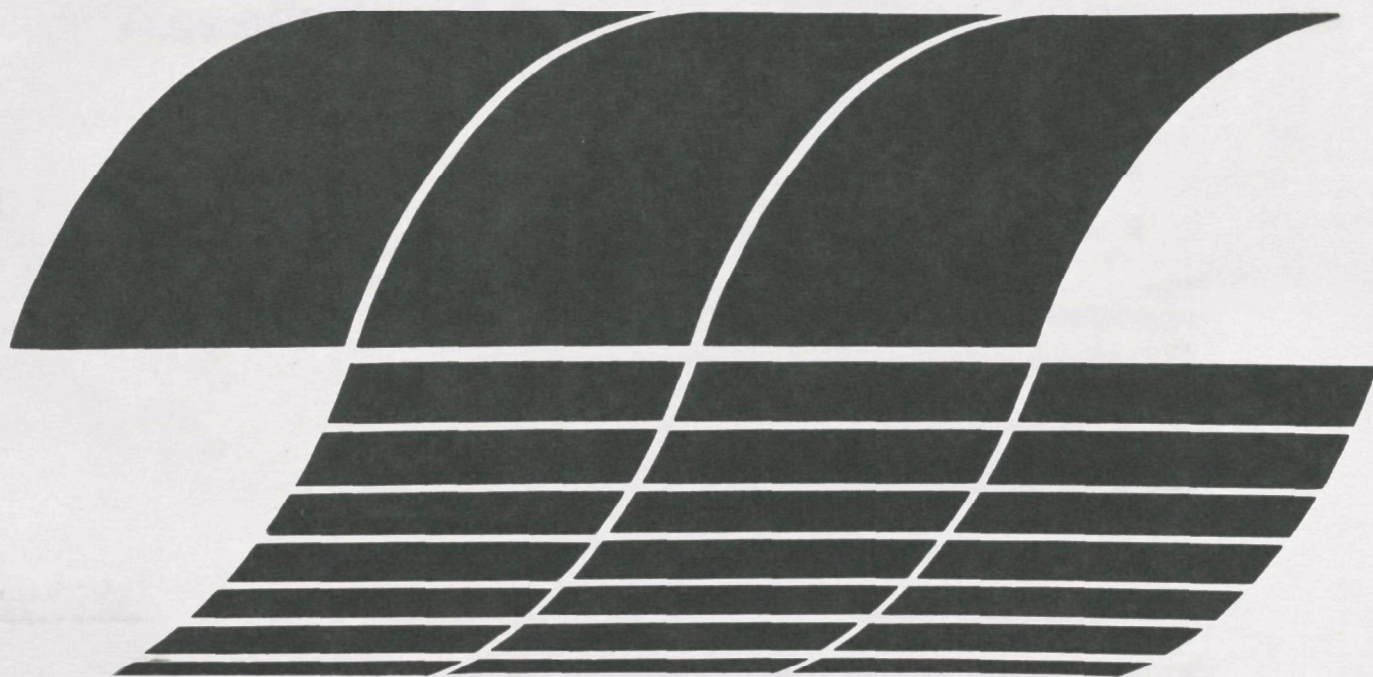




Level 1 Biological Testing Assessment and Data Formatting

Interagency
Energy/Environment
R&D Program Report



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Research reports of the Office of Research and Development, U.S. Environmental Protection Agency, have been grouped into nine series. These nine broad categories were established to facilitate further development and application of environmental technology. Elimination of traditional grouping was consciously planned to foster technology transfer and a maximum interface in related fields. The nine series are:

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2. Environmental Protection Technology
3. Ecological Research
4. Environmental Monitoring
5. Socioeconomic Environmental Studies
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7. Interagency Energy-Environment Research and Development
8. "Special" Reports
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Level 1 Biological Testing Assessment and Data Formatting

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ABSTRACT

A scheme was developed for the comparison of Health Effect and Ecological Bioassay Assessment Data. The scheme was based on the assumption that each test method had a maximum dose (concentration) which could be reliably applied and that effects based on fractions of the maximum applicable dose (MAD) can be designated as degrees of toxicity. The levels of toxicity based on fractions of the MAD are given as high, moderate, low, and nondetectable (i.e. no effect detected at the MAD).

Employing this scheme, data from three pilot studies including water effluent samples, fluidized bed combustion samples, and coal gasification samples were examined.

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I. INTRODUCTION

This report presents a system to convert results from Level 1 health effects, bioassays, and selected Level 1 ecological bioassays into a uniform format. This format is structured so that data can be converted from original study reports into nondetectable, low, moderate, and high designations. The evaluation of raw data and development of the final toxicity definitions require a set of ~~tables~~ and data transition sheets ~~tables~~ that provide a uniform method of data summarization.

The approach is based on the following rationale:

- Biological activity measured by lethality has sufficient, common phenomena to produce meaningful comparisons. The Ames Salmonella assay measures gene revertants rather than toxicity, but its results can be grouped in a similar fashion.
- Each assay, regardless of the type of response measured, has a maximum applicable dose above which the test data are virtually impossible to interpret because of nonspecific responses of the test organisms.
- A structure is needed for data assessment that can categorize toxicity data from diverse sources into a series of similar comparative categories. Without this, the comparison of these diverse assays would be difficult, and subject to variable interpretations.
- The categories low, moderate, and high are sufficiently broad to encompass normal variability and species differences, yet are narrow enough to provide meaningful categories for decision making.
- Level 1 testing is designed to rank sampling sites for further studies and is not intended for making human risk estimates.

Environmental assessment requires evaluation of data derived from chemistry, engineering, and biology. This data formatting program was developed to permit biological data to easily fit into the evaluation process without requiring the user to be an expert in numerous biological disciplines.

The data forms suggested here will be revised as Level 1 procedures are changed. Consult the most recent revision of "IERL-RTP Procedures Manual: Level 1 Biological Tests for Environmental Assessment" for current forms.

II. DEFINITION OF HIGH, MEDIUM, LOW, AND NONDETECTABLE TOXICITY

Definition of the above classes of toxicity would be as follows:

Nondetectable--no significant response as determined by a preestablished set of criteria. Toxicants could be present in a sample below the level of detectability (MDC) for the assay.

Low--a significant response or an LD50, LC50, or EC50 value ranging from the maximum dose applicable to the assay system (defined as MAD and established in advance) to one-tenth of that value. For example, if it is established that 10 g/kg will define the upper limit of sample application to rats in the acute rodent toxicity test, then any sample which has an LD50 value of 10 g/kg down to a concentration of 1 g/kg will be defined as having low toxicity.

Medium--a significant response or an LD50, LC50, or EC50 value ranging from a concentration less than the lower limit of the "low" toxicity (1/10 of the MAD) to 1/100 of the MAD. Using the same illustration, an LD50 (EC50) obtained at a concentration ranging from 0.1 g/kg to 1.0 g/kg in the rodent toxicity assay will be considered as moderate.

High--a significant response or an LD50 (EC50) value less than 1/100 of the MAD for the particular assay. Thus, any sample with an LD50 less than 0.1 g/kg in the rodent assay will be considered to have high toxicity. A summary of the toxicity levels and their associated definitions are shown in Table 1.

The degree of sample concentration will be factored into the calculation procedure and will reduce the level of toxicity accordingly.

Figures 1 to 3 illustrate the relationship of critical data (e.g. LD50) to high, moderate, low and nondetectable designations.

The MAD values are taken from current protocols used in conducting these studies. The values proposed for MAD in Level 1 screening were established from:

- pilot study data developed from Level 1 testing,
- scientific publications dealing with uniform testing methods (Science, 203:563, 1979), and
- standard operating procedures defined by testing laboratories experienced in conducting Level 1 bioassays.

TABLE 1

DEFINITION OF EFFECTIVENESS CATEGORIES

ASSAY	ACTIVITY MEASURED	UNITS	MAD	RANGE OF CONCENTRATION OR DOSAGE			
				HIGH	MODERATE	LOW	NOT DETECTABLE
Ames Test	Mutagenesis*	mg/plate	5	<0.05	0.05-0.5	0.5-5	ND at >5
		μl/plate	50	<0.5	0.5-5.0	5-50	ND at >50
RAM/WI-38 and CHO TOXICITY	Lethality EC50	mg/ml	1	<0.01	0.01-0.1	0.1-1	ND at >1
		μl/ml	600	<6.0	6.0-60	60-600	ND at >600
		μl/ml	20 **				ND at >20
RODENT TOXICITY	Lethality LD50	gm/kg	10	<0.1	0.1 -1	1 -10	ND at >10
Aquatic Tests							
Algae	Growth Inhibition EC50	gm/liter	1	<0.01	0.01-0.1	0.1-1	ND at >1
		%	100	<20	20-75	75-100	ND at >100
Fish	Lethality LC50	gm/liter	1	<0.01	0.01-0.1	0.1-1	ND at >1
		%	100	<20	20-75	75-100	ND at >100
Inverte- brate	Lethality LC50	gm/liter	1	<0.01	0.01-0.1	0.1-1	ND at >1
		%	100	<20	20-75	75-100	ND at >100

Abbreviations:

MAD = Maximum Applicable Dose (Technical Limitations)

LD50 = Calculated Dosage Expected to Kill 50% of Population

LC 50 = Calculated Concentration Expected to Kill 50% of Population

EC50 = Calculated Concentration Expected to Produce Effect in 50% of Population

ND = Not Detectable

*Negative response at 5 mg/plate or at level of toxicity is given as ND.

Positive response requires calculation of minimum effective concentration (MEC) to produce a positive mutagenic response. H, M and L designations are made from MEC values of positive agents.

**Volumes used for solvent exchange samples (This maximum keeps DMSO below level of toxicity).

III. DATA TRANSITION FORMS

In order to ensure uniform translation of raw data to definitions of toxicity, three intermediate forms were developed.

The Primary Data Transition Form (Table 2) records the basic information from a test by sample. The type of test, is respective MAD, and the calculated active dose are recorded on this table. Since the Ames Salmonella assay is a special case, the LC50 value is not directly applicable. The Ames test is evaluated as either positive (+) or negative (-). If it is (-), the level of activity is not as significant as the fact that the sample was not mutagenic. If the sample was (+), however, it is possible to assess the level of activity by defining the lowest concentration of the test sample at which mutagenicity could be detected (activity is defined by the preestablished criteria suggested in Appendix A).

The primary form is also used to record testing methods which concentrate a sample after receipt, such as solvent exchange. The concentration factor is the ratio of the total volume sampled to the final sample volume. There is also a place to record comments regarding the assay that provide backup documentation or explanations. In several tests (e.g., RAM), several parameters indicative of cell lethality are measured. Each of these should be recorded in the primary data transition form (Table 2) and the most sensitive parameter used to determine "Level of toxicity" for the Bioassay Summary (Table 6). Without some method of ranking each parameter, this is considered an adequate approach to uniform data interpretation.

The Primary Data Transition Forms are often used when results are submitted by more than one biological contractor. It is designed to provide a uniform base for assimilating data for transition to subsequent Level 1 formats.

Health and Ecological Effects Critical Data Summary Forms, (Tables 3, 4, and 5) combine the data from Level 1 tests conducted on samples from a single site. Tables 3 and 4 summarize responses from health effects tests and Table 5 summarizes the response from aquatic tests.

Tables 2 to 5 contain the responses which are finally summarized for all Level 1 tests in Table 6. It is a summary of the various tests using only ND, L, M, and H response designations. Table 6 allows rapid ranking of the streams evaluated by the variety of tests used in Level 1.

Table 2

PRIMARY DATA TRANSITION FORM

Assay: _____ Sample: _____

MAD: _____ Date Processers Name: _____

Results:

LD50 [EC50] Value^a _____ [or] Ames Response _____

MEC^b _____

Approximate Concentration Factor _____

Level of Toxicity^c _____

Comments:^d

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration [if response is positive].

^cSee Table 1 for definition of toxicity levels.

^dMay include;

Salmonella strains responding to a mutagenic substance.

Deviations from standard protocols.

Toxic signs in rodent assay.

Procedural problems.

HEALTH EFFECTS CRITICAL DATA
SUMMARY FORM: MUTAGENICITY

Technical Directive or Project No. _____

Sample Identification	Salmonella Plate Assay	
	Response ^a	LRPC ^b
1		
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99		
100		

[illegible]

^a given as + or - based on criteria in Section VII.
^b LRPC = lowest recorded positive concentration

TABLE 4

HEALTH EFFECTS CRITICAL DATA SUMMARY FORM: TOXICITY

Contract ID. _____ Technical Directive or Project ID. _____ Site ID. _____

[illegible]^d Viability Index (see EPA 600/7-77-043)

^bATP (see EPA 600/7-77-043 for derivation)

Protein (see EPA 600/7-77-043 for derivation)

^dToxic signs are identified in a numbered list in EPA 600/7-77-043. Only the number is reported here

TABLE 5

ECOLOGICAL EFFECTS CRITICAL DATA SUMMARY FORM

Contract No. _____

Technical Directive or Project No. _____

Site sampled _____

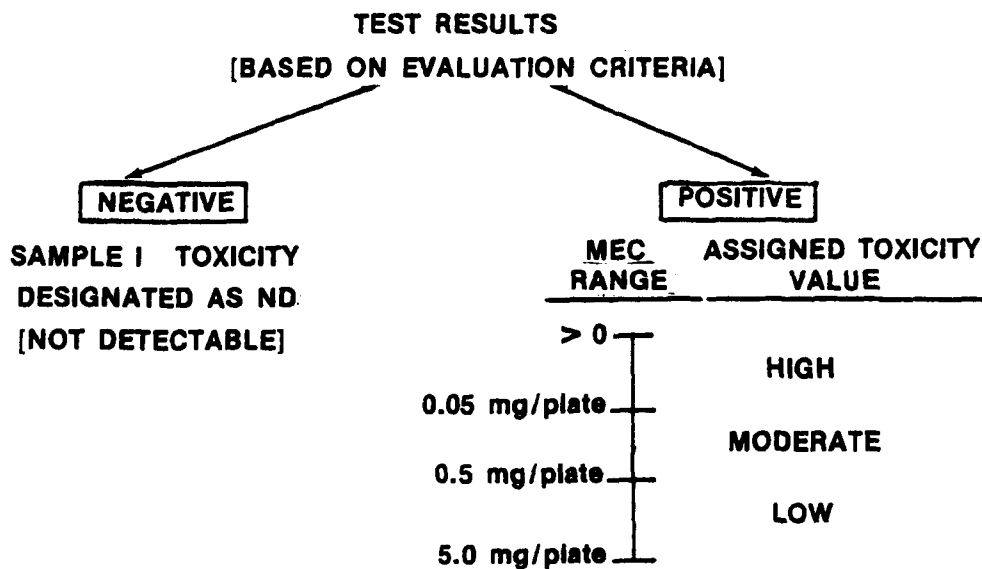
[illegible]

^a The derivations of all test values are explained in the text of the report of the Level 1 manual.

BIOASSAY SUMMARY

Contract No. _____

ND = No Detectable Toxicity
L = Low Toxicity
M = Moderate Toxicity
H = High Toxicity



The Maximum Applicable Dose [MAD] is set at 5 mg of substance per plate.

Figure 7.1 AMES TEST

<u>LD50 or EC50 RANGE</u>	<u>ASSIGNED TOXICITY VALUE</u>
> 0	HIGH
0.01 mg/ml	MODERATE
0.1 mg/ml	LOW
1.0 mg/ml	NOT DETECTABLE
>1.0 mg/ml	

1. CHO cells use viability index [EC50].
2. For RAM or WI-38 assays use the EC50 value from the most sensitive parameter [viability, ATP, Protein].

Figure 7.2 IN VITRO CYTOTOXICITY ASSAYS

LD50 [g/kg] RANGE	ASSIGNED TOXICITY VALUE
> 0	HIGH
0.1 g/kg	MODERATE
1.0 g/kg	LOW
10 g/kg	NOT DETECTABLE
>10 g/kg	

The Maximum Applicable Dose [MAD] is set at 10 grams of substance per kilogram of rat body weight.

Figure 7.3 RODENT TOXICITY ASSAY

IV. ABBREVIATIONS USED

MDC = Minimum Detectable Concentration
MAD = Maximum Applied Dose
LD50 = Lethal Dose to 50% of the Test Organisms
LC50 = Lethal Concentration for 50% of the Test Organisms
EC50 = Effective Concentration for 50% of the Test Organisms
MEC = Minimum Effective Concentration
LRPC = Lowest Recorded Positive Concentration

V. APPLICATION OF THE MAXIMUM APPLIED DOSE CONCEPT FOR ASSIGNMENT OF TOXIC LEVELS

By examination of already existing pilot study reports (supplied by EPA), test results were formatted using the methods described in this report. Numerous parameters could be considered in this data transfer, but we proposed a simple route relying primarily on LD50, LC50, EC50, or MEC. The data analyses given in reports submitted to EPA by various testing laboratories were amenable to this approach, and data conversion into toxic levels was accomplished readily.

Summaries of results from the application of this approach are shown in Tables 7, 11, and 15.

Legend to Tables 7, 11, and 15

These tables are a summary of EPA submitted data for Level 1 Pilot Study Bioassays.

The data were obtained from bioassays of various water effluents (Table 7), fluidized bed combustion samples (Table 11), and coal gasification procedures (Table 15).

Toxicity is defined as:

H = High

M = Moderate

L = Low

ND = Nondetectable

Tables 8, 9, 10, and 12, 13, 14, and 16, 17, 18 are intermediate summary tables. Reported values are given as concentration based on Table 1.

TABLE 7
BIOASSAY SUMMARY

Technical Directive or Project No. Water Effluent Study

Contract No. Pilot Study Results

Sample Identification	Fresh Water										Marine			Notes
	Salmonella	Ram Cytotoxicity	WI-38 Cytotoxicity	CHO Cytotoxicity	Rodent Toxicity	Fish	Invertebrate	Algal	Fish	Invertebrate	Algal	Plant Stress	Ethylene Soil Test	
A2	ND				ND	H	H	L	M	M				
B2	ND				ND	ND	ND	M	ND	ND	ND			
C2	ND	M			ND	M	M	ND	M	L	L			
D2	ND				ND									
E2	ND				ND	ND	H	H			M			
F2	ND				ND	ND	L		ND	ND	M			
G2	ND				ND	M	M	ND	ND	ND	M			
H2	ND				ND			L						
J2	ND				ND	ND	ND	ND						
K2	ND				ND	ND	ND	ND	ND	ND	L			
L2	ND	L	L	M	ND	M	M	M	L	ND	H			
M2	ND	L	ND	ND	ND	ND	M	ND						CHO >200 µl
N2	ND	H	L	L	ND	M	H	H	M	M	H			
P2	ND				ND	ND	ND	M			H			
R2	ND	ND	ND	M	ND	H	H	L						
S2	ND				ND	ND		ND	ND	ND	ND			
T2	ND				ND	M	ND	ND	M		L			
U2	ND				ND	ND	H	ND	ND	ND	ND			
V2	ND				ND	M	H	ND			L			
W2	ND				ND	M	H	L	M	M	M			
X2	ND				ND	ND	ND	ND	ND	ND	ND			
Y2	ND				ND	ND	ND	ND						
Z2	ND				ND	ND	M	H						

ND = No Detectable Toxicity
L = Low Toxicity
M = Moderate Toxicity
H = High Toxicity

Data transition sheets for all results not shown in Section VIII.

Contract No. Pilot Site Sampled WE
Technical Directive or Project No. NA

^a given as + or - based on criteria in Appendix A
^b LRPC = lowest recorded positive concentration (NA for negative data)

TABLE 9

ECOLOGICAL EFFECTS CRITICAL DATA SUMMARY FORM

Contract No. Pilot Technical Directive or Project No. NA Site sampled WE

Sample Identification	Fresh Water Toxicity Tests ^a			Marine Toxicity Tests ^a		
	Fish LC50	Invertebrate LC50	ALGAL EC50	Fish LC50	Invertebrate LC50	ALGAL EC50
A2	19%	9%	76%	60%	25%	
B2	>100%	>100%	30%	>100%	>100%	>100%
C2	46.5%	41.0%	>100%	70%	>100%	90%
D2						
E2	>100%	7.8%	2%			25%
F2	>100%	81.7%		>100%	>100%	70%
G2	64.7%	62.4%		>100%	>100%	59%
H2			96%			
J2	> 100%	>100%	>100%			
K2	>100%	>100%	>100%	>100%	>100%	77%
L2	25.5%	28.0%	42.0%	>100%	>100%	2%
M2	>100%	60%	>100%			
N2	48.8%	10%	2%	40%	25%	2%
P2	>100%	>100%	43%			9%
R2	16.5%	8%	93%			
S2	>100%		>100%	>100%	>>100%	>100%
T2	46.5%	>100%	>100%	70%		76%
U2	>100%	12.1%	>100%	>100%	>100%	>100%
V2	36%	9.4%	>100%			94%
W2	55.2%	6.3%	94%	56%	25%	50%
X2	>100%	>100%	>100%	>100%	>100%	>100%
Y2	>100%	>100%	>100%			
Z2	>100%	42.6%	18%			

^aThe derivations of all test values are explained in the test of the report of the Level I manual.

TABLE 10

HEALTH EFFECTS CRITICAL DATA SUMMARY FORM: TOXICITY

Contract ID. Pilot Technical Directive or Project ID. NA Site ID. WE

Sample Identification	CHO Toxicity	RAM Cell Toxicity			WI-38 Cell Toxicity			Rodent Toxicity	
	LC50	Viability ^a	ATP ^b	Protein ^c	Viability ^a	ATP ^b	Protein ^c	LD50	Toxic Signs ^d
A2								10g/kg	None
B2								10g/kg	None
C2			33.5 μ l					10g/kg	None
D2								10g/kg	None
E2								10g/kg	None
F2								10g/kg	None
G2								10g/kg	None
H2								10g/kg	None
J2								10g/kg	None
K2								10g/kg	None
L2	60 μ l		357 μ l			300 μ l	-	10g/kg	None
M2	>200 μ l	400 μ l			>600 μ l	>600 μ l	>600 μ l	10g/kg	None
N2	75 μ l	13.3 μ l	175 μ l			200 μ l		10g/kg	None
P2								10g/kg	None
R2	37 μ l	>600 μ l	>600 μ l	>600 μ l	>600 μ l	>600 μ l	>600 μ l	10g/kg	None
S2								10g/kg	None
T2								10g/kg	None
U2								10g/kg	None
V2								10g/kg	None
W2								10g/kg	None
X2								10g/kg	None
Y2								10g/kg	None
Z2								10g/kg	None

^aviability Index (see EPA 600/7-77-043)^bATP (see EPA 600/7-77-043 for derivation)^cProtein (see EPA 600/7-77-043 for derivation)^dToxic signs are identified in a numbered list in EPA 600/7-77-043. Only the number is reported here

TABLE 11
BIOASSAY SUMMARY

Technical Directive or Project No. Fluidized Bed Combustion (FBC)

Contract No. Pilot Study Data

[illegible]

ND = No Detectable Toxicity

L = Low Toxicity

M = Moderate Toxicity

H = High Toxicity

HEALTH EFFECTS CRITICAL DATA
SUMMARY FORM: MUTAGENICITY

Contract No. Pilot Site Sampled FBC
 Technical Directive or Project No. NA

Sample Identification	Salmonella Plate Assay	
	Response ^a	LRPC ^b
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[illegible]

^agiven as + or - based on criteria in Appendix A

^a given as + or - based on criteria in Appendix A
^b LRPC = lowest recorded positive concentration (Negative data = NA)

HEALTH EFFECTS CRITICAL DATA SUMMARY FORM: TOXICITY

[illegible]

^dToxic signs are identified in a numbered list in EPA 600/7-77-043. Only the number is reported here

TABLE 14

ECOLOGICAL EFFECTS CRITICAL DATA SUMMARY FORM

Contract No. Pilot Technical Directive or Project No. NA Site sampled FBC

Sample Identification	Fresh Water Toxicity Tests ^a			Marine Toxicity Tests ^a		
	Fish LC50	Invertebrate LC50	ALGAL EC50	Fish LC50	Invertebrate LC50	ALGAL EC50
Spent Bed Leachate	25%	40.9%	45%	>100%	>100%	>100%
Dolomite Leachate	>100%	>100%	>100%	>100%	74.8%	56%
Flyash Leachate	>100%	>100%	>100%	>100%	72.5%	>100%
Coal Leachate	92%	70.9%	19%	11.5%	66%	>100%
Slurry	67.9%	13.6%	9%	70.5%	18%	62%
Cyclone Discard	>100%	>100%	>100%			
Leachate						

^aThe derivations of all test values are explained in the text of the report of the Level 1 manual.

TABLE 15
BIOASSAY SUMMARY

Technical Directive or Project No. Coal Gasification (CG)

Contract No. Pilot Study Data

[illegible]

ND = No Detectable Toxicity
L = Low Toxicity
M = Moderate Toxicity
H = High Toxicity

*Data from all samples designated Separator Liquor combined.

Contract No. Pilot Site Sampled CG
 Technical Directive or Project No. NA

^a given as + or - based on criteria in Appendix A
^b LRPC = lowest recorded positive concentration (Negative data = NA)

TABLE 17

HEALTH EFFECTS CRITICAL DATA SUMMARY FORM: TOXICITY

Contract ID. Pilot Technical Directive or Project ID. NA Site ID. CG

[illegible]^d Viability Index (see EPA 600/7-77-043)

^bATP (see EPA 600/7-77-043 for derivation)

Protein (see EPA 600/7-77-043 for derivation)

^dToxic signs are identified in a numbered list in EPA 600/7-77-043. Only the number is reported here

TABLE 18

ECOLOGICAL EFFECTS CRITICAL DATA SUMMARY FORM

Contract No. Pilot Technical Directive or Project No. NA Site sampled CG

Sample Identification	Fresh Water Toxicity Tests ^a			Marine Toxicity Tests ^a		
	Fish LC50	Invertebrate LC50	ALGAL EC50	Fish LC50	Invertebrate LC50	ALGAL EC50
Separator Liquor	<1.0%	<1.0%	<1.0%	<1.0%	<1.0%	<1.0%

^aThe derivations of all test values are explained in the text of the report of the Level 1 manual.

VI. DATA ORIGIN

<u>SOURCE</u>	<u>CONTRACT #</u>	<u>DATE</u>
Northrop Services, Inc.	68-02-2566	1978
EG&G Bionomics	-	1977
SRI	68-01-2458	1977
SRI	-	1978
Battelle	68-02-2138	1977
Litton Bionetics, Inc.	-	1977
Monsanto Res. Corp.	68-02-1874	1978

VII. AMES TEST DATA INTERPRETATION CRITERIA

A. Evaluation Criteria

Plate test data consist of direct revertant colony counts obtained from a set of selective agar plates seeded with populations of mutant cells suspended in a semisolid overlay (Ames et al., 1975). Because the test chemical and the cells are incubated in the overlay for 2 days, and a few cell divisions occur during the incubation period, the test is semi-quantitative in nature. Although these features of the assay reduce the quantitation of results, they provide certain advantages not contained in a quantitative suspension test:

- The small number of cell divisions permits potential mutagens to act on replicating DNA, which is often more sensitive than nonreplicating DNA.
- The combined incubation of the compound and the cells in the overlay permits constant exposure of the indicator cells for 2 days.

1. Surviving Populations

Plate test procedures do not permit exact quantitation of the number of cells surviving chemical treatment. At low concentrations of the test chemical, the surviving population on the treatment plate is essentially the same as that on the negative control plate. At high concentrations, the surviving population is usually reduced by some fraction. Our protocol normally employs several doses ranging over 2 or 3 log concentrations, the highest of these doses being selected to show slight toxicity as determined by subjective criteria.

2. Dose-Response Phenomena

The demonstration of dose-related increases in mutant counts is an important criterion in establishing mutagenicity. A factor that might modify dose-response results for a mutagen would be the selection of doses that are too low (usually mutagenicity and toxicity are related). If the highest dose is far lower than a toxic concentration, no increases may be observed over the dose range selected. Conversely, if the lowest dose employed is highly cytotoxic, the test chemical may kill any mutants that are induced, and the compound will not appear to be mutagenic.

3. Control Tests

Positive and negative control assays are conducted with each experiment and consist of direct-acting mutagens for nonactivation assays and mutagens that require metabolic biotransformation in activation assay. Negative controls consist of the test compound solvent in the overlay agar together with the other essential components. The negative control plate for each strain gives a reference point to which the test data are compared. The positive control assay is conducted to demonstrate that the test systems are functional with known mutagens.

4. Evaluation Criteria for Ames Assay

Because the procedures used to evaluate the mutagenicity of the test chemical are semiquantitative, the criteria used to determine positive effects are inherently subjective and are based primarily on a historical data base. Most data sets are evaluated using the following criteria:

a. Strains TA-1535, TA-1537, and TA-1538

If the solvent control value is within the normal range, a chemical that produces a positive dose response over three concentrations with the lowest increase equal to twice the solvent control value is considered to be mutagenic.

b. Strains TA-98 and TA-100

If the solvent control value is within the normal range, a chemical that produces a positive dose response over three concentrations with the highest increase equal to twice the solvent control value for TA-100 and two to three times the solvent control value for strain TA-98 is considered to be mutagenic. For these strains, the dose-response increase should start at approximately the solvent control value.

c. Pattern

Because TA-1535 and TA-100 are both derived from the same parental strain (G-46) and because TA-1538 and TA-98 are both derived from the same parental strain (D3052), there is a built-in redundancy in the microbial assay. In general, the two strains of a set respond to the same mutagen and such a pattern is sought. It is also anticipated that if a given strain, e.g., TA-1537, responds to a mutagen in nonactivation tests, it will generally do so in activation tests. (The converse of this relationship is not expected). While similar response patterns are not required for all mutagens, they can be used to enhance the reliability of an evaluation decision.

d. Reproducibility

If a chemical produces a response in a single test that cannot be reproduced in one or more additional runs, the initial positive data lose significance.

The preceding criteria are not absolute, and other extenuating factors may enter into a final evaluation decision. However, these criteria are applied to the majority of situations and are presented to aid those individuals not familiar with this procedure. As the data base is increased, the criteria for evaluation can be more firmly established.

5. Relationship Between Mutagenicity and Carcinogenicity

It must be emphasized that the Ames Salmonella/Microsome Plate Test is not a definitive test for chemical carcinogens. It is recognized, however, that correlative and functional relationships have been demonstrated between these two end points. The results of comparative tests on 300 chemicals by McCann et al. (1975) show an extremely good correlation between results of microbial mutagenesis tests and in vivo rodent carcinogenesis assays.

B. References

Ames, B.N., McCann, J. and Yamasaki, E. (1975). Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test. Mutation Res. 31, 347-364.

McCann, J., Choi, E., Yamasaki, E. and Ames, B.N. (1975). Detection of carcinogens as mutagens in the Salmonella/microsome test: Assay of 300 chemicals. Proc. Nat. Acad. Sci. 72, 5135-5139.

VIII. DATA CONVERSION SHEETS FOR TABLES 7, 11, AND 15

Abbreviations of Sample Source

FBC = Fluidized Bed Combustion

CG = Coal Gasification

WE = Water Effluent Samples

Organisms Employed in Aquatic Tests

• Freshwater

Fish Fathead Minnow (Pimephates promelas)

Algae Selenastrum capricornutum

Invertebrate Daphnia Magna

• Marine

Fish Sheepshead Minnow (Cyprinodon variegatus)

Algae Skeletonema costatum

Invertebrate Grass Shrimp (Palaemonetes pugio)

PRIMARY
DATA TRANSITION FORM

FBC

ASSAY: Grass shrimp SAMPLE: Coal leachate
MAD: 100%

RESULTS:

LD50 (EC50) VALUE^a 96 hour LC50 = 66% (or) AMES RESPONSE _____
MEC^b _____

APPROXIMATE
CONCENTRATION FACTOR 0
LEVEL OF TOXICITY^c Moderate

COMMENTS:

24 hr toxicity = 75% effluent

48 hr toxicity = 72.5% effluent. Diss. O₂ = 42% of saturation

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

FBC

ASSAY: Grass shrimp SAMPLE: Flyash leachate
MAD: 100%

RESULTS:

LD50 (EC50) VALUE^a LC50 = 72.5% E at 96 hr. (or) AMES RESPONSE _____
MEC^b _____

APPROXIMATE
CONCENTRATION FACTOR 0
LEVEL OF TOXICITY^c Moderate

COMMENTS:

24 and 48 hr. LC50's = 75% E

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

FBC

ASSAY: Grass Shrimp SAMPLE: Dolomite Leachate
MAD: 100% E

RESULTS:

LD50 (EC50) VALUE ^a LC50 = 74.8% E at 96 hr. (or) AMES RESPONSE _____
MEC ^b _____

APPROXIMATE
CONCENTRATION FACTOR 0
LEVEL OF TOXICITY ^c L

COMMENTS:

24 and 48 hr. LC50's = 100% E.

E = Effluent

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

FBC

ASSAY: Grass Shrimp SAMPLE: N 9661 (Slurry)
MAD: 100% E

RESULTS:

LD50 (EC50) VALUE ^a LC50 = 18% at 96 hr. (or) AMES RESPONSE _____
MEC ^b _____

APPROXIMATE
CONCENTRATION FACTOR 0
LEVEL OF TOXICITY ^c High

COMMENTS:

48 hr. LC50 = 34.5% Effluent.

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

FBC

ASSAY: Grass shrimp SAMPLE: Spentbed leachate
MAD: 100% Effluent

RESULTS:

LD50 (EC50) VALUE^a Not Toxic >100% (or) AMES RESPONSE _____
MEC^b _____

APPROXIMATE
CONCENTRATION FACTOR 0
LEVEL OF TOXICITY^c ND

COMMENTS: Not detectable

Diss. O_2 = 55% of saturation at all test levels.

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

FBC

ASSAY: Fish toxicity SAMPLE: Dolomite leachate
MAD: 100%

RESULTS:

LD50 (EC50) VALUE^a Not toxic at 100% (or) AMES RESPONSE _____
MEC^b _____

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY^c ND

COMMENTS: Not detected

No toxicity after 96 hr. exposure. Diss. O₂ after 96 hrs. ranged from 26% of saturation in 10% of effluent to 48% of saturation in the control and 100% effluent.

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

FBC

ASSAY: Fish toxicity SAMPLE: N 9 661 (Slurry)
MAD: 100%

RESULTS:

LD50 (EC50) VALUE^a LC50 = 70.5% at 19 hr. (or) AMES RESPONSE _____
MEC^b _____

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY^c Moderate

COMMENTS:

Not toxic at 24 and 48 hr exposures.

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

FBC

ASSAY: Marine Fish toxicity SAMPLE: Coal leachate
MAD: 100%

RESULTS:

LD50 (EC50) VALUE^a LC50 = 71.5% effluent (or) AMES RESPONSE _____
MEC^b _____

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY^c Moderate

COMMENTS:

All three time periods: 24-, 48- and 96 hrs. gave LC50's of 71.5% effluent.
Diss. O₂ = 39% - 62% of saturation.

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

FBC

ASSAY: Marine Fish toxicity SAMPLE: Flyash leachate
MAD: 100%

RESULTS:

LD50 (EC50) VALUE^a Not toxic at 96 hrs. (>100%) (or) AMES RESPONSE _____
MEC^b _____

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY^c NB

COMMENTS: Not detectable

Diss O₂ = 48% of saturation in all tests.

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

FBC

ASSAY: Marine Fish toxicity SAMPLE: Spent bed leachate
MAD: 100%

RESULTS:

LD50 (EC50) VALUE^a Not toxic at 100% (or) AMES RESPONSE _____
MEC^b _____

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY^c ND

COMMENTS: Not detected

No mortality after 96 hr. exposure. Diss. O₂ \geq 57% of saturation
in all test levels.

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

FBC

Freshwater
ASSAY: Algal/DAPHNIA/Fish SAMPLE: Dolomite Leachate
MAD: 100%

RESULTS:

LD50 [EC50] VALUE^a > 100%/> 100%/ > 100% [or] AMES RESPONSE _____
MEC^b _____

APPROXIMATE
CONCENTRATION FACTOR 0 / 0 / 0

LEVEL OF TOXICITY^c ND / ND / ND

COMMENTS: Not detectable

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

FBC

Freshwater
ASSAY: Algal/DAPHNIA/Fish SAMPLE: Spent Bed Leachate
MAD: 100%

RESULTS:

LD50 [EC50] VALUE^a - 45%/40.9/25 [or] AMES RESPONSE _____
MEC^b _____

APPROXIMATE
CONCENTRATION FACTOR 0 / 0 / 0

LEVEL OF TOXICITY^c Moderate/Moderate/Moderate

COMMENTS:

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

FBC

ASSAY: Freshwater
Algal/DAPHNIA/Fish SAMPLE: Flyash Leachate
MAD: 100%

RESULTS:

LD50 [EC50] VALUE^a > 100% / > 100% / > 100% [or] AMES RESPONSE _____
MEC^b _____

APPROXIMATE
CONCENTRATION FACTOR 0 / 0 / 0

LEVEL OF TOXICITY^c ND / ND / ND

COMMENTS: Not detectable

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

FBC

ASSAY: Freshwater
Algal/DAPHNIA/Fish SAMPLE: Coal Leachate
MAD: 100%

RESULTS:

LD50 [EC50] VALUE^a 19% / 70.9 / 92% [or] AMES RESPONSE _____
MEC^b _____

APPROXIMATE
CONCENTRATION FACTOR 0 / 0 / 0

LEVEL OF TOXICITY^c High / Moderate / Low

COMMENTS:

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

FBC

ASSAY: Freshwater Algal/DAPHNIA/Fish SAMPLE: Scrubber Slurry
MAD: 100%

RESULTS:

LD50 (EC50) VALUE^a 9% / 31.6% / 67.9% (or) AMES RESPONSE _____
MEC^b _____

APPROXIMATE
CONCENTRATION FACTOR 0 / 0 / 0

LEVEL OF TOXICITY^c High / Moderate / Moderate

COMMENTS:

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

FBC

ASSAY: Marine Alga SAMPLE: Spent bed leachate
MAD: 100%

RESULTS:

LD50 [EC50] VALUE^a Not Toxic >100% (or) AMES RESPONSE _____
MEC^b _____

APPROXIMATE
CONCENTRATION FACTOR 0
LEVEL OF TOXICITY^c ND

COMMENTS:

Optical density increased by effluent.

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

FBC

ASSAY: Marine Alga SAMPLE: Coal leachate
MAD: 100%

RESULTS:

LD50 [EC50] VALUE^a Unaltered effluent
Not toxic (or) AMES RESPONSE _____
MEC^b _____

APPROXIMATE
CONCENTRATION FACTOR 0
LEVEL OF TOXICITY^c ND

COMMENTS: Not detectable

Filtered effluent gave EC50's at 98, 39 and 38% effluent for
4, 8 and 12-day exposures.

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

FBC

ASSAY: Marine Alga SAMPLE: N 9661
MAD: 100%

RESULTS:

LD50 [EC50] VALUE^a EC50 62% of effluent at 4 days (or) AMES RESPONSE _____
MEC^b _____

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY^c Moderate

COMMENTS:

The EC50's reported for unaltered effluent were 62% for 4 days and 66% for 12 days exposure. Filtered effluent was more active than unaltered effluent with EC50's of 22 and 15% for 4 and 12 days.

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

FBC

ASSAY: Marine alga SAMPLE: Dolomite leachate
MAD: 100%

RESULTS:

LD50 [EC50] VALUE^a EC50 = 56% effluent (or) AMES RESPONSE _____
MEC^b _____

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY^c Moderate

COMMENTS:

Filtered effluent gave EC50's at 19% and 24% for 8 and 12 days respectively.

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

FBC

ASSAY: Marine Alga SAMPLE: Flyash leachate
MAD: 100% effluent

RESULTS:

LD50 [EC50] VALUE^a Not toxic >100% (or) AMES RESPONSE _____
MEC^b _____

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY^c NO

COMMENTS: Not detectable

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

FBC

ASSAY: Rodent toxicity SAMPLE: Fly Ash
MAD: 10 g/kg

RESULTS:

LD50 [EC50] VALUE^a > 10 g/kg [or] AMES RESPONSE _____
MEC^b _____

APPROXIMATE
CONCENTRATION FACTOR 0
LEVEL OF TOXICITY^c Nondetectable

COMMENTS:

No toxic sign reported.

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

FBC

ASSAY: Rodent toxicity SAMPLE: Dolomite
MAD: 10 g/kg

RESULTS:

LD50 [EC50] VALUE^a 10 g/kg [or] AMES RESPONSE _____
MEC^b _____

APPROXIMATE
CONCENTRATION FACTOR 0
LEVEL OF TOXICITY^c Nondetectable

COMMENTS:

No toxic sign reported.

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

FBC

ASSAY: Rodent Toxicity SAMPLE: Spent Bed Dolomite
MAD: 10 g/kg

RESULTS:

LD50 [EC50] VALUE ^a > 10 g/kg (or) AMES RESPONSE _____
MEC ^b _____

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY ^c Nondetectable

COMMENTS:

No toxic signs reported.

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

FBC

ASSAY: Rodent Toxicity SAMPLE: Spent Bed Leachate
MAD: 10 g/kg or 10 ml/kg

RESULTS:

LD50 [EC50] VALUE ^a > 10 ml/kg (or) AMES RESPONSE _____
MEC ^b _____

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY ^c Nondetectable

COMMENTS:

No toxic signs reported.

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

FBC

ASSAY: RAM SAMPLE: Slurry (Fly Ash)
MAD: 600 μ l/ml

RESULTS:

LD50 [EC50] VALUE^a 146 μ l/ml (ATP fg/cell) [or] AMES RESPONSE _____
MEC^b _____

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY^c Low

COMMENTS:

Range of EC50s for all parameters = 146 - > 1000 μ l.

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

FBC

ASSAY: RAM SAMPLE: #7 - 3 μ Cyclone
MAD: 1000 μ g/ml

RESULTS:

LD50 [EC50] VALUE^a >1000 μ g/ml [or] AMES RESPONSE _____
MEC^b _____

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY^c Nondetectable

COMMENTS: All parameters

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

FBC

ASSAY: RAM SAMPLE: #6 - 1 μ filter Cyclone
MAD: 1000 μ g/ml

RESULTS:

LD50 [EC50] VALUE^a 596 μ g/ml (ATP fg/cell) [or] AMES RESPONSE _____
MEC^b _____

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY^c Low

COMMENTS: ND in other parameters

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive)

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

FBC

ASSAY: RAM SAMPLE: Dolomite
MAD: 600 μ l or 1000 μ g/ml

RESULTS:

LD50 [EC50] VALUE^a > 1000 μ g/ml [or] AMES RESPONSE _____
MEC^b _____

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY^c Nondetectable

COMMENTS: All parameters

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

FBC

ASSAY: RAM SAMPLE: Spent bed Dolomite
MAD: 600 μ l or 1000 μ g/ml

RESULTS:

LD50 [EC50] VALUE^a >1000 μ g/ml (or) AMES RESPONSE _____
MEC^b _____

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY^c Nondetectable

COMMENTS: All parameters

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

FBC

ASSAY: RAM SAMPLE⁽³⁾: Spent bed leachate
MAD: 600 μ l or 1000 μ g/ml Flyash leachate
Flyash

RESULTS:

LD50 [EC50] VALUE^a > 1000 μ g or μ l/ml (or) AMES RESPONSE _____
MEC^b _____

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY^c Nondetectable

COMMENTS: All parameters

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

FBC

ASSAY: Salmonella SAMPLE: Spent Bed Dolomite
MAD: 5 mg/plate

RESULTS:

LD50 (EC50) VALUE^a _____ (or) AMES RESPONSE Negative
MEC^b _____

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY^c Nondetectable

COMMENTS:

Maximum concentration tested was 5 mg/plate.

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

FBC

ASSAY: Salmonella SAMPLE: Fly Ash
MAD: 5 mg/plate

RESULTS:

LD50 (EC50) VALUE^a _____ (or) AMES RESPONSE Negative
MEC^b _____

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY^c Nondetectable

COMMENTS:

Maximum concentration used was 5 mg/plate.

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

FBC

ASSAY: Salmonella SAMPLE: Dolomite
MAD: 5 mg/plate

RESULTS:

LD50 (EC50) VALUE^a _____ (or) AMES RESPONSE Negative
MEC^b _____

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY^c Nondetectable

COMMENTS:

Maximum concentration tested was 5 mg/plate.

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

FBC

ASSAY: Salmonella SAMPLE: 3u Cyclone Coarse Particulate
MAD: 5 mg/plate

RESULTS:

LD50 (EC50) VALUE^a _____ (or) AMES RESPONSE Positive
MEC^b 2 mg/plate

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY^c Low

COMMENTS:

Active in strains TA-1538 and TA-98. Sample was more active without S9 than with S9 at equivalent concentrations.

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

FBC

RESULTS:

LEVEL OF TOXICITY^c Low

COMMENTS:

The sample was active in strains TA-1538 and TA-98. More activity was obtained in nonactivation tests (-S9) than in activation tests (+S9) at equivalent concentrations.

^aFor the NAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

CG

ASSAY: Marine Algal Assays SAMPLE: Holston Separator Liquor
MAD: 100 mg/l or 100%

RESULTS:

LD50 (EC50) VALUE^a 0.89% (4 days), 0.53% (8 days), 0.41% (12 days) [or] AMES RESPONSE _____
MEC^b _____

APPROXIMATE
CONCENTRATION FACTOR Filtered

LEVEL OF TOXICITY^c High

COMMENTS:

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

CG

ASSAY: FW Algal Assays SAMPLE: Radian Separator Liquor
MAD: 100 mg/liter or 100%

RESULTS:

LD50 [EC50] VALUE^a 0.1 - 1% [or] AMES RESPONSE _____
MEC^b _____

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY^c High

COMMENTS:

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

CG

ASSAY: FW Algal Assays SAMPLE: Holston Separator Liquor
MAD: 100 mg/liter or 100%

RESULTS:

LD50 [EC50] VALUE^a 1.9% (4 days), 0.57% (8 days),
0.53% (12 days) [or] AMES RESPONSE _____
MEC^b _____

APPROXIMATE
CONCENTRATION FACTOR Unfiltered

LEVEL OF TOXICITY^c High

COMMENTS:

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

CG

ASSAY: WI-38 SAMPLE: 655 - 5/6
MAD: 600 μ l or 1000 μ g/ml

RESULTS:

LD50 (EC50) VALUE^a $1.3 \times 10^{-4}\%$ (Viability Index) [or] AMES RESPONSE _____
MEC^b _____

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY^c (High)

COMMENTS:

Cannot interpret concentration relative to established MAD.

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration [if response is positive].

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

CG

ASSAY: WI-38 SAMPLE: 590 - 5/6
MAD: 600 μ l or 1000 μ g/ml

RESULTS:

LD50 (EC50) VALUE^a $5.1 \times 10^{-4}\%$ (Viability Index) [or] AMES RESPONSE _____
MEC^b _____

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY^c (High)

COMMENTS:

Cannot interpret concentration relative to established MAD.

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration [if response is positive].

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

CG

ASSAY: WI-38 SAMPLE: Separator Liquor
HB - 013
MAD: 600 μ l/ml or 1000 μ g/ml

RESULTS:

LD50 [EC50] VALUE^a 7.4 μ l/ml (Viability Index) [or] AMES RESPONSE _____
MEC^b _____

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY^c Moderate

COMMENTS:

MAD was 60 μ l/ml for this sample.

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration [N response is positive].

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

CG

ASSAY: FW Fish Test SAMPLE: Holston Separator Liquor
MAD: 100 mg/l or 100%

RESULTS:

LD50 (EC50) VALUE^a 0.23% (24 hrs), 0.20% (48 hrs), 0.16% (96 hrs) (or) AMES RESPONSE _____
MEC^b _____

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY^c High

COMMENTS:

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

CG

ASSAY: Freshwater Fish Test SAMPLE: Radian Separator Liquor
MAD: 100 mg/liter or 100%

RESULTS:

LD50 (EC50) VALUE^a 0.02% (96 hrs) (or) AMES RESPONSE _____
MEC^b _____

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY^c High

COMMENTS:

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

CG

ASSAY: Daphnia Assay SAMPLE: Radian Separator Liquor
MAD: 100 mg/liter or 100%

RESULTS:

LD50 [EC50] VALUE^a 0.51% (24 hrs) - 0.11% (48 hrs) [or] AMES RESPONSE _____
MEC^b _____

APPROXIMATE
CONCENTRATION FACTOR 0
LEVEL OF TOXICITY^c High

COMMENTS:

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (N response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

CG

ASSAY: Salmonella SAMPLE: HB-019 (HAAP cyclone dust)
MAD: 5 mg/plate

RESULTS:

LD50 (EC50) VALUE ^a _____ (or) AMES RESPONSE Negative
MEC ^b _____

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY ^c Nondetectable

COMMENTS:

Maximum concentration tested was 5 mg/plate.

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

CG

ASSAY: Salmonella SAMPLE: HB - 018 (HAAP ash)
MAD: 5 mg/plate

RESULTS:

LD50 (EC50) VALUE ^a _____ (or) AMES RESPONSE Negative
MEC ^b _____

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY ^c Nondetectable

COMMENTS:

Maximum concentrate tested was 5 mg/plate.

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive)

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

CG

ASSAY: Salmonella SAMPLE: Coal
MAD: 5 mg/plate

RESULTS:

LD50 (EC50) VALUE^a _____ (or) AMES RESPONSE Negative
MEC^b _____

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY^c Nondetectable

COMMENTS:

Maximum concentration tested was 5 mg/plate.

^aFor the NAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (H response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

CG

ASSAY: Salmonella SAMPLE: HB - 020 (HAAP Coal)
MAD: 5 mg/plate

RESULTS:

LD50 (EC50) VALUE^a _____ (or) AMES RESPONSE Negative
MEC^b _____

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY^c Nondetectable

COMMENTS:

Did not meet preestablished criteria for mutagenicity. However, the report indicated this sample was weakly mutagenic for strains TA-98 and TA-100. A concentration of 10 mg/plate produced less than 2-fold increases over the solvent control for both strains (1.5 - 1.8).

^aFor the NAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (H response is positive).

^cSee Table 1 for definition of toxicity levels.

CG

RESULTS:

LEVEL OF TOXICITY ^c Low

This sample was mutagenic for TA-1537, TA-1538, TA-98 and TA-100 only under conditions of activation (+S9).

^cSee Table 1 for definition of toxicity levels.

CG

RESULTS:

LEVEL OF TOXICITY^c Nondetectable

The sample was tested at a maximum concentration of 5 mg/plate. The submitter's report indicated marginal activity in strain TA-100; however, the increase was less than 1.5 times the spontaneous background and did not meet preestablished criteria for mutagenesis.

^aSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

CG

ASSAY: Salmonella SAMPLE: H8-017 (HAAP Separator liquor)
MAD: 5 mg/plate

RESULTS:

LD50 [EC50] VALUE^a _____ (or) AMES RESPONSE Negative
MEC^b _____

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY^c Nondetectable

COMMENTS:

The maximum concentration tested was 5 mg/plate.

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

CG

ASSAY: Salmonella SAMPLE: H-032 (HAAP Separator tar)
MAD: 5 mg/plate

RESULTS:

LD50 [EC50] VALUE^a _____ (or) AMES RESPONSE Positive
MEC^b 0.01 mg/plate

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY^c High

COMMENTS:

The sample was mutagenic for strains TA-1537, TA-1538,
TA-98 and TA-100.
Activity was observed only with S9 activation.

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

CG

ASSAY: Rodent Toxicity SAMPLE: HAAP Ash
MAD: 10 g/kg

RESULTS:

LD50 [EC50] VALUE^a > 10 g/kg (or) AMES RESPONSE _____
MEC^b _____

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY^c Nondetectable

COMMENTS:

No toxic sign reported.

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

CG

ASSAY: Rodent toxicity SAMPLE: HAAP coal
MAD: 10 g/kg

RESULTS:

LD50 [EC50] VALUE^a > 10 g/kg (or) AMES RESPONSE _____
MEC^b _____

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY^c Nondetectable

COMMENTS:

No toxic sign reported.

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

CG

ASSAY: Rodent Toxicity SAMPLE: Coal

MAD: 10 g/kg

RESULTS:

LD50 [EC50] VALUE^a > 10 g/kg [or] AMES RESPONSE

MEC^b

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY^c Nondetectable.

COMMENTS:

No toxic signs reported.

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

CG

ASSAY: Rodent Toxicity SAMPLE: HAAP Cyclone Dust

MAD: 10 g/kg

RESULTS:

LD50 [EC50] VALUE^a > 10 g/kg [or] AMES RESPONSE

MEC^b

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY^c Nondetectable

COMMENTS:

No toxic sign reported.

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

CG

ASSAY: Rodent Toxicity SAMPLE: HAAP Separator Liquor
MAD: 10 g/kg

RESULTS:

LD50 [EC50] VALUE^a > 10 g/kg (or) AMES RESPONSE
MEC^b

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY^c Nondetectable

COMMENTS:

No toxic signs reported.

^aFor the NAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

CG

ASSAY: RAM SAMPLE: 590-3
MAD: 600 μ l/ml

RESULTS:

LD50 [EC50] VALUE^a 5.3 μ l/ml (ATP fg/cell) (or) AMES RESPONSE _____
MEC^b _____

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY^c High

COMMENTS:

Several parameters showed toxicity in this range
(5.3 - 13.5 for all parameters).

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

CG

ASSAY: RAM SAMPLE: 655-3
MAD: 600 μ l/ml

RESULTS:

LD50 [EC50] VALUE^a 21.2 μ l/ml (Viab. Index) (or) AMES RESPONSE _____
MEC^b _____

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY^c Moderate

COMMENTS:

Range of EC50 for all parameters = 21.2 - 101.7.

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

CG

ASSAY: RAM SAMPLE: Coal

MAD: 600 μ l or 1000 μ g/ml

RESULTS:

LD50 [EC50] VALUE ^a > 1000 μ g/ml [or] AMES RESPONSE _____

MEC ^b _____

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY ^c Nondetectable

COMMENTS: All parameters

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

CG

ASSAY: RAM SAMPLE: 655 - 5/6

MAD: 600 μ l or 1000 μ g/ml

RESULTS:

LD50 [EC50] VALUE ^a 1.9×10^{-4} % viability [or] AMES RESPONSE _____

MEC ^b _____

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY ^c High

COMMENTS:

Cannot interpret data relative to established MAD.

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

CG

ASSAY: RAM SAMPLE: 590 - 5/6
MAD: 600 μ l or 1000 μ g/ml

RESULTS:

LD50 (EC50) VALUE^a > 4 x 10⁻³% (Cell count and protein) (or) AMES RESPONSE _____
MEC^b _____

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY^c (High)

COMMENTS:

Cannot interpret data relative to established MAD.

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

CG

ASSAY: RAM SAMPLE: Coal dust
MAD: 1000 μ g/ml

RESULTS:

LD50 (EC50) VALUE^a > 1000 μ g/ml (or) AMES RESPONSE _____
MEC^b _____

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY^c NO

COMMENTS: All parameters not detectable toxicity

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

CG

ASSAY: RAM SAMPLE: HAAP ASH
HB - 014
MAD: 600 μ l or 1000 μ g/ml

RESULTS:

LD50 [EC50] VALUE ^a > 300 μ g/ml (all parameters) (or) AMES RESPONSE _____
MEC ^b _____

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY ^c Low

COMMENTS:

No data > 300 μ g/ml; thus cannot interpret.

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

CG

ASSAY: RAM SAMPLE: HAAP COAL
HB - 016
MAD: 600 μ l or 1000 μ g/ml

RESULTS:

LD50 [EC50] VALUE ^a > 1000 μ g/ml (all) (or) AMES RESPONSE _____
MEC ^b _____

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY ^c Nondetectable

COMMENTS:

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

CG

ASSAY: RAM SAMPLE: HAAP Cyclone Dust
HB - 015
MAD: 600 μ l or 1000 μ g/ml

RESULTS:

LD50 [EC50] VALUE ^a > 1000 μ g/ml (all) (w) AMES RESPONSE _____
MEC ^b _____

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY ^c Nondetectable

COMMENTS:

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

CG

ASSAY: RAM SAMPLE: Separator Tar
HB - 026
MAD: 600 μ l/1000 μ g/ml

RESULTS:

LD50 [EC50] VALUE ^a 350 μ g/ml (Viab. Index) (w) AMES RESPONSE _____
MEC ^b _____

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY ^c Low

COMMENTS:

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

CG

ASSAY: RAM SAMPLE: Separator Liquor
HB - 013
MAD: 600 μ l or 1000 μ g/ml

RESULTS:

LD50 (EC50) VALUE^a 12 μ l/ml (Viability Index) (or) AMES RESPONSE _____
MEC^b _____

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY^c Moderate

COMMENTS:

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

CG

ASSAY: RAM SAMPLE: Cyclone Dust
MAD: 1000 μ g/ml

RESULTS:

LD50 (EC50) VALUE^a > 1000 μ g/ml (or) AMES RESPONSE _____
MEC^b _____

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY^c ND

COMMENTS: Non detectable toxicity all parameters

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

C6

ASSAY: Grass shrimp test SAMPLE: Holston separator liquor
MAD: 100 mg/liter or 100%

RESULTS:

LD50 (EC50) VALUE^a 0.41% (48 hrs), 0.25% (96 hrs) (or) AMES RESPONSE _____
MEC^b _____

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY^c High

COMMENTS:

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

WE

ASSAY: RAM SAMPLE: R-2-15
MAD: 600 μ l or 1000 μ g/ml

RESULTS:

LD50 [EC50] VALUE^a > 600 μ l/ml (or) AMES RESPONSE _____
MEC^b _____

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY^c Nondetectable

COMMENTS: All parameters

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

WE

ASSAY: RAM SAMPLE: M-2-15
MAD: 600 μ l or 1000 μ g/ml

RESULTS:

LD50 [EC50] VALUE^a ~ 400 μ l/ml (Viab. Index) (or) AMES RESPONSE _____
MEC^b _____

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY^c Low

COMMENTS:

EC50 estimated from raw data.

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

WE

ASSAY: RAM SAMPLE: N-2-15
MAD: 600 μ l or 1000 μ g/ml

RESULTS:

LD50 [EC50] VALUE^a ~ 175 μ l/ml (ATP) (or) AMES RESPONSE _____
MEC^b _____

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY^c Low

COMMENTS:

EC50 estimated from raw data showing EC50 >60 and <200.

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

WE

ASSAY: RAM SAMPLE: L-2-15
MAD: 600 μ l or 1000 μ g/ml

RESULTS:

LD50 [EC50] VALUE^a ~ 300 μ l/ml (ATP) (or) AMES RESPONSE _____
MEC^b _____

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY^c Low

COMMENTS:

EC 50 Estimated from raw data.

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

WE

ASSAY: CHO SAMPLE: R-2-15
MAD: 600 μ l/ml

RESULTS:

LD50 (EC50) VALUE^a 37 μ l/ml (or) AMES RESPONSE _____
MEC^b _____

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY^c Moderate

COMMENTS:

EC50 estimated from raw data.

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (H response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

WE

ASSAY: CHO SAMPLE: N-2-15
MAD: 600 μ l/ml

RESULTS:

LD50 (EC50) VALUE^a 75 μ l/ml (or) AMES RESPONSE _____
MEC^b _____

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY^c Low

COMMENTS:

EC50 estimated from raw data.

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (H response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

WE

ASSAY: CHO SAMPLE: M-2-15
MAD: 600 μ l/ml

RESULTS:

LD50 [EC50] VALUE ^a > 200 μ l/ml [or] AMES RESPONSE
MEC ^b

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY ^c Low

COMMENTS:

200 μ l/ml was highest concentration used. Thus could not determine
ED50 in context of MAD.

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

WE

ASSAY: CHO SAMPLE: L-2-15
MAD: 600 μ l/ml

RESULTS:

LD50 [EC50] VALUE ^a ~ 60 μ l/ml [or] AMES RESPONSE
MEC ^b

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY ^c Low

COMMENTS:

EC50 estimated from raw data. Value slightly > 60 μ l/ml.

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

WE

ASSAY: Marine Algae SAMPLE: F-2
MAD: 100%

RESULTS:

LD50 (EC50) VALUE^a 56 - 75% (or) AMES RESPONSE _____
MEC^b _____

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY^c Moderate

COMMENTS:

LC50 ~ 70% 90% mortality with 100% effluent.

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

WE

ASSAY: WI-38 SAMPLE: R-2-15
MAD: 600 μ l/ml

RESULTS:

LD50 [EC50] VALUE ^a > 600 μ l/ml (or) AMES RESPONSE _____

MEC ^b _____

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY ^c Nondetectable

COMMENTS: All parameters

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

WE

ASSAY: WI-38 SAMPLE: M-2-15
MAD: 600 μ l/ml

RESULTS:

LD50 [EC50] VALUE ^a > 600 μ l/ml (or) AMES RESPONSE _____

MEC ^b _____

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY ^c Nondetectable

COMMENTS: All parameters

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

WE

ASSAY: WI-38 SAMPLE: N-2-15
MAD: 600 μ l/ml

RESULTS:

LD50 (EC50) VALUE^a ~ 200 μ l/ml (ATP) (or) AMES RESPONSE _____
MEC^b _____

APPROXIMATE
CONCENTRATION FACTOR 0
LEVEL OF TOXICITY^c Low

COMMENTS:

EC50 estimated from raw data.

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

WE

ASSAY: WI-38 SAMPLE: L-2-15
MAD: 600 μ l/ml

RESULTS:

LD50 (EC50) VALUE^a ~ 300 μ l/ml (ATP) (or) AMES RESPONSE _____
MEC^b _____

APPROXIMATE
CONCENTRATION FACTOR 0
LEVEL OF TOXICITY^c Low

COMMENTS:

EC50 estimated from raw data showing EC50 >200 μ l and <600 μ l/ml.

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

WE

ASSAY: Grass shrimp SAMPLE: L-2
MAD: 100%

RESULTS:

LD50 [EC50] VALUE^a - >100% [or] AMES RESPONSE

MEC^b

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY^c ND

COMMENTS:

Nontoxic at 100%.

^aFor the NAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

WE

ASSAY: Grass shrimp SAMPLE: N-2
MAD: 100%

RESULTS:

LD50 [EC50] VALUE^a 10 - 32% [or] AMES RESPONSE

MEC^b

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY^c Moderate

COMMENTS:

LC50 about 25%

^aFor the NAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

WE

ASSAY: Grass Shrimp SAMPLE: S-2

MAD: 100%

RESULTS:

LD50 (EC50) VALUE^a - >100% (or) AMES RESPONSE

MEC^b

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY^c ND

COMMENTS:

20% toxicity at 100% effluent

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

WE

ASSAY: Grass shrimp SAMPLE: U - 2

MAD: 100%

RESULTS:

LD50 (EC50) VALUE^a - >100% (or) AMES RESPONSE

MEC^b

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY^c ND

COMMENTS:

Nontoxic at 100%.

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

ASSAY: Grass shrimp SAMPLE: W-2

MAD: 100%

RESULTS:

LD50 (EC50) VALUE^a 18 - 32% (or) AMES RESPONSE _____

MEC^b _____

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY^c Moderate

COMMENTS:

LC50 ~ 25% effluent.

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

WE

ASSAY: Grass shrimp SAMPLE: X-2

MAD: 100%

RESULTS:

LD50 (EC50) VALUE^a - >100% (or) AMES RESPONSE _____

MEC^b _____

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY^c NO

COMMENTS: Not detectable

Nontoxic at 100%

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

WE

ASSAY: Grass shrimp SAMPLE: A-2

MAD: 100%

RESULTS:

LD50 (EC50) VALUE ^a 32 - 18% (or) AMES RESPONSE

MEC ^b

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY ^c Moderate

COMMENTS:

LC50 at about 25% effluent conc.

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

WE

ASSAY: Grass shrimp SAMPLE: B-2

MAD: 100%

RESULTS:

LD50 (EC50) VALUE ^a >100% (or) AMES RESPONSE

MEC ^b

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY ^c ND

COMMENTS:

No toxicity at 100%.

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

WE

ASSAY: Grass shrimp SAMPLE: C-2
MAD: 100%

RESULTS:

LD50 [EC50] VALUE ^a 100% (or) AMES RESPONSE _____
MEC ^b _____

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY ^c LOW

COMMENTS:

100% = only 56% mortality.

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

WE

ASSAY: Grass shrimp SAMPLE: F-2
MAD: 100%

RESULTS:

LD50 [EC50] VALUE ^a >100% (or) AMES RESPONSE _____
MEC ^b _____

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY ^c ND

COMMENTS:

No toxicity at 100%.

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

WE

ASSAY: Grass shrimp SAMPLE: G-2
MAD: 100%

RESULTS:

LD50 (EC50) VALUE^a - >100% [or] AMES RESPONSE _____
MEC^b _____

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY^c ND

COMMENTS:

Only 20% mortality at 100% effluent.

^aFor the NAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

WE

ASSAY: Grass shrimp SAMPLE: K-2
MAD: 100%

RESULTS:

LD50 (EC50) VALUE^a - >100% [or] AMES RESPONSE _____
MEC^b _____

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY^c ND

COMMENTS:

Nontoxic at 100%

^aFor the NAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

WE

ASSAY: Marine Fish SAMPLE: X-2
MAD: 100%

RESULTS:

LD50 (EC50) VALUE^a - >100% (w) AMES RESPONSE _____
MEC^b _____

APPROXIMATE
CONCENTRATION FACTOR 0
LEVEL OF TOXICITY^c ND

COMMENTS: Not detectable
Nontoxic at 100%.

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

WE

ASSAY: Marine Fish SAMPLE: W-2
MAD: 100%

RESULTS:

LD50 (EC50) VALUE^a 32 - 56% (w) AMES RESPONSE _____
MEC^b _____

APPROXIMATE
CONCENTRATION FACTOR 0
LEVEL OF TOXICITY^c Moderate

COMMENTS:

56% effluent produced 100% lethality.

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

WE

ASSAY: Marine Fish SAMPLE: U-2
MAD: 100%

RESULTS:

LD50 (EC50) VALUE^a >100% (or) AMES RESPONSE _____
MEC^b _____

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY^c NO

Nontoxic at 100%.

COMMENTS: Not detectable

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

WE

ASSAY: Marine Fish SAMPLE: C-2
MAD: 100%

RESULTS:

LD50 (EC50) VALUE^a 56 - 75% (or) AMES RESPONSE _____
MEC^b _____

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY^c Moderate

COMMENTS:

LC50 ~ 70% 100% toxic at 100% effluent.

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

WE

ASSAY: Marine Fish SAMPLE: F-2
MAD: 100%

RESULTS:

LD50 [EC50] VALUE^a >100% (or) AMES RESPONSE
MEC^b

APPROXIMATE
CONCENTRATION FACTOR 0
LEVEL OF TOXICITY^c ND

COMMENTS:

Nontoxic at 100%

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

WE

ASSAY: Marine Fish SAMPLE: G-2
MAD: 100%

RESULTS:

LD50 [EC50] VALUE^a >100% (or) AMES RESPONSE
MEC^b

APPROXIMATE
CONCENTRATION FACTOR 0
LEVEL OF TOXICITY^c ND

COMMENTS: Non toxic

40% toxicity at 100%

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

WE

ASSAY: Marine Fish SAMPLE: K-2
MAD: 100%

RESULTS:

LD50 (EC50) VALUE^a >100% (or) AMES RESPONSE _____
MEC^b _____

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY^c NO

COMMENTS:

Nontoxic

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

WE

ASSAY: Marine Fish SAMPLE: L-2
MAD: 100%

RESULTS:

LD50 (EC50) VALUE^a 100% (or) AMES RESPONSE _____
MEC^b _____

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY^c Low 1

COMMENTS:

LC50 = 100%.

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

WE

ASSAY: Marine Fish SAMPLE: N-2
MAD: 100%

RESULTS:

LD50 [EC50] VALUE^a 32 - 56% (or) AMES RESPONSE _____
MEC^b _____

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY^c Moderate

COMMENTS:

LC50 - 40% Complete lethality at 100% effluent.

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

WE

ASSAY: Marine Fish SAMPLE: S-2
MAD: 100%

RESULTS:

LD50 [EC50] VALUE^a >100% (or) AMES RESPONSE _____
MEC^b _____

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY^c ND

COMMENTS: Not detectable

20% mortality at 100% effluent.

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

WE

ASSAY: Marine Fish SAMPLE: B-2
MAD: 100%

RESULTS:

LD50 (EC50) VALUE^a - >100% (or) AMES RESPONSE _____
MEC^b _____

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY^c NO

COMMENTS:

Nontoxic at 100%.

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

WE

ASSAY: Marine Fish SAMPLE: A-2
MAD: 100%

RESULTS:

LD50 (EC50) VALUE^a 56 - 75% (or) AMES RESPONSE _____
MEC^b _____

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY^c Moderate

COMMENTS:

LC50 ~ 60% effluent

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

WE

ASSAY: Salmonella SAMPLE: L-2-5 R-2-5 U-2-5 X-2-5
M-2-5 S-2-5 V-2-5 Y-2-5
MAD: 5 mg/plate N-2-5 T-2-5 W-2-5 Z-2-5

RESULTS:

LD50 (EC50) VALUE ^a _____ (or) AMES RESPONSE Negative
MEC ^b _____

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY ^c Nondetectable

COMMENTS:

All samples were tested at a maximum value of 1.0 ml/plate.

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

WE

ASSAY: Salmonella SAMPLE: P-2-5
MAD: 5 mg/plate

RESULTS:

LD50 (EC50) VALUE ^a _____ (or) AMES RESPONSE Negative
MEC ^b _____

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY ^c Nondetectable

COMMENTS:

Sample was tested at a maximum concentration (volume)
per plate of 500 μ l.

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

WE

ASSAY: Salmonella SAMPLE: C-2-5 G-2-5
D-2-5 H-2-5
MAD: 5 mg/plate E-2-5 J-2-5
F-2-5 K-2-5

RESULTS:

LD50 [EC50] VALUE ^a _____ (or) AMES RESPONSE Negative
MEC ^b _____

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY ^c Nondetectable

COMMENTS:

All samples employed 1.0 ml as the maximum concentration/plate.

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

WE

ASSAY: Salmonella SAMPLE: B2
MAD: 5 mg/plate

RESULTS:

LD50 (EC50) VALUE^a _____ (or) AMES RESPONSE Negative

MEC^b _____

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY^c Nondetectable

COMMENTS:

Maximum amount applied per plate was 1.0 ml.

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

WE

ASSAY: Salmonella SAMPLE: A-2-
MAD: 5 mg/kg

RESULTS:

LD50 (EC50) VALUE^a _____ (or) AMES RESPONSE Negative

MEC^b _____

APPROXIMATE
CONCENTRATION FACTOR 0

LEVEL OF TOXICITY^c ND Nondetectable

COMMENTS:

Maximum amount added per plate was 1.0 ml.

^aFor the RAM assay the EC50 for the most responsive parameter will be listed.

^bMEC = Minimum effective concentration (if response is positive).

^cSee Table 1 for definition of toxicity levels.

TECHNICAL REPORT DATA <i>(Please read Instructions on the reverse before completing)</i>		
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16. ABSTRACT The report gives results of an examination of data from three pilot studies including water effluent, fluidized-bed combustion, and coal gasification samples, using a scheme to compare health effects and ecological bioassay assessment data. The scheme is based on the assumption that each test method has a maximum dose (concentration) which can be reliably applied and that effects based on fractions of the maximum applicable dose (MAD) can be designated as degrees of toxicity. The levels of toxicity based on fractions of the MAD are given as high, moderate, low, and nondetectable (i.e., no effect detected at the MAD).		
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