions of S-9 (without, with 2%, 10%, and 30% S-9 mix) are applied strategically and cover a wide spectrum of activating enzyme requirements for a large number of chemicals. Sequential testing improves efficiency by eliminating the need for further large scale experiments once a sample has been found to be mutagenic, and the optimum testing condition has been established.

SECTION 3

RECOMMENDATIONS

TOPICS FOR FURTHER EVALUATION IN THE PREPARATION OF WASTEWATER SAMPLES

XAD-Resin Method

As described in Section 6.1.1.4, Industrial Wastewater Treatment Plant Effluent, the mutagenic activity initially was not detected in extracts from the industrial waste sample (No. AIHL-85-0406). The extracts tested were obtained by extraction of up to 3 L of sample with dichloromethane. One of the limitations of the liquid-liquid extraction method is that it can not be used to concentrate large samples. When mutagenic activity is below detection using liquid-liquid extraction of 3 L samples, it is useful to process larger quantities of material. In principal, this can be accomplished using sorbant resin concentration according to the EPA-consensus drinking water protocol (Appendix B). Several preliminary experiments were carried out applying XAD-2 and XAD-7 resin columns to concentrate larger volumes (10 L) of several samples. However, these pilot experiments did not always yield larger amounts of organics from the 10 L sample comparing with the liquid-liquid extraction of the 3 L sample. Furthermore, these extracts were either nonmutagenic

or toxic to the tester bacteria. Thus, extracting 10 L samples using the XAD method did not exhibit higher total mutagenic activity compared to the liquid-liquid extraction of 3 L samples. Future work is required to explore the problems of residue toxicity and of recovery in wastewater extracts isolated from XAD resin columns. The liquid-liquid extraction procedure and the XAD method should be compared for the preferential concentrating mutagens versus toxicants.

Sample Degradation

Among the six types of samples evaluated with the liquid-liquid extraction method, two samples exhibited significant decreases in mutagenicity over a period of time (See Section 6.1.1.7, Possible Changes in Sample Composition, for details): the surface runoff from a class I landfill (See Section 6.1.1.1, Surface Runoff from a Class I Landfill, for details) and the contaminated groundwater from the Stringfellow Hazardous Waste Disposal Facility (See Section 6.1.1.5, Contaminated Groundwater from the Stringfellow Hazardous Waste Disposal Facility, for details). These samples were highly mutagenic in the initial testing. After only approximately two to three weeks of storage at $4^{o}C$ in the dark, the activity was reduced by more than 50%.

The cause for these decreases is not known. It may be caused by combinations of chemical reactions, by microbial activities, or by storage conditions. A time-course study on several representative samples is also needed to evaluate the nature and extent of mutagen degradation. This will provide necessary information for determining the maximum sample storage time and the optimum storage conditions.

Extraction Efficiency

Three mutagens, 4NBA, B(a)P, 2AA, were used for evaluating the extraction efficiency of the liquid-liquid extraction procedure (See Section 6.1.3, Mutagen Extraction Efficiency, for details). The mutagen 4NBA is an acidic compound; B(a)P, a neutral compound; and 2AA, a basic compound. The recovery efficiencies of these chemicals spiked in distilled water varied from 60% to 80% for 4NBA to 80% to greater than 100% for B(a)P and 2AA. Similar recovery was obtained in the municipal wastewater sample (No. AIHL-85-0403) spiked with B(a)P. For 4NBA, the recovery was 40% to 60% in the wastewater sample. The recovery of 2AA was substantially

reduced to 25% to 35% in the municipal wastewater. Several possibilities may have contributed to the lower extraction efficiencies including chemical reactions or losses as a result of emulsion formation. These possibilities should be investigated further, and evaluation with other known mutagens is needed to verify the overall performance of the extraction method.

QUALITY ASSURANCE (QA) PLAN

In addition to the modifications in the sample preparation procedures suggested above, a number of steps are also recommended for the establishment of a QA program for sample preparation and mutagenicity testing of environmental wastewater samples.

The establishment of a QA plan makes possible the collection of consistent data through the use of systematic methods. QA represents the total integrated program for assuring the reliability of monitoring and measurement data (Booth, 1979). The assurance of this reliability is, in turn, maintained through the use of discrete quality control (QC) activities such as the chain-of-custody documentation of sample handling, the routine calibration of instruments, the purity and cleanness assurance of consumables and glassware, the establishment of method background, the construction of QC charts for precision and accuracy, the routine analyses of SPRM's, the monitoring of positive and negative controls, and the application of strain function check procedures for obtaining prescribed standards of performance.

There are several QA procedures that allow the project manager(s) to judge and to monitor the quality of the procedures performed by the analytical staff. The first of these procedures is planning the QA program. This planning covers all aspects of acquiring quality data. In general, it is essential that a QA project plan be written and approved before the initiation of sampling and of laboratory analysis. The plan should cover but not be limited to the following areas:

- Project description, objectives, and policy.
- Project organization and responsibilities.
- QA management.
- Personnel qualification, facilities, equipment, and services.

- QA objectives in terms of precision, accuracy, completeness, representativeness, and comparability.
- Sampling and analysis procedures assuring that only appropriate methods are used.
- Sample custody.
- Calibration procedures.
- Data generation and processing.
- Internal quality control checks.
- Data analysis, validation, and reporting.
- Data quality assessment.
- Performance and system audits.
- Preventive maintenance.
- Specific procedures to be used to routinely assess data precision, accuracy, and completeness.
- Corrective action.
- Quality assurance reports to management.

A second recommended QA method is the use of periodic on-site inspection and QA audits by the project manager. These activities are for the purpose of verifying that the QC procedures listed on the checklists are being performed and that they are sufficient. These inspections are performed for sampling, analysis, and data management activities.

SECTION 4

MATERIALS AND METHODS

APPARATUS, EQUIPMENT AND MATERIALS

The validation of the sample preparation protocol for environmental water and for wastewater involves both the wastewater protocol and the drinking water protocol. A complete copy of these two consensus protocols is attached as Appendix A and Appendix B. The apparatus and equipment listed on the original consensus protocols were applied in experiments. In addition, the following items were also used:

- ASTM Type I water prepared with a MILLI-Q® Water Purification System with three cartridges: ion-exchange, carbon, and prefilter; and a TWIN-90 filter unit (MILLIPORE CORPORATION, Bedford, Massachusetts) or equivalent. The American Society for Testing and Materials (ASTM) specifies four different grades of water for use in methods of chemical analysis and of physical testing. The ASTM Type I grade water is, by definition, prepared by the distillation of feed water having a maximum conductivity of 20 µmho/cm at 25°C followed by polishing with a mixed bed of ion-exchange materials and a 0.2-µm membrane filter. At AIHL, this water is obtained with a MILLIPORE MILLI-Q® water purification system.

- pH meter, CORNING Model 10 or equivalent.

- Standard buffer solutions for pH meter calibration, pHydrion Buffers (MICRO ESSENTIAL LABORATORY, Brooklyn, New York) or equivalent.

- Rotary evaporator, BUCHI Rotavapor R110 or equivalent.

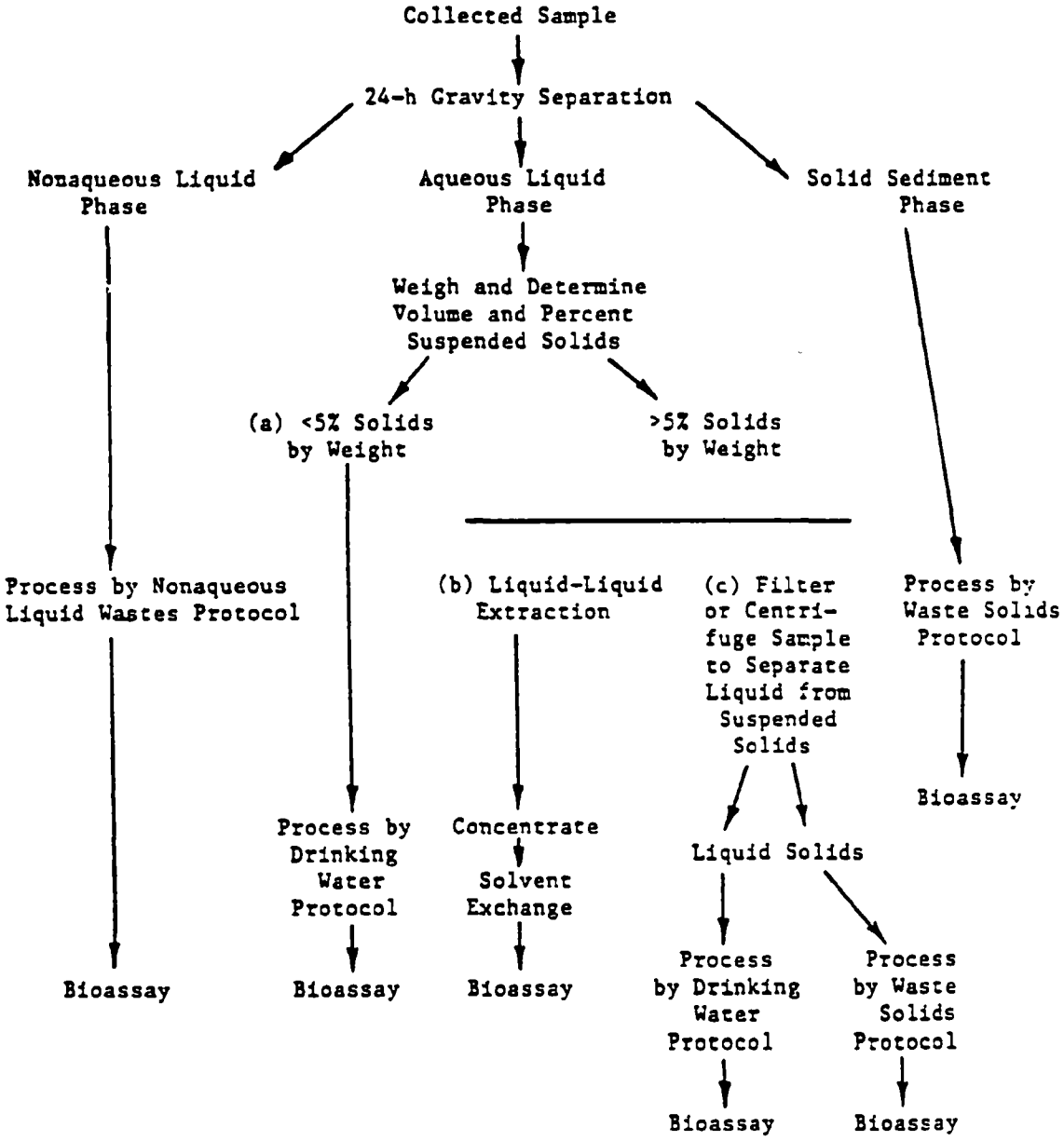
- Reference mutagen standards. In this project, the chemicals were provided by the EPA Chemical Repository through Dr. Llewellyn R. Williams, EPA, LV.

- The equipment and materials required for the Ames bioassay (Ames et al., 1975; Maron and Ames, 1983) were applied as specified in the EPA interim procedures (Williams and Preston, 1983).

- Reagents used for preparing the acetate buffer for TCLP extraction were all ACS (American Chemical Society) reagent grade: anhydrous sodium acetate, glacial acetic acid, and sodium hydroxide.

CONSENSUS PROTOCOL FOR ENVIRONMENTAL WATERS AND WASTEWATER

The consensus wastewater sample preparation protocol (See Appendix A) being validated in this project is summarized in the flow chart as shown on Figure 1. Wastewater samples may contain solids, nonaqueous liquids, and aqueous liquids. Each sample container should be stored in the dark, motionless at 4°C for a minimum of 24 hours after receipt. After the settling period, different phases are separated



Note: If results of (b) are negative (not mutagenic), it is recommended that the sample be subjected to (a) or (c) processing and that it be retested in the bioassay.

Figure 1. Wastewater sample processing flow diagram.

according to gravity. Any nonaqueous liquid phases identified in the liquid component of the sample are separated from the containers, are combined into one sample, and are processed as a nonaqueous liquid waste. Any solid sediment on the bottom of the sample container is collected, is combined into a single sample, and is processed as a solid waste. Each liquid or solid phase recovered must be weighed, and the volume must be determined before further processing. Like phases are combined into a common vessel for processing. Once combined, total weights and volumes are calculated. Storage conditions are the same as defined for the initial field sample.

The aqueous liquid phase recovered from the sample is processed by one of the following methods:

a. If the sample has < 5% suspended solids by weight (EPA Method 160.2, in USEPA, 1979b), the sample may be extracted and concentrated by the techniques described in the Drinking Water Protocol (XAD resin chromatography, see Appendix B for a complete copy) with the addition of a celite prefilter column to the concentrator apparatus.

b. If the sample has > 5% suspended solids, it may be processed by a liquid-liquid extraction method using a 3 L sample. If the bioassay result of the sample recovered from this method is negative, consideration should be given to processing a retained liquid phase (10 L) by XAD resin chromatography as described in the Drinking Water Protocol.

c. As an alternative to b., if the sample has > 5% suspended solids by weight, the sample may be further separated by high-pressure filtration or by high-speed centrifugation into liquid (< 5% suspended solids) and solids. These two phases can be processed further by the Drinking Water Protocol and by the Waste Solids Protocol, respectively.

SECTION 5

EXPERIMENTAL PROCEDURES - FIELD AND LABORATORY APPLICATIONS

The wastewater protocol validation process involves three aspects. First, the applicability of the protocol for providing sample extracts suitable for the Ames assay system is evaluated using various generic types of wastewater samples. Second, the adequacy of the protocol for extracting compounds representative of the original sample is studied by determining the extraction efficiency of SPRM's. Third, the validity of the protocol in avoiding the artificial generation of mutagens is established by developing the method background levels with blank water controls and by comparing these with the solvent and spontaneous mutation background in the Ames test.

SIX GENERIC SAMPLE TYPES

Since environmental water and wastewater may contain samples with a wide range of chemical constituents with different reactivities and extraction efficiencies, samples were collected which included most generic types of wastewater. The purpose is to prove that the protocol provides suitable extracts for the Ames testing from various types of wastewater samples.

Six types of wastewater samples were collected. These samples were the following:

- effluent from a municipal wastewater treatment plant;
- effluent from an industrial wastewater treatment plant;
- surface runoff from a hazardous waste landfill;
- aqueous leachate from solid waste extraction procedures;
- brackish estaurine surface water receiving industrial effluents and areal runoff;
- contaminated groundwater.

In collaboration with the California State Water Quality Control Board (CWQCB), San Francisco Regional Board, the following three samples were obtained:

1) Effluent from a municipal wastewater treatment plant (Sample No. AIHL-85-0403). The municipal wastewater was treated by chemical, physical, or biological means prior to discharge. Three stages of treatments, primary, secondary, and tertiary, were used at the municipal treatment plant. The wastewater was characteristic of that from an urban community. Various types of industrial discharges enter the system including those from some small businesses and light industries such as painting, paper decorating, roofing, sheet metal work, commercial printing, electroplating, metal finishing, machinery, electronic, gasoline service stations, auto repair shops, disinfecting and exterminating services, medical laboratories, etc. This sample may also contain products from cooking greases, household chemicals such as cleaners, detergents, auto/furniture polishes, drain openers, antifreeze, paints or thinners, wood preservatives, pesticides, herbicides, pool chemicals, motor oil, radiator flush, photographic chemicals, disinfectants, hair spray, lighter fluid, leather conditioner, spot remover, windshield cleaner, solvents, etc., and human and domestic wastes (Hathaway, 1980).

2) Effluent from an industrial wastewater treatment plant (Sample No. AIHL-85-0406). Industrial wastewaters vary significantly in pollutant characteristics according to the source of industry types, e.g., the industries in the neighborhood of Berkeley and the San Francisco Bay Area include large varieties of petroleum refineries, and chemical, pharmaceutical, agricultural, electronic, and genetic engineering firms. Thus, chemical composition of each industrial wastewater sample can be very different. The sample we evaluated was from a plant mainly treating wastewater from a refinery. The petroleum industry uses water for upgrading and refining crude oil. An average of 200 L of water is required to refine each 42 gallon barrel of crude oil (Metcalfe et al., 1985). The used water is then treated in an industrial wastewater treatment plant. Therefore, the effluent sample probably contains residues from the abovementioned processes (CDHS, 1983).

3) Surface runoff from a landfill (Sample No. AIHL-85-0405). This sample was collected from a class I hazardous waste disposal facility. The landfill is a disposal facility where hazarous waste is placed in or on the land; additionally, the landfill does not have a land treatment facility, surface impoundment, or

an injection well. By regulation, class I dump sites must provide complete protection for the quality of ground and surface waters from all wastes deposited therein and against hazard to public health and wildlife resources.

Wastes disposed of in a class I landfill contain toxic substances which could significantly impair the quality of usable waters. Examples include but are not limited to the following:

a) wastes of municipal origin, such as saline fluids from water or waste treatment and reclamation processes, community incinerator ashes, and toxic chemical toilet wastes;

b) wastes of industrial origin, such as brines from food processing, oil well production, water treatment, industrial processes, and geothermal plants; process ashes, chemical mixtures, mine tailings from which toxic materials can leach; and rotary drilling muds;

c) wastes of agricultural origin, such as pesticides, fertilizers, and discarded containers of chemicals;

d) other toxic wastes such as compounds of arsenic or mercury or chemical warfare agents (Franks, 1981).

Two samples were collected by AIHL staff:

1) Aqueous fraction from a solid waste extraction procedure. A Toxicity Characteristics Leachate Procedure (TCLP) was performed on a National Bureau of Standards (NBS) reference sludge sample. The aqueous extract of the TCLP procedure is used as a simulated landfill leachate wastewater sample (Sample No. AIHL-85-0401). TCLP is one of the solid waste extraction procedures developed under the Resource Conservation and Recovery Act (RCRA). The NBS sludge sample was provided by Dr. Llewellyn R. Williams, USEPA, Las Vegas, Nevada.

The TCLP is similar to the Extraction Procedure (EP, 40 CFR 261.24, specified in RCRA Section 3001) developed by the Office of Solid Wastes, EPA (USEPA, SW-846, 1982). EP toxicity is one of the four characteristics established by EPA for identifying hazardous waste (40 CFR 261.20). To emplement the EP toxicity characteristic, EPA developed a mandatory testing procedure which extracts the toxic constituents from a solid waste in a manner believed to simulate the leaching action that occurs in landfills. The EP toxicity testing protocol subjects a representative sample of a solid waste to an acidic leaching medium.

Like the EP, the TCLP is designed to simulate the leaching a waste will undergo if disposed of in a sanitary landfill. The following procedures (according to a draft of TKO703, revised on 3/2/85) were used in this project. (a) The liquid phase of the NBS sludge sample (16 bottles, labeled as: EPA/NBS Reference Sludge, RCRA EP-Inorganics, September 1981) was separated by centrifugation and filtration (MILLIPORE membrane filters, 0.45 μm pore size) from the solid phase and was stored in a refrigerator for later analysis. (b) The wet solid phase was weighed. If necessary, the particle size of the solids can be reduced by crushing or by grinding. (c) The solids were mixed with acetate buffer (0.1N, pH 5). Originally, the buffer was prepared according to the following formula by EG&G PRINCETON APPLIED RESEARCH: Dissolve 8.2 g of anhydrous sodium acetate in 800 mL deionized water and adjust to pH 5 with glacial acetic acid; then dilute to 1 L with deionized water. We replaced the deionized water with ASTM Type I water which is a better grade. (See Section 6.1.1.6, EPA/NBS Reference Sludge - TCLP Leachate, on discussions of problems encountered by using this formula). The volume of the buffer used equals 20 times the weight of the solids. (d) The extraction was performed at room temperature using a shaker table for 18 hours. (e) The sample was then centrifuged and filtered through a MILLIPORE membrane filter (0.45 μm pore size) using a sinter glass filter holder (See Section 6.1.1.6 for details of problems). (f) The liquid phase was combined with the original liquids from the sludge sample and served as the TCLP extract leachate. Freshly prepared TCLP leachate was used for each liquid-liquid extraction evaluation experiment.

2) Brackish surface water receiving industrial effluents and areal runoff. Two manual grab samples were collected as representatives of this type. One was a San Francisco Bay surface water collected at the Berkeley Marina (Sample No. AIHL-85-0404). Another was collected at the discharge site in the San Francisco Bay of an industrial wastewater treatment plant (Sample No. AIHL-85-044A). A boat was used to get access to the site. Each sample was collected from a pristine environment on a sunny autumn day in the morning.

San Francisco Bay is located at the mouth of the Sacramento-San Joaquin river system, a major estuary greatly modified by human activity. In 1978, wastes from more than 30 municipal and 40 industrial waste treatment facilities and from an additional 100 smaller industrial dischargers entered the bay at nearly 4 percent of the average annual freshwater inflow. Untreated urban runoff also enters the bay through more than 50 small local streams. Additional contamination results from daily accidental spills of industrial chemicals and oil (Nichols et al., 1986).

Two contaminated groundwater samples were obtained under the auspices of the Toxic Substances Control Division, CDHS, and the Hazardous Materials Laboratory (HML), CDHS. Two samples from the Stringfellow Quarry Hazardous Waste Disposal Site near Riverside, California, were sent to AIHL by the on-site contractor, Science Applications International Corporation (SAIC). One sample (Sample No. AIHL-85-0402) was collected from an on-site (OW-2) well. Another (Sample No. AIHL-85-042A) was from an upstream well (UGB-8).

The Stringfellow industrial waste disposal facility, situated in the Pyrite Canyon, is located in Riverside County, approximately five miles northwest of the city of Riverside. It is one of the most notorious uncontrolled hazardous waste sites in the nation (Ember, 1985). During the operation period from August 21, 1956, until voluntary closure in 1972, the site accepted about 34 million gallons of industrial, agricultural, and Defense Department wastes. Companies with products such as chemicals, solvents, pesticides, aircraft, steel, aluminum and other heavy metals,

and insulation materials dumped mixtures such as acid wastes, pesticides, chromate wastes, paint sludge and thinner, water softening brine, caustic soda, cyanides, starch sump residuals, etc., into surface ponds (Hatayama et al., 1979). The groundwaters in the neighborhood area were originally used for irrigation and for other domestic and industrial purposes. Analysis of the groundwater from nearby wells prior to establishment of the site indicated quality ranging from excellent to good for the beneficial uses. However, groundwater was contaminated as the result of contact with the wastes and the contaminated soils. The contaminated groundwater seeped through the fractured bedrock, and escaped the site.

CDHS, with the concurrence of EPA, has contracted with SAIC of La Jolla, California, to perform a remedial investigation/feasibility study on the site. SAIC collected two samples for AIHL to be used in this protocol evaluation study. One contaminated groundwater sample was collected from an on-site extraction well, OW-2, in Pyrite Canyon. Another was collected from an upgradient extraction well, UGB-8, outside the north boundary of the site. Previous chemical analysis results indicate that OW-2 is one of the most contaminated wells and that UGB-8 contains much lower concentrations of contaminants. The extraction wells are equipped with submersible pumps and are operated routinely. In all cases, wells were purged prior to sampling to ensure collection of representative samples. Sampling wells were purged until constant readings were obtained for pH, for electrical conductivity, for redox, and for temperature. After purging for approximately 20 gallons of discharge water, the sample was collected from the sampling spigot on the well-head. After collection, sample containers with appropriate labels were then inspected, were chain-of-custody sealed, and were stored in ice chests for transport (Shokes, 1984). The samples were shipped at ambient temperature to AIHL by Federal Express.

SAMPLE COLLECTION, LOG-IN, AND STORAGE

It is recommended that samples be taken which reflect the "normal" state of the sample site. Periodic sampling of the same site over several months may also be valuable in providing information on peak periods of activity (deVera et al., 1980). For aqueous samples, the most common sampling procedure is a manual grab collection of the volume needed for analysis. Amber glass bottles (1 gallon capacity) were most useful for handling, shipping, storage and processing. These bottles were rinsed

with ASTM Type I water, pesticide-grade methanol, hexane, and finally dichloromethane before use. Bottles were air-dried and were capped using the original bottle cap lined with a Teflon insert. All bottles were clearly labeled with the date and time of sampling, the site, and the field manager's name and telephone number if available. Once collected, samples were sealed in the amber glass bottles and were held at $4^{o}C$ during storage. The head space in the container was reduced by completely filling the container with the sample or by replacement of air with a nitrogen blanket. Table 2 lists the volume of sample required. At the time of collection, a sample collection record form was filled out by the field manager including any pertinent information relating to sample collection procedures, sample type and appearance, any necessary treatments, and storage and shipment methods. All of the completed forms obtained in this project are attached in Appendix C. A copy of the form accompanied the sample through all phases of the processing and the bioassay to insure the chain-of-custody.

Shipping and storage of the sample was planned so as to minimize the length of time from collection to processing. Shipping and storage at $4^{o}C$ is recommended, and protection from light sources is mandatory. It was originally recommended that all samples be processed (separated, extracted, and concentrated) within 14 days after collection. However, our results suggest that mutagens in some samples may decay during storage. Thus, it is recommended that the storage time should be reduced to the minimum time possible, e.g., the sample should be extracted 24 hours after receipt if possible. The 24-hour period is needed for gravity separation of solid and liquid phases. It is also recommended that samples be completely analyzed within 40 days of processing (See Section 6.1.1.7, Possible Changes in Sample Composition, for discussions).

PHASE SEPARATION

Each sample container was stored in the dark, motionless at $4^{o}C$ for 24 hours after receipt. During storage, a gravity separation of phases occurred. All the samples we collected so far do not contain any nonaqueous liquid phase and contain either no or less than 5% suspended particles without solid sediments at the bottom of the container. Therefore, the protocol evaluation effort has been concentrated on the liquid-liquid extraction process.

TABLE 2. RECOMMENDED VOLUMES AND STORAGE AND SAMPLES

Component	Volume or Weight	Storage Conditions
Collected Sample	Minimum of 30 L	4°C, dark
Gravity Separation Method	Minimum of 30 L	4°C, dark motionless
Sample for XAD-Resin Column Concentration	10 L	4°C, dark glass or Teflon-lined vessel
Liquid-Liquid Extraction Method	3 L	4°C, dark glass or Teflon-lined vessel
Solids for Solid Waste Extraction	500 g net weight	4°C, dark closed container
Extracted/Concentrated/ Solvent Exchanged Sample for Bioassay	10 mL	4°C, amber glass vial with Teflon cap liner

The XAD method as described in the Drinking Water Protocol (Appendix B) requires a 10 L sample and a set of expensive stainless steel columns. It is more time-consuming to process when compared to the liquid-liquid extraction method. In addition, the XAD resin requires extensive multi-steps of pre-cleaning. In principal, the XAD method can concentrate larger samples; and it may provide greater concentration than liquid-liquid extraction. However, as recommended in Section 3.1.1, XAD-Resin Method, further studies are needed to understand several problems with the XAD method. On the other hand, the liquid-liquid extraction procedure requires only a 3 L sample, is less expensive to set up, and takes less time to perform. Because of these advantages, the liquid-liquid extraction method was evaluated first with six generic types of wastewater samples.

Samples were processed based on the procedures indicated in the flow chart on Figure 2. The sample was separated into several aliquots. One aliquot of the sample was prepared according to the liquid-liquid extraction protocol. If the result from the Ames assay was positive, another aliquot of the sample was extracted using the identical procedures. The replicate, or triplicate when possible, results were compared for precision expressed by SD. A sample with relatively stable mutagenic activity was used as the base material for spike mutagen recovery studies. Field blanks and reagent blanks were evaluated to establish the background of preparation methods.

If the result of the first extraction and mutagen screening experiment was negative or equivocal, modifications of the extraction or mutagenicity testing procedures were made for the second experiment. If the results of the follow-up experiments were negative, another aliquot could be evaluated using the XAD-resin concentration protocol.

The liquid-liquid extraction methods for the processing of wastewater samples for the application to the Ames assay are described below essentially as they appear in the consensus protocol (Appendix A). Some minor revisions and modifications of the liquid-liquid extraction procedure have been suggested and have been validated. These changes are described in Section 6.1, Wastewater Protocol Evaluation, as part of the protocol validation.

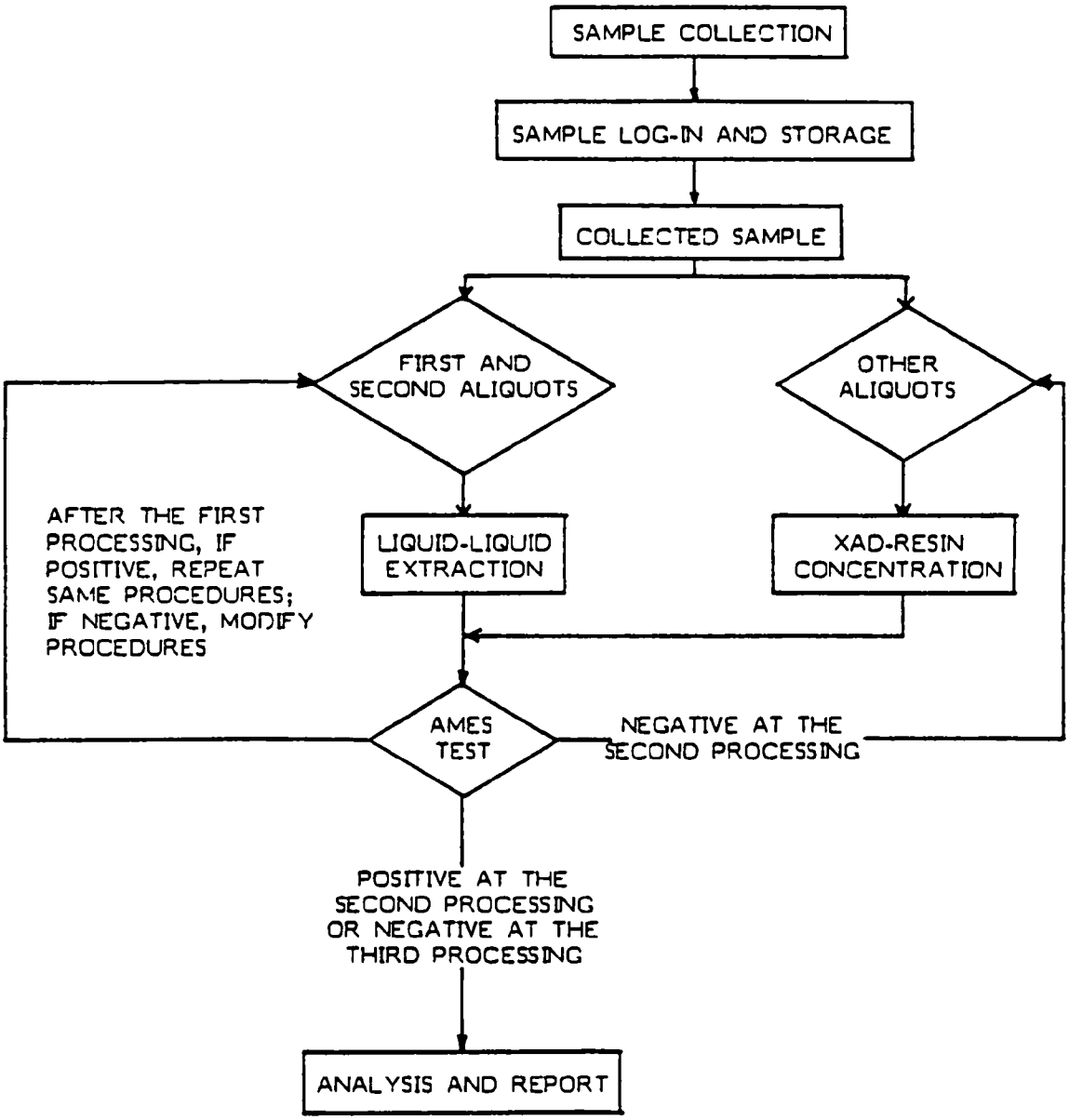


Figure 2. Sample processing scheme.

LIQUID-LIQUID EXTRACTION

Processing of wastewater samples by liquid-liquid extraction (See Figure 3) was carried out in a chemical fume hood and under yellow lights. Appropriate safety precautions as listed on the EPA-consensus protocol were complied with carefully. All processing was performed at room temperature. Two 1500 mL aliquots were measured from the aqueous sample. The sample was transferred into two 2000 mL glass beakers with each containing a 2" Teflon coated stir bar. The contents of both beakers were equilibrated to room temperature (approximately $20^{o}C$ to $25^{o}C$). The initial pH of the sample was determined using a pH meter with stirring. The pH of the water was adjusted to 11 with 5N NaOH. The aqueous phase was then transferred to two 2000 mL separatory funnels for extraction.

One hundred and fifty mL of dichloromethane was added to each separatory funnel after rinsing the beakers with the solvent. The basified sample was extracted by shaking the funnels for two minutes with periodic venting to release pressure. The organic layers were allowed to separate from the water phases for a minimum of ten minutes. The dichloromethane extracts were combined in a 1000 mL Erlenmeyer flask. The flask was kept in a hood. One hundred mL of dichloromethane was added to each separatory funnel, and the extraction procedure was repeated a second time. All extracts were combined in the 1000-mL Erlenmeyer flask. A third 100 mL extraction was performed in the same manner. The pH of the aqueous phase was adjusted to less than 2 using a diluted (1:1) sulfuric acid solution. The acidified sample was serially extracted with 150, 100, and 100 mL of dichloromethane per separatory funnel. All the extracts were combined in the 1000-mL Erlenmeyer flask. Notice that additional fractionation could be achieved at this stage by not combining these extracts (organic acids) with previous extracts (organic base/neutrals) but, rather, by processing each separately through the remaining procedure. Separate processing of acid and base/neutral fractions may be required with some samples.

A Kudema-Danish (K-D) concentrator was assembled by attaching a 25-mL concentrator tube to a 500-mL evaporative flask. Other concentration devices such as a rotary evaporator may be used in place of the K-D (See Section 6.1.2.4, Replace K-D Apparatus with Rotary Evaporator, for discussions). The combined extract was poured through a drying column containing about 10 cm of anhydrous sodium sulfate

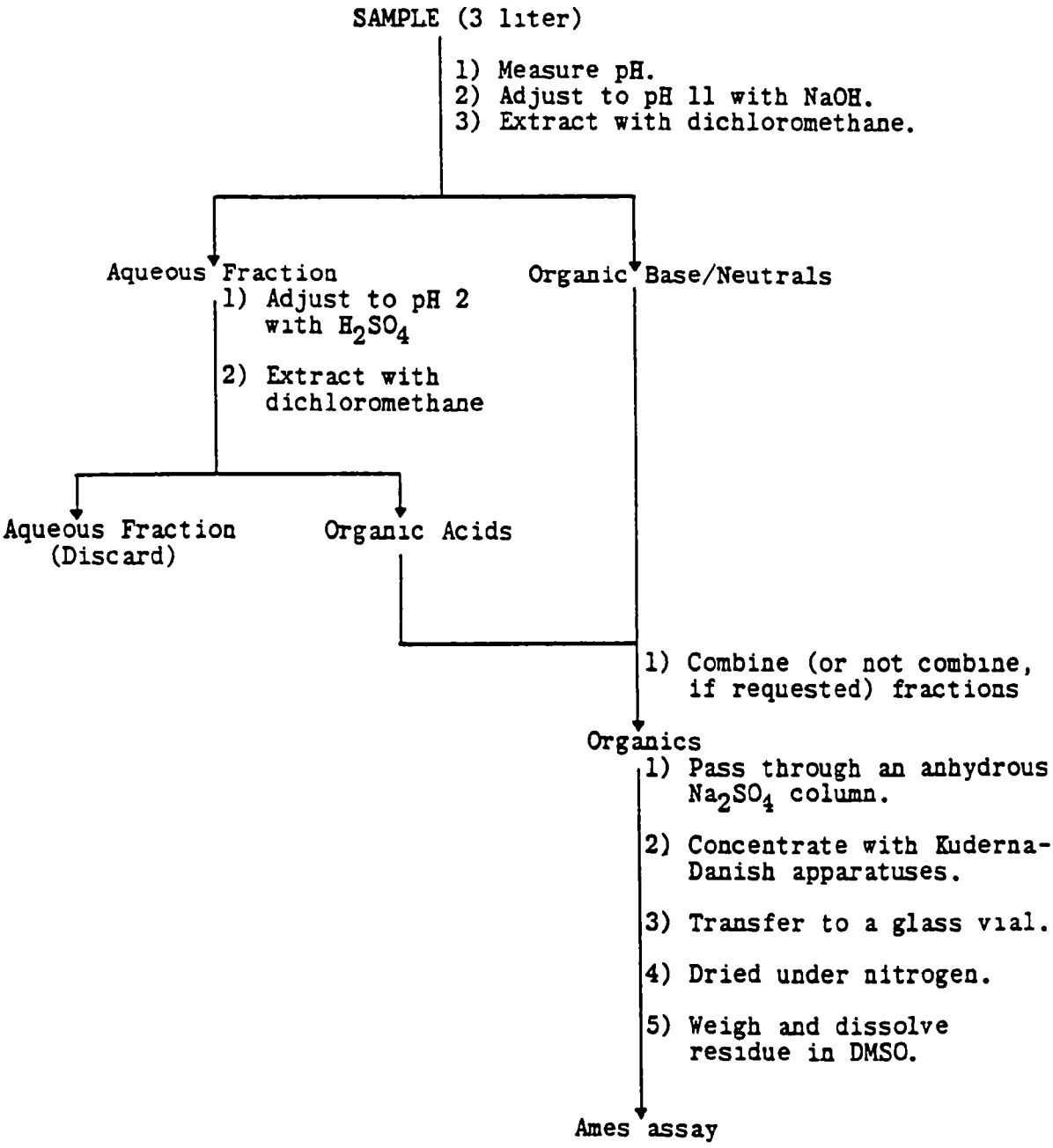


Figure 3. Liquid-liquid extraction scheme.

on top of 2 cm glass wool, and the extract was collected in the K-D concentrator. One or two clean Teflon-coated boiling chips were added, and a three-ball Snyder column was attached. The Snyder column was primed by adding about 1 mL of dichloromethane to the top of the column. The K-D apparatus was placed on a hot water bath (60°C to 70°C) so that the concentrator tube was partially immersed in the hot water, and the entire lower rounded surface of the flask was bathed with hot vapor. A 2-L beaker was a suitable water bath. In addition, the lower 500 mL evaporator flask was covered with aluminum foil forming a tent over the beaker water bath. In this way, the steam formed from the water bath will bathe the flask more efficiently. At the proper rate of distillation, the balls of the column will actively chatter, but the chambers will not flood with condensed solvent.

The Erlenmeyer flask and the sodium sulfate column were rinsed with 25 mL of dichloromethane to complete the quantitative transfer. Concentration on the water bath was continued until the liquid reached less than 10 mL. The K-D apparatus was removed from the water bath and was allowed to drain and to cool for at least 10 minutes. The Snyder column was removed, and the flask with its lower joint into the concentrator tube was rinsed with 1 to 2 mL of dichloromethane. The K-D apparatus was adequate for evaporation purposes. However, dichloromethane has been reported as an animal carcinogen and should be used with extra caution. Alternatively, the rotary evaporator technique is recommended so the potential carcinogenic solvent can be collected and can be disposed of properly.

The extract was transferred to a tared, 3-dram glass vial, and the remaining dichloromethane was evaporated under nitrogen using a heating block or water bath to prevent sample cooling. The temperature was not allowed to exceed 35°C. The vial and its contents was weighed, and the weight of the residue was recorded. If requested, the residue can be dissolved in 10 mL dichloromethane, and an aliquot can be removed for total organic carbon analysis (American Public Health Association et al., 1985), and an 1 mL aliquot can be analyzed by gas chromatography/mass spectrometry using EPA Method 625 (44 CFR 233, December 3, 1979; Longbottom and Lichtenberg, 1982).

The dried residue was stored in the dark at -20°C until application to the Ames assay. If no aliquot was removed for analysis by gas chromatography or for

total organic carbon analysis, resuspension of the residue in a 1.0 mL vehicle (e.g., DMSO for Ames assay) represents a concentration factor of 3000-fold.

AMES ASSAY

Assay Scheme

As shown in Figure 4, a strategic Ames assay scheme was developed (Zeiger et al., 1985b) for optimum cost- and effort-effectivness. All fractions were tested for mutagenic activity using Salmonella typhimurium strains TA98 and TA100 in the standard Ames plate incorporation assay. Due to limited sample size, only these two strains were selected for screening the complex wastewater samples. All samples were initially assayed in the presence and the absence of an exogenous metabolic activation system (2% and 10% S-9 fraction in the S-9 mix) derived from the livers of Aroclor 1254-induced Sprague-Dawley rats (purchased from LITTON BIONETICS, Kensington, Maryland). The protein concentration of the S-9 used in this project was 25 mg/mL as determined by the method of Lowry et al. (1951). All samples were evaluated by using a minimum of three dose levels at half-log intervals and by using triplicate or duplicate plates per dose whenever possible. Doses as high as 6 mg extract/plate were used when the sample quantity was sufficient.

If a positive response was seen in the initial screening test, only the strain giving the greatest response was used in the repeat assay with the same level of S-9 fraction. If a questionable mutagenic response was observed only in the presence of metabolic activation (2% or 10% S-9 mix), the strain(s) producing this response was retested using the same level of S-9 with higher nontoxic doses or with 30% S-9 mix. The dose ranges selected for the repeat test were based on the results obtained in the original test. If a questionable positive response was observed only in the absence of the metabolic activation system, the strain(s) producing this response was retested without S-9 by using an adjusted dose range. If a negative and toxic response was seen with and without the metabolic activation systems, the test was repeated by using 30% S-9 mix. If a negative and nontoxic response was seen at lower doses, the test was repeated with increasing doses up to 6 mg extract/plate both in the presence and in the absence of 10% S-9 mix.

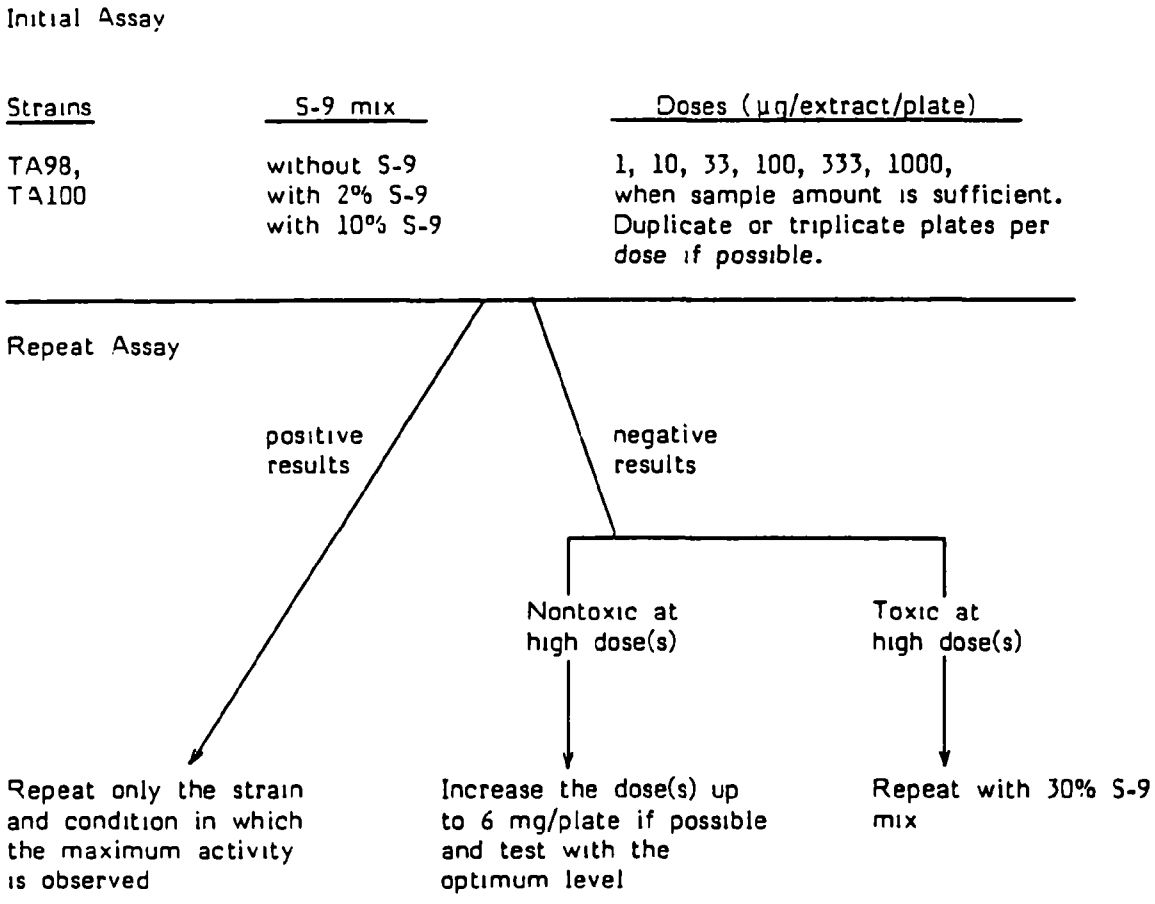


Figure 4. Ames assay scheme.

QC of the Ames Assay

The Salmonella typhimurium strains used are all histidine auxotrophs by virtue of mutations in the histidine operon. When these histidine-dependent cells are grown on minimal medium agar plates containing a trace of histidine, only those cells that revert to histidine independence (his$^+$) are able to form colonies. The small amount of histidine allows all the plated bacteria to undergo a few divisions; and this growth is essential for mutagenesis to occur. The his$^+$ revertants are easily visible as colonies against the slight background growth. The spontaneous mutation frequency of each strain is relatively constant (McCann et al., 1975a), but when a mutagen is added to the agar, the mutation frequency is increased usually in a dose-related manner.

The tester strains were obtained from Dr. Bruce N. Ames of the University of California at Berkeley (Ames et al., 1975). In addition to having mutations in the histidine operon, all the indicator strains have a mutation (rfa) that leads to a defective lipopolysaccharide coat; they also have a deletion that covers genes involved in the synthesis of the vitamin biotin (bio) and in the repair of ultraviolet (uv)-induced DNA damage (uvrB). The rfa mutation makes the strains more permeable to many large molecules, thereby increasing the mutagenic effect of these molecules. The uvrB mutation renders the bacteria unable to use the accurate excision repair mechanism.

Strains TA100 and TA98 were used in this project because of their high sensitivities for mutagens in complex environmental mixtures. Strain TA100 is derived from TA1535 by the introduction of the resistance transfer factor, plasmid pKM101. TA1535, carrying the base-pair substitution mutation at the hisG46 locus, is reverted to his$^+$ by many mutagens that cause base-pair substitutions. The plasmid pKM101 is believed to cause an increase in error-prone DNA repair that leads to many more mutations for a given dose of most mutagens (McCann et al., 1975b; Mortelmans and Stocker, 1979). In addition, the plasmid confers resistance to the antibiotic ampicillin. This antibiotic-resistant capability is a convenient marker to detect the presence of the plasmid in the cells. TA100 can detect mutagens such as benzyl chloride and 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (AF-2) that are not detected by TA1535. The presence of this plasmid also makes strain TA100 sensitive to some

frameshift mutagens, e.g., ICR-191, benzo(a)pyrene, aflatoxin B_1, and 7,12-dimethylbenz(a)anthracene. Strain TA98 is derived from strain TA1538 by the addition of plasmid pKM101. TA1538 carries a frameshift mutation at the hisD3052 locus.

All indicator strains are kept frozen in broth supplemented with 10% sterile glycerol or DMSO at -80°C in 1-mL aliquots containing about 10^9 cells. New frozen stock cultures are made approximately every six months from single colony isolates that have been checked for their genotypic characteristics (his, rfa, uvrB, bio) and for the presence of the plasmid.

Some variation has been found in the extent of growth of bacterial strains in overnight nutrient broth cultures. This seems to result from variability in the nutritional quality of the medium. There seem to be marked differences not only between media from different sources but also among batches from a single source. Overnight cultures which have just reached a density of (1-2) x 10^9 viable cells per mL are considered most desirable for mutagen testing.

For each experiment, the 1-mL frozen aliquots of the strain were allowed to thaw at room temperature before inoculation into 50 mL of Oxoid nutrient broth liquid medium. The cultures were grown at 37°C, were unshaken for up to 4 hours, and then were gently shaken (100-120 rpm) for approximately 12 hours. Inocula were taken directly from nutrient broth cultures. A fresh cell suspension was used for each day's experiments. Cell suspensions were maintained through the day at ice-bath temperature since storage at room temperature may result in loss of viability or mutagen sensitivity or both (CDHS, 1979; CDHS, 1985). For QC purposes, cell titer and viability was measured for each experiment (See Appendix F for data).

To ensure the validity of the test, the strain genotype function tests were performed for each experiment. The procedures outlined in the methods paper (Ames et al., 1975) are satisfactory with regard to confirming histidine requirement, deep rough character, and ultraviolet sensitivity of tester strains. All strains were genetically analyzed whenever experiments were performed (See Appendix F for data). Tests for the presence of the R factor conferring ampicillin resistance in strains TA100 and TA98 were conducted conveniently by using commercially available

filter paper disks containing 10 µg ampicillin (Zeiger et al., 1981). Ampicillin-containing disks were placed in the center of petri dishes which were overlaid with each of the tester strains. Zones of inhibition should be observed with strains TA98 and TA100. As this procedure will not determine what fraction of the culture has lost the R factor, it is important to check stock cultures periodically to ensure that close to 100% of the bacteria contain the R factor. This can be done by replica plating (preferably with 100 or more colonies) onto ampicillin-free plates and plates containing 25 µg ampicillin per mL of medium (CDHS, 1985).

Triplicate plates of the following compound were tested as positive controls: 2-nitrofluorene (2NF) for TA98 without S-9, 2-aminofluorene for TA98 with 2%, 10% and 30% S-9 mix, sodium azide for TA100 without S-9, and 2AA for TA100 with 2% or 10% S-9 mix and for TA98 with 30% S-9 mix. The data are recorded on the result form in Appendix D and also are listed on the cell function test form in Appendix F. Four conditions of S-9 were used to cover various requirements for optimum enzyme activation of different chemicals in the complex mixture wastewater samples (Sugimura and Nagao, 1980).

Several negative controls including the spontaneous mutation control and the solvent control were performed for each experiment. The extract of the laboratory distilled water which is used as the field, travel, and sample preparation method blank was also tested. Laboratory QC charts were established for these controls for accuracy and precision measurements. Statistical analyses were performed to establish the method background (See Section 6.2.3, Statistical QC Analysis and Records, for details).

As with the extraction procedures, mutagenicity testing was carried out in a room fitted with yellow fluorescent lights to minimize potential photooxidation. All the residues and chemicals were processed in an approved chemical fume hood. Safety rules described in the EPA-consensus protocol were complied with carefully.

EXTRACTION EFFICIENCY

Extraction efficiencies for the recovery of mutagens from an environmental water sample by the liquid-liquid extraction protocol can be assessed by two methods:

(1) by chemical analysis such as high pressure liquid chromatography (HPLC) for identification and quantitation of chemical standards; (2) by the Ames assay for the recovery of mutagenicity. Both methods quantitate the level of the spiked mutagens recovered in the final extract residue.

Chemical Analysis by HPLC

Chemical SPRM standards were provided by Dr. Llewellyn R. Williams, EPA. They were separated, identified, and quantitated by reversed-phase HPLC using a Varian model 5000 liquid chromatograph. The method used requires three reservoirs, and, therefore, only liquid chromatographs with this capability can be used. Five chemical mutagens with diverse chemical properties have been separated. They were 4-nitroquinoline-N-oxide (4NQO), 2NF, B(a)P, 2AA, and 4NBA. However, only three mutagens, B(a)P, 2AA, and 4NBA, were used in the recovery studies (See Section 6.1.3, Mutagen Extraction Efficiency, for details) because of time and resource limitations. The recovery of other chemical mutagens will be evaluated in the future. This HPLC scheme provides a basic method for the separation of numerous chemical standards. The method reported is a general protocol to adequately separate and quantitate the recovery standards reported in the results section.

All five of these compounds can be separated on an Altex ultrasphere-ODS-C18 column or its equivalent. Peaks were identified and were quantitated with a Varian UV-50 multiple wavelength spectrophotometer and a Varian Fluorichrom fluorimeter with a deuterium lamp supply. Filters were set for an excitation band of 340-380 nm and an emission cutoff of 460 nm for B(a)P fluorescense detection. The gradient used is shown in Table 3, and the flow rate is 1.5 mL/min. A sample chromatogram is shown in Figure 5.

Mutagenicity Recovery Analysis

Three SPRM's were used in the mutagenicity recovery studies: B(a)P, a neutral chemical; 2AA, a basic chemical; and 4NBA, an acidic chemical. These chemicals were chosen because of the fact that the liquid-liquid extraction is a method for selectively extracting acidic, neutral, and basic compounds.

TABLE 3. HPLC GRADIENT CONDITIONS FOR THE SEPARATION OF SPIKED REFERENCE MUTAGENS

Step	Time (min)	% Reservoir A	% Reservoir B	% Reservoir C	
1	0 (Initial)	100%	-	0%	Injection
2	5	100%	-	0%	
3	50	0%	-	100%	
4	60	-	0%	100%	
5	65	-	100%	0%	
6	75	-	100%	0%	
7	77	-	90%	10%	
8	78*	100%	-	0%	End

Stepwise gradient is dependent on a three-reservoir, paired system in which reservoir A is 10% acetonitrile in 0.02M $(NH_4)_2HPO_4$ (ammonium carbonate can be substituted), reservoir B is distilled water, and reservoir C is acetonitrile. All solvents and buffers are HPLC grade and should be filtered before use. Reservoir A should be degassed by continuously purging with helium during the run.

*Run until equilibrated before subsequent injection.

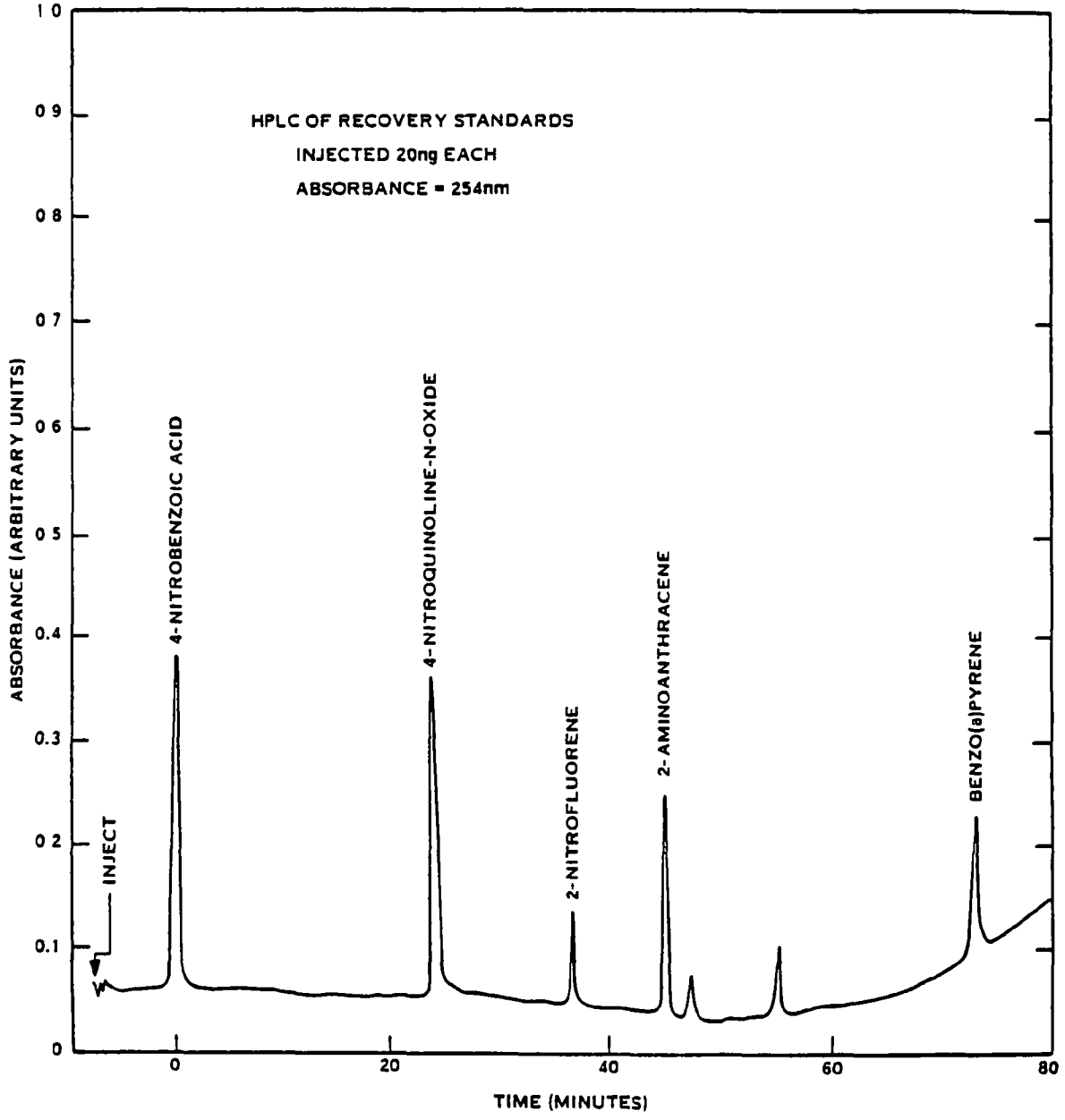


Figure 5. HPLC chromatograph of spiked reference mutagens.

Prior to extraction, a 1.5 L aliquot of water was spiked with a known quantity (0.25 µg-5 mg range) of one of the three chemical standards. The water was then processed essentially by following the procedures described in Section 5.4, Liquid-Liquid Extraction, and the modifications, when applicable, recommended in Section 6.1.2, Liquid-Liquid Extraction Results and Discussions. The doses (mg/plate) used in the Ames assay of the recovery study (See Appendix E for primary data) were estimated mg of the SPRM in the extract. The estimated doses were calculated from the original spiked dose and were based on the assumption of 100% recovery of the SPRM.

The percentage of recovery of the spiked mutagen was calculated by HPLC peak analysis of both a known standard and the unknown residue. When sufficient material was available, the mutagenicity recovery was also calculated from a dose-response curve of the pure chemical standard under the same conditions as the residue in the Ames assay. The conditions used in these studies for B(a)P and 2AA were TA98 with 2% and 10% S-9 mix, and for 4NBA, TA100 without S-9.

SECTION 6

RESULTS AND DISCUSSIONS

WASTEWATER PROTOCOL EVALUATION

Wastewater samples were collected with the extracts prepared following the procedures in the EPA proposed sample preparation protocol, and the extracts were tested in the Ames/Salmonella assay. Each sample was accompanied by a sample collection record that documented the sampling procedures (See Appendix C). For all wastewater samples, the EPA-suggested preparation protocol was carried out at least once, and additional procedures were developed to accommodate individual samples as needed. These modifications are described below.

Ames Assay Results and Interpretation

As listed on Table 1 in Section 2, Conclusions, mutagenic activity is detected in all the wastewater samples using the liquid-liquid extraction method. The primary

data are attached as part of Appendix D. All the samples are more mutagenic in bacterial strain TA98 than in TA100. The optimum S-9 condition for each sample is also listed on Table 1. The mutagenic activity expressed as revertants per liter of wastewater sample is obtained as follows: First the ASTM Type I blank water is used to establish the method background. It serves as the field and the travel blank as well as the method blank. Since the extract of the water is dissolved in DMSO, the water blank also includes the solvent (DMSO) blank (See Section 6.2.3, Statistical QC Analyses and Records, for statistical evaluation and Appendix G for primary data). A moderately conservative detection limit of two-fold of the method background is then established (Ames et al., 1975; Dunkel and Chu, 1980; Margolin, 1985; Williams and Preston, 1983). Responses below the two-fold detection limit are not considered for further evaluation. Positive responses are strengthened by dose-response relationships (deSerres and Shelby, 1979). This is established by plotting plate counts (y-axis, in revertants/plate) as a function of extract residue weight (x-axis, in mg/plate). The method background in revertants/plate is used as the response of the zero dose. The dose-response curves for all samples are also graphed and are attached as part of Appendix D. The slope (**b**, as shown in the linear regression calculation on the graphs in units of revertants/mg) of the linear portion of the curve is used to estimate the optimum specific activity of the sample. Only when the regression coefficient (**r**, as shown the the graphs) is $\geq$ 0.9, the maximum initial slope is considered acceptable. The residue weight (mg/L of original water sample) is obtained and is recorded in the laboratory notebook. The final mutagenic activity (revertants/L) is calculated by multiplying the slope (revertants/mg) times the residue weight per liter.

The detection limit for the specific activity in units of revertants/mg is calculated using net revertants/plate: Two times the background, corrected for the response of the zero dose, is then divided by the maximum nontoxic dose tested. The method background, established as described in Section 6.2.3, Statistical QC Analysis and Records, was the water blank value. One exception was the TCLP leachate. For that sample, the sodium acetate buffer blank value was used as the zero value for the dose-response curve.

Sample characteristics observed upon receipt and several parameters measured during sample preparation processing were tabulated for comparison (Table 4). The

TABLE 4. WASTEWATER SAMPLE DESCRIPTION AND CHARACTERIZATION

Characteristics	WASTEWATER SAMPLE TYPES					
	Industrial Effluent	Municipal Effluent	Contaminated Groundwater	Landfill Runoff	Estaurine Brackish	TCLP Leachate
pH Range	7.1-7.5	6.2-6.5	3.2-3.5	7.2-7.6	7.7-8.0	adjust to pH 5.0
Color	Lt. Yellow	Lt. Yellow	Lt. Brown	Lt. Yellow	None	None
Odor	Petroleum	Sewage	Sulfur	None	Briny	None
Emulsion[1]	Light	Heavy	Light	Moderate	Moderate	Moderate
Precipitation during Processing[2]	+	-	+++	++	+	++
Residue Weight (mg/3 L) (Range)	48-158	9-17	40-129	11-31	2-7	5-6

1. Relative degree of emulsion formed between the aqueous and the organic phases during processing. The pH at which the emulsion occurred varied with each sample.

2. Relative severity of precipitation for each sample as the pH was adjusted to 11. Samples labeled as negative (-) did not precipitate as the pH was raised.

sample collection records contain more detailed information on the collection, appearance, and storage of the sample (See Appendix C).

Surface Runoff from a Class I Landfill--

As summarized in Table 5, the surface runoff sample was extracted according to the original protocol (base extraction first). It was active in TA98 but not in TA100. The optimum condition was without S-9. The presence of 2% or 10% S-9 mix decreased the mutagenic activity by approximately 25%. There was no toxicity in the initial screening experiment at doses up to 0.666 mg/plate. Some elevated plate counts were observed in TA100 in the absence and presence of 2% S-9 mix but were not above the detection limit. However, modification of the extraction scheme because of the precipitation problem (See Section 6.1.2.2, Samples Form Precipitates, for details) in two later experiments resulted in a response below two fold of the background. Toxicity was observed in the last experiment which was performed approximately one month after receipt of the sample. The toxicity and loss of activity may be due to changes in sample composition (See Section 6.1.1.7, Possible Changes in Sample Composition, for discussions) which can be caused by factors such as microbial degradation of mutagens, microbial formation of antagonists (Alexander, 1974), chemical interference or chemical degradation, or combination of these factors.

Brackish Surface Water Receiving Industrial Effluents--

The brackish San Francisco Bay surface water is an example of samples requiring 2% S-9 mix for maximum activity. In the initial screening experiment, the results of all six conditions tested were below the detection limit. However, dose-related elevated colony counts were exhibited. Especially in the case of TA98 with 2% S-9 mix, the result (90 revertants/plate) was almost equal to the detection limit (93 revertants/plate). This condition was therefore used in the follow-up experiments. As shown on Table 6, a comparison of either acid first or base first extraction method produced similar results. The San Francisco Bay receives local industrial (mainly refinery) effluents which may be a major source of pollutants. A brackish surface water sample (No. AIHL-85-0404) at the discharge site of an industrial wastewater treatment plant was collected (No. AIHL-85-044A) and was tested. Both acid first and base first procedures produced results similar to those of the previous Bay water sample (Table 7).

TABLE 5. MUTAGENICITY OF A SURFACE RUNOFF FROM A CLASS I LANDFILL, SAMPLE NO. AIHL-85-0405

A. Results of the Initial Screening Experiment

Exp. Date	Extraction Method[1]	Residue Weight (mg)	Sample Volume (L)	Specific Mutagenic Activity (revertants/mg)[2,3] TA98 -S9	TA98 2%S9	TA98 10%S9	TA100 -S9	TA100 2%S9	TA100 10%S9
9/24/85	Base/Acid	15	3	122	88	86	< 242	< 230	< 200

B. Comparison of Mutagenic Response in TA98 without S-9[4]

Exp. Date	Extraction Method[1]	Residue Weight (mg)	Sample Volume (L)	Specific Mutagenic Activity (revertants/mg)[2,3]	Mutagenic Response per Unit Volume (revertants/L)
9/24/85	Base/Acid	15	3	122	614
10/4/85	Acid/Base	31	3	< 11	< 112
10/17/85	Acid/Base	11	3	< 32[5]	< 114

1. The extraction method indicates the order of pH in liquid-liquid extraction.

2. The specific activity of the mutagenicity dose response curve is represented by the slope (b as shown in the statistical analysis on graphs in Appendix D).

3. The spontaneous mutation and the positive control values are listed on the Strain Function Tests, Cell Titer, and Viability Record in Appendix F.

4. The optimum testing condition in the initial screening experiment was in TA98 without S-9 mix.

5. No revertant colonies on all the testing plates, background lawn was normal except the plates at the highest dose.

TABLE 6. MUTAGENICITY OF A BRACKISH SAN FRANCISCO BAY SURFACE WATER, SAMPLE NO. AIHL-85-0404

A. Results of the Initial Screening Experiment

Exp. Date	Extraction Method[1]	Residue Weight (mg)	Sample Volume (L)	Specific Mutagenic Activity (revertants/mg)[2,3] TA98 -S9	TA98 2%S9	TA98 10%S9	TA100 -S9	TA100 2%S9	TA100 10%S9
9/24/85	Base/Acid	6.5	3	< 106	< 139	< 162	< 484	< 460	< 401

B. *Comparison of Extraction Methods for Mutagenic Response in TA98 with 2% S-9 Mix*[4]

Exp. Date	Extraction Method[1]	Residue Weight (mg)	Sample Volume (L)	Specific Mutagenic Activity (revertants/mg)[2,3]	Mutagenic Response per Unit Volume (revertants/L)
10/4/85	Acid/Base	4.8	3	177	283
12/17/85	Base/Acid	1.2	1.5	218	176

1. The extraction method indicates the order of pH in liquid-liquid extraction.

2. The specific activity of the mutagenicity dose response curve is represented by the slope (b as shown in the statistical analysis on graphs in Appendix D).

3. The spontaneous mutation and positive control values are listed on the Strain Function Tests, Cell Titer, and Viability Record in Appendix F.

4. Although results of all six conditions in the initial screening experiment were below the detection limit, TA98 with 2% S-9 mix produced a questionable positive response close to the detection limit. This condition was therefore used in the follow-up experiments.

TABLE 7. MUTAGENICITY OF A BRACKISH RECEIVING SURFACE WASTEWATER FROM THE DISCHARGE SITE OF AN INDUSTRIAL WASTEWATER TREATMENT PLANT, SAMPLE NO. AIHL-85-044A

Comparison of Extraction Methods for Mutagenic Response in TA98 with 2% S-9 Mix[4]

Exp. Date	Extraction Method[1]	Residue Weight (mg)	Sample Volume (L)	Specific Mutagenic Activity (revertants/mg)[2,3]	Mutagenic Response per Unit Volume (revertants/L)
11/20/85	Base/Acid	1.5	3	465	232
12/17/85	Acid/Base	0.9	1.5	414	240

1. The extraction method indicates the order of pH in liquid-liquid extraction.

2. The specific activity of the mutagenicity dose response curve is represented by the slope (**b** as shown in the statistical analysis on graphs in Appendix D).

3. The spontaneous mutation and positive control values are listed on the Strain Function Tests, Cell Titer, and Viability Record in Appendix F.

4. The optimum testing condition for the San Francisco Bay water was in TA98 with 2% S-9 mix. The same condition was applied to the brackish receiving wastewater for comparison purposes.

Municipal Wastewater Treatment Plant Effluent--

As summarized in Table 8, the municipal effluent wastewater sample was mutagenic only in TA98 with 10% S-9 mix in the initial screening experiment. There was no activity in TA100. No toxicity was observed at doses up to 1 mg/plate. Similar results were obtained in two follow-up experiments. No toxicity was exhibited at 2 mg/plate. Even though there was no precipitation at pH 11, the acid first extraction scheme (See Section 6.1.2.2, Samples Form Precipitates, for details) was compared with the original base first extraction because of the potential of the municipal sample for stability in mutagen compositions. Both methods gave similar mutagenic responses.

Industrial Wastewater Treatment Plant Effluent--

Several problems were encountered in the liquid-liquid extraction where the EPA-suggested protocol was used without modification (See Section 6.1.2, Liquid-Liquid Extraction, for discussions). Interestingly, the only sample residue that was equivocal in the initial experiments without and with 2% and 10% S-9 mix (Table 9) was the only sample that did not exhibit any technical problems when using the original protocol. This sample, an industrial wastewater treatment effluent, was processed at first within three days of receipt. The sample had a strong petroleum-like odor (Table 4). Elevated colony counts were observed but mutagenicity was not significantly greater than twice that of background levels. The final residue (approximately 50 mg/3 L) was dark red in color and possessed a strong mildew odor. Toxicity was observed at the dose of 2 mg/plate. TA98 with 10% S-9 mix produced response close to the detection limit and was considered as the best among the six conditions. The residues obtained from the industrial wastewater sample within seven days of receipt dissolved readily in DMSO. However, when this sample was processed either by base first or acid first extraction again 24 days after receipt, the amount of residue was significantly increased (approximately 120-160 mg/3 L). Furthermore, the residue would not completely dissolve and this made analysis by the Ames assay extremely difficult. After sonication, a fine particulate suspension of the residue was applied for the Ames testing. However, there were no visible precipitates on the plates after incubation. The results were again equivocal. Two more experiments were attempted approximately four months after receipt. Because of light precipitation and emulsion formation (See Section 6.1.2, Liquid-Liquid Extraction, for

TABLE 8. MUTAGENICITY OF A MUNICIPAL WASTEWATER TREATMENT PLANT EFFLUENT, SAMPLE NO. AIHL-85-0403

A. Results of the Initial Screening Experiment

Exp. Date	Extraction Method[1]	Residue Weight (mg)	Sample Volume (L)	Specific Mutagenic Activity (revertants/mg)[2,3] TA98 -S9	TA98 2%S9	TA98 10%S9	TA100 -S9	TA100 2%S9	TA100 10%S9
8/2/85	Base/Acid	17	3	< 33	< 43	88	< 166	< 165	< 145

B. Comparison of Extraction Methods for Mutagenic Response in TA98 with 10% S-9 Mix[4]

Exp. Date	Extraction Method[1]	Residue Weight (mg)	Sample Volume (L)	Specific Mutagenic Activity (revertants/mg)[2,3]	Mutagenic Response per Unit Volume (revertants/L)
8/2/85	Base/Acid	17	3	88	491
8/13/85	Base/Acid	13	3	75	325
10/17/85	Acid/Base	9	3	148	443

1. The extraction method indicates the order of pH in liquid-liquid extraction.
2. The specific activity of the mutagenicity dose response curve is represented by the slope (b as shown in the statistical analysis on graphs in Appendix D).
3. The spontaneous mutation and positive control values are listed on the Strain Function Tests, Cell Titer and Viability Record in Appendix F.
4. The optimum testing condition in the initial screening experiment was in TA98 with 10% S-9 mix.

TABLE 9. MUTAGENICITY OF AN INDUSTRIAL WASTEWATER TREATMENT PLANT EFFLUENT, SAMPLE NO. AIHL-85-0406

A. Results of the Initial Screening Experiment

Exp. Date	Extraction Method[1]	Residue Weight (mg)	Sample Volume (L)	Specific Mutagenic Activity (revertants/mg)[2,3] TA98 -S9	TA98 2%S9	TA98 10%S9	TA100 -S9	TA100 2%S9	TA100 10%S9
9/24/85	Acid/Base	48	3	< 35	< 46	< 54	< 161	< 153	< 134

B. Results of Follow-up Experiments in TA98 with 10% S-9 Mix[4]

Exp. Date	Extraction Method[1]	Residue Weight (mg)	Sample Volume (L)	Specific Mutagenic Activity (revertants/mg)[2,3]
10/4/85	Base/Acid	52	3	< 53
10/17/85	Base/Acid	62	1.5	< 38
10/17/85	Acid/Base	79	1.5	< 38

C. Results of Follow-up Experiments in TA98 with 30% S-9 Mix[5]

Exp. Date	Extraction Method[1]	Residue Weight (mg)	Sample Volume (L)	Specific Mutagenic Activity (revertants/mg)[2,3]	Mutagenic Response per Unit Volume (revertants/L)
2/11/86	Acid/Base	28	1.1	63	1585
2/28/86	Acid/Base	57	3	95	1810

1. The extraction method indicates the order of pH in liquid-liquid extraction.

2. The specific activity of the mutagenicity dose-response curve is represented by the slope (b as shown in the statistical analysis on graphs in Appendix D).

3. The spontaneous mutation and positive control values are listed on the Strain Function Tests, Cell Titer and Viability Record in Appendix F.

4. Although results of all six conditions in the initial screening experiment were below the detection limit, TA98 with 10% S-9 mix was the best condition in which the result was closest to the detection limit. This condition was therefore used in the follow-up experiments.

5. Modification of the S-9 condition (30% S-9 mix) was applied because of equivocal results obtained in experiments with 10% S-9 mix.

discussions), acid first extraction was applied. There were no solubility problems, and the residue weights were 76 and 57 mg/3 L, respectively. Increasing the S-9 concentration to 30% was a necessary modification for detecting the mutagenicity. Petroleum distillate extracts have been reported to be exhibiting significant mutagenic response only at S-9 concentrations over 20% (Carver et al., 1985). Our results may indicate the possible existence of similar chemicals in the industrial wastewater treatment plant effluent sample.

Contaminated Groundwater from the Stringfellow Hazardous Waste Disposal Facility--

The Stringfellow on-site contaminated groundwater sample (No. AIHL-85-0402) produced approximately 3600 revertants/L. Table 10 summarizes the results of the four experiments performed on this sample. The residue contains both direct- and indirect-acting mutagens. Dose-related elevated colony counts were observed in TA100, but the numbers were less than twice the background. TA98 with 2% S-9 mix was the optimum Ames testing condition. Toxicity was not observed at doses up to 3 mg/plate in the initial screening experiment.

The sample was fairly acidic (approximately pH 3.5). It precipitated heavily at pH 11 which caused extreme difficulty in the basic extraction process (See Section 6.1.2.2, Samples Form Precipitates, for discussions). A modification in which the acid extraction was applied first allowed easier separation between the organic and aqueous phases. Three experiments using the modified extraction procedures were performed. In the first follow-up experiment, most of the mutagenic activity was found in the acid with neutral (pH 2) fraction. However, a large portion of the mutagenicity was lost in comparison with the initial screening experiment in TA98 with 2% S-9 mix. Mutagenicity was also found in TA100.

In addition to the sample precipitation problem, this sample may have undergone changes in chemical composition during storage (Table 10B, see Section 6.1.1.7, Possible Changes in Sample Composition, for discussions). The mutagenic activity gradually decreased over time to below the detection limit, and toxicity was observed at doses equal and higher than 3 mg/plate.

A chemical analysis by gas chromatography by J. Tang and H. Okamoto at HML, CDHS, according to EPA method 625 was performed on the Stringfellow

TABLE 10. MUTAGENICITY OF A CONTAMINATED GROUNDWATER FROM THE STRINGFELLOW HAZARDOUS WASTE DISPOSAL FACILITY ON-SITE WELL OW-2, SAMPLE NO. AIHL-85-0402

A. Results of the Initial Screening Experiment

Exp. Date	Extraction Method[1]	Residue Weight (mg)	Sample Volume (L)	Specific Mutagenic Activity (revertants/mg)[2,3] TA98 -S9	TA98 2%S9	TA98 10%S9	TA100 -S9	TA100 2%S9	TA100 10%S9
7/22/85	Base/Acid	129	3	58	84	79	< 57	< 61	< 56

B. Comparison of Mutagenic Response in TA98 with 2% S-9 Mix[4]

Exp. Date	Extraction Method[1]	Residue Weight (mg)	Sample Volume (L)	Specific Mutagenic Activity (revertants/mg)[2,3]	Mutagenic Response per Unit Volume (revertants/L)
7/22/85	Base/Acid	129	3	84	3598
8/2/85	Acid/Base pH 2 fraction only	43	3	48	685
8/2/85	Acid/Base pH 11 fraction only	7	3	< 41	< 95
9/24/85	Acid/Base	60	4.5	< 53	< 702
10/17/85	Acid/Base	67	3	< 28	< 629

1. The extraction method indicates the order of pH in liquid-liquid extraction.

2. The specific activity of the mutagenicity dose-response curve is represented by the slope (b as shown in the statistical analysis on graphs in Appendix D).

3. The spontaneous mutation and positive control values are listed on the Strain Function Tests, Cell Titer, and Viability Record in Appendix F.

4. The optimum testing condition in the initial screening experiment was in TA98 with 2% S-9 mix.

contaminated wastewater sample (No. AIHL-85-0402) and was confirmed by gas chromatography/mass spectrometry-mass selective detector by J. Hennings and T. Li at HML (CDHS, 1984). The following priority pollutants were searched and none was detected in the blank water extract control: bis(2-chloroethyl)ether, 1,3-dichlorobenzene, 1,4-dichlorobenzene, 1,2-dichlorobenzene, bis(2-chloroisopropyl)ether, hexachloroethane, N-nitrosodi-n-propylamine, nitrobenzene, isophrone, bis(2-chloroethoxy)methane, 1,2,4-trichlorobenzene, naphthalene, 2-chloronaphthalene, acenaphthylene, dimethylphthalate, 2,6-dinitrotoluene, acenaphthene, 2,4-dinitrotolune, fluorene, hexachlorobutadiene, 4-chlorophenylphenylether, 4-bromophenylphenylether, hexachlorocyclopentadiene, hexachlorobenzene, phenanthrene, anthracene, di-n-butylphthalate, fluoranthene, benzidine, pyrene, butylbenzylphthalate, 1,2-benzanthracene, 3,3'-dichlorobenzidine, chrysene, bis(2-ethylhexyl)phthalate, di-n-octylphthalate, benzo(a)pyrene, indeno(1,2,3-c,d)pyrene, 1,2:5,6-dibenzoanthracene, 1,12-benzoperylene, phenol, o-chlorophenol, o-cresol, p-cresol, 2-chloro-5-methylphenol, o-nitrophenol, 2,4-dimethylphenol, 4-ethylphenol, 2,4-dichlorophenol, 2,5-dichlorophenol, 3-chlorophenol, 2,6-dichlorophenol, 4-chloro-2-methylphenol, 4-chloro-3-methylphenol, 2,3,5-trichlorophenol, 2,4,6-trichlorophenol, 2,4,5-trichlorophenol, 2,3,4-trichlorophenol, 3,5-dichlorophenol, 2,3,6-trichlorophenol, 3,4-dichlorophenol, 2,4-dinitrophenol, 4-nitrophenol, 2,3,5,6-tetrachlorophenol, 2,3,4,5-tetrachlorophenol, 3,4,5-trichlorophenol, pentachlorophenol.

Only trace amounts of the following four among the abovementioned chemicals were identified in the Stringfellow sample: 1,2-dichlorobenzene, 1,4-dichlorobenzene, isophorone, and 3-chlorophenol. Haworth et al. (1983) reported 1,2-dichlorobenzene, 1,4-dichlorobenzene, and 3-chlorophenol as nonmutagens for Salmonella typhimurium. No mutagenicity test data was found for isophorone in a literature search by the Environmental Mutagen, Carcinogen, and Teratogen Information Program (EMIC), Oak Ridge, Tennessee. However, as an unsaturated ketone, isophorone is expected to have a strong tendency to behave as a direct alkylating agent. This possible alkylating activity suggests that this chemical may have mutagenic potential (Federal Register, 1979). Further investigation is needed to elucidate the mutagen identities in the Stringfellow sample.

Several metal scans which analyze 16 potentially toxic metals using induced coupled plasma atomic emission spectroscopy (USEPA, 1982) by M. Iskander at HML

(CDHS, 1984) indicated high levels of chromium, zinc, nickel, and copper in the original contaminated groundwater sample (No. AIHL-85-0402). However, the dichloromethane extract which was applied in the Ames assay did not contain metals at levels significantly higher than the distilled water blank control extract. Therefore, the mutagenic activity in the Stringfellow sample was not caused by metal salts (e.g. $CrO_4^{=}$).

The cause for the decrease of mutagenicity in the Stringfellow on-site well contaminated groundwater sample may be any combination of chemical reactions, microbial activities, or storage conditions during shipping and storage. A time-course study is needed to confirm the degree of the degradation problem and to evaluate the optimum storage condition and the maximum storage time. Before establishing these optimum conditions, it is recommended that the sample processing be started 24 hours after receipt. The 24-hour wait is needed for gravity separation as suggested in the original protocol.

The second Stringfellow groundwater sample (No. AIHL-85-042A) collected from an upgradient well exhibited much lower mutagenicity (Table 11) when it was compared to the on-site sample. No toxicity was exhibited at doses up to 0.666 mg/plate. This sample may serve as a background level of mutagenicity measurement for the Stringfellow site. Mutagenicity comparison of samples collected from different spots in a hazardous waste site may provide valuable information for potential health effect evaluation and for assisting in monitoring the spread of toxic contaminants.

EPA/NBS Reference Sludge - TCLP Leachate--

The TCLP leachate sample in the initial experiment exhibited high mutagenic acitivity in both TA98 and TA100 (Table 12). Both direct- and indirect-acting mutagens were detected. No toxicity was observed at doses up to 0.333 mg/plate. The optimum testing condition was in TA98 with 2% S-9 mix. The addition of 2% S-9 mix increased the direct-acting mutagenicity by approximately 45%. However, the extract of the sodium acetate buffer which was the medium for the TCLP extraction produced colony counts two to five fold of the water blank values. The activity was especially obvious in TA98 without S-9. An investigation tracing back through the source of the error was performed so that corrective actions could be taken before the data was accepted.

TABLE 11. MUTAGENICITY OF A CONTAMINATED GROUNDWATER FROM THE STRINGFELLOW HAZARDOUS WASTE DISPOSAL FACILITY UPGRADIENT WELL UGB-8, SAMPLE NO. AIHL-85-042A

Mutagenic Response in TA98 with 2% S-9 Mix[4]

Exp. Date	Extraction Method[1]	Residue Weight (mg)	Sample Volume (L)	Specific Mutagenic Activity (revertants/mg)[2,3]	Mutagenic Response per Unit Volume (revertants/L)
10/17/85	Acid/Base	3.8	3	138	174

1. The extraction method indicates the order of pH in liquid-liquid extraction.

2. The specific activity of the mutagenicity dose-response curve is represented by the slope (**b** as shown in the statistical analysis on graphs in Appendix D).

3. The spontaneous mutation and positive control values are listed on the Strain Function Tests, Cell Titer and Viability Record in Appendix F.

4. The optimum testing condition for the Stringfellow OW-2 sample was in TA98 with 2% S-9 mix. The same condition was applied to the UGB-8 sample for comparison purposes.

TABLE 12. MUTAGENICITY OF AN EPA/NBS REFERENCE SLUDGE - TCLP LEACHATE, SAMPLE NO. AIHL-85-0401

A. Results of Initial Screening Experiments

Exp. Date	Extraction Method[1]	Residue Weight (mg)	Sample Volume (L)	Specific Mutagenic Activity (revertants/mg)[2,3] TA98 -S9	TA98 2%S9	TA98 10%S9	TA100 -S9	TA100 2%S9	TA100 10%S9
7/9/85[5]	Base/Acid	5.3	3	887	1287	1071	674	600	812
9/24/85	Base/Acid	12.1	6	990	1149	918	< 460	< 483	< 502

B. Comparison of Mutagenic Response in TA98 with 2% S-9 Mix[4]

Exp. Date	Residue Weight (mg)	Sample Volume (L)	Specific Mutagenic Activity (revertants/mg)[2,3]	Mutagenic Response per Unit Volume (revertants/L)
7/9/85	5.3	3	1287	2274
9/24/85	12.1	6	1149	2308

1. The extraction method indicates the order of pH in liquid-liquid extraction.

2. The specific activity of the mutagenicity dose-response curve is represented by the slope (b as shown in the statistical analysis on graphs in Appendix D).

3. The spontaneous mutation and positive control values are listed on the Strain Function Tests, Cell Titer and Viability Record in Appendix F.

4. The optimum testing condition in the initial screening experiment was in TA98 with 2% S-9 mix.

5. Activity in the background control (sodium acetate butter) was observed; the screening experiment was repeated after making corrective actions.

Table 13 lists the steps of investigation and the resulting change in the mutagenicity comparison of water blank and sodium acetate buffer. An identical experiment following the procedures of the initial experiment was performed to confirm the existence of the problem. The sample preparation procedures involved both TCLP and liquid-liquid extraction. The reagent ingredients for preparation of the buffer were tested individually for mutagenicity. Negative responses were observed for both chemicals. The extract of the buffer which was processed through only the liquid-liquid extraction process was not mutagenic. The dichloromethane used for the liquid-liquid extraction was concentrated, and the residue was tested for mutagenicity. A negative response was obtained. Therefore, the mutagen was not produced during the liquid-liquid extraction procedures.

As described in Section 5.1, Six Generic Sample Types, the TCLP involved shaking, centrifugation, and filtration. Each step was evaluated separately. The buffer extract which was processed without filtration exhibited an elevated but below-thedetection-limit value. In contrast, the extract with the filtration produced mutagenicity that was more than twice that of the background. The filtration apparatus used had a sinter-glass filter holder. It was suspected that the sinter-glass was not clean because these glass particles can strongly adsorb potent mutagens such as nitroarenes in a way that is similar to that shown by the affinity of silica gel particles in a column. An all glass filtration apparatus with a stainless-steel (inert) screen filter holder (SUPELCO, No. 5-8062) was replaced. This substitution eliminated the mutagenicity problem.

In the meantime, a new recipe for the preparation of the sodium acetate buffer was developed in a revised version of the TCLP method (revised on 10/4/85, versus the previous draft on 3/2/85, see Section 5.1, Six Generic Sample Types, for details). A new batch of buffer was prepared by combining 5.7 mL glacial acetic acid and 64.3 mL 1N sodium hydroxide solution which was then diluted to a volume of 1 L. The pH was 4.93 $\pm$ 0.02. The new buffer processed through the TCLP procedures with the stainless-steel filter holder proved to be nonmutagenic.

A second screening experiment was performed on the TCLP leachate, and high mutagenicity was observed again. Some priority pollutants were identified in the leachate, and the concentrations were quantified. This information was provided by

TABLE 13. INVESTIGATION OF THE MUTAGENICITY OF SODIUM ACETATE BUFFER USED FOR THE TCLP LEACHATE PREPARATION

Exp. Date	Sample Preparation Procedures				Mutagenicity in TA98, -S9[1] (mean, revertants/plate)	
	TCLP			Liquid-Liquid Extraction[2]	Water blank	Sodium Acetate buffer
	Shaking	Centrifugation	Filtration			
7/9/85[3]	Yes	Yes	Yes[6]	Yes	36	183
7/22/85[3]	Yes	Yes	Yes[6]	Yes	35	81
8/2/85[3,4]	No	No	No	Yes	35	45
8/16/85[3]	Yes	Yes	No	Yes	38	71
8/23/85[3]	Yes	Yes	Yes[6]	Yes	30	80
9/24/85[3]	Yes	Yes	Yes[7]	Yes	37	54
11/22/85[5]	Yes	Yes	Yes[7]	Yes	38	68

1. The highest activity of the sodium acetate buffer was observed in TA98 without S-9 in the initial screening experiment on 7/9/85. This condition was therefore used for further investigation.

2. The dichloromethane residue was found to be nonmutagenic (See Appendix D).

3. The buffer was prepared by dissolving 8.2 g of anhydrous sodium acetate in 800 mL water by adjusting to pH 5 with glacial acetic acid and by diluting to a volume of 1 L.

4. Both ingredients for preparation of the abovementioned buffer were found to be nonmutagenic (See Appendix D).

5. The buffer was prepared by combining 5.7 mL glacial acetic acid and 64.3 mL 1N sodium hydroxide solution; dilution to a volume of 1 L followed. The pH was 4.93 $\pm$ 0.02.

6. An all glass filtration apparatus with a sinter-glass filter holder (MILLIPORE No. XX15-04700) was used for filtration.

7. An all glass filtration apparatus with a stainless-steel screen filter holder (SUPELCO No. 5-8062) was used for filtration.

Dr. L.R. Williams, EPA. A computer search by EMIC provided information on the mutagenicity of these chemicals in the Ames assay. Table 14 lists the chemical identities and their mutagenic activity and references.

Four chemicals were reported as mutagens: 3,3'-dichlorobenzidine, N-nitrosodiphenylamine, 2,6-dinitrotoluene, and acenaphthene. Reid et al. (1984), calculated the activity in TA98 for 3,3'-dichlorobenzidine as 68 revertants/n mole which equaled 68 revertants/0.25 µg. The concentration of the compound in the leachate was 4.4 µg/L. Therefore, the compound might produce approximately 1200 revertants/L based on a simple computation. Approximately 2300 revertants/L were detected (Table 12) in the leachate prepared for this project. Therefore, assuming no complicated synergistic or antagonistic reactions occurred, 3,3'-dichlorobenzidine accounts for approximately 52% of the activity in the leachate.

N-nitrosodiphenylamine and 2,6-dinitrotoluene are mainly active in TA100, and their potencies are not as high as the 3,3'-dichlorobenzidine. A discrepancy on the mutagenicity of the last chemical, acenaphthene, was found in the literature. Gatehouse (1980), Hermann (1981), and others reported negative results, but Epler et al. (1979), in citing results from other articles reported a positive result for the chemical. Further evaluation is needed to differentiate the activity of the compound.

Possible Changes in Sample Composition--

In addition to the technical difficulties encountered during the liquid-liquid extraction of the wastewater samples, some of the samples exhibited possible changes in composition after storage at $4^{o}C$. The changes in composition were characterized by any of the following: loss of mutagenic activity in the Ames assay; increase or decrease in the amount of residue obtained after extraction; change in solubility properties of the residue; change in odor; and change in appearance (color, particulate, etc.). In all cases there was relatively no change in the sample pH after storage, and there were no significant changes in the behavior of these samples during processing.

Several of the samples exhibited a loss of mutagenic activity during storage. The Stringfellow sample is a good example of this phenomenom (Figure 6). During a period of only 13 days storage at $4^{o}C$ (16 days after sample collection), the activity

TABLE 14. MUTAGENICITY OF CHEMICALS IDENTIFIED IN THE EPA/NBS REFERENCE SLUDGE - TCLP LEACHATE

Chemical Name	Concentration in the Leachate (µg/L)	Mutagenicity in the Ames Assay	Reference(s)
diethylphthalate	7.1	-	Zeiger et al., 1985a
bis-2-ethylhexylphthalate	961	-	Zeiger et al., 1985a
butylbenzylphthalate	5.3	-	Zeiger et al., 1985a
di-n-butylphthalate	21	-	Zeiger et al., 1985a
di-n-octylphthalate	8.9	-	Zeiger et al., 1985a
1,4-dichlorobenzene	2	-	Haworth et al., 1983
acenaphthene	3.6	-(+)	Gatehouse, 1980 (Epler et al., 1979)
acenaphthylene	0.89	-	Gatehouse, 1980
anthracene	6.2	-	McCann et al., 1975a
fluorene	5.3	-	McCann et al., 1975a
naphthalene	149	-	McCann et al., 1975a
2-methylnaphthalene	84	-	Hermann, 1981
phenanthrene	6.2	-	McCann et al., 1975a
dibenzofuran	5.3	-	Schoeny, 1982
nitrobenzene	5.3	-	Haworth et al., 1983
2,6-dinitrotoluene	16	+	Sandvall et al., 1984
3,3'-dichlorobenzidine	4.4	+	Reid et al., 1984
N-nitrosodiphenylamine	60	+	Haworth et al., 1983

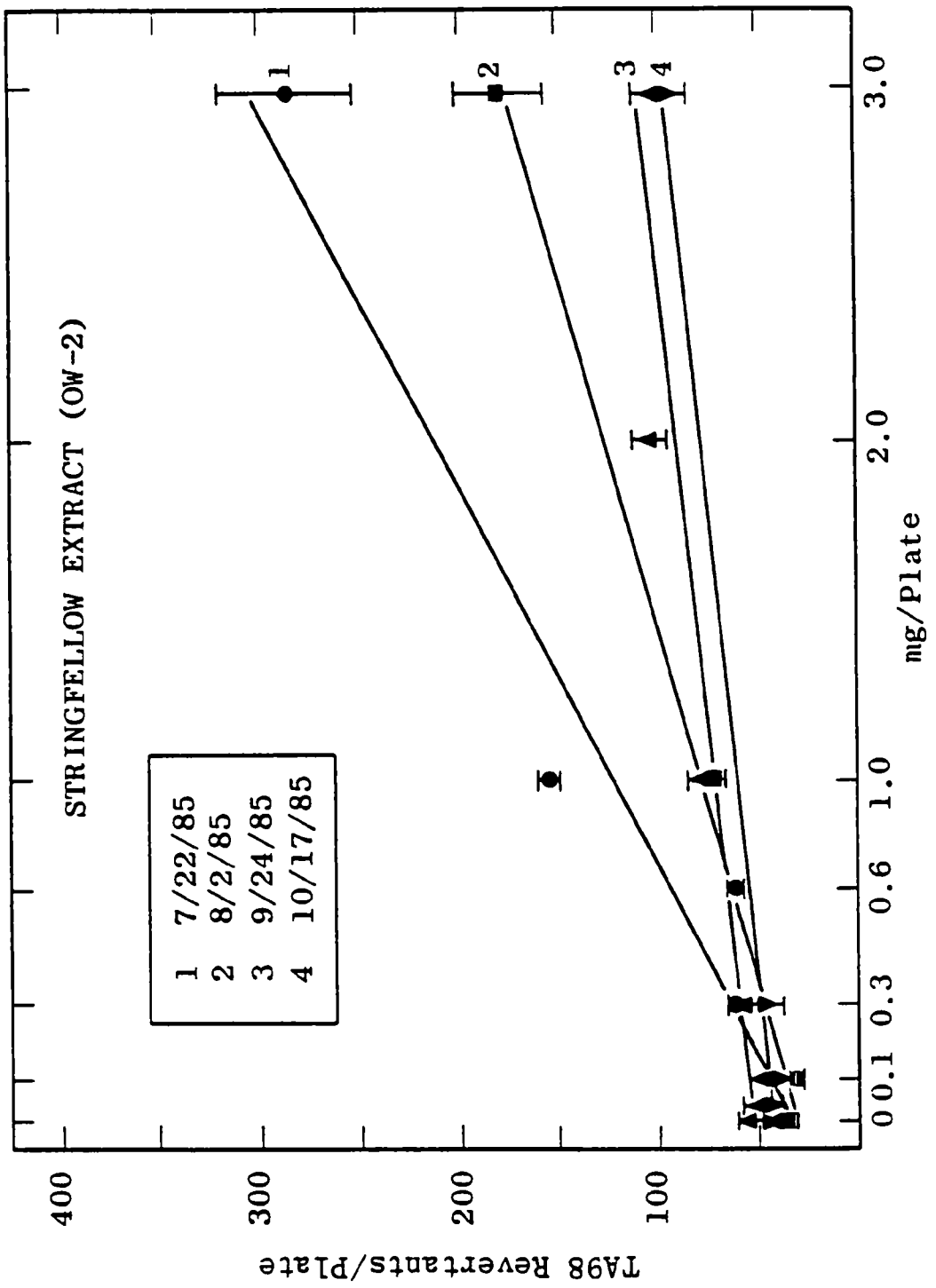


Figure 6. Dose-response curves showing degradation of mutagens in a Stringfellow contaminated groundwater sample.

initially observed in the Ames assay had been reduced by 50%. After almost three months of storage, the mutagenic activity in this sample was no longer detectable, and, in fact, the sample residue was highly toxic (See Appendix D).

Even more striking was the dramatic changes in the Stringfellow sample appearance. After two months storage, the color had turned from light brown to dark reddish brown and had become almost opaque. The odor was no longer sulfurous, and the sample had the aroma of an almond extract. The final residue obtained after liquid-liquid extraction had an oily property that could not be evaporated to complete dryness under nitrogen.

Similarly, the landfill surface runoff, exhibited loss of activity in the Ames assay. The sample lost approximately 80% of its mutagenic activity in a period of 20 days, and, after 30 days, no mutagenic activity was detected; the residue was extremely toxic to Salmonella typhimurium (Figure 7).

Not all samples exhibited changes in composition that were detected by the Ames assay or that were observed during sample processing. The municipal water effluent is an example of a highly stable wastewater in which the levels of mutagenic activity after 74 days of storage were comparable to the results obtained after the initial testing (Figure 8).

Because of the highly variable nature of these samples and because of the absence of predictability, it was concluded that all processing of wastewater samples should be completed within 14 days of receipt of the samples.

Liquid-Liquid Extraction

Samples Form Emulsions--

One common problem encountered by using the liquid-liquid extraction procedure was the formation of an emulsion between the aqueous phase and the organic phase during processing. The formation of an emulsion results in a less than efficient separation of the two phases and in invariably poor recovery yields. This problem is examplified by the municipal wastewater treatment plant effluent which was characterized by a notable odor of raw sewage (Table 4). During extraction,

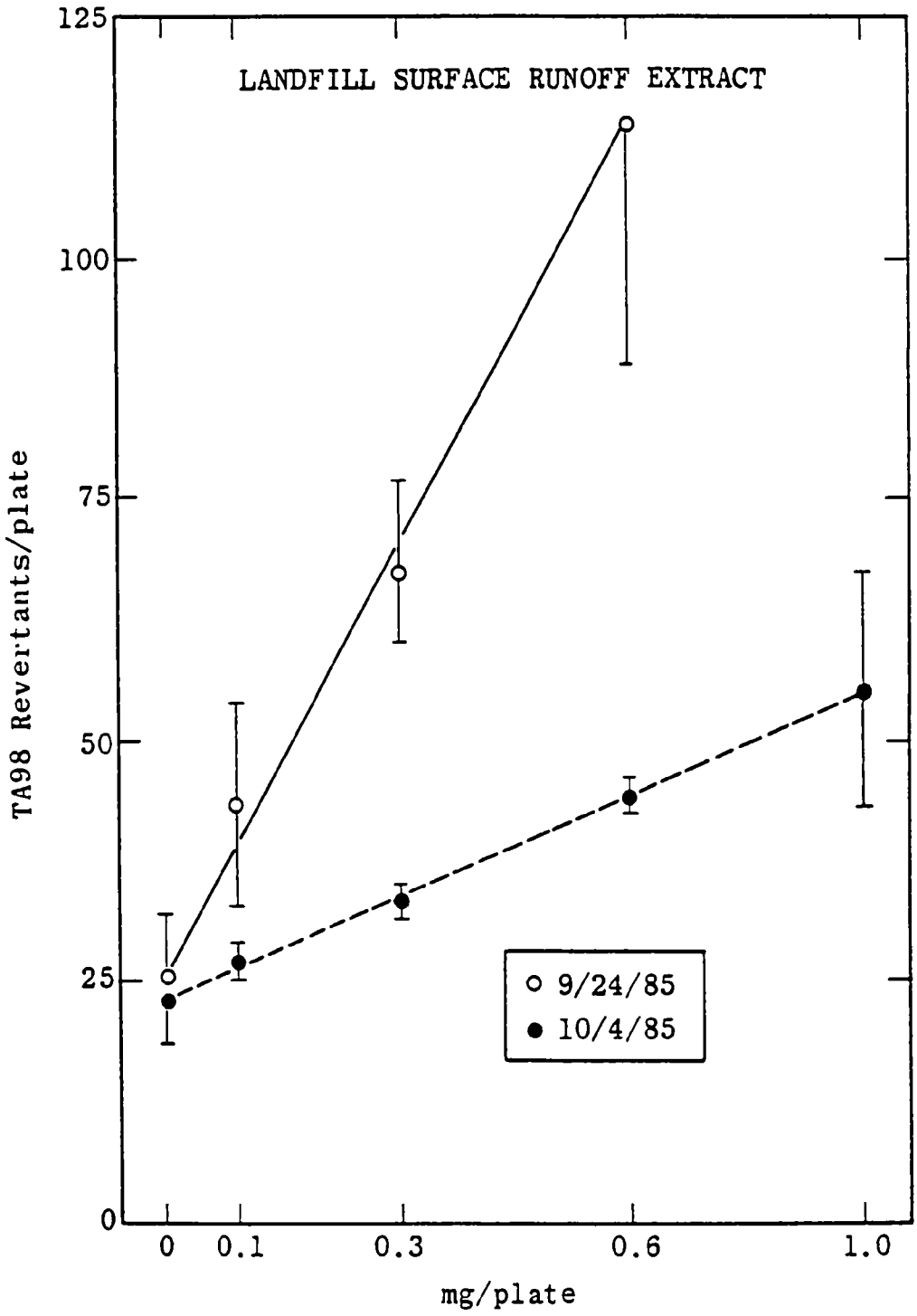


Figure 7. Dose-response curves showing degradation of mutagens in a surface runoff sample from a class I hazardous waste landfill.

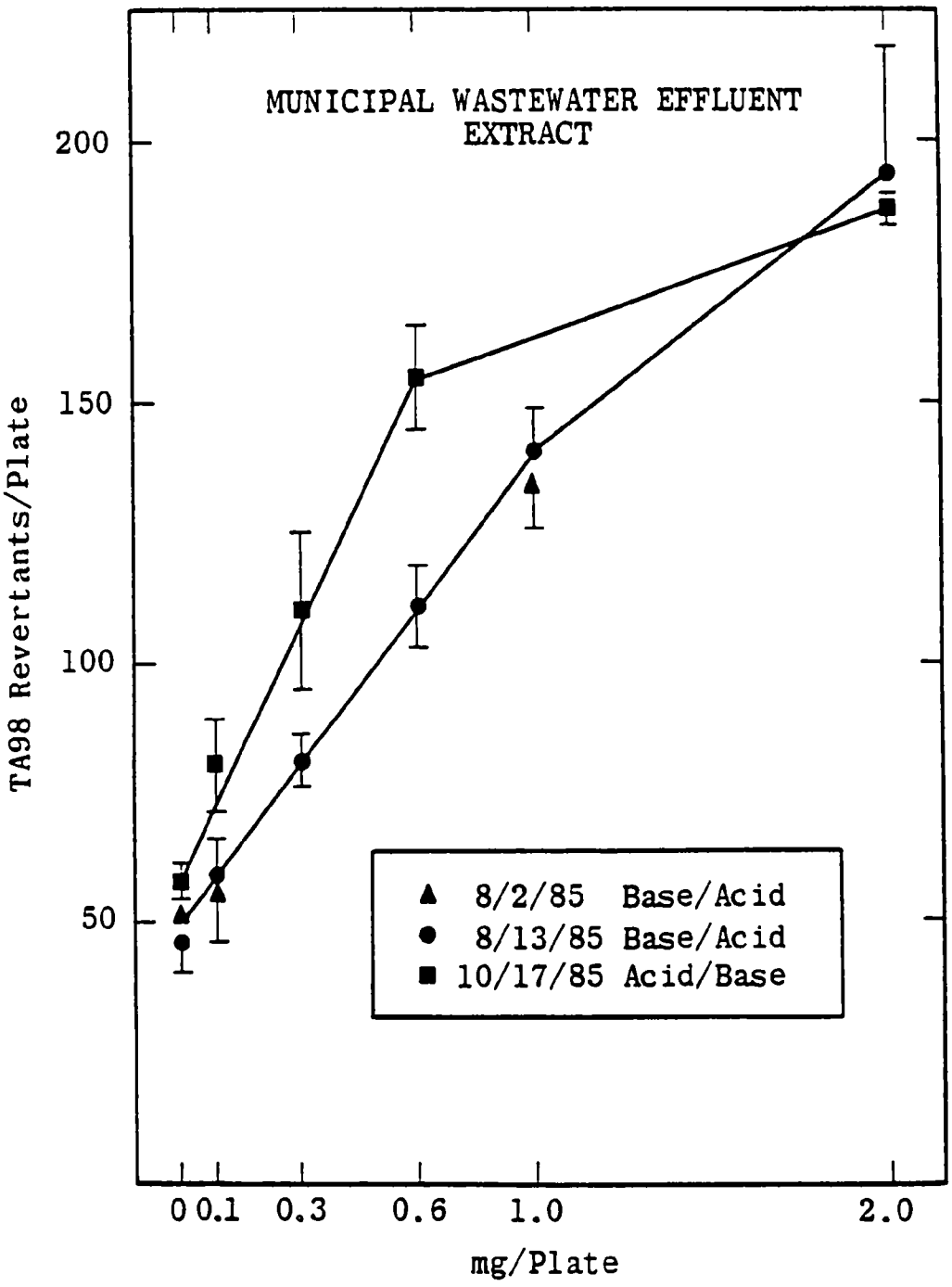


Figure 8. Dose-response curves for a municipal wastewater treatment plant effluent sample with stable mutagenicity.

a heavy emulsion formed which was independent of the extraction pH. The best separation of the aqueous-organic phases was accomplished by filtration through a funnel packed with glass wool when the organic phase was eluted. In some cases, a substantial quantity of the aqueous phase was included with the dichloromethane eluent. The two phases were easily separated in a clean separatory funnel. The resultant residue obtained from the liquid-liquid extraction of the municipal wastewater effluent (9-17 mg/3 L) was characterized by a light brownish red color, that dissolved readily in DMSO, and that exhibited significant mutagenic activity in the Ames assay (See Section 6.1.1.3, Municipal Wastewater Treatment Plant Effluent, for results). The addition of the glass wool filtration step was then included in the general liquid-liquid extraction protocol for all samples that formed emulsions during extractions.

Samples Form Precipitates--

Four of the six generic types of wastewater samples tested using the liquid-liquid extraction procedure formed a substantial precipitate when the pH was raised to a value approaching 11. This presented the most difficult problem associated with this extraction technique. Several solutions were evaluated; this included: changing the order of the pH extractions (i.e., acid extraction followed by the base extraction); filtration; centrifugation before extraction; and centrifugation after extraction. The following are examples of wastewater samples that formed a precipitate during liquid-liquid extraction: (1) contaminated groundwater samples from the Stringfellow acid pits; (2) estaurine brackish surface water from the San Francisco Bay and the brackish receiving water at the discharge site of an industrial wastewater treatment plant; (3) surface runoff from a class I hazardous waste landfill; and (4) TCLP extract leachate prepared from an EPA/NBS reference sludge. Those samples could not be processed by following the suggested EPA protocol for the preparation of wastewater exclusively. However, the inclusion of several steps was found to increase the utility of this procedure. Since these four types of samples precipitated only when the pH was raised to 11, it was logical to perform the first extraction at pH = 2. Following this extraction, the pH of the sample was adjusted to 11. The mixture was centrifuged, and the second extraction was performed on the alkaline supernatant. In this way, the majority of the extraction will not be affected by the precipitate since the mutagenic activity is predominantly extracted during the initial extraction (See Section 6.1.3, Mutagen Extraction Efficiency, for

recovery). In addition, if this procedure is followed, the difficulty in reconstituting the sample (i.e., recombining the precipitate with the aqueous portion) can be avoided, and a higher recovery would be expected.

The contaminated groundwater sample (Stringfellow) and the TCLP leachate both exhibited a precipitate that partitioned with the organic phase. The Stringfellow groundwater sample which was light brown in appearance and which was characterized by an acidic pH (Table 4) exhibited a strong sulfurous odor. Analysis of this sample revealed a high concentration of metal complexes (data not shown) that were insoluble at pH > 3.2. Similarly, the NBS sludge sample contained high levels of metals. Although the final residue weights of these two samples differed (Stringfellow approximately 40-129 mg/3 L and TCLP approximately 5-6 mg/3 L), the residues were soluble in DMSO, and they exhibited high mutagenic activity when applied to the Ames assay.

The two San Francisco Bay brackish water samples and the landfill runoff precipitated at pH 11, and this interfered with the extraction procedure. When the revised protocol was followed, the residues were easily obtained, were readily dissolved in DMSO, and both samples were found to be positive in the Ames assay.

Residual Water--

Because of the persistence of the organic phase to retain trace amounts of water, it was concluded that the anhydrous sodium sulfate column used before evaporation was not adequate to completely remove all water. Residual water in the concentrated extract and in vessels results in an incomplete evaporation process. In addition, the residual water will greatly affect the final residue weight determination used to calculate the final mutagenic potency of the sample. To remove small quantities of residual water, this final 10 mL of extract was passed over another sodium sulfate column (5 cm x 0.5 cm) before evaporation under nitrogen. This step has been added to the general protocol as a precaution against artifactual water contamination in the final residue.

Replace Kuderna-Danish (K-D) Apparatus with Rotary Evaporator--

In the original consensus protocol, a K-D apparatus was recommended for concentrating the extraction solvent, dichloromethane. However, dichloromethane

has been reported as a Salmonella mutagen and a suspected animal and human carcinogen. When the K-D concentrator is used, the solvent is heated and is vaporized in the chemical fume hood. This practice causes release of the dichloromethane to the general environment unless an activated-charcoal filter is placed properly at the top of the venting systems above the hood to adsorb the solvent. Therefore, a rotary evaporator was used as a concentrator instead of the K-D. The condensor of the evaporator cools the dichloromethane vapor, and the used solvents are collected in a waste reservoir. The solvent can then be treated as a carcinogen waste and can be disposed of properly.

Mutagen Extraction Efficiency

Determination of the extraction efficiency for the recovery of various types of mutagens from environmental water samples is critical in establishing a final protocol because each laboratory that uses this method is required to operate a formal QC program. The minimal requirements of this program include an initial demonstration of laboratory capability and the analysis of spiked samples as a continuing check on performance. Extraction efficiencies of the liquid-liquid extraction protocol can be assessed either by instrumentation such as HPLC for analytical identification and quantitation of the spiked standards or by the Ames assay for the recovery of mutagenicity.

The efficiency of the liquid-liquid extraction protocol was determined by measuring the recovery of three SPRM's in water. The three chemicals used in this study, B(a)P, 2AA, and 4NBA, were separated and were quantitated by HPLC (See Section 5.6.1, Chemical Analysis by HPLC, for details); they exhibited potent and characteristic mutagenicity in the Ames assay. In addition, these compounds were chosen because they represented three categories of chemicals; 4NBA represents an acidic compound, B(a)P represents a neutral compound, and 2AA represents a basic compound.

The overall efficiency of the liquid-liquid extraction protocol as outlined and revised in the results section was calculated from data obtained from blank water (ASTM Type I water) extraction. The recovery efficiencies for the three chemical mutagens, added at various doses to blank water prior to extraction, are shown in

Table 15. The recovery percentage was primarily calculated from HPLC analysis of the residue obtained after extraction. However, for those doses where sufficient amounts of the mutagen were used, the results obtained from the Ames assay were compared to those obtained from HPLC analysis. The recovery of the neutral compound, B(a)P, exhibited over 100% recovery at the highest spiked dose and fell to less than 50% when the dose was less than 25 µg/1.5 L water. High doses of 2AA exhibited similar recovery efficiencies when compared to B(a)P (over 100%). However, at doses less than 100 µg/1.5 L water, the efficiency decreased rapidly. The recovery efficiency of 25 µg 2AA was approximately only one fifth of that for B(a)P at the same dose. The recovery of the acidic compound, 4NBA, was not as complete at the highest dose tested (approximately 60% to 80% recovery) when it was compared with B(a)P or 2AA. In addition, a substantial decrease in recovery efficiency for 4NBA was observed only when the dose (2.5 µg) was 2000-fold less than the maximum dose (5000 µg). Also, the sequence of acid first followed by the base extraction produced higher recovery (60% to 80%) than the recovery of base first extraction (49% to 60%) in blank water.

These data suggest that low or trace levels of mutagens will be extracted poorly relative to high levels of mutagens in an environmental sample. However, because of the nature of this liquid-liquid extraction procedure extracting larger quantities of an environmental wastewater sample to increase recovery efficiency may not always be practical. Therefore, problems such as poor recovery efficiencies of certain chemicals in the liquid-liquid extraction should be considered when assessing unknown environmental complex samples to avoid underestimating the hazardous potential.

The efficiency of the liquid-liquid extraction procedure for recovering these three chemical mutagens was determined in a wastewater sample. The municipal wastewater treatment effluent sample (No. AIHL-85-0403) was chosen because of its stability and its representative behavior when processed by liquid-liquid extraction. A maximum dose of each chemical mutagen was used to evaluate optimal conditions. Two hundred fifty µg B(a)P and 2AA were added in 1.5 L of the water sample whereas 5000 µg 4NBA was used because of the larger amounts needed for the Ames assay. The percentage recovery of those three compounds is shown in Table 16. As expected from blank water efficiencies, B(a)P was recovered with

TABLE 15. LIQUID-LIQUID EXTRACTION RECOVERY OF THREE SPIKED REFERENCE MUTAGENS, BENZO(A)PYRENE (B(A)P), 2-AMINOANTHRACENE (2AA), AND 4-NITROBENZOIC ACID (4NBA) ADDED TO LABORATORY DISTILLED WATER

Mutagen Added	Extraction Method[1]	Spiked Dose (µg/1.5 L water)	% Recovery Ames Assay -S9	Ames Assay 2%S9	Ames Assay 10%S9	HPLC
B(a)P (neutral)	Base/Acid	500	-	79	185	110
		250 (pH 11 fraction)	-	48	131	85[3]
		250 (pH 2 fraction)	-	16	36	-
		25	-	-	-	58
		0.25	-	-	-	50
2AA (base)	Base/Acid	500	-	121	119	105
		250 (pH 11 fraction)	-	86[2]	64[2]	80[3]
		250 (pH 2 fraction)	-	< 2[2]	< 4[2]	-
		100	-	66	49	40
		25	-	-	-	12
4NBA (acid)	Acid/Base	5000	80	-	-	60
	Base/Acid	5000	60[2]	-	-	49
		2500	< 56[2]	-	-	55
		250	0	-	-	50
		25	-	-	-	45
		2.5	-	-	-	25

1. The extraction method indicates the order of pH in liquid-liquid extraction.

2. The number was calculated by using the method background value. The actual plate counts were below the detection limit.

3. A combined extract of the pH 11 fraction and of the pH 2 fraction was analyzed.

TABLE 16. LIQUID-LIQUID EXTRACTION RECOVERY OF THREE SPRM'S, B(A)P, 2AA, AND 4NBA ADDED TO THE MUNICIPAL WASTEWATER EFFLUENT SAMPLE (NO. AIHL-85-0403)[1]

Extraction Method[2]	Mutagen Added	% Recovery	
		Ames	HPLC
Base/Acid	B(a)P	90	103
	2AA	35[3]	29
	4NBA	< 56[3], Toxic	42
Acid/Base	B(a)P	105	100
	2AA	27[3]	25
	4NBA	< 56[3]	58

1. B(a)P or 2AA (250 µg/1.5 L wastewater, TA98, 10% S9 mix) or 4NBA (5000 µg/1.5 L wastewater, TA100, -S9) was added to a 1.5 L aliquot of the Municipal Wastewater Sample, was extracted with dichloromethane following the procedure outlined in Section 5.4, and the recovery was analyzed by both the Ames assay and HPLC.

2. The extraction method indicates the order of pH in the liquid-liquid extraction.

3. The number was calculated using the method background. The actual plate counts were below the detection limit.

approximately 100% efficiency regardless of the order of extraction (i.e., base first or acid first). Surprisingly, the recovery of 2AA was significantly reduced to only approximately 25% to 35% which is about half of the recovery efficiency in distilled water. The order of extraction did not significantly affect the extraction efficiency. The recovery of 4NBA in the Ames assay was incomplete due to the below-the-detection-limit response, and the response was also below the maximum 60% to 80% recovery observed for blank water. The order of extraction may have some effect on the recovery efficiencies of certain toxic chemicals since toxicity was observed in the base first extract and not in the acid first extract. However, the HPLC results indicated the 4NBA recovery in the municipal wastewater (42% to 58%) was similar to that in blank water (49% to 60%). The sequence of acid first when it is compared to the base first extraction effected an increase in recovery from 60% to 80%.

It is concluded that the recovery percentage of a neutral compound such as B(a)P, and maybe an acidic compound as 4NBA, is approximately the same in blank water and in an environmental sample with the extraction characteristics of the municipal wastewater effluent sample. The recovery of 2AA, a basic compound, was substantially reduced in the municipal wastewater by using either the base first or the acid first extraction scheme. Several possibilities may have contributed to the lower extraction efficiencies of 2AA from the municipal wastewater sample when it was compared to blank water; these include: chemical degradation; losses sustained because of the formation of an emulsion; or loss of extraction efficiency as a result of chemical-chemical interactions inherent in the sample (negative interference). Further evaluation of the extraction efficiencies of various chemicals is needed to verify the overall performance of this extraction procedure.

DEVELOPMENT OF QA PROGRAM

To ensure the production of data of continuing high validity, an intralaboratory QA program has been developed.

General Goal and Specific Objectives

The primary goal of establishing the QA program is to provide guidance to ensure that all environmental sample preparation procedures and mutagenicity measurements sponsored by EPA or other participating laboratories under regulations such as the Toxic Substances Control Act (TSCA), the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), the Federal Food, Drug, and Cosmetic Act, and RCRA, produce data of known quality. The quality of data is known and is reliable when all components associated with its derivation are thoroughly documented and when such documentation is both verifiable and defensible. The purpose of laboratory analyses is to provide qualitative and quantitative data for use in decision-making by management. The quality of the data must be specified, and sufficient resources must be provided to assure that an adequate level of QA is performed. All tasks should be undertaken with an adequate QA plan that specifies data quality goals acceptable to the data user and that assigns responsibility for achieving these goals (USEPA, 1984). In this fashion, the data will provide accurate and precise indications for evaluating the situation and effect of the environment and will not lead to faulty interpretation.

Specific objectives are as follows:

- To develop or put into service methods capable of meeting the users' needs for precision, accuracy, sensitivity, and specificity.

- To establish the level of quality for routine performance of the laboratory and to maintain a continuing assessment of the accuracy and the precision of analysts within the laboratory group.

- To monitor the routine operational performance of the laboratory through an appropriate intralaboratory program, to identify weak methodology, and to provide sources for corrective actions as necessary.

- To detect training needs within the analytical group.

- To provide a permanent record of instrument performance as a basis for validating data and for projecting repair or replacement needs.

- To upgrade the overall quality of laboratory performance.

This program includes several key areas such as the periodic analysis and interpretation of results of SPRM's, instrumental maintenance and calibration, and monitoring of the quality of various reagents and solvents used in the sample preparation and mutagenicity testing scheme.

The intralaboratory SPRM program provides a continuing measurement of the performance capability of each analyst. Each person can be constantly aware of his strengths and weaknesses, and corrective steps can be undertaken when necessary before serious problems occur and before erroneous data are reported out of the laboratory (Sherma, 1976).

Sufficient analyses are made on the unspiked subsamples so as to be satisfied with the reproducibility of results from the same analyst and among all participating analysts. When necessary, the sample may be sent to an outside laboratory (e.g., EPA-assigned reference laboratory) with experience in performing the analysis in question for verification. A NBS standard complex mixture sample (e.g., NBS reference sludge, TCLP leachate) with known stable chemical compositions may be used to prepare the unspiked and spiked SPRM's. When reproducibility is sufficient to establish a reliable mutagenicity profile in the unspiked sample, the other half is spiked to produce residues with higher mutagenicity. The spiked sample is thoroughly mixed, is transferred to small amber glass bottles with Teflon-lined caps, and is stored in a freezer. These spiked samples serve to test the capability of the analyst for recovery of higher mutagenicity levels. If the compound(s) and media are known to be fully stable at room temperature or at refrigerator temperature, freezer storage is not required.

For both the unspiked and spiked SPRM's, at least a dozen replications of the analysis on the same sample should be conducted by chemists with recognized competence. From this data, the percentage relative standard deviation is

calculated and is used in construction of control charts as described later in Section 6.2.3.2, QC Charts for Accuracy and Precision.

QC Approach

The approach to be implemented in order to achieve the objectives listed in Section 6.2.1, General Goals and Specific Objectives, is recommended in this section. These suggested policies (USEPA, 1979b) include:

- Publication, distribution, and maintenance of current and complete Laboratory Sample Preparation and Mutagenicity Testing Methods and Procedures, Sample Collection Information Record sheets, Strain Function, Cell Titer and Viability Record sheets, QC Charts for Accuracy and Precision Measurements, Calibration Data Sheets and Analytical Instrument Operating Instructions.

- Promulgation, distribution, and retention of laboratory reports with provision for administrative/technical review.

- Periodic calibration of instruments and equipment, both in the laboratory and in the field, QC checks on instruments for sample preparation and mutagenicity testing procedures to ensure proper function at all times; and a preventive maintenance program.

- Routine evaluations and maintenance of bacterial strains and the mammalian enzyme systems with positive and negative controls.

- Assurance of appropriate, fresh reagents and chemicals and for appropriate, calibrated glassware.

- Establishment and maintenance of total QC systems to assure continued precision and accuracy of laboratory reports, including, as appropriate, requirements that:

    a. Each procedure shall be checked on each day of use.

    b. At least one standard (may be an instrument standard) and one control

sample (working value established and run through the entire test procedure) shall be included with each run of unknown samples. A blank sample (no added amount of the constituent being determined) may be a combination of field blank, travel blank, reagent blank, and method blank; it shall be run to aid in detecting reagent contamination and other problems important near the lower limit of operation of the method.

c. If the results on the standard, control, or blank samples are not within acceptable limits, the entire batch of analyses must be repeated and control must be verified before reports are issued. Serious consideration should be given to the nonacceptance of samples where there is only enough material for a single analysis. There may be situations where this policy is waived. Consideration of the consequences of reporting results when the analytical system is apparently "out-of-control" should minimize such waivers.

- Requirements for participation in interlaboratory QC evaluation programs.

- Requirements for training and qualifying personnel in QC techniques prior to running new procedures. This qualification test is to be statistically valid and is to include evaluation of precision and accuracy. The qualification standard shall be the established level of quality of the laboratory.

Statistical QC Analyses and Records

There are two principal kinds of statistical tools available for use in QC analyses: tests for differences including analysis of variance, and control charts. For the protocol validation study, several statistical analyses were performed to establish the method background; control charts for the background control were then constructed for routine QC.

Method Background--

Since the mutagenicity results were used as the endpoint for data reporting and evaluation, the protocol validation study involved both the sample preparation

and the Ames testing procedures. Therefore, assuming that the basic variables such as laboratory services, instrumentation, glassware, reagents, solvents, and gases etc., were under QC, the method background included three negative controls: the spontaneous mutation revertants (SR) as the blank control for the mutagenicity measurement of the bacterial strain, the dimethylsulfoxide (DMSO) revertants as the solvent vehicle control in the Ames assay, and the revertants of blank water extract representing the laboratory, field, travel, sample preparation method, and mutagenicity procedure blanks.

Analysis of variance with two factor block design ($\alpha = 0.05$) was used to test the similarities among these three controls in TA98 under three S-9 conditions: without, with 2% S-9 mix, and with 10% S-9 mix. Data from eight to nine experiments on different dates were analyzed. Detailed calculations and primary data were listed on the worksheet and were attached as Appendix G. The main effects of daily variations and control conditions were tested using the F-test. If there was any difference either among day-to-day or among three treatments, another statistical analysis (t-test) was used to find out where the difference was (Dunn and Clark, 1974). Only TA98 data were evaluated because all samples contained mutagens detectable in TA98 in this study and because the sample size for TA100 data were too small for evaluation. This was the same reason that the data for TA98 with 30% S-9 mix were not analyzed.

The results in TA98 without S-9 showed that there was no difference among daily measurements ($p > 0.5$), but there was significant difference among treatments ($p < 0.05$). Data from each two controls were then compared by paired t-test ($\alpha = 0.05$). Significant differences were found between SR and DMSO ($p < 0.05$), between SR and water ($p < 0.05$), and between DMSO and water ($p < 0.05$). The average plate count (revertants/plate) was 27 for DMSO, 30 for SR, and 35 for water blank.

In the case of TA98 with 2% S-9 mix, the results showed that there was significant difference either among daily measurements or among three treatments ($p < 0.05$). The differences may be attributed to unstable data that results from combinations of variation in bacteria, in testing conditions, and/or in preparation procedures. The t-test comparison indicates similarity between SR and DMSO ($p >$

0.5), and significant difference between SR and water ($p < 0.05$) and between DMSO and water ($p < 0.05$). The average plate count (revertants/plate) was 40 for DMSO, 44 for SR, and 49 for water blank.

In TA98 with 10% S-9 mix, the results indicated that there was no significant difference among daily measurements ($p > 0.5$), but there was a significant difference among three controls ($p < 0.05$). Again, the t-test results showed similarity between SR and DMSO ($p > 0.05$), and significant difference between SR and water ($p < 0.05$) and between DMSO and water ($p < 0.05$). The average plate count (revertants/plate) was 44 for DMSO, 45 for SR, and 51 for water blank.

In conclusion, the three types of controls were different from each other in most of the cases. The SR and DMSO values could not be included as part of the method background. The water blank values including the effect of the laboratory process, field and travel procedure, sample preparation method, and mutagenicity testing, were accepted as the method background. The value of method background was 35 (without S-9), 49 (2% S-9), 51 (10% S-9), and 39 (30% S-9). The detection limit for the Ames assay, which was twice the background, as discussed previously in Section 6.1.1, Ames Assay Results and Interpretation, would be twice that of the water blank value.

QC Charts for Accuracy and Precision--

In order to establish the method background, valid precision and accuracy data must be developed in addition to the abovementioned statistical analyses. Accuracy and precision are two aspects of the possible error of every scientific measurement.

Accuracy relates to the closeness of approach of a single measurement, or of the average of a series of measurements, to the true value. The true value is incapable of being measured exactly, but in many cases it can be estimated very closely. By using calibrated equipment, by performing the work extremely carefully, by executing a very large number of measurements, and then by applying statistics to the results, reasonable approximations of the true value can be obtained. Frequently, the true value is estimated from the results of different analysts in different laboratories. Precision describes the closeness of approach of replicate results to a common value. Repeated measurements of the same quantity will usually

not be identical but will scatter around some common value. Precision describes the reproducibility or the scatter of a series of measurements or results.

One commonly used method for analyzing potential errors of any measurement is the construction of tion of Quality Control Charts. That is, in addition to recording numerical results of each analysis, the result is plotted as a point in a chronological sequence on a chart.

The purpose of this chart is to provide graphic assessment of accuracy and precision for each analysis and to provide instant detection of erroneous data. The charts allow quick observation of trends for a particular analysis and have long term value for the self evaluation of analytical output by staff personnel. Another significant value of the charts is that of providing a laboratory administrator with a rapid assessment of the continuing analytical capability of the staff scientists as related to the output of valid analytical data. The essential elements common to control charts include an expected value (the central line) and an acceptable range of occurrence (the region between upper and lower control limits), as shown in Figures 9, 10, and 11 (Software Consulting Group, 1984).

Figures 9, 10, and 11 are QC charts of the method background (i.e., water blank control) in TA98 in the absence and in the presence of 2% and 10% S-9 mix. Nine to 11 sets of data were collected from different experiments in this project and were used for construction of these QC charts.

The upper graphs represent the QC charts for accuracy measurements. Accuracy is the difference between a measurement and a true value. The calculated mean value (XBAR, or $\bar{\bar{X}}$) was assumed as the true value. The upper control limit ($UCL_{\bar{\bar{X}}}$) and lower control value ($LCL_{\bar{\bar{X}}}$) for the 99% confidence interval was then computed. The calculation process was recorded on the worksheets attached as Appendix H. The control chart is then the graphical presentation of the experimental results plotted in relation to these calculated limits. If the results fall within the limits, the experimental process is considered "in control." Otherwise, the process is judged as "out of control."

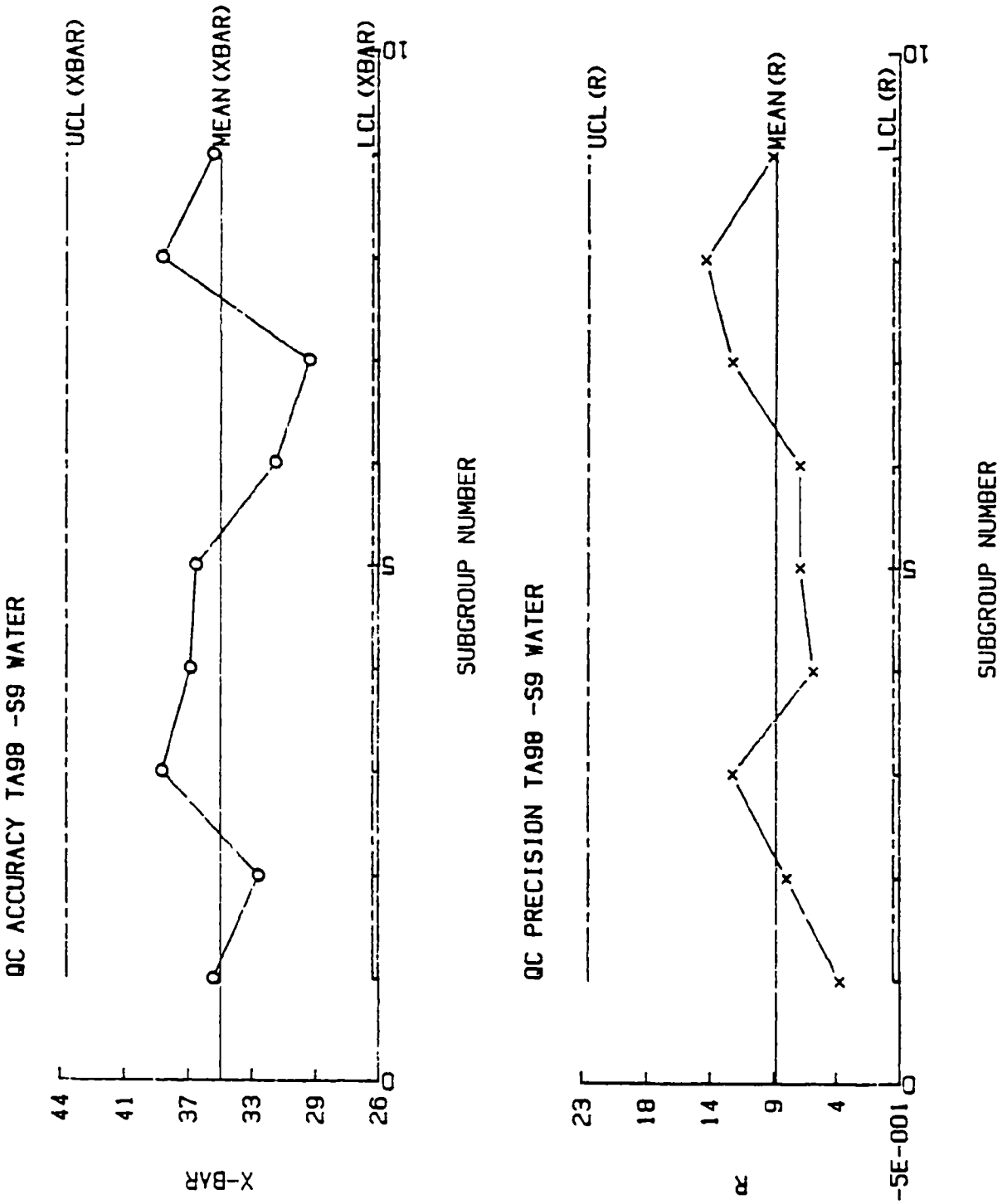


Figure 9. Control charts of the method background in TA98 without S-9.

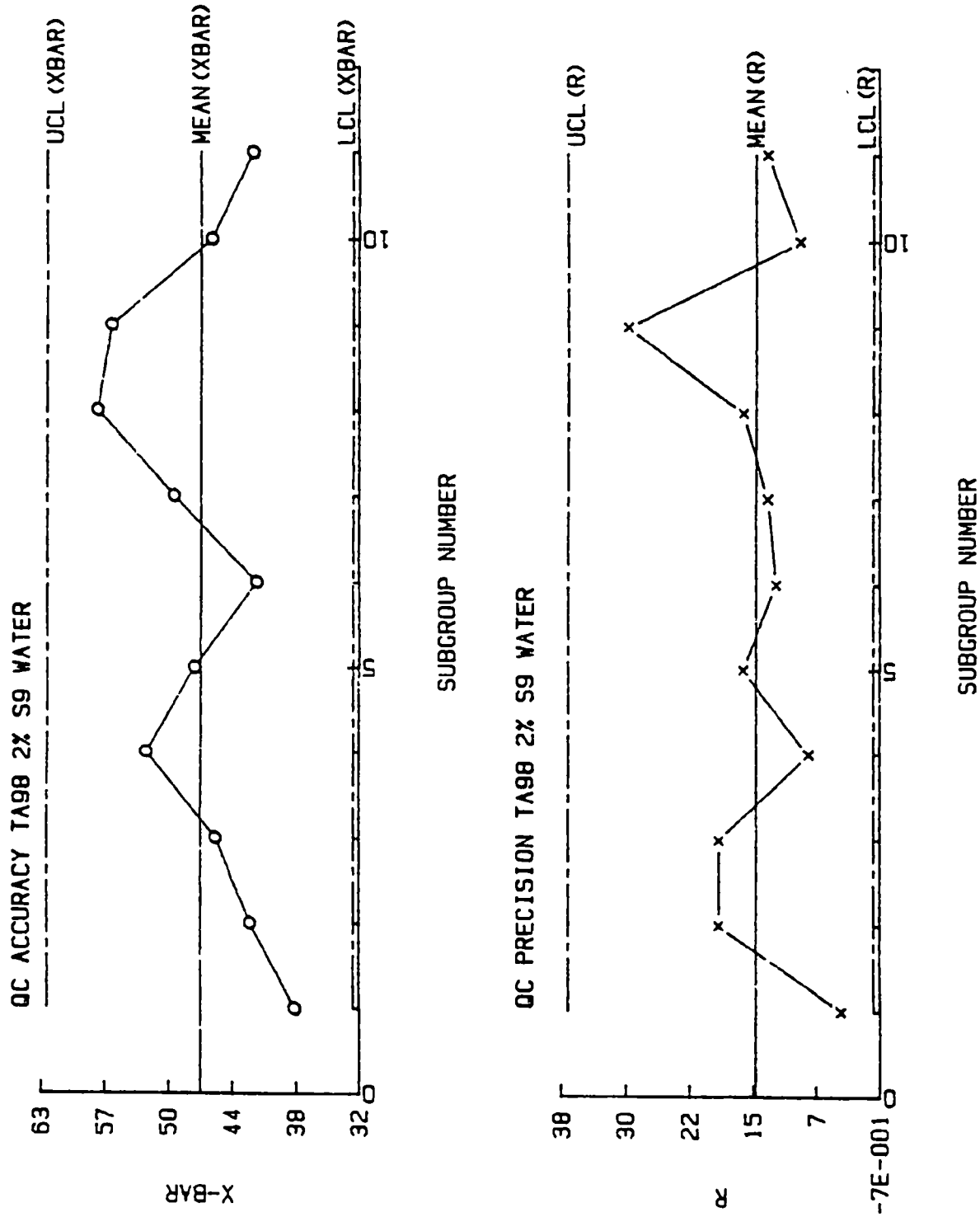


Figure 10. Control charts of the method background in TA98 with 2% S-9 mix.

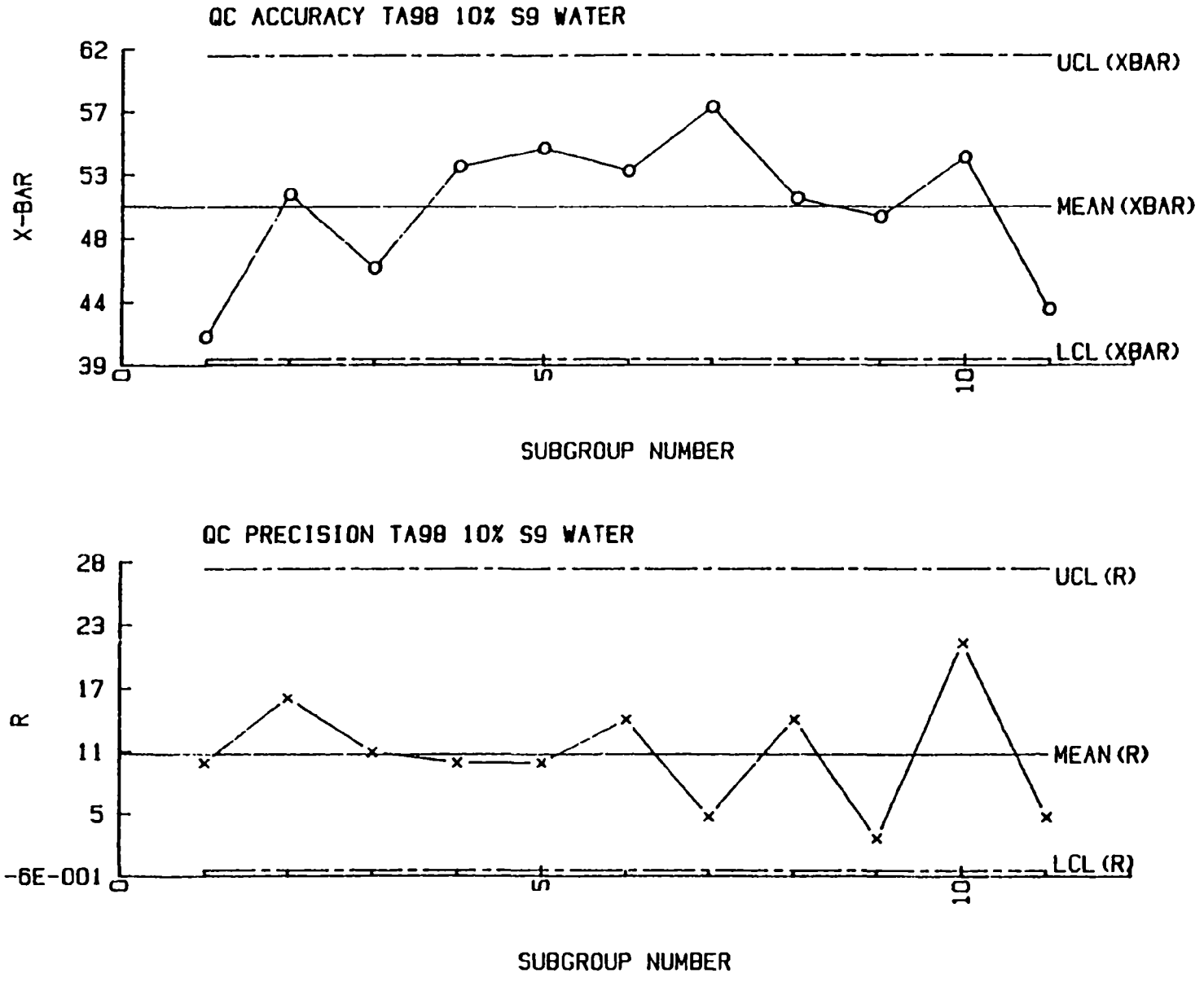


Figure 11. Control charts of the method background in TA98 with 10% S-9 mix.

The lower graphs represent the QC charts for precision measurements. Precision is a measure of reproducibility of results. It can be expressed as SD, variance (V), or range (R). When the number of replicates (n) is small as in this project (n = 9-11), the range chart is the most efficient way to measure precision. Range is the difference between the highest and the lowest measurements. The upper control limit ($UCL_{\bar{R}}$) and lower control limit ($LCL_{\bar{R}}$) were computed (Appendix H). Again, 99% of the calculated range values are expected to be within the boundary of these control limits. If eight or more successive values appear on one side of the mean value line, the process is considered as biased. The source of error which causes the bias should be traced. If the experimental results fall outside of the control limits, the process is judged as "out of control."

Graphs on Figures 9, 10, and 11 indicate that the water blank values in all three conditions are all within the control limits of accuracy and precision measurements. Continuation of these analyses and systematic routine evaluations will establish a large data base and will ensure the validity of the sample preparation and of the mutagenicity testing procedures.

The upper limit for accuracy of the method background was 44 (without S-9), 62 (2% S-9), and 62 (10% S-9). The lower limit was 26 (without S-9), 33 (2% S-9), and 39 (10% S-9). The range for precision of the method background was 0-23 (without S-9), 0-37 (2% S-9), and 0-28 (10% S-9).

Sample sizes are related to the number of replications. Since triplicate plate counts are used as a single control chart point, the chances of picking up small changes in the process average are increased. The protection against not detecting small changes in the process increases as the sample size increases. The use of frequent sampling will detect changes more quickly with time. The ultimate goal would be large sample sizes and measurements taken frequently. However, an economic decision has to be made as to the value of closer control versus the increased cost of attaining that degree of control.

In environmental measurements, separate control charts are recommended for each parameter, for each instrument, and for each analyst. However, the true value of the investigated parameter may vary considerably among samples. This variability

in true value means there are no expected numbers for randomly selected samples so that the accuracy of testing methodology must be evaluated indirectly through the recovery of standards and of spikes as described previously in Sections 5.6.2, Mutagenicity Recovery Analysis, and 6.1.3, Mutagen Extraction Efficiency.

Once the valid precision and accuracy data are developed for each method and each analyst, to insure that valid data continue to be produced, systematic routine checks must show that the test results remain reproducible and that the methodology is actually measuring the quantity in each sample. In addition, QC must begin with sample collection and must not end until the resulting data have been reported. QC of analytical performance within the laboratory is thus but one vital link in the dissemination of valid data to the public. Understanding and conscientious use of QC among all field sampling personnel, analytical personnel, and management personnel is imperative to the success of this project.

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APPENDIX A

ORIGINAL PROTOCOL FOR ENVIRONMENTAL WATERS AND WASTEWATER

The original consensus protocol for preparing environmental water and wastewater samples, published as part of the meeting proceedings (ICAIR ed., 1985), is attached in this report as Appendix A for detail descriptions of the samle preparation procedures.

PROTOCOL FOR THE PREPARATION OF ENVIRONMENTAL WATERS AND WASTEWATER FOR MUTAGENICITY TESTING

1.0 Scope and Application

1.1 This is a general purpose method which covers the preparation of wastewater samples for solvent-extractable organic compounds that are amenable to Ames mutagenicity assay.

1.2 The method is applicable only to solvent-extractable organic compounds, and not all chemicals in the sample are stable or recoverable by this procedure.

1.3 The final extract will be subjected to the Ames mutagenicity assay using the procedure of Williams and Preston (1982).

2.0 Summary of Method

Genotoxic activity of wastewater samples is assessed on the liquid and solid phases of wastewater grab samples. The discrete phases are collected, quantified, extracted and concentrated, and the resultant concentrates are transferred to DMSO and subjected to bioassay evaluation using a standard Ames test (Ames et al. 1975, Brusick and Young 1981, Williams and Preston 1982).

3.0 Definitions

Environmental Waters and Wastewater - Water not intended for human consumption, containing less than 50% suspended solids by weight, including water from industrial emissions, rivers, lakes and ponds. Wastewater may contain nonaqueous liquids in addition to the aqueous and solid phases.

Internal Standard - A pure compound added to a sample in known amounts and used to calibrate concentration measurements of other compounds that are sample components.

Field Duplicates - Two samples taken at the same time, placed under identical circumstances and treated exactly the same throughout field and laboratory procedures. Analysis of field duplicates indicates the precision associated with sample collection, preservation and storage, as well as with laboratory procedures.

Reagent Blank - Reagent water placed in a sample container in the laboratory and treated as a sample in all respects, including exposure to sampling site conditions, storage, preservation and all analytical procedures.

Nonaqueous Liquids Phase - Liquids whose major component is not water and which form discrete zones in an aqueous medium.

Sediment Solid Phase - The solid or semisolid phase recovered from a 24-h resting wastewater sample.

4.0 Interferences

4.1 Samples may change or become contaminated during transport from the collection site to the laboratory. Sample custody and shipping/storage conditions must be fully described and documented.

4.2 Emissions from wastewater sources change with time. Sufficient sample to complete all phases of the evaluation should be collected at the time of sampling. Date, time and exact location of sampling must be fully described and documented.

4.3 Method interferences may be caused by contaminants in solvents, reagents, glassware and other sample processing hardware that lead to discrete artifacts and/or elevated baselines in the total ion current profiles. All of these materials must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks.

4.4 Matrix interferences may be caused by contaminants that are coextracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality being sampled. An internal standard should be employed to evaluate matrix interference.

4.5 Methylene chloride can interfere with the mutagenicity assay, so care must be exercised to completely remove all methylene chloride prior to dilution with dimethyl sulfoxide.

4.6 The sample will be supplied for Ames testing in its most concentrated form in dimethyl sulfoxide, because additional sample concentration in the presence of dimethyl sulfoxide (B.P. 189 C) is prohibitive due to potential thermal or evaporative loss of sample organic constituents. If the extract is not soluble in its concentrated form, dilution with additional dimethyl sulfoxide or other appropriate solvents is advised.

4.7 Some extracted concentrated samples in DMSO may be highly cytotoxic in the Ames test. If a sample cannot be tested up to a level of 1,000 µg extracted organics per plate, it should be considered "toxic," and alternative treatment or bioassay methods should be performed, including chromatographic separation of toxic compounds from nontoxic compounds (HPLC or acid/base/neutral fractionation) (Hughes et al. 1980, Tabor and Loper 1980). In the case of limited sample size, the maximum level will depend only on sample availability.

5.0 Safety

5.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available.

5.2 The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method (NIOSH 1977, OSHA 1976). A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified for the information of the analyst (ACS 1979).

5.3 Solvent rinsing of vessels with methylene chloride may result in excessive exposure to this solvent. All rinsing should be performed in an approved chemical fume hood, and appropriate personal safety apparel should be worn.

5.4 Internal control samples may be spiked with chemicals demonstrating carcinogenic activity in animals. Internal control samples should be handled according to safety procedures consistent with this fact (NCI 1981).

5.5 Care should be used when pressurizing or vacuum-filtering samples. Excessive pressure or vacuum might cause vessel or filter breakage and expulsion of concentrated sample into the laboratory environment.

5.6 Since some wastewater samples may come from sources containing human or domesticated animal excrement, communicable disease risk is present. Laboratory personnel should be adequately immunized for communicable diseases, and all samples should be considered potentially contaminated and handled accordingly.

6.0 Apparatus and Equipment

6.1 Grab Sample Bottle--
Amber glass wide-mouth, 3.8-L or 1-gal volume, fitted with screw caps lined with Teflon. Aluminum foil may be substituted for Teflon if the sample is not corrosive. Alternatively, Amicon stainless steel 20-L carboys may be used (see Drinking Water Protocol).

6.2 Pressure Filter--
Millipore unit fitted with a glass fiber filter (1-μ pore size).

6.3 Centrifuge--
High-speed, refrigerated sample centrifuge capable of handling 500-ml centrifuge tubes.

6.4 Celite column to trap particulate matter prior to resin concentration (acceptable devices are commercially available).

6.5 Glassware (all specifications are suggested. Catalog numbers are included for illustration only).

6.5.1 Separatory funnel--2,000 ml, with Teflon stopcock.

6.5.2 Drying column--9-mm ID chromatographic column with coarse frit.

6.5.3 Concentrator tube, Kuderna-Danish--25 mL, graduated (Kontes K-570050-2525 or equivalent). Calibration must be checked at the volumes employed in the test. Ground glass stopper is used to prevent evaporation of extracts.

6.5.4 Evaporative flask, Kuderna-Danish--500 mL (Kontes K-570001-0500 or equivalent). Attach to concentrator tube with springs.

6.5.5 Snyder column, Kuderna-Danish--Three-ball macro (Kontes K-503000-0121 or equivalent).

6.5.6 Vials--Amber glass, 10- to 5-mL capacity, with Teflon-lined screw cap.

6.5.7 Continuous liquid-liquid extractors--Equipped with Teflon or glass connecting joints and stopcocks requiring no lubrication. (Hershberg-Wolf Extractor-Ace Glass Company, Vineland, N.J. P/N 6B4-0 or equivalent).

6.6 Boiling Chips--
Approximately 10/40 mesh. Heat to 400 C for 30 min or Soxhlet extract with methylene chloride.

6.7 Water Bath--
Heated, with concentric ring cover, capable of temperature control (± 2 C). The bath should be used in a chemical fume hood.

6.8 Balance--
Analytical, capable of accurately weighing 0.0001 g.

6.9 Nitrogen Evaporator--
Equipped with Teflon or glass jets and temperature control. (Meyer N-evap Model 112 or equivalent - Organomation Assoc., Inc., Northborough, MA).

6.10 Standard amber glass storage containers, 10-mL bottles with Teflon-lined screw caps.

7.0 Reagents and Consumable Materials

7.1 Reagent Water--
Reagent water is defined as a water in which an interferent is not observed at the method detection limit of each parameter of interest. It consists of XAD-2 cleaned tap water.

7.2 Sodium Hydroxide Solution (10 N)--
Dissolve 40 g NaOH in reagent water and dilute to 100 mL.

7.3 Sodium Thiosulfate (ACS), granular.

7.4 Sulfuric Acid Solution (1+1)--
Slowly add 50 mL of H_2SO_4 (sp. gr. 1.84) to 50 mL of reagent water.

7.5 Acetone, methanol, methylene chloride, dimethyl sulfoxide (pesticide-quality or equivalent).

7.6 Sodium Sulfate (ACS) granular, anhydrous--
Purify by heating at 400 C for 4 h in a shallow tray.

7.7 Internal Standard Mutagen--
Use 4-nitroquinoline-N-oxide dissolved at 100 µg/L of liquid sample or other acceptable mutagens.

8.0 Quality Control

8.1 Each laboratory that uses this method is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the analysis of spiked samples as a continuing check on performance. The laboratory is required to maintain performance records to define the quality of data that is generated. Ongoing performance checks must be compared with established performance criteria to determine if the results of analyses are within accuracy and precision limits expected of the method.

8.1.1 Before performing any analyses, the analyst must demonstrate the ability to generate acceptable accuracy and precision with this method.

8.1.2 In recognition of the rapid advances that are occurring in chromatography, the analyst is permitted certain options to improve the separations or lower the cost of measurements. Each time such modifications are made to the method, the analyst is required to repeat the procedure in Section 8.2.

8.2 To establish the ability to generate acceptable accuracy and precision, the analyst must perform the following operations.

8.2.1 Select a representative spike concentration for each parameter to be measured. Using stock standards, prepare a quality control check sample concentrate in acetone 1,000 times more concentrated than the selected concentrations. Quality control check sample concentrates, appropriate for use with this method, will be available from the U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268.

8.2.2 Using a pipet, add 1.00 mL of the check sample concentrate to each of a minimum of four 1,000-mL aliquots of reagent water. A representative wastewater should be used in place of the reagent water, but one or more additional aliquots must be analyzed to determine background levels, and the spike level must exceed twice the background level for the test to be valid. Analyze the aliquots.

8.3 It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Field duplicates may be analyzed to monitor the precision of the sampling technique. Whenever possible, the laboratory should perform analysis of standard reference materials and participate in relevant performance evaluation studies.

9.0 Procedure

9.1 Sample Collection--

9.1.1 Wastewater collection methods--It is recommended that samples be taken which reflect the "normal" state of the sample site. Periodic sampling of the same site over several months may also be valuable in providing information on peak periods of activity. For aqueous samples, the most common sampling procedure is a manual grab collection of the volume needed for analysis. Once collected, samples should be placed in the sealed amber glass bottles and held at 4 C during any storage or shipping. The head space in the container should be reduced by complete filling of the container or by replacement with a N_2 blanket. Table 5 identifies the volume of sample required.

9.1.2 Wastewater sample custody--An example of a sample custody form is shown in Figure 12. This form is from EPA document EPA-600/8?1-024 (Brusick and Young 1981). Either this EPA form or its equivalent should be prepared at the time the sample is collected, and a copy of the form should accompany the sample through all phases of processing and bioassay.

9.1.3 Storage and use of sample--It is strongly recommended that all samples be processed (separated, extracted and concentrated) within 14 days after collection and completely analyzed within 40 days of concentration and solvent exchange.

9.2 Sample Separation into Component Phases--

9.2.1 Each sample container is stored motionless at 4 C for 24 h after receipt. At this point, a gravity separation of phases will occur. Any nonaqueous liquid phases identified in the liquid component of the sample will be separated from the containers, combined into one sample and processed as a nonaqueous liquid waste.

Any solid sediment on the bottom of the sample container will be collected, combined into a single sample and processed as a waste solid.

The aqueous phase, with or without suspended particles will be recovered and treated in one of three methods (see Section 9.2.3).

9.2.2 Each liquid phase recovered must be weighed to the nearest gram and the volume measured. The solid sediment is weighed (net weight to the nearest gram). Like phases are combined into a common vessel for processing. Once combined, total weights and/or volumes are calculated. Storage conditions are the same as defined for the initial collected sample. No attempts to control

TABLE 5. RECOMMENDED VOLUMES AND STORAGE OF SAMPLES

Component	Volume/Weight	Storage Conditions
Collected Sample	Minimum of 30 L	4 C, dark
Gravity Separation Method	Minimum of 30 L	4 C, dark motionless
Sample for Resin Column Concentration	10 L	4 C, dark glass or Teflon-lined vessel
Liquid/Liquid Extraction Method	3 L	4 C, dark glass or Teflon-lined vessel
Solids for Solid Waste Extraction	500 g net weight	4 C, dark closed container
Extracted/Concentrated/ Solvent Exchanged Sample for Bioassay	10 mL	4 C, amber glass vial with Teflon cap liner

I. SAMPLE INFORMATION

1. Sample No. _____ Collection Date _____
2. Sampling Site[a] _____
3. Field Sampling Manager (on-site) _____
4. Contractor _____ Contract No. _____
5. EPA Project Officer _____ Program Name _____
6. Source Sampled _____
7. Discharge Rate of Source (Volume/Time) _____
8. Quantity Sampled/Units _____
9. Sample Description (liquid, slurry, solid, extract, appearance, etc.)

_____

10. Other Information as Applicable

Collection temp. _____ Sampling location _____
pH _____ Sampling technique _____
Other _____

II. HANDLING & SHIPPING

1. Describe Sample Treatment Prior to Shipping (e.g., transfers, extractants, stored undiluted, grinding, solvents used) _____

_____

2. Field Storage and Shipping Conditions

Container	Temperature	Light
___ Amber Glass	___ Ambient	___ Shield from light
___ Polyethylene Bottle	___ Refrigerate (0 to 4 C)	
___ Coated Bag or Bottle	___ Freeze (-20 C)	
___ Teflon or Tedlar Bags	___ Dry Ice	
___ Other _____		

3. Approximate Time in Storage and Time in Shipping _____
4. Sample Shipped to _____
5. Mode and Carrier for Shipping _____
6. Comments _____

_____

(This form should be completed by the on-site sampling manager and accompany each sample.)

(a) Graphically illustrate site of collection.

Figure 12. Sample information.
(Extracted from: IERL-RTP Procedures Manual Level I Environmental Assessment) Biological Tests EPA-600/8-81-024, October, 1981. pp. 138.)

pH or change the pH of the sample are required. The initial sample containers should be rinsed three times with methylene chloride. The final rinse collection will be concentrated and added to the final extract sample prior to solvent exchange.

9.2.3 The liquid phase recovered from the sample will be processed by one of the following methods:

a. If the sample has <5% suspended solids by weight (reference for method TSS), the sample may be extracted and concentrated by the techniques described in the Drinking Water Protocol, with the addition of ASTM Celite prefilter to the concentrator apparatus.

b. If the sample has >5% suspended solids by weight, the sample may be further separated by high-pressure filtration or high-speed centrifugation into liquid (<5% suspended solids) and solids. These two phases can be processed further by the Drinking Water Protocol and Solid Waste Protocol, respectively.

c. If the sample has >5% suspended solids by weight, it may be processed by a liquid/liquid extraction method (Section 9.3). If the bioassay from the sample recovered from this method is negative, consideration should be given to processing a retained liquid phase (10 L) by the method described in Section 9.2.3.b.

9.3 Separatory Funnel Liquid-Liquid Extraction Method--

9.3.1 Shake the sample, contained in a gallon amber glass bottle, to assure homogeneity. Samples are usually extracted using separatory funnel techniques. If emulsions will prevent achieving acceptable solvent recovery with separatory funnel extractions, continuous extraction may be used.

9.3.2 Measure two 1,500-mL sample aliquots into two 2000-mL separatory funnels. Check the pH of the sample with wide-range pH paper and adjust to pH 11 with 10N sodium hydroxide.

9.3.3 Add 150 mL of methylene chloride to each separatory funnel, and extract the sample by shaking the funnels for two minutes with periodic venting to release excess pressure. Allow the organic layers to separate from the water phases for a minimum of ten minutes If the emulsion interface between layers is more than one-third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may including stirring, filtration of the emulsion through glass wool, centrifugation or other physical methods. Combine the methylene chloride extracts in a 1,000-mL Erlenmeyer flask. If the emulsion cannot be broken (recovery of less than 80% of the methylene chloride, corrected for the water solubility of methylene chloride), transfer the sample, solvent and emulsion into the extraction chamber of a continuous extractor and proceed as described in Section 10.

9.3.4 Add 100 mL of methylene chloride to each separatory funnel, and repeat the extraction procedure a second time, combining all extracts in the 1,000-mL Erlenmeyer flask. Perform another 100-mL extraction in the same manner.

9.3.5 Adjust the pH of the aqueous phases to less than 2 using the sulfuric acid solution. Serially extract the sample with 150, 100 and 100 mL of methylene chloride per separatory funnel. Combine the extracts with previous extracts in the 1,000-mL Erlenmeyer flask. Notice that additional fractionation could be achieved here, if requested, by not combining these extracts (organic acids) with previous extracts (organic base/neutrals) but, rather, processing each separately through the remaining procedure.

9.3.6 Assemble a Kuderna-Danish (K-D) concentrator by attaching a 25-mL concentrator tube to a 500-mL evaporative flask. Other concentration devices or techniques may be used in place of the Kuderna-Danish. Pour one half of the combined extract through a drying column containing about 10 cm of anhydrous sodium sulfate, and collect the extract in the K-D concentrator. Add one or two clean boiling chips and attach a three-ball Snyder column. Prewet the Snyder column by adding about 1 mL of methylene chloride to the top of the column. Place the K-D apparatus on a hot water bath (60 to 65 C) so that the concentrator tube is partially immersed in the hot water, and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 minutes. At the proper rate of distillation, the balls of the column will actively chatter, but the chambers will not flood with condensed solvent.

9.3.7 When the apparent volume of liquid reaches approximately 10 mL, remove the K-D apparatus from the water bath and add the remaining combined extract through the drying column and into the K-D apparatus. Rinse the Erlenmeyer flask and column with 25 mL of methylene chloride to complete the quantitative transfer. Add two clean boiling chips and continue concentration on the water bath until the liquid reaches 10 mL. Remove the K-D apparatus from the water bath and allow it to drain and cool for at least 10 minutes. Remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of methylene chloride.

9.3.8 Concentrate the methylene chloride extract to 10 mL using the nitrogen evaporator. Do not allow the bath temperature to exceed 35 C. An aliquot should be removed for total organic carbon (TOC) analysis (Lentzen et al. 1978). If requested, at this point a 1-mL aliquot may be removed for analysis by gas chromatograph/mass spectrometer using Method 625 (44 FR 233, December 3, 1979). Transfer the extract to a tared, 3-dram glass vial that is etched at the 5-mL mark. Weigh the vial and its contents and record the weight of the residue. Dilute the sample extract to the 10-mL mark with dimethyl sulfoxide and attach the Teflon-lined screw cap to the vial. From the TOC determination, the DMSO extract should be recorded as mg organics/mL DMSO. Refrigerate the extract until ready for Ames testing.

9.3.9 If no aliquot was removed for analysis by gas chromatograph/mass spectrometer, the DMSO extract represents a concentration factor of

approximately 600x. If an aliquot was removed for analysis by gas chromatograph/mass spectrometer, the DMSO extract represents a concentration factor of approximately 540x.

10.0 Calculations

All measurements of mutagenic activity should be expressed as revertants per liter of emission and as a rate (revertants/liter/hour). In order to generate these values, data for revertants/mg organics extracted by each sample phase depicted in Figure 11 are needed.

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APPENDIX B

ORIGINAL CONSENSUS PROTOCOL FOR DRINKING WATER

The original consensus protocol for preparing water samples, published as part of the meeting proceedings (ICAIR ed., 1985) is attached in this report as Appendix B for detail descriptions of the sample preparation procedures.

PROTOCOL FOR THE PREPARATION OF DRINKING WATER FOR MUTAGENICITY TESTING

1.0 Scope and Application

1.1 This method is used for the isolation/preparation of residue organics from drinking water for mutagenicity testing. Drinking water is defined as water intended for human consumption.

1.2 This method is applicable to the isolation of residue organics from drinking waters purified from both surface and ground sources. The method may be applicable to other waters (e.g., see Wastewater Protocol).

1.3 The method provides for the reproducible qualitative recovery of mutagenic residue organics absorbed by XAD resins from drinking water. Residue organics are defined in Section 3.2.

1.4 This method does not provide information on volatile organics or on highly polar and/or ionic organics that may be present in the water sample.

1.5 The apparatus employed in this method is portable, and its use is straightforward. This allows for application to a wide variety of drinking water sources in the field, and thus does not require the transport of water samples to the laboratory.

1.6 This method is restricted to use by, or under the supervision of, analysts experienced in chromatography and properly trained in the handling and use of biohazardous materials.

2.0 Summary of Methods

2.1 A field duplicate sample of drinking water is passed through specially designed chromatography columns containing polystyrenedivinylbenzene copolymers and polymethacrylate polymer stationary phases. Following passage of the sample through the columns, the residue organics are eluted from the collection system components with organic solvents, the solvents are removed by evaporation and the remaining nonvolatile residue organics are stored under nitrogen until mutagenicity testing is conducted.

2.2 The method described in this protocol is based on reports by LeBel et al. (1979), Loper et al. (1983, 1984), Tabor and Loper (1984) Baird et al. (1981), Jenkins et al. (1983) and Nellor et al. (1984) for the isolation of residue organics from drinking water. For the purposes of this method, residue organics are those which are absorbed by resins under the conditions described herein and recovered by the solvent elution method of this protocol. The procedure may not recover the highly polar and ionic organic species or the highly volatile, low molecular weight organics. The design and testing of

the large-volume, >50 L and small-volume, <50 L sampling apparatuses is given in Loper et al. (1982, 1984), Tabor and Loper (1984) and Nellor et al. (1984). These tests, for example, have shown that the large sampling apparatus is capable of accommodating 1,100 L of low total organic carbon water (i.e., drinking water), and that the small sampling apparatus is capable of accommodating more than 200 L of similar water, both without apparent breakthrough of mutagenic residue organics. Additionally, the passage of chlorinated, 2-ppm, ASTM Type I water through the system did not produce residue organics that gave positive results in the Salmonella mutagenicity test using strains TA98 and TA100 in the absence and presence of metabolic activation. These studies established operation parameters such as flow rates and line pressures. The results of these studies show that the method provides reproducible qualitative recoveries of mutagenic residue organics from a wide variety of drinking waters prepared from ground and surface sources.

3.0 Definitions

3.1 ASTM Type I Water--

The American Society for Testing Materials (ASTM) defines Type I water as having a maximum total matter of 0.1 mg/L, a maximum electrical conductivity at 25 C of 0.06 μmho/cm, a minimum electrical resistivity at 25 C of 16.67 Mohm·cm and a minimum color retention time for potassium permanganate of 60 min.

3.2 Drinking Water--

Water intended for human consumption.

3.3 Field Duplicate Samples--

Two samples taken at the same time and place, under identical circumstances, and treated exactly the same throughout field and laboratory procedures. Analysis of field duplicates indicates the precision associated with sample collection, preservation and storage, as well as with laboratory procedures.

3.4 Liter Equivalent--

The amount of residue organics concentrated from one liter of water.

3.5 Resin Blanks--

Two types of XAD resin blanks are required. The first, a chemical contamination blank, is determined on an extract of the resin via gas chromatography. The second, a mutagen contamination blank, is determined by mutagenesis assay of residue organics eluted from the resins following passage of ASTM Type I water through the assembled collection system.

3.6 Residue Organics--

Those organics adsorbed by XAD resins under the conditions described herein and recovered by the solvent elution method of this protocol.

3.7 Solvent Blank--

Elution solvents are concentrated 1,000 times, and the concentrates are bioassayed for mutagenesis.

4.0 Interferences

4.1 The polystyrenedivinylbenzene copolymer stationary phase, XAD-2, and the polymethacrylate polymer stationary phase, XAD-7, should be cleaned extensively prior to use according to the methods described in Section 7.1 and analyzed according to the methods in Section 9.2.

4.2 Interferences to the mutagenicity tests from elution solvents will vary from supplier to supplier and from grade to grade. Therefore, it is recommended that pesticide-grade or equivalent solvents are used and that appropriate tests of each lot of solvent are conducted according to the procedures described in Section 9.1.

4.3 Interferences to mutagenicity tests and subsequent chemical analyses from the apparatus can be avoided by using the stainless steel apparatus or its equivalent, described in Section 6.1, allowing the sample and solvents to come into contact only with properly cleaned glassware, described in Section 6.11, and utilizing only TFE tubing and TFE sealants where required.

5.0 Safety

5.1 The toxicity and carcinogenicity of the residue organic samples generated in this method have not been defined; however, each sample should be treated as a potential health hazard. Procedures for handling such materials have been described (USNCI 1981).

6.0 Apparatus and Equipment

6.1. Apparatus—
The general design of the sampling apparatus is shown in Figure 9, and specifics of the design are shown in Figure 10.

6.1.1 Gauge, 0-100 psi, stainless steel with TFE diaphragm (Veriflo Model IR-101S25DG or equivalent).

6.1.2 Flow control valve, one-way, needle, stainless steel (Whitey SS-IRM4-S4 or equivalent).

6.1.3 Bacterial filter holder—

6.1.3.1 For high total organic carbon (20 ppm) or particulate-laden water, a 142-mm sanitary filter holder, 316 stainless steel (Millipore YY30 142 36 or equivalent) fitted with 1/4-in stainless steel pipe to tube male couplings (Swagelok SS400-1-4-316 or equivalent).

6.1.3.2 For low total organic carbon (20 ppm) or low particulate-laden water, a 47-mm high-pressure filter holder, 316 stainless steel (Millipore XX45 047 00 or equivalent) fitted with 1/4-in stainless steel pipe to tube male couplings (Swagelok SS4DO-1-4-316 or equivalent).

6.1.4 Bacterial filters—

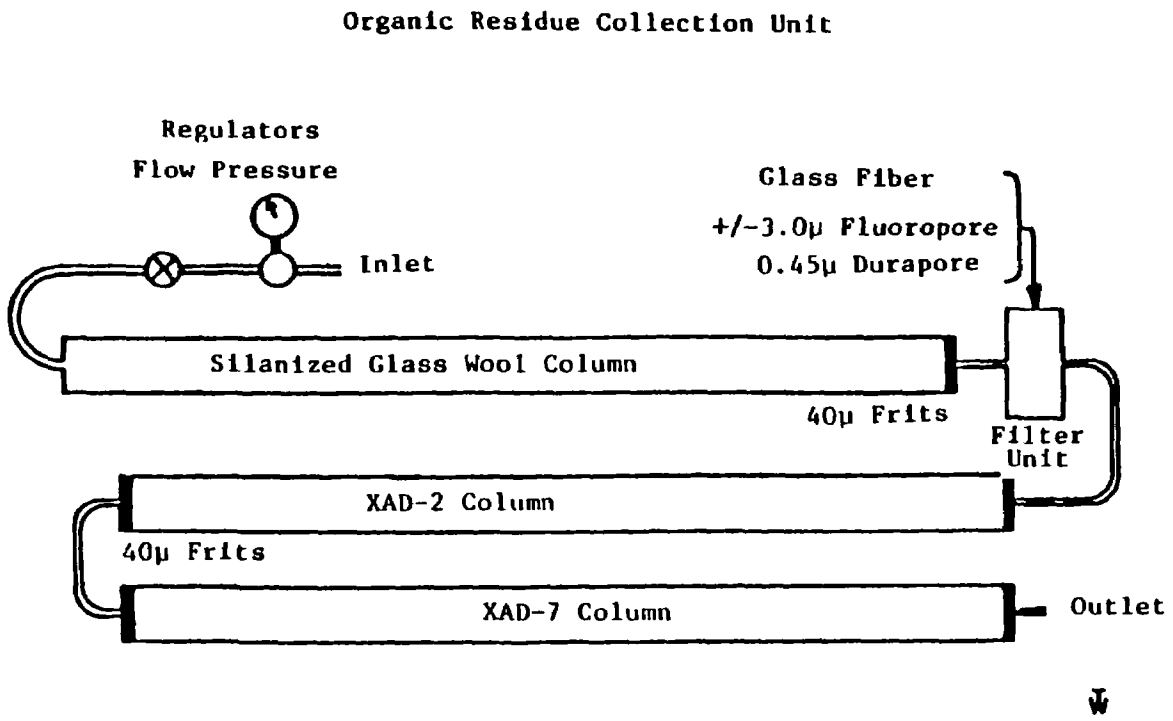

Figure 9. Schematic of nonvolatile residue organics concentration apparatus.

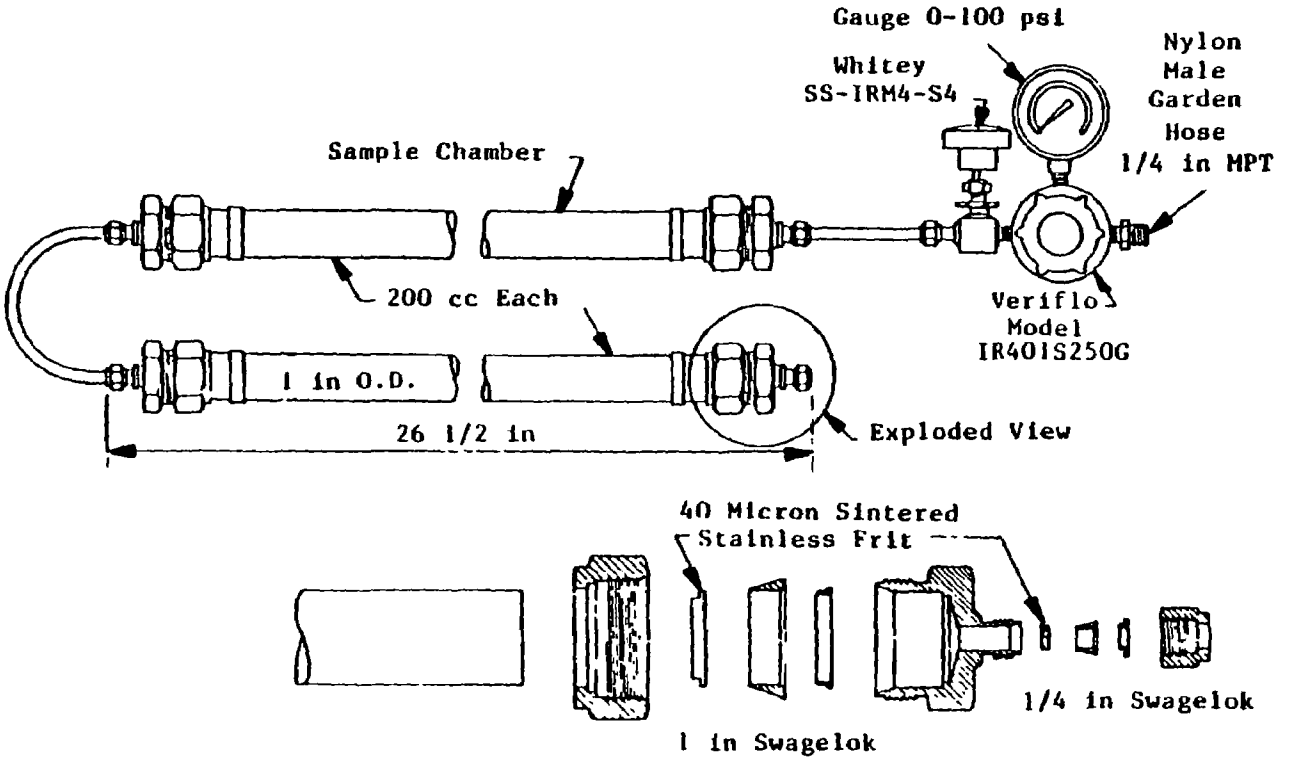

Figure 10. Details of nonvolatile residue organics concentration apparatus. (Note: prefiltration units are not shown.)

6.1.4.1 Microfilter glass discs without binder resin (Millipore Type AP40, AP40 047 05) for low total organic carbon filter holder.

6.1.4.2 Hydrophilic 0.45-micron Durapore filter (Millipore NVLP D4700 or equivalent) for low total organic carbon filter holder.

6.1.4.3 Hydrophilic 0.45-micron Durapore filter (Millipore HVLP 142 5D or equivalent) for high total organic carbon filter holder.

6.1.5 Connecting tubing, 316 stainless steel fitted with 1/4-in female Swagelok stainless steel fittings (Swagelok SS402-1-316, SS403-1-316, SS404-1-316 or equivalent). Any source of 316 stainless steel 1/4-in tubing acceptable.

6.1.6 Resin and glass wool columns--

6.1.6.1 Large sample volume (>50 L) columns of 200-cc bed volumes are constructed, as shown in Figure 10, of 1-in by 26.5-in 316 stainless steel tubing fitted with 1-in female Swagelok stainless steel fittings (Swagelok SS1012-1-316, SS1613-1-316, SS1614-1-316 or equivalent) and a 1-in male Swagelok stainless steel cap (Swagelok SS1610-C-316 or equivalent) modified as follows. The center of the 1-in cap is tapped and threaded to accept a 1/4-in pipe male coupling. The cap is fitted with a 1/4-in stainless steel pipe to tube male coupling (Swagelok SS400-1-4-316 or equivalent) which is silver-soldered in place. Prior to installation, the 1/4-in coupling is machined internally from the tubing side for an opening 3/16-in wide and 3/4-in deep. This opening is then fitted with a 5/32-in diameter by 1/8-in 40-micron sintered 316 stainless steel frit. Both column ends are fitted with the 40-micron sintered 316 stainless steel frits. This entire column assembly is available from Tristate Controls, Inc., 4303 Kellogg Avenue, Cincinnati, OH 45226.

6.1.6.2 Small sample volume (<50 L) columns of 25-cc bed volumes are constructed as shown in Figure 2 and described above, except that the columns are constructed of 1/2-in by 13-in 316 stainless steel tubing fitted with 1/2-in female Swagelok stainless steel fittings (Swagelok SS812-1-316, SS813-1-316, SS814-1-316 or equivalent), the 40-micron sintered 316 stainless steel frits and 1/2-in male Swagelok stainless steel caps (Swagelok SS810-C-316) modified as described above. This entire column system is available from Tristate Controls, Inc., 4303 Kellogg Avenue, Cincinnati, OH 45226.

6.2 Miscellaneous Apparatus Components--
(when line pressure of drinking water exceeds supplies 40 psi).

6.2.1 Pumping--The low line pressure drinking water sample source may be pumped through the columns by connecting a control volume TFE diaphragm pump (Milton Roy Co., model NR-117S or equivalent) between the drinking water outlet tap and the collection apparatus (Section 6.1.1). The pump should be capable of delivering 2.5 U.S. gal/h and developing a test pressure of 1,000 psi. The pump is fitted with a flow control valve, described in Section

6.1.2, to regulate flow rates. The pump is usually used when large sample volumes (>60 L) are to be collected.

6.2.2 Positive displacement--The low line pressure drinking water samples are collected in 20-L stainless steel reservoirs (Amicon Corporation model RS20 stainless steel reservoir or equivalent). The apparatus (Section 6.1.) is connected to the outlet of the reservoir by stainless steel tubing/ fittings. The inlet is connected to a nitrogen or helium gas cylinder fitted with a pressure regulator. The reservoir system is usually used when small volumes (<60 L) are to be collected.

6.3 Sample Storage Vials (Wheaton "500" amber serum bottles Nos. 223778, 223779, 223785 or 223787 or equivalent), sealable with Teflon-faced septa (Wheaton No. 224167 or 224172 or equivalent).

6.4 Resin Eluate Solvent Evaporator for initial reduction of volume (Buchi/Brinkman Rotary Evaporator, Model 15 00 500-9 or equivalent).

6.5 Sample Evaporator (Organomation Associates, Inc.) N-Evaps Model 111 or equivalent).

6.6 Analytical Balance - Readable to 0.01 mg with a precision of ± 0.01 mg.

6.7 Evaporation, displacement and sample storage gas, nitrogen or helium, water compressed, high-purity grade.

6.8 Special Glassware--

6.8.1 Evaporative concentrator, modified micro Snyder, 4-mL tube (Kontes No. K-569250 or equivalent).

6.8.2 Soxhlet extraction apparatus, Corning series 3840 or equivalent.

6.9 Muffle Furnace capable of sustaining 500 C, for use in glassware decontamination.

6.10 Solvent Reservoir 4-L stainless steel (Amicon Corporation, Model RS4 stainless steel reservoir or equivalent).

6.11 Flame Ionization Gas Chromatographic Unit fitted with a 6-ft by 4-mm glass column containing 10% OV-101 (or equivalent) on 100/120 mesh GAS-CHROM-Q. This unit must be capable of temperature programming.

6.12 Cleaning of Apparatus and Glassware--

6.12.1 Apparatus--Detergent wash, rinse with tap and ASTM Type I water, followed by successive rinses with pesticide-grade acetone and hexane.

6.12.2 Forty-micron sintered 316 stainless steel frits. Clean by sonication for 15 min in 6N nitric acid, followed by three successive 15-min sonications in ASTM Type I water. The frits are rinsed successively with pesticide-grade acetone and hexane.

6.12.3 Sample vials—Detergent wash, rinse with tap and ASTM Type I water, followed by drying/muffling overnight at 500 C. Tightly wrap vials in aluminum foil for storage until use.

6.12.4 Septum—Clean as for apparatus, Section 6.12.1.

6.12.5 General glassware—Clean as recommended in 44 FR 69464, December 3, 1979.

7.0 Reagents and Consumable Materials

7.1 Stationary Phases for Concentrating Nonvolatile Residue Organics—
Polystyrenedivinylbenzene copolymer, XAD-2, resin (Rohm and Haas Co.) and polymethacrylate polymer, XAD-7, resin (Rohm and Haas Co.) are available from numerous distributors. The XAD resins, as supplied, are contaminated with extractable monomeric and polymeric species that must be removed before use. The XAD-2 and XAD-7 resins are cleaned individually, but using the same procedure. The resin clean-up, detailed below, involves removal of fines, followed by Soxhlet extraction using a series of organic solvents. The purified resins are stored as an acetone slurry in amber bottles until use. Alternatively, the XAD resins, purified according to recommended methods and specifications, are available with certification of analysis from the Munhall Company, 5850 High Street, Worthington, OH 43085.

7.1.1 Resin cleaning—The resins can be cleaned in batches of 500 g, enough for one sampling plus required blanks, or multiples thereof. The following procedure is for 500-g batches, but can be scaled up as required.

7.1.1.1 To wash the resins and remove fines, transfer 500 g of the resin to a 1-L beaker and fill with ASTM Type I water. Slurry and allow to settle. Decant the supernatant fluid containing the fines and repeat until supernatant fluid is clear. This process may have to be repeated as many as ten times to obtain a clear supernatant fluid.

7.1.1.2 Following removal of the fines, the moist resin is transferred to a glass extraction thimble fitted with a fritted disc, and then the thimble is inserted into the Soxhlet extraction apparatus. For each 500 g of resin, the following extraction sequence is conducted using 1 L of solvent for each extraction. First the resin is extracted with 1 L of methanol for 8 h, followed by a 14-h extraction with an additional 1 L of fresh methanol. The 22-h methanol extraction is followed by extraction with two 1-L portions of methylene chloride, 8 h and 14 h, respectively. The 22-h methylene chloride extraction is followed by extraction with two 1-L portions of hexane, 8 h and 14 h, respectively. Finally, the resin is extracted with two 1-L portions of acetone, 8 h and 14 h, respectively. The resin is rinsed from the thimble into an amber bottle using a fresh portion of acetone. At this point, 20 cc of resin is taken for resin blank analysis, as described in Section 9.2 (see USEPA 1978). The resin is stored under acetone.

7.2 Glass Wool—
Silanized (Supelco No. 2-0411 or equivalent)

7.3 Solvents—

Methanol, methylene chloride, hexane and acetone, pesticide-grade or equivalent, and dimethylsulfoxide, DMSO, reagent-grade or equivalent, stored in original containers and used as received.

7.4 ASTM Type I water, generated by a Continental/Millipore Water Conditioning system (Tabor and Loper 1980) or equivalent.

7.5. Celite 545—

Prewashed as follows: Slurry 75 g of the filter aid in 500 mL ASTM Type I water by swirling, then settle briefly and decant the supernatant fluid containing the fine particles; repeat the process with a second aliquot of ASTM Type I water. Pack a 25-cc stainless steel column, fitted at the outlet end with a frit, with a 2-g plug of glass wool, then fill partly with ASTM Type I water. Slurry pack the column with the Celite 545, using Type I water as a liquid vehicle. Fit the inlet end of the column with a stainless steel frit, followed by the Swagelok fittings. Connect the inlet end of the column to a 4-L stainless steel reservoir (Amicon Corporation, model RS4 or equivalent). Successively wash the column with one liter each of pesticide-grade acetone, hexane and acetone, according to the procedure in Section 6.12.2. Following the last acetone wash, cap the column with 1/4-in stainless steel Swagelok plugs for storage of the column until use (within one week).

8.0 Residue Organics Isolation Procedure

8.1 Packing Procedure for 200-cc and 25-cc Columns—

The XAD-2 resin and XAD-7 resin columns are slurry packed, using acetone as a liquid vehicle. After packing, label flow direction and seal columns with 1/4-in stainless steel Swagelok plugs (Swagelok ss-400-P-316) for storage at 4 C until use (within one week). Do not allow the resins to dry; keep them covered with solvent. The glass wool prefiltration column is firmly but not tightly dry packed with 25 g of silanized glass wool, using a clean glass rod to position the packing material. Note: the stainless steel frits are omitted from the inlet end of the glass wool column. Label the flow direction of the column.

8.2 Apparatus for Drinking Water Sources with Line Pressure >40 psi—

The apparatus is assembled with proper flow directions for the columns, as shown in Figure 9, and is connected to the sample source via an appropriate fitting to the pressure regulator. When the Celite 545 column is required, e.g., for wastewaters, insert this column in-line between the glass wool column and the bacterial filter. When all of the filters and columns are connected in-line, turn on the water and partially open the valve to gently displace the acetone. Following the passage of one system volume of water through the apparatus, open the valve fully and adjust the flow rate. If using the large columns, set the regulator to 30 to 35 psi. For use of the large columns, adjust the flow rate to no greater than 250 mL/min by setting the pressure regulator to no greater than 30 psi (Tabor and Loper 1984, Loper et al. 1984). For use of the small columns, adjust the flow rate to no greater than 100 mL/min.

8.3 Apparatus for Drinking Water Sources at Line Pressures <40 psi--
The apparatus is assembled with proper flow directions for the columns, as shown in Figure 9.

8.3.1 Displacement of drinking water through the collection apparatus using nitrogen or helium--Connect the gas line to the inlet of a 20-L stainless steel reservoir containing the sample. Open the gas flow partially to gently displace the acetone. Following the passage of one system volume of water through the apparatus, further open the gas flow and adjust the tank pressure regulator to 30 psi. Flow rates can be regulated via the in-line valve. If the water sample is larger than 20 L or if the filters need to be changed, the flow may be stopped by closing the nitrogen tank and releasing the pressure via the pressure release valve on the reservoir. Following the required operation, changing of filters or connection to a new sample reservoir, close the pressure release valve and open the gas tank to resume flow; continue until all the sample has been displaced over the system.

8.3.2 Use of the in-line pump--The drinking water supply is connected to the pump inlet with appropriate fittings. This connecting line must be filled with water before the pump is turned on. The apparatus is connected to the pump, and the drinking water sample is pumped through the system. Note that a gentle flow of water is required in the beginning (Section 8.2) to displace the acetone.

8.4 Bacterial Filters--
When the flow rate decreases to approximately 50% of the initial rate, the bacterial filters probably need to be replaced. If the majority of the sample, >90%, has been concentrated, continue collection. If not, then discontinue the concentration operation, disassemble the bacterial filter holder and replace the filter(s) with fresh ones. The used filters are placed in amber bottles for storage until extraction. Handle these filters with forceps, since they are contaminated with bacteria, etc.

8.5 Volume Measurement--
The flow from the apparatus is collected in an appropriate measuring container. Usually 55-gal drums or smaller containers are used. At the end of the collection process, the total collected volume is recorded.

8.6 At the completion of a collection, the apparatus is disassembled in the order from the XAD-7 column, the last component in the system, to the drinking water source. All columns are sealed with stainless steel Swagelok plugs (Swagelok ss-400-P-316). Bacterial filters are removed from the holder and stored in amber bottles until extraction. Water in the glass wool column is allowed to drain, and the column is plugged as with the XAD columns. Columns and filters are stored at 4 C until extraction, usually within 3 days. The system should not be subjected to extremes of pressure or temperature.

8.7 Extraction of Residue Organics--
Resin columns and other system components, i.e., glass wool and filters, are extracted with a hexane:acetone solvent system, 85:15 by volume, according to the methods of LeBel et al. (1979), Loper et al. (1983, 1984) and Tabor and Loper (1984).

8.7.1 XAD columns—The XAD columns are individually mounted in an upright position so that the effluent end of the column is at the top. The end plugs on the top of the columns are removed, and the column is immediately connected via the top fitting to a pressure reservoir containing four to eight column volumes of hexane:acetone, 85:15 by volume, solvent. Following removal of the caps from the bottom of the column (i.e., the original inlet end), a length, 6 to 10 in, of 1/4-in stainless steel tubing is connected immediately to the bottom, and the free end of this tube is inserted into a cleaned glass separatory funnel to collect the water in the column. Pressure is gently applied (1 to 3 psi) to the system, and the column is allowed to fill with solvent. After the solvent has filled the column, stop the flow for equilibration for 15 to 20 min. The eluate tube is removed from the separatory funnel and inserted into a cleaned glass receiver. The system is pressurized for a flow rate not greater than one column volume per 20 min. The aqueous sample in the separatory funnel is extracted three times with equal volumes of pesticide-grade methylene chloride. These extracts are added to the solvent eluates from the column. The solvent eluates from the column are collected, passed through a small column (1 cm by 3 cm for the 25-cc system and 1 cm by 10 cm for the 200-cc system) of anhydrous sodium sulfate, and the eluates are collected in a cleaned round-bottom flask for initial concentration via rotary evaporation.

8.7.2 Glass wool columns—The columns are mounted upright and connected to a solvent reservoir containing four column volumes of hexane:acetone, 85:15 by volume, solvent. The top of the column is fitted to a tube in a manner similar to the XAD columns. The column is filled with solvent, which is allowed to equilibrate for 20 min. Following the equilibration, the solvent is replaced with fresh solvent, and the process is repeated. The extracts are combined for concentration via rotary evaporation. The glass wool is removed for further extraction. This is accomplished by Soxhlet extraction for 10 h using a ratio of five volumes of the hexane:acetone solvent for each cc volume of glass wool. The Soxhlet extracts are dried as before (Section 8.7.1) and are reduced in volume by rotary evaporation.

8.7.3 Bacterial filters—Each filter change group is extracted with 100 ml of the hexane:acetone solvent by soaking the filter in the solvent contained in a beaker for 20 min. This process is repeated two more times. The combined extracts are reduced in volume by rotary evaporation.

8.8 Concentration of Reduced Volume Extracts—

Following rotary evaporation, the volume of each extract is measured and recorded. The volume of each extract is reduced further using the micro-Snyder apparatus. Usually a measured aliquot of an extract is concentrated at one time, rather than concentrating the whole extract. The sample is gently heated using the N-Evap[a] bath. As the solution is concentrated, some constituents may come out of solution. If so, a small volume of acetone may be added to keep the components in solution until sufficient evaporation of the solvent has occurred to azeotrope the remaining hexane from the sample.

(a) Registered trademark.

Usually three to four additions of acetone are required. Final volumes of the acetone concentrates of the residue organic samples are recorded, and these samples are stored in Teflon-capped amber vials at -20 C until mutagenicity testing. At the time of bioassay, an aliquot of the residue solution is removed from the sample vial; typically, this aliquot is adjusted to necessary bioassay volume with DMSO. If it is necessary to know the mass of residue organics per dose, the amount of organics should be determined gravimetrically on a separate aliquot of the acetone solution of residue organics.

9.0 Quality Control

9.1 Solvent Blanks--

Samples of each lot of the hexane and acetone elution solvents and the methylene chloride extraction solvent are concentrated for mutagenesis testing. Two liters of each solvent and 2 L of the 85:15 hexane:acetone solvent are reduced in volume via rotary evaporation to 20 mL. Each sample is further concentrated to 0.2 mL via the micro-Snyder evaporative concentrator. The residue concentrate is mixed with 400 µL of DMSO and submitted for mutagenicity testing, two doses in duplicate, with Salmonella tester strains TA98 and TA100, in the absence and presence of metabolic activation (Loper et al. 1982, 1984). If the mutagenic response for any of the bioassay tests is equal to or greater than double the spontaneous rate, the solvent lot is rejected.

9.2 Resin Blanks--

9.2.1 Chemical contamination--The general procedure (USEPA 1978) for residual extractable organics is followed to determine contamination of the cleaned XAD resins. For each resin, a 20-g sample of resin is extracted for 22 h with 200 mL of acetone using a Soxhlet extractor. The 200-mL extracts are reduced in volume to 10 mL via evaporation under nitrogen, as described in Section 8.8. The concentrated extracts are analyzed by gas chromatography according to the USEPA total chromatographable organics analysis procedure (USEPA 1978). In this procedure, 5 µL of the extract are injected into a flame ionization gas chromatographic unit fitted with a 6-ft by 4-mm glass column containing 10% OV-101 (or equivalent) on 100/120 mesh GAS-CHROM Qm. Gas chromatography conditions: injector temperature 300 C, initial oven temperature 50 C, final oven temperature 250 C, temperature program rate 20 C/min, nitrogen carrier gas flowing at 40 mL/min, sensitivity 8 x, recorder range 1 mV. Resins are recleaned if the chromatograms of resin extracts show peaks greater than 10% full-scale eluting 5 min or later after injection.

The small sample volume apparatus (Section 6.1.6.2) is assembled as described. Two liters of the 85:15 hexane:acetone are eluted through the system and concentrated. Repeat this process until a gas chromatographic run of background is constant ±10%. Pass 1 L acetone through the columns, followed by 40 L ASTM Type I water. Elute columns per Section 8.7, and bioassay this residue. Mutagenic response of 1,000:1 concentrates should be less than 2x spontaneous reversion rate in the assay.

In cases where the organic residue is to be used for chemical analysis, it is desirable to characterize the solvent and water blank elutions by GC/MS or other specific analyses. In these cases, it is also recommended

that chlorinated waters be dechlorinated with ferrous citrate (Cheh et al. 1979) prior to concentration in order to prevent resin artifacts from interfering with chemical analyses.

10.0 Sample Storage

10.1 Acetone concentrates of the residue organics are stored in Teflon-capped amber vials containing an inert gas (nitrogen or helium) atmosphere at -20 C until mutagenicity testing or further chemical analysis. The initial mutagenicity testing should be conducted within two weeks, since it has been noted that the bioactivity of some residue organics decreases with time (Loper and Tabor, unpublished).

10.2 If the initial bioassay of the residue organics results in toxicity or a no-dose response, a second portion (50- to 100-L equivalents) of residue organics is separated via HPLC, and the collected fractions of eluates are bioassayed for mutagenicity. The HPLC methodology is detailed in the protocol attendant to this overall document on the preparation of residue organics.

11.0 Data Records

11.1 Records to be maintained include a general description of the practices used in water treatment at the purification plant supplying the drinking water being sampled; time and location of sample collection; operation parameters for sample concentration, including flow rates, pressures, filter changes and volumes; details of resin preparation; data on quality control of resins and solvents; volumes of concentrated extracts and storage data and any unusual occurrences during the collection operation. Chain-of-custody forms should be executed for each sample from the time of preparation of the columns and of concentration of the water residue organics through the mutagenesis testing.

11.2 These records will be maintained for the glass wool extracts, the bacterial filter extracts, each XAD resin extract, the companion blank water system sample and each lot of solvents used in the extraction of a sample set.

12.0 Calculations

12.1 From data records, the final volume and/or weight of each concentrate is related directly back to the number of liters of drinking water concentrated. Mutagenesis data will be reported in terms of number of revertants or mutations per liter equivalent of water. Therefore, it is imperative that accurate records of all volumes be maintained throughout all operations.

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APPENDIX C

SAMPLE COLLECTION INFORMATION CENTER

For the purpose of chain-of-custody and sample collection information, a sample collection record form was filled out by the field manager at the time of collection. The record included pertinent information relating to sample collection procedures, sample type and appearance, any treatments performed, and storage and shipment methods. All of the completed forms obtained in this project are attached to this report as Appendix C.

SAMPLE COLLECTION RECORD

I. SAMPLE INFORMATION

1. Sample No. NBS Reference Sludge-Toxicity Characteristics Leachate Procedure (TCLP) Extract Leachate, AIHL-85-0401 Collection Date 7/9/85 & 9/24/85
2. Sampling Site (a) AIHL
3. Field Sampling Manager (on-site) Yi Y. Wang, AIHL
4. Contractor AIHL-CPHF Contract No. CR810022-02-0
5. EPA Project Officer L.R. Williams Program Name Quality Assurance
6. Source Sampled 16 bottles of EPA/NBS reference sludge (RCRA EP-Inorganics, Sept., 1981, 100 gm/bottle. No.111401-111416) provided by L.R. Williams, EPA
7. Discharge Rate of Source (Volume/Time) N/A
8. Quantity Sampled/Units 3 to 6 L of TCLP leachate, freshly prepared for each liquid-liquid extraction method evaluation experiment
9. Sample description (liquid, slurry, solid extract, appearance, etc.) odorless transparent aqueous liquid without visible suspended solids, no sediments
10. Other Information as Applicable

    Collection Temp. room temperature Sampling Location AIHL

    pH adjust to 5.0 Sampling Technique TCLP extraction

    Other none

II. HANDLING & SHIPPING

1. Describe Sample Treatment Prior to Shipping (e.g., transfers, extractrants, stored undiluted, grinding, solvents used) TCLP extraction: sodium acetate buffer extraction with shaking, centrifugation, filtration; prepared at AIHL, no transportation involved
2. Field Storage and Shipping Conditions

Container	Temperature	Light
X Amber Glass	X Ambient	X Shield from light
___ Polyethylene Bottle	___ Refrigerate (0° to 4°C)	
___ Coated Bag or Bottle	___ Freeze (-20°C)	
___ Teflon or Tedlar Bags	___ Dry Ice	
___ Other ___		

3. Approximate Time in Storage and Time in Shipping none
4. Sample shipped to N/A
5. Mode and Carrier for Shipping N/A
6. Comments The EPA/NBS sludge sample was shipped by Federal Express from EPA, Las Vegas, to AIHL. Once received, it was stored in a refrigerator (0 to 4°C).

(This form should be completed by the on-site sampling manager and accompany each sample.)

(a) Graphically illustrate site of collection.

N/A

SAMPLE COLLECTION RECORD

I. SAMPLE INFORMATION

1. Sample No. Stringfellow contaminated groundwater from an on-site well, OW-2, AIHL-85-0402 Collection Date 7/9/85
2. Sampling Site [a] Stringfellow Hazardous Waste Site
3. Field Sampling Manager (on-site) Wilson Hom, SAIC
4. Contractor SAIC/CDHS-TSCD Contract No. collaboration
5. EPA Project Officer L.R. Williams Program Name Quality Assurance
6. Source Sampled on-site extraction well, OW-2
7. Discharge Rate of Source (Volume/Time) N/A
8. Quantity Sampled/Units collected in 10 1-gallon bottles
9. Sample description (liquid, slurry, solid extract, appearance, etc.) clear light brown aqueous liquid without visible suspended solids, no sediments, foamed when poured
10. Other Information as Applicable

Collection Temp. ambient Sampling Location OW-2

pH 3.2-3.5 Sampling Technique spigot on the well-head

Other slight sulfurous odor, collected after purge

II. HANDLING & SHIPPING.

1. Describe Sample Treatment Prior to Shipping (e.g., transfers, extractrants, stored undiluted, grinding, solvents used) shipped in the original collection containers at ambient temperature, stored at 4°C at AIHL-CDHS
2. Field Storage and Shipping Conditions

Container	Temperature	Light
X Amber Glass	X Ambient (collection & shipping)	X Shield from light
___ Polyethylene Bottle	X Refrigerate (0° to 4°C) (storage at CDHS)	
___ Coated Bag or Bottle	___ Freeze (-20°C)	
___ Teflon or Tedlar Bags	___ Dry Ice	
___ Other ___		

3. Approximate Time in Storage and Time in Shipping storage approximately 4 hr. before shipping, approximately 36 hr. shipping
4. Sample shipped to AIHL-CDHS, 2151 Berkeley Way, Berkeley, CA 94704
5. Mode and Carrier for Shipping Federal Express- Aircraft with air freight coolers
6. Comments 5 1-gallon bottles of ASTM Type I distilled water were provided by SAIC as the field and travel blank, and processed at AIHL later as the method blank.

(This form should be completed by the on-site sampling manager and accompany each sample.)

(a) Graphically illustrate site of collection.

SAMPLE COLLECTION RECORD

I. SAMPLE INFORMATION

1. Sample No. ~~AIHL-83-042A~~ Stringfellow contaminated groundwater from an upgradient well, UGB-8, Collection Date ~~9/27/83~~
2. Sampling Site Stringfellow Hazardous Waste Site
3. Field Sampling Manager (on-site) Wilson Hom, SAIC
4. Contractor SAIC/CDHS-TSCD Contract No. collaboration
5. EPA Project Officer L.R. Williams Program Name Quality Assurance
6. Source Sampled upgradient well, UGB-8
7. Discharge Rate of Source (Volume/Time) 30 gallons per minute
8. Quantity Sampled/Units collected in 10 1-gallon containers
9. Sample description (liquid, slurry, solid extract, appearance, etc.) clear colorless odorless aqueous liquid without visible suspended solids, no ~~sediments, no foaming when poured~~
10. Other Information as Applicable

Collection Temp. ambient Sampling Location UGB-8

pH 7.1-7.3 Sampling Technique hosing with a pump

Other hosing directly after 4000 gallon purge

II. HANDLING & SHIPPING

1. Describe Sample Treatment Prior to Shipping (e.g., transfers, extractants, stored undiluted, grinding, solvents used) no sample treatment, shipped in the original collection containers at ambient temperature, stored at 4°C at AIHL-CDHS
2. Field Storage and Shipping Conditions

Container	Temperature	Light
X Amber Glass	X Ambient collection & shipping	X Shield from light
___ Polyethylene Bottle	X Refrigerate (0° to 4°C) (storage)	
___ Coated Bag or Bottle	___ Freeze (-20°C)	
___ Teflon or Tedlar Bags	___ Dry Ice	
___ Other ___		

3. Approximate Time in Storage and Time in Shipping ~~before~~ stored for approximately 3 days Shipping, approximately 3 days shipping
4. Sample shipped to AIHL-CDHS, 2151 Berkeley Way, Berkeley, CA 94704
5. Mode and Carrier for Shipping UPS with air freight coolers
6. Comments SAIC laboratory distilled ASTM Type I water was served as the field, travel, and method blank.

(This form should be completed by the on-site sampling manager and accompany each sample.)

(a) Graphically illustrate site of collection.

SAMPLE COLLECTION RECORD

I. SAMPLE INFORMATION

1. Sample No. ~~AIHL-85-0403~~ Effluent from a municipal wastewater treatment plant, Collection Date 7/24/85
2. Sampling Site [a] a local municipal wastewater treatment plant
3. Field Sampling Manager (on-site) Harold J. Singer, CWQCB
4. Contractor Calif. State Water Quality Control Board Contract No. collaboration
5. EPA Project Officer L.R. Williams Program Name Quality Assurance
6. Source Sampled municipal wastewater effluents
7. Discharge Rate of Source (Volume/Time) N/A
8. Quantity Sampled/Units collected in 2 5-gallon containers
9. Sample description (liquid, slurry, solid extract, appearance, etc.) clear aqueous liquid with light yellow tint, some fine particulate suspension, no sediments
10. Other Information as Applicable

Collection Temp. ambient Sampling Location on-site pipe/faucet

pH 6.2-6.5 Sampling Technique faucet

Other swampy sewage odor

II. HANDLING & SHIPPING

1. Describe Sample Treatment Prior to Shipping (e.g., transfers, extractrants, stored undiluted, grinding, solvents used) shipped in the original collection containers at ambient temperature, stored at 4°C at AIHL-CDHS
2. Field Storage and Shipping Conditions

Container	Temperature	Light
X Amber Glass	X Ambient (collection & shipping)	X Shield from light
____ Polyethylene Bottle	X Refrigerate (0° to 4°C) (storage at CDHS)	
____ Coated Bag or Bottle	____ Freeze (-20°C)	
____ Teflon or Tedlar Bags	____ Dry Ice	
____ Other ____		

3. Approximate Time in Storage and Time in Shipping no storage, 10 min. shipping
4. Sample shipped to AIHL-CDHS, 2151 Berkeley Way, Berkeley, CA 94704
5. Mode and Carrier for Shipping automobile
6. Comments 2 1-gallon ASTM Type I distilled water, provided by AIHL, was brought to the site and returned to AIHL as the field, travel, and method blank.

(This form should be completed by the on-site sampling manager and accompany each sample.)

(a) Graphically illustrate site of collection.

N/A

SAMPLE COLLECTION RECORD

I. SAMPLE INFORMATION

1. Sample No. ~~AIHL-85-0404~~ Brackish San Francisco Bay surface water Collection Date 9/4/85
2. Sampling Site (a) Berkeley Marina pier
3. Field Sampling Manager (on-site) Yi Y. Wang, AIHL and M.J. DiBartolomeis, CPHF
4. Contractor AIHL-CPHF Contract No. CR810022-02-0
5. EPA Project Officer L.R. Williams Program Name Quality Assurance
6. Source Sampled San Francisco Bay water
7. Discharge Rate of Source (Volume/Time) N/A
8. Quantity Sampled/Units collected in 12 1-gallon bottles
9. Sample description (liquid, slurry, solid extract, appearance, etc.) clear salty aqueous liquid without visible suspended solids, no debris, no sediments, ~~no unusual odor~~
10. Other Information as Applicable

Collection Temp. ambient Sampling Location end of the pier, northern side

pH 7.8-8.0 Sampling Technique grab

Other briny odor

II. HANDLING & SHIPPING

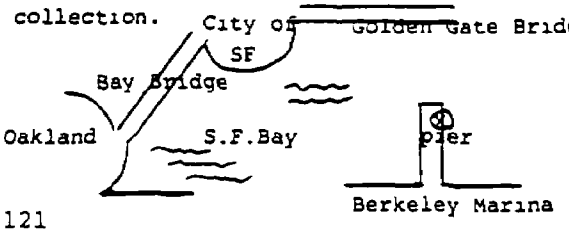
1. Describe Sample Treatment Prior to Shipping (e.g., transfers, extractrants, stored undiluted, grinding, solvents used) no sample treatment, shipped in the original collection containers at ambient temperature, storage at 4°C at AIHL-CDHS
2. Field Storage and Shipping Conditions

Container	Temperature	Light
X Amber Glass	X Ambient (collection & shipping)	X Shield from light
____ Polyethylene Bottle	X Refrigerate (0° to 4°C) (storage)	
____ Coated Bag or Bottle	____ Freeze (-20°C)	
____ Teflon or Tedlar Bags	____ Dry Ice	
____ Other ____		

3. Approximate Time in Storage and Time in Shipping no storage, 10 min. shipping
4. Sample shipped to AIHL-CDHS, 2151 Berkeley Way, Berkeley, CA 94704
5. Mode and Carrier for Shipping automobile
6. Comments AIHL laboratory distilled ASTM Type I water was served as the field, travel, and method blank.

(This form should be completed by the on-site sampling manager and accompany each sample.)

(a) Graphically illustrate site of collection.



SAMPLE COLLECTION RECORD

I. SAMPLE INFORMATION

1. Sample No. ~~AIHL 85 0405~~ Surface runoff from a class I hazardous waste landfill Collection Date 9/12/85
2. Sampling Site (a) an on-site pond
3. Field Sampling Manager (on-site) Harold J. Singer, CWQCB
4. Contractor Calif. State Water Quality Control Board Contract No. collaboration
5. EPA Project Officer L.R. Williams Program Name Quality Assurance
6. Source Sampled runoff from a hazardous waste disposal facility
7. Discharge Rate of Source (Volume/Time) none
8. Quantity Sampled/Units collected in 10 1-gallon containers
9. Sample description (liquid, slurry, solid extract, appearance, etc.) clear light yellow odorless aqueous liquid without visible suspended solids, no ~~sediments~~
10. Other Information as Applicable

Collection Temp. ambient Sampling Location on-site pond

pH 7.2-7.6 Sampling Technique grab

Other none

II. HANDLING & SHIPPING

1. Describe Sample Treatment Prior to Shipping (e.g., transfers, extractrants, stored undiluted, grinding, solvents used) no sample treatment, shipped in the original collection containers at ambient temperature, storage at 4°C at AIHL-CDHS
2. Field Storage and Shipping Conditions

Container	Temperature	Light
X Amber Glass	X Ambient (collection & shipping)	X Shield from light
____ Polyethylene Bottle	X Refrigerate (0° to 4°C) (storage)	
____ Coated Bag or Bottle	____ Freeze (-20°C)	
____ Teflon or Tedlar Bags	____ Dry Ice	
____ Other ____		

3. Approximate Time in Storage and Time in Shipping no storage, 45 min. shipping
4. Sample shipped to AIHL-CDHS, 2151 Berkeley Way, Berkeley, CA 94704
5. Mode and Carrier for Shipping automobile
6. Comments AIHL laboratory distilled ASTM Type I water was served as the field, travel, and method blank.

(This form should be completed by the on-site sampling manager and accompany each sample.)

(a) Graphically illustrate site of collection.

N/A

SAMPLE COLLECTION RECORD

I. SAMPLE INFORMATION

1. Sample No. Brackish surface water at the discharge site of an industrial wastewater ~~treatment plant, AIHL 85-044A~~ Collection Date 11/13/85
2. Sampling Site [a] effluent discharge site in the San Francisco Bay
3. Field Sampling Manager (on-site) Yi Y. Wang, AIHL
4. Contractor AIHL-CPHF Contract No. CR810022-02-0
5. EPA Project Officer L.R. Williams Program Name Quality Assurance
6. Source Sampled mix of the brackish bay water and industrial effluents
7. Discharge Rate of Source (Volume/Time) more than 1 million gallon per day
8. Quantity Sampled/Units collected in 3 1-gallon bottles
9. Sample description (liquid, slurry, solid extract, appearance, etc.) clear salty aqueous liquid without visible suspended solids, no sediments, ~~lightly oily odor~~
10. Other Information as Applicable

    Collection Temp. ambient Sampling Location mixing point of ~~the discharge site~~

    pH 7.7 Sampling Technique grab

    Other briny, slightly oily odor

II. HANDLING & SHIPPING

1. Describe Sample Treatment Prior to Shipping (e.g., transfers, extractrants, stored undiluted, grinding, solvents used) shipped in the original collection containers at ambient temperature, storage at 4°C at AIHL-CDHS
2. Field Storage and Shipping Conditions

Container	Temperature	Light
X Amber Glass	X Ambient (collection & shipping)	X Shield from light
____ Polyethylene Bottle	X Refrigerate (0° to 4°C) (storage at CDHS)	
____ Coated Bag or Bottle	____ Freeze (-20°C)	
____ Teflon or Tedlar Bags	____ Dry Ice	
____ Other ____		

3. Approximate Time in Storage and Time in Shipping storage approximately 3 hr. before ~~shipping, 30 min. shipping~~
4. Sample shipped to AIHL-CDHS, 2151 Berkelev Wav, Berkeley, CA 94704
5. Mode and Carrier for Shipping automobile
6. Comments AIHL laboratory distilled ASTM Type I water was served as the field, travel, and method blank.

(This form should be completed by the on-site sampling manager and accompany each sample.)

(a) Graphically illustrate site of collection.

N/A

SAMPLE COLLECTION RECORD

I. SAMPLE INFORMATION

1. Sample No. Effluent from an industrial wastewater treatment plant AIHL-83-0406 Collection Date 9/17/85
2. Sampling Site [a] a local industrial wastewater treatment plant
3. Field Sampling Manager (on-site) Harold J. Singer, CWQCB
4. Contractor Calif. State Water Quality Control Board Contract No. collaboration
5. EPA Project Officer L.R. Williams Program Name Quality Assurance
6. Source Sampled industrial wastewater effluents
7. Discharge Rate of Source (Volume/Time) 2 million gallons per day
8. Quantity Sampled/Units collected in 10 1-gallon containers
9. Sample description (liquid, slurry, solid extract, appearance, etc.) clear light yellow aqueous liquid without visible suspended solids, no sediments
10. Other Information as Applicable

Collection Temp. ambient Sampling Location on-site pipe

pH 7.1-7.5 Sampling Technique grab

Other musty with oily/petroleum odor

II. HANDLING & SHIPPING

1. Describe Sample Treatment Prior to Shipping (e.g., transfers, extractrants, stored undiluted, grinding, solvents used) no sample treatment, shipped in the original collection containers at ambient temperature, storage at 4°C at AIHL-CDHS
2. Field Storage and Shipping Conditions

Container	Temperature	Light
x Amber Glass	x Ambient (collection & shipping)	x Shield from light
_____ Polyethylene Bottle	x Refrigerate (0° to 4°C) (storage)	
_____ Coated Bag or Bottle	_____ Freeze (-20°C)	
_____ Teflon or Tedlar Bags	_____ Dry Ice	
_____ Other _____		

3. Approximate Time in Storage and Time in Shipping no storage, 1 hr. shipping
4. Sample shipped to AIHL-CDHS, 2151 Berkeley Way, Berkeley, CA 94704
5. Mode and Carrier for Shipping automobile
6. Comments AIHL laboratory distilled ASTM Type I water was served as the field, travel, and method blank.

(This form should be completed by the on-site sampling manager and accompany each sample.)

(a) Graphically illustrate site of collection.

N/A

APPENDIX D

PRIMARY AMES BIOASSAY DATA AND DOSE-RESPONSE CURVES FOR THE PROTOCOL VALIDATION STUDY

The primary data of the Ames assay and graphs of the dose-response curves for the protocol evaluation study using six generic types of environmental water and wastewater samples are attached as Appendix D. The data are recorded on the EPA-HERL (Health Effects Research Laboratory) In Vitro Result Form obtained from Dr. Larry D. Claxton, USEPA, Research Triangle Park, North Carolina. An explanation for the codes used on this form is listed in the following:

Research Lab ID: CPHF = California Public Health Foundation
AIHL = Air and Industrial Hygiene Laboratory

Experiment Date: Month, day, year of the date of the Ames assay performed

Test Sample Identification: See the Sample Identification Form for details

Activation Batch: CPHF-AIHL S-9 batch number

Test Type: 01 = standard plate incorporation assay

Strain Batch Number: CPHF-AIHL bacterial strain batch number

Mammalian S-9: R = male Sprague-Dawley rats
L = liver
A = Aroclor 1254-induced

Remarks Made: 2 = no remarks

Phenotype Check Conclusion: 1 = true mutants

Technician/Personnel: KIC = Kuo-In Chang, the technical leader for the Ames assay
YYW = Yi Y. Wang, the project manager

Solvent: 51 = distilled water
54 = dimethylsulfoxide

Positive Controls: 01 = sodium azide
03 = 2-nitrofluorene
04 = 2-aminoanthracene
07 = 2-aminofluorene
10 = benzo(a)pyrene
11 = 4-nitrobenzoic acid

Background: blank = normal background lawn
2 = partially clear, sparse lawn
3 = clear, no lawn
4 = contaminated
5 = precipitation and normal background lawn
6 = precipitation and partially clear
7 = precipitation and clear
8 = tiny pinpoint colonies with partially clear lawn
9 = tiny pinpoint colonies without background lawn

CR 810022-02-0

HERL IN VITRO SYSTEM
SAMPLE IDENTIFICATION FORM (INTERIM)

SECTION 4. ENVIRONMENTAL WATERS AND WASTEWATER.

System ID: IVS (2-4)

Lab (5-8)	Sample ID Yr (9-10)	No (11-14)	Sample Description (15-56)
AIHL	85	0401	NBS REFERENCE SLUDGE-TCLP LEACHATE
AIHL	85	0402	STRINGFELLOW CONTAMINATED GROUNDWATER OW2
AIHL	85	042A	STRINGFELLOW UPGRADIENT GROUNDWATER UGW8
AIHL	85	0403	MUNICIPAL WASTEWATER PLANT EFFLUENT
AIHL	85	0404	SAN FRANCISCO BAY BRACKISH SURFACE WATER
AIHL	85	044A	BRACKISH RECEIVING INDUSTRIAL EFFLUENT
AIHL	85	0405	SURFACE RUNOFF FROM A CLASS-I LANDFILL
AIHL	85	0406	INDUSTRIAL WASTEWATER PLANT EFFLUENT

Forms Completion

TABLE 12. MUTAGENICITY OF AN EPA/NBS REFERENCE SLUDGE - TCLP LEACHATE, SAMPLE NO. AIHL-85-0401

A. Results of Initial Screening Experiments

Exp. Date	Extraction Method[1]	Residue Weight (mg)	Sample Volume (L)	Specific Mutagenic Activity (revertants/mg)[2,3] TA98 -S9	TA98 2%S9	TA98 10%S9	TA100 -S9	TA100 2%S9	TA100 10%S9
7/9/85[5]	Base/Acid	5.3	3	887	1287	1071	674	600	812
9/24/85	Base/Acid	12.1	6	990	1149	918	< 460	< 483	< 502

B. Comparison of Mutagenic Response in TA98 with 2% S-9 Mix[4]

Exp. Date	Residue Weight (mg)	Sample Volume (L)	Specific Mutagenic Activity (revertants/mg)[2,3]	Mutagenic Response per Unit Volume (revertants/L)
7/9/85	5.3	3	1287	2274
9/24/85	12.1	6	1149	2308

1. The extraction method indicates the order of pH in liquid-liquid extraction.

2. The specific activity of the mutagenicity dose-response curve is represented by the slope (**b** as shown in the statistical analysis on graphs in Appendix D).

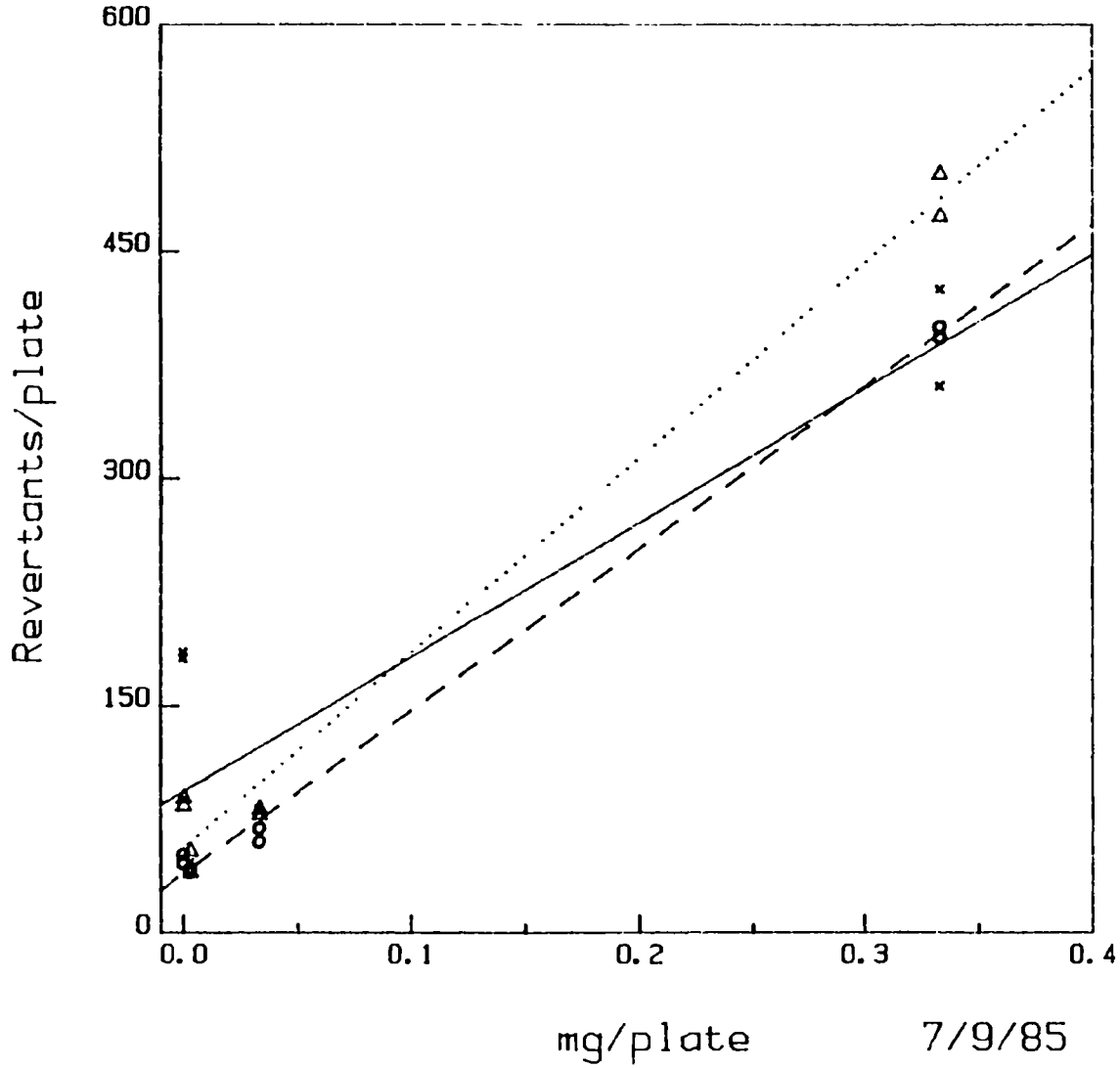
3. The spontaneous mutation and positive control values are listed on the Strain Function Tests, Cell Titer and Viability Record in Appendix F.

4. The optimum testing condition in the initial screening experiment was in TA98 with 2% S-9 mix.

5. Activity in the background control (sodium acetate butter) was observed; the screening experiment was repeated after making corrective actions.

TCLP-NBS WASTEWATER

Dichloromethane Extract



TA 98, -S9
SYMBOL=×
LINETYPE=————
y=a+b*x
n=8
a=93.0471 s_a=28.6295
b=887.0238 s_b=171.1038
$s_{y.x}$=67.5575 r=0.9042

TA 98, 2% S9
SYMBOL=△
LINETYPE=.....
y=a+b*x
n=8
a=56.3834 s_a=10.0801
b=1287.1720 s_b=60.2435
$s_{y.x}$=23.7862 r=0.9935

TA 98, 10% S9
SYMBOL=o
LINETYPE=— — — —
y=a+b*x
n=8
a=38.9795 s_a=3.8028
b=1070.6829 s_b=22.7274
$s_{y.x}$=8.9735 r=0.9987

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER	Activation Batch	Test Type (Table 10)	Strain	Batch No
	IVR	CPVHF	07 09 85	AIHL	85	0401		01	TA98	5501
				Test Sample Identification					Microorganism	

Animal
Organ
Inducer
[redacted] -59

A 64	B 65	C 66	D 67 70	E 71	F 72 73 74 75	G 76 78	80
2 Remarks Made? Yes 1	1 Phenocopy Check Conclusion (Table 13)	Sterility S 9 Mix 1 Not Contam 2 Contam 3 Not Checked	Activation Mixture Per Plate (µl)	1 Sample Sterility Check 1 Not Contam 2 Contam 3 Not Checked	Time (min) Temp (Cent) Pre-Incubation	Technician KIC	A Card Code

		Dose Level			Plate A		Plate B		Plate C		Plate D		Plate E		
	H Solvent Positive (Table 11)	I Units of Concentration Blank = mg/ml 2 = µg/ml	J Stock concentration (µg/plate)	K Amt Per Plate (µl)	L Count	M BG	N Count	O BG	P Count	Q BG	R Count	S BG	T Count	U BG	Card Code
	43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL	54			50	29		36		34						B
Pos	03		40	50	1606		1563		1621						C
			30	50	46		39								D
			330	50	80		82								E
			3330	50	361		425								F
buffer blank				50	185		181								G
water blank				50	38		34								H
															I
															J

Remarks Indicate Item Code and Card Code

Forms Completion
Initials

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER	Activation Batch	Test Type (Table 10)	Strain	Batch No
	IVR	CPHF	07 07 85	AIHL	85	0401	03122	01	TA98	8501

Microorganism

Animal	R
Organ	L
Inducer	A
[redacted]	2 % S9 Mix

(A) 64 Remarks Made? Yes 1: 2

(B) 65 Phenocopy Check Conclusion (Table 13): 1

(C) 66 Sterility S 9 Mix: 1 (1 Not Contam, 2 Contam, 3 Not Checked)

(D) 67 70 Activation Mixture Per Plate (µl): 500

(E) 71 Sample Sterility Check: 1 (1 Not Contam, 2 Contam, 3 Not Checked)

(F) 72 73 74 75 Pre-Incubation: Time (min) | Temp (Cent)

(G) 76 78 Technician: KIC

80 Card Code: A

(H) Solvent Positive (Table 11)	Dose Level: (I) Units of Concentration Blank = mg/ml 2 = µg/ml	Dose Level: (J) Stock concentration (µg/plate)	Dose Level: (K) Amt Per Plate (µl)	Plate A: (L) Count	Plate A: (M) BG	Plate B: (N) Count	Plate B: (O) BG	Plate C: (P) Count	Plate C: (Q) BG	Plate D: (R) Count	Plate D: (S) BG	Plate E: (T) Count	Plate E: (U) BG	Card Code
	43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL	54			50	42		32		25						B
Pos	07		10	50	610		535		517						C
			30	50	54		40								D
			330	50	82		78								E
			3330	50	473		501								F
buffer blank				50	87		84								G
water blank				50	40		42								H
															I
															J

Remarks Indicate Item Code and Card Code

Forms Completion Initials

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER	Activation Batch	Test Type (Table 10)	Strain	Batch No
	IVR	CPHF	07 09 85	AIHL	85	0401	03122	01	TA98	8501

Test Sample Identification

Microorganism

		A 64	B 65	C 66	D 67 70	E 71	F 72 73 74 75	G 76 78	80
Animal	R	2 Remarks Made? Yes 1	1 Phenocopy Check Conclusion (Table 13)	1 Sterility S 9 Mix 1 Not Contam 2 Contam 3 Not Checked	500 Activation Mixture Per Plate (µl)	1 Sample Sterility Check 1 Not Contam 2 Contam 3 Not Checked	Pre-Incubation Time (min) Temp (Cent)	KIC Technician	A Card Code
Organ	L								
Inducer	A								
[redacted]	10 % S9 Mix								

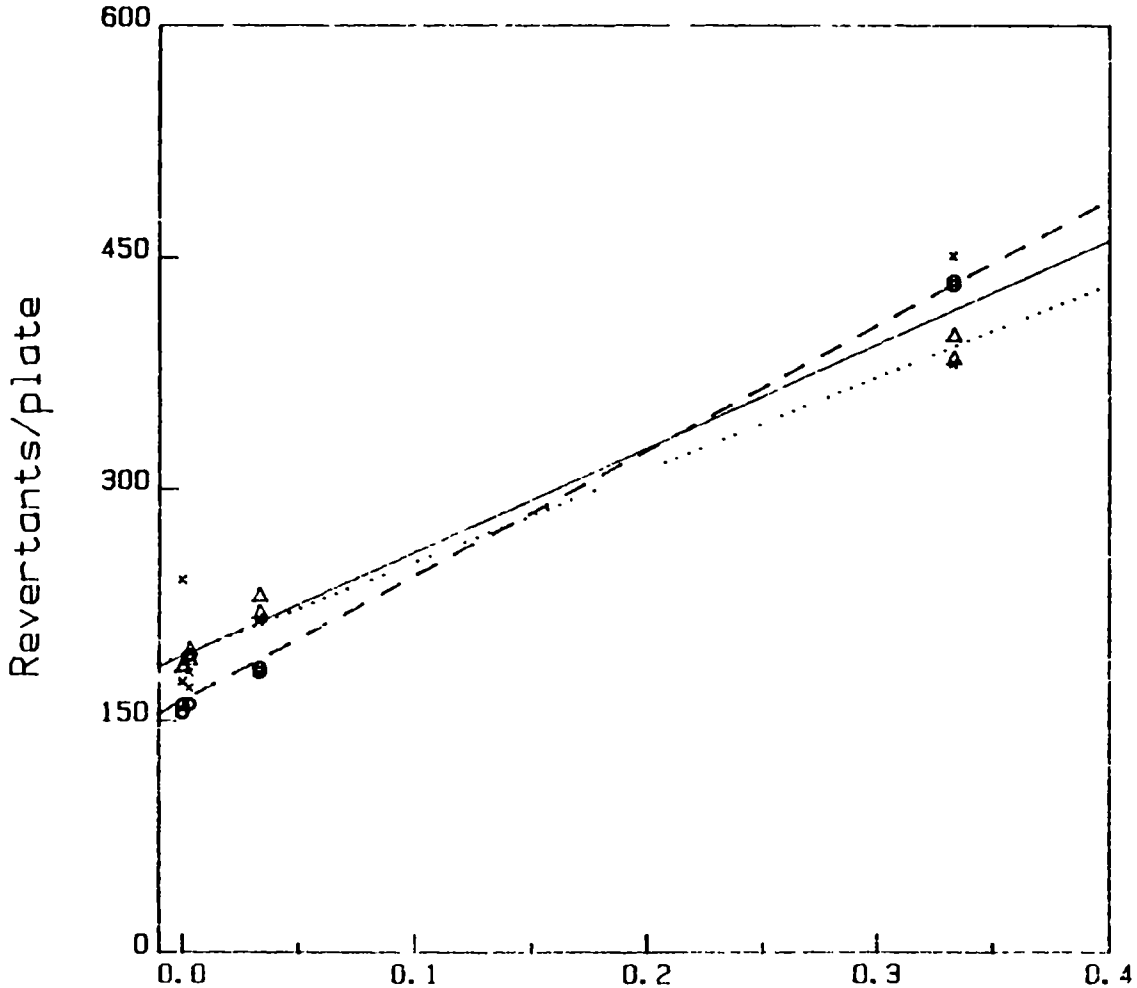
	Dose Level			Plate A		Plate B		Plate C		Plate D		Plate E		
H Solvent Positive (Table 11)	I Units of Concentration Blank = mg/ml 2 = µg/ml	J Stock concentration (µg/plate)	K Amt Per Plate (µl)	L Count	M BG	N Count	O BG	P Count	Q BG	R Count	S BG	T Count	U BG	Card Code
43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL 54			50	53		46		35						B
Pos 07		10	50	3579		326		418						C
		30	50	42		40								D
		330	50	69		60								E
		3330	50	393		400								F
buffer blank			50	52		46								G
water blank			50	48		54								H
														I
														J

Remarks Indicate Item Code and Card Code

Forms Completion Initials

TCLP-NBS WASTEWATER

Dichloromethane Extract



TA 100, -S9
SYMBOL=×
LINETYPE= ————
y=a+b*x
n=8
a=191.5958 s_a=13.2231
b=673.7580 s_b=79.0276
$s_{y.x}$=31.2027 r=0.9611

TA 100, 2% S9
SYMBOL=Δ
LINETYPE= .. ..
y=a+b*x
n=8
a=192.7421 s_a=4.3370
b=600.3570 s_b=25.9199
$s_{y.x}$=10.2341 r=0.9945

TA 100, 10% S9
SYMBOL=o
LINETYPE= — — — —
y=a+b*x
n=8
a=162.4421 s_a=5.2516
b=812.2805 s_b=31.3860
$s_{y.x}$=12.3922 r=0.9956

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER Test Sample Identification	Activation Batch	Test Type (Table 10)	Strain . Microorganism	Batch No
	IVR	CPHF	07 09 85	AIHL	85	0401		01	TA100	J505

Animal	(A) 64	(B) 65	(C) 66	(D) 67 70	(E) 71	(F) 72 73 74 75		(G) 76 78	80
Organ	2 Remarks Made? Yes 1	1 Phenocopy Check Conclusion (Table 13)	Sterility S 9 Mix 1 Not Contam 2 Contam 3 Not Checked	Activation Mixture Per Plate (µl)	1 Sample Sterility Check 1 Not Contam 2 Contam 3 Not Checked	Time (min)	Temp (Cent) Pre-Incubation	Technician	Card Code
Inducer									
[redacted] -59								KIC	A

	Dose Level				Plate A		Plate B		Plate C		Plate D		Plate E		
(H) Solvent Positive (Table 11)	(I) Units of Concentration Blank = mg/ml 2 = µg/ml	(J) Stock concentration (µg / plate)	(K) Amt Per Plate (µl)	(L) Count	(M) BG	(N) Count	(O) BG	(P) Count	(Q) BG	(R) Count	(S) BG	(T) Count	(U) BG	Card Code
43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL 54			50	1167		1177		2162						B
Pos 01		05	50	907		1040		952						C
		30	50	181		171								D
		330	50	216		214								E
		3330	50	451		381								F
buffer blank			50	175		241								G
water blank			50	178		185								H
														I
														J

Remarks Indicate Item Code and Card Code

Forms Completion
Initials

HERL IN VITRO RESULTS FORM

1	2-4	5-8	9-14	15-18	19-20	21-24	25-30	31-32	33-38	39-42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER Test Sample Identification	Activation Batch	Test Type (Table 10)	Strain	Batch No Microorganism
	IVR	CPHF	07 07 85	ALHL	85	0401	03122	01	TA100	8505

Animal	R
Organ	L
Inducer	A
[redacted]	2 % S9 Mix

(A) 64 Remarks Made? Yes 1: 2

(B) 65 Phenocopy Check Conclusion (Table 13): 1

(C) 66 Sterility S 9 Mix: 1 (1 Not Contam, 2 Contam, 3 Not Checked)

(D) 67-70 Activation Mixture Per Plate (µl): 500

(E) 71 Sample Sterility Check: 1 (1 Not Contam, 2 Contam, 3 Not Checked)

(F) 72-75 Pre-Incubation: Time (min) [ ] Temp (Cent) [ ]

(G) 76-78 Technician: KIC

80 Card Code: A

(H) Solvent Positive (Table 11)	43-44	(I) Units of Concentration Blank = mg/ml 2 = µg/ml	45	(J) Stock concentration (mg/plate)	46-50	(K) Amt Per Plate (µl)	51-54	Plate A (L) Count	55-58	Plate A (M) BG	59	Plate B (N) Count	60-63	Plate B (O) BG	64	Plate C (P) Count	65-68	Plate C (Q) BG	69	Plate D (R) Count	70-73	Plate D (S) BG	74	Plate E (T) Count	75-78	Plate E (U) BG	79	Card Code	80
SOL	54					50		199				170				164												B	
Pos	04			05		50		2547				2095				2724												C	
				30		50		189				195																D	
				330		50		219				230																E	
				3330		50		384				397																F	
buffer blank						50		185				184																G	
water blank						50		188				184																H	
																												I	
																												J	

Remarks Indicate Item Code and Card Code

Forms Completion Initials [ ]

HERL IN VITRO RESULTS FORM

1	2-4	5-8	9-14	15-18	19-20	21-24	25-30	31-32	33-38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER Test Sample Identification	Activation Batch	Test Type (Table 10)	Strain	Batch No
	IVR	CPHF	07 09 85	AIHL	85	0401	03122	01	7A100	8505

Microorganism

Animal	R
Organ	L
Inducer	A
[redacted]	10 % S9 Mix

(A) 64	(B) 65	(C) 66	(D) 67-70	(E) 71	(F) 72-73	74-75	(G) 76-78	80
Remarks Made? Yes-1	Phenocopy Check Conclusion (Table 13)	Sterility S-9 Mix 1-Not Contam 2-Contam 3-Not Checked	Activation Mixture Per Plate (µl)	Sample Sterility Check 1-Not Contam 2-Contam 3-Not Checked	Pre-Incubation Time (min)	Temp (Cent)	Technician	Card Code
2	1	1	500	1			KIC	A

	Dose Level				Plate A		Plate B		Plate C		Plate D		Plate E		
(H) Solvent Positive (Table 11)	(I) Units of Concentration Blank = mg/ml 2 = µg/ml	(J) Stock concentration (mg/plate)	(K) Amt. Per Plate (µl)	(L) Count	(M) BG	(N) Count	(O) BG	(P) Count	(Q) BG	(R) Count	(S) BG	(T) Count	(U) BG	Card Code
43-44	45	46-50	51-54	55-58	59	60-63	64	65-68	69	70-73	74	75-78	79	80
SOL 54			50	1146		1168		1155						B
Pos. 04		05	50	1002		867		800						C
		30	50	192		160								D
		330	50	184		181								E
		3330	50	432		435								F
buffer blank			50	155		160								G
water blank			50	168		175								H
														I
														J

Remarks: Indicate Item Code and Card Code

Forms Completion Initials

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER Test Sample Identification	Activation Batch	Test Type (Table 10)	Strain	Batch No
	IVR	CPHF	07 22 85	AIHL	85	0401		01	TA98	8501

Microorganism

Animal
Organ
Inducer
[redacted] −S9

(A) 64 Remarks Made? Yes 1: 2

(B) 65 Phenocopy Check Conclusion (Table 13): 1

(C) 66 Sterility S 9 Mix: 1 Not Contam, 2 Contam, 3 Not Checked:

(D) 67 70 Activation Mixture Per Plate (µl):

(E) 71 Sample Sterility Check: 1 Not Contam, 2 Contam, 3 Not Checked: 1

(F) 72 73 74 75 Pre-Incubation: Time (min) | Temp (Cent)

(G) 76 78 Technician: KIC

80 Card Code: A

		Dose Level			Plate A		Plate B		Plate C		Plate D		Plate E		
	(H) Solvent Positive (Table 11)	(I) Units of Concentration Blank = mg/ml 2 = µg/ml	(J) Stock concentration (µg/plate)	(K) Amt Per Plate (µl)	(L) Count	(M) BG	(N) Count	(O) BG	(P) Count	(Q) BG	(R) Count	(S) BG	(T) Count	(U) BG	Card Code
	43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL	54			50	36		28		235						B
Pos	03		40	50	1202		1525		1355						C
buffer blank				50	63		79		103		84		76		D
water blank				50	33		36		37						E
			1												F
															G
															H
															I
															J

Remarks Indicate Item Code and Card Code

Forms Completion
Initials

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER Test Sample Identification	Activation Batch	Test Type (Table 10)	Strain Microorganism	Batch No
	IVR	CPHF	072285	AIVHL	85	0401		01	1TA98	8501

Animal / Organ / Inducer	(A) 64 Remarks Made? Yes 1	(B) 65 Phenocopy Check Conclusion (Table 13)	(C) 66 Sterility S 9 Mix 1 Not Contam 2 Contam 3 Not Checked	(D) 67 70 Activation Mixture Per Plate (µl)	(E) 71 Sample Sterility Check 1 Not Contam 2 Contam 3 Not Checked	(F) 72 73 74 75 Time (min) Temp (Cent) Pre-Incubation	(G) 76 78 Technician	80 Card Code
[redacted] -S9	2	1			1		KIC	A

(H) Solvent Positive (Table 11)	(I) Units of Concentration Blank = mg/ml 2 = µg/ml	(J) Stock concentration (µg/plate)	(K) Amt Per Plate (µl)	Plate A (L) Count	Plate A (M) BG	Plate B (N) Count	Plate B (O) BG	Plate C (P) Count	Plate C (Q) BG	Plate D (R) Count	Plate D (S) BG	Plate E (T) Count	Plate E (U) BG	Card Code
43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL 54			50	36		28		23						B
Pos 03		40	50	1202		1525		1355						C
cHaCl2 K-D residue			50	30		30		31		27		26		D
														E
														F
														G
														H
														I
														J

Remarks Indicate Item Code and Card Code

Forms Completion
Initials

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER Test Sample Identification	Activation Batch	Test Type (Table 10)	Strain Microorganism	Batch No
	IVR	CPHF	072285	AIHL	85	0401		01	TA100	8505

Animal	(A) 64	(B) 65	(C) 66	(D) 67 70	(E) 71	(F) 72 73 74 75	(G) 76 78	80
Organ	Remarks Made? Yes 1	Phenocopy Check Conclusion (Table 13)	Sterility S 9 Mix 1 Not Contam 2 Contam 3 Not Checked	Activation Mixture Per Plate (µl)	Sample Sterility Check 1 Not Contam 2 Contam 3 Not Checked	Time (min) Temp (Cent) Pre-Incubation	Technician	Card Code
Inducer	2	1			1		KIC	A
[redacted] -S9								

(H) Solvent Positive (Table 11)	Dose Level: (I) Units of Concentration Blank = mg/ml 2 = µg/ml	Dose Level: (J) Stock concentration (µg/plate)	Dose Level: (K) Amt Per Plate (µl)	Plate A: (L) Count	Plate A: (M) BG	Plate B: (N) Count	Plate B: (O) BG	Plate C: (P) Count	Plate C: (O) BG	Plate D: (R) Count	Plate D: (S) BG	Plate E: (T) Count	Plate E: (U) BG	Card Code
43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL 54			50	160		178		158						B
Pos 01		05	50	899		870		879						C
Chaetomium K-Residue			50	174		166		159		177		160		D
														E
														F
														G
														H
														I
														J

Remarks Indicate Item Code and Card Code

Forms Completion
Initials

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER Test Sample Identification	Activation Batch	Test Type (Table 10)	Strain	Batch No Microorganism
	IVR	CPHF	08 02 85	ALHL	85	0401		01	TA98	8507

Animal	(A) 64	(B) 65	(C) 66	(D) 67 70	(E) 71	(F) 72 73 74 75	(G) 76 78	80
Organ	Remarks Made? Yes 1	Phenocopy Check Conclusion (Table 13)	Sterility S 9 Mix 1 Not Contam 2 Contam 3 Not Checked	Activation Mixture Per Plate (µl)	Sample Sterility Check 1 Not Contam 2 Contam 3 Not Checked	Time (min) Temp (Cent) Pre-Incubation	Technician	Card Code
Inducer	2	1			1		KJC	A
[redacted] -59								

	Dose Level				Plate A		Plate B		Plate C		Plate D		Plate E		
(H) Solvent Positive (Table 11)	(I) Units of Concentration Blank = mg/ml 2 = µg/ml	(J) Stock concentration (µg/plate)	(K) Amt Per Plate (µl)	(L) Count	(M) BG	(N) Count	(O) BG	(P) Count	(Q) BG	(R) Count	(S) BG	(T) Count	(U) BG	Card Code
83 84	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL 54			50	23		35		23						B
Pos 03		40	50	1128		1230		1045						C
buffer residue without TCLP extraction			50	42		44		44		39		46		D
water blank			50	29		37		32						E
														F
														G
														H
														I
														J

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Remarks Indicate Item Code and Card Code

Forms Completion
Initials

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER Test Sample Identification	Activation Batch	Test Type (Table 10)	Strain .	Batch No Microorganism
	IVR	CPHI-	08 02 85	AIHL	85	0401		01	TA100	85 05

Animal
Organ
Inducer
[redacted] -59

(A) 64	(B) 65	(C) 66	(D) 67 70	(E) 71	(F) 72 73 74 75	(G) 76 78	80
2 Remarks Made? Yes 1	1 Phenocopy Check Conclusion (Table 13)	1 Sterility S 9 Mix 1 Not Contam 2 Contam 3 Not Checked	Activation Mixture Per Plate (µl)	1 Sample Sterility Check 1 Not Contam 2 Contam 3 Not Checked	Time (min) Temp (Cent) Pre-Incubation	Technician	A Card Code

	Dose Level			Plate A		Plate B		Plate C		Plate D		Plate E		
(H) Solvent Positive (Table 11)	(I) Units of Concentration Blank = mg/ml 2 = µg/ml	(J) Stock concentration (mg/plate)	(K) Amt Per Plate (µl)	(L) Count	(M) BG	(N) Count	(O) BG	(P) Count	(Q) BG	(R) Count	(S) BG	(T) Count	(U) BG	Card Code
43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL 54			50	1458		1482		1488						B
Pos 01		05	50	7118		6833		6881						C
buffer residue without TCLP extraction			30	1558		1655		1558		1633		1611		D 163
water blank			50	1738		1538		1712						E
														F
														G
														H
														I
														J

Remarks Indicate Item Code and Card Code

Forms Completion
Initials

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER Test Sample Identification	Activation Batch	Test Type (Table 10)	Strain .	Batch No
	IVR	CPHF	08 02 85	AIHL	85	0401		01	1 7A 78	85 07
									Microorganism	

Animal	(A) 64	(B) 65	(C) 66	(D) 67 70	(E) 71	(F) 72 73 74 75	(G) 76 78	80
Organ	2 Remarks Made? Yes 1	1 Phenocopy Check Conclusion (Table 13)	Sterility S 9 Mix 1 Not Contam 2 Contam 3 Not Checked	Activation Mixture Per Plate (µl)	1 Sample Sterility Check 1 Not Contam 2 Contam 3 Not Checked	Time (min) Temp (Cent) Pre-Incubation	Technician	A Card Code
Inducer								
[redacted] -S9								

	(H) Solvent Positive (Table 11)	(I) Units of Concentration Blank = mg/ml 2 = µg/ml	(J) Stock concentration (mg/plate)	(K) Amt Per Plate (µl)	Plate A (L) Count	Plate A (M) BG	Plate B (N) Count	Plate B (O) BG	Plate C (P) Count	Plate C (Q) BG	Plate D (R) Count	Plate D (S) BG	Plate E (T) Count	Plate E (U) BG	Card Code
	43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL	51			50	26		30		28						B
Pos	03		40	50	1128		1230		1045						C
CHSCO14 in dist'd H2O			10	50	24		31		24						D
			100	50	29		18		37						E
			3300	50	27		40		15						F
			1000	50	39		31		38						G
			3330	50	29		31		43						H
		1	0000	50	33		39		34						I
		3	0000	50	34		28		28						J

Remarks Indicate Item Code and Card Code

Forms Completion
Initials

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER Test Sample Identification	Activation Batch	Test Type (Table 10)	Strain .	Batch No Microorganism
	IVR	CPHE	08 02 85	AIHL	85	0401	0312 22	01	1 7A98	8507

Animal	R
Organ	L
Inducer	A
[redacted]	2% S9 Mix

A 64	B 65	C 66	D 67 70	E 71	F 72 73 74 75	G 76 78	80
2 Remarks Made? Yes 1	1 Phenocopy Check Conclusion (Table 13)	1 Sterility S 9 Mix 1 Not Contam 2 Contam 3 Not Checked	500 Activation Mixture Per Plate (µl)	1 Sample Sterility Check 1 Not Contam 2 Contam 3 Not Checked	Time (min) Temp (Cent) Pre-Incubation	KIC Technician	A Card Code

H	I	J	K	L	M	N	O	P	Q	R	S	T	U	
Solvent Positive (Table 11)	Units of Concentration Blank = mg/ml 2 = µg/ml	Dose Level: Stock concentration (mg/plate)	Dose Level: Amt Per Plate (µl)	Plate A: Count	Plate A: BG	Plate B: Count	Plate B: BG	Plate C: Count	Plate C: BG	Plate D: Count	Plate D: BG	Plate E: Count	Plate E: BG	Card Code
43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL 51			50	36		38		27						B
Pos 07		10	50	399		395		416						C
CH_3COONa Indistilled H_2O		100	50	30		47								D
		1000	50	38		31								E
		330	50	35		41								F
		100	50	44		50								G
		3330	50	42		42								H
	1	0000	50	53		45								I
	3	0000	50	55		40								J

Remarks Indicate Item Code and Card Code

D-142

Forms Completion
Initials

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER Test Sample Identification	Activation Batch	Test Type (Table 10)	Strain	Batch No Microorganism
	IVR	CPHF	08 02 85	AIVL	85	0401		01	TA100	8505

Animal	(A) 64	(B) 65	(C) 66	(D) 67 70	(E) 71	(F) 72 73 74 75	(G) 76 78	80
Organ	Remarks Made? Yes 1	Phenocopy Check Conclusion (Table 13)	Sterility S 9 Mix 1 Not Contam 2 Contam 3 Not Checked	Activation Mixture Per Plate (µl)	Sample Sterility Check 1 Not Contam 2 Contam 3 Not Checked	Time (min) Temp (Cent) Pre-Incubation	Technician	Card Code
Inducer								
[redacted] -39	2	1			1		KIC	A

	Dose Level				Plate A		Plate B		Plate C		Plate D		Plate E		
(H) Solvent Positive (Table 11)	(I) Units of Concentration Blank = mg/ml 2 = µg/ml	(J) Stock concentration (µg/plate)	(K) Amt Per Plate (µl)	(L) Count	(M) BG	(N) Count	(O) BG	(P) Count	(Q) BG	(R) Count	(S) BG	(T) Count	(U) BG	Card Code
43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL 51			50	1179		1161		1166						B
Pos 01		05	50	711		683		681						C
CH_3COONa in distilled H_2O		10	50	208		182								D
		100	50	165										E
		330	50	134		151								F
		1000	50	163		169								G
		3330	50	154		141								H
	1	0000	50	157		185								I
	3	0000	50	164		145								J

Remarks Indicate Item Code and Card Code

Forms Completion Initials

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER Test Sample Identification	Activation Batch	Test Type (Table 10)	Strain .	Batch No Microorganism
	IVR	CPHF	08 02 85	AZHL	85	0401	0312 2	01	TA100	8305

Animal	R
Organ	L
Inducer	A
[redacted]	2 % S9 Mix

(A) 64 Remarks Made? Yes 1: 2

(B) 65 Phenocopy Check Conclusion (Table 13): 1

(C) 66 Sterility S 9 Mix: 1 (1 Not Contam, 2 Contam, 3 Not Checked)

(D) 67 70 Activation Mixture Per Plate (µl): 500

(E) 71 Sample Sterility Check: 1 (1 Not Contam, 2 Contam, 3 Not Checked)

(F) 72 73 74 75 Time (min) Temp (Cent) Pre-Incubation

(G) 76 78 Technician: KIC

80 Card Code: A

(H) Solvent Positive (Table 11)	(I) Units of Concentration Blank = mg/ml 2 = µg/ml	(J) Stock concentration (x1 /plate)	(K) Amt Per Plate (µl)	Plate A (L) Count	Plate A (M) BG	Plate B (N) Count	Plate B (O) BG	Plate C (P) Count	Plate C (Q) BG	Plate D (R) Count	Plate D (S) BG	Plate E (T) Count	Plate E (U) BG	Card Code
	43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL	31			50	1351		1320		1432						B
Pos	04		05	50	2676		2870		2762						C
CH_3COONa in distilled H_2O			1.0	50	1774		1431								D
			10.0	50	1254		1411								E
			33.0	50	1599		1554								F
			100.0	50	1577		1755								G
			333.0	50	1588		1602								H
	1		0.0	50	1281		1304								I
	3		0.0	50	1354		1344								J

Remarks Indicate Item Code and Card Code

Forms Completion
Initials

D-144

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER Test Sample Identification	Activation Batch	Test Type (Table 10)	Strain Microorganism	Batch No
	IVR	CPHF	08 02 85	AZHL	85	0401		01	1 TA98	8507

Animal	(A) 64	(B) 65	(C) 66	(D) 67 70	(E) 71	(F) 72 73 74 75	(G) 76 78	80
Organ	2	1			1		KIC	A
Inducer	Remarks Made? Yes 1	Phenocopy Check Conclusion (Table 13)	Sterility S 9 Mix 1 Not Contam 2 Contam 3 Not Checked	Activation Mixture Per Plate (µl)	Sample Sterility Check 1 Not Contam 2 Contam 3 Not Checked	Time (min) Temp (Cent) Pre-Incubation	Technician	Card Code
[redacted] -S9								

	(H) Solvent Positive (Table 11)	(I) Units of Concentration Blank = mg/ml 2 = µg/ml	(J) Stock concentration (µg/plate)	(K) Amt Per Plate (µl)	Plate A (L) Count	Plate A (M) BG	Plate B (N) Count	Plate B (O) BG	Plate C (P) Count	Plate C (Q) BG	Plate D (R) Count	Plate D (S) BG	Plate E (T) Count	Plate E (U) BG	Card Code
	43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL	51			50	26		30		28						B
Pos	03		40	50	1128		1230		1045						C
CH3COOH in distilled H2O			(percent)/(plate)												D
			2.0 03	50	31		28								E
			1.0 3	50	28		17		31		15				F
			0.3	50	38		31								G
			3.	50	26	2	31	2							H
			30.	50	22	2	22	2							I
															J

Remarks Indicate Item Code and Card Code

Forms Completion
Initials

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER Test Sample Identification	Activation Batch	Test Type (Table 10)	Strain Microorganism	Batch No
	IVR	CAHF	08 02 85	AZVHL	85	0401	03122	01	1TA98	8507

Animal	R
Organ	L
Inducer	A
Microorganism	2% S9 Mix

(A) 64	(B) 65	(C) 66	(D) 67 70	(E) 71	(F) 72 73 74 75	(G) 76 78	80
Remarks Made? Yes 1	Phenocopy Check Conclusion (Table 13)	Sterility S 9 Mix 1 Not Contam 2 Contam 3 Not Checked	Activation Mixture Per Plate (µl)	Sample Sterility Check 1 Not Contam 2 Contam 3 Not Checked	Pre-Incubation: Time (min) Temp (Cent)	Technician	Card Code
2	1	1	500	1		KIC	A

(H) Solvent Positive (Table 11)	Dose Level: (I) Units of Concentration Blank = mg/ml 2 = µg/ml	(J) Stock concentration (µg/plate)	(K) Amt Per Plate (µl)	Plate A: (L) Count	(M) BG	Plate B: (N) Count	(O) BG	Plate C: (P) Count	(Q) BG	Plate D: (R) Count	(S) BG	Plate E: (T) Count	(U) BG	Card Code
43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL 51			500	336		338		229						B
Pos 07		10	50	399		375		416						C
CH3COOH in distilled H2O		(percent/plate)												D
		0.03	50	433		566								E
		0.03	50	355		466		433		566				F
		0.3	50	405		511								G
		3	50	782	2	312	2							H
		30	50	302	2	255	2							I
														J

Remarks Indicate Item Code and Card Code

Forms Completion Initials	

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER Test Sample Identification	Activation Batch	Test Type (Table 10)	Strain	Batch No Microorganism
	IVR	CMH	080285	ALHL	85	0401		01	TA100	8505

Animal	(A) 64	(B) 65	(C) 66	(D) 67 70	(E) 71	(F) 72 73 74 75	(G) 76 78	80
Organ	Remarks Made? Yes 1	Phenocopy Check Conclusion (Table 13)	Sterility S 9 Mix 1 Not Contam 2 Contam 3 Not Checked	Activation Mixture Per Plate (µl)	Sample Sterility Check 1 Not Contam 2 Contam 3 Not Checked	Time (min) Temp (Cent) Pre-Incubation	Technician	Card Code
Inducer	2	1			1		KIC	A
[redacted] — S9								

	Dose Level				Plate A		Plate B		Plate C		Plate D		Plate E		
(H) Solvent Positive (Table 11)	(I) Units of Concentration Blank = mg/ml 2 = µg/ml	(J) Stock concentration (µg/plate)	(K) Amt Per Plate (µl)	(L) Count	(M) BG	(N) Count	(O) BG	(P) Count	(Q) BG	(R) Count	(S) BG	(T) Count	(U) BG	Card Code
43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL 51			50	1179		1161		1166						B
Pos 01		05	50	711		653		631						C
CH_3COOH in distilled H_2O		(green update)												D
		0.03	50	150		149								E
		0.03	50	157		170								F
		0.3	50	145		150								G
		3	50	160	2	168	2							H
		30.	50	103	2	68	2							I
														J

Remarks Indicate Item Code and Card Code

Forms Completion
Initials

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER Test Sample Identification	Activation Batch	Test Type (Table 10)	Strain Microorganism	Batch No
	IVR	CFHF	08 02 85	AIHL	85	0401	03122	01	TA100	8505

		(A) 64	(B) 65	(C) 66	(D) 67 70	(E) 71	(F) 72 73 74 75	(G) 76 78	80
Animal	R	Remarks Made? Yes 1	Phenocopy Check Conclusion (Table 13)	Sterility S 9 Mix 1 Not Contam 2 Contam 3 Not Checked	Activation Mixture Per Plate (µl)	Sample Sterility Check 1 Not Contam 2 Contam 3 Not Checked	Time (min) Temp (Cent) Pre-Incubation	Technician	Card Code
Organ	L								
Inducer	A								
[redacted]	2% S9 Mix	2	1	1	500	1		KIC	A

	(H) Solvent Positive (Table 11)	(I) Units of Concentration Blank = mg/ml 2 = µg/ml	(J) Stock concentration (ug/plate)	(K) Amt Per Plate (µl)	Plate A (L) Count	Plate A (M) BG	Plate B (N) Count	Plate B (O) BG	Plate C (P) Count	Plate C (Q) BG	Plate D (R) Count	Plate D (S) BG	Plate E (T) Count	Plate E (U) BG	Card Code
	43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL	51			50	1151		1132		1143						B
Pos	04		05	50	2676		2870		2762						C
CH_3COOH in distilled H_2O			(percent/plate)												D
			0.003	50	1145		1143								E
			0.03	50	1158		1163								F
			0.3	50	1156		1145								G
			3.	50	1312	2	1116	2							H
			30.	50	1098	2	1125	2							I
															J

Remarks Indicate Item Code and Card Code

Forms Completion
Initials

HEAL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER Test Sample Identification	Activation Batch	Test Type (Table 10)	Strain (Microorganism)	Batch No
	IVR	CFHF	081685	AZHL	85	0401		01	7A98	0785

Animal	(A) 64	(B) 65	(C) 66	(D) 67 70	(E) 71	(F) 72 73 74 75	(G) 76 78	80
Organ	2 Remarks Made? Yes 1	1 Phenocopy Check Conclusion (Table 13)	Sterility S 9 Mix 1 Not Contam 2 Contam 3 Not Checked	Activation Mixture Per Plate (µl)	1 Sample Sterility Check 1 Not Contam 2 Contam 3 Not Checked	Time (min) Temp (Cent), Pre-Incubation	Technician	Card Code
Inducer							KZC	A
[redacted] – S9								

(H) Solvent Positive (Table 11)	(I) Units of Concentration Blank = mg/ml 2 = µg/ml	(J) Stock concentration (µg/plate)	(K) Amt Per Plate (µl)	Plate A (L) Count	Plate A (M) BG	Plate B (N) Count	Plate B (O) BG	Plate C (P) Count	Plate C (Q) BG	Plate D (R) Count	Plate D (S) BG	Plate E (T) Count	Plate E (U) BG	Card Code
	Dose Level													
	43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL	54			50	27		33		37						B
Pos	03		40	50	1035		1052		1368						C
buffer blank		TCLF without filtration		50	85		66		62						D
water blank				50	44		32		39						E
															F
															G
															H
															I
															J

Remarks Indicate Item Code and Card Code

Forms Completion
Initials

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER	Activation Batch	Test Type (Table 10)	Strain	Batch No
	IVR	CPHF	08/16/86	AZVHL	85	0401	03630	01	TA98	0785

Microorganism

		A 64	B 65	C 66	D 67 70	E 71	F 72 73 74 75	G 76 78	80
Animal	R	Remarks Made? Yes 1	Phenocopy Check Conclusion (Table 13)	Sterility S 9 Mix 1 Not Contam 2 Contam 3 Not Checked	Activation Mixture Per Plate (µl)	Sample Sterility Check 1 Not Contam 2 Contam 3 Not Checked	Time (min) Temp (Cent) Pre-Incubation	Technician	Card Code
Organ	L	2	1	1	300	1		KIC	A
Inducer	A								
[redacted]	2% S9 Mix								

	Dose Level			Plate A		Plate B		Plate C		Plate D		Plate E		
H Solvent Positive (Table 11)	I Units of Concentration Blank = mg/ml 2 = µg/ml	J Stock concentration (µg/plate)	K Amt Per Plate (µl)	L Count	M BG	N Count	O BG	P Count	O BG	R Count	S BG	T Count	U BG	Card Code
43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL 54			50	448		444		388						B
Pos 07		10	30	479		478		526						C
buffer blank	TCL Pwithout filtration		50	496		495		492						D
water blank			50	553		536		447						E
														F
														G
														H
														I
														J

Remarks Indicate Item Code and Card Code

Forms Completion
Initials

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER Test Sample Identification	Activation Batch	Test Type (Table 10)	Strain	Batch No Microorganism
	IVR	CHHF	082385	AIHL	85	0401		01	TA98	0785

Animal	(A) 64	(B) 65	(C) 66	(D) 67 70	(E) 71	(F) 72 73 74 75	(G) 76 78	80
Organ	Remarks Made? Yes 1	Phenocopy Check Conclusion (Table 13)	Sterility S 9 Mix 1 Not Contam 2 Contam 3 Not Checked	Activation Mixture Per Plate (µl)	Sample Sterility Check 1 Not Contam 2 Contam 3 Not Checked	Time (min) Temp (Cent) Pre-Incubation	Technician	Card Code
Inducer								
[redacted] -S9	2	1			1		KIC	A

	Dose Level				Plate A		Plate B		Plate C		Plate D		Plate E		
(H) Solvent Positive (Table 11)	(I) Units of Concentration Blank = mg/ml 2 = µg/ml	(J) Stock concentration (µg/plate)	(K) Amt. Per Plate (µl)	(L) Count	(M) BG	(N) Count	(O) BG	(P) Count	(Q) BG	(R) Count	(S) BG	(T) Count	(U) BG	Card Code
43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL 54			50	33		34		22						B
Pos 03		40	50	1088		1113		1021						C
buffer blank	TCLP with sinter-glass filtration		50	70		82		87						D
water blank			50	36		29		24						E
														F
														G
														H
														I
														J

Remarks Indicate Item Code and Card Code

Forms Completion
Initials

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER Test Sample Identification	Activation Batch	Test Type (Table 10)	Strain	Batch No Microorganism
	IVR	CPHV=	08 23 85	AIHL	85	0401	03630	01	17A98	07855

Animal	R
Organ	L
Inducer	A
[redacted]	2% S9 Mix

(A) 64	(B) 65	(C) 66	(D) 67 70	(E) 71	(F) 72 73 74 75	(G) 76 78	80
2 Remarks Made? Yes 1	1 Phenocopy Check Conclusion (Table 13)	1 Sterility S 9 Mix 1 Not Contam 2 Contam 3 Not Checked	500 Activation Mixture Per Plate (µl)	1 Sample Sterility Check 1 Not Contam 2 Contam 3 Not Checked	Time (min) Temp (Cent) Pre-Incubation	KIC Technician	A Card Code

(H) Solvent Positive (Table 11)	43 44	(I) Units of Concentration Blank = mg/ml 2 = µg/ml	45	Dose Level (J) Stock concentration (µg/plate)	46 50	(K) Amt Per Plate (µl)	51 54	Plate A (L) Count	55 58	(M) BG	59	Plate B (N) Count	60 63	(O) BG	64	Plate C (P) Count	65 68	(Q) BG	69	Plate D (R) Count	70 73	(S) BG	74	Plate E (T) Count	75 78	(U) BG	79	Card Code	80
SOL	54					50		553				453				544												B	
Pos	07			10		50		459				473				446												C	
buffer blank		TCHP with single-glass filtration				50		156				105				129												D	
water blank						50		45				58				47												E	
																												F	
																												G	
																												H	
																												I	
																												J	

Remarks Indicate Item Code and Card Code

Forms Completion
Initials

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER Test Sample Identification	Activation Batch	Test Type (Table 10)	Strain Microorganism	Batch No
	IVR	CPHF	08 23 85	AIHL	85	0401	03630	01	17A98	0785

Animal	12	(A) 64	(B) 65	(C) 66	(D) 67 70	(E) 71	(F) 72 73 74 75	(G) 76 78	80
Organ	L	2 Remarks Made? Yes 1	1 Phenocopy Check Conclusion (Table 13)	1 Sterility S 9 Mix 1 Not Contam 2 Contam 3 Not Checked	500 Activation Mixture Per Plate (µl)	1 Sample Sterility Check 1 Not Contam 2 Contam 3 Not Checked	Time (min) Temp (Cent) Pre-Incubation	KIC Technician	A Card Code
Inducer	A								
[redacted]	10% S9 Mix								

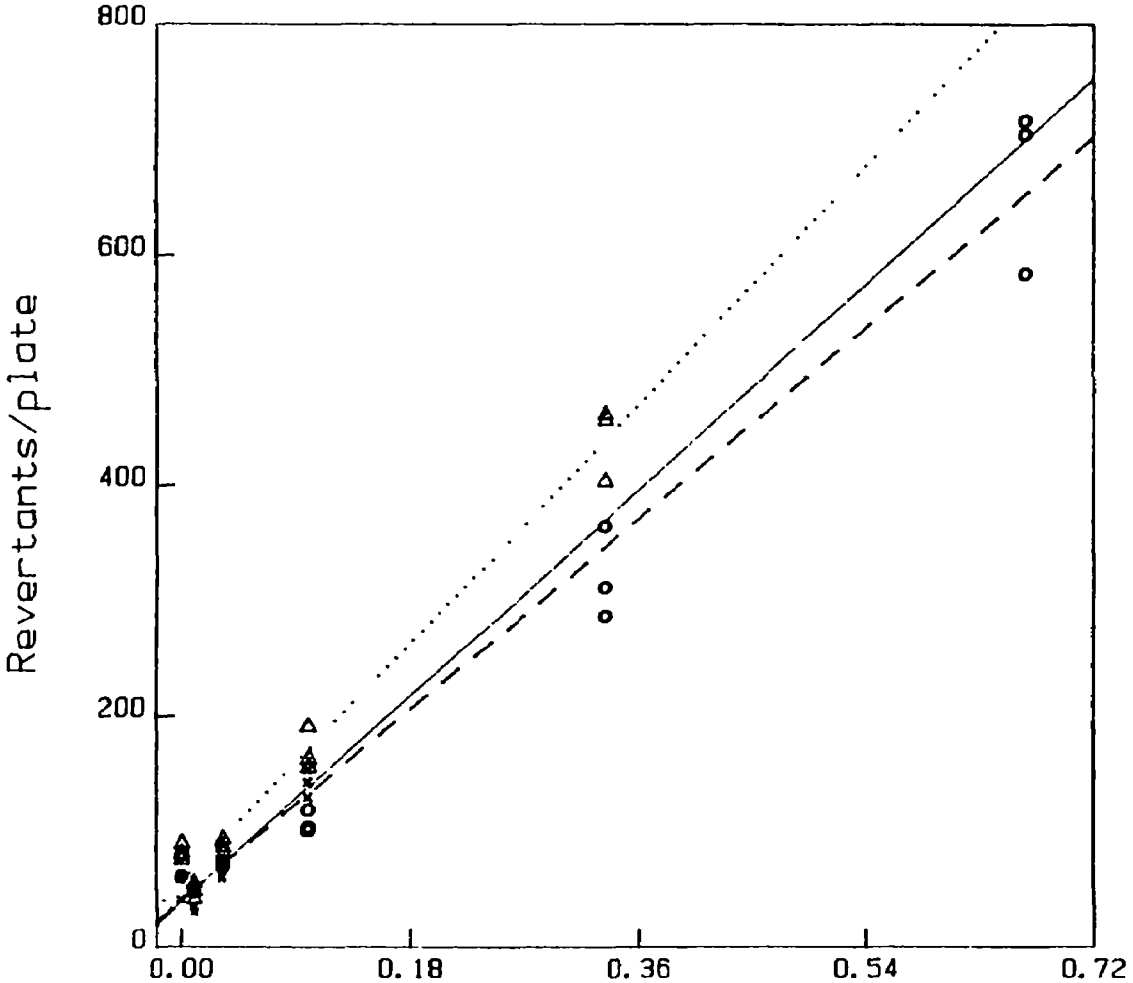
		Dose Level			Plate A		Plate B		Plate C		Plate D		Plate E		
	(H) Solvent Positive (Table 11)	(I) Units of Concentration Blank = mg/ml 2 = µg/ml	(J) Stock concentration (µg/plate)	(K) Amt Per Plate (µl)	(L) Count	(M) BG	(N) Count	(O) BG	(P) Count	(Q) BG	(R) Count	(S) BG	(T) Count	(U) BG	Card Code
	43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL	54			50	48		51		42						B
Pos	07		10	50	437		446		472						C
buffer blank		TCLA sterile - glass filtration		50	96		79		67						D
water blank				50	59		49		52						E
															F
															G
															H
															I
															J

Remarks Indicate Item Code and Card Code

Forms Completion
Initials

TCLP-NBS WASTEWATER

Dichloromethane Extract



TA 98, -S9
SYMBOL=×
LINETYPE=————
y=a+b*x
n=12
a=38.5069 s_a=5.6222
b=990.4829 s_b=106.3014
$s_{y.x}$=14.3528 r=0.9470

TA 98, 2% S9
SYMBOL=△
LINETYPE=····· ·· ··
y=a+b*x
n=15
a=55.5910 s_a=7.1453
b=1149.2546 s_b=45.7287
$s_{y.x}$=21.9445 r=0.9899

TA 98, 10% S9
SYMBOL=o
LINETYPE=— — — —
y=a+b*x
n=18
a=39.9381 s_a=11.1771
b=918.0136 s_b=36.4028
$s_{y.x}$=37.2097 r=0.9877

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER Test Sample Identification	Activation Batch	Test Type (Table 10)	Strain .	Batch No Microorganism
	IVR	CPHF	09 24 85	AIHL	85	0401		01	TA98	8501

Animal	(A) 64	(B) 65	(C) 66	(D) 67 70	(E) 71	(F) 72 73 74 75	(G) 76 78	80
Organ	2 Remarks Made? Yes 1	1 Phenocopy Check Conclusion (Table 13)	Sterility S 9 Mix 1 Not Contam 2 Contam 3 Not Checked	Activation Mixture Per Plate (µl)	1 Sample Sterility Check 1 Not Contam 2 Contam 3 Not Checked	Time (min) Temp (Cent) Pre-Incubation	Technician	Card Code
Inducer								
[redacted] -59								A

		Dose Level			Plate A		Plate B		Plate C		Plate D		Plate E		
	(H) Solvent Positive (Table 11)	(I) Units of Concentration Blank = mg/ml 2 = µg/ml	(J) Stock concentration (µg/plate)	(K) Amt Per Plate (µl)	(L) Count	(M) BG	(N) Count	(O) BG	(P) Count	(Q) BG	(R) Count	(S) BG	(T) Count	(U) BG	Card Code
	43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL	54			50	21		33		20						B
Pos	03		40	50	1155		1257		1310						C
			100	50	30		34		49						D
			330	50	68		59		62						E
			1000	50	1A2		153		129						F
water blank				50	38		33		39						G
buffer blank				50	62		58		41						H
															I
															J

Remarks Indicate Item Code and Card Code

Forms Completion
Initials

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER Test Sample Identification	Activation Batch	Test Type (Table 10)	Strain .	Batch No Microorganism
	IVR	CPHA	09 24 85	AIHL	85	0401	0363 0	01	1 TA98	8507

Animal	R
Organ	L
Inducer	A
[redacted]	2% S9 Mix

A 64	B 65	C 66	D 67 70	E 71	F 72 73 74 75	G 76 78	80
Remarks Made? Yes 1	Phenocopy Check Conclusion (Table 13)	Sterility S 9 Mix 1 Not Contam 2 Contam 3 Not Checked	Activation Mixture Per Plate (µl)	Sample Sterility Check 1 Not Contam 2 Contam 3 Not Checked	Time (min) Temp (Cent) Pre-Incubation	Technician	Card Code
2	1	1	500	1		KIC	A

		Dose Level			Plate A		Plate B		Plate C		Plate D		Plate E		
	H Solvent Positive (Table 11)	I Units of Concentration Blank = mg/ml 2 = µg/ml	J Stock concentration (mg/plate)	K Amt Per Plate (µl)	L Count	M BG	N Count	O BG	P Count	Q BG	R Count	S BG	T Count	U BG	Card Code
	43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL	54			50	36		37		47						B
Pos	07		10	50	284		360		322						C
			100	50	41		48		55						D
			330	50	81		86		93						E
			1000	50	190		155		162						F
			333	50	403		456		461						G
water blank				50	57		49		52						H
buffer blank				50	89		80		75						I
															J

Remarks Indicate Item Code and Card Code

Forms Completion
Initials

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER	Activation Batch	Test Type (Table 10)	Strain	Batch No
	IVR	CPHF	09 24 85	ALVAL	85	0401	03630	01	1TA98	35057

Microorganism

		(A) 64	(B) 65	(C) 66	(D) 67 70	(E) 71	(F) 72 73 74 75	(G) 76 78	80
Animal	R	2	1	1	500	1		KIC	A
Organ	L	Remarks Made? Yes 1	Phenocopy Check Conclusion (Table 13)	Sterility S 9 Mix 1 Not Contam 2 Contam 3 Not Checked	Activation Mixture Per Plate (µl)	Sample Sterility Check 1 Not Contam 2 Contam 3 Not Checked	Time (min) Temp (Cent) Pre-Incubation	Technician	Card Code
Inducer	A								
[redacted]	10% S9 Mix								

		Dose Level			Plate A		Plate B		Plate C		Plate D		Plate E		
	(H) Solvent Positive (Table 11)	(I) Units of Concentration Blank = mg/ml 2 = µg/ml	(J) Stock concentration (µg/plate)	(K) Amt Per Plate (µl)	(L) Count	(M) BG	(N) Count	(O) BG	(P) Count	(Q) BG	(R) Count	(S) BG	(T) Count	(U) BG	Card Code
	43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL	54			500	31		52		45						B
Pos	07		10	50	462		350		381						C
			100	50	51		52		47						D
			330	50	68		74		70						E
			1000	50	118		103		100						F
			3330	50	311		286		364						G
			6660	50	704		716		583						H
water blank				50	61		51		52						I
buffer blank				50	79		78		60						J

Remarks Indicate Item Code and Card Code

Forms Completion Initials

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER	Activation Batch	Test Type (Table 10)	Strain . Batch No	
	IVR	CPHF	09 24 85	AIVHL	85	0401		01	TA100	850 8
				Test Sample Identification					Microorganism	

Animal	(A) 64	(B) 65	(C) 66	(D) 67 70	(E) 71	(F) 72 73 74 75	(G) 76 78	80
Organ	Remarks Made? Yes 1	Phenocopy Check Conclusion (Table 13)	Sterility S 9 Mix 1 Not Contam 2 Contam 3 Not Checked	Activation Mixture Per Plate (µl)	Sample Sterility Check 1 Not Contam 2 Contam 3 Not Checked	Time (min) Temp (Cent) Pre-Incubation	Technician	Card Code
Inducer	2	1			1		KIC	A
[redacted] -59								

	Dose Level			Plate A		Plate B		Plate C		Plate D		Plate E		
(H) Solvent Positive (Table 11)	(I) Units of Concentration Blank = mg/ml 2 = µg/ml	(J) Stock concentration (µg/plate)	(K) Amt Per Plate (µl)	(L) Count	(M) BG	(N) Count	(O) BG	(P) Count	(Q) BG	(R) Count	(S) BG	(T) Count	(U) BG	Card Code
43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL 54			50	1173		1417		1445						B
Pos 01		05	50	1539		1649		1744						C
		100	50	1855		1788		1833						D
		330	50	1910		2022		1558						E
		1000	50	2011		2111		2223						F
		3330	50	2834		2444		2511						G
water blank			50	1722		1719		1777						H
buffer blank			50	1555		1555		1477						I
														J

Remarks Indicate Item Code and Card Code

Forms Completion
Initials

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER Test Sample Identification	Activation Batch	Test Type (Table 10)	Strain	Batch No Microorganism
	IVR	CPHF	09 24 85	AIVH	L 85	0401	0363U	01	TA100	5508

Animal	R
Organ	L
Inducer	A
[redacted]	2% S9 Mix

(A) 64	(B) 65	(C) 66	(D) 67 70	(E) 71	(F) 72 73 74 75	(G) 76 78	80
2 Remarks Made? Yes 1	1 Phenocopy Check Conclusion (Table 13)	1 Sterility S 9 Mix 1 Not Contam 2 Contam 3 Not Checked	500 Activation Mixture Per Plate (µl)	1 Sample Sterility Check 1 Not Contam 2 Contam 3 Not Checked	Time (min) Temp (Cent) Pre-Incubation	KLC Technician	A Card Code

		Dose Level			Plate A		Plate B		Plate C		Plate D		Plate E		
	(H) Solvent Positive (Table 11)	(I) Units of Concentration Blank = mg/ml 2 = µg/ml	(J) Stock concentration (µg/plate)	(K) Amt Per Plate (µl)	(L) Count	(M) BG	(N) Count	(O) BG	(P) Count	(Q) BG	(R) Count	(S) BG	(T) Count	(U) BG	Card Code
	43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL	54			50	1162		1161		1422						B
Pos	0A		05	50	2435		2147		2522						C
			100	50	1600		1178		1811						D
			330	50	1754		1854		1853						E
			1000	50	2074		2224		2400						F
			3330	50	3078		2622		3498						G
water blank				50	1611		1553		1585						H
buffer blank				50	1611		1779		1453						I
															J

Remarks Indicate Item Code and Card Code

Forms Completion	
Initials	

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33-38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER Test Sample Identification	Activation Batch	Test Type (Table 10)	Strain . Batch No Microorganism	
	IVR	CPHF	09 24 85	A2HL	85	0401	036300	01	TA100	8508

Animal	R
Organ	L
Inducer	A
[redacted]	10% S9 Mix

A 64	B 65	C 66	D 67 70	E 71	F 72 73 74 75	G 76 78	80
Remarks Made? Yes 1	Phenocopy Check Conclusion (Table 13)	Sterility S 9 Mix 1 Not Contam 2 Contam 3 Not Checked	Activation Mixture Per Plate (µl)	Sample Sterility Check 1 Not Contam 2 Contam 3 Not Checked	Time (min) Temp (Cent) Pre-Incubation	Technician	Card Code
2	1	1	500	1		KLC	A

		Dose Level			Plate A		Plate B		Plate C		Plate D		Plate E		
	H Solvent Positive (Table 11)	I Units of Concentration Blank = mg/ml 2 = µg/ml	J Stock concentration (µg/plate)	K Amt Per Plate (µl)	L Count	M BG	N Count	O BG	P Count	Q BG	R Count	S BG	T Count	U BG	Card Code
	43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL	54			50	1136		1163		1155						B
Pos	04		05	50	1037		832		855						C
			100	50	1150		1152		1162						D
			330	50	1163		1163		1151						E
			1000	50	1196		1186		1190						F
			3330	50	2296		2287		3199						G
water blank				50	1142		1151		1151						H
buffer blank				50	1166		1168		1167						I
															J

Remarks Indicate Item Code and Card Code

D-160

Forms Completion Initials

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER	Activation Batch	Test Type (Table 10)	Strain	Batch No
	1VR	CPHF	112285	AIHL	85	0401		01	1TA98	07835

Microorganism

Animal	(A) 64	(B) 65	(C) 66	(D) 67 70	(E) 71	(F) 72 73 74 75	(G) 76 78	80
Organ	2	1			1		KIC	A
Inducer	Remarks Made? Yes 1	Phenocopy Check Conclusion (Table 13)	Sterility S 9 Mix 1 Not Contam 2 Contam 3 Not Checked	Activation Mixture Per Plate (µl)	Sample Sterility Check 1 Not Contam 2 Contam 3 Not Checked	Time (min) Temp (Cent) Pre-Incubation	Technician	Card Code
[redacted] -59								

	Dose Level				Plate A		Plate B		Plate C		Plate D		Plate E		
(H) Solvent Positive (Table 11)	(I) Units of Concentration Blank = mg/ml 2 = µg/ml	(J) Stock concentration (mg/plate)	(K) Amt Per Plate (µl)	(L) Count	(M) BG	(N) Count	(O) BG	(P) Count	(Q) BG	(R) Count	(S) BG	(T) Count	(U) BG	Card Code
43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL 54			50	32		23		28						B
Pos 03		40	50	1158		1166		1266						C
buffer blank	New buffer formula		50	62		62		79						D
water blank			50	41		44		30						E
														F
														G
														H
														I
														J

Remarks Indicate Item Code and Card Code

Forms Completion
Initials

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	IVR	CPHF	112285	AiHL	85	0401	04605	01	174985	0785
	System ID	Research Lab ID	MO DA YR Experiment Date		LAB	YR	NUMBER Test Sample Identification	Activation Batch	Test Type (Table 10)	Strain / Batch No Microorganism

		(A) 64	(B) 65	(C) 66	(D) 67 70	(E) 71	(F) 72 73 74 75		(G) 76 78	80
Animal	R	2	1	1	500	1			KIC	A
Organ	L	Remarks Made? Yes 1	Phenocopy Check Conclusion (Table 13)	Sterility S 9 Mix 1 Not Contam 2 Contam 3 Not Checked	Activation Mixture Per Plate (µl)	Sample Sterility Check 1 Not Contam 2 Contam 3 Not Checked	Time (min)	Temp (Cent) Pre-Incubation	Technician	Card Code
Inducer	A									
[redacted]	2% S9 Mix									

	(H) Solvent Positive (Table 11)	(I) Units of Concentration Blank = mg/ml 2 = µg/ml	(J) Stock concentration (µg/plate)	(K) Amt Per Plate (µl)	Plate A (L) Count	Plate A (M) BG	Plate B (N) Count	Plate B (O) BG	Plate C (P) Count	Plate C (Q) BG	Plate D (R) Count	Plate D (S) BG	Plate E (T) Count	Plate E (U) BG	Card Code
	43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL	54			50	53		44		50						B
Pos	07		10	50	848		877		838						C
buffer blank		New buffer formula		50	76		77		76						D
water blank				50	66		50		56						E
															F
															G
															H
															I
															J

Remarks Indicate Item Code and Card Code

Forms Completion
Initials

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER	Activation Batch	Test Type (Table 10)	Strain	Batch No
	IVR	CPHF	11 22 85	AZ HL	85	0401	04605		17A78	0785

Microorganism

		A 64	B 65	C 66	D 67 70	E 71	F 72 73 74 75	G 76 78	80
Animal	R	2	1	1	500	1		KIC	A
Organ	L	Remarks Made? Yes 1	Phenocopy Check Conclusion (Table 13)	Sterility S 9 Mix 1 Not Contam 2 Contam 3 Not Checked	Activation Mixture Per Plate (µl)	Sample Sterility Check 1 Not Contam 2 Contam 3 Not Checked	Time (min) Temp (Cent) Pre-Incubation	Technician	Card Code
Inducer	A								
[redacted]	10% S9 Mix								

H	I	J	K	L	M	N	O	P	Q	R	S	T	U	
Solvent Positive (Table 11)	Units of Concentration Blank = mg/ml 2 = µg/ml	Dose Level: Stock concentration (µg/plate)	Dose Level: Amt Per Plate (µl)	Plate A: Count	Plate A: BG	Plate B: Count	Plate B: BG	Plate C: Count	Plate C: BG	Plate D: Count	Plate D: BG	Plate E: Count	Plate E: BG	Card Code
43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL 54			50	41		56		37						B
Pos 07		10	50	262		301		261						C
buffer blank	New buffer formula		50	63		68		74						D
water blank			50	50		48		51						E
														F
														G
														H
														I
														J

Remarks Indicate Item Code and Card Code

Forms Completion
Initials

TABLE 10. MUTAGENICITY OF A CONTAMINATED GROUNDWATER FROM THE STRINGFELLOW HAZARDOUS WASTE DISPOSAL FACILITY ON-SITE WELL OW-2, SAMPLE NO. AIHL-85-0402

A. Results of the Initial Screening Experiment

Exp. Date	Extraction Method[1]	Residue Weight (mg)	Sample Volume (L)	Specific Mutagenic Activity (revertants/mg)[2,3] TA98 -S9	TA98 2%S9	TA98 10%S9	TA100 -S9	TA100 2%S9	TA100 10%S9
7/22/85	Base/Acid	129	3	58	84	79	< 57	< 61	< 56

B. Comparison of Mutagenic Response in TA98 with 2% S-9 Mix[4]

Exp. Date	Extraction Method[1]	Residue Weight (mg)	Sample Volume (L)	Specific Mutagenic Activity (revertants/mg)[2,3]	Mutagenic Response per Unit Volume (revertants/L)
7/22/85	Base/Acid	129	3	84	3598
8/2/85	Acid/Base pH 2 fraction only	43	3	48	685
8/2/85	Acid/Base pH 11 fraction only	7	3	< 41	< 95
9/24/85	Acid/Base	60	4.5	< 53	< 702
10/17/85	Acid/Base	67	3	< 28	< 629

1. The extraction method indicates the order of pH in liquid-liquid extraction.

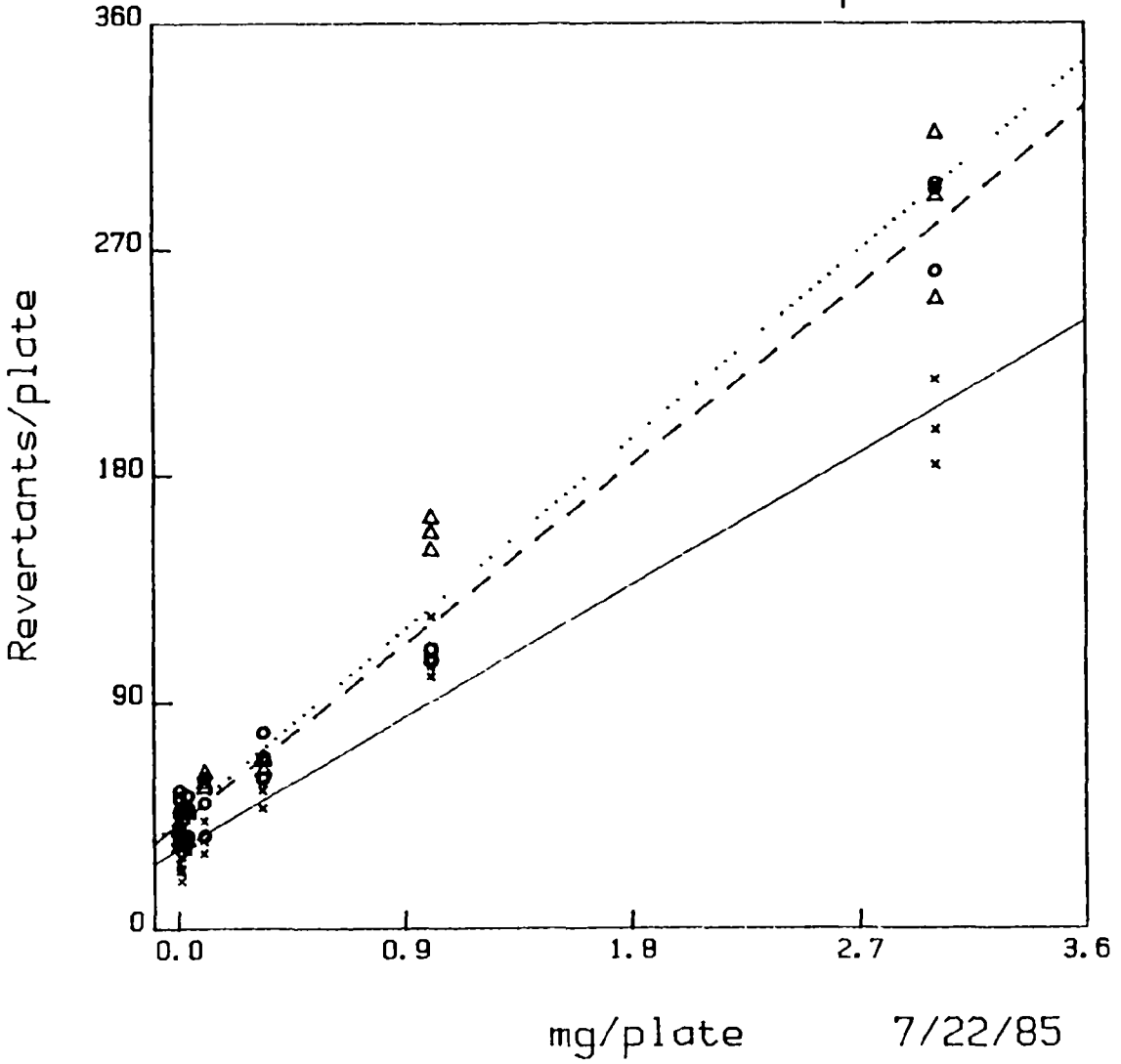
2. The specific activity of the mutagenicity dose-response curve is represented by the slope (b as shown in the statistical analysis on graphs in Appendix D).

3. The spontaneous mutation and positive control values are listed on the Strain Function Tests, Cell Titer, and Viability Record in Appendix F.

4. The optimum testing condition in the initial screening experiment was in TA98 with 2% S-9 mix.

STRINGFELLOW WASTEWATER

Dichloromethane Extract (Sample 0402)



TA 98, -S9
SYMBOL=×
LINETYPE=————
y=a+b*x
n=26
a=31.8582 s_a=2.4370
b=58.2372 s_b=2.2550
$s_{y.x}$=10.9046 r=0.9825

TA 98, 2% S9
SYMBOL=Δ
LINETYPE=·····
y=a+b*x
n=24
a=43.6639 s_a=3.8608
b=83.7664 s_b=3.4324
$s_{y.x}$=16.4072 r=0.9820

TA 98, 10% S9
SYMBOL=o
LINETYPE=— — — —
y=a+b*x
n=24
a=41.9181 s_a=2.4864
b=79.1426 s_b=2.2105
$s_{y.x}$=10.5665 r=0.9915

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER Test Sample Identification	Activation Batch	Test Type (Table 10)	Strain	Batch No Microorganism
	IVR	CPHF	072285	AIHL	85	0402		01	TA98	8501

Animal	(A) 64	(B) 65	(C) 66	(D) 67 70	(E) 71	(F) 72 73 74 75	(G) 76 78	80
Organ	Remarks Made? Yes 1	Phenocopy Check Conclusion (Table 13)	Sterility S 9 Mix 1 Not Contam 2 Contam 3 Not Checked	Activation Mixture Per Plate (µl)	Sample Sterility Check 1 Not Contam 2 Contam 3 Not Checked	Time (min) Temp (Cent) Pre-Incubation	Technician	Card Code
Inducer								
[redacted] -59	2	1			1		KIC	A

	(H) Solvent Positive (Table 11)	(I) Units of Concentration Blank = mg/ml 2 = µg/ml	Dose Level (J) Stock concentration (µg/plate)	Dose Level (K) Amt Per Plate (µl)	Plate A (L) Count	Plate A (M) BG	Plate B (N) Count	Plate B (O) BG	Plate C (P) Count	Plate C (Q) BG	Plate D (R) Count	Plate D (S) BG	Plate E (T) Count	Plate E (U) BG	Card Code
	43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL	54			50	36		28		23						B
Pos	03		4000	50	1202		1525		1355						C
			1000	50	26		35		23						D
			10000	50	19		24		29		23				E
			33000	50	32		35		33		31				F
			100000	50	35		43		30						G
			333000	50	55		58		48						H
		1	00000	50	104		100		124						I
		3	00000	50	218		198		184						J

Remarks Indicate Item Code and Card Code

(Continued)

Forms Completion
Initials

HERL IN VITRO RESULTS CONTINUATION FORM

1 Update Code	2 4 System ID	5 8 Research Lab ID	9 14 MO DA YR Experiment Date	15 18 Lab	19 20 YR	21 24 Number Test Sample Identification	25 30 Activation Batch	31 32 Test Type (Table 10)	33 38 Strain	39 42 Batch No Microorganism
	IVR	CPHF	072285	AIHL	85	0402	-59	01	TA98	S301

		Dose Level		Plate A		Plate B		Plate C		Plate D		Plate E		
(H) Solvent Positive (Table 11)	(I) Units of Concentration Blank = mg/ml 2 = µg/ml	(J) Stock Concentration (µg / plate)	(K) Amt Per Plate (µl)	(L) Count	(M) BG	(N) Count	(O) BG	(P) Count	(Q) BG	(R) Count	(S) BG	(T) Count	(U) BG	Card Code
43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
water blank			50	33		36		37						K
														L
														M
														N
														O
														P
														Q
								1						R
														S
														T
														U
														V
														W
														X
														Y

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER Test Sample Identification	Activation Batch	Test Type (Table 10)	Strain .	Batch No Microorganism
	IVR	CPHF	072285	HLH	85	0402	031222	01	TA98	8501

Animal	R
Organ	L
Inducer	A
[redacted]	2% S9 Mix

(A) 64 Remarks Made? Yes 1: 2

(B) 65 Phenocopy Check Conclusion (Table 13): 1

(C) 66 Sterility S 9 Mix: 1 (1 Not Contam, 2 Contam, 3 Not Checked)

(D) 67 70 Activation Mixture Per Plate (µl): 500

(E) 71 Sample Sterility Check: 1 (1 Not Contam, 2 Contam, 3 Not Checked)

(F) 72 73 74 75 Pre-Incubation: Time (min) | Temp (Cent), Technician: KLC

(G) 76 78

80 Card Code: A

	Dose Level				Plate A		Plate B		Plate C		Plate D		Plate E		
(H) Solvent Positive (Table 11)	(I) Units of Concentration Blank = mg/ml 2 = µg/ml	(J) Stock concentration (mg/plate)	(K) Amt Per Plate (µl)	(L) Count	(M) BG	(N) Count	(O) BG	(P) Count	(Q) BG	(R) Count	(S) BG	(T) Count	(U) BG	Card Code
	43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL	54			50	35		32		32						B
Pos	07		10	50	497		452		472						C
			10	50	35		33		35						D
			100	50	44		44		38						E
			330	50	33		46		47						F
			1000	50	58		56		62						G
			3330	50	63		67		68						H
	1		0000	50	163		150		157						I
	3		0000	50	316		291		250						J

Remarks Indicate Item Code and Card Code

(continued)

Forms Completion Initials

HERL IN VITRO RESULTS CONTINUATION FORM

1 Update Code	2 4 System ID	5 8 Research Lab ID	9 14 MO DA YR Experiment Date	15 18 19 20 21 24 Lab YR Number Test Sample Identification	25 30 Activation Batch	31 32 Test Type (Table 10)	33 38 Strain / Microorganism	39 42 Batch No
	IVR	CPHF	072285	AZHL-85-0402	03122	01	TA98	8501

		Dose Level		Plate A		Plate B		Plate C		Plate D		Plate E		
(H) Solvent Positive (Table 11)	(I) Units of Concentration Blank = mg/ml 2 = µg/ml	(J) Stock Concentration (µg/plate)	(K) Amt Per Plate (µl)	(L) Count	(M) BG	(N) Count	(O) BG	(P) Count	(Q) BG	(R) Count	(S) BG	(T) Count	(U) BG	Card Code
43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
Water Blank			50	39		36		40						K
														L
														M
														N
														O
														P
														Q
														R
														S
														T
														U
														V
														W
														X
														Y

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER	Activation Batch	Test Type (Table 10)	Strain	Batch No
	IVR	CPHF	07 22 85	AIHL	85	0402	03122	01	1 7A98	8501
				Test Sample Identification					Microorganism	

Animal	R
Organ	L
Inducer	A
[redacted]	10 % S9 Mix

A 64	B 65	C 66	D 67 70	E 71	F 72 73 74 75	G 76 78	80
2 Remarks Made? Yes 1	1 Phenocopy Check Conclusion (Table 13)	1 Sterility S 9 Mix 1 Not Contam 2 Contam 3 Not Checked	500 Activation Mixture Per Plate (µl)	1 Sample Sterility Check 1 Not Contam 2 Contam 3 Not Checked	Time (min) Temp (Cent) Pre-Incubation	KIC Technician	A Card Code

		Dose Level			Plate A		Plate B		Plate C		Plate D		Plate E		
	H Solvent Positive (Table 11)	I Units of Concentration Blank = mg/ml 2 = µg/ml	J Stock concentration (µg/plate)	K Amt Per Plate (µl)	L Count	M BG	N Count	O BG	P Count	Q BG	R Count	S BG	T Count	U BG	Card Code
	43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL	54			500	37		36		47						B
Pos	07		10	50	338		360		372						C
			10	50	55		47		52						D
			100	50	47		48		31						E
			330	50	48		37		53						F
			1000	50	60		50		37						G
			333	50	60		78		68						H
		1	000	50	107		111		106						I
		3	000	50	274		276		261						J

Remarks Indicate Item Code and Card Code

(continued)

Forms Completion Initials

HERL IN VITRO RESULTS CONTINUATION FORM

1 Code / Update	2 4 System ID	5 8 Research Lab ID	9 14 MO DA YR Experiment Date	15 18 - 19 20 - 21 24 Lab YR Number Test Sample Identification	25 30 Activation Batch	31 32 Test Type (Table 10)	33 38 Strain	39 42 Batch No Microorganism
	IVR	CPHF	072285	AZVL-85-0402	10% S9 03122	01	TA98	8501

		Dose Level		Plate A		Plate B		Plate C		Plate D		Plate E		
H Solvent Positive (Table 11)	I Units of Concentration Blank = mg/ml 2 = µg/ml	J Stock Concentration (µl/plate)	K Amt Per Plate (µl)	L Count	M BG	N Count	O BG	P Count	Q BG	R Count	S BG	T Count	U BG	Card Code
43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
water blank			50	46		41		36						K
														L
														M
														N
														O
														P
														Q
														R
														S
														T
														U
														V
														W
														X
														Y

HERL IN VITRO RESULTS FORM

1	2-4	5-8	9-14	15-18	19-20	21-24	25-30	31-32	33-38	39-42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB Test Sample Identification	YR	NUMBER	Activation Batch	Test Type (Table 10)	Strain . Microorganism	Batch No
	IVR	CPHF	07 22 85	AIHL	85	0402		01	TA100	8505

Animal	A 64	B 65	C 66	D 67-70	E 71	F 72-73-74-75	G 76-78	80
Organ	2 Remarks Made? Yes 1	1 Phenocopy Check Conclusion (Table 13)	Sterility S 9 Mix 1 Not Contam 2 Contam 3 Not Checked	Activation Mixture Per Plate (µl)	1 Sample Sterility Check 1 Not Contam 2 Contam 3 Not Checked	Time (min) Temp (Cent) Pre-Incubation	KIC Technician	A Card Code
Inducer								
[redacted] -S9								

	Dose Level				Plate A		Plate B		Plate C		Plate D		Plate E		
H Solvent Positive (Table 11)	I Units of Concentration Blank = mg/ml 2 = µg/ml	J Stock concentration (µl/plate)	K Amt Per Plate (µl)	L Count	M BG	N Count	O BG	P Count	Q BG	R Count	S BG	T Count	U BG	Card Code
43-44	45	46-50	51-54	55-58	59	60-63	64	65-68	69	70-73	74	75-78	79	80
SOL 54			50	1160		1178		1158						B
Pos 01		05	50	879		870		879						C
		10	50	1170		1147		1155						D
		100	50	1151		1173		1168						E
		330	50	1162		1167		1155						F
		1000	50	1183		1195		1157						G
		333	50	1189		208		1176						H
	1	000	50	226		222		249						I
	3	000	50	266		249		335						J

Remarks Indicate Item Code and Card Code

(continued)

Forms Completion
Initials

HERL IN VITRO RESULTS CONTINUATION FORM

1 Update Code	2-4 System ID	5-8 Research Lab ID	9-14 Experiment Date (MO DA YR)	15-18 Lab	19-20 YR	21-24 Number	25-30 Activation Batch	31-32 Test Type (Table 10)	33-38 Strain	39-42 Batch No
	IVR	CPHF	072285	AIHL	85	0402	-S7	01	TA100	8505
									Microorganism	

		Dose Level		Plate A		Plate B		Plate C		Plate D		Plate E		
(H) Solvent Positive (Table 11)	(I) Units of Concentration Blank = mg/ml 2 = µg/ml	(J) Stock Concentration	(K) Amt Per Plate (µl)	(L) Count	(M) BG	(N) Count	(O) BG	(P) Count	(Q) BG	(R) Count	(S) BG	(T) Count	(U) BG	Card Code
43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
Water Blank			50	156		165		179						K
														L
														M
														N
														O
														P
														Q
														R
														S
														T
														U
														V
														W
														X
														Y

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER Test Sample Identification	Activation Batch	Test Type (Table 10)	Strain .	Batch No Microorganism
	IVR	CPHF	07 22 85	AIHL	85	0402	03122	01	TA100	85 05

Animal	R
Organ	L
Inducer	A
[redacted]	2 % S9 Mix

(A) 64	(B) 65	(C) 66	(D) 67 70	(E) 71	(F) 72 73 74 75	(G) 76 78	80
2 Remarks Made? Yes 1	1 Phenocopy Check Conclusion (Table 13)	1 Sterility S 9 Mix 1 Not Contam 2 Contam 3 Not Checked	500 Activation Mixture Per Plate (µl)	1 Sample Sterility Check 1 Not Contam 2 Contam 3 Not Checked	Time (min) Temp (Cent) Pre-Incubation	KIC Technician	A Card Code

	Dose Level			Plate A		Plate B		Plate C		Plate D		Plate E		
(H) Solvent Positive (Table 11)	(I) Units of Concentration Blank = mg/ml 2 = µg/ml	(J) Stock concentration (µg/plate)	(K) Amt Per Plate (µl)	(L) Count	(M) BG	(N) Count	(O) BG	(P) Count	(Q) BG	(R) Count	(S) BG	(T) Count	(U) BG	Card Code
	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL 54			50	1698		1552		1774						B
Pos 04		05	50	2389		2305		2444						C
		1.0	50	1551		1711		1844						D
		10.0	50	1422		1466		1677						E
		3.0	50	222		1771		1833						F
		1.00	50	1906		1866		229						G
		333.0	50	1977		1912		1855						H
	/	0.00	50	228		237		248						I
	3	0.00	50	326		326		351						J

Remarks Indicate Item Code and Card Code

(continued)

Forms Completion Initials

HERL IN VITRO RESULTS CONTINUATION FORM

1 Update Code	2 4 System ID	5 8 Research Lab ID	9 14 MO DA YR Experiment Date	15 18 Lab	19 20 YR	21 24 Number Test Sample Identification	25 30 Activation Batch	31 32 Test Type (Table 10)	33 38 Strain	39 42 Batch No Microorganism
	IVR	CPHE	072285	ALHL	85	0402	2% S9	01	TA100	355

		Dose Level		Plate A		Plate B		Plate C		Plate D		Plate E		
(H) Solvent Positive (Table 11)	(I) Units of Concentration Blank = mg/ml 2 = µg/ml	(J) Stock Concentration	(K) Amt Per Plate (µl)	(L) Count	(M) BG	(N) Count	(O) BG	(P) Count	(Q) BG	(R) Count	(S) BG	(T) Count	(U) BG	Card Code
43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
water blank			50	172		1086		1173						K
														L
														M
														N
														O
														P
														Q
														R
														S
														T
														U
														V
														W
														X
														Y

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER Test Sample Identification	Activation Batch	Test Type (Table 10)	Strain . Batch No Microorganism	
	IVR	CPHF	07 22 85	AIHL	85	0402	03122	01	TA100	8505

Animal	R
Organ	L
Inducer	A
[redacted]	10 % S9 Mix

(A) 64	(B) 65	(C) 66	(D) 67 70	(E) 71	(F) 72 73 74 75	(G) 76 78	80
2 Remarks Made? Yes 1	1 Phenocopy Check Conclusion (Table 13)	1 Sterility S 9 Mix 1 Not Contam 2 Contam 3 Not Checked	500 Activation Mixture Per Plate (µl)	1 Sample Sterility Check 1 Not Contam 2 Contam 3 Not Checked	Time (min) Temp (Cent) Pre-Incubation	KIC Technician	A Card Code

		Dose Level			Plate A		Plate B		Plate C		Plate D		Plate E		
	(H) Solvent Positive (Table 11)	(I) Units of Concentration Blank = mg/ml 2 = µg/ml	(J) Stock concentration (mg / plate)	(K) Amt Per Plate (µl)	(L) Count	(M) BG	(N) Count	(O) BG	(P) Count	(Q) BG	(R) Count	(S) BG	(T) Count	(U) BG	Card Code
	43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL	54			50	189		183		127						B
Pos	04		05	50	975		970		981						C
			10	50	150		167		183						D
			100	50	161		153		157						E
			330	50	159		150		126						F
			1000	50	150		183		174						G
			3330	50	211		212		191		172				H
		1	0.00	50	191		208		227						I
		.3	0.00	50	305		278		277						J

Remarks Indicate Item Code and Card Code

(continued)

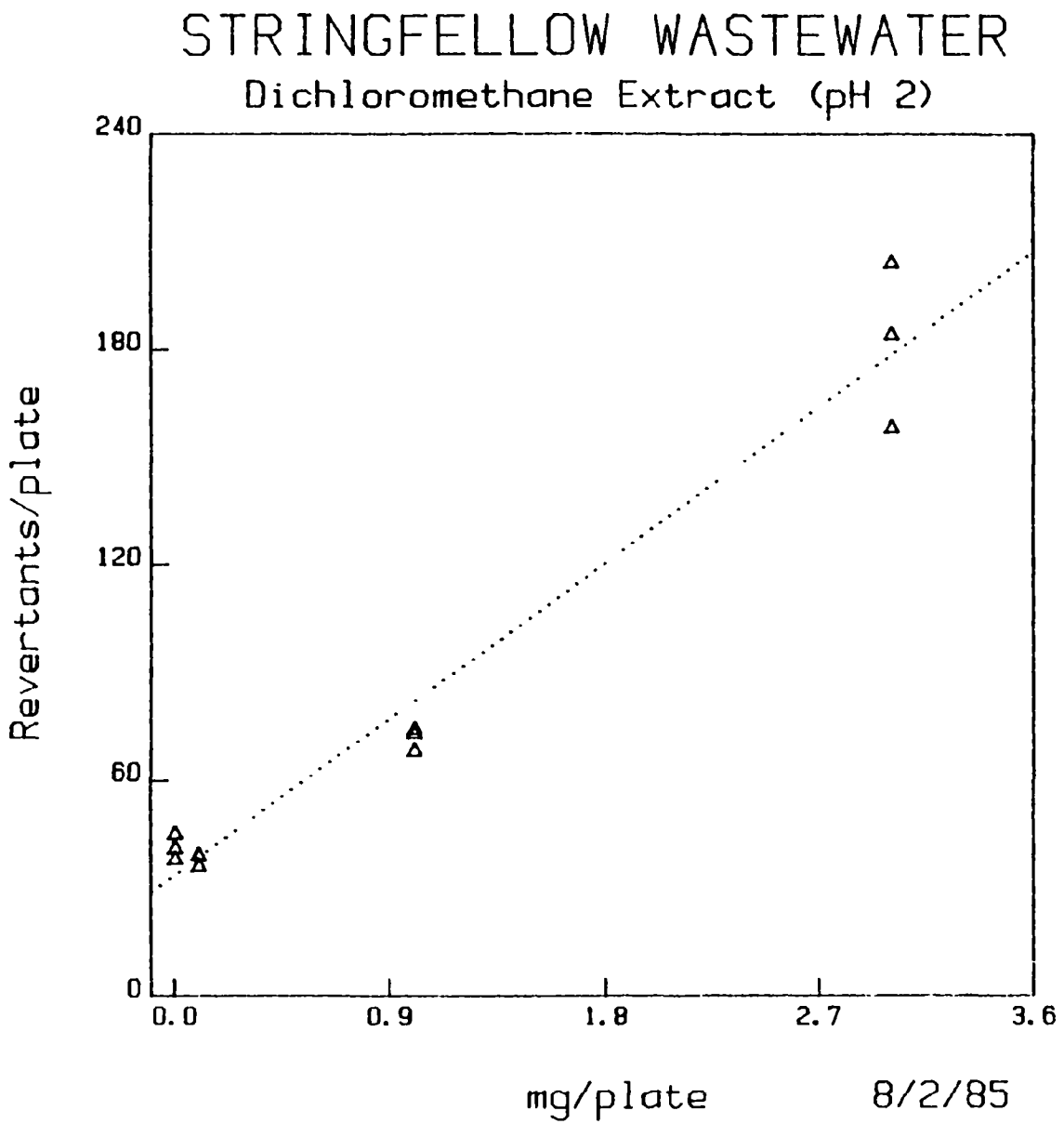
Forms Completion
Initials

D-176

HERL IN VITRO RESULTS CONTINUATION FORM

1 Update Code	2 4 System ID	5 8 Research Lab ID	9 14 MO DA YR Experiment Date	15 18 19 20 21 24 Lab YR Number Test Sample Identification	25 30 Activation Batch	31 32 Test Type (Table 10)	33 38 Strain	39 42 Batch No
	IVR	CPHF	072285	AIVHL-85-0402	0 % S9	01	TA100	8505
							Microorganism	

		Dose Level		Plate A		Plate B		Plate C		Plate D		Plate E		
H Solvent Positive (Table 11)	I Units of Concentration Blank = mg/ml 2 = µg/ml	J Stock Concentration	K Amt Per Plate (µl)	L Count	M BG	N Count	O BG	P Count	Q BG	R Count	S BG	T Count	U BG	Card Code
43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
water blank			50	161		179		166						K
														L
														M
														N
														O
														P
														Q
														R
														S
														T
														U
														V
														W
														X
														Y



TA 98, 2% S9
SYMBOL=△
LINETYPE=.....
y=a+b*x
n=12
a=33.8061 s_a=4.8622
b=48.2379 s_b=3.0736
$s_{y.x}$=12.8293 r=0.9803

X(I)	Y(I)
0.0000	41.0000
0.0000	45.0000
0.0000	38.0000
.1000	36.0000
.1000	39.0000
.1000	39.0000
1.0000	73.0000
1.0000	68.0000
1.0000	74.0000
3.0000	204.0000
3.0000	184.0000
3.0000	158.0000

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER Test Sample Identification	Activation Batch	Test Type (Table 10)	Strain . Microorganism	Batch No
	IVR	CPHF	08 02 85	AIHL	85	0402		01	TA98	8507

Animal / Organ / Inducer	(A) 64 Remarks Made? Yes 1	(B) 65 Phenocopy Check Conclusion (Table 13)	(C) 66 Sterility S 9 Mix 1 Not Contam 2 Contam 3 Not Checked	(D) 67 70 Activation Mixture Per Plate (µl)	(E) 71 Sample Sterility Check 1 Not Contam 2 Contam 3 Not Checked	(F) 72 73 74 75 Time (min) Temp (Cent) Pre-Incubation	(G) 76 78 Technician	80 Card Code
-59	2	1			1		KIC	A

	(H) Solvent Positive (Table 11)	(I) Units of Concentration Blank = mg/ml 2 = µg/ml	(J) Stock concentration (mg/plate)	(K) Amt Per Plate (µl)	Plate A (L) Count	Plate A (M) BG	Plate B (N) Count	Plate B (O) BG	Plate C (P) Count	Plate C (Q) BG	Plate D (R) Count	Plate D (S) BG	Plate E (T) Count	Plate E (U) BG	Card Code
	43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL	54			50	23		35		23						B
Pos	03		40	50	1128		1230		1045						C
pH 2 fraction			1000	50	31		35		30						D
only, Acid/Base		1	0000	50	59		62		69						E
		3	0000	50	95	2	83	2	70	2					F
water blank				50	35		32		38						G
															H
															I
															J

Remarks Indicate Item Code and Card Code

Forms Completion Initials	

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER Test Sample Identification	Activation Batch	Test Type (Table 10)	Strain	Batch No Microorganism
	IVR	CPHF	08 02 83	AIHL	85	0402	03122	01	TA98	8507

Animal	Organ	Inducer	Activation Mixture Per Plate (µl)	Pre-Incubation	80
R	L	A	2 % S9 Mix		

(A) 64 Remarks Made? Yes 1: 2

(B) 65 Phenocopy Check Conclusion (Table 13): 1

(C) 66 Sterility S 9 Mix: 1 (1 Not Contam, 2 Contam, 3 Not Checked)

(D) 67 70: 500

(E) 71 Sample Sterility Check: 1 (1 Not Contam, 2 Contam, 3 Not Checked)

(F) 72 73 74 75 Time (min) Temp (Cent):

(G) 76 78 Technician: KIC

80 Card Code: A

	Dose Level			Plate A		Plate B		Plate C		Plate D		Plate E		
(H) Solvent Positive (Table 11)	(I) Units of Concentration Blank = mg/ml 2 = µg/ml	(J) Stock concentration (mg/plate)	(K) Amt Per Plate (µl)	(L) Count	(M) BG	(N) Count	(O) BG	(P) Count	(Q) BG	(R) Count	(S) BG	(T) Count	(U) BG	Card Code
43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL 54			50	27		37		34						B
Pos 07		10	50	399		395		416						C
pH 2 fraction		1000	50	36		39		39						D
only Acid/Base	1	0000	50	73		68		74						E
	3	0000	50	204		184		158						F
water blank.	pH 2 fraction only		50	41		45		38						G
														H
														I
														J

Remarks Indicate Item Code and Card Code

Forms Completion Initials

HERL IN VITRO RESULTS FORM

1	2-4	5-8	9-14	15-18	19-20	21-24	25-30	31-32	33-38	39-42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER Test Sample Identification	Activation Batch	Test Type (Table 10)	Strain	Batch No Microorganism
	IVR	CPHF	08 02 85	AIHL	85	0402	03122	01	TA98	8507

Animal	Organ	Inducer	Solvent
R	L	A	2% S9 Mix

A 64	B 65	C 66	D 67-70	E 71	F 72-73-74-75	G 76-78	80
Remarks Made? Yes 1	Phenocopy Check Conclusion (Table 13)	Sterility S9 Mix: 1 Not Contam, 2 Contam, 3 Not Checked	Activation Mixture Per Plate (µl)	Sample Sterility Check: 1 Not Contam, 2 Contam, 3 Not Checked	Pre-Incubation: Time (min), Temp (Cent)	Technician	Card Code
2	1	1	500	1		KIC	A

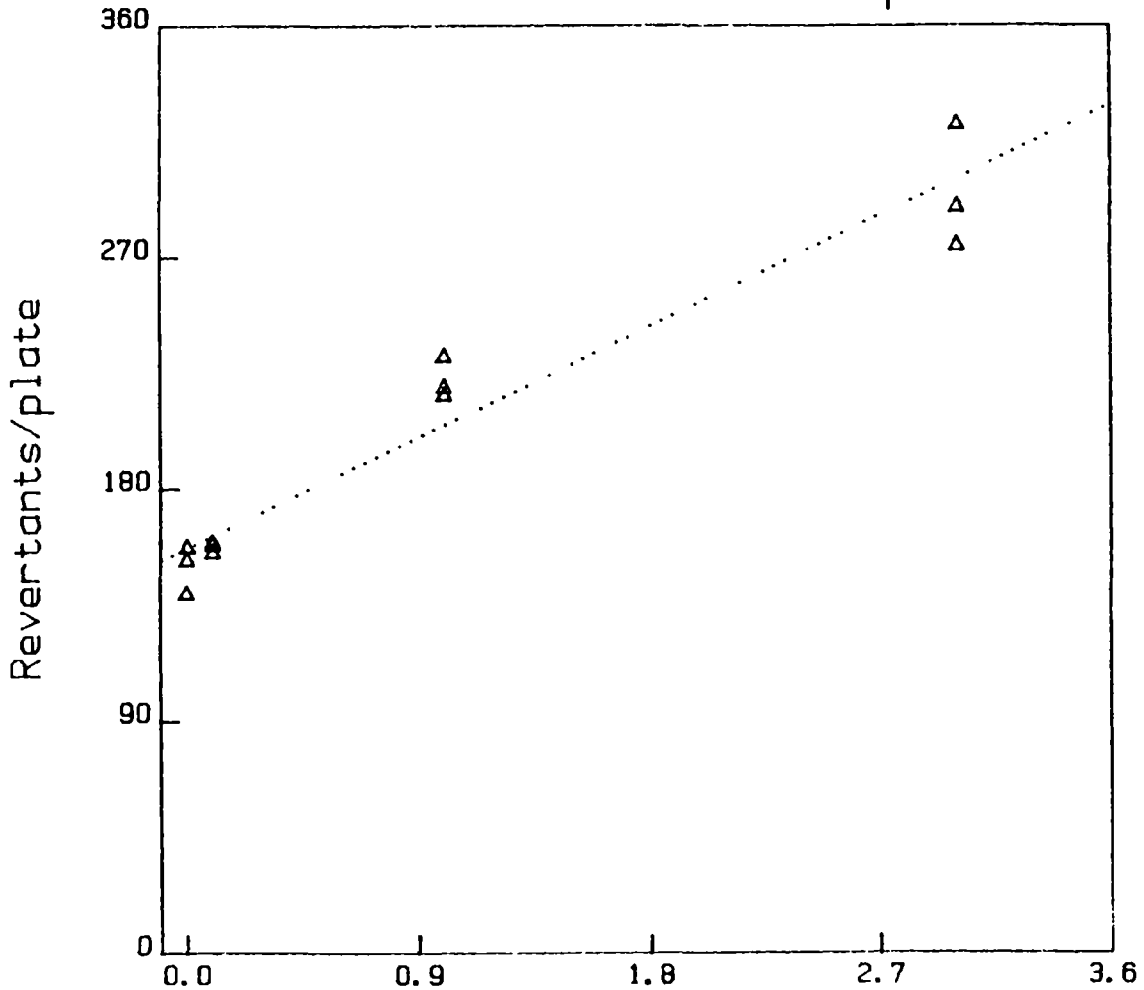
H Solvent Positive (Table 11)	43-44	I Units of Concentration Blank = mg/ml 2 = µg/ml	45	J Stock concentration (mg/plate)	46-50	K Amt Per Plate (µl)	51-54	Plate A L Count	55-58	M BG	59	Plate B N Count	60-63	O BG	64	Plate C P Count	65-68	Q BG	69	Plate D R Count	70-73	S BG	74	Plate E T Count	75-78	U BG	79	Card Code	80
SOL	54					50		27				37				34												B	
Pos	07			10		50		397				395				416												C	
pH 11 fraction only		Acid/Base	1	0000		50		36				39				49												D	
water blank		pH 11 fraction only				50		39				34				45												E	
																												F	
																												G	
																												H	
																												I	
																												J	

Remarks Indicate Item Code and Card Code

Forms Completion
Initials

STRINGFELLOW WASTEWATER

Dichloromethane Extract (pH 2)



TA 100, 2% S9
SYMBOL=△
LINETYPE=.....
y=a+b*x
n=12
a=156.7998 s_a=6.2061
b=47.8376 s_b=3.9231
$s_{y.x}$=16.3751 r=0.9680

X(I)	Y(I)
0 0000	157 0000
0 0000	152 0000
0 0000	139 0000
1000	155 0000
1000	158 0000
1000	159.0000
1 0000	216.0000
1 0000	231.0000
1 0000	219 0000
3.0000	321.0000
3 0000	289.0000
3 0000	274 0000

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER Test Sample Identification	Activation Batch	Test Type (Table 10)	Strain	Batch No Microorganism
	IVR	CPHF	08 02 85	AIHL	85	0402	03122	01	TA100	8505

Animal	R
Organ	L
Inducer	A
[redacted]	2 % S9 Mix

(A) 64	(B) 65	(C) 66	(D) 67 70	(E) 71	(F) 72 73 74 75	(G) 76 78	80
2 Remarks Made? Yes-1	1 Phenocopy Check Conclusion (Table 13)	1 Sterility S 9 Mix 1 Not Contam 2 Contam 3 Not Checked	500 Activation Mixture Per Plate (µl)	1 Sample Sterility Check 1 Not Contam 2 Contam 3 Not Checked	Time (min) Temp (Cent) Pre-Incubation	KIC Technician	A Card Code

	Dose Level			Plate A		Plate B		Plate C		Plate D		Plate E		
(H) Solvent Positive (Table 11)	(I) Units of Concentration Blank = mg/ml 2 = µg/ml	(J) Stock concentration (mg/plate)	(K) Amt Per Plate (µl)	(L) Count	(M) BG	(N) Count	(O) BG	(P) Count	(Q) BG	(R) Count	(S) BG	(T) Count	(U) BG	Card Code
43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL 54			50	1126		1132		1135						B
Pos 04		05	50	2870		2762		2676						C
pH 2 fraction only, Acid/Base		1000.0	30	153		158		159						D
	1	0000	50	216		231		219						E
	3	0000	50	321		289		274						F
water blank	pH 2 fraction only		50	157		152		139						G
														H
														I
														J

Remarks Indicate Item Code and Card Code

Forms Completion
Initials

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER	Activation Batch	Test Type (Table 10)	Strain .	Batch No
	IVR	CPHF	08 02 85	AIHL	85	0402	033122	01	TA100	8505

Microorganism

Animal	R
Organ	L
Inducer	A
[redacted]	2% S9 Mix

(A) 64	(B) 65	(C) 66	(D) 67 70	(E) 71	(F) 72 73 74 75	(G) 76 78	80
Remarks Made? Yes 1	Phenocopy Check Conclusion (Table 13)	Sterility S 9 Mix 1 Not Contam 2 Contam 3 Not Checked	Activation Mixture Per Plate (µl)	Sample Sterility Check 1 Not Contam 2 Contam 3 Not Checked	Time (min) Temp (Cent) Pre-Incubation	Technician	Card Code
2	1	1	500	1		KLC	A

	Dose Level			Plate A		Plate B		Plate C		Plate D		Plate E		
(H) Solvent Positive (Table 11)	(I) Units of Concentration Blank = mg/ml 2 = µg/ml	(J) Stock concentration (µg/plate)	(K) Amt Per Plate (µl)	(L) Count	(M) BG	(N) Count	(O) BG	(P) Count	(Q) BG	(R) Count	(S) BG	(T) Count	(U) BG	Card Code
43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL 54			50	1126		1132		1135						B
Pos 04		05	50	2870		2762		2676						C
pH 11 fraction only	Acid/Base 1	0000	50	160		175		150						D
water blank	pH 11 fraction only		50	137		155		158						E
														F
														G
														H
														I
														J

Remarks Indicate Item Code and Card Code

Forms Completion
Initials

HERL IN VITRO RESULTS FORM

1	2-4	5-8	9-14	15-18	19-20	21-24	25-30	31-32	33-38	39-42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER Test Sample Identification	Activation Batch	Test Type (Table 10)	Strain	Batch No Microorganism
	IVR	CRVHF	09 24 85	AIVHL	85	0402	0363O	01	ITA98	8507

Animal	Organ	Inducer	(A) 64	(B) 65	(C) 66	(D) 67-70	(E) 71	(F) 72-75	(G) 76-78	80
R	L	A	Remarks Made? Yes 1	Phenocopy Check Conclusion (Table 13)	Sterility S 9 Mix 1 Not Contam 2 Contam 3 Not Checked	Activation Mixture Per Plate (µl)	Sample Sterility Check 1 Not Contam 2 Contam 3 Not Checked	Time (min) Temp (Cent) Pre-Incubation	Technician	Card Code
[redacted] 2% S9 Mix			2	1	1	500	1		KIC	A

	(H) Solvent Positive (Table 11)	43-44	Dose Level (I) Units of Concentration Blank = mg/ml 2 = µg/ml	45	(J) Stock concentration (µg/plate)	46-50	(K) Amt Per Plate (µl)	51-54	Plate A (L) Count	55-58	(M) BG	59	Plate B (N) Count	60-63	(O) BG	64	Plate C (P) Count	65-68	(Q) BG	69	Plate D (R) Count	70-73	(S) BG	74	Plate E (T) Count	75-78	(U) BG	79	Card Code	80
SOL		54					50		36				37				47												B	
Pos		07			10		50		284				360				322												C	
Acid/Base					330		50		55				40				47												D	
					1000		50		31				47				35												E	
					3330		50		47				59				38												F	
				1	0000		50		71				75				77												G	
				3	0000		50		114		2		91		2		89		2										H	
				6	0000		50		0		3		0		3		0		3										I	
water blank							50		57				49				52												J	

Remarks Indicate Item Code and Card Code

Forms Completion Initials	

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER Test Sample Identification	Activation Batch	Test Type (Table 10)	Strain	Batch No
	IVR	CPHF	10 17 85	AZHL	85	0402	03630	01	TA98	8507

Microorganism

Animal	R
Organ	L
Inducer	A
[redacted]	2% S9 Mix

A 64	B 65	C 66	D 67 70	E 71	F 72 73 74 75	G 76 78	80
Remarks Made? Yes 1	Phenocopy Check Conclusion (Table 13)	Sterility S 9 Mix 1 Not Contam 2 Contam 3 Not Checked	Activation Mixture Per Plate (µl)	Sample Sterility Check 1 Not Contam 2 Contam 3 Not Checked	Time (min) Temp (Cent) Pre-Incubation	Technician	Card Code
2	1	1	500	1		KIC	A

	Dose Level				Plate A		Plate B		Plate C		Plate D		Plate E		
H Solvent Positive (Table 11)	I Units of Concentration Blank = mg/ml 2 = µg/ml	J Stock concentration (µg/plate)	K Amt Per Plate (µl)	L Count	M BG	N Count	O BG	P Count	Q BG	R Count	S BG	T Count	U BG	Card Code
43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL 54			500	391		430		287						B
Pos 07		10	500	562		470		479						C
Acid/Base		330	500	591		422		488						D
		1000	500	411		491		466						E
		3330	500	601		577		600						F
	/	0000	500	811		822		799						G
	2	0003	500	113		902		118						H
	3	0000	500	116	2	1022	2	712	2					I
	4	5000	500	837	3	711	3	813	3					J

Remarks Indicate Item Code and Card Code

(continued)

Forms Completion Initials	

HERL IN VITRO RESULTS CONTINUATION FORM

1 Update Code	2 4 System ID	5 8 Research Lab ID	9 14 MO DA YR Experiment Date	15 18 Lab	19 20 YR	21 24 Number Test Sample Identification	25 30 Activation Batch	31 32 Test Type (Table 10)	33 38 Strain	39 42 Batch No Microorganism
	IVR	CPHF	10 17 85	AZHL	- 85	- 0402	2% S9 Mix 0360	01	TA98	8507

		Dose Level		Plate A		Plate B		Plate C		Plate D		Plate E		
(H) Solvent Positive (Table 11)	(I) Units of Concentration Blank = mg/ml 2 = µg/ml	(J) Stock Concentration (µg/plate)	(K) Amt Per Plate (µl)	(L) Count	(M) BG	(N) Count	(O) BG	(P) Count	(Q) BG	(R) Count	(S) BG	(T) Count	(U) BG	Card Code
43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
Water Blank			50	46		54		69						K
														L
														M
														N
														O
														P
														Q
														R
														S
														T
														U
														V
														W
														X
														Y

TABLE 11. MUTAGENICITY OF A CONTAMINATED GROUNDWATER FROM THE STRINGFELLOW HAZARDOUS WASTE DISPOSAL FACILITY UPGRADIENT WELL UGB-8, SAMPLE NO. AIHL-85-042A

Mutagenic Response in TA98 with 2% S-9 Mix[4]

Exp. Date	Extraction Method[1]	Residue Weight (mg)	Sample Volume (L)	Specific Mutagenic Activity (revertants/mg)[2,3]	Mutagenic Response per Unit Volume (revertants/L)
10/17/85	Acid/Base	3.8	3	138	174

1. The extraction method indicates the order of pH in liquid-liquid extraction.

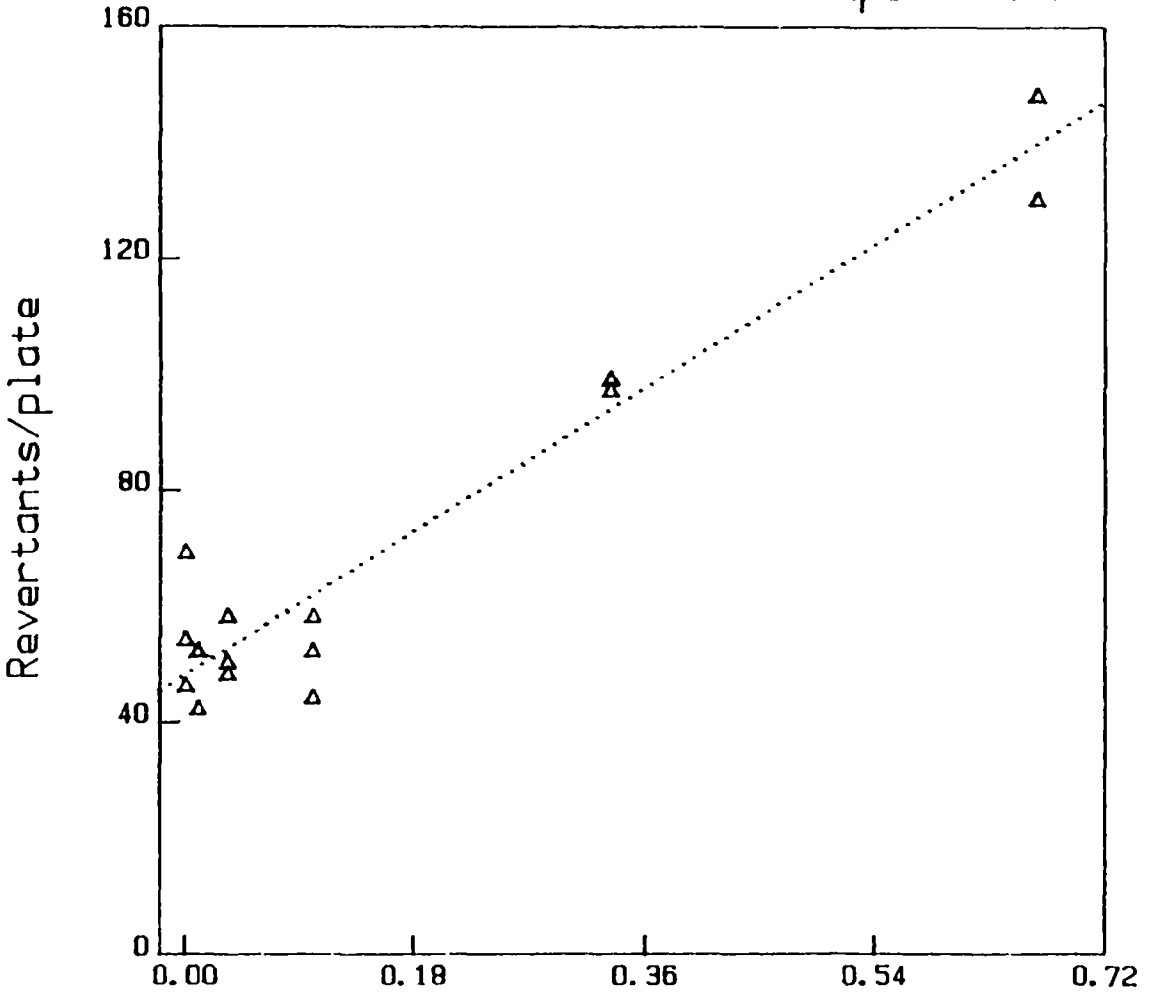
2. The specific activity of the mutagenicity dose-response curve is represented by the slope (**b** as shown in the statistical analysis on graphs in Appendix D).

3. The spontaneous mutation and positive control values are listed on the Strain Function Tests, Cell Titer and Viability Record in Appendix F.

4. The optimum testing condition for the Stringfellow OW-2 sample was in TA98 with 2% S-9 mix. The same condition was applied to the UGB-8 sample for comparison purposes.

STRINGFELLOW WASTEWATER

Dichloromethane Extract (Sample 042A)

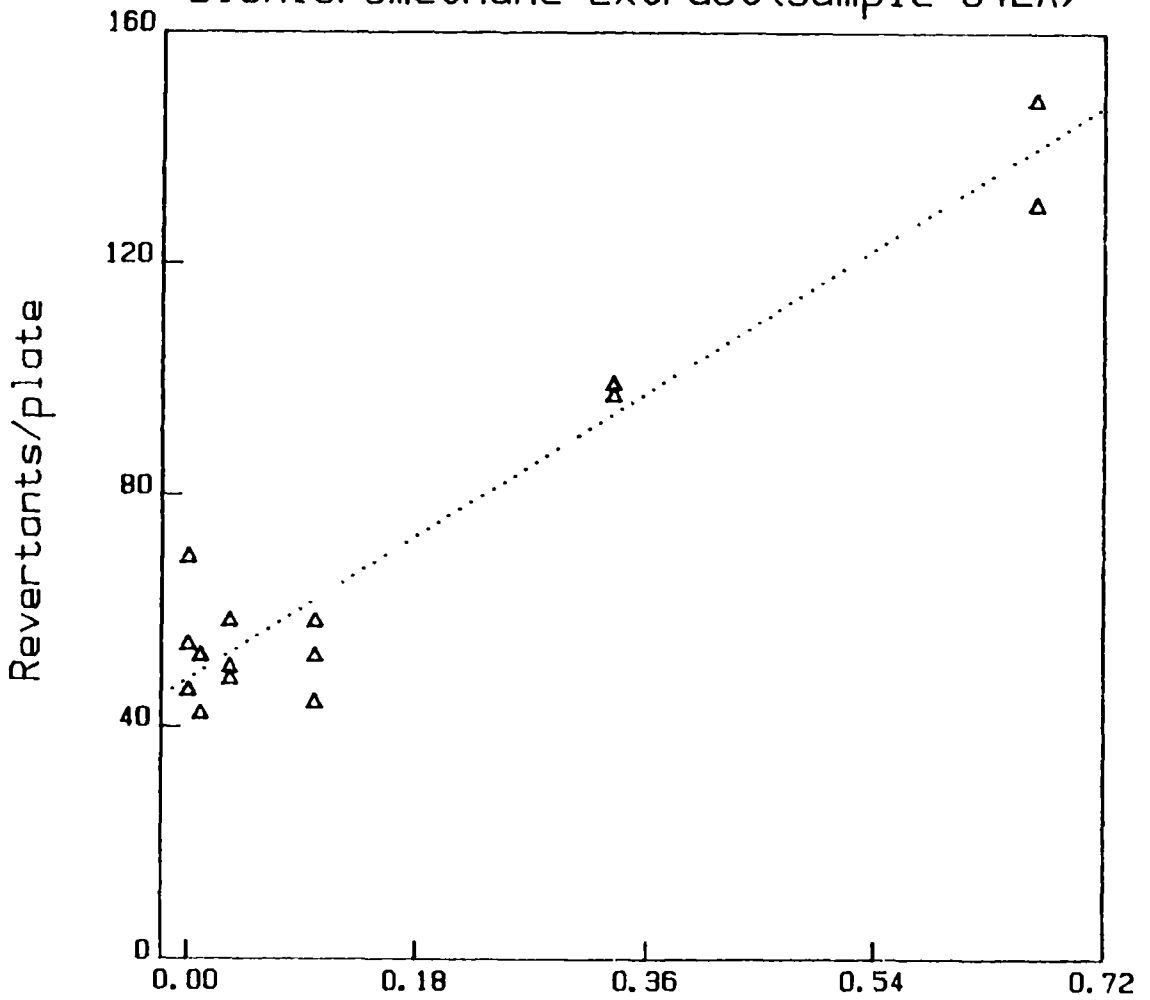


TA 98, 2% S9
SYMBOL=Δ
LINETYPE=.....
y=a+b*x
n=17
a=48.1128 s_a=2.7701
b=137.7112 s_b=10.2019
$s_{y.x}$=9.1548 r=0.9612

X(I)	Y(I)
0.0000	46.0000
0.0000	54.0000
0.0000	69.0000
.0100	52.0000
.0100	42.0000
.0100	52.0000
.0330	48.0000
.0330	58.0000
.0330	50.0000
.1000	58.0000
.1000	52.0000
.1000	44.0000
.3330	99.0000
.3330	99.0000
.3330	97.0000
.6660	130.0000
.6660	148.0000

STRINGFELLOW WASTEWATER

Dichloromethane Extract (Sample 042A)



TA 98, 2% S9
SYMBOL=Δ
LINETYPE=.....
y=a+b*x
n=17
a=48.1128 s_a=2.7701
b=137.7112 s_b=10.2019
$s_{y.x}$=9.1548 r=0.9612

X(I)	Y(I)
0.0000	46.0000
0.0000	54.0000
0.0000	69.0000
.0100	52.0000
.0100	42.0000
.0100	52.0000
.0330	48.0000
.0330	58.0000
.0330	50.0000
.1000	58.0000
.1000	52.0000
.1000	44.0000
.3330	99.0000
.3330	99.0000
.3330	97.0000
.6660	130.0000
.6660	148.0000

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER Test Sample Identification	Activation Batch	Test Type (Table 10)	Strain	Batch No
	IVR	CPHF	10 17 85	ALVL	85	0426A	03630	01	TA98	8507

Microorganism

Animal	R
Organ	L
Inducer	A
[redacted]	2% S9 Mix

(A) 64	(B) 65	(C) 66	(D) 67 70	(E) 71	(F) 72 73 74 75	(G) 76 78	80
2 Remarks Made? Yes 1	1 Phenocopy Check Conclusion (Table 13)	1 Sterility S 9 Mix 1 Not Contam 2 Contam 3 Not Checked	500 Activation Mixture Per Plate (µl)	1 Sample Sterility Check 1 Not Contam 2 Contam 3 Not Checked	Time (min) Temp (Cent) Pre-Incubation	KLC Technician	A Card Code

		Dose Level			Plate A		Plate B		Plate C		Plate D		Plate E		
	(H) Solvent Positive (Table 11)	(I) Units of Concentration Blank = mg/ml 2 = µg/ml	(J) Stock concentration (µg/plate)	(K) Amt Per Plate (µl)	(L) Count	(M) BG	(N) Count	(O) BG	(P) Count	(Q) BG	(R) Count	(S) BG	(T) Count	(U) BG	Card Code
	43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL	54			500	313		443		288						B
Pos	07		10	500	562		470		479						C
			100	500	552		442		552						D
			330	500	488		558		550						E
			1000	500	558		552		444						F
			333	500	997		997		997						G
			666	500	130		148								H
water blank				500	466		554		689						I
															J

Remarks Indicate Item Code and Card Code

Forms Completion Initials	

TABLE 8. MUTAGENICITY OF A MUNICIPAL WASTEWATER TREATMENT PLANT EFFLUENT, SAMPLE NO. AIHL-85-0403

A. Results of the Initial Screening Experiment

Exp. Date	Extraction Method[1]	Residue Weight (mg)	Sample Volume (L)	Specific Mutagenic Activity (revertants/mg)[2,3] TA98 -S9	TA98 2%S9	TA98 10%S9	TA100 -S9	TA100 2%S9	TA100 10%S9
8/2/85	Base/Acid	17	3	< 33	< 43	88	< 166	< 165	< 145

B. Comparison of Extraction Methods for Mutagenic Response in TA98 with 10% S-9 Mix[4]

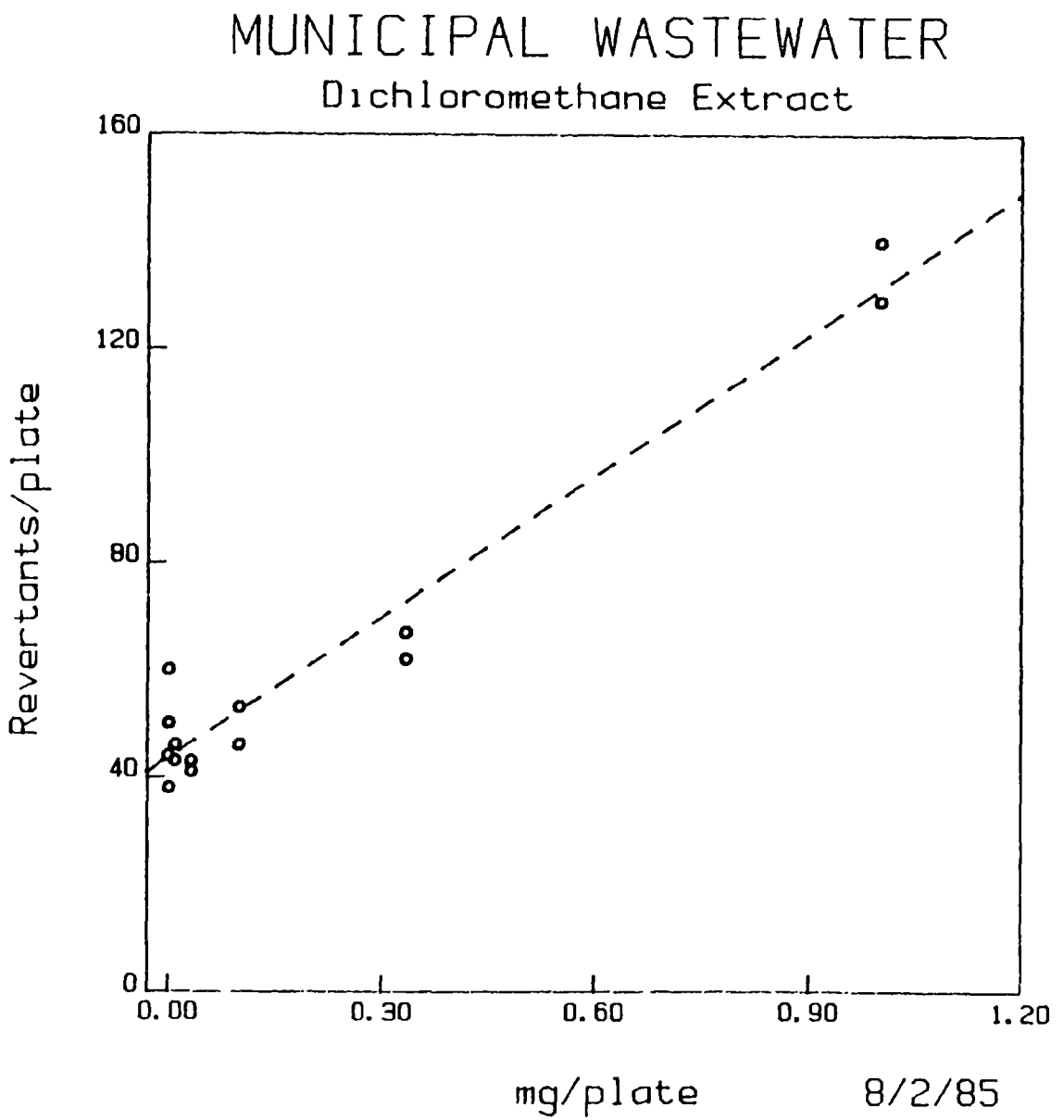
Exp. Date	Extraction Method[1]	Residue Weight (mg)	Sample Volume (L)	Specific Mutagenic Activity (revertants/mg)[2,3]	Mutagenic Response per Unit Volume (revertants/L)
8/2/85	Base/Acid	17	3	88	491
8/13/85	Base/Acid	13	3	75	325
10/17/85	Acid/Base	9	3	148	443

1. The extraction method indicates the order of pH in liquid-liquid extraction.

2. The specific activity of the mutagenicity dose response curve is represented by the slope (b as shown in the statistical analysis on graphs in Appendix D).

3. The spontaneous mutation and positive control values are listed on the Strain Function Tests, Cell Titer and Viability Record in Appendix F.

4. The optimum testing condition in the initial screening experiment was in TA98 with 10% S-9 mix.



TA 98, 10% S9
SYMBOL=o
LINETYPE= — — — —
y=a+b*x
n=15
a=43.5041 s_e=2.2036
b=87.8262 s_b=5.6971
$s_{y.x}$=7.3455 r=0.9737

X(I)	Y(I)
0.0000	60.0000
0.0000	44.0000
0.0000	50.0000
.0010	50.0000
.0010	38.0000
.0100	46.0000
.0100	43.0000
.0330	43.0000
.0330	41.0000
.1000	46.0000
.1000	53.0000
.3330	67.0000
.3330	62.0000
1.0000	140.0000
1.0000	129.0000

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER	Activation Batch	Test Type (Table 10)	Strain	Batch No
	IVR	CPHF	08 02 85	AZHL	85	0403		01	1798	8507

Microorganism

Animal	(A) 64	(B) 65	(C) 66	(D) 67 70	(E) 71	(F) 72 73 74 75	(G) 76 78	80
Organ	Remarks Made? Yes 1	Phenocopy Check Conclusion (Table 13)	Sterility S 9 Mix 1 Not Contam 2 Contam 3 Not Checked	Activation Mixture Per Plate (µl)	Sample Sterility Check 1 Not Contam 2 Contam 3 Not Checked	Time (min) Temp (Cent) Pre-Incubation	Technician	Card Code
Inducer								
[redacted] -59	2	1			1		KIC	A

	Dose Level				Plate A		Plate B		Plate C		Plate D		Plate E		
(H) Solvent Positive (Table 11)	(I) Units of Concentration Blank = mg/ml 2 = µg/ml	(J) Stock concentration (MG/plate)	(K) Amt Per Plate (µl)	(L) Count	(M) BG	(N) Count	(O) BG	(P) Count	(Q) BG	(R) Count	(S) BG	(T) Count	(U) BG	Card Code
43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL 54			050	023		035		023						B
Pos 03		40	050	1128		1230		1045						C
		10	050	029		020		023						D
		100	050	025		026		021						E
		330	050	029		033		030						F
		1000	050	038		023		026						G
		3300	050	042		037		041						H
	/	0000	030	053		051								I
water blank			050	029		037		032						J

Remarks Indicate Item Code and Card Code

Forms Completion Initials

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER	Activation Batch	Test Type (Table 10)	Strain	Batch No
	IVR	CPHF	08 02 85	AIHL	85	0403	03122	01	TA98	85 07
				Test Sample Identification					Microorganism	

Animal	(A) 64	(B) 65	(C) 66	(D) 67 70	(E) 71	(F) 72 73 74 75	(G) 76 78	80
R	Remarks Made? Yes 1	Phenocopy Check Conclusion (Table 13)	Sterility S 9 Mix 1 Not Contam 2 Contam 3 Not Checked	Activation Mixture Per Plate (µl)	Sample Sterility Check 1 Not Contam 2 Contam 3 Not Checked	Pre-Incubation: Time (min) Temp (Cent)	Technician	Card Code
Organ L								
Inducer A								
[redacted] 2% S9 Mix	2	1	1	500	1		KIC	A

	(H) Solvent Positive (Table 11)	(I) Units of Concentration Blank = mg/ml 2 = µg/ml	(J) Stock concentration (mg/plate)	(K) Amt Per Plate (µl)	Plate A (L) Count	Plate A (M) BG	Plate B (N) Count	Plate B (O) BG	Plate C (P) Count	Plate C (Q) BG	Plate D (R) Count	Plate D (S) BG	Plate E (T) Count	Plate E (U) BG	Card Code
	43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL	54			50	227		377		374						B
Pos	07		1.0	50	319		375		416						C
			1.0	50	322		422								D
			10.0	50	336		300								E
			33.0	50	446		444								F
			100.0	50	337		422								G
			333.0	50	511		661								H
		1	1000.0	50	661		655								I
water blank				50	335		397		354						J

Remarks Indicate Item Code and Card Code

Forms Completion
Initials

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER Test Sample Identification	Activation Batch	Test Type (Table 10)	Strain	Batch No Microorganism
	IVR	CPHF	08 02 85	AIHL	85	0403	03122	01	1 T A 98	8507

Animal	R
Organ	L
Inducer	A
[redacted]	10 % S9 Mix

(A) 64 — 2 — Remarks Made? Yes 1

(B) 65 — 1 — Phenocopy Check Conclusion (Table 13)

(C) 66 — 1 — Sterility S 9 Mix: 1 Not Contam, 2 Contam, 3 Not Checked

(D) 67 70 — 500 — Activation Mixture Per Plate (µl)

(E) 71 — 1 — Sample Sterility Check: 1 Not Contam, 2 Contam, 3 Not Checked

(F) 72 73 74 75 — Time (min) Temp (Centi) — Pre-Incubation

(G) 76 78 — KIC — Technician

80 — A — Card Code

	(H) Solvent Positive (Table 11)	(I) Units of Concentration Blank = mg/ml 2 = µg/ml	(J) Stock concentration (µg/plate)	(K) Amt Per Plate (µl)	Plate A (L) Count	Plate A (M) BG	Plate B (N) Count	Plate B (O) BG	Plate C (P) Count	Plate C (Q) BG	Plate D (R) Count	Plate D (S) BG	Plate E (T) Count	Plate E (U) BG	Card Code
	43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL	54			50	33		46		44						B
Pos	07		10	50	313		283		370						C
			10	50	50		38								D
			100	50	46		43								E
			330	50	43		41								F
			1000	50	46		53								G
			3330	50	67		62								H
		1	0000	50	140		129								I
water blank				50	60		44		50						J

Remarks Indicate Item Code and Card Code

Forms Completion	
Initials	

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER	Activation Batch	Test Type (Table 10)	Strain	Batch No
	IVR	CPHF	08 02 85	AIHL	85	0403		01	TA100	85052

Microorganism

Animal	(A) 64	(B) 65	(C) 66	(D) 67 70	(E) 71	(F) 72 73 74 75	(G) 76 78	80
Organ	2 Remarks Made? Yes 1	1 Phenocopy Check Conclusion (Table 13)	Sterility S 9 Mix 1 Not Contam 2 Contam 3 Not Checked	Activation Mixture Per Plate (µl)	Sample Sterility Check 1 Not Contam 2 Contam 3 Not Checked	Time (min) Temp (Cent) Pre-Incubation	Technician	Card Code
Inducer								
[redacted] -59								A

(H) Solvent Positive (Table 11)	(I) Units of Concentration Blank = mg/ml 2 = µg/ml	Dose Level: (J) Stock concentration (µg/plate)	Dose Level: (K) Amt Per Plate (µl)	Plate A: (L) Count	Plate A: (M) BG	Plate B: (N) Count	Plate B: (O) BG	Plate C: (P) Count	Plate C: (Q) BG	Plate D: (R) Count	Plate D: (S) BG	Plate E: (T) Count	Plate E: (U) BG	Card Code
43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL 54			50	1148		1142		1148						B
Pos 01		05	50	711		683		681						C
		10	50	132		142								D
		100	50	149		154								E
		330	50	146		165								F
		1000	50	167		152								G
		330	50	143		137								H
	/	000	50	140										I
water blank			50	173		133		172						J

Remarks Indicate Item Code and Card Code

Forms Completion
Initials

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER Test Sample Identification	Activation Batch	Test Type (Table 10)	Strain	Batch No
	IVR	CPHF	08 02 85	AEHL	85	0403	03122	01	TA100	8505

Microorganism

Animal	R
Organ	L
Inducer	A
[redacted]	2% S9 Mix

(A) 64	(B) 65	(C) 66	(D) 67 70	(E) 71	(F) 72 73 74 75		(G) 76 78	80
Remarks Made? Yes-1	Phenocopy Check Conclusion (Table 13)	Sterility S 9 Mix 1 Not Contam 2 Contam 3 Not Checked	Activation Mixture Per Plate (µl)	Sample Sterility Check 1 Not Contam 2 Contam 3 Not Checked	Time (min)	Temp (Cent) Pre-Incubation	Technician	Card Code
2	1	1	500	1			KIC	A

(H) Solvent Positive (Table 11)	Dose Level: (I) Units of Concentration Blank = mg/ml 2 = µg/ml	Dose Level: (J) Stock concentration (mg/plate)	Dose Level: (K) Amt Per Plate (µl)	Plate A: (L) Count	Plate A: (M) BG	Plate B: (N) Count	Plate B: (O) BG	Plate C: (P) Count	Plate C: (O) BG	Plate D: (R) Count	Plate D: (S) BG	Plate E: (T) Count	Plate E: (U) BG	Card Code
	43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL	54			50	126		132		135						B
Pos	04		05	50	2676		2870		2762						C
			10	50	144		139								D
			100	50	125		133								E
			330	50	144		154								F
			1000	50	136		153								G
			3330	50	136		149								H
		1	0000	50	160										I
water blank				50	170		181		144						J

Remarks Indicate Item Code and Card Code

Forms Completion
Initials

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER Test Sample Identification	Activation Batch	Test Type (Table 10)	Strain Microorganism	Batch No
	IVR	CPHF	08 02 85	AIHL	85	0403	03122	01	TA100	8505

Animal	R	(A) 64	(B) 65	(C) 66	(D) 67 70	(E) 71	(F) 72 73 74 75	(G) 76 78	80
Organ	L	2 Remarks Made? Yes 1	1 Phenocopy Check Conclusion (Table 13)	1 Sterility S 9 Mix 1 Not Contam 2 Contam 3 Not Checked	500 Activation Mixture Per Plate (µl)	1 Sample Sterility Check 1 Not Contam 2 Contam 3 Not Checked	Time (min) Temp (Cent) Pre-Incubation	KIC Technician	A Card Code
Inducer	A								
[redacted]	10 % S9 Mix								

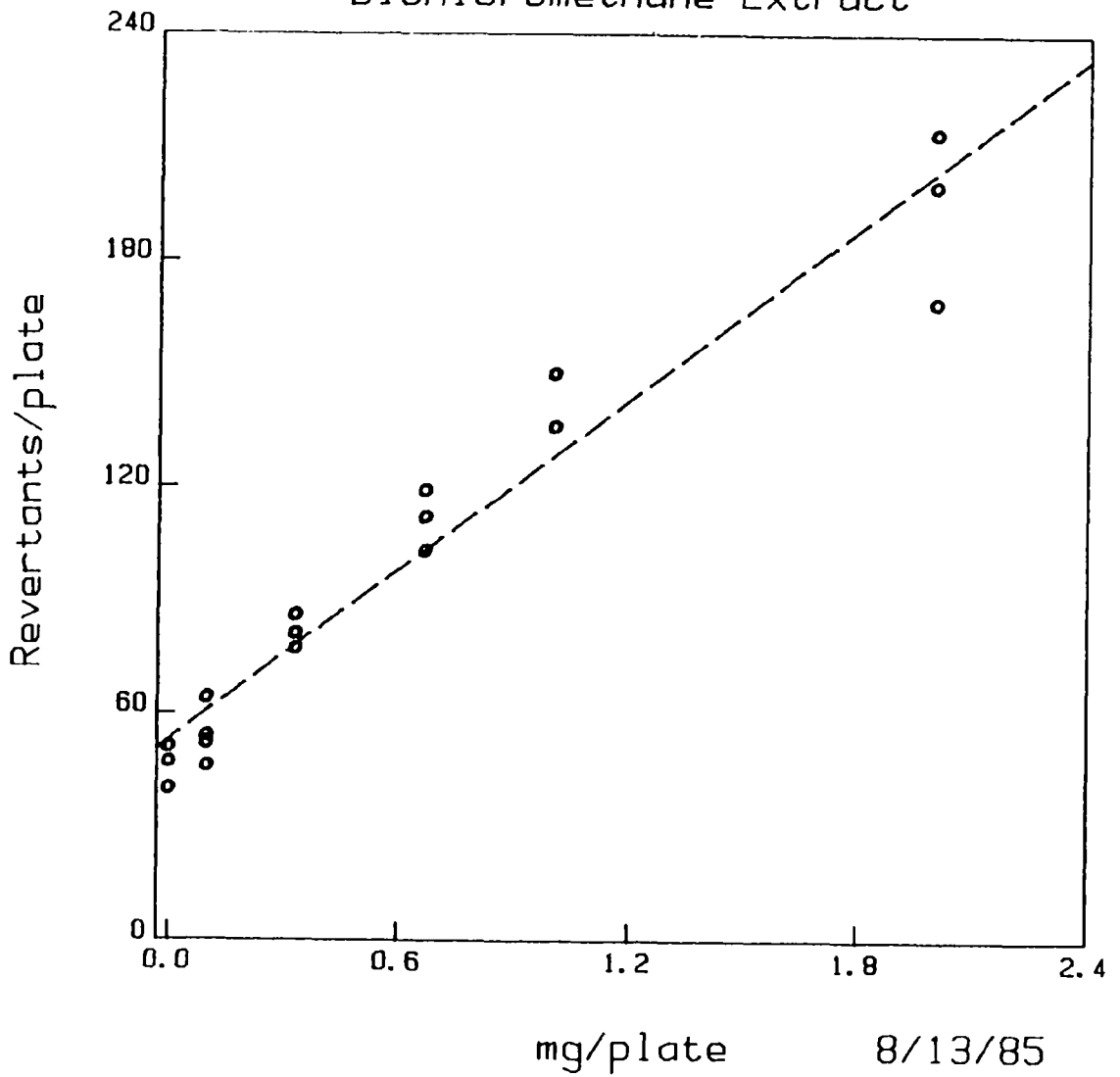
		Dose Level			Plate A		Plate B		Plate C		Plate D		Plate E		
	(H) Solvent Positive (Table 11)	(I) Units of Concentration Blank = mg/ml 2 = µg/ml	(J) Stock concentration (µg/plate)	(K) Amt Per Plate (µl)	(L) Count	(M) BG	(N) Count	(O) BG	(P) Count	(Q) BG	(R) Count	(S) BG	(T) Count	(U) BG	Card Code
	43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL	54			50	1297		1266		1204						B
Pos	04		05	50	1510		1482		1435						C
			10	50	1118										D
			100	50	1222		1223								E
			330	50	1330		1336								F
			1000	50	1148		1140								G
			3330	50	1155		1162								H
		1	0000	50	1164										I
water blnk				50	1144		1352		1440						J

Remarks Indicate Item Code and Card Code

Forms Completion
Initials

MUNICIPAL WASTEWATER

Dichloromethane Extract



TA 98, 10% S9
SYMBOL=o
LINETYPE= — — — —
y=a+b*x
n=19
a=52.7575 s_a=4.1411
b=75.3898 s_b=4.4166
$s_{y.x}$=12.9631 r=0.9720

X(I)	Y(I)
0.0000	40.0000
0.0000	47.0000
0.0000	51.0000
.1000	54.0000
.1000	46.0000
.1000	64.0000
.1000	52.0000
.3330	81.0000
.3330	86.0000
.3330	77.0000
.6660	119.0000
.6660	103.0000
.6660	112.0000
1.0000	150.0000
1.0000	136.0000
1.0000	136.0000
2.0000	200.0000
2.0000	214.0000
2.0000	199.0000

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	Experiment Date	Test Sample Identification			Activation Batch	Test Type (Table 10)	Strain	Batch No
	IVR	CPHF	08/13/85	AIHL	85	0403	03122	01	17A98	85072
			MO DA YR	LAB	YR	NUMBER			Microorganism	

Animal	R
Organ	L
Inducer	A
[redacted]	10% S9 Mix

(A) 64 Remarks Made? Yes 1: 2

(B) 65 Phenocopy Check Conclusion (Table 13): 1

(C) 66 Sterility S 9 Mix: 1 (1 Not Contam, 2 Contam, 3 Not Checked)

(D) 67 70 Activation Mixture Per Plate (µl): 300

(E) 71 Sample Sterility Check: 1 (1 Not Contam, 2 Contam, 3 Not Checked)

(F) 72 73 74 75 Pre-Incubation: Time (min) | Temp (Cent)

(G) 76 78 Technician: KIC

80 Card Code: A

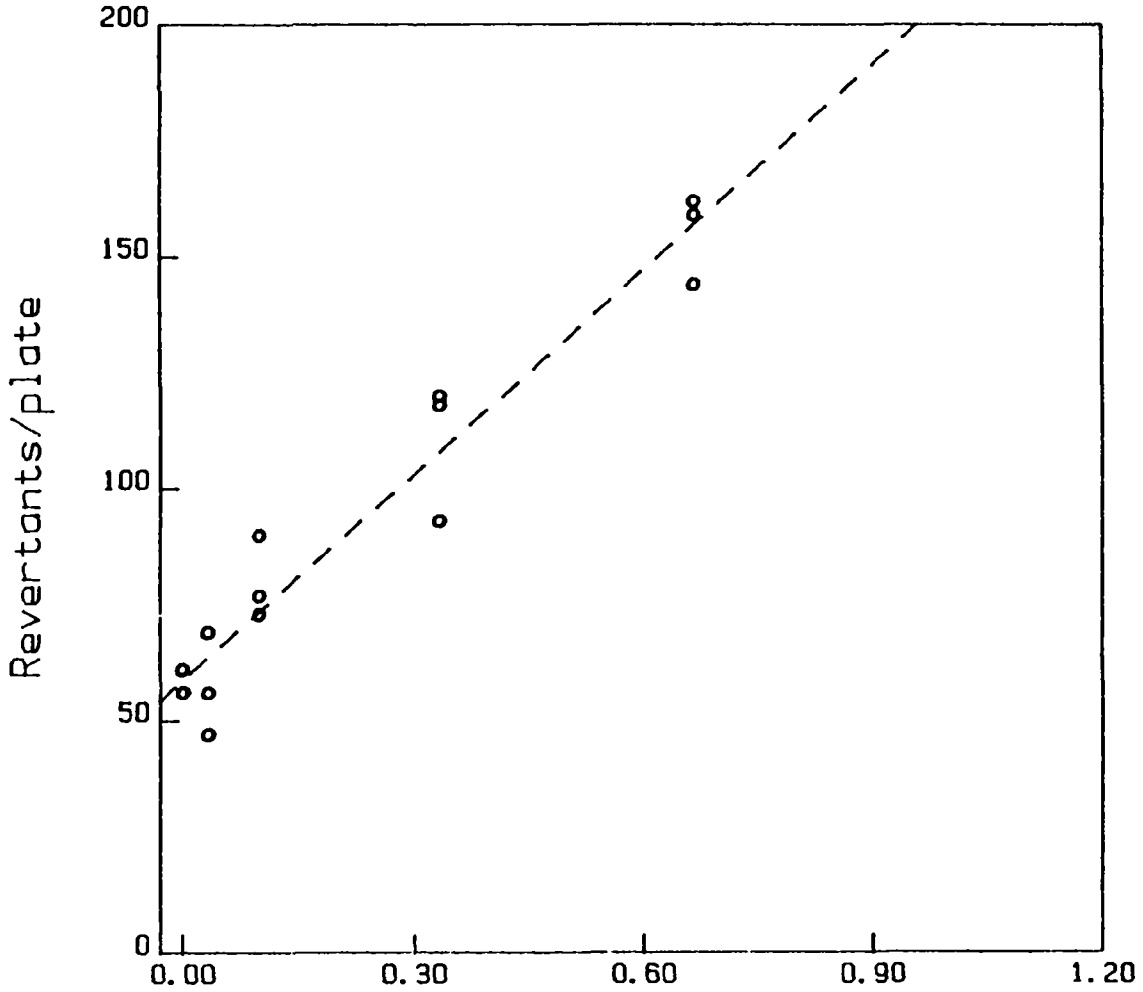
(H) Solvent Positive (Table 11)	Dose Level: (I) Units of Concentration Blank = mg/ml 2 = µg/ml	Dose Level: (J) Stock concentration (µg/plate)	Dose Level: (K) Amt Per Plate (µl)	Plate A: (L) Count	Plate A: (M) BG	Plate B: (N) Count	Plate B: (O) BG	Plate C: (P) Count	Plate C: (Q) BG	Plate D: (R) Count	Plate D: (S) BG	Plate E: (T) Count	Plate E: (U) BG	Card Code
43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL 54			50	47		36		47						B
Pos 07		10000	50	2038		1845		2095						C
		10000	50	54		46		64		52				D
		3330	50	81		86		77						E
		6660	50	119		103		112						F
	1	0000	50	150		136		136						G
	2	0000	30	200		214		169						H
water blank			50	40		47		51						I
														J

Remarks Indicate Item Code and Card Code

Forms Completion
Initials

MUNICIPAL WASTEWATER

Dichloromethane Extract



TA 98, 10% S9
SYMBOL=o
LINETYPE= — — — —
y=a+b*x
n=15
a=58.6512 s_a=3.5406
b=147.5947 s_b=10.5277
$s_{y.x}$=10.1402 r=0.9685

X(I)	Y(I)
0 0000	56 0000
0 0000	56.0000
0 0000	61.0000
0330	47 0000
0330	56 0000
0330	69 0000
1000	73 0000
1000	77 0000
1000	90 0000
3330	118 0000
3330	120.0000
3330	93.0000
6660	159 0000
6660	162 0000
6660	144 0000

HERL IN VITRO RESULTS FORM

1	2-4	5-8	9-14	15-18	19-20	21-24	25-30	31-32	33-38	39-42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER Test Sample Identification	Activation Batch	Test Type (Table 10)	Strain	Batch No Microorganism
	IVR	CPHF	10 17 85	AZHL	85	0403	03630	01	TA98	8507

Animal	R
Organ	L
Inducer	A
[redacted]	10% S9 Mix

(A) 64	(B) 65	(C) 66	(D) 67-70	(E) 71	(F) 72-73-74-75	(G) 76-78	80
2 Remarks Made? Yes 1	1 Phenocopy Check Conclusion (Table 13)	1 Sterility S9 Mix 1 Not Contam 2 Contam 3 Not Checked	500 Activation Mixture Per Plate (µl)	1 Sample Sterility Check 1 Not Contam 2 Contam 3 Not Checked	Time (min) Temp (Cent) Pre-Incubation	KIC Technician	A Card Code
	1	1	500	1		KIC	A

	Dose Level				Plate A		Plate B		Plate C		Plate D		Plate E		
(H) Solvent Positive (Table 11)	(I) Units of Concentration Blank = mg/ml 2 = µg/ml	(J) Stock concentration (µg/plate)	(K) Amt Per Plate (µl)	(L) Count	(M) BG	(N) Count	(O) BG	(P) Count	(Q) BG	(R) Count	(S) BG	(T) Count	(U) BG	Card Code
43-44	45	46-50	51-54	55-58	59	60-63	64	65-68	69	70-73	74	75-78	79	80
SOL 54			500	53		455		465						B
Pos 07		10	500	326		359		367						C
Acid/Base		3300	500	477		556		669						D
		1000	500	773		777		990						E
		3330	500	1118		1120		993						F
		6660	500	1579		1626		1444						G
	2	0000	500	1855		1899								H
Water blank			500	556		556		661						I
														J

Remarks Indicate Item Code and Card Code

Forms Completion
Initials

TABLE 6. MUTAGENICITY OF A BRACKISH SAN FRANCISCO BAY SURFACE WATER, SAMPLE NO. AIHL-85-0404

A. Results of the Initial Screening Experiment

Exp. Date	Extraction Method[1]	Residue Weight (mg)	Sample Volume (L)	Specific Mutagenic Activity (revertants/mg)[2,3] TA98 -S9	TA98 2%S9	TA98 10%S9	TA100 -S9	TA100 2%S9	TA100 10%S9
9/24/85	Base/Acid	6.5	3	< 106	< 139	< 162	< 484	< 460	< 401

B. Comparison of Extraction Methods for Mutagenic Response in TA98 with 2% S-9 Mix[4]

Exp. Date	Extraction Method[1]	Residue Weight (mg)	Sample Volume (L)	Specific Mutagenic Activity (revertants/mg)[2,3]	Mutagenic Response per Unit Volume (revertants/L)
10/4/85	Acid/Base	4.8	3	177	283
12/17/85	Base/Acid	1.2	1.5	218	176

1. The extraction method indicates the order of pH in liquid-liquid extraction.

2. The specific activity of the mutagenicity dose response curve is represented by the slope (**b** as shown in the statistical analysis on graphs in Appendix D).

3. The spontaneous mutation and positive control values are listed on the Strain Function Tests, Cell Titer, and Viability Record in Appendix F.

4. Although results of all six conditions in the initial screening experiment were below the detection limit, TA98 with 2% S-9 mix produced a questionable positive response close to the detection limit. This condition was therefore used in the follow-up experiments.

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER Test Sample Identification	Activation Batch	Test Type (Table 10)	Strain	Batch No
	IVR	CPHF	09 24 85	AIHL	85	0404		01	TA98	85 07
									Microorganism	

Animal	(A) 64	(B) 65	(C) 66	(D) 67 70	(E) 71	(F) 72 73 74 75	(G) 76 78	80
Organ	2 Remarks Made? Yes 1	1 Phenocopy Check Conclusion (Table 13)	Sterility S 9 Mix 1 Not Contam 2 Contam 3 Not Checked	Activation Mixture Per Plate (µl)	1 Sample Sterility Check 1 Not Contam 2 Contam 3 Not Checked	Time (min) Temp (Cent) Pre-Incubation	Technician	Card Code
Inducer								
[redacted] -59							KZC	A

	Dose Level				Plate A		Plate B		Plate C		Plate D		Plate E		
(H) Solvent Positive (Table 11)	(I) Units of Concentration Blank = mg/ml 2 = µg/ml	(J) Stock concentration (µg / plate)	(K) Amt Per Plate (µl)	(L) Count	(M) BG	(N) Count	(O) BG	(P) Count	(Q) BG	(R) Count	(S) BG	(T) Count	(U) BG	Card Code
43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL 54			050	221		333		220						B
Pos 03		4000	050	1155		1257		1310						C
		1000	050	222		225								D
		3300	050	330		258								E
		1000	050	448		433								F
		3330	050	666		551								G
Water Blank			050	330		337		337						H
														I
														J

Remarks Indicate Item Code and Card Code

Forms Completion
Initials

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER Test Sample Identification	Activation Batch	Test Type (Table 10)	Strain .	Batch No Microorganism
	IVR	CPHF	09 24 85	AIHL	85	0404	03630	01	1 7A 78	85 07

Animal	(A) 64	(B) 65	(C) 66	(D) 67 70	(E) 71	(F) 72 73 74 75	(G) 76 78	80
Organ	2 Remarks Made? Yes 1	1 Phenocopy Check Conclusion (Table 13)	1 Sterility S 9 Mix 1 Not Contam 2 Contam 3 Not Checked	500 Activation Mixture Per Plate (µl)	1 Sample Sterility Check 1 Not Contam 2 Contam 3 Not Checked	Time (min) Temp (Cent) Pre-Incubation	KLC Technician	A Card Code
Inducer								
[redacted]								
2% S9 Mix								

	Dose Level			Plate A		Plate B		Plate C		Plate D		Plate E		
(H) Solvent Positive (Table 11)	(I) Units of Concentration Blank = mg/ml 2 = µg/ml	(J) Stock concentration (µg/plate)	(K) Amt Per Plate (µl)	(L) Count	(M) BG	(N) Count	(O) BG	(P) Count	(Q) BG	(R) Count	(S) BG	(T) Count	(U) BG	Card Code
43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL 54			50	36		37		47						B
Pos 07		10	50	284		360		322						C
		100	50	53		38								D
		330	50	31		44								E
		1000	50	46		52								F
		333	50	89		90								G
water blank			50	43		52		44						H
														I
														J

Remarks Indicate Item Code and Card Code

Forms Completion
Initials

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER Test Sample Identification	Activation Batch	Test Type (Table 10)	Strain	Batch No Microorganism
	IVR	CPHF	07 24 85	AIHL	85	0404	03630	01	TA98	8507

Animal	(A) 64	(B) 65	(C) 66	(D) 67 70	(E) 71	(F) 72 73 74 75	(G) 76 78	80
Organ	2 Remarks Made? Yes 1	1 Phenocopy Check Conclusion (Table 13)	1 Sterility S 9 Mix 1 Not Contam 2 Contam 3 Not Checked	500 Activation Mixture Per Plate (µl)	1 Sterility Check 1 Not Contam 2 Contam 3 Not Checked	Time (min) Temp (Cent) Pre-Incubation	KIC Technician	A Card Code
Inducer								
[redacted]	10 % S9 Mix							

	Dose Level			Plate A		Plate B		Plate C		Plate D		Plate E		
(H) Solvent Positive (Table 11)	(I) Units of Concentration Blank = mg/ml 2 = µg/ml	(J) Stock concentration (mg/plate)	(K) Amt. Per Plate (µl)	(L) Count	(M) BG	(N) Count	(O) BG	(P) Count	(Q) BG	(R) Count	(S) BG	(T) Count	(U) BG	Card Code
43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL 54			50	31		52		45						B
Pos 07		10	50	462		350		381						C
		100	30	59		58								D
		330	50	46		46								E
		1000	50	61		50								F
		3330	50	90		70								G
Water blank			50	42		57		63						H
														I
														J

Remarks Indicate Item Code and Card Code

Forms Completion
Initials

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER Test Sample Identification	Activation Batch	Test Type (Table 10)	Strain	Batch No
	IVR	CPHF	09 24 85	AZHL	85	0404		01	TA100	8508

Microorganism

Animal	(A) 64	(B) 65	(C) 66	(D) 67 70	(E) 71	(F) 72 73 74 75	(G) 76 78	80
Organ	2	1			1		KIC	A
Inducer	Remarks Made? Yes 1	Phenocopy Check Conclusion (Table 13)	Sterility S 9 Mix 1 Not Contam 2 Contam 3 Not Checked	Activation Mixture Per Plate (µl)	Sample Sterility Check 1 Not Contam 2 Contam 3 Not Checked	Time (min) Temp (Cent) Pre-Incubation	Technician	Card Code
[redacted] -S9								

	Dose Level			Plate A		Plate B		Plate C		Plate D		Plate E		
(H) Solvent Positive (Table 11)	(I) Units of Concentration Blank = mg/ml 2 = µg/ml	(J) Stock concentration (µg/plate)	(K) Amt Per Plate (µl)	(L) Count	(M) BG	(N) Count	(O) BG	(P) Count	(Q) BG	(R) Count	(S) BG	(T) Count	(U) BG	Card Code
43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL 54			50	173		147		145						B
Pos 01		05	50	1339		1649		1744						C
		100	50	181		164								D
		330	50	193		177								E
		1000	50	173		189								F
		333	50	216		183								G
water blank			50	181		148		155						H
														I
														J

Remarks Indicate Item Code and Card Code

Forms Completion
Initials

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER Test Sample Identification	Activation Batch	Test Type (Table 10)	Strain	Batch No Microorganism
	IVR	CPHF	09 24 85	AIHL	85	0404	03630	01	TA100	8508

Animal	(A) 64	(B) 65	(C) 66	(D) 67 70	(E) 71	(F) 72 73 74 75	(G) 76 78	80
Organ	2	1	1	500	1		KIC	A
Inducer	Remarks Made? Yes 1	Phenocopy Check Conclusion (Table 13)	Sterility S 9 Mix 1 Not Contam 2 Contam 3 Not Checked	Activation Mixture Per Plate (µl)	Sample Sterility Check 1 Not Contam 2 Contam 3 Not Checked	Pre-Incubation: Time (min) Temp (Cent)	Technician	Card Code
[redacted] 2% S9 Mix								

	(H) Solvent Positive (Table 11)	(I) Units of Concentration Blank = mg/ml 2 = µg/ml	Dose Level: (J) Stock concentration (µg/plate)	Dose Level: (K) Amt Per Plate (µl)	Plate A: (L) Count	Plate A: (M) BG	Plate B: (N) Count	Plate B: (O) BG	Plate C: (P) Count	Plate C: (Q) BG	Plate D: (R) Count	Plate D: (S) BG	Plate E: (T) Count	Plate E: (U) BG	Card Code
	43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL	54			50	1162		1161		1422						B
Pos	04		05	50	2435		2147		2522						C
			10	50	1597		1331								D
			330	50	1781		1811								E
			100	30	1511		1710								F
			333	50	2022		2111								G
water blank				50	1605		1455		1555						H
															I
															J

Remarks Indicate Item Code and Card Code

Forms Completion
Initials

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER Test Sample Identification	Activation Batch	Test Type (Table 10)	Strain	Batch No Microorganism
	IVR	CPHF	09 24 83	AZHL	83	0404	03630	01	TA100	8508

Animal	(A) 64	(B) 65	(C) 66	(D) 67 70	(E) 71	(F) 72 73 74 75	(G) 76 78	80
Organ	2 Remarks Made? Yes 1	1 Phenocopy Check Conclusion (Table 13)	1 Sterility S 9 Mix 1 Not Contam 2 Contam 3 Not Checked	500 Activation Mixture Per Plate (µl)	1 Sterility Check 1 Not Contam 2 Contam 3 Not Checked	Time (min) Temp (Cent) Pre-Incubation	KIC Technician	A Card Code
Inducer								
[redacted]	10% S9 Mix							

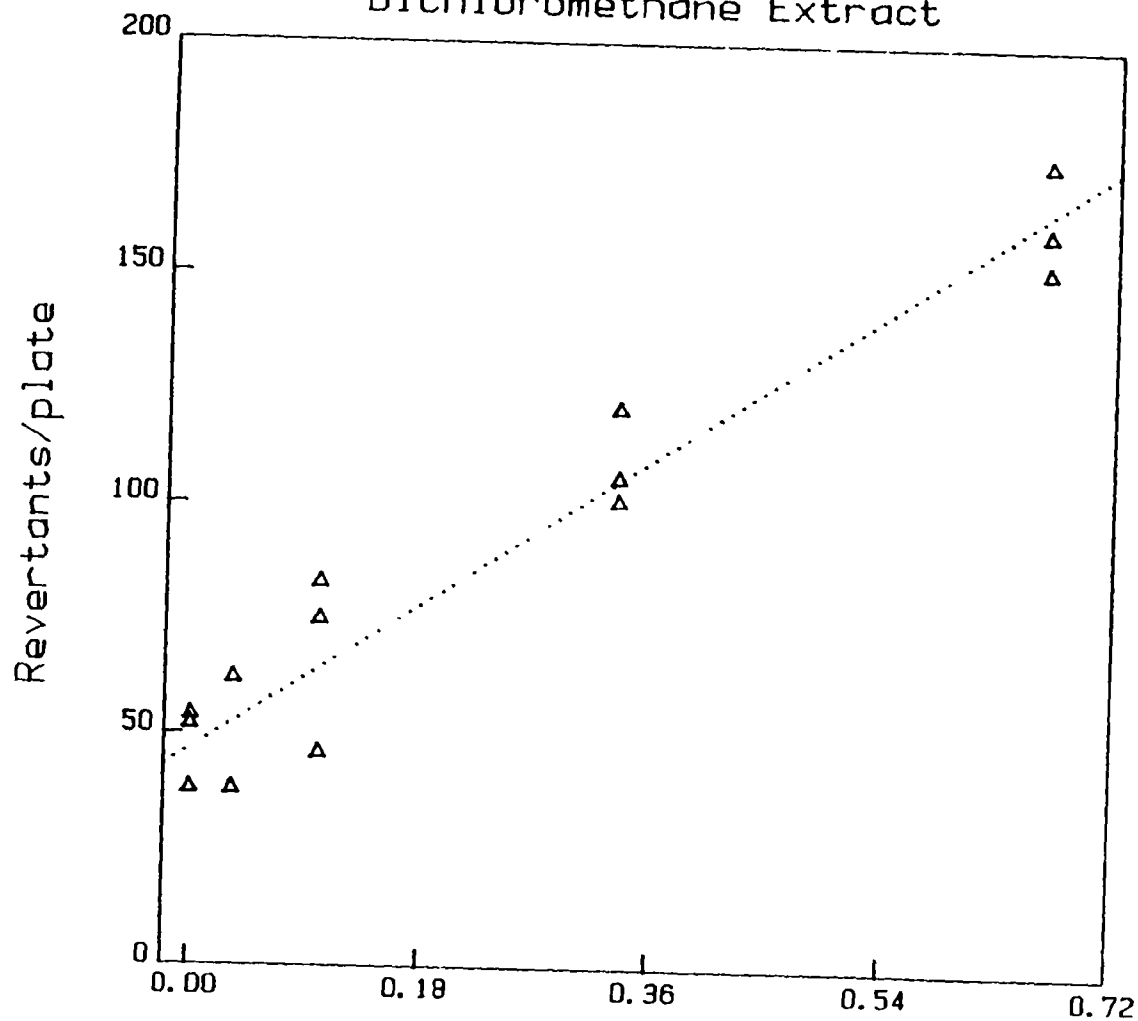
		Dose Level			Plate A		Plate B		Plate C		Plate D		Plate E		
	(H) Solvent Positive (Table 11)	(I) Units of Concentration Blank = mg/ml 2 = µg/ml	(J) Stock concentration (µg/plate)	(K) Amt Per Plate (µl)	(L) Count	(M) BG	(N) Count	(O) BG	(P) Count	(Q) BG	(R) Count	(S) BG	(T) Count	(U) BG	Card Code
	43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL	54			50	136		163		155						B
Pos	04		05	50	1037		732		953						C
			100	50	120		130								D
			330	50	156		147								E
			1000	50	151		158								F
			3330	50	161		166								G
water blank				50	147		129		125						H
															I
															J

Remarks Indicate Item Code and Card Code

Forms Completion	
Initials	

BAY WASTEWATER

Dichloromethane Extract



TA 98, 2% S9
SYMBOL=Δ
LINETYPE=.....
y=a+b*x
n=15
a=46.7185
b=176.7438
$s_{y.x}$=12.4395
s_a=4.3434
s_b=12.9148
r=0.9670

X(I)	Y(I)
0.0000	38.0000
0.0000	54.0000
0.0000	52.0000
.0330	62.0000
.0330	38.0000
.0330	38.0000
.1000	46.0000
.1000	83.0000
.1000	75.0000
.3330	101.0000
.3330	121.0000
.3330	106.0000
.6660	175.0000
.6660	160.0000
.6660	152.0000

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER	Activation Batch	Test Type (Table 10)	Strain	Batch No
	IVR	CPHF	10 04 85	AIHL	85	0404	0363	01	1 TA98	8507

Test Sample Identification

Microorganism

Animal	(A) 64	(B) 65	(C) 66	(D) 67 70	(E) 71	(F) 72 73 74 75	(G) 76 78	80
Organ	2	1	1	500	1		KZC	A
Inducer	Remarks Made? Yes 1	Phenocopy Check Conclusion (Table 13)	Sterility S 9 Mix 1 Not Contam 2 Contam 3 Not Checked	Activation Mixture Per Plate (µl)	Sample Sterility Check 1 Not Contam 2 Contam 3 Not Checked	Time (min) Temp (Cent) Pre-Incubation	Technician	Card Code
[redacted] 2% S9 Mix								

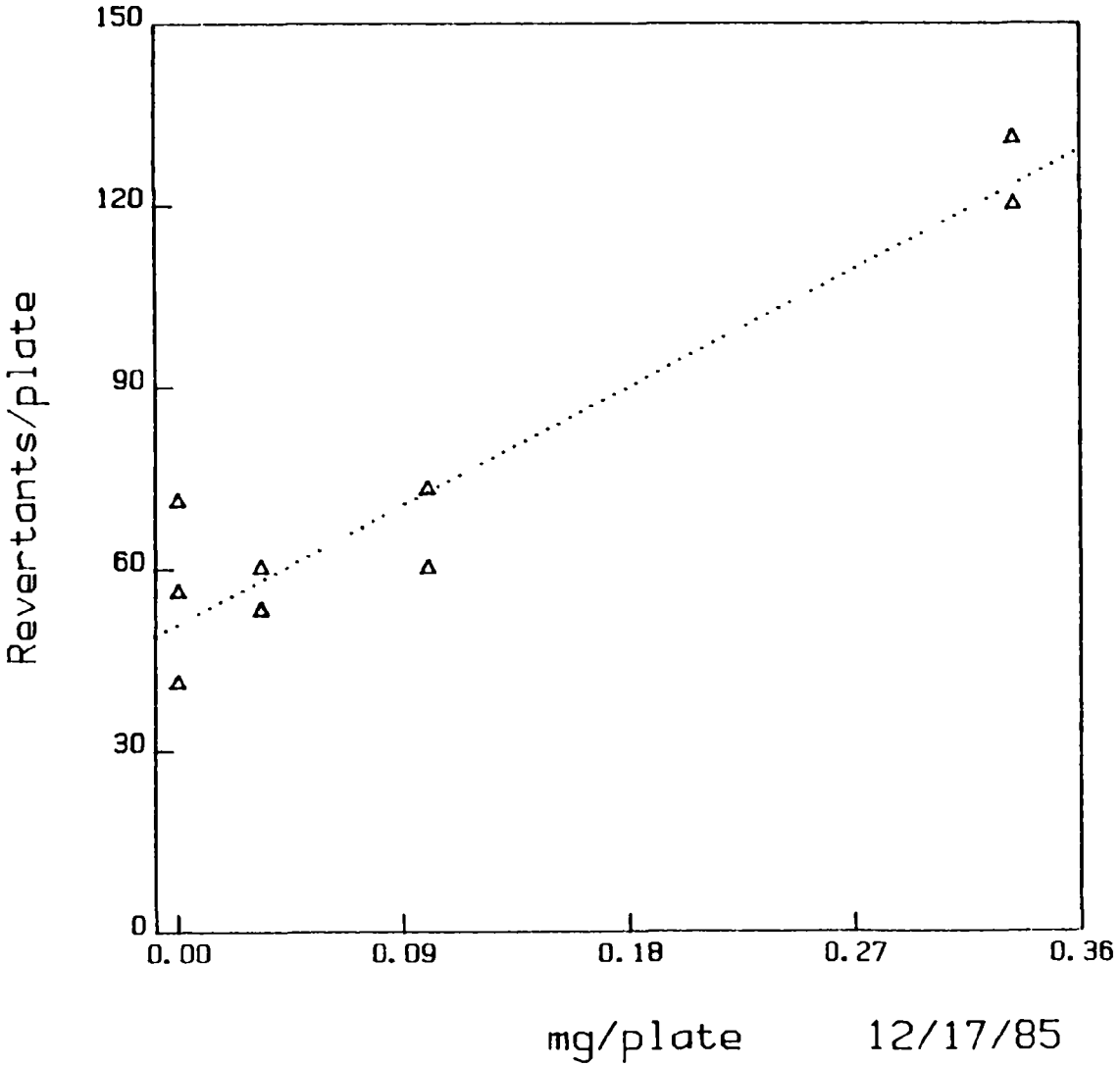
	Dose Level			Plate A		Plate B		Plate C		Plate D		Plate E		
(H) Solvent Positive (Table 11)	(I) Units of Concentration Blank = mg/ml 2 = µg/ml	(J) Stock concentration (µg/plate)	(K) Amt Per Plate (µl)	(L) Count	(M) BG	(N) Count	(O) BG	(P) Count	(Q) BG	(R) Count	(S) BG	(T) Count	(U) BG	Card Code
43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL 34			50	36		45		31						B
Pos 07		10	50	395		393		342						C
Acid/Base		330	50	62		38		38						D
		1000	50	46		83		75						E
		333	50	101		121		106						F
		666	50	175		160		152						G
Water blank			50	38		54		52						H
														I
														J

Remarks Indicate Item Code and Card Code

Forms Completion
Initials

BAY WASTEWATER

Dichloromethane extract



TA 98, 2% S9
SYMBOL=Δ
LINETYPE=.....
y=a+b*x
n=10
a=50.7917 s_a=4.0494
b=217.7031 s_b=25.8682
$s_{y.x}$=10.0827 r=0.9479

X(I)	Y(I)
0.0000	56.0000
0.0000	71.0000
0.0000	41.0000
.0330	53.0000
.0330	60.0000
.0330	53.0000
.1000	73.0000
.1000	60.0000
.3330	131.0000
.3330	120.0000

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER Test Sample Identification	Activation Batch	Test Type (Table 10)	Strain	Batch No
	IVR	CPHF	12 17 85	AIHL	85	0404	04E05	01	T A 78	8502

Microorganism

Animal	R
Organ	L
Inducer	A
Microorganism	2 % S9 Mix

(A) 64 Remarks Made? Yes 1: 2

(B) 65 Phenocopy Check Conclusion (Table 13): 1

(C) 66 Sterility S 9 Mix: 1 Not Contam, 2 Contam, 3 Not Checked: 1

(D) 67 70 Activation Mixture Per Plate (µl): 500

(E) 71 Sample Sterility Check: 1 Not Contam, 2 Contam, 3 Not Checked: 1

(F) 72 73 74 75 Pre-Incubation: Time (min) | Temp (Cent)

(G) 76 78 Technician: KIC

80 Card Code: A

(H) Solvent Positive (Table 11) 43 44	(I) Units of Concentration Blank = mg/ml 2 = µg/ml 45	(J) Stock concentration (µg/plate) 46 50	(K) Amt Per Plate (µl) 51 54	Plate A: (L) Count 55 58	(M) BG 59	Plate B: (N) Count 60 63	(O) BG 64	Plate C: (P) Count 65 68	(Q) BG 69	Plate D: (R) Count 70 73	(S) BG 74	Plate E: (T) Count 75 78	(U) BG 79	Card Code 80
SOL 54			50	42		38		36						B
Pos 07		10	50	763		739		728						C
		330	50	53		60		53						D
		1000	50	73		60								E
		3330	50	131		120								F
water blank			50	56		71		41						G
														H
														I
														J

Remarks Indicate Item Code and Card Code

Forms Completion
Initials

TABLE 7. MUTAGENICITY OF A BRACKISH RECEIVING SURFACE WASTEWATER FROM THE DISCHARGE SITE OF AN INDUSTRIAL WASTEWATER TREATMENT PLANT, SAMPLE NO. AIHL-85-044A

Comparison of Extraction Methods for Mutagenic Response in TA98 with 2% S-9 Mix[4]

Exp. Date	Extraction Method[1]	Residue Weight (mg)	Sample Volume (L)	Specific Mutagenic Activity (revertants/mg)[2,3]	Mutagenic Response per Unit Volume (revertants/L)
11/20/85	Base/Acid	1.5	3	465	232
12/17/85	Acid/Base	0.9	1.5	414	240

1. The extraction method indicates the order of pH in liquid-liquid extraction.

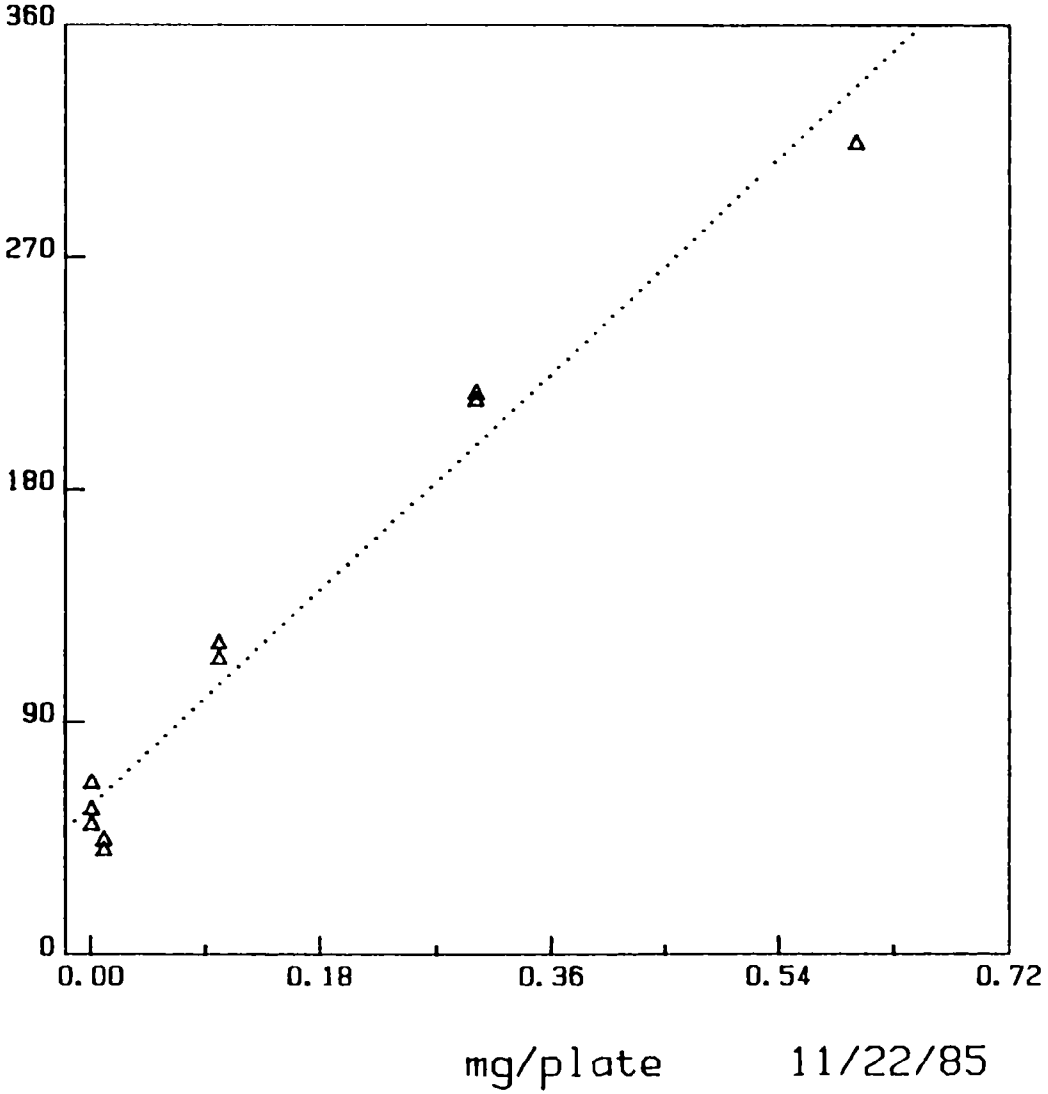
2. The specific activity of the mutagenicity dose response curve is represented by the slope (b as shown in the statistical analysis on graphs in Appendix D).

3. The spontaneous mutation and positive control values are listed on the Strain Function Tests, Cell Titer, and Viability Record in Appendix F.

4. The optimum testing condition for the San Francisco Bay water was in TA98 with 2% S-9 mix. The same condition was applied to the brackish receiving wastewater for comparison purposes.

BRACKISH WASTEWATER

Dichloromethane Extract



TA 98, 2% S9
SYMBOL=Δ
LINETYPE=..... .....
y=a+b*x
n=10
a=57.5335 s_a=6.9901
b=464.5527 s_b=29.5335
$s_{y.x}$=17.6846 r=0.9842

X(I)	Y(I)
0.0000	66.0000
0.0000	50.0000
0.0000	56.0000
.0100	40.0000
.0100	44.0000
.1000	120.0000
.1000	114.0000
.3000	217.0000
.3000	214.0000
.6000	314.0000

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER	Activation Batch	Test Type (Table 10)	Strain	Batch No
	IVR	CPHF	11 22 85	AZHL	85	0446	0460 5	01	1 TA98	8507

Microorganism

Animal	R
Organ	L
Inducer	A
[illegible]	2% S9 Mix

(A) 64 Remarks Made? Yes 1: 2

(B) 65 Phenocopy Check Conclusion (Table 13): 1

(C) 66 Sterility S 9 Mix: 1 (1 Not Contam, 2 Contam, 3 Not Checked)

(D) 67 70 Activation Mixture Per Plate (µl): 500

(E) 71 Sample Sterility Check: 1 (1 Not Contam, 2 Contam, 3 Not Checked)

(F) 72 73 74 75 Pre-Incubation: Time (min) | Temp (Cent) | Technician

(G) 76 78: KLC

80 Card Code: A

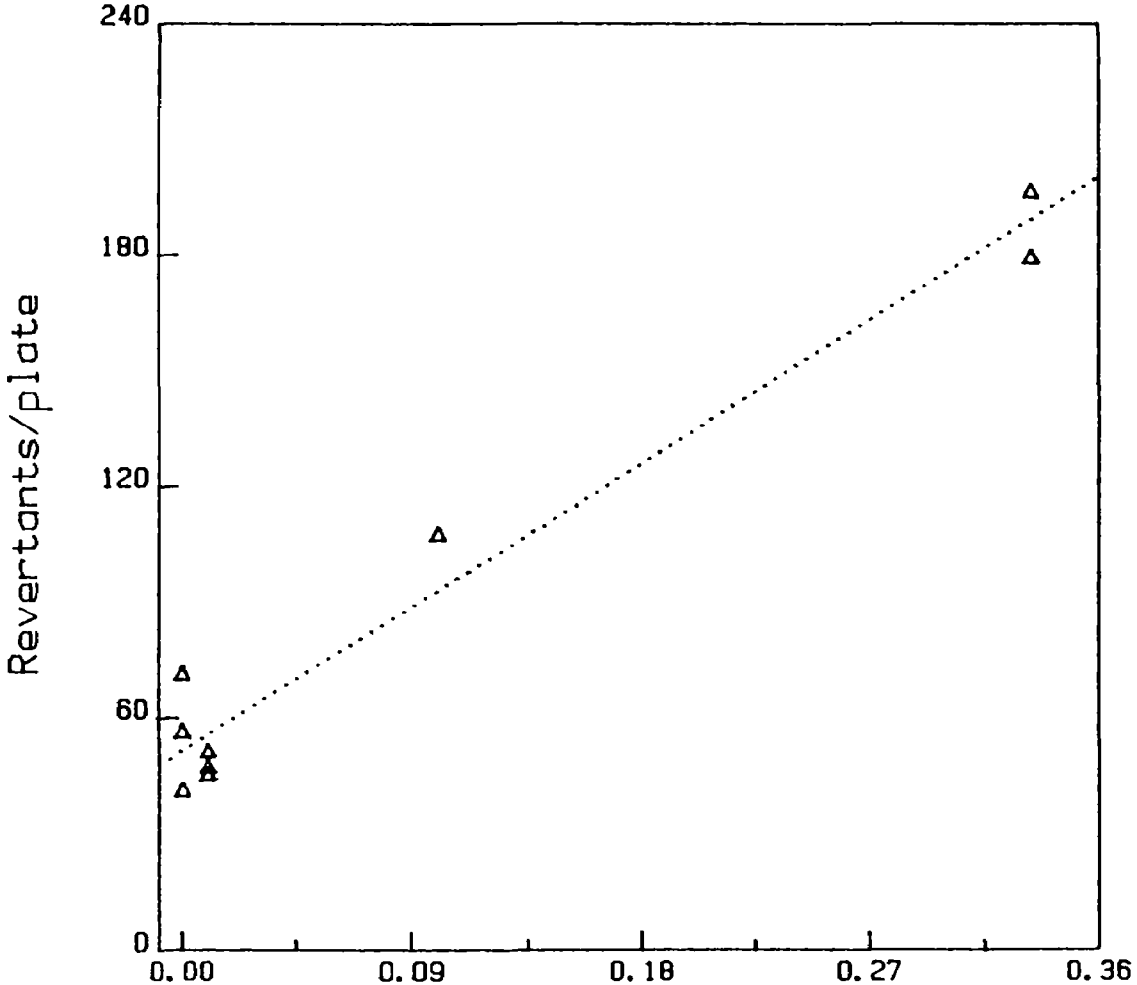
	Dose Level				Plate A		Plate B		Plate C		Plate D		Plate E		
(H) Solvent Positive (Table 11)	(I) Units of Concentration Blank = mg/ml 2 = µg/ml	(J) Stock concentration (µg/plate)	(K) Amt Per Plate (µl)	(L) Count	(M) BG	(N) Count	(O) BG	(P) Count	(Q) BG	(R) Count	(S) BG	(T) Count	(U) BG	Card Code
43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL 54			50	53		44		50						B
Pos 07		10	50	848		877		838						C
		100	50	40		44								D
		1000	50	120		114								E
		3000	50	217		214								F
		6000	50	314										G
water blank			50	66		50		56						H
														I
														J

Remarks Indicate Item Code and Card Code

Forms Completion
Initials

BRACKISH WASTEWATER

Dichloromethane Extract



TA 98, 2% S9
SYMBOL=Δ
LINETYPE=.....
y=a+b*x
n=9
a=51.5309 s_a=4.9384
b=413.5955 s_b=30.7533
s_{y*x}=12.3656 r=0.9812

X(I)	Y(I)
0.0000	56.0000
0.0000	71.0000
0.0000	41.0000
.0100	45.0000
.0100	47.0000
.0100	51.0000
.1000	107.0000
.3330	179.0000
.3330	196.0000

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER Test Sample Identification	Activation Batch	Test Type (Table 10)	Strain	Batch No Microorganism
	IVR	CPHF	12 17 85	AIHL	85	0444 1	04605	01	TA98	8507

Animal	R
Organ	L
Inducer	A
[redacted]	10% S9 Mix

A 64	B 65	C 66	D 67 70	E 71	F 72 73 74 75	G 76 78	80
2 Remarks Made? Yes 1	1 Phenocopy Check Conclusion (Table 13)	1 Sterility S 9 Mix 1 Not Contam 2 Contam 3 Not Checked	500 Activation Mixture Per Plate (µl)	1 Sterility Check 1 Not Contam 2 Contam 3 Not Checked	Time (min) Temp (Cent) Pre-Incubation	KKC Technician	A Card Code

H	I	J	K	Plate A		Plate B		Plate C		Plate D		Plate E		
Solvent Positive (Table 11)	Units of Concentration Blank = mg/ml 2 = µg/ml	Stock concentration (µg/plate)	Amt Per Plate (µl)	L Count	M BG	N Count	O BG	P Count	Q BG	R Count	S BG	T Count	U BG	Card Code
43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL 54			50	42		38		36						B
Pos 07		10	50	763		739		728						C
Acid/Base		100	50	45		47		51						D
		1000	50	107										E
		3330	50	178		196								F
water blank			50	56		71		41						G
														H
														I
														J

Remarks Indicate Item Code and Card Code

Forms Completion
Initials

TABLE 5. MUTAGENICITY OF A SURFACE RUNOFF FROM A CLASS I LANDFILL, SAMPLE NO. AIHL-85-0405

A. Results of the Initial Screening Experiment

Exp. Date	Extraction Method[1]	Residue Weight (mg)	Sample Volume (L)	Specific Mutagenic Activity (revertants/mg)[2,3] TA98 -S9	TA98 2%S9	TA98 10%S9	TA100 -S9	TA100 2%S9	TA100 10%S9
9/24/85	Base/Acid	15	3	122	88	86	< 242	< 230	< 200

B. Comparison of Mutagenic Response in TA98 without S-9[4]

Exp. Date	Extraction Method[1]	Residue Weight (mg)	Sample Volume (L)	Specific Mutagenic Activity (revertants/mg)[2,3]	Mutagenic Response per Unit Volume (revertants/L)
9/24/85	Base/Acid	15	3	122	614
10/4/85	Acid/Base	31	3	< 11	< 112
10/17/85	Acid/Base	11	3	< 32[5]	< 114

1. The extraction method indicates the order of pH in liquid-liquid extraction.

2. The specific activity of the mutagenicity dose response curve is represented by the slope (b as shown in the statistical analysis on graphs in Appendix D).

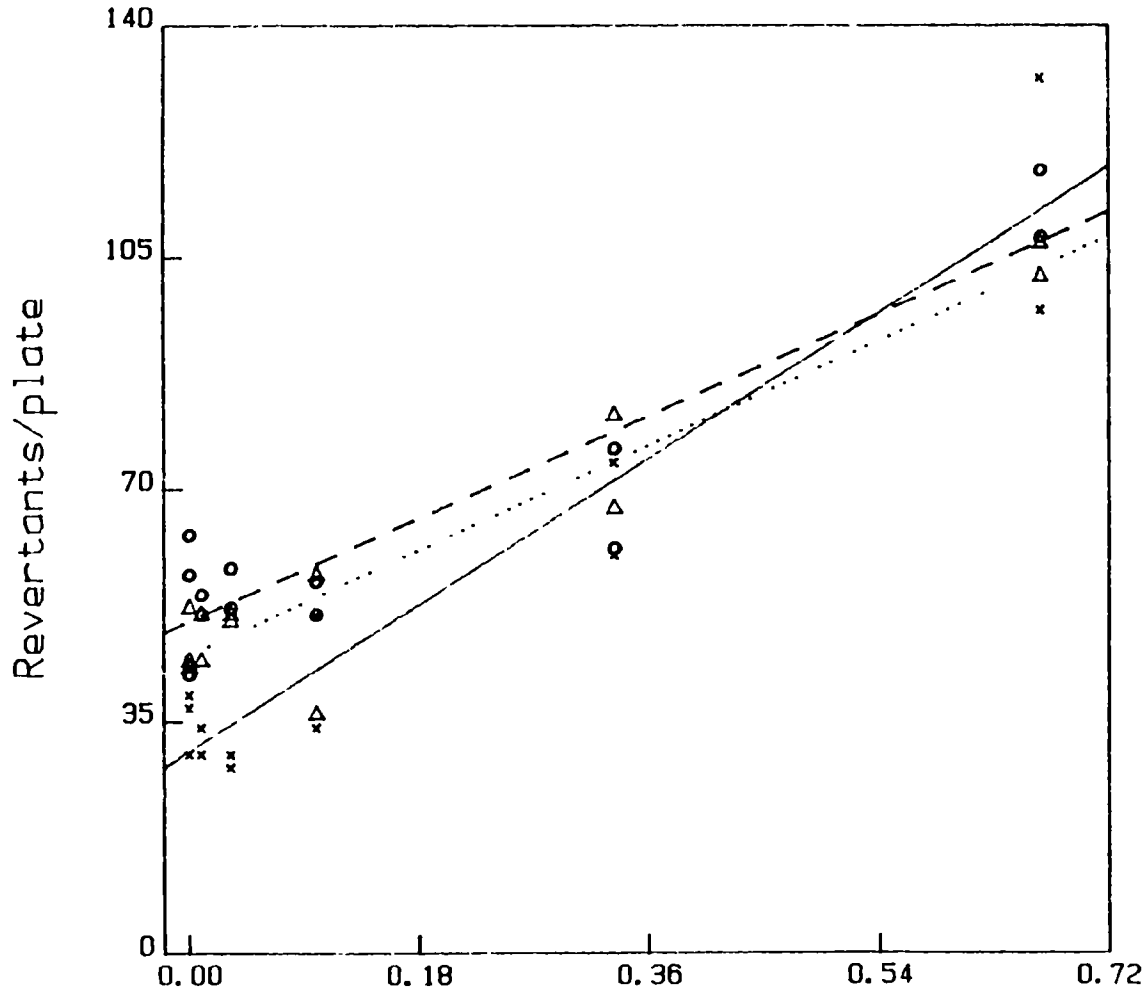
3. The spontaneous mutation and the positive control values are listed on the Strain Function Tests, Cell Titer, and Viability Record in Appendix F.

4. The optimum testing condition in the initial screening experiment was in TA98 without S-9 mix.

5. No revertant colonies on all the testing plates, background lawn was normal except the plates at the highest dose.

SURFACE RUNOFF

Dichloromethane Extract



TA 98, -S9
SYMBOL=×
LINETYPE=————
y=a+b*x
n=13
a=30.4780 s_a=3.4369
b=122.4981 s_b=11.6508
$s_{y.x}$=9.9543 r=0.9537

TA 98, 2% S9
SYMBOL=△
LINETYPE=.....
y=a+b*x
n=13
a=44.9469 s_a=2.4124
b=87.8681 s_b=8.1777
$s_{y.x}$=6.9870 r=0.9555

TA 98, 10% S9
SYMBOL=o
LINETYPE=— — — —
y=a+b*x
n=13
a=50.0619 s_a=2.9761
b=85.8997 s_b=10.0887
$s_{y.x}$=8.6198 r=0.9318

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER Test Sample Identification	Activation Batch	Test Type (Table 10)	Strain	Batch No
	IVR	CPHF	09 24 85	AIHL	85	0405		01	1 TA98	8507

Microorganism

Animal	(A) 64	(B) 65	(C) 66	(D) 67 70	(E) 71	(F) 72 73 74 75	(G) 76 78	80
Organ	2	1			1		KLC	A
Inducer	Remarks Made? Yes-1	Phenocopy Check Conclusion (Table 13)	Sterility S 9 Mix 1 Not Contam 2 Contam 3 Not Checked	Activation Mixture Per Plate (µl)	Sample Sterility Check 1 Not Contam 2 Contam 3 Not Checked	Time (min) Temp (Cent) Pre-Incubation	Technician	Card Code
[redacted] -59								

		Dose Level			Plate A		Plate B		Plate C		Plate D		Plate E		
	(H) Solvent Positive (Table 11)	(I) Units of Concentration Blank = mg/ml 2 = µg/ml	(J) Stock concentration (µg/plate)	(K) Amt Per Plate (µl)	(L) Count	(M) BG	(N) Count	(O) BG	(P) Count	(Q) BG	(R) Count	(S) BG	(T) Count	(U) BG	Card Code
	43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL	54			50	21		33		20						B
Pos	03		40	50	1155		1257		1310						C
			100	50	34		30								D
			330	50	30		28								E
			1000	50	51		34								F
			3330	50	74		60								G
			6660	50	97		132								H
water blank				50	30		39		37						I
															J

Remarks Indicate Item Code and Card Code

Forms Completion
Initials

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER Test Sample Identification	Activation Batch	Test Type (Table 10)	Strain	Batch No Microorganism
	IVR	CPHF	092485	ALHL	85	0405	036300	01	TA98	8507

Animal	R
Organ	L
Inducer	A
[redacted]	2% S9 Mix

(A) 64	(B) 65	(C) 66	(D) 67 70	(E) 71	(F) 72 73 74 75	(G) 76 78	80
2 Remarks Made? Yes 1	1 Phenocopy Check Conclusion (Table 13)	1 Sterility S9 Mix 1 Not Contam 2 Contam 3 Not Checked	500 Activation Mixture Per Plate (µl)	1 Sample Sterility Check 1 Not Contam 2 Contam 3 Not Checked	Time (min) Temp (Cent) Pre-Incubation	KZC Technician	A Card Code

		Dose Level			Plate A		Plate B		Plate C		Plate D		Plate E		
	(H) Solvent Positive (Table 11)	(I) Units of Concentration Blank = mg/ml 2 = µg/ml	(J) Stock concentration (µg/plate)	(K) Amt Per Plate (µl)	(L) Count	(M) BG	(N) Count	(O) BG	(P) Count	(Q) BG	(R) Count	(S) BG	(T) Count	(U) BG	Card Code
	43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL	54			50	36		37		47						B
Pos	07		10	50	284		360		322						C
			100	50	51		44								D
			330	50	50		51								E
			1000	50	57		36								F
			3330	50	67		81								G
			666	50	107		102								H
water blank				50	43		52		44						I
															J

Remarks Indicate Item Code and Card Code

Forms Completion
Initials

HERL IN VITRO RESULTS FORM

1	2-4	5-8	9-14	15-18	19-20	21-24	25-30	31-32	33-38	39-42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER Test Sample Identification	Activation Batch	Test Type (Table 10)	Strain Microorganism	Batch No
	IVR	CPHF	09 24 85	AZHL	85	0405	036.30	01	1 TA98	6507

Animal	R
Organ	L
Inducer	A
[redacted]	10 % S9 Mix

(A) 64	(B) 65	(C) 66	(D) 67 70	(E) 71	(F) 72 73 74 75	(G) 76 78	80
2 Remarks Made? Yes 1	1 Phenocopy Check Conclusion (Table 13)	1 Sterility S 9 Mix 1 Not Contam 2 Contam 3 Not Checked	500 Activation Mixture Per Plate (µl)	1 Sample Sterility Check 1 Not Contam 2 Contam 3 Not Checked	Time (min) Temp (Cent) Pre-Incubation	KZC Technician	A Card Code

(H) Solvent Positive (Table 11)	(I) Units of Concentration Blank = mg/ml 2 = µg/ml	(J) Stock concentration (mg/µl/plate)	(K) Amt Per Plate (µl)	Plate A (L) Count	Plate A (M) BG	Plate B (N) Count	Plate B (O) BG	Plate C (P) Count	Plate C (Q) BG	Plate D (R) Count	Plate D (S) BG	Plate E (T) Count	Plate E (U) BG	Card Code
43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL 54			50	31		52		45						B
Pos 07		10	50	462		350		381						C
		100	50	54		51								D
		330	30	58		52								E
		1000	50	56		51								F
		3330	50	61		76								G
		6660	50	108		118								H
water blank			50	42		57		63						I
														J

Remarks Indicate Item Code and Card Code

Forms Completion
Initials

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER Test Sample Identification	Activation Batch	Test Type (Table 10)	Strain Microorganism	Batch No
	IVR	CPHF	09 24 85	AIHL	85	0405		01	TA100	85086

Animal
Organ
Inducer
[redacted] -59

(A) 64	(B) 65	(C) 66	(D) 67 70	(E) 71	(F) 72 73 74 75	(G) 76 78	80
Remarks Made? Yes 1	Phenocopy Check Conclusion (Table 13)	Sterility S 9 Mix 1 Not Contam 2 Contam 3 Not Checked	Activation Mixture Per Plate (µl)	Sample Sterility Check 1 Not Contam 2 Contam 3 Not Checked	Time (min) Temp (Cent) Pre-Incubation	Technician	Card Code
2	1			1		KIC	A

	Dose Level			Plate A		Plate B		Plate C		Plate D		Plate E		
(H) Solvent Positive (Table 11)	(I) Units of Concentration Blank = mg/ml 2 = µg/ml	(J) Stock concentration (µg/plate)	(K) Amt Per Plate (µl)	(L) Count	(M) BG	(N) Count	(O) BG	(P) Count	(Q) BG	(R) Count	(S) BG	(T) Count	(U) BG	Card Code
	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL 54			50	173		147		145						B
Pos 01		05	50	1539		1649		1744						C
		100	50	169		179		167						D
		330	50	158		159								E
		1000	50	178		166								F
		333	50	214		216								G
		666	50	246		259								H
water blank			50	181		148		155						I
														J

Remarks Indicate Item Code and Card Code

Forms Completion
Initials

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	Experiment Date (MO DA YR)	Test Sample Identification (LAB)	(YR)	(NUMBER)	Activation Batch	Test Type (Table 10)	Microorganism: Strain	Microorganism: Batch No
	IVR	CPHF	09 24 85	AZHL	85	0405	03630	01	TA100	8508

Animal	R
Organ	L
Inducer	A
[illegible]	2% S9 Mix

(A) 64	(B) 65	(C) 66	(D) 67 70	(E) 71	(F) 72 73 74 75	(G) 76 78	80
2	1	1	500	1		KZC	A
Remarks Made? Yes-1	Phenocopy Check Conclusion (Table 13)	Sterility S 9 Mix: 1 Not Contam, 2 Contam, 3 Not Checked	Activation Mixture Per Plate (µl)	Sample Sterility Check: 1 Not Contam, 2 Contam, 3 Not Checked	Time (min) Temp (Cent) Pre-Incubation	Technician	Card Code

	Dose Level			Plate A		Plate B		Plate C		Plate D		Plate E		
(H) Solvent Positive (Table 11)	(I) Units of Concentration Blank = mg/ml 2 = µg/ml	(J) Stock concentration (µg/plate)	(K) Amt. Per Plate (µl)	(L) Count	(M) BG	(N) Count	(O) BG	(P) Count	(Q) BG	(R) Count	(S) BG	(T) Count	(U) BG	Card Code
43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL 54			50	1162		1161		1422						B
Pos 04		05	50	2435		2147		2522						C
		3330	50	1396		1500								D
		1000	50	1730		1700								E
		3330	50	1633		1788								F
		6660	50	233		1776								G
water blank			50	1600		1450		1555						H
														I
														J

Remarks Indicate Item Code and Card Code

Forms Completion
Initials

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER Test Sample Identification	Activation Batch	Test Type (Table 10)	Strain Microorganism	Batch No
	IVR	CPHF	092485	ALHL	85	0405	036300	01	TA100	8508

Animal	R
Organ	L
Inducer	A
[redacted]	10% S9 Mix

(A) 64 Remarks Made? Yes 1: 2

(B) 65 Phenocopy Check Conclusion (Table 13): 1

(C) 66 Sterility S 9 Mix: 1 (1 Not Contam, 2 Contam, 3 Not Checked)

(D) 67 70 Activation Mixture Per Plate (µl): 500

(E) 71 Sample Sterility Check: 1 (1 Not Contam, 2 Contam, 3 Not Checked)

(F) 72 73 74 75 Pre-Incubation: Time (min) | Temp (Cent)

(G) 76 78 Technician: KIC

80 Card Code: A

	Dose Level				Plate A		Plate B		Plate C		Plate D		Plate E		
(H) Solvent Positive (Table 11)	(I) Units of Concentration Blank = mg/ml 2 = µg/ml	(J) Stock concentration (µg/plate)	(K) Amt Per Plate (µl)	(L) Count	(M) BG	(N) Count	(O) BG	(P) Count	(Q) BG	(R) Count	(S) BG	(T) Count	(U) BG	Card Code
43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL 34			500	1136		1163		1355						B
Pos 04		05	500	1037		932		935						C
		10.0	500	162		157								D
		33.0	500	149		189								E
		10.0	500	180		160								F
		3.30	500	163		141								G
		6.60	500	188										H
water blank			500	147		129		125						I
														J

Remarks Indicate Item Code and Card Code

Forms Completion	
Initials	

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER Test Sample Identification	Activation Batch	Test Type (Table 10)	Strain Microorganism	Batch No
	IVR	CPHF	10 04 85	AIHL	85	0405		01	TA98	8507

Animal	(A) 64	(B) 65	(C) 66	(D) 67 70	(E) 71	(F) 72 73 74 75	(G) 76 78	80
Organ	2 Remarks Made? Yes 1	1 Phenocopy Check Conclusion (Table 13)	Sterility S 9 Mix 1 Not Contam 2 Contam 3 Not Checked	Activation Mixture Per Plate (µl)	1 Sample Sterility Check 1 Not Contam 2 Contam 3-Not Checked	Time (min) Temp (Cent) Pre-Incubation	Technician	Card Code
Inducer								
[redacted] -59								A

	Dose Level				Plate A		Plate B		Plate C		Plate D		Plate E		
(H) Solvent Positive (Table 11)	(I) Units of Concentration Blank = mg/ml 2 = µg/ml	(J) Stock concentration (µg/plate)	(K) Amt Per Plate (µl)	(L) Count	(M) BG	(N) Count	(O) BG	(P) Count	(Q) BG	(R) Count	(S) BG	(T) Count	(U) BG	Card Code
43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL 54			50	28		18		24						B
Pos 03		40	50	861		903		605						C
Acid/Base		330	50	34		22		30						D
		1000	50	25		26		29						E
		3330	50	31		35		33						F
		666 0	50	45		42		45						G
	1	000 0	50	68		54		43						H
	3	333 0	50	74	2	66	2	74	2					I
water blank			50	39		38		32						J

Remarks Indicate Item Code and Card Code

D-228

Forms Completion	
Initials	

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER	Activation Batch	Test Type (Table 10)	Strain	Batch No
	IVR	CFHF	10 17 85	AZHL	85	0405		01	TA98	6507

Test Sample Identification — Microorganism

Animal	(A) 64	(B) 65	(C) 66	(D) 67 70	(E) 71	(F) 72 73 74 75	(G) 76 78	80
Organ	2 Remarks Made? Yes 1	1 Phenocopy Check Conclusion (Table 13)	Sterility S 9 Mix: 1 Not Contam, 2 Contam, 3 Not Checked	Activation Mixture Per Plate (µl)	Sample Sterility Check: 1 Not Contam, 2 Contam, 3 Not Checked	Pre-Incubation: Time (min) Temp (Cent)	Technician	Card Code
Inducer								
[redacted] -59								A

(H) Solvent Positive (Table 11)	(I) Units of Concentration Blank = mg/ml 2 = µg/ml	(J) Stock concentration (mg/plate)	(K) Amt Per Plate (µl)	Plate A: (L) Count	Plate A: (M) BG	Plate B: (N) Count	Plate B: (O) BG	Plate C: (P) Count	Plate C: (Q) BG	Plate D: (R) Count	Plate D: (S) BG	Plate E: (T) Count	Plate E: (U) BG	Card Code
43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL 54			50	25		24		28						B
Pos 03		4.0	50	1236		1132		1180						C
Acid/Base		33.0	50	0		0		0						D
		100.0	50	0		0		0						E
		333.0	50	0		0		0						F
		666.0	50	0		0		0						G
	1	0000	50	0	8	0	8	0	8					H
	2	00.0	50	0	3	0	3							I
Water Blank			50	36		29		30						J

Remarks Indicate Item Code and Card Code

Forms Completion
Initials

TABLE 9. MUTAGENICITY OF AN INDUSTRIAL WASTEWATER TREATMENT PLANT EFFLUENT, SAMPLE NO. AIHL-85-0406

A. Results of the Initial Screening Experiment

Exp. Date	Extraction Method[1]	Residue Weight (mg)	Sample Volume (L)	Specific Mutagenic Activity (revertants/mg)[2,3] TA98 -S9	TA98 2%S9	TA98 10%S9	TA100 -S9	TA100 2%S9	TA100 10%S9
9/24/85	Acid/Base	48	3	< 35	< 46	< 54	< 161	< 153	< 134

B. Results of Follow-up Experiments in TA98 with 10% S-9 Mix[4]

Exp. Date	Extraction Method[1]	Residue Weight (mg)	Sample Volume (L)	Specific Mutagenic Activity (revertants/mg)[2,3]
10/4/85	Base/Acid	52	3	< 53
10/17/85	Base/Acid	62	1.5	< 38
10/17/85	Acid/Base	79	1.5	< 38

C. Results of Follow-up Experiments in TA98 with 30% S-9 Mix[5]

Exp. Date	Extraction Method[1]	Residue Weight (mg)	Sample Volume (L)	Specific Mutagenic Activity (revertants/mg)[2,3]	Mutagenic Response per Unit Volume (revertants/L)
2/11/86	Acid/Base	28	1.1	63	1585
2/28/86	Acid/Base	57	3	95	1810

1. The extraction method indicates the order of pH in liquid-liquid extraction.

2. The specific activity of the mutagenicity dose-response curve is represented by the slope (**b** as shown in the statistical analysis on graphs in Appendix D).

3. The spontaneous mutation and positive control values are listed on the Strain Function Tests, Cell Titer and Viability Record in Appendix F.

4. Although results of all six conditions in the initial screening experiment were below the detection limit, TA98 with 10% S-9 mix was the best condition in which the result was closest to the detection limit. This condition was therefore used in the follow-up experiments.

5. Modification of the S-9 condition (30% S-9 mix) was applied because of equivocal results obtained in experiments with 10% S-9 mix.

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER	Activation Batch	Test Type (Table 10)	Strain	Batch No
	IVR	CPHF	09 24 85	ALHL	85	0406		01	TA98	85017
				Test Sample Identification					Microorganism	

Animal	(A) 64	(B) 65	(C) 66	(D) 67 70	(E) 71	(F) 72 73 74 75	(G) 76 78	80
Organ	2	1			1		KLC	A
Inducer	Remarks Made? Yes 1	Phenocopy Check Conclusion (Table 13)	Sterility S 9 Mix 1 Not Contam 2 Contam 3 Not Checked	Activation Mixture Per Plate (µl)	Sample Sterility Check 1 Not Contam 2 Contam 3 Not Checked	Time (min) Temp (Cent.) Pre-Incubation	Technician	Card Code
[redacted] -59								

	(H) Solvent Positive (Table 11)	(I) Units of Concentration Blank mg/ml 2 µg/ml	(J) Stock concentration (µg/plate)	(K) Amt Per Plate (µl)	Plate A (L) Count	Plate A (M) BG	Plate B (N) Count	Plate B (O) BG	Plate C (P) Count	Plate C (Q) BG	Plate D (R) Count	Plate D (S) BG	Plate E (T) Count	Plate E (U) BG	Card Code
	43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL	54			50	21		33		20						B
Pos	03		40	50	1155		1257		1310						C
Acid/Base			100	50	26		27								D
			330	50	30		36								E
			1000	50	52		34								F
			3330	50	39		58								G
		1	0000	50	69		53								H
		2	0000	50	0	3	0	3							I
Water blank				50	30		39		37						J

Remarks Indicate Item Code and Card Code

Forms Completion
Initials

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HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER Test Sample Identification	Activation Batch	Test Type (Table 10)	Strain	Batch No
	IVR	CPHI	09 24 85	AIHL	85	0406	0 3 6 3 0	01	1 7A 9 8	85 07
									Microorganism	

Animal	Organ	Inducer	Solvent	A 64 Remarks Made? Yes 1	B 65 Phenocopy Check Conclusion (Table 13)	C 66 Sterility S 9 Mix 1 Not Contam 2 Contam 3 Not Checked	D 67 70 Activation Mixture Per Plate (µl)	E 71 Sample Sterility Check 1 Not Contam 2 Contam 3 Not Checked	F 72 73 74 75 Time (min) Temp (Cent) Pre-Incubation	G 76 78 Technician	80 Card Code
		R	2 % S9 Mix	2	1	1	500	1		KIC	A
		L									
		A									

	Dose Level				Plate A		Plate B		Plate C		Plate D		Plate E		
H Solvent Positive (Table 11)	I Units of Concentration Blank = mg/ml 2 = µg/ml	J Stock concentration (µg/plate)	K Amt Per Plate (µl)	L Count	M BG	N Count	O BG	P Count	Q BG	R Count	S BG	T Count	U BG	Card Code
43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL 54			500	356		337		447						B
Pos 07		10	500	284		360		322						C
Acid/Base		100	500	400		477								D
		330	500	397		422								E
		1000	500	333		411								F
		3330	500	553		533								G
	1	000	500	655		777								H
	2	000	500	80	2	834	2							I
water blank			500	443		532		444						J

Remarks Indicate Item Code and Card Code

Forms Completion
Initials

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER	Activation Batch	Test Type (Table 10)	Strain	Batch No
	IVR	CPHF	09 24 85	AIHL	85	0406	0 0 3 6 3 0	01	TA98	8507
				Test Sample Identification					Microorganism	

Animal	R
Organ	L
Inducer	A
[redacted]	10% S9 MIX

(A) 64	(B) 65	(C) 66	(D) 67 70	(E) 71	(F) 72 73 74 75	(G) 76 78	80
2 Remarks Made? Yes 1	1 Phenocopy Check Conclusion (Table 13)	1 Sterility S 9 Mix 1 Not Contam 2 Contam 3 Not Checked	500 Activation Mixture Per Plate (µl)	1 Sample Sterility Check 1 Not Contam 2 Contam 3 Not Checked	Time (min) Temp (Cent) Pre-Incubation	KIC Technician	A Card Code

		Dose Level			Plate A		Plate B		Plate C		Plate D		Plate E		
	(H) Solvent Positive (Table 11)	(I) Units of Concentration Blank = mg/ml 2 = µg/ml	(J) Stock concentration (µg / plate)	(K) Amt Per Plate (µl)	(L) Count	(M) BG	(N) Count	(O) BG	(P) Count	(Q) BG	(R) Count	(S) BG	(T) Count	(U) BG	Card Code
	43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL	54			500	3 1		5 2		4 5						B
Pos	07		10	500	462		350		381						C
Acid/Base			100	500	44		48								D
			330	500	35		53								E
			1000	500	56		68								F
			3330	500	81		87								G
		1	0000	500	78		83								H
		2	0000	500	52	2	72	2							I
Water blank				500	42		57		63						J

Remarks Indicate Item Code and Card Code

Forms Completion	
Initials	

HERL IN VITRO RESULTS FORM

1	2-4	5-8	9-14	15-18	19-20	21-24	25-30	31-32	33-38	39-42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER Test Sample Identification	Activation Batch	Test Type (Table 10)	Strain	Batch No Microorganism
	IVR	CPHF	09 24 85	AZHL	85	0406		01	TA100	5508

Animal
Organ
Inducer
[redacted] -59

A 64	B 65	C 66	D 67-70	E 71	F 72-73-74-75	G 76-78	80
Remarks Made? Yes-1	Phenocopy Check Conclusion (Table 13)	Sterility S 9 Mix 1 Not Contam 2 Contam 3 Not Checked	Activation Mixture Per Plate (µl)	Sample Sterility Check 1 Not Contam 2 Contam 3 Not Checked	Time (min) Temp (Cent.) Pre-Incubation	Technician	Card Code
2	1			1		KIC	A

	H	I	J	K	Plate A L	M	Plate B N	O	Plate C P	Q	Plate D R	S	Plate E T	U	
	Solvent Positive (Table 11)	Units of Concentration Blank = mg/ml 2 = µg/ml	Stock concentration (µg/plate)	Amt. Per Plate (µl)	Count	BG	Count	BG	Count	BG	Count	BG	Count	BG	Card Code
	43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL	54			50	173		147		145						B
Pos	01		05	50	1539		1649		1744						C
Acid/Base			100	50	175		161								D
			330	50	162		170								E
			1000	50	197		168								F
			333	50	187		203								G
		1	0000	50	236		249								H
		2	000.0	50	210	2	208	2							I
Water blank				50	181		148		155						J

Remarks Indicate Item Code and Card Code

Forms Completion
Initials

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER Test Sample Identification	Activation Batch	Test Type (Table 10)	Strain	Batch No Microorganism
	IVR	CPHF	09 24 85	AIHL	85	0406	03630	01	TA100	8508

Animal	R
Organ	L
Inducer	A
[redacted]	2 % S9 Mix

(A) 64 Remarks Made? Yes 1: 2

(B) 65 Phenocopy Check Conclusion (Table 13): 1

(C) 66 Sterility S 9 Mix: 1 (1 Not Contam, 2 Contam, 3 Not Checked)

(D) 67 70 Activation Mixture Per Plate (µl): 500

(E) 71 Sample Sterility Check: 1 (1 Not Contam, 2 Contam, 3 Not Checked)

(F) 72 73 74 75 Pre-Incubation: Time (min); Temp (Cent)

(G) 76 78 Technician: KIC

80 Card Code: A

	Dose Level				Plate A		Plate B		Plate C		Plate D		Plate E		
(H) Solvent Positive (Table 11)	(I) Units of Concentration Blank = mg/ml 2 = µg/ml	(J) Stock concentration (mg/plate)	(K) Amt Per Plate (µl)	(L) Count	(M) BG	(N) Count	(O) BG	(P) Count	(Q) BG	(R) Count	(S) BG	(T) Count	(U) BG	Card Code
	43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL	54			50	1162		161		142						B
Pos	04		05	50	2435		2147		2322						C
Acid/Base			100	50	1151		140								D
			330	50	1157		173								E
			1000	50	204		173								F
			3330	50	177		164								G
		1	0000	50	203		218								H
		2	0000	50	227	2	241	2							I
water blank				50	1160		145		1355						J

Remarks Indicate Item Code and Card Code

Forms Completion
Initials

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER Test Sample Identification	Activation Batch	Test Type (Table 10)	Strain (Microorganism)	Batch No
	IVR	CPHV-	09 24 85	AIHL-	85	04 06	03630	01	TA100	8508

Animal	R
Organ	L
Inducer	A
[redacted]	10% S9 Mix

(A) 64 Remarks Made? Yes 1: 2

(B) 65 Phenocopy Check Conclusion (Table 13): 1

(C) 66 Sterility S 9 Mix: 1 (1 Not Contam, 2 Contam, 3 Not Checked)

(D) 67 70 Activation Mixture Per Plate (µl): 500

(E) 71 Sample Sterility Check: 1 (1 Not Contam, 2 Contam, 3 Not Checked)

(F) 72 73 74 75 Pre-Incubation: Time (min) | Temp (Cent) | Technician: KIC

80 Card Code: A

	Dose Level				Plate A		Plate B		Plate C		Plate D		Plate E		
(H) Solvent Positive (Table 11)	(I) Units of Concentration Blank = mg/ml 2 = µg/ml	(J) Stock concentration (µg/plate)	(K) Amt Per Plate (µl)	(L) Count	(M) BG	(N) Count	(O) BG	(P) Count	(Q) BG	(R) Count	(S) BG	(T) Count	(U) BG	Card Code
43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL 54			500	1136		1163		1155						B
Pos 04		05	500	1037		932		955						C
Acid/Base		100	500	1500		1136								D
		330	500	1446		1500								E
		1000	500	1183		1685								F
		3330	500	1689		2112								G
	1	0000	500	2411		2117								H
	2	0000	500	2155	2	2522	2							I
water blank			500	1477		1299		1125						J

Remarks Indicate Item Code and Card Code

Forms Completion	
Initials	
Date	

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER Test Sample Identification	Activation Batch	Test Type (Table 10)	Strain Microorganism	Batch No
	IVR	CPHF	10 04 85	AZHL	85	0406	03630	01	TA98	8307

Animal	R
Organ	L
Inducer	A
[redacted]	10 % S9 Mix

(A) 64 Remarks Made? Yes 1: 2

(B) 65 Phenocopy Check Conclusion (Table 13): 1

(C) 66 Sterility S 9 Mix: 1 (1 Not Contam, 2 Contam, 3 Not Checked)

(D) 67 70 Activation Mixture Per Plate (µl): 500

(E) 71 Sample Sterility Check: 1 (1 Not Contam, 2 Contam, 3 Not Checked)

(F) 72 73 74 75 Pre-Incubation: Time (min) | Temp (Cent)

(G) 76 78 Technician: KZC

80 Card Code: A

(H) Solvent Positive (Table 11)	Dose Level: (I) Units of Concentration Blank = mg/ml 2 = µg/ml	(J) Stock concentration (mg/plate)	(K) Amt Per Plate (µl)	Plate A: (L) Count	(M) BG	Plate B: (N) Count	(O) BG	Plate C: (P) Count	(Q) BG	Plate D: (R) Count	(S) BG	Plate E: (T) Count	(U) BG	Card Code
	43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL	54			500	446		451		442						B
Pos	07		10	500	485		403		427						C
			330	500	666		622		579						D
			1000	500	544		600		611						E
			1660	500	633		534		530						F
			3330	500	711		655		666						G
			6660	500	533		499		755						H
		1	0000	500	798		844		788						I
water blank				500	499		602		488						J

Remarks Indicate Item Code and Card Code

Forms Completion
Initials

HERL IN VITRO RESULTS FORM

1 Update Code	2 4 System ID	5 8 Research Lab ID	9 14 MO DA YR Experiment Date	15 18 LAB	19 20 YR	21 24 NUMBER Test Sample Identification	25 30 Activation Batch	31 32 Test Type (Table 10)	33 38 Strain	39 42 Batch No Microorganism
	IVR	CPHF	10 17 85	ALHL	85	0406	03630	01	17A78	8507

Animal	R
Organ	L
Inducer	A
[redacted]	10% S9 Mix

A 64 Remarks Made? Yes 1	B 65 Phenocopy Check Conclusion (Table 13)	C 66 Sterility S 9 Mix 1 Not Contam 2 Contam 3 Not Checked	D 67 70 Activation Mixture Per Plate (µl)	E 71 Sample Sterility Check 1 Not Contam 2 Contam 3 Not Checked	F 72 73 74 75 Time (min) Temp (Cent) Pre-Incubation	G 76 78 Technician	80 Card Code
2	1	1	500	1		KIC	A

		Dose Level			Plate A		Plate B		Plate C		Plate D		Plate E		
	H Solvent Positive (Table 11) 43 44	I Units of Concentration Blank = mg/ml 2 = µg/ml 45	J Stock concentration (µg/plate) 46 50	K Amt Per Plate (µl) 51 54	L Count 55 58	M BG 59	N Count 60 63	O BG 64	P Count 65 68	Q BG 69	R Count 70 73	S BG 74	T Count 75 78	U BG 79	Card Code 80
SOL	54			50	53		45		45						B
Pos	07		10	50	326		359		367						C
			330	50	69		56		73						D
			1000	50	53		49		45						E
			3330	50	57		62		68						F
			666	50	77		54		61						G
		1	0000	50	65		90		82						H
		1	5000	50	89		104		92						I
		2	0000	50	55	2	71	2	57	2					J

Remarks Indicate Item Code and Card Code

(continued)

Forms Completion Initials	

HERL IN VITRO RESULTS CONTINUATION FORM

1 Update Code	2 4 System ID	5 8 Research Lab ID	9 14 MO DA YR Experiment Date	15 18 Lab	19 20 YR	21 24 Number Test Sample Identification	25 30 Activation Batch (10% S9 Mix)	31 32 Test Type (Table 10)	33 38 Strain	39 42 Batch No Microorganism
	IVR	CPHF	10 17 85	AIHL	85	0406	0360	01	TA98	5507

		Dose Level		Plate A		Plate B		Plate C		Plate D		Plate E		
(H) Solvent Positive (Table 11)	(I) Units of Concentration Blank = mg/ml 2 = µg/ml	(J) Stock Concentration (mg/plate)	(K) Amt Per Plate (µl)	(L) Count	(M) BG	(N) Count	(O) BG	(P) Count	(Q) BG	(R) Count	(S) BG	(T) Count	(U) BG	Card Code
43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
	3	0000	50	52	2	60	2	28	2					K
water blank			50	56		56		61						L
														M
														N
														O
														P
														Q
														R
														S
														T
														U
														V
														W
														X
														Y

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER Test Sample Identification	Activation Batch	Test Type (Table 10)	Strain	Batch No Microorganism
	IVR	CPHF	10 17 85	AIHL	85	0406	03630	01	1 7A98	8507

Animal	R
Organ	L
Inducer	A
[redacted]	10% S9 Mix

(A) 64	(B) 65	(C) 66	(D) 67 70	(E) 71	(F) 72 73 74 75	(G) 76 78	80
2 Remarks Made? Yes 1	1 Phenocopy Check Conclusion (Table 13)	1 Sterility S 9 Mix 1 Not Contam 2 Contam 3 Not Checked	500 Activation Mixture Per Plate (µl)	1 Sample Sterility Check 1 Not Contam 2 Contam 3 Not Checked	Time (min) Temp (Cent) Pre-Incubation	KZC Technician	A Card Code

(H) Solvent Positive (Table 11)	Dose Level: (I) Units of Concentration Blank = mg/ml 2 = µg/ml	Dose Level: (J) Stock concentration (mg/plate)	Dose Level: (K) Amt Per Plate (µl)	Plate A: (L) Count	Plate A: (M) BG	Plate B: (N) Count	Plate B: (O) BG	Plate C: (P) Count	Plate C: (Q) BG	Plate D: (R) Count	Plate D: (S) BG	Plate E: (T) Count	Plate E: (U) BG	Card Code
	43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL	54			50	353		455		455						B
Pos	07		10	50	326		357		367						C
Acid/Base			330	50	433		553		533						D
			1000	50	510		430		466						E
			3330	50	775		711		644						F
			6660	50	799		666		644						G
		1	0000	50	688		533		688						H
		1	5000	50	774		850		879						I
		2	0000	50	110	2	555	2	755	2					J

Remarks Indicate Item Code and Card Code

(continued)

Forms Completion
Initials

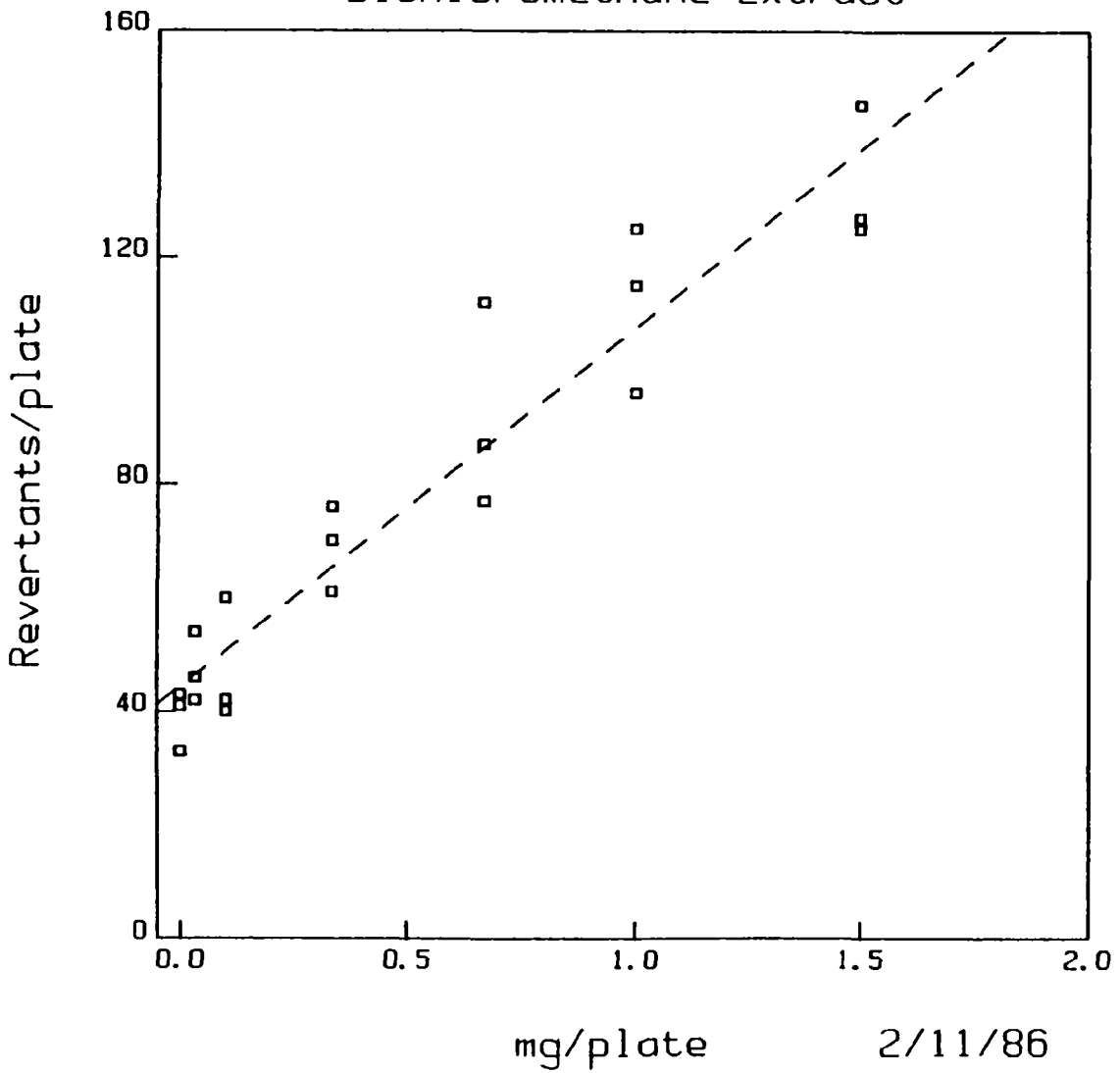
HERL IN VITRO RESULTS CONTINUATION FORM

1 Update Code	2 4 System ID	5 8 Research Lab ID	9 14 MO DA YR Experiment Date	15 18 Lab	19 20 YR	21 24 Number Test Sample Identification	25 30 Activation Batch	31 32 Test Type (Table 10)	33 38 Strain Microorganism	39 42 Batch No
	IVR	CPHF	10 7 85	AZHL	-85	-0406	0536/0 % S9 Mix	01	TA98	8507

Ⓗ Solvent Positive (Table 11)	Ⓘ Units of Concentration Blank = mg/ml 2 = µg/ml	Dose Level Ⓙ Stock Concentration (µg/plate)	Dose Level Ⓚ Amt Per Plate (µl)	Plate A Ⓛ Count	Plate A Ⓜ BG	Plate B Ⓝ Count	Plate B Ⓞ BG	Plate C Ⓟ Count	Plate C Ⓠ BG	Plate D Ⓡ Count	Plate D Ⓢ BG	Plate E Ⓣ Count	Plate E Ⓤ BG	Card Code
43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
	3	0000	50	98	2	109	2	89	2					K
water blank			50	56		36		61						L
														M
														N
														O
														P
														Q
														R
														S
														T
														U
														V
														W
														X
														Y

INDUSTRIAL WASTEWATER

Dichloromethane Extract



TA 98, 30% S9
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y=a+b*x
n=21
a=44.3055 s_a=3.3701
b=63.1961 s_b=4.5648
$s_{y.x}$=10.9869 r=0.9538

0 0000	73 0000
0 0000	41 0000
0 0000	43 0000
0330	42 0000
0330	46 0000
0330	54 0000
1000	60 0000
1000	40 0000
1000	42 0000
3330	76 0000
3330	70 0000
3330	61 0000
6660	77 0000
6660	87 0000
6660	113 0000
1 0000	125 0000
1 0000	115 0000
1 0000	95 0000
1 5000	137 0000
1 5000	147 0000
1 5000	135 0000

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER Test Sample Identification	Activation Batch	Test Type (Table 10)	Strain Microorganism	Batch No
	IVR	CPHF	02 11 86	AIHL	85	0406	04605	01	TA98	8507

Animal	Organ	Inducer	(A) 64	(B) 65	(C) 66	(D) 67 70	(E) 71	(F) 72 73 74 75	(G) 76 78	80
R	L	A	Remarks Made? Yes 1	Phenocopy Check Conclusion (Table 13)	Sterility S 9 Mix 1 Not Contam 2 Contam 3 Not Checked	Activation Mixture Per Plate (µl)	Sample Sterility Check 1 Not Contam 2 Contam 3 Not Checked	Time (min) Temp (Cent) Pre-Incubation	Technician	Card Code
[redacted] 30% S9 Mix			2	1	1	500	1		KIC	A

	Dose Level			Plate A		Plate B		Plate C		Plate D		Plate E		
(H) Solvent Positive (Table 11)	(I) Units of Concentration Blank = mg/ml 2 = µg/ml	(J) Stock concentration (µg/plate)	(K) Amt Per Plate (µl)	(L) Count	(M) BG	(N) Count	(O) BG	(P) Count	(Q) BG	(R) Count	(S) BG	(T) Count	(U) BG	Card Code
43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL 54			50	45		36		37						B
Pos 07		10	50	142		106		107						C
04		05	50	253		235		246						D
		330	50	42		46		54						E
		1000	50	60		40		42						F
		3330	50	76		70		61						G
		6660	50	77		87		112						H
	/	0000	50	125		115		96						I
	/	5000	50	127		147		125						J

Remarks Indicate Item Code and Card Code

(continued)

Forms Completion
Initials

HERL IN VITRO RESULTS CONTINUATION FORM

1 Update Code	2 4 System ID	5 8 Research Lab ID	9 14 MO DA YR Experiment Date	15 18 19 20 21 24 Lab YR Number Test Sample Identification	25 30 Activation Batch	31 32 Test Type (Table 10)	33 38 Strain	39 42 Batch No	Microorganism
	IVR	CPVF	02/18/86	AIVL-85-0406	30% S9 Mix	01	TA98	8507	

		Dose Level		Plate A		Plate B		Plate C		Plate D		Plate E		
H Solvent Positive (Table 11)	I Units of Concentration Blank = mg/ml 2 = µg/ml	J Stock Concentration (µg/plate)	K Amt Per Plate (µl)	L Count	M BG	N Count	O BG	P Count	Q BG	R Count	S BG	T Count	U BG	Card Code
43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
	3	0000	50	117	2	128	2	165	2					K
	water blank		50	33		41		43						L
														M
														N
														O
														P
														Q
														R
														S
														T
														U
														V
														W
														X
														Y

HERL IN VITRO RESULTS FORM

1	2-4	5-8	9-14	15-18	19-20	21-24	25-30	31-32	33-38	39-42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER Test Sample Identification	Activation Batch	Test Type (Table 10)	Strain	Batch No Microorganism
	IVR	CPHF	02 28 86	AIHL	85	0406	04603	01	1 TA98	8507

		64 (A)	65 (B)	66 (C)	67-70 (D)	71 (E)	72-73-74-75 (F)	76-78 (G)	80
Animal	R	2 Remarks Made? Yes 1	1 Phenocopy Check Conclusion (Table 13)	1 Sterility S 9 Mix 1 Not Contam 2 Contam 3 Not Checked	500 Activation Mixture Per Plate (µl)	1 Sample Sterility Check 1 Not Contam 2 Contam 3 Not Checked	Time (min) Temp (Cent) Pre-Incubation	KIC Technician	A Card Code
Organ	L								
Inducer	A								
[redacted]	10% S9 Mix								

	Dose Level			Plate A		Plate B		Plate C		Plate D		Plate E		
(H) Solvent Positive (Table 11)	(I) Units of Concentration Blank = mg/ml 2 = µg/ml	(J) Stock concentration (mg/plate)	(K) Amt Per Plate (µl)	(L) Count	(M) BG	(N) Count	(O) BG	(P) Count	(Q) BG	(R) Count	(S) BG	(T) Count	(U) BG	Card Code
43-44	45	46-50	51-54	55-58	59	60-63	64	65-68	69	70-73	74	75-78	79	80
SOL 54			50	41		47		61						B
Pos 07		10	50	355		301		375						C
		330	50	60		44		58						D
		1000	50	65		67		75						E
		3330	50	84		97		83						F
		6660	50	89		91		125						G
	1	0000	50	106		94		107						H
	1	3330	50	104		104		102						I
	2	0000	50	106	2	111	2	109	2					J

Remarks Indicate Item Code and Card Code

(continued)

Forms Completion	
Initials	

D-245

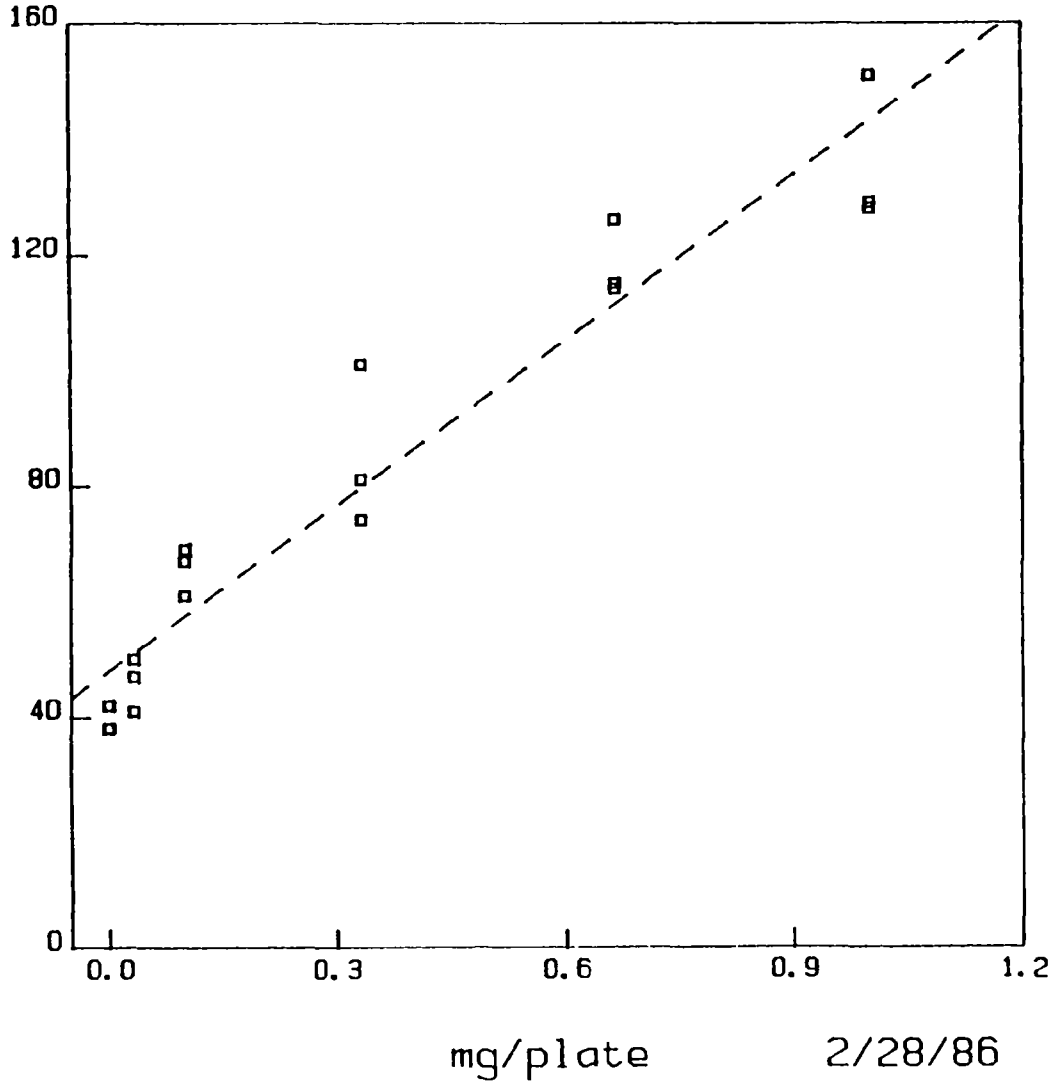
HERL IN VITRO RESULTS CONTINUATION FORM

1 Update Code	2-4 System ID	5-8 Research Lab ID	9-14 MO DA YR Experiment Date	15-18 Lab	19-20 YR	21-24 Number Test Sample Identification	25-30 Activation Batch	31-32 Test Type (Table 10)	33-38 Strain	39-42 Batch No Microorganism
	IVR	CPHE	021886	AIHL	85	0406	10% S9 Mix	01	TA98	8507

		Dose Level		Plate A		Plate B		Plate C		Plate D		Plate E		
H Solvent Positive (Table 11)	I Units of Concentration Blank = mg/ml 2 = µg/ml	J Stock Concentration (ug/plate)	K Amt. Per Plate (µl)	L Count	M BG	N Count	O BG	P Count	Q BG	R Count	S BG	T Count	U BG	Card Code
43-44	45	46-50	51-54	55-58	59	60-63	64	65-68	69	70-73	74	75-78	79	80
Water	blank		50	58		54		50						K
														L
														M
														N
														O
														P
														Q
														R
														S
														T
														U
														V
														W
														X
														Y

INDUSTRIAL WASTEWATER

Dichloromethane Extract



TA 98, 30% S9
SYMBOL=□
LINETYPE= — — — —
y=a+b*x
n=18
a=47.9314 s_a=3.4849
b=95.2524 s_b=6.8224
$s_{y.x}$=10.6216 r=0.9613

X(I)	Y(I)
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0.0000	42.0000
0.0000	38.0000
.0330	47.0000
.0330	41.0000
.0330	50.0000
.1000	67.0000
.1000	61.0000
.1000	69.0000
.3330	101.0000
.3330	81.0000
.3330	74.0000
.6660	115.0000
.6660	114.0000
.6660	136.0000
1.0000	128.0000
1.0000	139.0000
1.0000	151.0000

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER Test Sample Identification	Activation Batch	Test Type (Table 10)	Strain Microorganism	Batch No
	IVR	CPHF	02 28 86	AIHL	85	0406	04605	01	TA98	8507

Animal	Organ	Inducer	30% S9 Mix	(A) 64 Remarks Made? Yes 1	(B) 65 Phenocopy Check Conclusion (Table 13)	(C) 66 Sterility S 9 Mix 1 Not Contam 2 Contam 3 Not Checked	(D) 67 70 Activation Mixture Per Plate (µl)	(E) 71 Sample Sterility Check 1 Not Contam 2 Contam 3 Not Checked	(F) 72 73 74 75 Pre-Incubation Time (min) Temp (Cent)	(G) 76 78 Technician	80 Card Code
	R	L	A	2	1	1	500	1		KIC	A

	Dose Level				Plate A		Plate B		Plate C		Plate D		Plate E		
(H) Solvent Positive (Table 11)	(I) Units of Concentration Blank = mg/ml 2 = µg/ml	(J) Stock concentration (µg/plate)	(K) Amt Per Plate (µl)	(L) Count	(M) BG	(N) Count	(O) BG	(P) Count	(Q) BG	(R) Count	(S) BG	(T) Count	(U) BG	Card Code
	43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL	54			50	45		52		41						B
Pos	07		10	50	89		82		86						C
	04		05	50	248		315		129						D
			330	50	47		41		50						E
			100	50	67		61		69						F
			333	50	101		81		74						G
			666	50	115		114		126						H
		/	0000	50	128		129		151						I
		/	333	50	155		136		120						J

Remarks Indicate Item Code and Card Code

(continued)

Forms Completion
Initials

HERL IN VITRO RESULTS-CONTINUATION FORM

1 Update Code	2 4 System ID	5 8 Research Lab ID	9 14 Experiment Date (MO DA YR)	15 18 Lab	19 20 YR	21 24 Number (Test Sample Identification)	25 30 Activation Batch	31 32 Test Type (Table 10)	33 38 Strain (Microorganism)	39 42 Batch No
	IVR	CPHF	02 28 86	AZHL	85	0406	0465 30% S9 Mix	01	TA98	0307

		Dose Level		Plate A		Plate B		Plate C		Plate D		Plate E		
H Solvent Positive (Table 11)	I Units of Concentration Blank = mg/ml 2 = µg/ml	J Stock Concentration (µg/plate)	K Amt Per Plate (µl)	L Count	M BG	N Count	O BG	P Count	Q BG	R Count	S BG	T Count	U BG	Card Code
43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
	2	0000	50	1129	2	1112	2	1511	2					K
	water blank		50	38		42		38						L
														M
														N
														O
														P
														Q
														R
														S
														T
														U
														V
														W
														X
														Y

APPENDIX E

PRIMARY AMES BIOASSAY DATA FOR THE RECOVERY STUDY

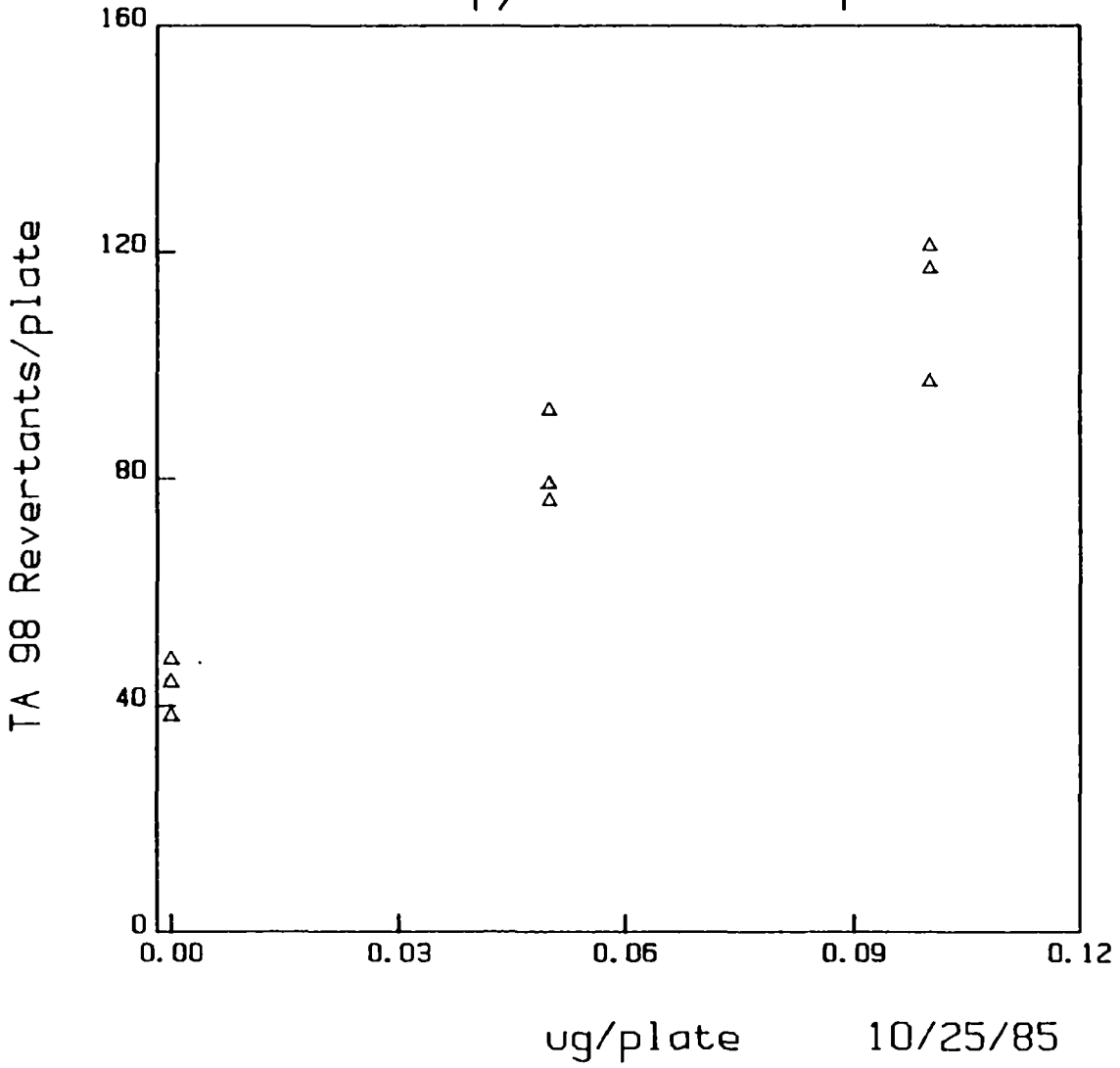
The worksheets, primary data, and dose-response curve graphs for the mutagenicity recovery of three spiked reference mutagens: benzo(a)pyrene, 2-aminoanthracene, and 4-nitrobenzoic acid, are attached as Appendix E.

WORKSHEET FOR THE SPRM B(a)P RECOVERY STUDY IN THE AMES ASSAY IN STRAIN TA98

Extraction Method	Spiked Dose (ug/1.5 L water)	% Recovery 2% S-9 Mix	10% S-9 Mix
Base/Acid	250 in blank water, pH 11 fraction	327/683 = 48%	401/306 = 131%
	250 in blank water, pH 2 fraction	106/683 = 16%	109/306 = 36%
Base/Acid	500 in blank water	331/419 = 79%	777/419 = 185%
Base/Acid	250 in the Municipal Wastewater Sample	—	274/306 = 90%
Acid/Base	250 in the Municipal Wastewater Sample	—	321/306 = 105%

RECOVERY EFFICIENCY

Benzo(a)pyrene Dose-Response

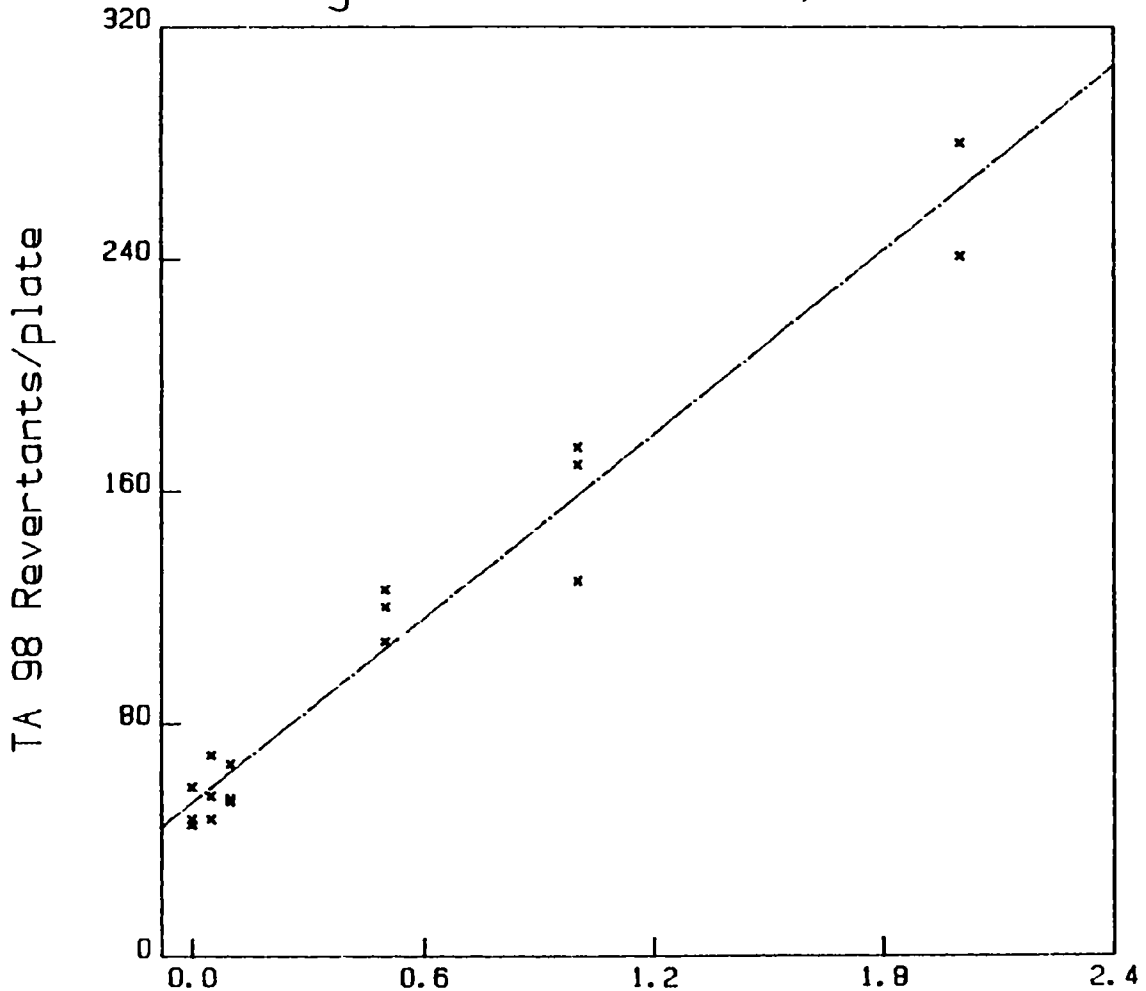


2% S9
SYMBOL=Δ
LINETYPE=
y=a+b*x
n=9
a=44.9444 s_a=4.7673
b=683.3333 s_b=73.8546
$s_{y.x}$=9.0453 r=0.9615

X: I:	Y: I:
0.0000	48.0000
0.0000	44.0000
0.0000	38.0000
0.0500	73.0000
0.0500	76.0000
0.0500	92.0000
0.1000	121.0000
0.1000	117.0000
0.1000	97.0000

RECOVERY EFFICIENCY

250 ug B(a)P/1.5 l Blank, Base/Acid

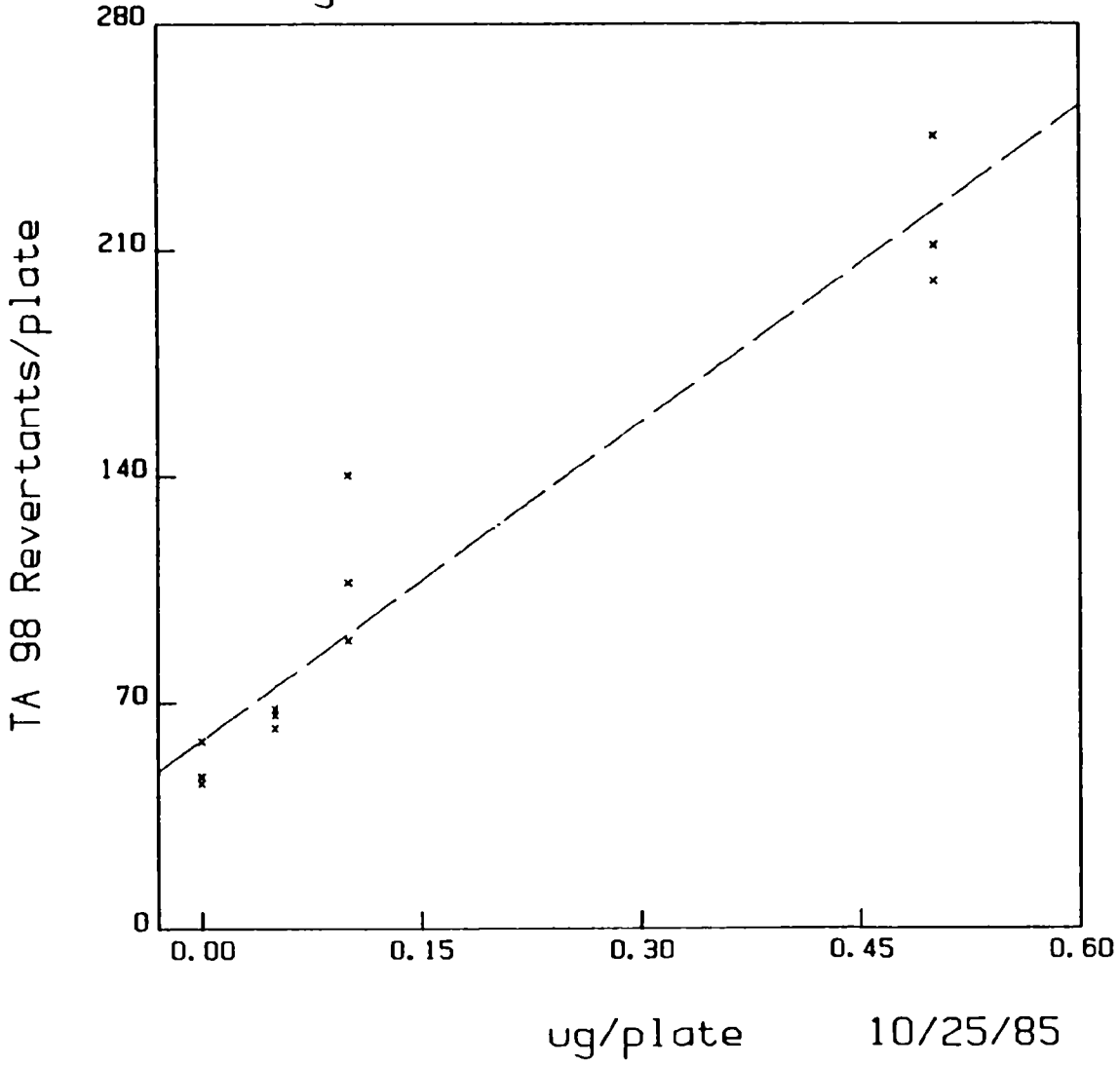


2% S9, pH2 fraction
SYMBOL=×
LINETYPE=————————
y=a+b*x
n=17
a=52.6900 s_e=4.6017
b=105.7285 s_b=5.5263
$s_{y.x}$=14.7000 r=0.9801

0.0000	45.0000
0.0000	50.0000
0.0000	47.0000
.0500	69.0000
.0500	55.0000
.0500	47.0000
.1000	66.0000
.1000	54.0000
.1000	53.0000
.5000	108.0000
.5000	136.0000
.5000	120.0000
1.0000	129.0000
1.0000	169.0000
1.0000	175.0000
2.0000	241.0000
2.0000	280.0000

RECOVERY EFFICIENCY

250 ug B(a)P/1.5 l Blank. Base/Acid



2% S9, pH11 fraction
SYMBOL=×
LINETYPE= — - — - — —
y=a+b*x
n=12
a=58.3347 s_a=7.8105
b=327.1713 s_b=30.4889
$s_{y.x}$=20.9160 r=0.9592

X	Y
0.0000	45.0000
0.0000	58.0000
0.0000	47.0000
.0500	63.0000
.0500	66.0000
.0500	68.0000
.1000	107.0000
.1000	140.0000
.1000	89.0000
.5000	345.0000
.5000	311.0000
.5000	300.0000

HERL IN VITRO RESULTS FORM

1	2-4	5-8	9-14	15-18	19-20	21-24	25-30	31-32	33-38	39-42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER	Activation Batch	Test Type (Table 10)	Strain	Batch No
	IVR	CPHF	10 25 85	AZHL	85	0123	03630	01	TA98	8307
				Test Sample Identification					Microorganism	

Animal	Organ	Inducer	A (64)	B (65)	C (66)	D (67-70)	E (71)	F (72-73-74-75)	G (76-78)	80
K	L	A	2	1	1	500	1		KIC	A
2% S9 Mix			Remarks Made? Yes 1	Phenocopy Check Conclusion (Table 13)	Sterility S9 Mix 1 Not Contam 2 Contam 3 Not Checked	Activation Mixture Per Plate (µl)	Sample Sterility Check 1 Not Contam 2 Contam 3 Not Checked	Time (min) Temp (Cent) Pre-Incubation	Technician	Card Code

	Dose Level				Plate A		Plate B		Plate C		Plate D		Plate E		
	(H) Solvent Positive (Table 11)	(I) Units of Concentration Blank = mg/ml 2 = µg/ml	(J) Stock concentration (µg/plate)	(K) Amt Per Plate (µl)	(L) Count	(M) BG	(N) Count	(O) BG	(P) Count	(Q) BG	(R) Count	(S) BG	(T) Count	(U) BG	Card Code
	43-44	45	46-50	51-54	55-58	59	60-63	64	65-68	69	70-73	74	75-78	79	80
SOL	54			50	48		44		38						B
Pos	10		005	50	79		76		92						C
	10		010	50	121		117		97						D
	10		050	50	223		185		213						E
	10		1000	50	234		195		210						F
	10		2000	50	337		315		326						G
water blank				50	45		58		47						H
spiked water blank 2µH2	10		005	50	69		55		47						I
fraction only, Base/Acid	10		010	50	66		54		53						J

Remarks Indicate Item Code and Card Code

(continued)

Forms Completion
Initials

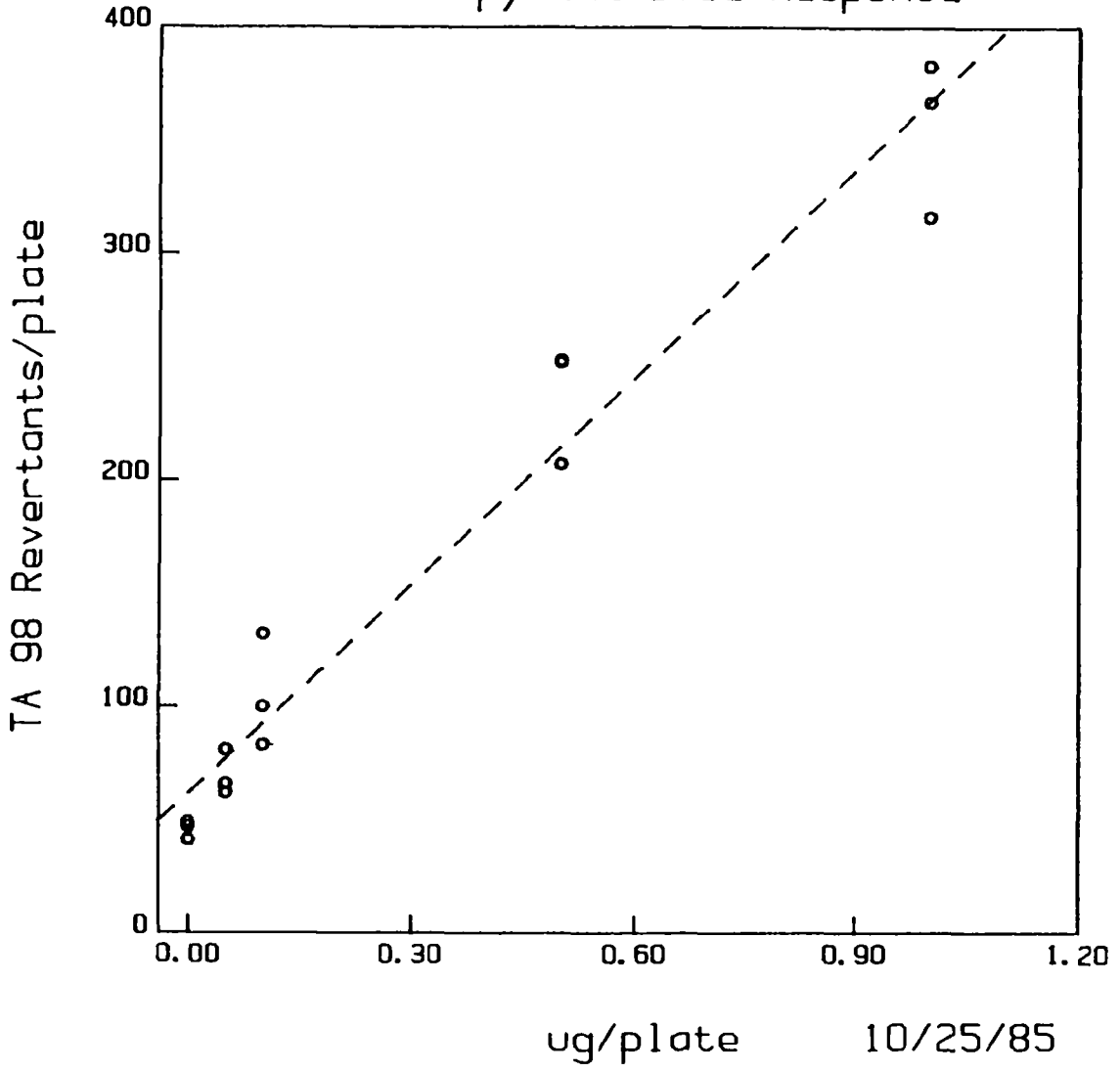
HERL IN VITRO RESULTS CONTINUATION FORM

1 Update Code	2 4 System ID	5 8 Research Lab ID	9 14 Experiment Date (MO DA YR)	15 18 Lab	19 20 YR	21 24 Number (Test Sample Identification)	25 30 Activation Batch	31 32 Test Type (Table 10)	33 38 Strain (Microorganism)	39 42 Batch No
	IVR	CPHF	10 25 85	AZHL	85	0703	036JO	01	TA98	8507

		Dose Level		Plate A		Plate B		Plate C		Plate D		Plate E		
(H) Solvent Positive (Table 11)	(I) Units of Concentration Blank = mg/ml 2 = µg/ml	(J) Stock Concentration (µg/plate)	(K) Amt Per Plate (µl)	(L) Count	(M) BG	(N) Count	(O) BG	(P) Count	(O) BG	(R) Count	(S) BG	(T) Count	(U) BG	Card Code
43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
10		050	50	1108		126		120						K
10		100	50	129		169		175						L
10		200	50	241		280		161	4					M
10	spiked water blank 3	005	50	62		66		68						N
Base/Acid	pH 11 titration only													
10		010	50	107		140		87						O
10		050	50	245		211		200						P
10		100	50	271		268		268						Q
10		200	50	370		405		374						R
														S
														T
														U
														V
														W
														X
														Y

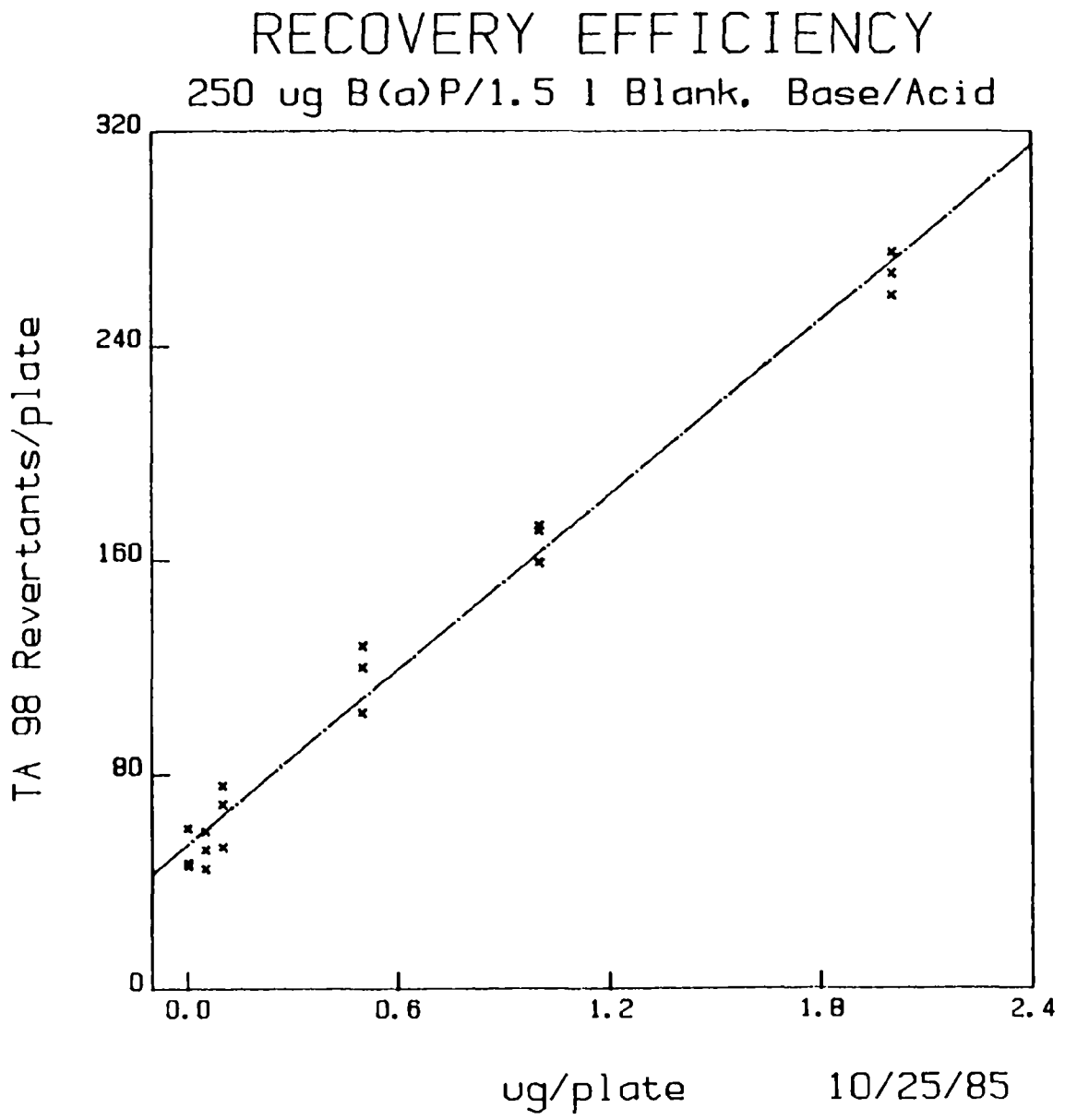
RECOVERY EFFICIENCY

Benzo(a)pyrene Dose-Response



10% S9
SYMBOL=o
LINETYPE= — — — —
y=a+b*x
n=15
a=61.6267 s_a=8.8646
b=305.9796 s_b=17.6412
$s_{y.x}$=25.8912 r=0.9791

X: I:	Y: I:
0 0000	41 0000
0 0000	47 0000
0 0000	49 0000
0500	81 0000
0500	62 0000
0500	66 0000
1000	100 0000
1000	83 0000
1000	132 0000
5000	252 0000
5000	253 0000
5000	207 0000
1 0000	363 0000
1 0000	216 0000
1 0000	357 0000

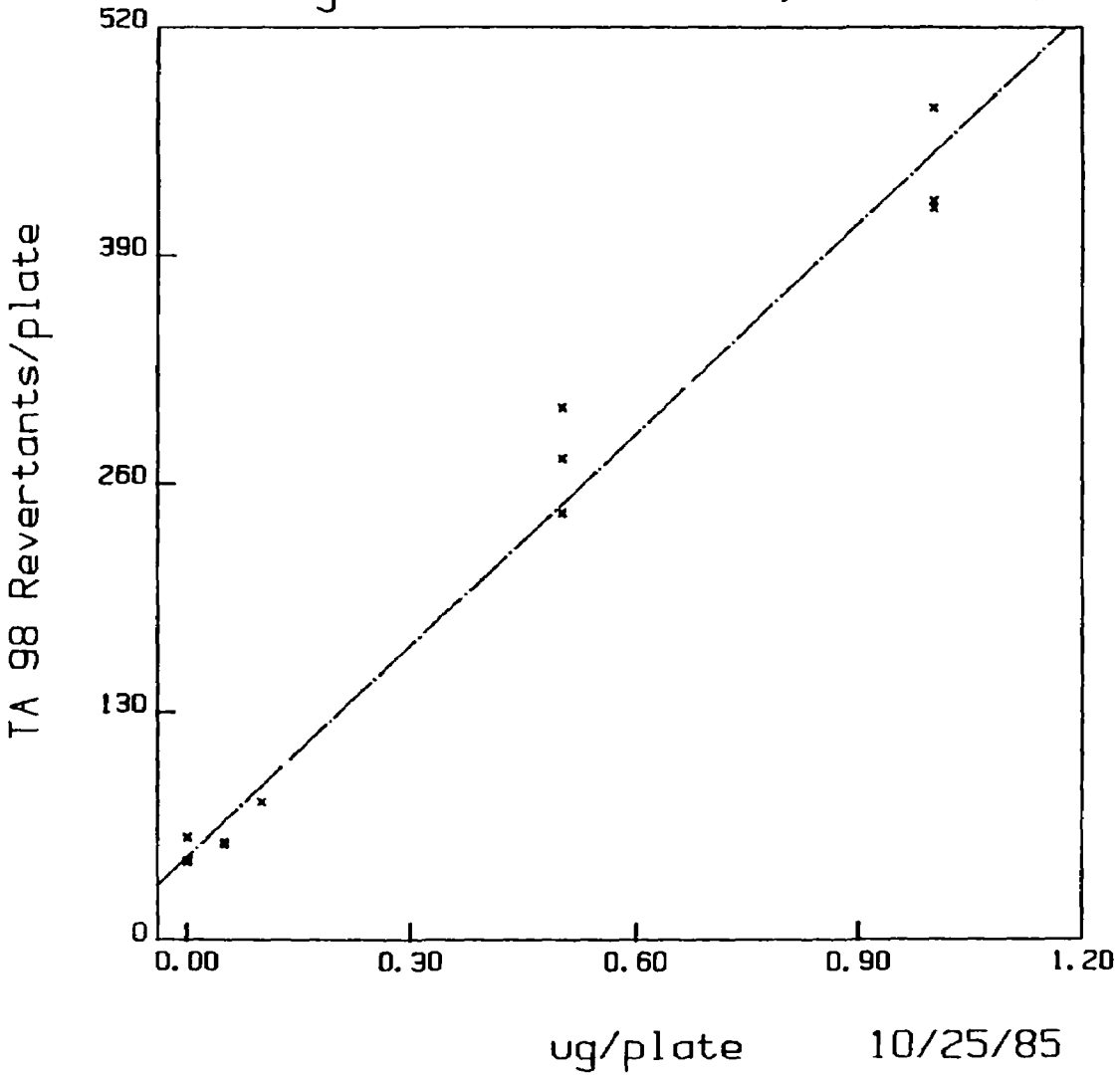


10% S9, pH2 fraction
SYMBOL=×
LINETYPE= ——·——·——
y=a+b*x
n=18
a=53.9280 s_a=3.1192
b=108.7942 s_b=3.3306
$s_{y.x}$=10.0617 r=0.9926

X(I)	Y(I)
0.0000	47.0000
0.0000	46.0000
0.0000	60.0000
.0500	45.0000
.0500	59.0000
.0500	52.0000
.1000	69.0000
.1000	53.0000
.1000	76.0000
.5000	120.0000
.5000	128.0000
.5000	103.0000
1.0000	173.0000
1.0000	159.0000
1.0000	171.0000
2.0000	259.0000
2.0000	275.0000
2.0000	267.0000

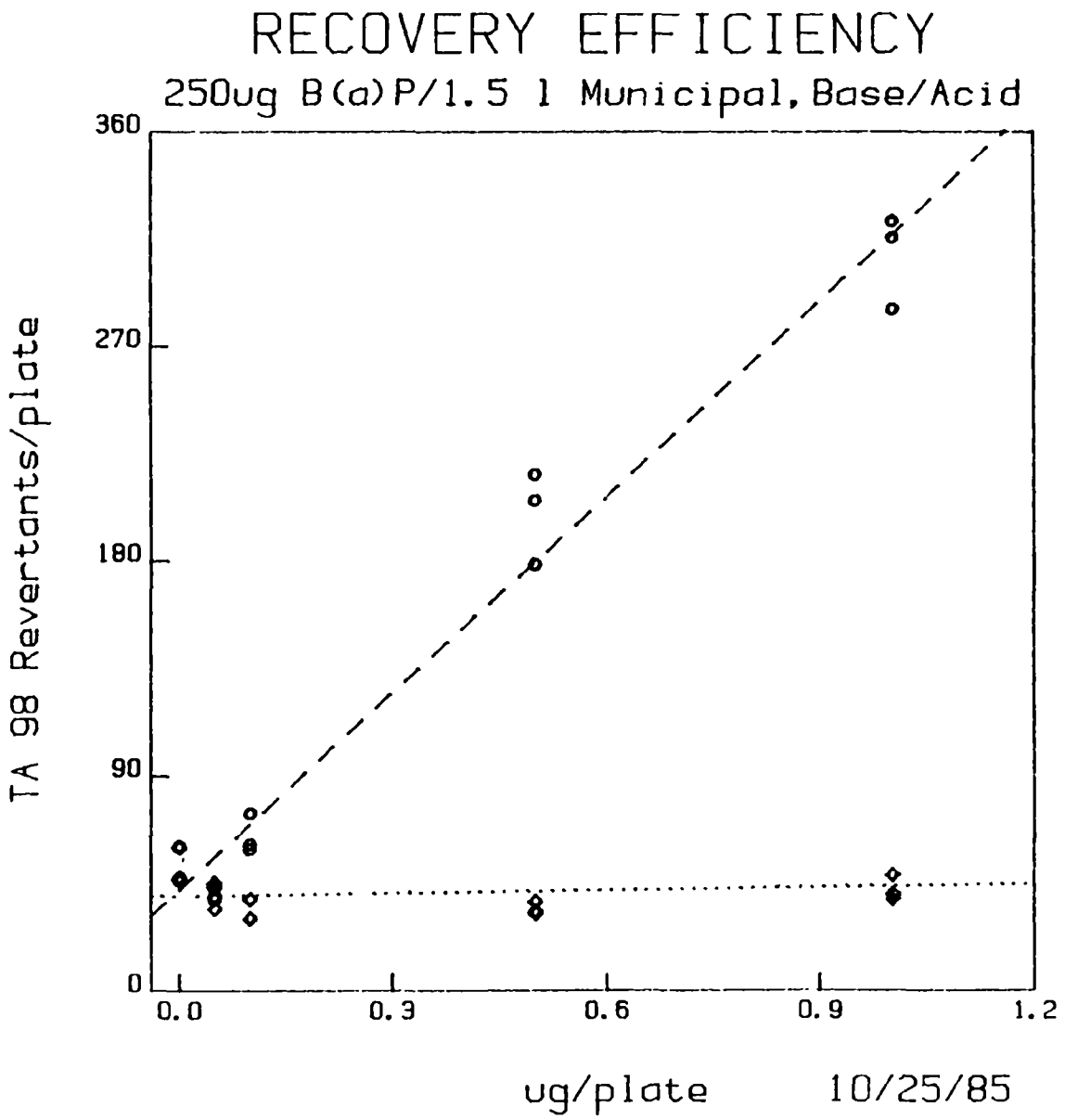
RECOVERY EFFICIENCY

250 ug B(a)P/1.5 l Blank, Base/Acid



10% S9, pH11 fraction
SYMBOL=×
LINETYPE= ——————
y=a+b*x
n=13
a=48.9861 s_a=9.3630
b=400.8802 s_b=17.3925
$s_{y.x}$=24.7922 r=0.9898

0.0000	47.0000
0.0000	46.0000
0.0000	60.0000
.0500	57.0000
.0500	57.0000
.0500	56.0000
.1000	80.0000
.5000	244.0000
.3000	304.0000
.5000	275.0000
1.0000	418.0000
1.0000	422.0000
1.0000	475.0000

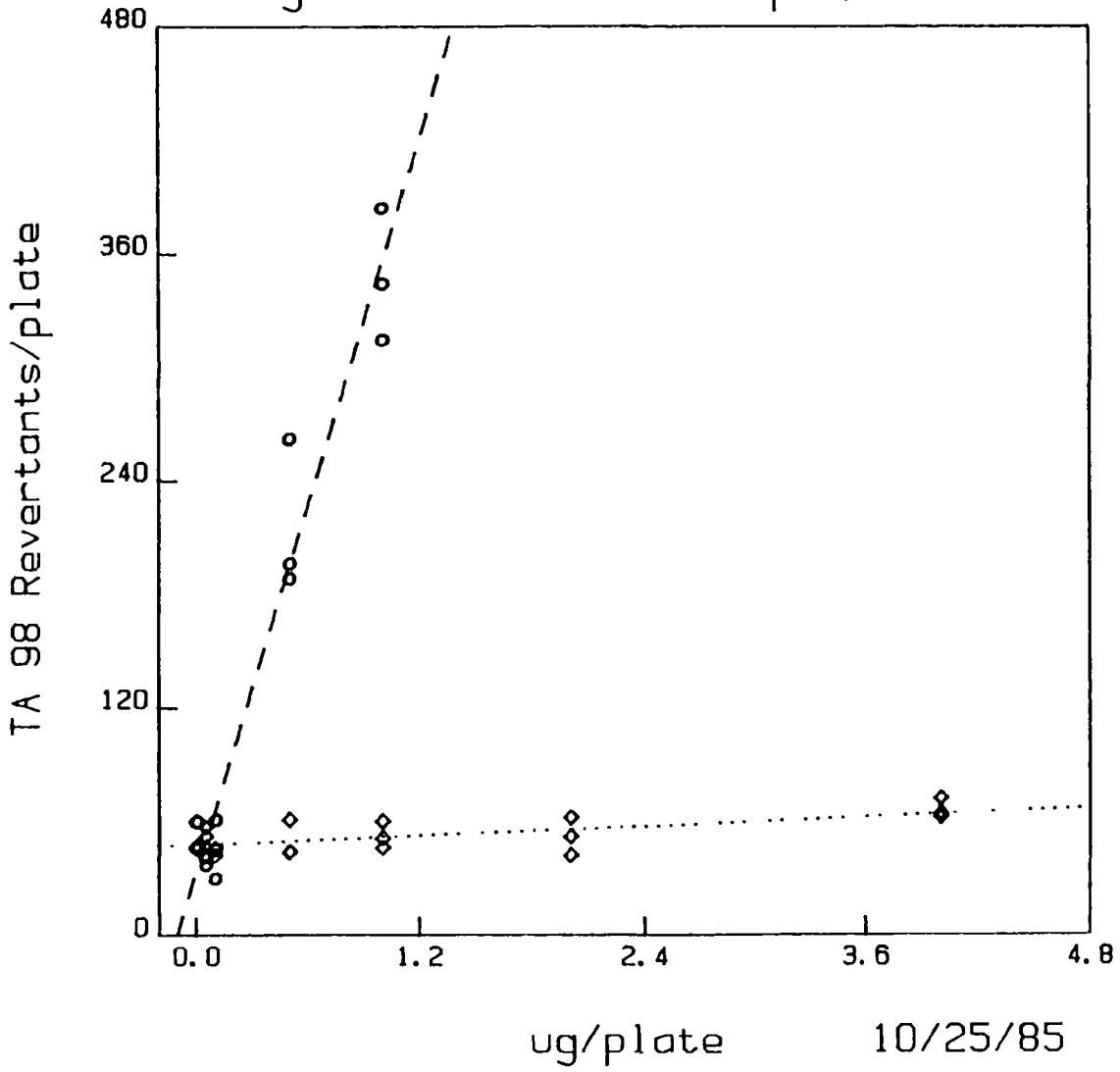


10% S9 unspiked municipal
SYMBOL=◇
LINETYPE=.....
y=a+b*x
n=21
a=39.3700 s_a=2.4675
b=4.4109 s_b=1.4158
$s_{y.x}$=8.8084 r=0.5815

10% S9, spiked municipal
SYMBOL=o
LINETYPE=— — — —
y=a+b*x
n=15
a=42.0116 s_a=6.2716
b=274.3083 s_b=12.4809
$s_{y.x}$=18.3176 r=0.9868

RECOVERY EFFICIENCY

250 ug B(a)P/1.5 l Municipal, Acid/Base



10% S9, unspiked municipal
SYMBOL=◇
LINETYPE=.....
y=a+b*x
n=21
a=47.8672 s_a=1.9720
b=4.0431 s_b=1.1315
$s_{y.x}$=7.0396 r=0.6340

10% S9, spiked municipal
SYMBOL=o
LINETYPE= — — — —
y=a+b*x
n=15
a=33.6929 s_a=9.6482
b=321.5367 s_b=19.2006
$s_{y.x}$=28.1798 r=0.9776

HERL IN VITRO RESULTS FORM

1	2-4	5-8	9-14	15-18	19-20	21-24	25-30	31-32	33-38	39-42
Update Code	System ID: IVR	Research Lab ID: CPHF	MO DA YR: 10 25 85	LAB: AZVL	YR: 85	NUMBER: 0403	Activation Batch: 03630	Test Type (Table 10): 01	Strain: 1712	Batch No: 8507
			Experiment Date	Test Sample Identification					Microorganism	

Animal: R
Organ: L
Inducer: A
Monomycin: 10% S9 Mix

(A) 64	(B) 65	(C) 66	(D) 67-70	(E) 71	(F) 72-73-74-75	(G) 76-78	80
2	1	1	500	1		KLC	A
Remarks Made? Yes 1	Phenocopy Check Conclusion (Table 13)	Sterility S9 Mix: 1 Not Contam, 2 Contam, 3 Not Checked	Activation Mixture Per Plate (µl)	Sample Sterility Check: 1 Not Contam, 2 Contam, 3 Not Checked	Time (min) Temp (Cent), Pre-Incubation	Technician	Card Code

(H) Solvent Positive (Table 11)	(I) Units of Concentration Blank = mg/ml 2 = µg/ml	(J) Stock concentration (µg/plate)	(K) Amt Per Plate (µl)	Plate A: (L) Count	(M) BG	Plate B: (N) Count	(O) BG	Plate C: (P) Count	(Q) BG	Plate D: (R) Count	(S) BG	Plate E: (T) Count	(U) BG	Card Code
	43-44	45	46-50	51-54	55-58	59	60-63	64	65-68	69	70-73	74	75-78	79	80
SOL	54			50	41		47		49						B
Pos	10		00500	50	81		62		66						C
	10		00100	50	100		83		132						D
	10		00500	50	252		253		207						E
	10		01000	50	383		316		367						F
	10		05000	50	432										G
water blank				50	477		466		602						H
spiked water blank, pH2	10		00500	50	455		599		522						I
fraction #1 Base/Acid	10		00100	50	699		533		776						J

Remarks Indicate Item Code and Card Code

(continued)

Forms Completion
Initials

HERL IN VITRO RESULTS CONTINUATION FORM

1 Update Code	2 4 System ID	5 8 Research Lab ID	9 14 Experiment Date (MO DA YR)	15 18 - 19 20 - 21 24 Test Sample Identification (Lab / YR / Number)	25 30 Activation Batch	31 32 Test Type (Table 10)	33 38 Strain	39 42 Batch No
	IVR	CPHF	10 23 85	ALHL - 85 - 0403	036 30 10% S9 Mix	01	TA98	8507
							Microorganism	

(H) Solvent Positive (Table 11)	(I) Units of Concentration Blank = mg/ml 2 = µg/ml	Dose Level (J) Stock Concentration (mg/plate)	Dose Level (K) Amt Per Plate (µl)	Plate A (L) Count	Plate A (M) BG	Plate B (N) Count	Plate B (O) BG	Plate C (P) Count	Plate C (Q) BG	Plate D (R) Count	Plate D (S) BG	Plate E (T) Count	Plate E (U) BG	Card Code
43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
10		050	050	120		128		103						K
10		100	050	173		159		171						L
10		200	050	259		275		267						M
10 Base/Acid	spikal water blank 2 pH titration only	005	050	57		57		56						N
10		010	050	180										O
10		050	050	244		304		275						P
10		100	050	418		422		475						Q
10		200	050	448		497		547						R
Acid/Base	unspiked mouse liver S9 sample	005	050	57		52		42						S
		010	050	46		46		42						T
		050	050	44		44		61						U
		100	050	51		46		60						V
		200	050	42		62		52						W
		400	050	22		62		64						X
Base/Acid	unspiked mouse liver S9 sample	005	050	34		43		45						Y

(continued)

HERL IN VITRO RESULTS CONTINUATION FORM

1 Update Code	2 4 System ID	5 8 Research Lab ID	9 14 MO DA YR Experiment Date	15 18 - 19 20 - 21 24 Lab YR Test Sample Identification	25 30 Activation Batch (S9 Mix)	31 32 Test Type (Table 10)	33 38 Strain / Microorganism	39 42 Batch No
	IVRI	CPVHF	10 25 85	AHL - 85 - 0403	036300	01	TA98	6307

		Dose Level		Plate A		Plate B		Plate C		Plate D		Plate E		
H Solvent Positive (Table 11)	I Units of Concentration Blank = mg/ml 2 = µg/ml	J Stock Concentration (µg/plate)	K Amt Per Plate (µl)	L Count	M BG	N Count	O BG	P Count	Q BG	R Count	S BG	T Count	U BG	Card Code
43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
		010	50	38		30		30						K
		050	50	32		37		33						L
		100	50	48		38		40						M
		200	50	67		47		44						N
		400	50	55		62		52						O
10 Aid/Boss	spiked mutagen sample	005	50	42		37		41						P
10		010	50	61		45		30						Q
10		050	50	262		196		188						R
10		100	50	344		314		384						S
10		200	50	316		352		325						T
10		400	50	335		316		330						U
10 Boss/Aid	spiked mutagen sample	005	50	38		43		39						V
10		010	50	74		59		61						W
10		050	50	216		205		178						X
10		100	50	322		315		285						Y

(continued)

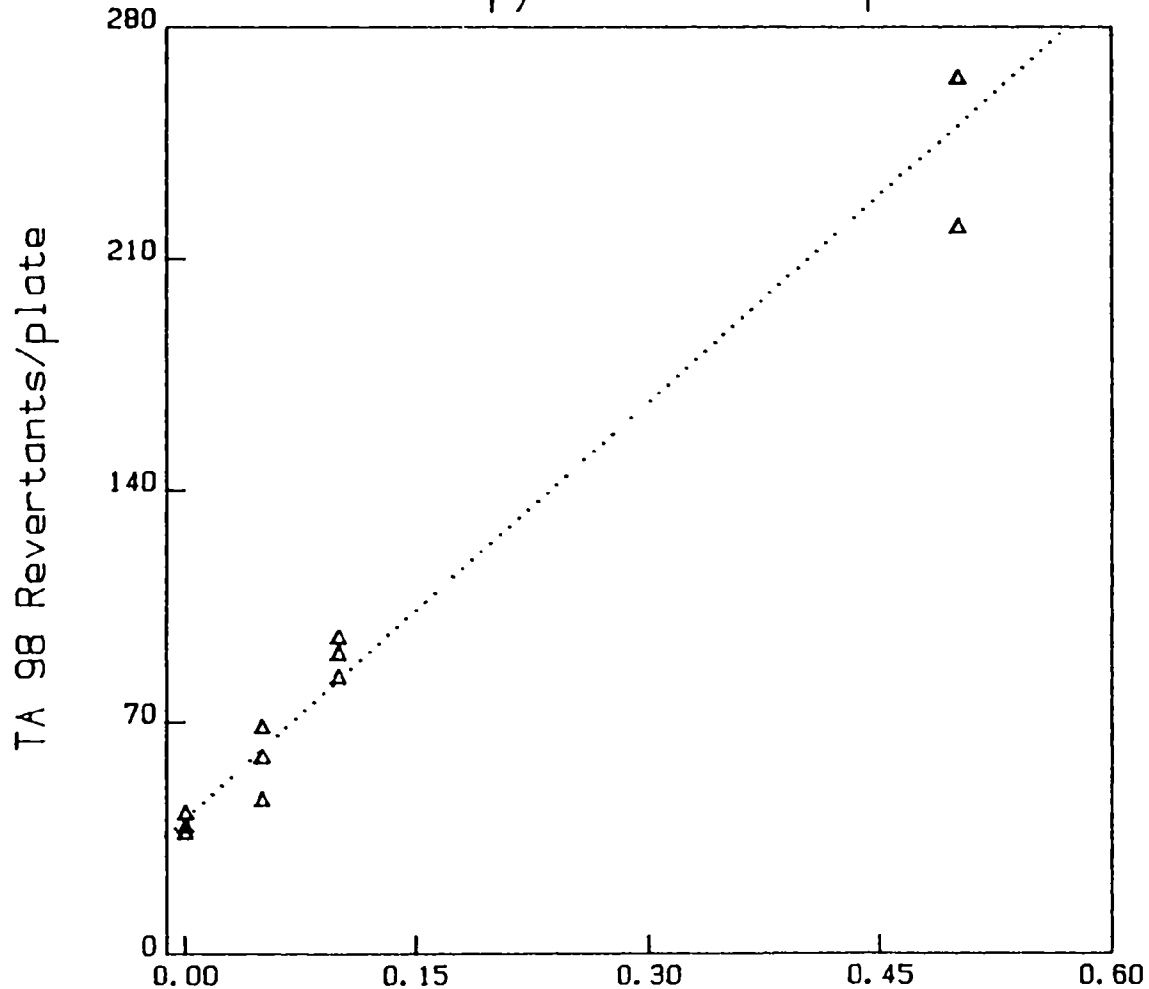
HERL IN VITRO RESULTS CONTINUATION FORM

1 Update Code	2 4 System ID	5 8 Research Lab ID	9 14 Experiment Date (MO DA YR)	15 18 Lab	19 20 Test Sample Identification (YR)	21 24 Number	25 30 Activation Batch	31 32 Test Type (Table 10)	33 38 Strain	39 42 Batch No
	IVR	CPHE	10 05 85	AZHL	85	0404	05360	01	TA98	8507
							10% S9 Mix		Microorganism	

		Dose Level		Plate A		Plate B		Plate C		Plate D		Plate E		
(H) Solvent Positive (Table 11)	(I) Units of Concentration Blank = mg/ml 2 = µg/ml	(J) Stock Concentration (mg/plate)	(K) Amt Per Plate (µl)	(L) Count	(M) BG	(N) Count	(O) BG	(P) Count	(Q) BG	(R) Count	(S) BG	(T) Count	(U) BG	Card Code
43 44	45	46-50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
10		200	50	357		377								K
10		400	50	279		320								L
														M
														N
														O
														P
														Q
														R
														S
														T
														U
														V
														W
														X
														Y

RECOVERY EFFICIENCY

Benzo(a)pyrene Dose-Response

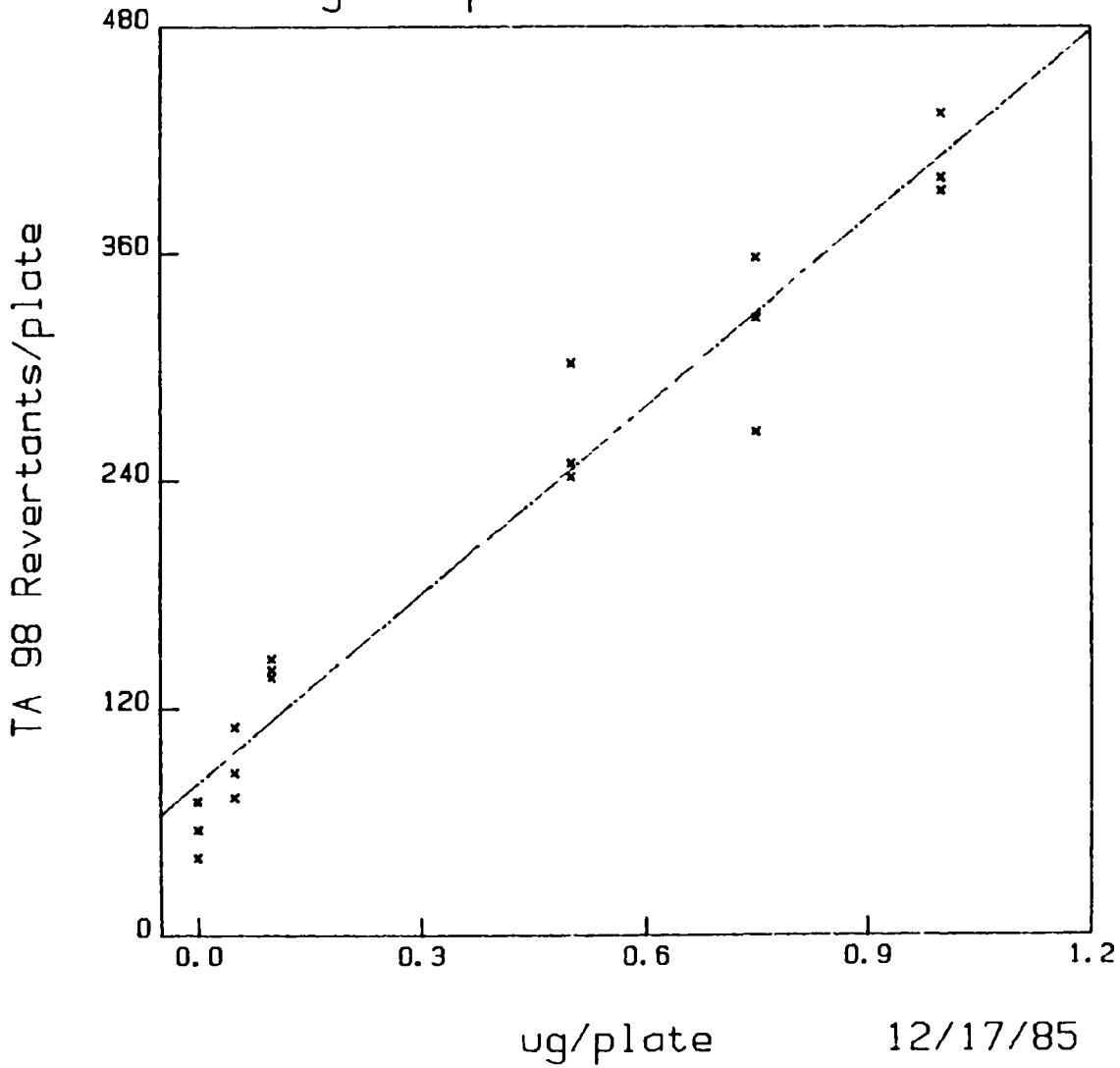


2% S9
SYMBOL=Δ
LINETYPE=. ... .
y=a+b*x
n=12
a=40.6282 s_a=5.1284
b=418.6985 s_b=20.0192
$s_{y.x}$=13.7336 r=0.9888

X(I)	Y(I)
0.0000	42.0000
0.0000	38.0000
0.0000	36.0000
.0500	59.0000
.0500	46.0000
.0500	68.0000
.1000	90.0000
.1000	95.0000
.1000	83.0000
.5000	264.0000
.5000	264.0000
.5000	219.0000

RECOVERY EFFICIENCY

500 ug B(a)p/1.5 l Blank. Base/Acid



2% S9
SYMBOL=×
LINETYPE=————·————
y=a+b*x
n=18
a=80.1519 s_a=10.2383
b=331.4258 s_b=18.5641
$s_{y.x}$=29.9049 r=0.9758

X(I)	Y(I)
0.0000	56.0000
0.0000	71.0000
0.0000	41.0000
.0500	73.0000
.0500	110.0000
.0500	86.0000
.1000	140.0000
.1000	146.0000
.1000	136.0000
.5000	302.0000
.5000	249.0000
.5000	242.0000
.7500	266.0000
.7500	358.0000
.7500	336.0000
1.0000	434.0000
1.0000	400.0000
1.0000	393.0000

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER	Activation Batch	Test Type (Table 10)	Strain	Batch No
	IVR	CPHF	12 17 85	AZVL	85	0403	04605	01	17A98	85057
				Test Sample Identification					Microorganism	

Animal	R
Organ	L
Inducer	A
[redacted]	2% S9 Mix

(A) 64 Remarks Made? Yes 1: 2

(B) 65 Phenocopy Check Conclusion (Table 13): 1

(C) 66 Sterility S 9 Mix: 1 (1 Not Contam, 2 Contam, 3 Not Checked)

(D) 67 70 Activation Mixture Per Plate (µl): 500

(E) 71 Sample Sterility Check: 1 (1 Not Contam, 2 Contam, 3 Not Checked)

(F) 72 73 74 75 Pre-Incubation: Time (min) | Temp (Cent)

(G) 76 78 Technician: AZC

80 Card Code: A

(H) Solvent Positive (Table 11)	Dose Level: (I) Units of Concentration Blank = mg/ml 2 = µg/ml	(J) Stock concentration (µg/plate)	(K) Amt. Per Plate (µl)	Plate A: (L) Count	(M) BG	Plate B: (N) Count	(O) BG	Plate C: (P) Count	(Q) BG	Plate D: (R) Count	(S) BG	Plate E: (T) Count	(U) BG	Card Code
	43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL	34			500	412		338		336						B
Pos	10		00.05	50	597		466		688						C
	10		0.10	50	990		775		833						D
	10		0.50	50	264		264		219						E
	10		0.75	50	262		300		252						F
	10		1.00	50	292		268		312						G
water blank				50	556		711		411						H
spiked water blanks	10		0.05	50	773		1110		886						I
50 mg/ml blank water	10		0.10	50	1450		1466		1366						J

Remarks Indicate Item Code and Card Code

(continued)

Forms Completion	
Initials	

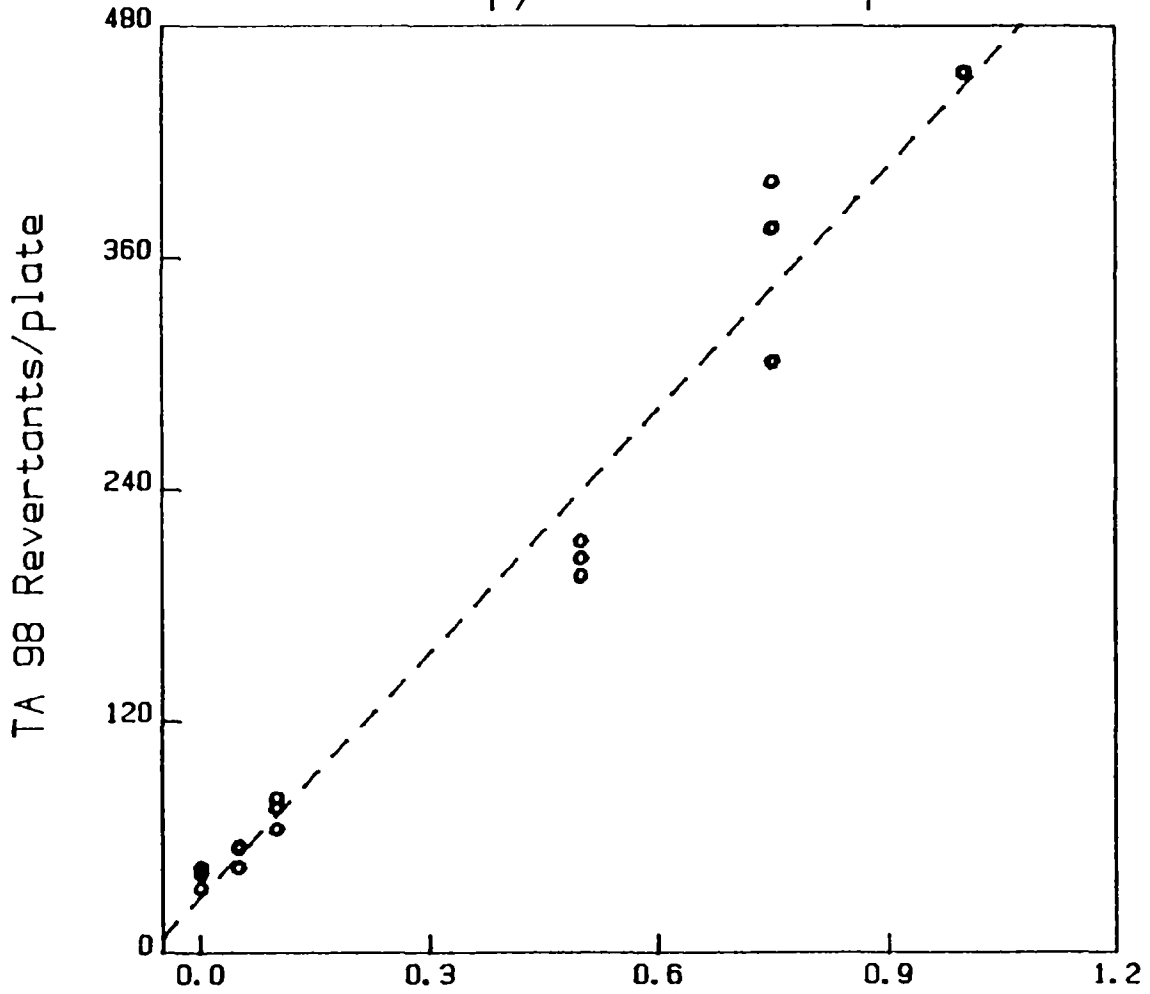
HERL IN VITRO RESULTS CONTINUATION FORM

1 Update Code	2 4 System ID	5 8 Research Lab ID	9 14 MO DA YR Experiment Date	15 18 Lab	19 20 YR	21 24 Number Test Sample Identification	25 30 Activation Batch	31 32 Test Type (Table 10)	33 38 Strain Microorganism	39 42 Batch No
	IVR	CPHF	12/78 5	AZHL	- 85	0403	2% S9 Mix	01	TA98	837

		Dose Level		Plate A		Plate B		Plate C		Plate D		Plate E		
(H) Solvent Positive (Table 11)	(I) Units of Concentration Blank = mg/ml 2 = µg/ml	(J) Stock Concentration (mg/plate)	(K) Amt. Per Plate (µl)	(L) Count	(M) B G	(N) Count	(O) B G	(P) Count	(Q) B G	(R) Count	(S) B G	(T) Count	(U) B G	Card Code
43 44	45	46-50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
10		050	050	302		247		242						K
10		075	050	266		358		326						L
10		100	050	434		400		393						M
														N
														O
														P
														Q
														R
														S
														T
														U
														V
														W
														X
														Y

RECOVERY EFFICIENCY

Benzo(a)pyrene Dose-Response

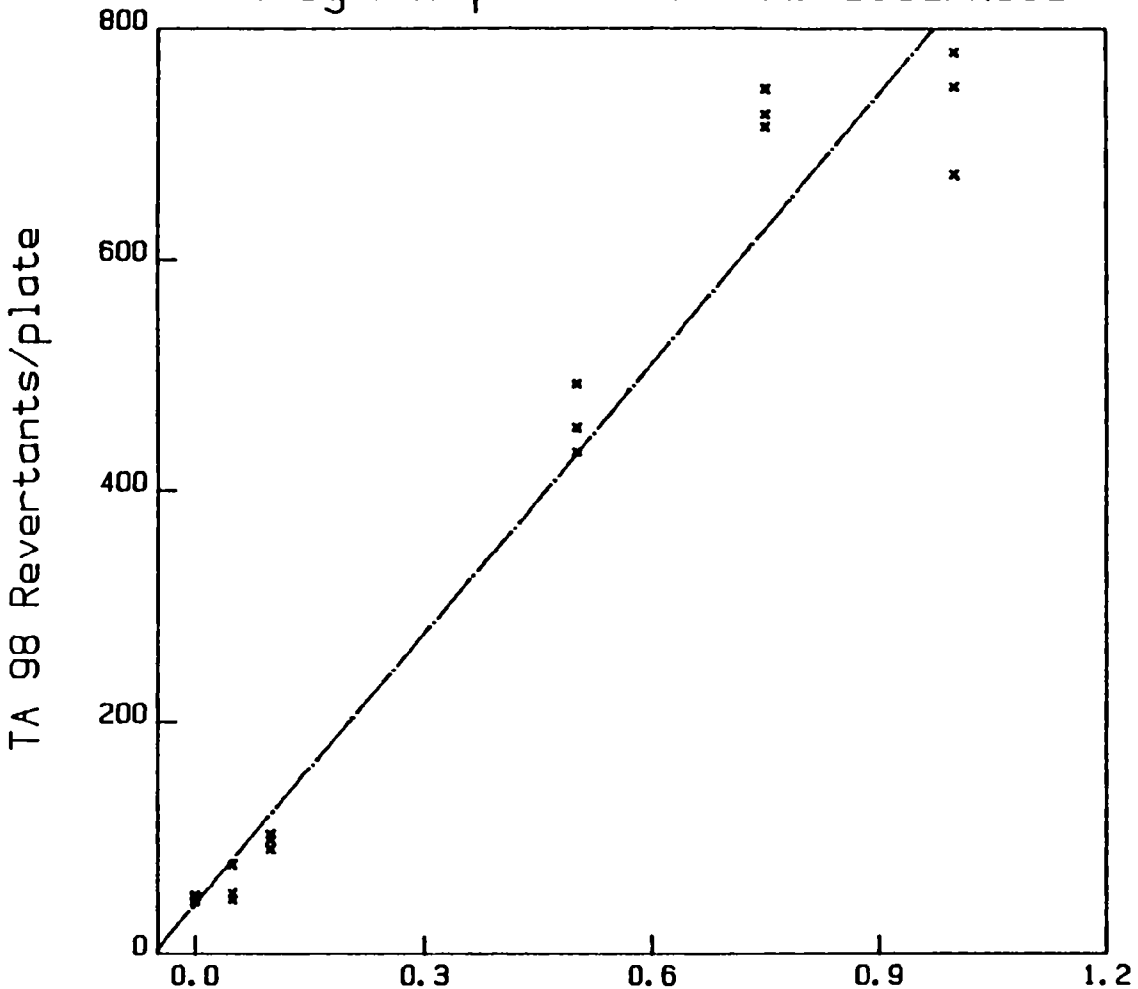


10% S9
SYMBOL=o
LINETYPE= — — — —
y=a+b*x
n=17
a=28.9774 s_a=8.8848
b=419.4168 s_b=17.3171
$s_{y.x}$=25.7660 r=0.9875

X(I)	Y(I)
0 0000	41 0000
0 0000	33 0000
0 0000	44 0000
0500	55 0000
0500	54 0000
0500	44 0000
1000	80 0000
1000	64 0000
1000	75.0000
5000	204 0000
5000	213 0000
.5000	195.0000
7500	306.0000
7500	375 0000
7500	399 0000
1 0000	455 0000
1 0000	456 0000

RECOVERY EFFICIENCY

500 ug B(a)p/1.5 l Blank. Base/Acid



10% S9
SYMBOL=×
LINETYPE=————·————·—
y=a+b*x
n=18
a=44.3568 s_a=22.4543
b=777.3025 s_b=40.7140
$s_{y.x}$=65.5862 r=0.9787

X(I)	Y(I)
0 0000	51 0000
0 0000	50.0000
0 0000	46.0000
0500	78.0000
0500	53 0000
0500	48 0000
1000	99.0000
1000	104.0000
1000	91 0000
5000	493 0000
5000	455.0000
5000	434 0000
7500	726 0000
7500	715 0000
7500	748 0000
1 0000	750 0000
1 0000	674 0000
1 0000	780 0000

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER	Activation Batch	Test Type (Table 10)	Strain	Batch No
	IVR	CFHF	12 17 75	AZHL	75	0403	0465	01	1718	8501
				Test Sample Identification					Microorganism	

Animal	R
Organ	L
Inducer	A
[redacted]	10% S9 Mix

A 64	B 65	C 66	D 67 70	E 71	F 72 73 74 75	G 76 78	80
Remarks Made? Yes 1	Phenocopy Check Conclusion (Table 13)	Sterility S 9 Mix 1 Not Contam 2 Contam 3 Not Checked	Activation Mixture Per Plate (µl)	Sample Sterility Check 1 Not Contam 2 Contam 3 Not Checked	Time (min) Temp (Cent) Pre-Incubation	Technician	Card Code
2	1	1	500	1		KZC	A

	Dose Level				Plate A		Plate B		Plate C		Plate D		Plate E		
	H Solvent Positive (Table 11)	I Units of Concentration Blank = mg/ml 2 = µg/ml	J Stock concentration (µg/plate)	K Amt Per Plate (µl)	L Count	M BG	N Count	O BG	P Count	Q BG	R Count	S BG	T Count	U BG	Card Code
	43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL	54			50	41		33		44						B
Pos	10		005	50	555		544		44						C
	10		010	50	800		644		75						D
	10		050	50	204		213		195						E
	10		075	50	306		375		399						F
	10		100	50	455		456								G
water blank				50	51		50		46						H
spiked water blank	10		005	50	788		538		488						I
Soong/ISI blank water	10		010	50	999		1004		991						J

Remarks Indicate Item Code and Card Code

(continued)

Forms Completion Initials

HERL IN VITRO RESULTS CONTINUATION FORM

1 Update Code	2 4 System ID	5 8 Research Lab ID	9 14 Experiment Date MO DA YR	15 18 - 19 20 - 21 24 Test Sample Identification Lab - YR - Number	25 30 Activation Batch (10% S9 Mix)	31 32 Test Type (Table 10)	33 38 Microorganism Strain	39 47 Batch No
	IVR	CPHF	12/17/85	AZVH-85-0403	04650	01	TA78	830702

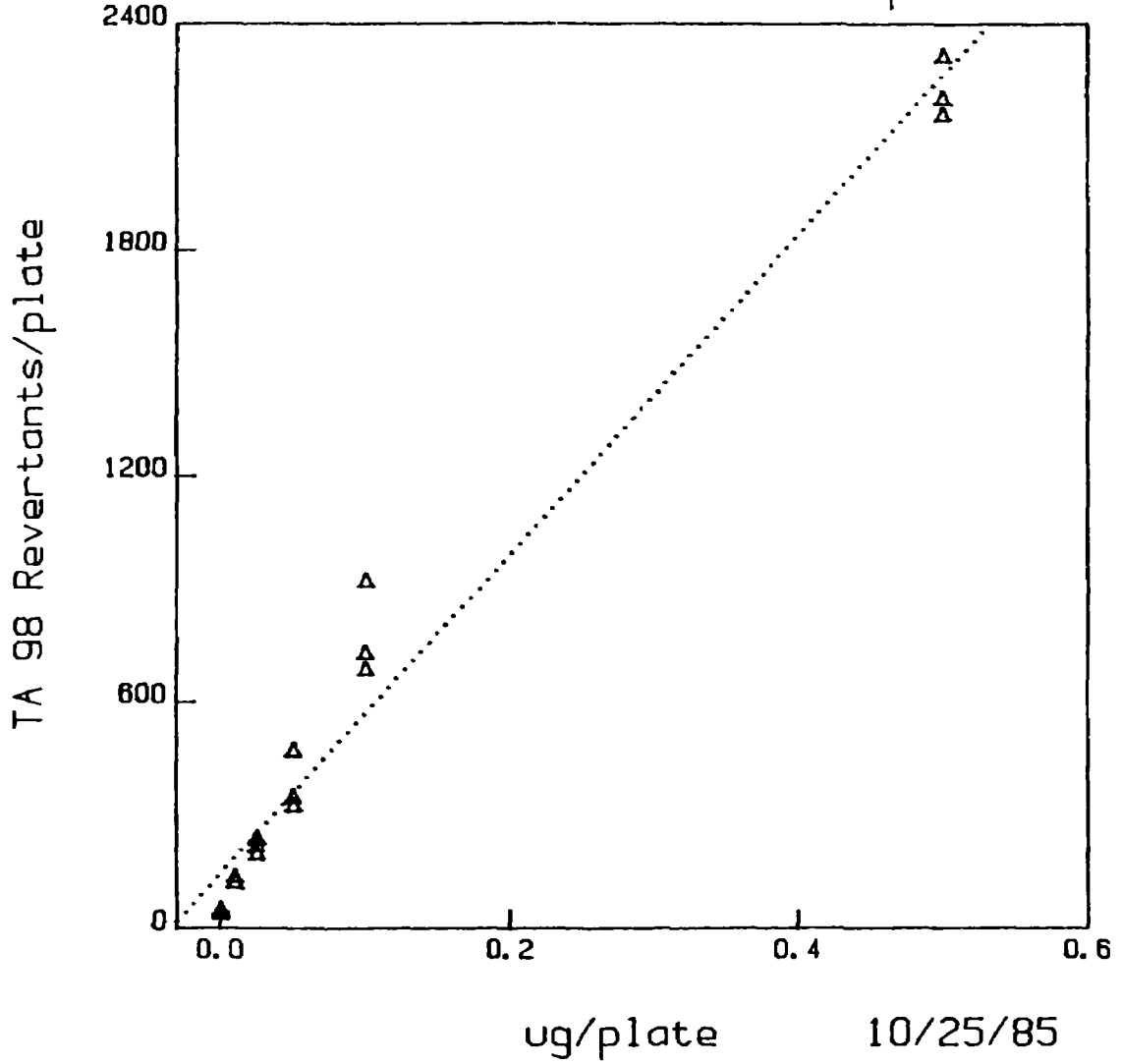
		Dose Level		Plate A		Plate B		Plate C		Plate D		Plate E		
H Solvent Positive (Table 11)	I Units of Concentration Blank = mg/ml 2 = µg/ml	J Stock Concentration (µg/plate)	K Amt. Per Plate (µl)	L Count	M BG	N Count	O BG	P Count	Q BG	R Count	S BG	T Count	U BG	Card Code
43 44	45	46-50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
10		0050	0050	4713		4555		4344						K
10		0075	0050	7216		7115		7448						L
10		0100	0050	7810		7350		6744						M
														N
														O
														P
														Q
														R
														S
														T
														U
														V
														W
														X
														Y

WORKSHEET FOR THE SPRM 2AA RECOVERY STUDY IN THE AMES ASSAY
IN STRAIN TA98

Extraction Method	Spiked Dose (ug/1.5 L water)	% Recovery 2% S-9 Mix	10% S-9 Mix
Base/Acid	250 in blank water pH 11 fraction	$3664/4233 = 86\%$	$1494/2342 = 64\%$
	250 in blank water pH 2 fraction	$< \frac{50/a5}{4233} = 2\%$	$< \frac{51/0.5}{2342} = 4\%$
Base/Acid	500 in blank water	$3701/3058 = 121\%$	$1175/986 = 119\%$
Base/Acid	100 in blank water	$2022/3058 = 66\%$	$484/986 = 49\%$
Base/Acid	250 in the Municipal Wastewater Sample	—	$828/2342 = 35\%$
Acid/Base	250 in the Municipal Wastewater Sample	—	$637/2342 = 27\%$

RECOVERY EFFICIENCY

2-Aminoanthracene Dose-Response

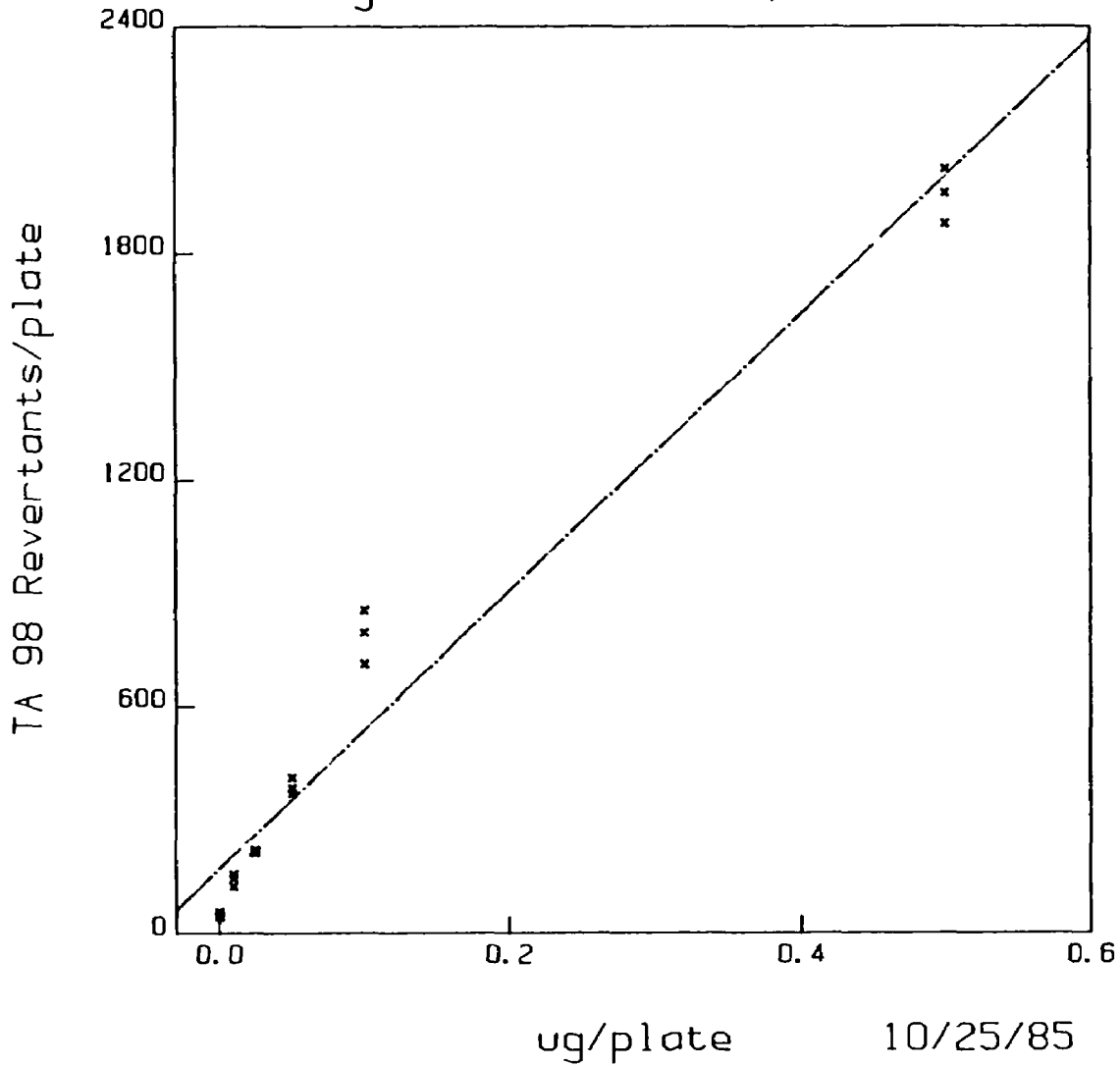


2% S9
SYMBOL=Δ
LINETYPE=.....
y=a+b*x
n=18
a=146.7108 s_a=34.1785
b=4233.4329 s_b=163.1250
$s_{y.x}$=121.2130 r=0.9883

X(I)	Y(I)
0.0000	13.0000
0.0000	44.0000
0.0000	38.0000
.0100	135.0000
.0100	120.0000
.0250	216.0000
.0250	235.0000
.0250	237.0000
.0250	196.0000
.0500	468.0000
.0500	322.0000
.0500	346.0000
.1000	918.0000
.1000	684.0000
.1000	728.0000
.5000	2157.0000
.5000	1312.0000
.5000	1290.0000

RECOVERY EFFICIENCY

250 ug 2AA/1.5 l Blank, Base/Acid



2% S9, pH11 fraction
SYMBOL=×
LINETYPE=————
y=a+b*x
n=18
a=171.9618 s_a=37.4502
b=3664.0814 s_b=178.7993
$s_{y.x}$=133.2100 r=0.9815

0 0000	45 0000
0 0000	58 0000
0 0000	47 0000
0100	142 0000
0100	135 0000
0100	158 0000
0250	317 0000
0250	333 0000
0250	318 0000
0500	413 0000
0500	734 0000
0500	770 0000
1000	736 0000
1000	855 0000
1000	713 0000
5000	1379 0000
5000	2734 0000
5000	2??? 0000

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER Test Sample Identification	Activation Batch	Test Type (Table 10)	Strain	Batch No
	IVR	CHHF	10 25 85	ALHL	85	0403	03630	01	1 TA98	8507

Microorganism

Animal	R	(A) 64	(B) 65	(C) 66	(D) 67 70	(E) 71	(F) 72 73 74 75	(G) 76 78	80
Organ	L	2 Remarks Made? Yes 1	1 Phenocopy Check Conclusion (Table 13)	1 Sterility S 9 Mix 1 Not Contam 2 Contam 3 Not Checked	500 Activation Mixture Per Plate (µl)	1 Sample Sterility Check 1 Not Contam 2 Contam 3 Not Checked	Time (min) Temp (Cent) Pre-Incubation	KLC Technician	A Card Code
Inducer	A								
[redacted]	2% S9 Mix								

	Dose Level			Plate A		Plate B		Plate C		Plate D		Plate E		
(H) Solvent Positive (Table 11)	(I) Units of Concentration Blank = mg/ml 2 = µg/ml	(J) Stock concentration (µg/plate)	(K) Amt Per Plate (µl)	(L) Count	(M) BG	(N) Count	(O) BG	(P) Count	(Q) BG	(R) Count	(S) BG	(T) Count	(U) BG	Card Code
43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL 54			50	48		44		38						B
Pos. 04		0 01	50	135		120								C
04		0 025	50	216		235		237		196				D
04		0 05	50	468		322		346						E
04		10 10	50	918		684		728						F
04		0 50	50	2157		2312		2200						G
water blank			50	45		58		47						H
spiked water blank pH2 04		0 01	50	47		52		49						I
fraction only/ Bio/A.id 04		0 025	50	62		49		60						J

Remarks Indicate Item Code and Card Code

(continued)

Forms Completion
Initials

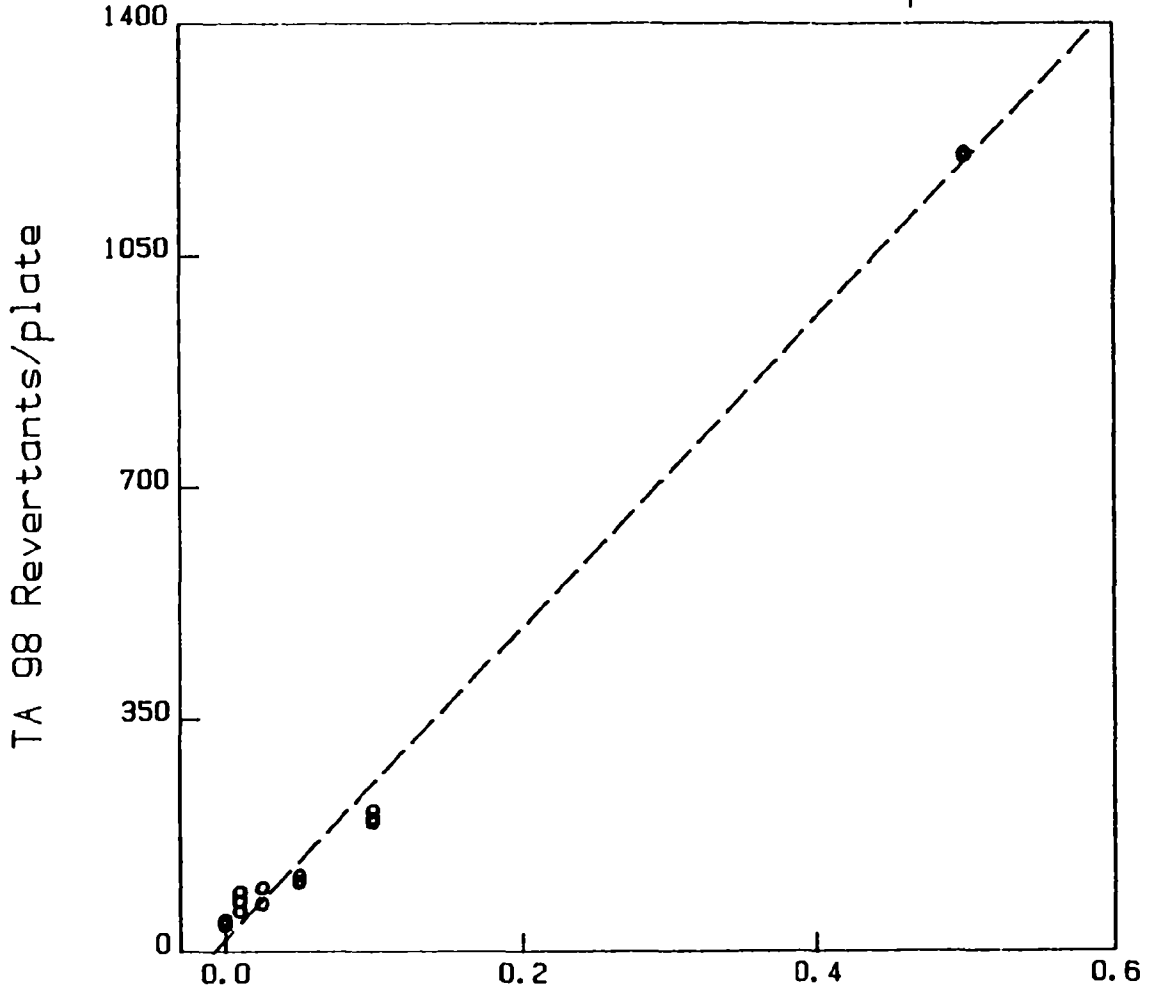
HERL IN VITRO RESULTS CONTINUATION FORM

1 Update Code	2 4 System ID	5 8 Research Lab ID	9 14 Experiment Date (MO DA YR)	15 18 Lab	19 20 Test Sample Identification YR	21 24 Number	25 30 Activation Batch	31 32 Test Type (Table 10)	33 38 Microorganism: Strain	39 42 Microorganism: Batch No
	IVR	CPVHF	10 25 85	AIHL	85	0403	03630 2% S9 Mix	01	TA98	0507

		Dose Level		Plate A		Plate B		Plate C		Plate D		Plate E		
(H) Solvent Positive (Table 11)	(I) Units of Concentration Blank = mg/ml 2 = µg/ml	(J) Stock Concentration (µg/plate)	(K) Amt Per Plate (µl)	(L) Count	(M) BG	(N) Count	(O) BG	(P) Count	(Q) BG	(R) Count	(S) BG	(T) Count	(U) BG	Card Code
43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
04		0050	050	533		443		541						K
04		0010	050	772		554		554						L
04		0050	050	445		556		556						M
04 Base/Acid	spiked lake blank / pH 11 freshw. only	0001	050	1446		1225		1558						N
04		0025	050	2133		2222		2218						O
04		0005	050	4112		3844		3700						P
04		0016	050	7936		8555		7713						Q
04		0050	050	1077		2024		1960						R
														S
														T
														U
														V
														W
														X
														Y

RECOVERY EFFICIENCY

2-Aminoanthracene Dose-Response

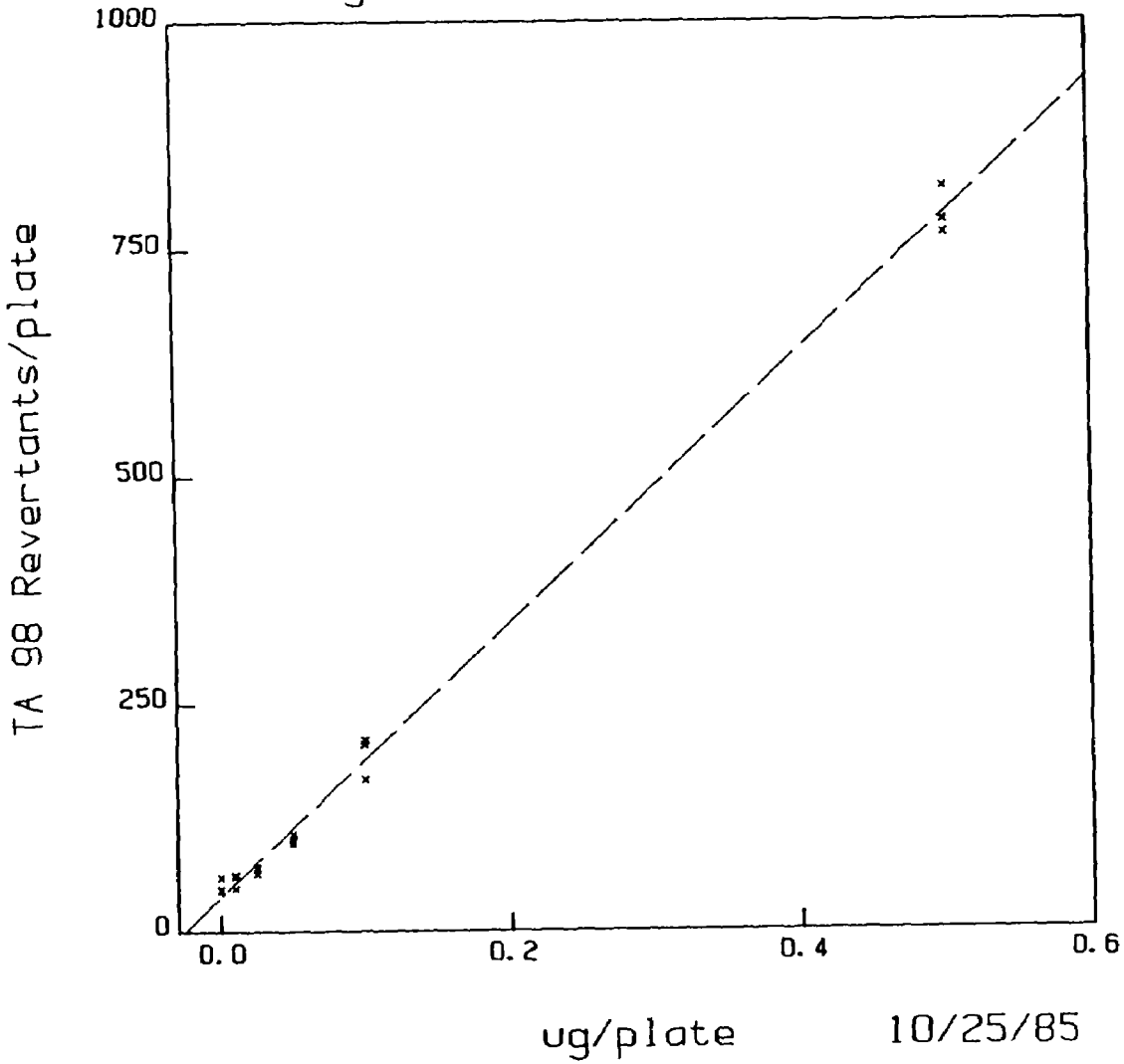


10% S9
SYMBOL=o
LINETYPE= — — — —
y=a+b*x
n=17
a=19.0050 s_a=9.7823
b=2342.3229 s_b=45.4060
$s_{y.x}$=33.5711 r=0.9972

X(I)	Y(I)
0.0000	41.0000
0.0000	47.0000
0.0000	49.0000
.0100	77.0000
.0100	61.0000
.0100	91.0000
.0250	97.0000
.0250	73.0000
.0500	107.0000
.0500	115.0000
.0500	105.0000
.1000	201.0000
.1000	213.0000
.1000	195.0000
.5000	1159.0000
.5000	1205.0000
.5000	1202.0000

RECOVERY EFFICIENCY

250 ug 2AA/1.5 l Blank. Base/Acid

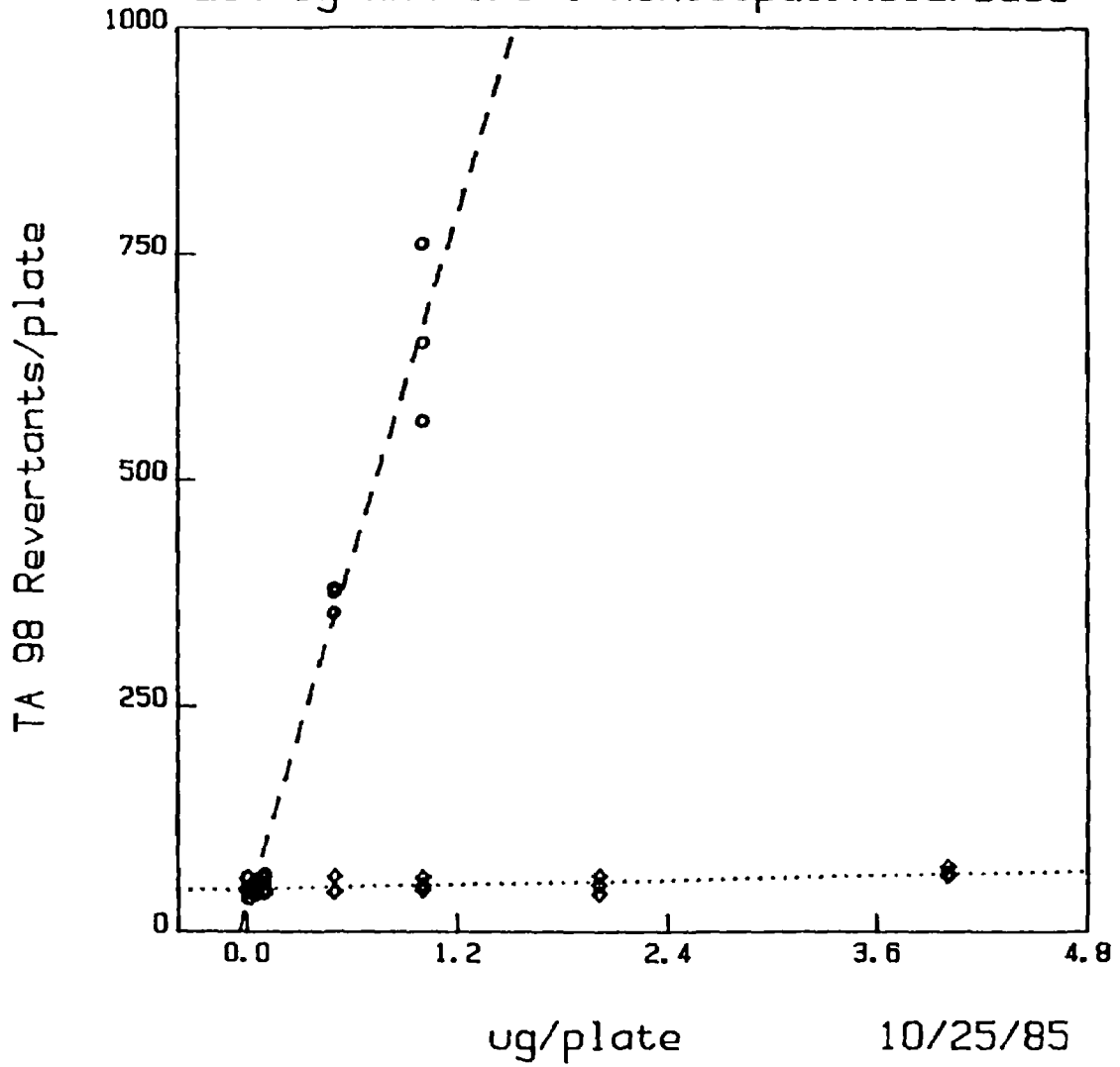


10% S9, pH11 fraction
SYMBOL= ×
LINETYPE= — — — —
y=a+b*x
n=18
a=39.3045 s_a=4.4543
b=1493.6831 s_b=21.2663
$s_{y.x}$=15.8439 r=0.9984

0 0000	47 0000
0 0000	46 0000
0 0000	50 0000
0100	48 0000
0100	61 0000
0100	67 0000
0250	64 0000
0250	71 0000
0250	79 0000
0500	107 0000
0500	101 0000
0500	97 0000
1000	205 0000
1000	210 0000
1000	167 0000
5000	779 0000
5000	755 0000
5000	816 0000

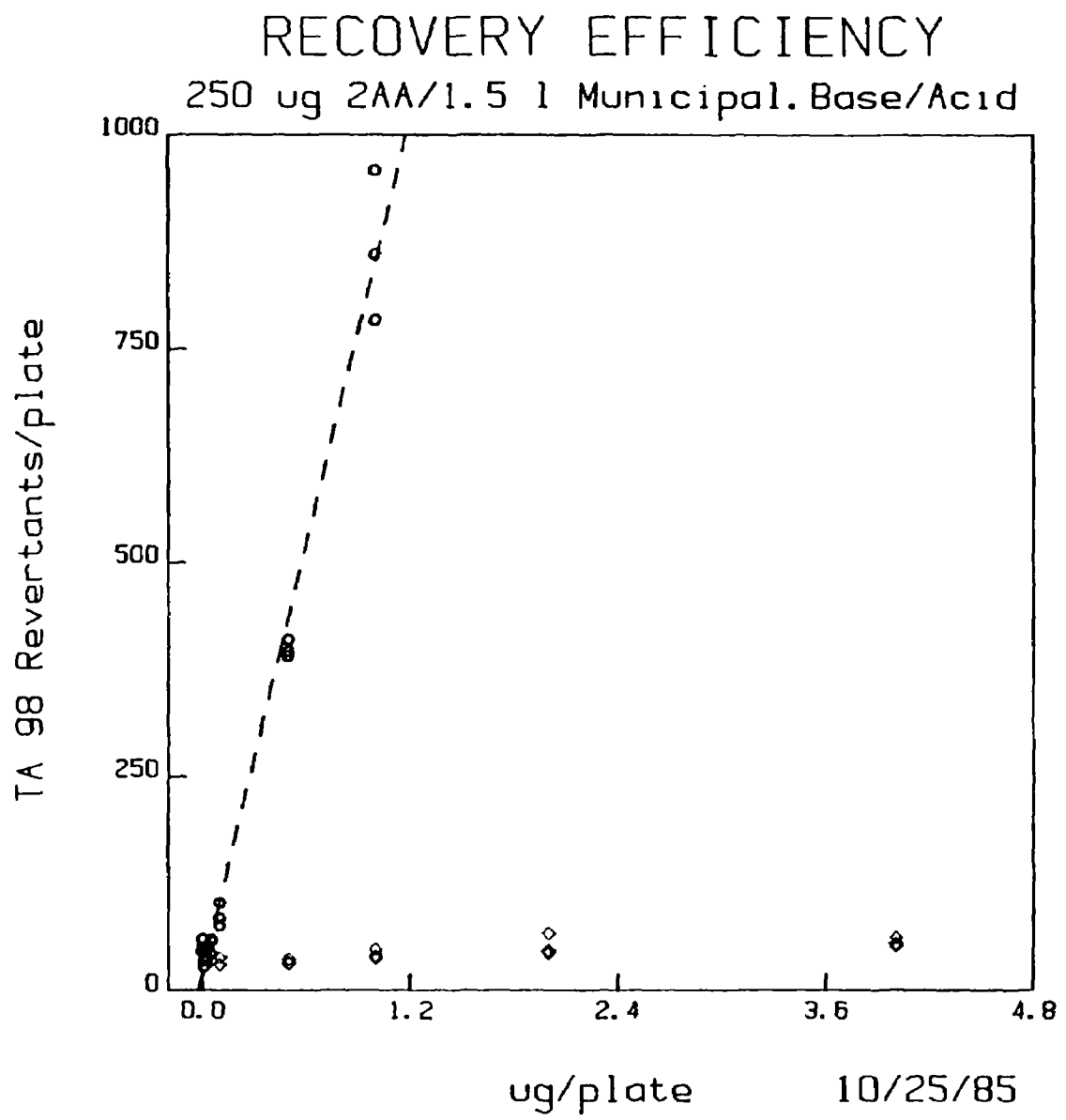
RECOVERY EFFICIENCY

250 ug 2AA/1.5 l Municipal. Acid/Base



10% S9, unspiked municipal
SYMBOL=◇
LINETYPE=·····
y=a+b*x
n=21
a=47.8672 s_a=1.9720
b=4.0431 s_b=1.1315
$s_{y.x}$=7.0396 r=0.6340

10% S9, spiked municipal
SYMBOL=o
LINETYPE=— — — —
y=a+b*x
n=21
a=28.7590 s_a=10.0454
b=636.8075 s_b=23.6470
$s_{y.x}$=37.9302 r=0.9872



10% S9. unspiked municipal
SYMBOL=◇
LINETYPE=
y=a+b*x
n=21
a=39.3700 s_a=2.4675
b=4.4109 s_b=1.4158
$s_{y.x}$=8.8084 r=0.5815

10% S9. spiked municipal
SYMBOL=o
LINETYPE= — — — —
y=a+b*x
n=21
a=19.7495 s_a=9.5959
b=828.1426 s_b=22.5888
$s_{y.x}$=36.2328 r=0.9930

HERL IN VITRO RESULTS FORM

1	2-4	5-8	9-14	15-18	19-20	21-24	25-30	31-32	33-38	39-42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB Test Sample Identification	YR	NUMBER	Activation Batch	Test Type (Table 10)	Strain Microorganism	Batch No
	IVR	CFHF	10 25 85	AZHL	85	0403	0365C	01	TA98	8507

Animal	R
Organ	L
Inducer	A
[redacted]	10% S9 Mix

A 64	B 65	C 66	D 67-70	E 71	F 72-75	G 76-78	80
Remarks Made? Yes 1	Phenocopy Check Conclusion (Table 13)	Sterility S 9 Mix 1 Not Contam 2 Contam 3 Not Checked	Activation Mixture Per Plate (µl)	Sample Sterility Check 1 Not Contam 2 Contam 3 Not Checked	Time (min) Temp (Cent) Pre-Incubation	Technician	Card Code
2	1	1	500	1		KIC	A

	Dose Level				Plate A		Plate B		Plate C		Plate D		Plate E		
H Solvent Positive (Table 11)	I Units of Concentration Blank = mg/ml 2 = µg/ml	J Stock concentration (µl/plate)	K Amt Per Plate (µl)	L Count	M BG	N Count	O BG	P Count	Q BG	R Count	S BG	T Count	U BG	Card Code
43-44	45	46-50	51-54	55-58	59	60-63	64	65-68	69	70-73	74	75-78	79	80
SOL 54			50	41		47		49						B
Pos 04		001	50	77		61		91						C
04		025	50	97		73								D
04		005	50	107		115		105						E
04		010	50	201		213		195						F
04		050	50	1197		1205		1202						G
water blank			50	47		46		60						H
spiked water blank, pH2 04		001	50	61		43		40						I
fraction only, Biol/Acid 04		025	50	59		45		69						J

Remarks Indicate Item Code and Card Code

(continued)

Forms Completion
Initials

HERL IN VITRO RESULTS CONTINUATION FORM

1 Update Code	2 4 System ID	5 8 Research Lab ID	9 14 Experiment Date (MO DA YR)	15 18 Lab	19 20 Test Sample Identification YR	21 24 Number	25 30 Activation Batch	31 32 Test Type (Table 10)	33 38 Microorganism: Strain	39 42 Batch No
	IVR	CPHE	10 25 85	AZHL	85	0401	02, S9 Mix	01	TA98	8507

		Dose Level		Plate A		Plate B		Plate C		Plate D		Plate E		
H Solvent Positive (Table 11)	I Units of Concentration Blank = mg/ml 2 = µg/ml	J Stock Concentration (µg/plate)	K Amt Per Plate (µl)	L Count	M BG	N Count	O BG	P Count	Q BG	R Count	S BG	T Count	U BG	Card Code
43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
04		005	50	49		47		50						K
04		010	50	51		60		43						L
04		050	50	58		50		60						M
04	spiked water blank = 2	001	50	48		61		63						N
Bis/Acid														
04		005	50	64		71		70						O
04		005	50	107		101		97						P
04		010	50	205		210		167						Q
04		050	50	779		765		816						R
	unspiked water sample = 1	005	50	57		52		42						S
Acid/Bis														
		010	50	46		46		42						T
		050	50	44		44		61						U
		100	50	51		46		60						V
		200	50	42		62		52						W
		400	50	72		62		64						X
	unspiked water sample = 1	005	50	34		43		45						Y
Bis/Acid														

(continued)

HERL IN VITRO RESULTS CONTINUATION FORM

1 Update Code	2 4 System ID	5 8 Research Lab ID	9 14 MO DA YR Experiment Date	15 18 Lab	19 20 YR	21 24 Number Test Sample Identification	25 30 Activation Batch	31 32 Test Type (Table 10)	33 38 Strain	39 42 Batch No Microorganism
	IVR	CVAF	10 25	AIVAL	85	0403	036300	01	TA1538	8507

		Dose Level		Plate A		Plate B		Plate C		Plate D		Plate E		
H Solvent Positive (Table 11)	I Units of Concentration Blank = mg/ml 2 = µg/ml	J Stock Concentration (mg/plate)	K Amt Per Plate (µl)	L Count	M BG	N Count	O BG	P Count	Q BG	R Count	S BG	T Count	U BG	Card Code
43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
		0100	50	38		30		30						K
		0500	50	32		37		33						L
		1000	50	48		38		40						M
		2000	50	67		47		44						N
		4000	50	55		62		52						O
04 Acid/Base	spiked mammalian Sample #1	0001	50	47		36		47						P
04		0025	50	44		48		42						Q
04		0050	50	47		55		40						R
04		0100	50	54		64		60						S
04		0500	50	352		375		377						T
04		1000	50	761		564		651						U
04 Base/Acid	spiked mammalian Sample #1	0001	50	28		34		28						V
04		0025	50	46		51		36						W
04		0050	50	58		60		43						X
04		0100	50	76		65		103						Y

(continued)

HERL IN VITRO RESULTS CONTINUATION FORM

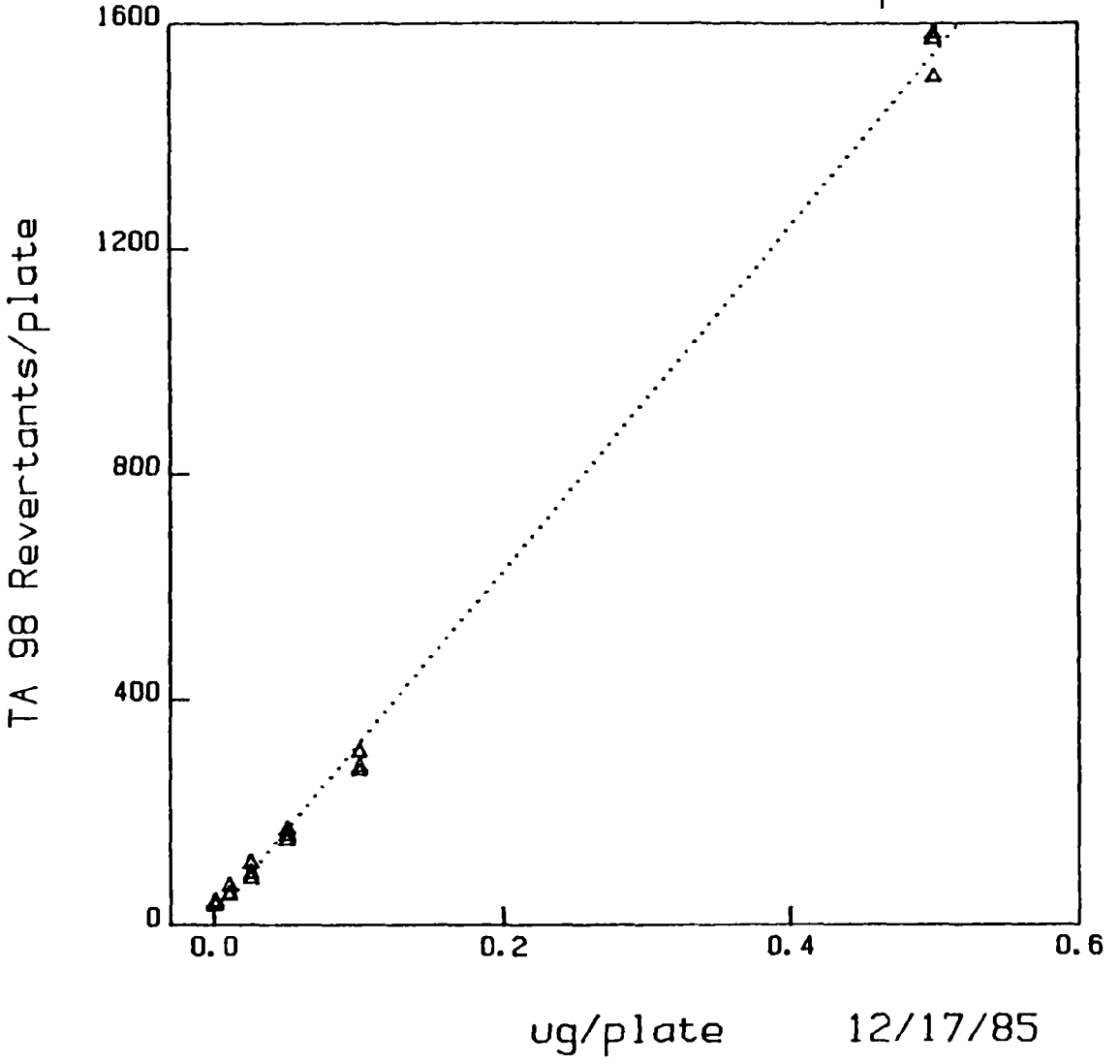
Update Code (1)	System ID (2-4)	Research Lab ID (5-8)	Experiment Date MO DA YR (9-14)	Test Sample Identification Lab (15-18)	YR (19-20)	Number (21-24)	Activation Batch (25-30)	Test Type (Table 10) (31-32)	Microorganism Strain (33-38)	Batch No (39-42)
	IVR	CPHF	10 25 85	AIHL	85	0403	03630	01	TA98	8507

Activation Batch: 10% S9 Mix

Solvent Positive (Table 11) (43-44)	Units of Concentration Blank = mg/ml 2 = µg/ml (45)	Dose Level: Stock Concentration (mg/plate) (46-50)	Dose Level: Amt Per Plate (µl) (51-54)	Plate A Count (55-58)	Plate A BG (59)	Plate B Count (60-63)	Plate B BG (64)	Plate C Count (65-68)	Plate C BG (69)	Plate D Count (70-73)	Plate D BG (74)	Plate E Count (75-78)	Plate E BG (79)	Card Code (80)
04		050	50	410		391		396						K
04		100	50	958		860		783						L
														M
														N
														O
														P
														Q
														R
														S
														T
														U
														V
														W
														X
														Y

RECOVERY EFFICIENCY

2-Aminoanthracene Dose-Response

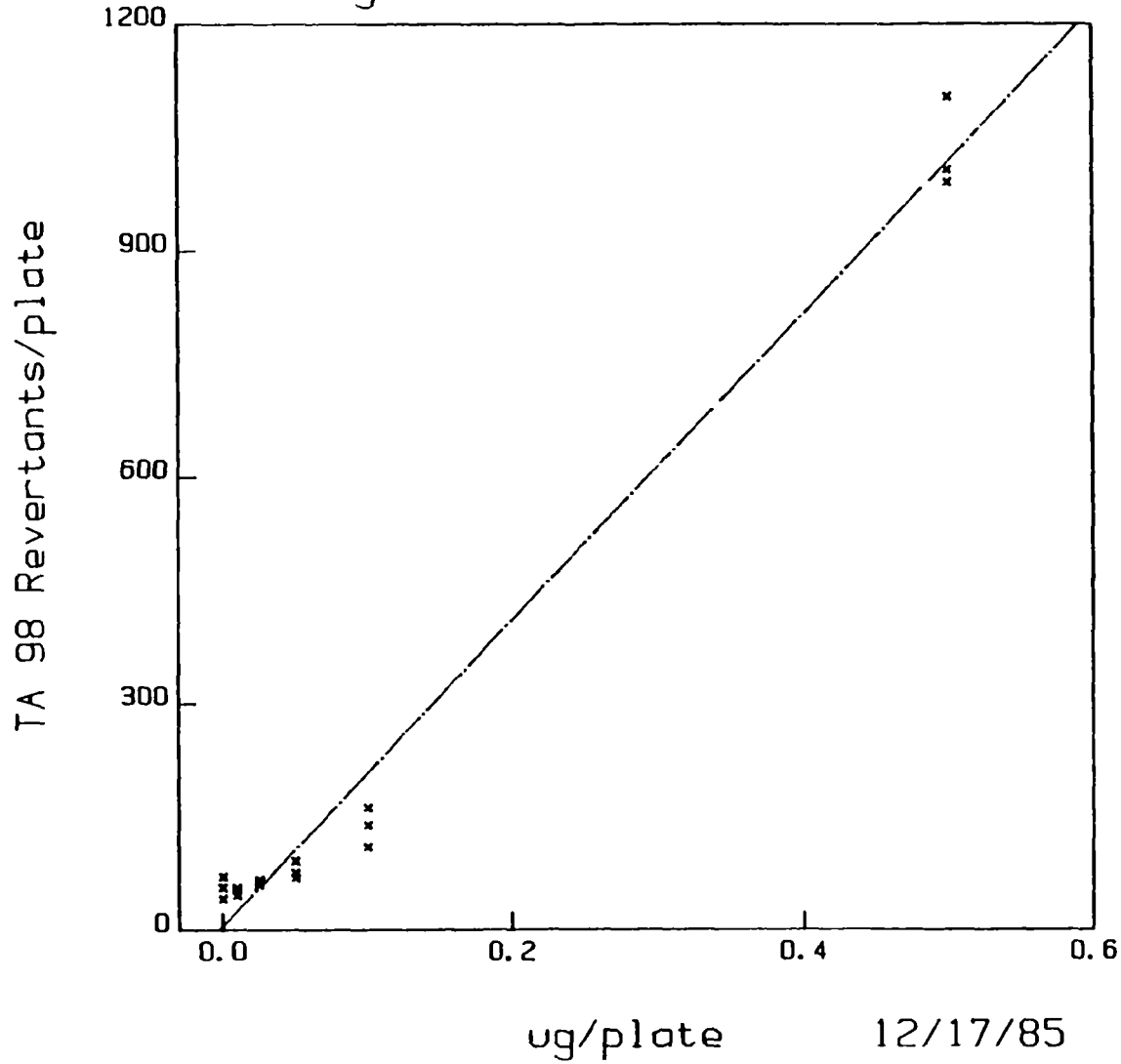


2% S9
SYMBOL=Δ
LINETYPE=.....
y=a+b*x
n=18
a=16.4959 s_a=7.3820
b=3058.4304 s_b=35.2440
$s_{y.x}$=26.2577 r=0.9989

X(I)	Y(I)
0 0000	42.0000
0 0000	38 0000
0.0000	36 0000
0100	55 0000
0100	54.0000
0100	70 0000
0250	83 0000
0250	110 0000
0250	92.0000
0500	160.0000
0500	170.0000
0500	151 0000
1000	280.0000
1000	274.0000
1000	307.0000
5000	1573 0000
5000	1582.0000
5000	1505 0000

RECOVERY EFFICIENCY

100 ug 2AA/1.5 l Blank, Base/Acid

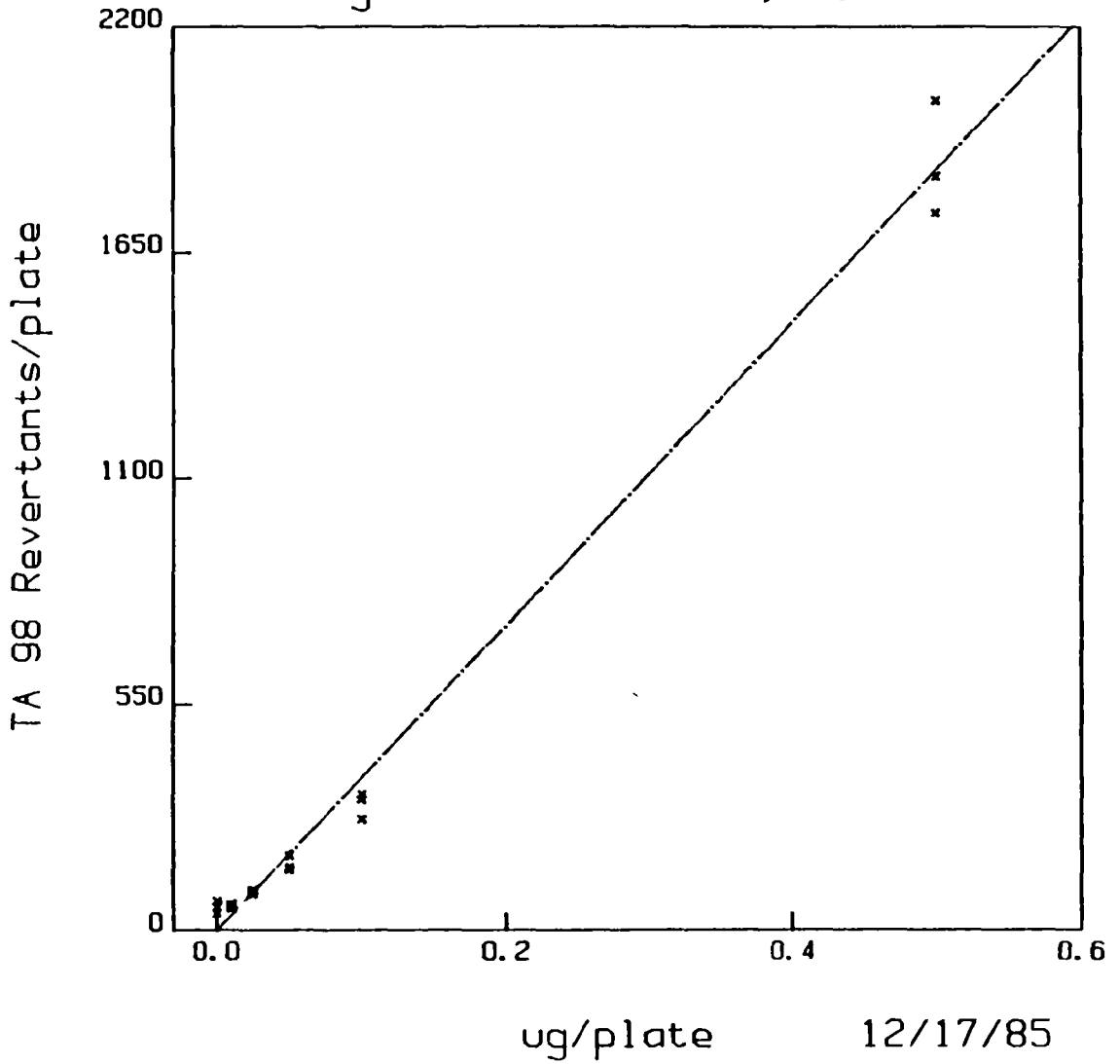


2% S9
SYMBOL=×
LINETYPE=——·——·——
y=a+b*x
n=18
a=6.0251 s_a=13.6144
b=2021.6777 s_b=64.9995
$s_{y.x}$=48.4263 r=0.9918

0 0000	56 0000
0 0000	71 0000
0 0000	41 0000
0100	57 0000
0100	56 0000
0100	46 0000
0250	87 0000
0250	80 0000
0250	83 0000
0500	70 0000
0500	76 0000
0500	92 0000
1000	133 0000
1000	110 0000
1000	152 0000
5000	990 0000
5000	1103 0000
5000	1006 0000

RECOVERY EFFICIENCY

500 ug 2AA/1.5 l Blank, Base/Acid



2% S9
SYMBOL= ×
LINETYPE= ——·——·——
y=a+b*x
n=18
a=0.9197 s_a=18.4091
b=3700.9466 s_b=87.8910
$s_{y.x}$=65.4810 r=0.9955

X: I\	Y: I\
0 0000	56 0000
0 0000	71 0000
0 0000	41 0000
0100	63 0000
0100	53 0000
0100	61 0000
0250	93 0000
0250	89 0000
0250	94 0000
0500	181 0000
0500	153 0000
0500	147 0000
1000	330 0000
1000	317 0000
1000	269 0000
5000	2030 0000
5000	1237 0000
5000	1745 0000

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER	Activation Batch	Test Type (Table 10)	Strain	Batch No
	IVR	CPHF	12 17 85	AZHL	85	0403	04605	01	17498	8507
				Test Sample Identification					Microorganism	

Animal	R
Organ	L
Inducer	A
[redacted]	2% S9 Mix

A 64	B 65	C 66	D 67 70	E 71	F 72 73 74 75	G 76 78	80
Remarks Made? Yes 1	Phenocopy Check Conclusion (Table 13)	Sterility S 9 Mix 1 Not Contam 2 Contam 3 Not Checked	Activation Mixture Per Plate (µl)	Sample Sterility Check 1 Not Contam 2 Contam 3 Not Checked	Time (min) Temp (Cent) Pre-Incubation	Technician	Card Code
2	1	1	500	1		KLC	A

H Solvent Positive (Table 11)	I Units of Concentration Blank = mg/ml 2 = µg/ml	J Stock concentration (µg/plate)	K Amt Per Plate (µl)	Plate A L Count	Plate A M BG	Plate B N Count	Plate B O BG	Plate C P Count	Plate C Q BG	Plate D R Count	Plate D S BG	Plate E T Count	Plate E U BG	Card Code
	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL 54			50	42		38		36						B
Pos 04		001	50	55		54		79						C
04		003	50	83		110		92						D
04		005	50	160		170		151						E
04		010	50	280		274		307						F
04		050	50	1573		1582		1505						G
water blank			50	56		71		41						H
spiked water blank. 04		001	50	63		52		61						I
5000µg/ml blank water 04		003	50	98		87		94						J

Remarks Indicate Item Code and Card Code

(continued)

Forms Completion Initials

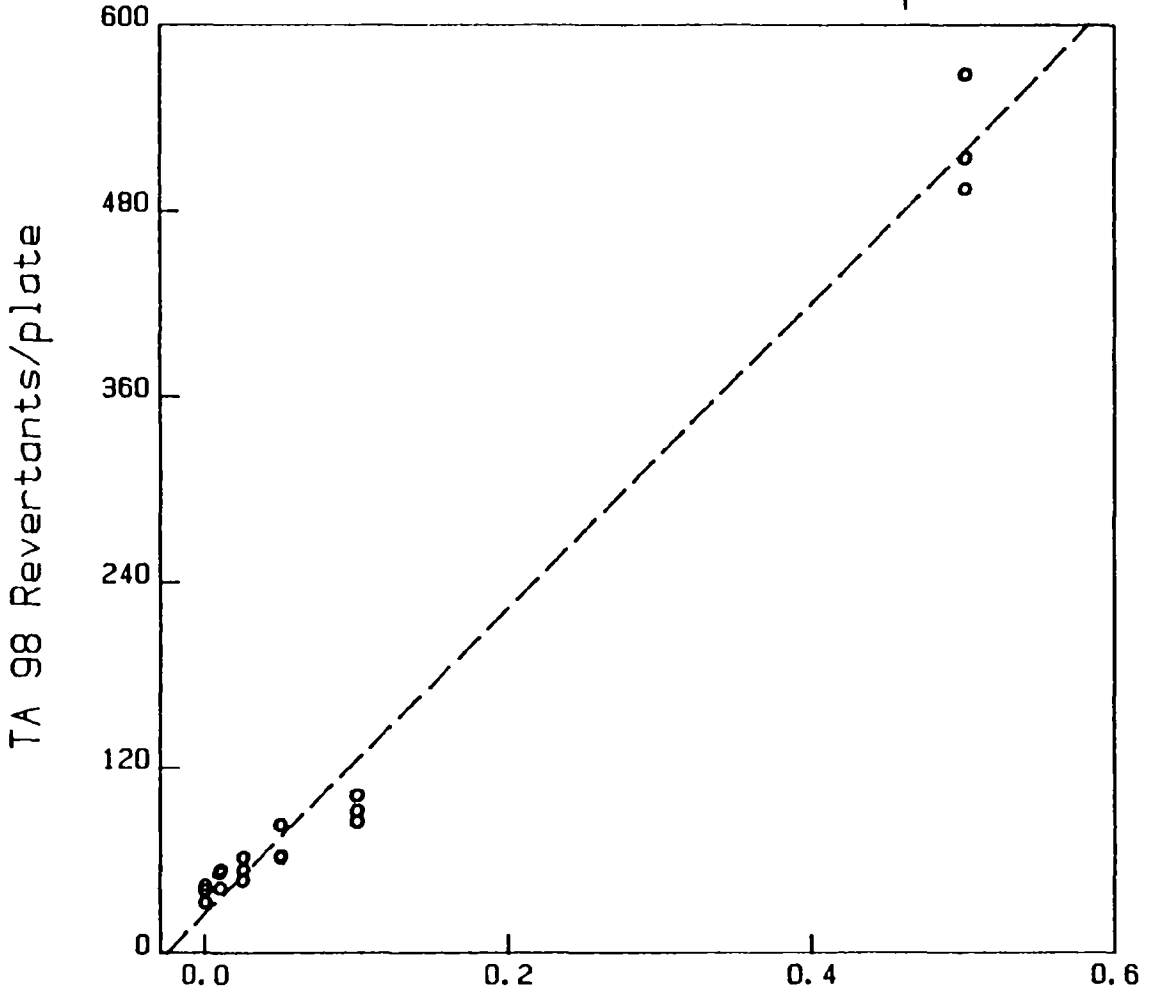
HERL IN VITRO RESULTS CONTINUATION FORM

1 Update Code	2 4 System ID	5 8 Research Lab ID	9 14 Experiment Date (MO DA YR)	15 18 Lab	19 20 Test Sample Identification (YR)	21 24 Number	25 30 Activation Batch	31 32 Test Type (Table 10)	33 38 Microorganism (Strain)	39 42 Batch No
	IVR	CPHF	121785	AZVL	85	0403	04605 2% S9 Mix	01	TA98	8507

		Dose Level		Plate A		Plate B		Plate C		Plate D		Plate E		
H Solvent Positive (Table 11)	I Units of Concentration Blank = mg/ml 2 = µg/ml	J Stock Concentration (µg/plate)	K Amt. Per Plate (µl)	L Count	M BG	N Count	O BG	P Count	Q BG	R Count	S BG	T Count	U BG	Card Code
43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
04		00050	0050	1810		1530		1470						K
04		00010	0050	3300		3170		2690						L
04		00050	0050	2020		1835		1745						M
04	spiked water blank	00001	0050	0053		0056		0046						N
Boric Acid	2 µg/ml 1.5 l blank	Test												
04		00005	0050	0067		0060		0065						O
04		00005	0050	0070		0076		0092						P
04		00010	0050	0139		0110		0162						Q
04		00050	0050	0990		1103		1006						R
														S
														T
														U
														V
														W
														X
														Y

RECOVERY EFFICIENCY

2-Aminoanthracene Dose-Response

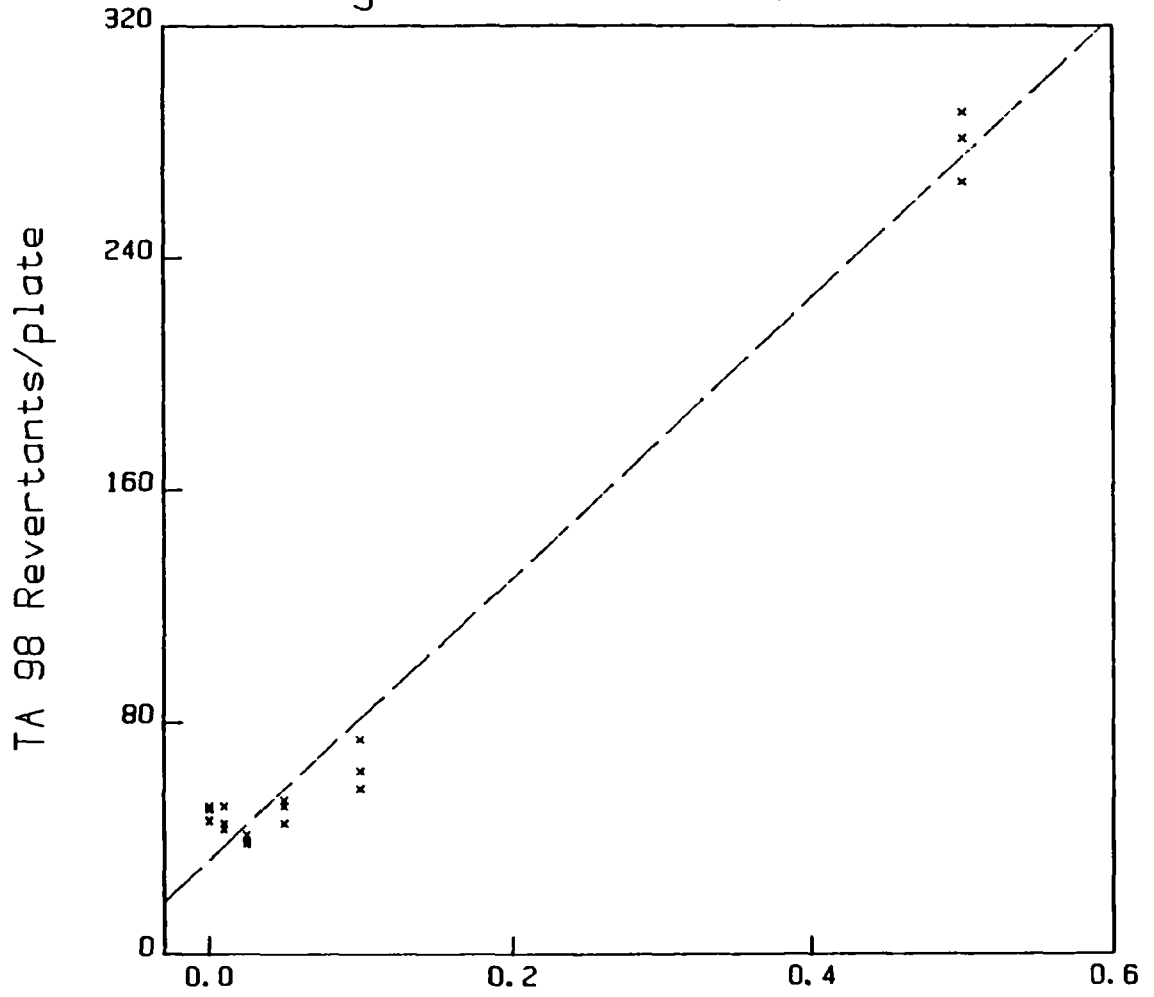


10% S9
SYMBOL=o
LINETYPE= — — — —
y=a+b*x
n=18
a=25.8638 s_a=6.2701
b=986.1082 s_b=29.9354
$s_{y.x}$=22.3026 r=0.9927

X(I)	Y(I)
0 0000	41 0000
0 0000	33 0000
0 0000	44 0000
0100	42.0000
0100	54.0000
0100	52.0000
0250	54 0000
0250	47 0000
0250	62.0000
0500	83 0000
0500	63 0000
0500	62 0000
1000	85 0000
1000	102 0000
1000	92.0000
5000	569.0000
5000	494 0000
5000	514 0000

RECOVERY EFFICIENCY

100 ug 2AA/1.5 l Blank, Base/Acid

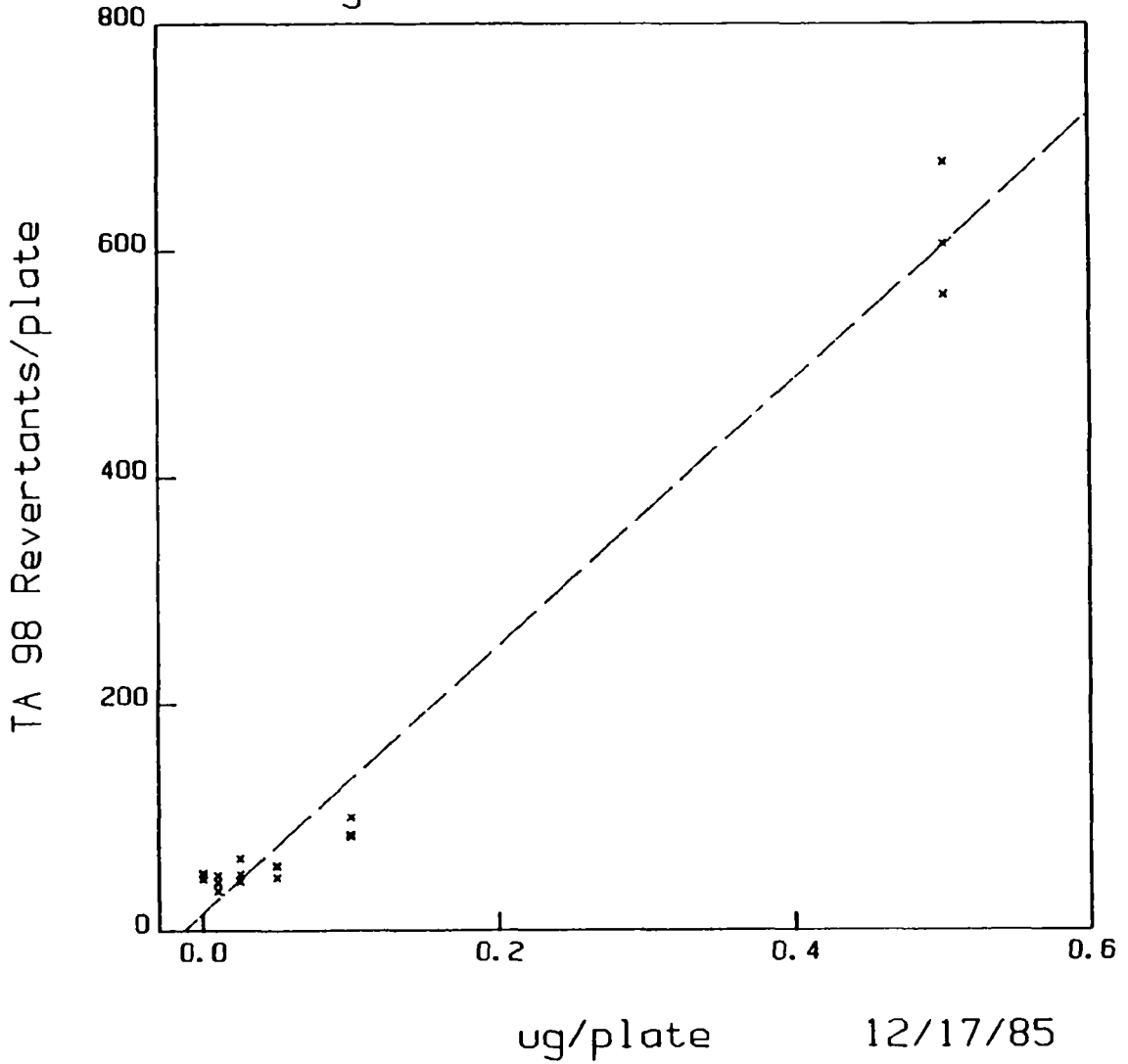


10% S9
SYMBOL=×
LINETYPE= —— —— ——
y=a+b*x
n=18
a=32.7022 s_a=3.6330
b=484.3599 s_b=17.3450
$s_{y.x}$=12.9225 r=0.9899

X: I:	Y: I:
0.0000	51.0000
0.0000	50.0000
0.0000	46.0000
.0100	51.0000
.0100	43.0000
.0100	45.0000
.0250	38.0000
.0250	39.0000
.0250	41.0000
.0500	53.0000
.0500	51.0000
.0500	45.0000
.1000	63.0000
.1000	57.0000
.1000	74.0000
.5000	266.0000
.5000	290.0000
.5000	281.0000

RECOVERY EFFICIENCY

500 ug 2AA/1.5 l Blank, Base/Acid



10% S9
SYMBOL= ×
LINETYPE= —— —— ——
y=a+b*x
n=18
a=16.0322 s_a=9.7071
b=1175.3872 s_b=46.3447
$s_{y.x}$=34.5279 r=0.9878

X	Y
0.0000	51.0000
0.0000	50.0000
0.0000	46.0000
.0100	49.0000
.0100	43.0000
.0100	35.0000
.0250	44.0000
.0250	64.0000
.0250	50.0000
.0500	57.0000
.0500	47.0000
.0500	58.0000
.1000	100.0000
.1000	85.0000
.1000	83.0000
.5000	605.0000
.5000	560.0000
.5000	677.0000

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER Test Sample Identification	Activation Batch	Test Type (Table 10)	Strain	Batch No
	IVR	CHHF	12 17 85	AIHL	85	0403	04605	01	1 TA78	8507

Microorganism

Animal	K
Organ	L
Inducer	A
[redacted]	10% S9 Mix

(A) 64 Remarks Made? Yes 1: 2

(B) 65 Phenocopy Check Conclusion (Table 13): 1

(C) 66 Sterility S 9 Mix: 1 (1 Not Contam, 2 Contam, 3 Not Checked)

(D) 67 70 Activation Mixture Per Plate (µl): 500

(E) 71 Sample Sterility Check: 1 (1 Not Contam, 2 Contam, 3 Not Checked)

(F) 72 73 74 75 Time (min) Temp (Cent), Pre-Incubation

(G) 76 78 Technician: KIC

80 Card Code: A

(H) Solvent Positive (Table 11)	43 44	Dose Level: (I) Units of Concentration Blank = mg/ml 2 = µg/ml	45	(J) Stock concentration (µg/plate)	46 50	(K) Amt. Per Plate (µl)	51 54	Plate A: (L) Count	55 58	(M) BG	59	Plate B: (N) Count	60 63	(O) BG	64	Plate C: (P) Count	65 68	(Q) BG	69	Plate D: (R) Count	70 73	(S) BG	74	Plate E: (T) Count	75 78	(U) BG	79	Card Code	80
SOL	54					50		41				33				44												B
Pos	04			001		50		42				54				52												C
	04			0025		50		54				47				62												D
	04			005		50		83				63				62												E
	04			010		50		85				102				92												F
	04			050		50		568				494				514												G
water blank						50		51				50				46												H
spiked water blank,	04			001		50		49				43				35												I
500 µg/plate blank water	04			0025		50		44				64				50												J

Remarks Indicate Item Code and Card Code

(continued)

Forms Completion Initials

HERL IN VITRO RESULTS CONTINUATION FORM

1 Code	Update	2 4 System ID	5 8 Research Lab ID	9 14 Experiment Date (MO DA YR)	15 18 Lab	19 20 Test Sample Identification YR	21 24 Number	25 30 Activation Batch	31 32 Test Type (Table 10)	33 38 Microorganism: Strain	39 42 Batch No
		IVR	CRVAF	12/17/85	ALVAL	85	0403	10% S9 Mix	01	TA98	8507

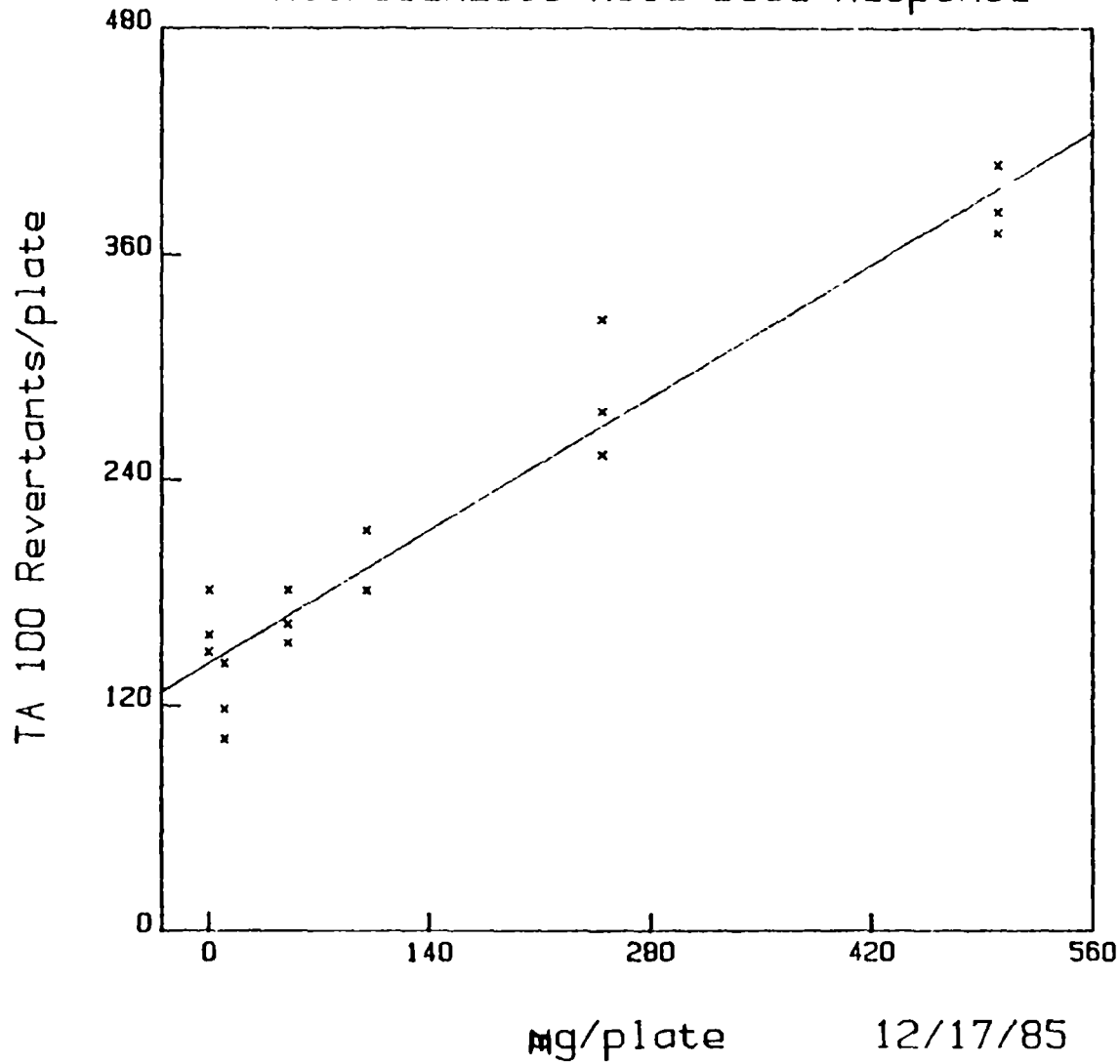
		Dose Level		Plate A		Plate B		Plate C		Plate D		Plate E		
Ⓗ Solvent Positive (Table 11)	Ⓘ Units of Concentration Blank = mg/ml 2 = µg/ml	Ⓙ Stock Concentration (MG/PLATE)	Ⓚ Amt Per Plate (µl)	Ⓛ Count	Ⓜ BG	Ⓝ Count	Ⓞ BG	Ⓟ Count	Ⓠ BG	Ⓡ Count	Ⓢ BG	Ⓣ Count	Ⓤ BG	Card Code
43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
0H		005	50	57		47		58						K
0H		010	50	101		85		83						L
0H		050	50	605		560		677						M
0H	spiking water blank	001	50	51		43		45						N
Base/Acid	1.5 ml Blank water													
0H		003	50	38		39		41						O
0H		005	50	53		51		45						P
0H		010	50	63		57		74						Q
0H		050	50	266		270		281						R
														S
														T
														U
														V
														W
														X
														Y

WORKSHEET FOR THE SPRM 4NBA RECOVERY STUDY IN THE AMES ASSAY IN STRAIN TA100

Extraction Method	Spiked Dose (ug/1.5 L water)	-S9 % Recovery
Acid/Base	5000 in blank water	$0.4012/0.5039 = 80\%$
Base/Acid	5000 in blank water	$0.3008/0.5039 = 60\%$
Base/Acid	2500 in blank water	$< \frac{141/500}{0.5039} = 56\%$
Base/Acid	5000 in the Municipal Wastewater Sample	$< \frac{141/500}{0.5039} = 56\%$
Acid/Base	5000 in the Municipal Wastewater Sample	$< \frac{141/500}{0.5039} = 56\%$

RECOVERY EFFICIENCY

4-Nitrobenzoic Acid Dose-Response

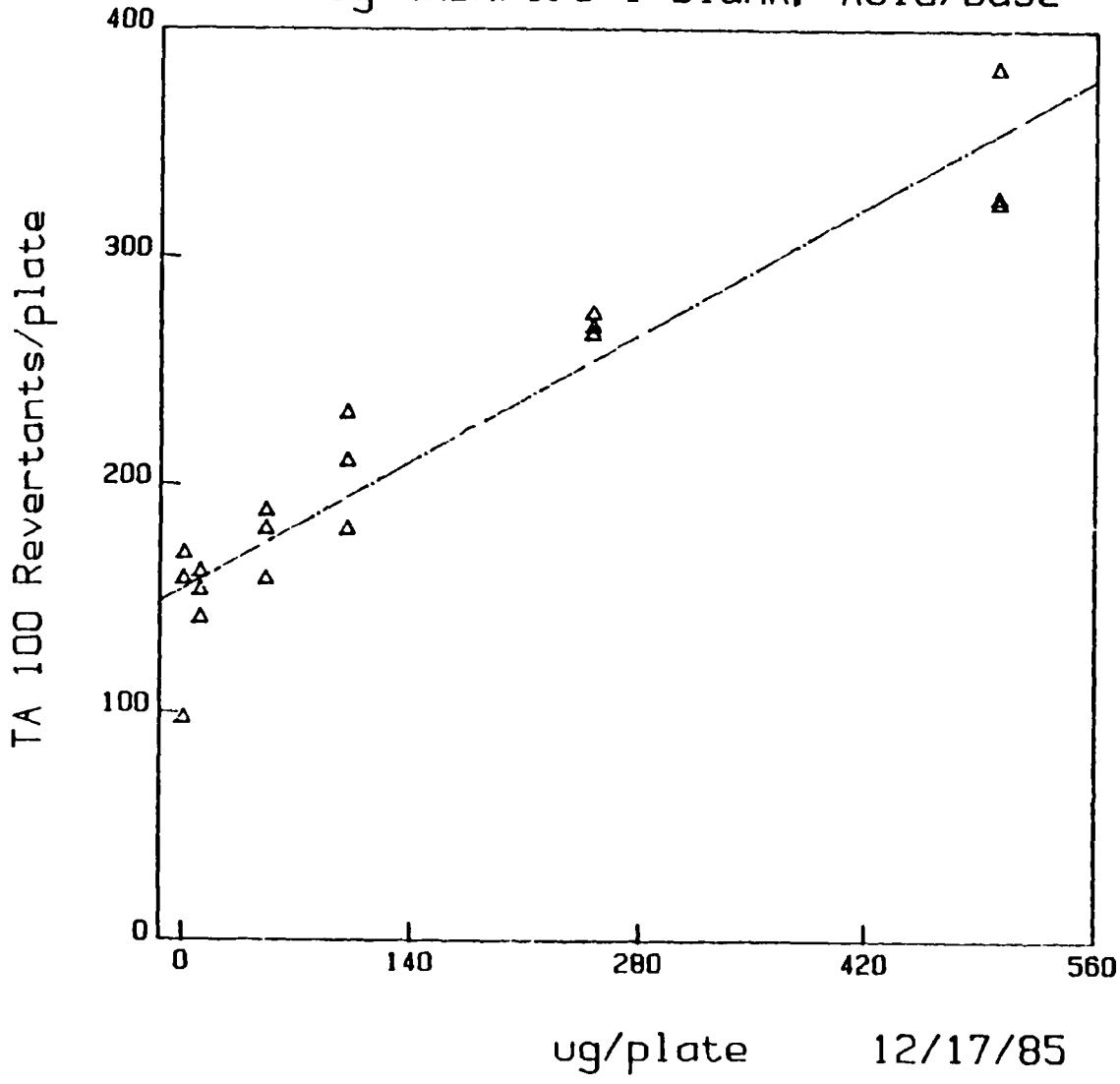


-S9
SYMBOL=×
LINETYPE= – – – – – – –
y=a+b*x
n=18
a=142.1309 s_a=7.8270
b=0.5039 s_b=0.0336
$s_{y.x}$=25.1906 r=0.9662

X(I)	Y(I)
0.0000	148.0000
0.0000	157.0000
0.0000	181.0000
10.0000	142.0000
10.0000	102.0000
10.0000	118.0000
50.0000	163.0000
50.0000	181.0000
50.0000	153.0000
100.0000	213.0000
100.0000	181.0000
100.0000	181.0000
250.0000	253.0000
250.0000	276.0000
250.0000	325.0000
500.0000	371.0000
500.0000	407.0000
500.0000	382.0000

RECOVERY EFFICIENCY

5000 ug 4NBA/1.5 l Blank. Acid/Base

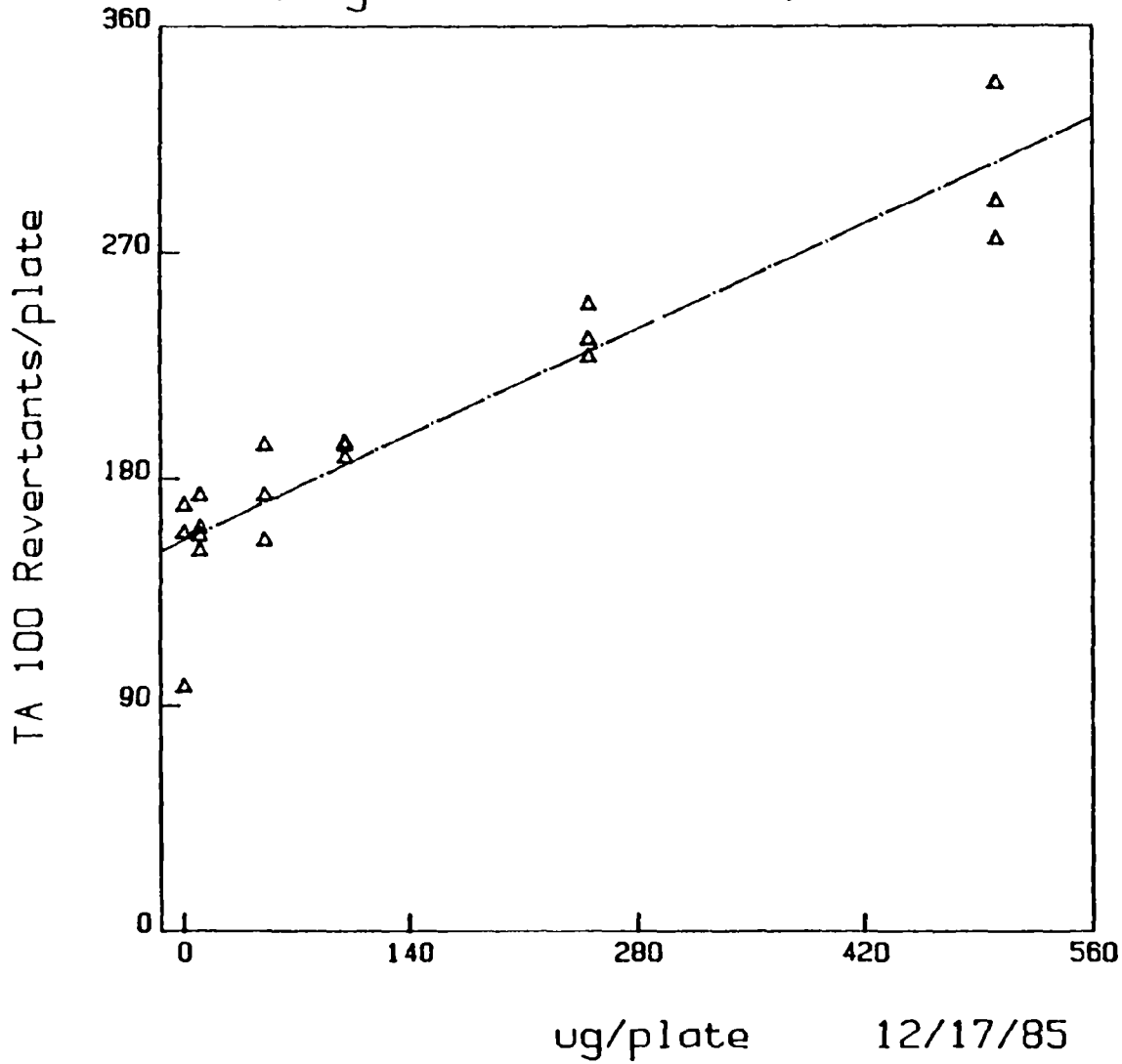


-S9
SYMBOL=Δ
LINETYPE= — · — · —
y=a+b*x
n=18
a=154.1017 s_a=7.5530
b=0.4012 s_b=0.0324
$s_{y.x}$=24.3090 r=0.9514

X(I)	Y(I)
0 0000	169 0000
0 0000	158 0000
0 0000	97 0000
10 0000	141 0000
10 0000	153 0000
10 0000	161.0000
50.0000	153 0000
50 0000	180 0000
50 0000	188 0000
100 0000	180 0000
100 0000	210.0000
100 0000	231.0000
250 0000	269 0000
250 0000	266 0000
250 0000	275.0000
500.0000	383 0000
500 0000	326.0000
500 0000	324 0000

RECOVERY EFFICIENCY

5000 ug 4NBA/1.5 l Blank, Acid/Base



-S9
SYMBOL=△
LINETYPE=——·——·——
y=a+b*x
n=19
a=155.3002 s_a=6.1638
b=0.3008 s_b=0.0272
$s_{y.x}$=20.7226 r=0.9370

X(I)	Y(I)
0.0000	169.0000
0.0000	158.0000
0.0000	97.0000
10.0000	151.0000
10.0000	173.0000
10.0000	157.0000
10.0000	160.0000
50.0000	155.0000
50.0000	173.0000
50.0000	193.0000
100.0000	188.0000
100.0000	193.0000
100.0000	194.0000
250.0000	235.0000
250.0000	249.0000
250.0000	228.0000
500.0000	337.0000
500.0000	275.0000
500.0000	290.0000

HERL IN VITRO RESULTS FORM

1	2 4	5 8	9 14	15 18	19 20	21 24	25 30	31 32	33 38	39 42
Update Code	System ID	Research Lab ID	MO DA YR Experiment Date	LAB	YR	NUMBER Test Sample Identification	Activation Batch	Test Type (Table 10)	Strain	Batch No
	IVR	CPHF	12 17 85	AIHL	85	0403		01	TA100	851 1

Microorganism

Animal
Organ
Inducer
[redacted] -S9

(A) 64 Remarks Made? Yes 1: 2

(B) 65 Phenocopy Check Conclusion (Table 13): 1

(C) 66 Sterility S 9 Mix: 1 Not Contam 2 Contam 3 Not Checked:

(D) 67 70 Activation Mixture Per Plate (µl):

(E) 71 Sample Sterility Check: 1 Not Contam 2 Contam 3 Not Checked: 1

(F) 72 73 74 75 Pre-Incubation: Time (min) | Temp (Cent)

(G) 76 78 Technician: KIC

80 Card Code: A

	Dose Level				Plate A		Plate B		Plate C		Plate D		Plate E		
(H) Solvent Positive (Table 11)	(I) Units of Concentration Blank = mg/ml 2 = µg/ml	(J) Stock concentration (µg/plate)	(K) Amt Per Plate (µl)	(L) Count	(M) BG	(N) Count	(O) BG	(P) Count	(Q) BG	(R) Count	(S) BG	(T) Count	(U) BG	Card Code
43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
SOL 54			50	1148		1157		1181						B
Pos 11		1000	50	1142		1102		1118						C
11		5000	50	1163		1181		1153						D
11		10000	50	2133		1811		1811						E
11		25000	50	2533		2766		3255						F
11		50000	50	3711		4007		3822						G
Water Blank			50	1697		1581		1977						H
spiked water blank 5ug/pl 5 black water Acid / Base 11		1000	50	1411		1531		1611						I
11		5000	50	1588		1810		1885						J

Remarks Indicate Item Code and Card Code

(continued)

Forms Completion
Initials

HERL IN VITRO RESULTS CONTINUATION FORM

1 Update Code	2 4 System ID	5 8 Research Lab ID	9 14 Experiment Date (MO DA YR)	15 18 Lab	19 20 Test Sample Identification YR	21 24 Number	25 30 Activation Batch	31 32 Test Type (Table 10)	33 38 Microorganism Strain	39 42 Microorganism Batch No
	IVR	CPHF	12/78	AIHL	83	0403		01	TA100	8311

		Dose Level		Plate A		Plate B		Plate C		Plate D		Plate E		
Ⓗ Solvent Positive (Table 11)	Ⓘ Units of Concentration Blank = mg/ml 2 = µg/ml	Ⓙ Stock Concentration (µg/plate)	Ⓚ Amt Per Plate (µl)	Ⓛ Count	Ⓜ BG	Ⓝ Count	Ⓞ BG	Ⓟ Count	Ⓠ BG	Ⓡ Count	Ⓢ BG	Ⓣ Count	Ⓤ BG	Card Code
43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
11		1000	50	180		210		231						K
11		2500	50	269		266		275						L
11		5000	50	383		326		324						M
11	spiked water blank, 50 mg/ml DMSO, blank water	1000	50	151		173		157		160				N
11		5000	50	155		173		193						O
11		1000	50	188		193		194						P
11		2500	50	235		249		228						Q
11		5000	50	337		275		290						R
11	spiked water blank, 50 mg/ml DMSO, blank water	1000	50	162		208		162						S
11		5000	50	163		166		153						T
11		1000	50	135		125		137						U
11		2500	50	184		136		141						V
11		5000	50	223		231		242						W
	unspiked untreated sample,	100	50	137		142		132						X
		1000	50	147		151		154						Y

(continued)

HERL IN VITRO RESULTS CONTINUATION FORM

1 Update Code	2 4 System ID	5 8 Research Lab ID	9 14 MO DA YR Experiment Date	15 18 Lab	19 20 YR	21 24 Number Test Sample Identification	25 30 Activation Batch	31 32 Test Type (Table 10)	33 38 Strain	39 42 Batch No Microorganism
	IVR	CPHF	12/75	AZHL	55	0430		01	TA100	551 1

		Dose Level		Plate A		Plate B		Plate C		Plate D		Plate E		
H Solvent Positive (Table 11)	I Units of Concentration Blank = mg/ml 2 = µg/ml	J Stock Concentration (µg/plate)	K Amt Per Plate (µl)	L Count	M BG	N Count	O BG	P Count	Q BG	R Count	S BG	T Count	U BG	Card Code
43 44	45	46 50	51 54	55 58	59	60 63	64	65 68	69	70 73	74	75 78	79	80
		3300	50	1146		1165		1155						K
		1000	50	1152		1158		1167						L
		333 0	50	1143		1140		1137						M
	2 = µg/ml	100	50	196		1122		1127						N
Acid/Base		500	50	219		1183		1184						O
		1000	50	1167		1133		1157						P
		250	50	1191		2337		2399						Q
		500	50	1178		2755		2266						R
	2 = µg/ml	100	50	1169		1158		1148						S
Base/Acid		500	50	1191		1179								T
		1000	50	1150		1160		1172						U
		250	50	1177	2	1181	2	1177	2					V
		500	50	1177	3	8 5	3	5 6	3					W
														X
														Y

APPENDIX F

STRAIN FUNCTION, CELL TITER, AND CELL VIABILITY RECORD

For the purpose of quality control of the Ames assay, the bacterial strains used were checked for their genotype functions. These characteristics were analyzed for each experiment. The cell titer and cell viability were measured whenever an experiment was performed. The record of these analyses and measurements is attached to this report as Appendix F.

STRAIN FUNCTION TESTS, CELL TITER AND VIABILITY RECORD

Experiment Date 7/9/85 Initial KLC

Test		TA98	TA100
His^+ Bio^+ [1]		+	+
His^+ Bio^-		+	+
His^- Bio^+		−	−
His^- Bio^-		−	−
Ampicillin Sensitivity[1]		−	−
Crystal Violet Sensitivity[1] (Inhibition Zone, mm)		21	21
UV Sensitivity, 9 sec.[2] (Irradiated/non-irradiated)		−/+++	−/+++
Negative Control[3]: Spontaneous Mutation	1)	30	1) 180
	2)	33	2) 222
	3)	27	3) 152

Positive Controls[3]:

2-Nitrofluorene (TA98, -S9, 4 μg/plate)
1) 1606
2) 1563
3) 1621

Sodium Azide (TA100, -S9, 0.5 μg/plate)
1) 907
2) 1040
3) 952

2-Aminofluorene (TA98, +S9, 1 μg/plate)
1) 610 351
2) 535 326
3) 517 418
(2% S9) (10% S9)

2-Aminoanthracene (TA100, +S9, 2.5 μg/plate)
1) 2547 1032
2) 2015 867
3) 2724 866
(2% S9) (10% S9)

O.D. Reading for 10^{-1} Dilution 0.48 0.49

Viable Cell Count (x 10^9 cells/ml) 1.9 2.2

[1] + = Growth, no inhibition zone.
- = No growth, record the diameter of the inhibition zone, in mm.

[2] +++ = Full growth, non-irradiated control.
++ = 10^{-1} to 10^{-2} x control.
+ = 10^{-2} to >0 x control.
- = No growth.

[3] Plate count, revertants/plate.

STRAIN FUNCTION TESTS, CELL TITER AND VIABILITY RECORD

Experiment Date 7/22/85 Initial KIC

Test	TA98	TA100
His^+ Bio^+ [1]	+	+
His^+ Bio^-	+	+
His^- Bio^+	−	−
His^- Bio^-	−	−
Ampicillin Sensitivity[1]	−	−
Crystal Violet Sensitivity[1] (Inhibition Zone, mm)	19	17
UV Sensitivity, 9 sec.[2] (Irradiated/non-irradiated)	−/+++	+/+++

Negative Control[3]: Spontaneous Mutation

TA98	TA100
1) 24	1) 170
2) 29	2) 144
3) 27	3) 156

Positive Controls[3]:

2-Nitrofluorene (TA98, -S9, 4 ug/plate)

TA98
1) 1202
2) 1525
3) 1355

Sodium Azide (TA100, -S9, 2.5 ug/plate)

TA100
1) 599
2) 870
3) 879

2-Aminofluorene (TA98, +S9, 1 ug/plate)

1)	497	308
2)	452	360
3)	472	372
	(2% S9)	(10% S9)

2-Aminoanthracene (TA100, +S9, 2.5 ug/plate)

1)	2387	995
2)	2305	970
3)	2444	981
	(2% S9)	(10% S9)

O.D. Reading for 10^{-1} Dilution	0.47	0.32
Viable Cell Count (x 10^9 cells/ml)	2.0	3.1

[1] + = Growth, no inhibition zone.
- = No growth, record the diameter of the inhibition zone, in mm.

[2] +++ = Full growth, non-irradiated control.
++ = 10^{-1} to 10^{-2} x control.
+ = 10^{-2} to >0 x control.
- = No growth.

[3] Plate count, revertants/plate.

STRAIN FUNCTION TESTS, CELL TITER AND VIABILITY RECORD

Experiment Date 8/2/85 Initial KLC

Test	TA98	TA100
His^+ Bio^+ [1]	+	+
His^+ Bio^-	+	+
His^- Bio^+	−	−
His^- Bio^-	−	−
Ampicillin Sensitivity[1]	−	−
Crystal Violet Sensitivity[1] (Inhibition Zone, mm)	19	17
UV Sensitivity, 9 sec.[2] (Irradiated/non-irradiated)	−/+++	−/+++
Negative Control[3]: Spontaneous Mutation	1) 26 2) 30 3) 28	1) 179 2) 161 3) 166

Positive Controls[3]:

2-Nitrofluorene (TA98, -S9, 4 µg/plate)	1) 1125 2) 1230 3) 1045	Sodium Azide (TA100, -S9, 0.5 µg/plate) 1) 711 2) 683 3) 681
2-Aminofluorene (TA98, +S9, 1 µg/plate)	1) 379 313 2) 395 283 3) 416 370 (2% S9) (10% S9)	2-Aminoanthracene (TA100, +S9, 2.5 µg/plate) 1) 2676 1510 2) 2870 1452 3) 2762 1435 (2% S9) (10% S9)
O.D. Reading for 10^{-1} Dilution	0.48	0.42
Viable Cell Count (x 10^9 cells/ml)	1.6	2.5

[1] + = Growth, no inhibition zone.
- = No growth, record the diameter of the inhibition zone, in mm.

[2] +++ = Full growth, non-irradiated control.
++ = 10^{-1} to 10^{-2} x control.
+ = 10^{-2} to >0 x control.
- = No growth.

[3] Plate count, revertants/plate.

STRAIN FUNCTION TESTS, CELL TITER AND VIABILITY RECORD

Experiment Date 8/13/85 Initial KIC

Test	TA98	TA100
His^+ Bio^+ [1]	+	
His^+ Bio^-	+	
His^- Bio^+	−	
His^- Bio^-	−	
Ampicillin Sensitivity[1]	−	
Crystal Violet Sensitivity[1] (Inhibition Zone, mm)	21	
UV Sensitivity, 9 sec.[2] (Irradiated/non-irradiated)	−/+++	
Negative Control[3]: Spontaneous Mutation	1) 27 2) 29 3) 21	1) 2) 3)

Positive Controls[3]:

2-Nitrofluorene (TA98, -S9, 4 µg/plate)	1) 1540 2) 1521 3) 1490	Sodium Azide (TA100, -S9, µg/plate) 1) 2) 3)
2-Aminofluorene (TA98, +S9, 1 µg/plate)	1) 2038 2) 1845 3) 2095 (10%S9)	2-Aminoanthracene (TA100, +S9, µg/plate) 1) 2) 3)

O.D. Reading for 10^{-1} Dilution 0.47

Viable Cell Count (x 10^9 cells/ml) 1.6

[1] + = Growth, no inhibition zone.
- = No growth, record the diameter of the inhibition zone, in mm.

[2] +++ = Full growth, non-irradiated control.
++ = 10^{-1} to 10^{-2} x control.
+ = 10^{-2} to >0 x control.
- = No growth.

[3] Plate count, revertants/plate.

STRAIN FUNCTION TESTS, CELL TITER AND VIABILITY RECORD

Experiment Date 8/23/85 Initial KJC

Test	TA98	TA100
His^+ Bio^+ [1]	+	+
His^+ Bio^-	+	+
His^- Bio^+	-	-
His^- Bio^-	-	-
Ampicillin Sensitivity[1]	-	-
Crystal Violet Sensitivity[1] (Inhibition Zone, mm)	19	18
UV Sensitivity, 9 sec.[2] (Irradiated/non-irradiated)	-/+++	-/+++
Negative Control[3]: Spontaneous Mutation	1) 30 2) 30 3) 32	1) 347 2) 344 3) 318

Positive Controls[3]:

2-Nitrofluorene (TA98, -S9, 4 µg/plate)
1) 1088
2) 1113
3) 1021

Sodium Azide (TA100, -S9, 0.5 µg/plate)
1) 1355
2) 1297
3) 1346

2-Aminofluorene (TA98, +S9, 1 µg/plate)
1) 459 437
2) 473 446
3) 446 472
(2% S9) (10% S9)

2-Aminoanthracene (TA100, +S9, 2.5 µg/plate)
1) 2525 1278
2) 2325 1353
3) 2719 1297
(2% S9) (10% S9)

O.D. Reading for 10^{-1} Dilution 0.42 0.27

Viable Cell Count (x 10^9 cells/ml) 2.0 3.2

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[1] + = Growth, no inhibition zone.
- = No growth, record the diameter of the inhibition zone, in mm.

[2] +++ = Full growth, non-irradiated control.
++ = 10^{-1} to 10^{-2} x control.
+ = 10^{-2} to >0 x control.
- = No growth.

[3] Plate count, revertants/plate.

STRAIN FUNCTION TESTS, CELL TITER AND VIABILITY RECORD

Experiment Date 9/24/85 Initial KLC

Test	TA98	TA100
His^+ Bio^+ [1]	+	+
His^+ Bio^-	+	+
His^- Bio^+	−	−
His^- Bio^-	−	−
Ampicillin Sensitivity[1]	−	−
Crystal Violet Sensitivity[1] (Inhibition Zone, mm)	19	19
UV Sensitivity, 9 sec.[2] (Irradiated/non-irradiated)	−/+++	−/+++
Negative Control[3]: Spontaneous Mutation	1) 32 2) 24 3) 24	1) 139 2) 183 3) 170

Positive Controls[3]:

2-Nitrofluorene (TA98, -S9, 4 µg/plate)	Sodium Azide (TA100, -S9, .5µg/plate)
1) 1155	1) 1539
2) 1257	2) 1649
3) 1310	3) 1744

2-Aminofluorene (TA98, +S9, 1 µg/plate)		2-Aminoanthracene (TA100, +S9, .5µg/plate)	
1) 284	462	1) 2435	1037
2) 360	350	2) 2147	932
3) 322	381	3) 2522	955
(2%S9)	(10%S9)	(2%S9)	(10%S9)

O.D. Reading for 10^{-1} Dilution	0.41	0.38
Viable Cell Count (x 10^9 cells/ml)	1.4	0.9

[1] + = Growth, no inhibition zone.
- = No growth, record the diameter of the inhibition zone, in mm.

[2] +++ = Full growth, non-irradiated control.
++ = 10^{-1} to 10^{-2} x control.
+ = 10^{-2} to > 0 x control.
- = No growth.

[3] Plate count, revertants/plate.

STRAIN FUNCTION TESTS, CELL TITER AND VIABILITY RECORD

Experiment Date 10/4/85 Initial KLC

Test	TA98	TA100
His^+ Bio^+ [1]	+	+
His^+ Bio^-	+	+
His^- Bio^+	−	−
His^- Bio^-	−	−
Ampicillin Sensitivity[1]	−	−
Crystal Violet Sensitivity[1] (Inhibition Zone, mm)	20	17
UV Sensitivity, 9 sec.[2] (Irradiated/non-irradiated)	−/+++	+/+++
Negative Control[3]: Spontaneous Mutation	1) 28 2) 34 3) 28	1) 181 2) 143 3) 212

Positive Controls[3]:

2-Nitrofluorene (TA98, -S9, 4 µg/plate)
1) 903
2) 861
3) 882

Sodium Azide (TA100, -S9, .5 µg/plate)
1) 352
2) 381
3) 359

2-Aminofluorene (TA98, +S9, 1 µg/plate)
1) 375 485
2) 393 403
3) 342 427
(2% S9) (10% S9)

2-Aminoanthracene (TA100, +S9, .5 µg/plate)
1) 2003 1014
2) 2050 1262
3) 1828 1257
(2% S9) (10% S9)

O.D. Reading for 10^{-1} Dilution 0.45 0.35

Viable Cell Count (x 10^9 cells/ml) 1.5 1.4

[1] + = Growth, no inhibition zone.
- = No growth, record the diameter of the inhibition zone, in mm.

[2] +++ = Full growth, non-irradiated control.
++ = 10^{-1} to 10^{-2} x control.
+ = 10^{-2} to >0 x control.
- = No growth.

[3] Plate count, revertants/plate.

STRAIN FUNCTION TESTS, CELL TITER AND VIABILITY RECORD

Experiment Date 10/17/85 Initial KIC

Test	TA98	TA100
His^+ Bio^+ [1]	+	+
His^+ Bio^-	+	+
His^- Bio^+	–	–
His^- Bio^-	–	–
Ampicillin Sensitivity[1]	–	–
Crystal Violet Sensitivity[1] (Inhibition Zone, mm)	17	16
UV Sensitivity, 9 sec.[2] (Irradiated/non-irradiated)	–/+++	–/+++
Negative Control[3]: Spontaneous Mutation	1) 34 2) 30 3) 38	1) 183 2) 169 3) 157

Positive Controls[3]:

2-Nitrofluorene (TA98, -S9, 4 ug/plate)
1) 1236
2) 1132
3) 1130

Sodium Azide (TA100, -S9, 0.5ug/plate)
1) 1497
2) 1364
3) 1451

2-Aminofluorene (TA98, +S9, 1 ug/plate)
1) 562 326
2) 470 359
3) 479 367
(2%S9) (10%S9)

2-Aminoanthracene (TA100, +S9, 2.5ug/plate)
1) 1757 946
2) 2149 964
3) 1935 954
(2%S9) (10%S9)

O.D. Reading for 10^{-1} Dilution 0.48 0.36

Viable Cell Count (x 10^9 cells/ml) 1.2 1.9

[1] + = Growth, no inhibition zone.
- = No growth, record the diameter of the inhibition zone, in mm.

[2] +++ = Full growth, non-irradiated control.
++ = 10^{-1} to 10^{-2} x control.
+ = 10^{-2} to >0 x control.
- = No growth.

[3] Plate count, revertants/plate.

STRAIN FUNCTION TESTS, CELL TITER AND VIABILITY RECORD

Experiment Date 10/25/85 Initial KLC

Test	TA98	TA100
His^+ Bio^+ [1]	+	+
His^+ Bio^-	+	+
His^- Bio^+	-	-
His^- Bio^-	-	-
Ampicillin Sensitivity[1]	-	-
Crystal Violet Sensitivity[1] (Inhibition Zone, mm)	18	17
UV Sensitivity, 9 sec.[2] (Irradiated/non-irradiated)	-/+++	-/+++

Negative Control[3]: Spontaneous Mutation

TA98	TA100
1) 25	1) 147
2) 34	2) 159
3) 35	3) 153

Positive Controls[3]:

2-Nitrofluorene (TA98, -S9, 4 ug/plate)

1) 1035
2) 1052
3) 1368

Sodium Azide (TA100, -S9, 2.5ug/plate)

1) 579
2) 510
3) 564

2-Aminofluorene (TA98, +S9, 1 ug/plate)

1) 479	391
2) 478	360
3) 526	464
(2% S9)	(10% S9)

2-Aminoanthracene (TA100, +S9, 0.5ug/plate)

1) 2352	1707
2) 2700	1537
3) 2501	1807
(2% S9)	(10% S9)

O.D. Reading for 10^{-1} Dilution	0.43	0.39
Viable Cell Count (x 10^9 cells/ml)	1.0	1.7

[1] + = Growth, no inhibition zone.
- = No growth, record the diameter of the inhibition zone, in mm.

[2] +++ = Full growth, non-irradiated control.
++ = 10^{-1} to 10^{-2} x control.
+ = 10^{-2} to >0 x control.
- = No growth.

[3] Plate count, revertants/plate.

STRAIN FUNCTION TESTS, CELL TITER AND VIABILITY RECORD

Experiment Date 11/22/85 Initial KIC

Test	TA98	TA100
His^+ Bio^+ [1]	+	+
His^+ Bio^-	+	+
His^- Bio^+	−	−
His^- Bio^-	−	−
Ampicillin Sensitivity[1]	−	−
Crystal Violet Sensitivity[1] (Inhibition Zone, mm)	21	16
UV Sensitivity, 9 sec.[2] (Irradiated/non-irradiated)	−/+++	−/+++

Negative Control[3]: Spontaneous Mutation

TA98	TA100
1) 37	1) 155
2) 33	2) 186
3) 39	3) 158

Positive Controls[3]:

2-Nitrofluorene (TA98, -S9, 4 ug/plate)

1) 1158
2) 1166
3) 1266

Sodium Azide (TA100, -S9, 5 ug/plate)

1) 695
2) 670
3) 689

2-Aminofluorene (TA98, +S9, 1 ug/plate)

	(2%S9)	(10%S9)
1)	848	262
2)	877	301
3)	838	261

2-Aminoanthracene (TA100, +S9, 5 ug/plate)

	(2%S9)	(10%S9)
1)	2344	890
2)	2289	793
3)	2279	807

O.D. Reading for 10^{-1} Dilution	0.46	0.35
Viable Cell Count (x 10^9 cells/ml)	1.7	2.1

[1] + = Growth, no inhibition zone.
- = No growth, record the diameter of the inhibition zone, in mm.

[2] +++ = Full growth, non-irradiated control.
++ = 10^{-1} to 10^{-2} x control.
+ = 10^{-2} to >0 x control.
- = No growth.

[3] Plate count, revertants/plate.

STRAIN FUNCTION TESTS, CELL TITER AND VIABILITY RECORD

Experiment Date 12/17/85 Initial KIC

Test	TA98	TA100
His^+ Bio^+ [1]	+	+
His^+ Bio^-	+	+
His^- Bio^+	–	–
His^- Bio^-	–	–
Ampicillin Sensitivity[1]	–	–
Crystal Violet Sensitivity[1] (Inhibition Zone, mm)	19	18
UV Sensitivity, 9 sec.[2] (Irradiated/non-irradiated)	–/+++	–/+++
Negative Control[3]: Spontaneous Mutation	1) 34 2) 23 3) 37	1) 178 2) 171 3) 157

Positive Controls[3]:

2-Nitrofluorene (TA98, -S9, 4 µg/plate)
1) 1091
2) 1085
3) 1105

Sodium Azide (TA100, -S9, 2.5µg/plate)
1) 688
2) 686
3) 670

2-Aminofluorene (TA98, +S9, 1 µg/plate)
1) 763 252
2) 739 251
3) 728 261
(2%S9) (1%S9)

2-Aminoanthracene (TA100, +S9, 2.5µg/plate)
1) 1923 786
2) 2150 973
3) 2023 551
(2%S9) (1%S9)

O.D. Reading for 10^{-1} Dilution	0.21	0.38
Viable Cell Count (x 10^9 cells/ml)	1.8	2.0

[1] + = Growth, no inhibition zone.
- = No growth, record the diameter of the inhibition zone, in mm.

[2] +++ = Full growth, non-irradiated control.
++ = 10^{-1} to 10^{-2} x control.
+ = 10^{-2} to >0 x control.
- = No growth.

[3] Plate count, revertants/plate.

STRAIN FUNCTION TESTS, CELL TITER AND VIABILITY RECORD

Experiment Date 2/11/86 Initial K.I.C.

Test	TA98	TA100
His^+ Bio^+ [1]	+	
His^+ Bio^-	+	
His^- Bio^+	−	
His^- Bio^-	−	
Ampicillin Sensitivity[1]	−	
Crystal Violet Sensitivity[1] (Inhibition Zone, mm)	18	
UV Sensitivity, 9 sec.[2] (Irradiated/non-irradiated)	−/+++	
Negative Control[3]: Spontaneous Mutation	1) 35 2) 30 3) 28	1) 2) 3)
Positive Controls[3]:		
2-Nitrofluorene (TA98, -S9, µg/plate)	1) 2) 3)	Sodium Azide (TA100, -S9, µg/plate) 1) 2) 3)
2-Aminofluorene (TA98, +S9, 1 µg/plate)	1) 142 2) 106 3) 107 (30% S-9 Mix)	2-Aminoanthracene (TA[redacted]8, +S9, 2.5µg/plate) 1) 253 2) 235 3) 246 (30% S-9 Mix)
O.D. Reading for 10^{-1} Dilution	0.43	
Viable Cell Count (x 10^9 cells/ml)	1.2	

[1] + = Growth, no inhibition zone.
- = No growth, record the diameter of the inhibition zone, in mm.

[2] +++ = Full growth, non-irradiated control.
++ = 10^{-1} to 10^{-2} x control.
+ = 10^{-2} to >0 x control.
- = No growth.

[3] Plate count, revertants/plate.

STRAIN FUNCTION TESTS, CELL TITER AND VIABILITY RECORD

Experiment Date 2/28/86 Initial K.L.C.

Test	TA98	TA100
His^+ Bio^+ [1]	+	
His^+ Bio^-	+	
His^- Bio^+	−	
His^- Bio^-	−	
Ampicillin Sensitivity[1]	−	
Crystal Violet Sensitivity[1] (Inhibition Zone, mm)	20	
UV Sensitivity, 9 sec.[2] (Irradiated/non-irradiated)	−/+++	
Negative Control[3]: Spontaneous Mutation	1) 31 2) 31 3) 25	1) 2) 3)

Positive Controls[3]:

2-Nitrofluorene (TA98, -S9, µg/plate)	1) 2) 3)	Sodium Azide (TA100, -S9, µg/plate) 1) 2) 3)
2-Aminofluorene (TA98, +S9, 1 µg/plate)	1) 89 2) 82 3) 86	2-Aminoanthracene (TA98, +S9, 0.5 µg/plate) 1) 248 2) 315 3) 129 (dense distribution of colo... on one s... of the pl...
1) 355 2) 321 3) 375 (10% S-9 Mix)	(30% S-9 Mix)	(30% S-9 Mix)
O.D. Reading for 10^{-1} Dilution	0.54	
Viable Cell Count (x 10^9 cells/ml)	1.1	

[1] + = Growth, no inhibition zone.
- = No growth, record the diameter of the inhibition zone, in mm.

[2] +++ = Full growth, non-irradiated control.
++ = 10^{-1} to 10^{-2} x control.
+ = 10^{-2} to >0 x control.
- = No growth.

[3] Plate count, revertants/plate.

APPENDIX G

PRIMARY DATA WORK SHEET FOR STATISTICAL ANALYSIS

For the protocol validation study, several statistical analyses were performed to establish the method background. The study included both the sample preparation and the Ames assay procedures. Therefore, three negative controls were involved: (1) the revertant counts of the blank water extract representing the laboratory, field, travel, sample preparation method, and mutagenicity testing procedure blanks; (2) the revertant counts of the DMSO solvent vehicle control in the Ames assay; and, (3) the spontaneous mutation revertant counts as the blank control for the mutagenicity measurement of the bacterial strain. An F-test with two factor block analysis of variance was used to analyze the similarities among these three controls. If there was any difference among these controls, a paired t-test was used to find out where the difference was. The primary data and calculation processes are recorded on the work sheets which are attached to this report as Appendix G.

WORK SHEET FOR STATISTICAL ANALYSIS

TA98, — S-9

a= 8 , b=3, n=3

Exp. Date	Y_{ij} DMSO	SR	WATER	$\bar{Y}_i$
7/22/85	26	27	35	29
8/2/85	27	28	33	29
8/13/85	—	—	—	—
8/23/85	29	31	38	33
9/24/85	25	27	35	29
10/4/85	23	30	36	30
10/17/85	26	34	32	31
10/25/85	32	31	30	31
11/22/85	28	36	38	34
12/17/85	—	—	—	—
$\bar{Y}_j$	27	30	35	$\bar{Y}$= 31

Days (D) $SS_a = bn \sum_{i=1}^{a} (\bar{Y}_i - \bar{Y})^2 = 3\times3\,(4+4+4+4+1+9) = 9\times26 = 234$

Treatments(T) $SS_b = an \sum_{j=1}^{b} (\bar{Y}_j - \bar{Y})^2 = 8\times3\,(16+1+16) = 792$

D x T $SS_{ab} = n \sum_{i=1}^{a}\sum_{j=1}^{b} (\bar{Y}_{ij} - \bar{Y}_i - \bar{Y}_j + \bar{Y})^2 = 3\times[(26-29-27+31)^2 + \cdots] = 3(121)\cdot 36$

Residual $SS_r = \sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{k=1}^{n} (Y_{ijk} - \bar{Y}_{ij})^2 = 1044$

F-test:

	SS	df	MS(SS/df)	F(MS/s^2)	Tabulate (α= 0.05)
		(a-1)			
D	234	7	33	1.5	2.22
		(b-1)			
T	792	2	396	18.0	3.2
		(a-1)(b-1)			
D x T	363	14	26	1.2	1.92
		ab(n-1)			
Residual	1044	48	22 (s^2)		

t-test:

	t	Tabulate (α= 0.05)	H_A
DMSO vs. SR	-2.76	1.895	SR > DMSO
DMSO vs. WATER	-4.79	1.895	H_2O > DMSO
SR vs. WATER	-2.93	1.895	H_2O > SR

WORK SHEET FOR STATISTICAL ANALYSIS

TA98, 2%S-9

a= 9, b=3, n=3

Exp. Date	Y_{ij} DMSO	SR	WATER	$\bar{Y}_i$
7/22/85	33	45	38	39
8/2/85	33	34	43	37
8/13/85	—	—	—	—
8/23/85	51	39	47	46
9/24/85	40	39	46	42
10/4/85	37	54	48	46
10/17/85	37	43	56	45
10/25/85	43	49	50	47
11/22/85	49	44	57	50
12/17/85	39	45	56	47
$\bar{Y}_j$	40	44	49	$\bar{Y}$= 44

Days (D) $SS_a = bn \sum_{i=1}^{a} (\bar{Y}_i - \bar{Y})^2 = 3 \times 3\,(141) = 1269$

Treatments (T) $SS_b = an \sum_{j=1}^{b} (\bar{Y}_j - \bar{Y})^2 = 9 \times 3\,(41) = 1107$

D x T $SS_{ab} = n \sum_{i=1}^{a} \sum_{j=1}^{b} (\bar{Y}_{ij} - \bar{Y}_i - \bar{Y}_j + \bar{Y})^2 = 3(497) = 1491$

Residual $SS_r = \sum_{i=1}^{a} \sum_{j=1}^{b} \sum_{k=1}^{n} (\bar{Y}_{ijk} - \bar{Y}_{ij})^2 = 2704$

F-test:

	SS	df	MS(SS/df)	F(MS/s^2)	Tabulate (α= 0.05)
D	1269	8 (a-1)	159	3.18	2.12
T	1107	2 (b-1)	554	11.08	3.17
D x T	1491	16 (a-1)(b-1)	93	1.86	1.84
Residual	2704	54 ab(n-1)	50 (s^2)		

t-test:

	t	Tabulate (α= 0.05)	H_A
DMSO vs. SR	−1.14	1.895	DMSO = SR
DMSO vs. WATER	−3.55	1.895	H_2O > DMSO
SR vs. WATER	−1.97	1.895	H_2O > SR

WORK SHEET FOR STATISTICAL ANALYSIS

TA98, 10%S-9

a= 9, b=3, n=3

Exp. Date	Y_{ij} DMSO	SR	WATER	$\bar{Y}_i$
7/22/85	40	47	41	43
8/2/85	41	36	51	43
8/13/85	43	43	46	44
8/23/85	47	49	53	50
9/24/85	43	49	54	49
10/4/85	46	49	53	49
10/17/85	48	48	58	51
10/25/85	46	45	51	47
11/22/85	45	41	50	45
12/17/85	—	—	—	—
$\bar{Y}_j$	44	45	51	$\bar{Y}$= 47

Days (D) $SS_a = bn\sum_{i=1}^{a}(\bar{Y}_i - \bar{Y})^2 = 9(78) = 702$

Treatments (T) $SS_b = an\sum_{j=1}^{b}(\bar{Y}_j - \bar{Y})^2 = 27(29) = 783$

D x T $SS_{ab} = n\sum_{i=1}^{a}\sum_{j=1}^{b}(\bar{Y}_{ij} - \bar{Y}_i - \bar{Y}_j + \bar{Y})^2 = 513$

Residual $SS_r = \sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{k=1}^{n}(Y_{ijk} - \bar{Y}_{ij})^2 = 2783$

F-test:

	SS	df	MS(SS/df)	F(MS/s^2)	Tabulate (α= 0.05)
D	702	(a-1) 8	88	1.69	2.12
T	783	(b-1) 2	392	7.54	3.17
D x T	513	(a-1)(b-1) 16	32	0.62	1.84
Residual	2783	ab(n-1) 54	52(s^2)		

t-test:

	t	Tabulate (α= 0.05)	H_A
DMSO vs. SR	-0.65	1.895	DMSO = SR
DMSO vs. WATER	-5.69	1.895	H_0 > DMSO
SR vs. WATER	-2.89	1.895	H_0 > SR

APPENDIX H

PRIMARY DATA WORK SHEET FOR QUALITY CONTROL CHART CALIBRATION

Quality control charts were constructed for the method background to provide graphic assessment of accuracy and precision for each analysis and to provide instant detection of unacceptable data. The calculation process for establishing these data are recorded on the work sheets which are attached to this report as Appendix H. The calculated mean value (X) was assumed as the true value for accuracy evaluation. The range (R) chart was used for precision measurements. The upper control limit (UCL) and lower control limit (LCL) represented the 99% confidence interval.

WORK SHEET FOR DATA QUALITY CONTROL CHART

Strain TA98 Sample H_2O Activation $-S9$

Exp. Date	Plate Count (1)	(2)	(3)	Average $\bar{X}$	SD σ	Variance σ^2	CV $\frac{\sigma}{\bar{X}} \times 100\%$	Range
7/9/85	—	—	—	—	—	—	—	—
7/22/85	33	36	37	35	2.1	4.3	6.0	4
8/2/85	29	37	32	33	4.0	16.0	12.2	8
8/13/85	—	—	—	—	—	—	—	—
8/23/85	44	32	37	38	6.0	36.0	15.9	12
9/24/85	38	33	37	37	3.2	10.3	8.7	6
10/4/85	39	38	32	36	3.8	14.3	10.5	7
10/17/85	36	29	30	32	3.8	14.3	11.8	7
10/25/85	36	29	24	30	6.0	36.0	20.0	12
11/22/85	41	44	30	38	7.4	54.3	17.4	14
12/17/85	—	—	—	—	—	—	—	—
9/24/85	30	39	37	35	4.7	22.3	13.5	9

Total n= 9
$\Sigma\bar{X}$= 314
ΣR= 79

1. $\bar{\bar{X}} = \Sigma\bar{X} \div n = 34.9$
2. $UCL_{\bar{X}} = \bar{\bar{X}} + CL_{\bar{X}} = 34.9 + 9.0 = 43.9$
3. $LCL_{\bar{X}} = \bar{\bar{X}} - CL_{\bar{X}} = 34.9 - 9.0 = 25.9$
4. $\bar{R} = \Sigma R \div n = 8.8$
5. $UCL_{\bar{R}} = D_4 \times \bar{R} = 2.575 \times 8.8 = 22.7$
6. $LCL_{\bar{R}} = D_3 \times \bar{R} = 0$

SD: Standard Deviation
CV: Coefficient of Variance
CL: Control Limit
UCL: Upper Control Limit
LCL: Lower Control Limit
$CL_{\bar{X}}$: $A_2 \times \bar{R} = 1.023 \times 8.8 = 9.0$
A_2: Factors for Average = 1.023 (3 counts)
D_4: Factors for Range = 2.575 (3 counts)
D_3: Factors for Range = 0 (3 counts)

WORK SHEET FOR DATA QUALITY CONTROL CHART

Strain TA98 Sample H2O Activation 2% S9

Exp. Date	Plate Count (1)	(2)	(3)	Average $\bar{X}$	SD σ	Variance σ^2	CV $\frac{\sigma}{\bar{x}} \times 100\%$	Range
7/9/85	—	—	—	—	—	—	—	—
7/22/85	37	36	40	38	2.1	4.3	5.5	4
8/2/85	35	39	54	43	10.0	100	23 3	19
8/13/85	—	—	—	—	—	—	—	—
8/23/85	55	36	47	46	9.5	91.0	20 7	19
9/24/85	57	49	52	53	4.0	16.3	7.6	8
10/4/85	38	54	52	48	8.7	76.0	18.2	16
10/17/85	49	40	37	42	6.2	39.0	14.9	12
10/25/85	45	58	47	50	7.0	49.0	14.0	13
11/22/85	66	50	56	57	8.1	65.3	14.2	16
12/17/85	56	71	41	56	15.0	225.0	26.8	30
9/24/85	43	52	44	46	4.9	24.3	10.7	9
10/25/85	35	44	48	42	6.7	44.3	15.9	13

Total n= 11
$\Sigma\bar{X}$= 521
ΣR= 159

SD: Standard Deviation
CV: Coefficient of Variance

1. $\bar{\bar{X}} = \Sigma\bar{X} \div n = 47.4$

2. $UCL_{\bar{X}} = \bar{\bar{X}} + CL_{\bar{X}} = 47.4 + 14.7 = 62.1$

3. $LCL_{\bar{X}} = \bar{\bar{X}} - CL_{\bar{X}} = 47.4 - 14.7 = 32.7$

4. $\bar{R} = \Sigma R \div n = 14.4$

5. $UCL_{\bar{R}} = D_4 \times \bar{R} = 2.575 \times 14.4 = 37.1$

6. $LCL_{\bar{R}} = D_3 \times \bar{R} = 0$

CL: Control Limit

UCL: Upper Control Limit

LCL: Lower Control Limit

$CL_{\bar{X}}$: $A_2 \times \bar{R} = 1.023 \times 14.4 = 14.7$

A_2: Factors for Average = 1.023 (3 counts)

D_4: Factors for Range = 2.575 (3 counts)

D_3: Factors for Range = 0 (3 counts)

WORK SHEET FOR DATA QUALITY CONTROL CHART

Strain TA98 Sample H20 Activation 10% S9

Exp. Date	Plate Count (1)	(2)	(3)	Average $\bar{X}$	SD σ	Variance σ^2	CV $\frac{\sigma}{\bar{X}} \times 100\%$	Range
7/9/85	—	—	—	—	—	—	—	—
7/22/85	46	41	36	41	5.0	25	12.2	10
8/2/85	60	44	50	51	8.1	65	15.8	16
8/13/85	40	47	51	46	5.6	31	12.1	11
8/23/85	59	49	52	53	5.1	26	9.7	10
9/24/85	61	51	52	55	5.5	30	10.0	10
10/4/85	49	62	48	53	7.8	61	14.7	14
10/17/85	56	56	61	58	2.9	8	5.0	5
10/25/85	47	46	60	51	7.8	61	15.3	14
11/22/85	50	48	51	49	1.5	2	3.1	3
12/17/85	—	—	—	—	—	—	—	—
9/24/85	42	57	63	54	10.8	117	20.0	21
10/25/85	46	42	41	43	2.6	7	6.1	5

Total n= 11
$\Sigma\bar{X}$= 554
ΣR= 119

SD: Standard Deviation
CV: Coefficient of Variance

1. $\bar{\bar{X}} = \Sigma\bar{X} \div n = 50.4$

CL: Control Limit

2. $UCL_{\bar{X}} = \bar{\bar{X}} + CL_{\bar{X}} = 50.4 + 11.1 = 61.5$

UCL: Upper Control Limit

3. $LCL_{\bar{X}} = \bar{\bar{X}} - CL_{\bar{X}} = 50.4 - 11.1 = 39.3$

LCL: Lower Control Limit

4. $\bar{R} = \Sigma R \div n = 10.8$

$CL_{\bar{X}}$: $A_2 \times \bar{R} = 1.023 \times 10.8 = 11.1$

5. $UCL_{\bar{R}} = D_4 \times \bar{R} = 2.575 \times 10.8 = 27.8$

A_2: Factors for Average = 1.023 (3 counts)

6. $LCL_{\bar{R}} = D_3 \times \bar{R} = 0$

D_4: Factors for Range = 2.575 (3 counts)

D_3: Factors for Range = 0 (3 counts)