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6 THE MUTAGENIC POTENTIAL OF: 4-nitrophenyl methyl phenyl phosphinate, 4-nitrophenyl diphenyl phosphinate, 4-nitrophenyl dimethyl phosphinate, 4-chlorophenyl methyl phenyl phosphinate, 4-chlorophenyl diphenyl phosphinate

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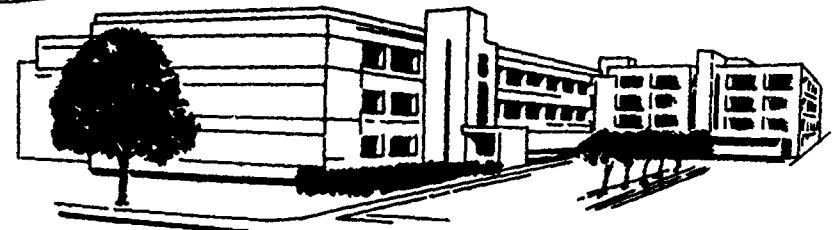
TOXICOLOGY SERVICES GROUP,
DIVISION OF RESEARCH SUPPORT

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) The Mutagenic Potential of 4-nitrophenyl methyl phenyl phosphinate; 4-nitrophenyl diphenyl phosphinate; 4-nitrophenyl dimethyl phosphinate; 4-chlorophenyl methyl phenyl phosphinate; 4-chlorophenyl diphenyl phosphinate was assessed using the Ames Salmonella/Mammalian Microsome Mutagenicity Assay. .0000032 Tester Strains TA 98, TA 100, TA 1535, TA 1537, TA 1538 were exposed to doses ranging from 0.01 mg/plate to 3.2×10^{-6} mg/plate for 4-chlorophenyl diphenyl phosphinate and 1 mg/plate to 3.2×10^{-4} mg/plate for all other test compounds. It was determined that none of the tested substances had mutagenic potential.		

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ABSTRACT

The mutagenic potential of:

4-nitrophenyl methyl phenyl phosphinate 37
4-nitrophenyl diphenyl phosphinate 73A
4-nitrophenyl dimethyl phosphinate 83
4-chlorophenyl methyl phenyl phosphinate 53
4-chlorophenyl diphenyl phosphinate 91

was assessed by the Ames Salmonella/Mammalian Microsome Assay.

Tester strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538 were exposed to doses ranging from 0.01 mg/plate to 3.2×10^{-6} mg/plate for 4 chlorophenyl diphenyl phosphinate and 1 mg/plate to 3.2×10^{-4} mg/plate for all other test compounds. It was determined that none of the tested substances had mutagenic potential.

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PREFACE


	SUBSTANCE	Code No.
AMES ASSAY REPORT:	4-nitrophenyl methyl phenyl phosphinate	37
	4-nitrophenyl diphenyl phosphinate	73A
	4-nitrophenyl dimethyl phosphinate	83
	4-chlorophenyl methyl phenyl phosphinate	53
	4-chlorophenyl diphenyl phosphinate	91
TESTING FACILITY:	Letterman Army Institute of Research Presidio of San Francisco, CA 94129	
SPONSOR:	Biomedical Laboratory, Aberdeen Proving Grounds Aberdeen, MD 21005	
PROJECT:	Toxicity Testing of Phosphinate Compounds - 612772.875	
GLP STUDY NUMBER:	80012	
STUDY DIRECTOR:	LTC John T. Fruin D.V.M., PhD.	
CO-PRINCIPAL INVESTIGATORS:	SSG Freddica R. Pulliam, B.S. SP5 Leonard J. Sauers, B.A.	
RAW DATA:	A copy of the final report, study protocol and retired SOPs will be maintained in the LAIR archives. Test compounds were provided by sponsor. Chemical, analytical, stability, purity, etc. data available from sponsor.	
PURPOSE:	To determine the mutagenic potential of the above compounds using Ames Salmonella/Mammalian Microsome Mutagenicity Assay. Tester strains TA 98, TA 100, TA 1535, TA 1537, and TA 1538 were used.	

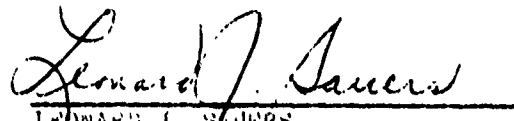
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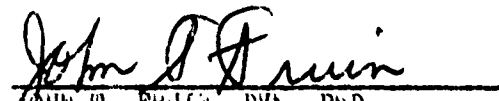
The authors wish to thank SP5 Lon Kincannon, BA; and SP5 Robert Summers for their assistance in performing the research.

Signatures of Principal Scientists Involved
In The Study

We, the undersigned, believe the study described in this report to be scientifically sound and the results and interpretation to be valid. The study was conducted to comply to the best of our ability, with the Good Laboratory Practice Regulations outlined by the Environmental Protection Agency.


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REPLY TO
ATTENTION OF.
SGRD-ULZ-QA

8 January 1981

MEMORANDUM FOR RECORD

SUBJECT: Report of GLP Compliance

I hereby certify that in relation to LAIR GLP study 80012 the following inspections were made:

28 October 1980
30 October 1980
18 November 1980
20 November 1980

Routine inspections with no adverse findings are reported quarterly, thus these inspections are also included in the December 1980 report to management and the Study Director.

A handwritten signature in cursive script, reading "John L. Szurek", is positioned above the typed name.

JOHN L. SZUREK
MAJ, MS
Quality Assurance Officer

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Rationale for using the Ames Assay

The Ames Salmonella/Mammalian Microsome Mutagenicity Test is one of a standard bank of tests used by our laboratory for the assessment of the mutagenic potential of a test substance. It is a short-term screening assay for the prediction of potential mutagenic agents in mammals. It is inexpensive when compared to in vivo tests, yet is highly predictive and reliable in its ability to detect mutagenic activity and therefore carcinogenic probability (1). It relies on basic genetic principles and allows for the incorporation of a mammalian microsome enzyme system to increase sensitivity through enzymatically altering the test substance into an active metabolite. It has proven highly effective in assessing human risk (1).

Description of Test (Rationale for the selection of strains)

The test was developed by Bruce Ames, Ph.D. from the University of California-Berkeley. The test involves the use of several different genetically altered strains of Salmonella typhimurium, each with a specific mutation in the histidine operon (2). The test substance demonstrates mutagenic potential if it is able to revert the mutation in the bacterial histidine operon back to the wild type and thus reestablish prototrophic growth within the test strain. This reversion also can occur spontaneously due to a random mutational event. If, after adding a test substance, the number of revertants is significantly greater than the spontaneous reversion rate, then the test substance physically altered the locus involved in the operon's mutation and is able to induce point mutations and genetic damage (2).

In order to increase the sensitivity of the test system, two other mutations in the Salmonella are used (2). To insure a higher probability of uptake of test substance, the genome for the lipopolysaccharide layer (LP) is mutated and allows larger molecules to enter the bacteria. Each strain has another induced mutation which causes loss of excision repair mechanisms. Since many chemicals are not by themselves mutagenic but have to be activated by an enzymatic process, a mammalian microsome system is incorporated. These microsomal enzymes are obtained from livers of rats induced with Aroclor 1254; the enzymes allow for the expression of the metabolites in the mammalian system. This activated rat liver microsomal enzyme homogenate is termed S-9.

Description of Strains (History of the strains used, methods to monitor the integrity of the organisms, and data pertaining to current and historical controls and spontaneous reversion rates)

The test consists of using five different strains of Salmonella typhimurium that are unable to grow in absence of histidine because of a specific mutation in the histidine operon. This histidine requirement is verified by attempting to grow the tester strains on minimal glucose agar (MGA) plates, both with and without histidine. The dependence on this amino acid is shown when growth occurs only in its presence. The plasmids in strains TA 98 and TA 100 contain an ampicillin resistant R factor. Strains deficient in this plasmid demonstrate a zone of growth inhibition around an ampicillin impregnated disc. The alteration of the LP layer allows uptake by the Salmonella of larger molecules. If a crystal violet impregnated disc is placed onto a plate containing any one of the bacterial strains, a zone of growth inhibition will occur because the LP layer is altered. The absence of excision repair mechanisms can be determined by using ultraviolet (UV) light. These mechanisms function primarily by repairing photodimers between pyrimidine bases; exposure of bacteria to UV light will activate the formation of these dimers and cause cell lethality, since excision of these photodimers can not be made. The genetic mutation resulting in UV sensitivity also induces a dependence by the Salmonella to biotin. Therefore, this vitamin must be added. In order to prove that the bacteria are responsive to the mutation process, positive controls are run with known mutagens. If after exposure to the positive control substance, a larger number of revertants are obtained, then the bacteria are adequately responsive. Sterility controls are performed to determine the presence of contamination. Sterility of the test compound is also confirmed in each first dilution. Verification of the tester strains occurs spontaneously with the running of each assay. The value of the spontaneous reversion rate is obtained using the same inoculum of bacteria that is used in the assay (3).

Strains were obtained directly from Dr. Ames, University of California, Berkeley, propagated and then maintained at -80 C in our laboratory. Before any substance was tested, quality controls were run on the bacterial strains to establish the validity of their special features and also to determine the spontaneous reversion rate (2). Records are maintained of all the data, to determine if deviations from the set trends have occurred.

We compared the spontaneous reversion values with our own historical values and those cited by Ames et al (2). Our conclusions are based on the spontaneous reversion rate compared to the experimentally induced rate of mutation. When operating effectively, these strains detect substances that cause base pair

mutations (TA 1535, TA 100) and frameshift mutations (TA 1537, TA 1538 and TA 98) (2).

METHODS (3)

Rationale for Dosage Levels and Dose Response Tabulations

To insure readable and reliable results, a sublethal concentration of the test substance had to be determined. This toxicity level was found by using MGA plates, various concentrations of the substance, and approximately 10^8 cells of TA 100 per plate, unless otherwise specified. Top agar containing trace amounts of histidine and biotin were placed on MGA plates. TA 100 is used because it is the most sensitive strain. Strain verification was confirmed on the bacteria, along with a determination of the spontaneous reversion rate. After incubation, the growth was observed on the plates. (The auxotrophic Salmonella will replicate a few times and potentially express a mutation. When the histidine and biotin supplies are exhausted, only those bacteria that reverted to the prototrophic phenotype will continue to reproduce and form macrocolonies; the remainder of the bacteria comprises the background lawn. The minimum toxic level is defined as the lowest serial dilution at which decreased macrocolony formation, below that of the spontaneous revertant rate, and an observable reduction in the density of the background lawn occurs.) A maximum dose of 1 mg/plate is used when no toxicity is observed. The densities were recorded as normal slight, and no growth.

Test Format

After we validated our bacterial strains and determined the optimal dosage of the test substance, we began the Ames Assay. In the actual experiment, 0.1ml of the particular strain of Salmonella (10^8 cells) and the specific dilutions of the test substance were added to 2 ml of molten top agar, which contained trace amounts of histidine and biotin. Since survival is better from cultures which have just passed the log phase, the Salmonella strains were used 16 hours (maximum) after initial inoculation into nutrient broth. The dose of the test substance spanned more than a 1000-fold, decreasing from the minimum toxic level by a dilution factor of 5. All the substances were tested with and without S-9 microsome fraction. The S-9 mixture which was previously titered at an optimal strength was added to the molten top agar. After all the ingredients were added, the top agar was vortexed, then overlaid on minimum glucose agar plates. These plates contained 2% glucose and Vogel Bonner "E" Concentrate (4). The water used in this medium and all reagents came from a polymetric system. Plates were incubated, upside down in the dark at 37 C for 48 hours. Plates were prepared in triplicate and the average revertant counts were recorded. The corresponding number of revertants obtained was compared to the number of spontaneous

revertants; the conclusions were recorded statistically. A correlated dose response is considered necessary to declare a substance as a mutagen. Commoner (5), in his report, "Reliability of Bacterial Mutagenesis Techniques to Distinguish Carcinogenic and Non-Carcinogenic Chemicals," and McCann et al (1) in their paper, "Detection of Carcinogens as Mutagen: Assay of over 300 Chemicals," have concurred on the test's ability to detect mutagenic potential.

Statistical Analysis

Quantitative evaluation was ascertained by two independent methods. Ames et al (2) assumed that a compound which caused twice the spontaneous reversion rate is mutagenic. Commoner (5), developed the MUTAR Ratio, which is stated in the following equation:

$$\text{MUTAR} = (E - C)/C_{AV}$$

Here, C is the number of spontaneous revertant colonies on control plates obtained on the same day and with the same treatment and strains. E is the number of revertants in response to the compound; C_{AV} is the number of spontaneous revertants on control plates calculated from historical records. The explanation of the results of this equation can be determined by the method of Commoner (5). This variation determines the probability of correctly classifying substances as carcinogens on the basis of their mutagenic activity. The E values were recorded by strain, with and without S-9. Values for C and C_{AV} were recorded separately.

We used the formula and logged all values for our permanent records.

RESULTS AND DISCUSSION

Throughout this report, all the test substances will be referred to by their respective code numbers:

<u>Substance</u>	<u>Code No.</u>
4-nitrophenyl methyl phenyl phosphinate	37
4-nitrophenyl diphenyl phosphinate	73A
4-nitrophenyl dimethyl phosphinate	83
4-chlorophenyl methyl phenyl phosphinate	53
4-chlorophenyl diphenyl phosphinate	91

A series of assays was run to conclusively determine the mutagenic potential of the five substances. Data from tests that were determined to be invalid due to medium preparation errors, inadequate inoculum or control failures are not reported but are retained in the LAIR archives. On 4 Nov 80, the Ames Test was performed on 73A and 37. Due to an error in medium preparation, no

growth was present after the 48-hour incubation. This assay was repeated on 12 Nov 80. On 18 Nov 80, substances 53, 83 and 91 were tested. Throughout the assays of 12 and 18 Nov, we observed uneven lawns on plates containing test strain TA 1537. We suspected that the TA 1537 inoculum was insufficient; therefore, all five chemicals were retested on 2 Dec 80, using an inoculum of TA 1537 prepared from parent culture stock. A plating error resulted in a lack of growth on the positive control plates. The test was done again on 9 Dec 80. The spontaneous reversion level was below our historical data for nonactivated TA 98 and nonactivated TA 1538 from the 18 Nov 80 assay. The experiment was repeated on 16 Dec 80 with only TA 98 and TA 1538.

Strain verification and sterility controls were normal for all assays reported (Tables 1A - 1E). The assay of 12 Nov 80 showed a spontaneous reversion rate below that suggested by Ames et al (2) on both activated and nonactivated TA 98, TA 100, and TA 1538 also for activated TA 1535 (Table 1A). On 18 Nov 80, all the spontaneous reversion rates for the nonactivated strains were below that suggested range along with activated TA 1535 and TA 1538. Nonactivated TA 98 and TA 1538 (Table 1B) were significantly below our historical data values. The spontaneous reversion rate was low for TA 98 nonactivated on 16 Dec 80 (Table 1E). Spontaneous reversion values below that suggested by Ames et al (2) are indicative of high quality water, materials, techniques, etc. Counts higher than those suggested by Ames et al (2) are indicators of serious performance

The effects of the positive control chemicals are reported in Tables 2A - 2D. Positive control values below that expected were observed for TA 98, TA 1537 and TA 1538 to dimethyl-benzanthracene (DMBA) on 12 Nov 80 (Table 2A). On 18 Nov 80, the same results were seen for TA 98, TA 100, TA 1537 and TA 1538 (Table 2B). Below par value were also evident on 9 Dec 80 for TA 1537 to DMBA. The same was true on 16 Dec 80 for TA 98 and TA 1537 (Table 2D). DMBA functions as a frameshift mutagen and is used to determine if strains TA 98, TA 1537 and TA 1538 are functioning properly. Although the strains did not respond to DMBA, they did respond to aminofluorene (AF) and benzo(a)pyrene (BP), both of which are also frameshift mutagens. In all instances when n-methyl-n nitro-N-nitrosoquinidine (MNNG) was the positive control, test strains responded as anticipated.

The Minimum Toxicity Level Determination Assay was performed on 28 Oct 80. Our quality control showed that we had incurred experimental contamination on the test plates (Table 3). By observing the condition of the background lawn, the optimal sublethal dose was determined, even though extraneous growth was present. Sparse or no growth of the background lawn signified toxicity. The optimal sublethal dose was chosen at a point where a lawn having normal growth became evident (Table 4A - 4E).

The data for the mutagenic potential are reported in Tables 5A - 5J. Data for test compound 37 were collected on 12 Nov 80 and 11 Dec 80. On 12 Nov 80 (Table 5A), two isolated incidences of a more than doubling of the spontaneous reversion rate occurred: activated TA 1535 at the 0.0016 mg/plate dose and activated TA 1537 at the 0.04 mg/plate level. The assay of strain TA 1537 was performed again on test substance 37 on 11 Dec 80 (Table 5G). No mutagenic activity was demonstrated. It is concluded that the response of activated TA 1537 on 12 Nov 80 for the 0.04 mg/plate dose was unexplainable and probably due to experimental error since the results could not be reproduced. The activity found in TA 1535 was disregarded due to the lack of correlation with dose response.

The data for test substance 73A were obtained on 12 Nov 80 (Table 5B) and 11 Dec 80 (Table 5G). In all occurrences, no evidence of mutagenic activity was found.

Compound 83 was tested on 18 Nov 80 (Table 5C), 11 Dec 80 (Table 5G) and 16 Dec 80 (Table 5H). On 18 Nov 80, all TA 98 dose levels showed doubling or greater of the spontaneous reversion rate. This was also true for nonactivated TA 1538 at the 0.04 mg/plate dose level through the 0.00032 mg/plate dose. The spontaneous reversion rate for these nonactivated strains was below that suggested by Ames et al (2) as indicative of mutagenicity. Test substance 83 was assayed using only strains TA 98 and TA 1538 on 16 Dec 80. No mutagenic activity was presented. It was concluded that the 18 Nov 80, suggestion of mutagenic activity was due to the spontaneous reversion rate for TA 98 and TA 1538 which was far below the historical average. The MUTAR values were also insignificant.

Test substance 53 was tested on 18 Nov 80 (Table 5D), 11 Dec 80 (Table 5G), and 16 Dec 80 (Table 5I). On 18 Nov 80, a greater than twice the spontaneous reversion rate was observed for all dose levels containing nonactivated TA 98 and nonactivated TA 1538. The same occurred for nonactivated TA 1535 at the 0.008 and 0.00032 mg/plate doses. Nonactivated TA 1537 showed possible mutagenic activity at the 0.008 and 0.0016 mg/plate dose levels. On 11 Dec 80, the TA 1537 assay was repeated; no evidence of mutagenic activity was present. It was concluded that the mutagenicity initially presented with TA 98, TA 1537 and TA 1538 was due to low spontaneous reversion rates. The activity found in TA 1535 was disregarded due to the low spontaneous reversion rate and the lack of correlation to dose response.

Test substance 91 was assayed on 18 Nov 80 (Table 5E), 11 Dec 80 (Table 5G) and 16 Dec 80 (Table 5J). In the assay of 18 Nov 80, a doubling or greater spontaneous reversion rate was seen for nonactivated TA 1538 and nonactivated TA 98 for all dose levels. The spontaneous reversion values were low for both of these nonactivated strains. When the assay was repeated on 11 Dec 80, no mutagenic

activity was seen. It was concluded that the initial observation of mutagenicity was due to the low spontaneous reversion values. All calculated MUTAR values were below the 1.5 threshold value necessary to declare a substance as a mutagen (Tables 6A-6M).

CONCLUSION

To declare that a substance is a mutagen through the Ames Test, two criteria must be met: a more than doubling of the spontaneous reversion rate and an obvious dose response. Since only a few scattered incidences of twice the spontaneous reversion rate were observed, it was concluded that compounds 37, 73A, 83, 53 and 91 are not mutagenic.

RECOMMENDATION

We recommend that organo-phosphinate compounds 37, 73A, 83, 53, and 91 be tested using other toxicological testing systems if efficacy tests show those chemicals to be promising antidotes.

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Table 1-A

QUALITY CONTROL OF TESTER STRAINS WORKSHEET
Salmonella/Microsome Assay

Strain No.	Histidine (a) Requirements	Ampicillin (b) Resistance	uvr-B (c) Deletion	rfa Crystal Violet (d)	Sterility Control (e)
TA 98	+	+	+	16.27 mm	NG
TA 100	+	+	+	16.65 mm	NG
TA 1535	+	NA	+	20.35 mm	NG
TA 1537	+	26.05 mm	+	18.34 mm	NG
TA 1538	+	NA	+	20.30 mm	NG
WT	growth	NA	NA	NA	NA

QUALITY CONTROL (e)

His-Bio mix Initial: NT End: NT Test Compound 1: + (73A)
 Top Agar Initial: + End: + Test Compound 2: + (37)
 S - 9 Initial: + End: + Test Compound 3: NA
 Diluent: + Nutrient Broth: + Test Compound 4: NA
 MGA Plate w/ bacteria: + MGA Plate: + Test Compound 5: NA

(a) + = no growth (requires histidine for growth); (b) + = no zone of inhibition, - = zone of inhibition of approximately 16mm; (c) + = no growth on irradiated side of plate; (d) + = zone of inhibition approximately 14mm diameter; (e) + = no growth (growth indicates contamination); NT=not tested; NG=no growth; WT=wild type. NA = not applicable.

Spontaneous Revertants (1)

Strain (1)	Avg	Range	No S-9			Avg	S-9			Avg
			25	24	18		22	32	24	
TA 98	40	30-50	25	24	18	22	32	24	23	26
TA 100	160	120-200	102	121	122	115	112	102	123	112
TA 1535	20	10-35	14	16	8	13	6	3	5	5
TA 1537	7	3-15	14	11	8	11	15	4	10	10
TA 1538	25	15-35	6	14	11	10	13	13	15	14

Ames, B.N., J. McCann and E. Yamasaki. Mutat. Res. 31:347

Test Inoculated By: Summers, Sauers, Pulliam, Kincannon Date: 12 Nov 80
 Test Read By: Pulliam Date: 14 Nov 80

Table-1-B

QUALITY CONTROL OF TESTER STRAINS WORKSHEET
Salmonella/Microsome Assay

Strain No.	Histidine (a) Requirements	Ampicillin (b) Resistance	uvr-B (c) Deletion	rfa Crystal Violet (d)	Sterility Control (e)
TA 98	+	+	+	15.33mm	NG
TA 100	+	+	+	17.22mm	NG
TA 1535	+	NA	+	16.40mm	NG
TA 1537	+	22.43	+	17.44mm	NG
TA 1538	+	NA	+	20.0mm	NG
WT	Growth	NA	NA	NA	NT

QUALITY CONTROL (e)

His-Bio mix Initial: + End: + Test Compound 1: 83- NG
 Top Agar Initial: + End: + Test Compound 2: 91- NG
 S - 9 Initial: + End: + Test Compound 3: 53- NG
 Diluent: + Nutrient Broth: + Test Compound 4: NA
 MGA Plate w/ bacteria: Growth MGA Plate: + Test Compound 5: NA

(a) + = no growth (requires histidine for growth); (b) + = no zone of inhibition, - = zone of inhibition of approximately 16mm; (c) + = no growth on irradiated side of plate; (d) + = zone of inhibition approximately 14mm diameter; (e) + = no growth (growth indicates contamination); NT=not tested; NG=no growth; WT=wild type NA=not applicable.

Spontaneous Revertants (1)

Strain (1)	Avg	Range	No S-9			Avg	S-9			Avg
			4	0	3		2	29	41	
TA 98	40	30-50	4	0	3	2	29	41	38	36
TA 100	160	120-200	78	63	88	76	133	140	118	130
TA 1535	20	10-35	8	6	2	5	12	2	3	6
TA 1537	7	3-15	*3	0	7	3	1	7	8	5
TA 1538	25	15-35	1	4	1	2	6	13	13	11

Ames, B.N., J. McCann and E. Yamasaki Mutat. Res. 31:347

Test Inoculated By: Sauers, Summers, Pulliam Date: 18 Nov 80

Test Read By: Pulliam Date: 20 Nov 80

* Sparse lawn

Table-1-C

QUALITY CONTROL OF TESTER STRAINS WORKSHEET
Salmonella/Microsome Assay

Strain No.	Histidine (a) Requirements	Ampicillin (b) Resistance	uvr-B (c) Deletion	rfa Crystal Violet (d)	Sterility Control (e)
TA 98	NA	NA	NA	NA	NA
TA 100	NA	NA	NA	NA	NA
TA 1535	+	NA	NA	NA	NA
TA 1537	+	18mm	+	20mm	NG
TA 1538	NA	NA	NA	NA	NA
WT	Growth	NA	NA	NA	NA

QUALITY CONTROL (e)

His-Bio mix Initial: + End: + Test Compound 1: NG
 Top Agar Initial: + End: + Test Compound 2: NA
 S - 9 Initial: + End: + Test Compound 3: NA
 Diluent: NT Nutrient Broth: + Test Compound 4: NA
 MGA Plate w/ bacteria: + MGA Plate: + Test Compound 5: NA

(a) + = no growth (requires histidine for growth); (b) + = no zone of inhibition, - = zone of inhibition of approximately 16mm; (c) + = no growth on irradiated side of plate; (d) + = zone of inhibition approximately 14mm diameter; (e) + = no growth (growth indicates contamination); NT=not tested; NG=no growth; WT=wild type NA=not applicable.

Spontaneous Revertants (1)

Strain (1)	Avg	Range	No S-9			Avg	S-9			Avg
TA 98	40	30-50				NA				NA
TA 100	160	120-200				NA				NA
TA 1535	20	10-35				NA				NA
TA 1537	7	3-15	4	4	3	4	7	8	2	6
TA 1538	25	15-35				NA				NA

Ames, B.N., J. McCann and E. Yamasaki. Mutat. Res. 31:347

Test Inoculated By: Sauers, Summers, Pulliam Date: 2 Dec 80

Test Read By: Pulliam Date: 4 Dec 80

* Tested when batch first made, see data dated 18 Nov 80

Table-1-D

QUALITY CONTROL OF TESTER STRAINS WORKSHEET
Salmonella/Microsome Assay

Strain No.	Histidine (a) Requirements	Ampicillin (b) Resistance	uvr-B (c) Deletion	rfa Crystal Violet (d)	Sterility Control (e)
TA 98	NA	NA	NA	NA	NA
TA 100	NA	NA	NA	NA	NA
TA 1535	NA	NA	NA	NA	NA
TA 1537	+	16mm	+	17mm	NG
TA 1538	NA	NA	NA	NA	NA
WT	NA	NA	NA	NA	NA

QUALITY CONTROL (e)

His-Bio mix Initial: + End: + Test Compound 1: NG
 Top Agar Initial: 1 colony End: + Test Compound 2: NG
 S - 9 Initial: + End: + Test Compound 3: NG
 Diluent: + Nutrient Broth: + Test Compound 4: NG
 MGA Plate w/ bacteria: (WT) growth MGA Plate: + Test Compound 5: NG

(a) + = no growth (requires histidine for growth); (b) + = no zone of inhibition, - = zone of inhibition of approximately 16mm; (c) + = no growth on irradiated side of plate; (d) + = zone of inhibition approximately 14mm diameter; (e) + = no growth (growth indicates contamination); NT=not tested; NG=no growth; WT=wild type
 NA= not applicable.

Spontaneous Revertants (1)

Strain (1)	Avg	Range	No S-9			Avg	S-9			Avg
TA 98	40	30-50				NA				NA
TA 100	160	120-200				NA				NA
TA 1535	20	10-35				NA				NA
TA 1537	7	3-15	9	8	5	7	9	8	6	8
TA 1538	25	15-35				NA				NA

Ames, B.N., J. McCann and E. Yamasaki. Mutat. Res. 31:347

Test Inoculated By: Pulliam, Sauer Date: 9 Dec 80

Test Read By: Pulliam Date: 11 Dec 80

Table-1-E

QUALITY CONTROL OF TESTER STRAINS WORKSHEET
Salmonella/Microsome Assay

Strain No.	Histidine (a) Requirements	Ampicillin (b) Resistance	uvr-B (c) Deletion	rfa Crystal Violet (d)	Sterility Control (e)
TA 98	+	+	+	17.43mm	NG
TA 100	+	NA	+	NA	NA
TA 1535	NA	NA	NA	NA	NA
TA 1537	NA	NA	NA	NA	NA
TA 1538	+	23.45	+	17.21mm	NG
WT	NA	NA	Growth	NA	NA

QUALITY CONTROL (e)

His-Bio mix Initial: + End: + Test Compound 1: +(53)
 Top Agar Initial: + End: + Test Compound 2: +(91)
 S - 9 Initial: + End: + Test Compound 3: +(33)
 Diluent: + Nutrient Broth: + Test Compound 4: NA
 MGA Plate w/ bacteria: + MGA Plate: + Test Compound 5: NA

(a) + = no growth (requires histidine for growth); (b) + = no zone of inhibition, - = zone of inhibition of approximately 16mm; (c) + = no growth on irradiated side of plate; (d) + = zone of inhibition approximately 14mm diameter; (e) + = no growth (growth indicates contamination); NT=not tested; NG=no growth; WT=wild type NA=not applicable.

Spontaneous Revertants (1)

Strain (1)	Avg	Range	No S-9			S-9				Avg
TA 98	40	30-50	23	24	28	25	25	34	32	30
TA 100	160	120-200				NA				NA
TA 1535	20	10-35				NA				NA
TA 1537	7	3-15				NA				NA
TA 1538	25	15-35	11	14	19	15	20	16	17	18

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Test Inoculated By: Pulliam, Summers, Sauer Date: 16 Dec 89

Test Read By: Sauers, Summers Date: 19 Dec 89

Table-3

STRAIN VERIFICATION FOR TOXICITY LEVEL DETERMINATION
Salmonella/Microsome Assay

Strain No.	Histidine (a) Requirements	Ampicillian (b) Resistance	uvr=B (c) Deletion	rfa Crystal Violet (d)	Sterility Control (e)
TA 100	+	+	+	15mm	NG
TA 1537	NT	24 mm	NT	NT	NG
WT	Growth	NT	Growth	NT	NT
Diluent	NT	NT	NT	NT	NG
Test Compound (s)					<i>Contamination</i>
#1 _____	NT	NT	NT	NT	
#2 _____	NT	NT	NT	NT	
#3 _____	NT	NT	NT	NT	
#4 _____	NT	NT	NT	NT	
#5 _____	NT	NT	NT	NT	
<p>(a) + = no growth (requires histidine for growth); (b) + = no zone of inhibition, - = zone of inhibition of approximately 16mm; (c) + = no growth on irradiated side of plate; (d) + = zone of inhibition approximately 14mm diameter; (e) + = no growth (growth indicates contamination); NT=not tested; WT= wild type.</p>					
Spontaneous Revertants					
Strain	Average	Range			Average
TA 100	160	120-200	<i>Contamination</i>		

Test Inoculated By: Sauers, Summers, Kellner Date: 28 Oct 90

Test Read By: Pulliam Date: 31 Oct 90

Table 4-A
 TOXICITY LEVEL DETERMINATION
 Salmonella/Microsome Assay

Substance assayed: (1) Code # 37 (2) _____

(3) _____ (4) _____ (5) _____

Date: 28 Oct 80 Performed by: Sauers, Kincannon, Pulliam, Summers

Substance dissolved in: (1) DMSO (2) _____ (3) _____

(4) _____ (5) _____

Visual estimation of background lawn on
 Nutrient Agar Plates: NG = no growth
 ST = slight growth
 NL = normal growth

TA 100
 Revertant Plate Count

Test Compound Concentration	Plate #1	Plate #2	Plate #3	Average	Background Lawn
1.0 mg/plate					NL
0.1 mg/plate					NL
0.01 mg/plate					NI
0.001 mg/plate					NI
0.000,1 mg/plate					NI
0.000,01 mg/plate					NI
0.000,001 mg/plate					NI
0.000,000,1 mg/plate					NI

Table 4-B

TOXICITY LEVEL DETERMINATION
Salmonella/Microsome Assay

Substance assayed: (1) Code #73 (2) _____

(3) _____ (4) _____ (5) _____

Date: 28 Oct 80 Performed by: Sauers, Kincannon, Pulliam, Summers

Substance dissolved in: (1) DMSO (2) _____ (3) _____

(4) _____ (5) _____

Visual estimation of background lawn on
Nutrient Agar Plates: NG = no growth
ST = slight growth
NL = normal growth

TA 100
Revertant Plate Count

Test Compound Concentration	Plate #1	Plate #2	Plate #3	Average	Background Lawn
1.0 mg/plate					ST
0.1 mg/plate					NL
0.01 mg/plate					NL
0.001 mg/plate					NL
0.000,1 mg/plate					NL
0.000,01 mg/plate					NL
0.000,001 mg/plate					NL
0.000,000,1 mg/plate					NL

Table 4-C

TOXICITY LEVEL DETERMINATION
Salmonella/Microsome Assay

Substance assayed: (1) Code #83 (2) _____

(3) _____ (4) _____ (5) _____

Date: 28 Oct 80 Performed by: Sauers, Kincannon, Pullian, Summers

Substance dissolved in: (1) DMSO (2) _____ (3) _____

(4) _____ (5) _____

Visual estimation of background lawn on
Nutrient Agar Plates: NG = no growth
ST = slight growth
NL = normal growth

TA 100
Revertant Plate Count

Test Compound Concentration	Plate #1	Plate #2	Plate #3	Average	Background Lawn
1.0 mg/plate	<i>Contaminated</i>				NL
0.1 mg/plate					NL
0.01 mg/plate					NL
0.001 mg/plate					NL
0.000,1 mg/plate					NL
0.000,01 mg/plate					NL
0.000,001 mg/plate					NL
0.000,000,1 mg/plate					NL

Table 4-D
 TOXICITY LEVEL DETERMINATION
 Salmonella/Microsome Assay

Substance assayed: (1) Code #53 (2) _____

(3) _____ (4) _____ (5) _____

Date: 28 Oct 80 Performed by: Sauers, Kincannon, Pulliam, Summers

Substance dissolved in: (1) DMSO (2) _____ (3) _____

(4) _____ (5) _____

Visual estimation of background lawn on
 Nutrient Agar Plates: NG = no growth
 ST = slight growth
 NL = normal growth

TA 100
 Revertant Plate Count

Test Compound Concentration	Plate #1	Plate #2	Plate #3	Average	Background Lawn
1.0 mg/plate					NL
0.1 mg/plate					NL
0.01 mg/plate					NL
0.001 mg/plate					NL
0.000,1 mg/plate					NL
0.000,01 mg/plate					NL
0.000,001 mg/plate					NL
0.000,000,1 mg/plate					NL

Table 4-E
 TOXICITY LEVEL DETERMINATION
 Salmonella/Microsome Assay

Substance assayed: (1) Code #91 (2) _____

(3) _____ (4) _____ (5) _____

Date: 28 Oct 80 Performed by: Sauers, Kincannon, Pulliam, Summers

Substance dissolved in: (1) DMSO (2) _____ (3) _____

(4) _____ (5) _____

Visual estimation of background lawn on
 Nutrient Agar Plates: NG = no growth
 ST = slight growth
 NL = normal growth

TA 100
 Revertant Plate Count

Test Compound Concentration	Revertant Plate Count				Background Lawn
	Plate #1	Plate #2	Plate #3	Average	
1.0 mg/plate	<i>Contaminated</i>				NG
0.1 mg/plate					ST
0.01 mg/plate					NL
0.001 mg/plate					NL
0.000,1 mg/plate					NL
0.000,01 mg/plate					NL
0.000,001 mg/plate					NL
0.000,000,1 mg/plate					NL

Table-5-C
SALMONELLA/MICROSOME ASSAY WORKSHEET
(POSITIVE CONTROLS/TEST COMPOUND)

Substance Assayed: (1) Code #83 (2) _____
 (3) _____ (4) _____ (5) _____

Date: 18 Nov 80 Performed By: Kincannon, Summers, Sauer, Pulliam

Substance dissolved in: (1) DMSO (2) _____
 (3) _____ (4) _____ (5) _____

Revertant/Plate

Sub	Conc	98	98A	100	100A	1535	1535A	1537	1537A	1538	1538A
83	1.0 mg/pl	11	10	47	40	4	6	1	1	4	7
83	0.2 mg/pl	19	22	81	71	9	7	3	4	3	12
83	0.04 mg/pl	17	19	84	96	5	7	5	3	6	12
83	0.008 mg/pl	19	22	85	84	6	6	3	3	7	15
83	0.0016 mg/pl	20	17	66	60	6	4	1	4	13	7
83	0.00032 mg/pl	12	15	51	83	6	7	3	2	6	11
	Spon. rev.	2	36	76	130	5	6	3	5	2	11

Table-5-F
SALMONELLA/MICROSOME ASSAY WORKSHEET
(POSITIVE CONTROLS/TEST COMPOUND)

Substance Assayed: (1) Code #37 (2) Code #73A
 (3) Code #83 (4) Code #53 (5) Code #91
 Date: 4 Dec 80 Performed By: Kincannon, Summers, Sauers, Pulliam
 Substance dissolved in: (1) DMSO (2) _____
 (3) _____ (4) _____ (5) _____

Revertant/Plate

Sub	Conc	98	98A	100	100A	1535	1535A	1537	1537A	1538	1538A
37	1.0 mg/pl							Toxic	Toxic		
37	0.2 mg/pl							1	1		
37	0.04 mg/pl							3	3		
37	0.008 mg/pl							2	3		
37	0.0016 mg/pl							3	3		
37	0.00032 mg/pl			1				4	2		
73A	1.0 mg/pl							Toxic	Toxic		
73A	0.2 mg/pl							2	2		
73A	0.04 mg/pl							4	4		
73A	0.008 mg/pl							3	2		
73A	0.0016 mg/pl							2	3		
73A	0.00032 mg/pl							4	3		
83	1.0 mg/pl							3	1		
83	0.2 mg/pl							3	3		
83	0.04 mg/pl							4	3		
83	0.008 mg/pl							4	5		
83	0.0016 mg/pl							5	2		
83	0.00032 mg/pl							2	3		

SALMONELLA/ MICROSOME ASSAY WORKSHEET
 (POSITIVE CONTROLS/TEST COMPOUND)
 Table-5-F
 Continuation Page

Revertant/Plate

Sub	Conc	98	98A	100	100A	1535	1535A	1537	1537A	1538	1538A
53	1.0 mg/pl							4	2		
53	0.02 mg/pl							3	3		
53	0.04 mg/pl							4	3		
53	0.008 mg/pl							2	2		
53	0.0016 mg/pl							2	3		
53	0.00032 mg/pl							5	3		
91	0.01 mg/pl							3	2		
91	0.002 mg/pl							3	2		
91	0.0004 mg/pl							4	3		
91	0.00008 mc/pl							2	2		
91	0.000016mc/pl							2	2		
91	0.0000032ng/pl							2	2		
	Spon. Re /							4	6		

Table-5-G
SALMONELLA/MICROSOME ASSAY WORKSHEET
(POSITIVE CONTROLS/TEST COMPOUND)

Substance Assayed: (1) Code #37 (2) Code #73A

(3) Code #83 (4) Code #53 (5) Code #91

Date: 11 Dec 80 Performed By: Sauers, Summers, Kincannon, Pulliam

Substance dissolved in: (1) DMSO (2) _____

(3) _____ (4) _____ (5) _____

Revertant/Plate

Sub	Conc	98	98A	100	100A	1535	1535A	1537	1537A	1538	1538A
37	1.0 mg/pl							3	6		
37	0.2 mg/pl							4	4		
37	0.04 mg/pl							5	9		
37	0.008 mg/pl							4	7		
37	0.0016 mg/pl							7	7		
37	0.00032 mg/pl							4	4		
73A	1.0 mg/pl							7	6		
73A	0.2 mg/pl							6	5		
73A	0.04 mg/pl							4	6		
73A	0.008 mg/pl							4	9		
73A	0.0016 mg/pl							6	7		
73A	0.00032 mg/pl							4	7		
83	1.0 mg/pl							6	4		
83	0.2 mg/pl							4	6		
83	0.04 mg/pl							4	7		
83	0.008 mg/pl							7	8		
83	0.0016 mg/pl							5	5		
83	0.00032 mg/pl							7	7		

Table-5-H
 SALMONELLA/MICROSOME ASSAY WORKSHEET
 (POSITIVE CONTROLS/TEST COMPOUND)

Substance Assayed: (1) Code # 83 (2) _____

(3) _____ (4) _____ (5) _____

Date: 16 Dec 80 Performed By: Pulliam, Summers, Sauers, Kellner

Substance dissolved in: (1) DMSO (2) _____

(3) _____ (4) _____ (5) _____

Revertant/Plate

Sub	Conc	98	98A	100	100A	1535	1535A	1537	1537A	1538	1538A
83	1.0 mg/pl	9	16							10	
83	0.2 mg/pl	11	17							11	
83	0.04 mg/pl	16	19							14	
83	0.008 mg/pl	14	21							8	
83	0.0016 mg/pl	17	20							6	
83	0.00032 mg/pl	17	16							6	
	Spon Rev.	25	30							15	

Table-5-1
SALMONELLA/MICROSOME ASSAY WORKSHEET
(POSITIVE CONTROLS/TEST COMPOUND)

Substance Assayed: (1) Code #53 (2) _____

(3) _____ (4) _____ (5) _____

Date: 16 Dec 80 Performed By: Pulliam, Kellner, Sauer, Summers

Substance dissolved in: (1) DMSO (2) _____

(3) _____ (4) _____ (5) _____

Revertant/Plate

Sub	Conc	98	98A	100	100A	1535	1535A	1537	1537A	1538	1538A
53	1.0 mg/pl	14	18							9	
53	0.2 mg/pl	16	17							14	
53	0.04 mg/pl	19	17							10	
53	0.008 mg/pl	15	10							7	
53	0.0016 mg/pl	17	20							9	
53	0.00032 mg/pl	11	8							7	
	Spon. Rev.	25	30							15	

Table-5-J
SALMONELLA/MICROSOME ASSAY WORKSHEET
(POSITIVE CONTROLS/TEST COMPOUND)

Substance Assayed: (1) Code #91 (2) _____

(3) _____ (4) _____ (5) _____

Date: 16 Dec 80 Performed By: Pulliam, Sauers, Summers, Kellner

Substance dissolved in: (1) DMSO (2) _____

(3) _____ (4) _____ (5) _____

Revertant/Plate

Sub	Conc	98	98A	100	100A	1535	1535A	1537	1537A	1538	1538A
91	0.01 mg/p1	8	19							6	
91	0.002 mg/p1	14	22							10	
91	0.0004 mg/p1	11	18							12	
91	0.00008 mg/p1	13	19							10	
91	0.000016mg/p1	14	23							14	
91	0.0000032mg/p1	13	14							8	
	Spon. Rev.	25	30							15	

Table-6-A

MUTAGENIC ACTIVITY RATIO WORKSHEET

Salmonella/Microsome Assay

Substance Assayed: Code #37 Dissolved In: DMSO

Date: 12 Nov 80 Performed By: Pulliam, Sauers

Test Compound and Concentration	TA (a)	E (b)	E act	C (c)	C act	E-C (d)	E-C act	CAV (e)	CAV act	MUTAR (f)	MUTA-act
1.0 mg/pl	TA 98	12	13	22	26						
0.2 mg/pl	TA 98	12	19	22	26						
0.04 mg/pl	TA 98	14	28	22	26						
0.008 mg/pl	TA 98	21	23	22	26						
0.0016 mg/pl	TA 98	18	22	22	26						
0.00032 mg/pl	TA 98	18	19	22	26						
1.0 mg/pl	TA 100	60	66	115	112						
0.2 mg/pl	TA 100	83	92	115	112						
0.04 mg/pl	TA 100	89	97	115	112						
0.008 mg/pl	TA 100	75	88	115	112						
0.0016 mg/pl	TA 100	84	77	115	112						
0.00032 mg/pl	TA 100	112	89	115	112						

(a)= tester strain: (b)=no. of experimental revertant colony forming units. (c)=no. of assayed spontaneous revertants: (d)=no. revertants in excess of the assayed spontaneous revertant rate: (e)=spontaneous reversion rate calculated from historical data: (f) = E-C/CAV: act - activation with S-9

Table-6-A

MUTAGENIC ACTIVITY RATIO WORKSHEET

Salmonella/Microsome Assay

Substance Assayed: Code #37 Dissolved In: DMSODate: 12 Nov 80 Performed By: Pulliam, Sauer

Test Compound and Concentration	TA (a)	E (b)	E act	C (c)	C act	E-C (d)	E-C act	C _{AV} (e)	C _{AV} act	MUTAR (f)	MUTA-act
1.0 mg/pl	TA 1535	6	5	13	5						
0.2 mg/pl	TA 1535	10	4	13	5						
0.0004 mg/pl	TA 1535	6	7	13	5		2		13.3		0.15
0.008 mg/pl	TA 1535	11	6	13	5		1		13.3		0.8
0.0016 mg/pl	TA 1535	10	12	13	5		7		13.3		0.53
0.00032 mg/pl	TA 1535	12	6	13	5		1		13.3		0.8
1.0 mg/pl	TA 1537	6	7	11	10						
0.2 mg/pl	TA 1537	5	7	11	10						
0.04 mg/pl	TA 1537	9	110	11	10		100		7.5		13.3
0.008 mg/pl	TA 1537	8	8	11	10						
0.0016 mg/pl	TA 1537	7	12	11	10		2		7.5		0.27
0.00032 mg/pl	TA 1537	6	7	11	10						

(a)=tester strain: (b)= no. of experimental revertant colony forming units: (c)=no of assayed spontaneous revertants: (d)= no. revertants in excess of the assayed spontaneous revertant rate: (e)=spontaneous reversion rate calculated from historical data: (f) = E-C/C_{AV}: act = activation with S-9

Table-6-A

MUTAGENIC ACTIVITY RATIO WORKSHEET

Salmonella/Microsome Assay

Substance Assayed: Code #37 Dissolved In: DMSO

Date: 12 Nov 80 Performed By: Kincannon, Sauer, Summers, Pulliam

Test Compound and Concentration	TA (a)	E (b)	E act	C (c)	C act	E-C (d)	E-C act	C _{AV} (e)	C _{AV} act	MUTAR (f)	MUTAR act
1.0 mg/pl	TA 1538	5	7	10	14						
0.2 mg/pl	TA 1538	10	21	10	14		7		17.1		0.41
0.04 mg/pl	TA 1538	10	25	10	14		11		17.1		0.64
0.008 mg/pl	TA 1538	9	17	10	14		3		17.1		0.17
0.0016 mg/pl	TA 1538	12	14	10	14	2		8.3		0.24	
0.00032 mg/pl	TA 1538	5	13	10	14						

(a)- tester strain: (b)-no. of experimental revertant colony forming units: (c)-no. of assayed spontaneous revertants: (d)-no. revertants in excess of the assayed spontaneous revertant rate: (e)=spontaneous reversion rate calculated from historical data: (f) - E-C/C_{AV} act = activation with S-9

Table-6-B

MUTAGENIC ACTIVITY RATIO WORKSHEET

Salmonella/Microsome Assay

Substance Assayed: Code #73A Dissolved In: DMSO

Date: 12 Nov 80 Performed By: Pulliam, Sauers

Test Compound and Concentration	TA (a)	E (b)	E act	C (c)	C act	E-C (d)	E-C act	C _{AV} (e)	C _{AV} act	MUTAR (f)	MUT-act
1.0 mg/pl	TA 98	9	14	22	26						
0.2 mg/pl	TA 98	14	21	22	26						
0.04 mg/pl	TA 98	16	21	22	26						
0.008 mg/pl	TA 98	14	16	22	26						
0.0016 mg/pl	TA 98	12	24	22	26						
0.00032 mg/pl	TA 98	13	15	22	26						
1.0 mg/pl	TA 100	47	54	115	112						
0.2 mg/pl	TA 100	63	68	115	112						
0.04 mg/pl	TA 100	80	90	115	112						
0.008 mg/pl	TA 100	65	90	115	112						
0.0016 mg/pl	TA 100	74	95	115	112						
0.00032 mg/pl	TA 100	76	80	115	112						

(a)= tester strain: (b)=no. of experimental revertant colony forming units: (c)-no. of assayed spontaneous revertants: (d)=no. revertants in excess of the assayed spontaneous revertant rate: (e)=spontaneous reversion rate calculated from historical data. (f) = E-C/C_{AV}: act = activation with S-9

Table-6-B

MUTAGENIC ACTIVITY RATIO WORKSHEET

Salmonella/Microsome Assay

Substance Assayed: Code #73A Dissolved In: DMSO

Date: 12 Nov 80 Performed By: Pulliam, Sayers

Test Compound and Concentration	TA (a)	E (b)	E act	C (c)	C act	E-C (d)	E-C act	C _{AV} (e)	C _{AV} act	MUTAR (f)	MUTAR act
1.0 mg/pl	TA 1535	9	5	13	5						
0.2 mg/pl	TA 1535	8	7	13	5		2		13.3		0.15
0.04 mg/pl	TA 1535	10	7	13	5		2		13.3		0.15
0.008 mg/pl	TA 1535	7	8	13	5		3		13.3		0.45
0.0016 mg/pl	TA 1535	7	8	13	5		3		13.3		0.45
0.00032 mg/pl	TA 1535	5	9	13	5		4		13.3		0.61
1.0 mg/pl	TA 1537	4	2	11	10						
0.2 mg/pl	TA 1537	5	6	11	10						
0.04 mg/pl	TA 1537	8	6	11	10						
0.008 mg/pl	TA 1537	10	6	11	10						
0.0016 mg/pl	TA 1537	7	9	11	10						
0.00032 mg/pl	TA 1537	5	7	11	10						

(a)- tester strain: (b)=no. of experimental revertant colony forming units: (c)=no. of assayed spontaneous revertants: (d)=no. revertants in excess of the assayed spontaneous revertant rate: (e)=spontaneous reversion rate calculated from historical data (f) = E-C/C_{AV} act = activation with S-9

Table-6-B

MUTAGENIC ACTIVITY RATIO WORKSHEET

Salmonella/Microsome Assay

Substance Assayed: Code #73A Dissolved In: DMSO

Date: 12 Nov 80 Performed By: Sauers

Test Compound and Concentration	TA (a)	E (b)	E act	C (c)	C act	E-C (d)	E-C act	CAY (e)	CAY act	MUTAR. (f)	MUTAR. act
1.0 mg/pl	TA 1538	8	15	10	14		1		17.1		0.06
0.2 mg/pl	TA 1538	10	14	10	14						
0.04 mg/pl	TA 1538	8	22	10	14		6		17.1		0.35
0.008 mg/pl	TA 1538	6	12	10	14						
0.0016 mg/pl	TA 1538	10	14	10	14						
0.00032 mg/pl	TA 1538	8	14	10	14						

(a)= tester strain: (b)=no. of experimental revertant colony forming units: (c)=no. of assayed spontaneous revertants: (d)=no. revertants in excess of the assayed spontaneous revertant rate: (e)=spontaneous reversion rate calculated from historical data: (f) = E-C/CAY: act = activation with S-9

Table-6-C
MUTAGENIC ACTIVITY RATIO WORKSHEET
Salmonella/Microsome Assay

Substance Assayed: Code #83 Dissolved In: DMSO
Date: 18 Nov 80 Performed By: Pulliam, Sauers

Test Compound and Concentration	TA (a)	E (b)	E act	C (c)	C act	E-C (d)	E-C act	C _{AV} (e)	C _{AV} act	MUTAR: (f)	MUTA act
1.0 mg/pl	TA 98	11	10	2	36	9		23.1		0.39	
0.2 mg/pl	TA 98	19	22	2	36	17		23.1		0.74	
0.04 mg/pl	TA 98	17	19	2	36	15		23.1		0.65	
0.008 mg/pl	TA 98	19	22	2	36	17		23.1		0.74	
0.0016 mg/pl	TA 98	20	17	2	36	18		23.1		0.78	
0.00032 mg/pl	TA 98	12	15	2	36	10		23.1		0.43	
1.0 mg/pl	TA 100	47	40	76	130						
0.2 mg/pl	TA 100	81	71	76	130	5		106		0.05	
0.04 mg/pl	TA 100	84	96	76	130	8		106		0.07	
0.008 mg/pl	TA 100	85	84	76	130	9		106		0.08	
0.0016 mg/pl	TA 100	66	60	76	130						
0.00032 mg/pl	TA 100	51	83	76	130						

(a)= tester strain: (b)=no. of experimental revertant colony forming units: (c)=no. of assayed spontaneous revertants: (d)=no. revertants in excess of the assayed spontaneous revertant rate: (e)=spontaneous reversion rate calculated from historical data: (f) - E-C/C_{AV}: act - activation with S-9

Table-6-C

MUTAGENIC ACTIVITY RATIO WORKSHEET

Salmonella/Microsome Assay

Substance Assayed: Code #83 Dissolved In: DMSO

Date: 13 Nov 80 Performed By: Pulliam, Sauers

Test Compound and Concentration	TA (a)	E (b)	E act	C (c)	C act	E-C (d)	E-C act	C _{AV} (e)	C _{AV} act	MUTAR (f)	MUTA act
1.0 mg/pl	TA 1535	4	6	5	6						
0.2 mg/pl	Ta 1535	9	7	5	6	4	1	9.4	13.3	0.43	0.08
0.04 mg/pl	TA 1535	5	7	5	6		1		13.3		0.08
0.008 mg/pl	TA 1535	6	6	5	6	1		9.4		0.11	
0.0016 mg/pl	TA 1535	6	4	5	6	1		9.4		0.11	
0.00032 mg/pl	TA 1535	6	7	5	6	1	1	9.4	13.3	0.11	0.08
1.0 mg/pl	TA 1537	1	1	3	5						
0.2 mg/pl	TA 1537	3	4	3	5						
0.04 mg/pl	TA 1537	5	3	3	5	2		6.1		0.33	
0.008 mg/pl	TA 1537	3	3	3	5						
0.0016 mg/pl	TA 1537	1	4	3	5						
0.00032 mg/pl	TA 1537	3	2	3	5						

(a)= tester strain: (b)=no. of experimental revertant colony forming units: (c)=no. of assayed spontaneous revertants: (d)=no. revertants in excess of the assayed spontaneous revertant rate: (e)=spontaneous reversion rate calculated from historical data. (f) = E-C/C_{AV}: act = activation with S-9

Table 6-C

MUTAGENIC ACTIVITY RATIO WORKSHEET

Salmonella/Microsome Assay

Substance Assayed: Code #83 Dissolved In: DMSO

Date: 18 Nov 80 Performed By: Sauers

Test Compound and Concentration	TA (a)	E (b)	E act	C (c)	C act	E-C (d)	E-C act	CAV (e)	CAV act	MUTAR (f)	MUTA act
1.0 mg/pl	TA 1538	4	7	2	11	2		8.3		0.24	
0.2 mg/pl	TA 1538	3	12	2	11	1	1	8.3	17.1	0.12	0.06
0.04 mg/pl	TA 1538	6	12	2	11	4	1	8.3	17.1	0.48	0.06
0.008 mg/pl	TA 1538	7	15	2	11	5	4	8.3	17.1	0.61	0.23
0.0016 mg/pl	TA 1538	13	7	2	11	11		8.3		1.32	
0.00032 mg/pl	TA 1538	6	11	2	11	4		8.3		0.48	

(a)= tester strain. (b)=no. of experimental revertant colony forming units: (c)=no. of assayed spontaneous revertants: (d)=no. revertants in excess of the assayed spontaneous revertant rate: (e)=spontaneous reversion rate calculated from historical data: (f) = E-C, CAV act = activation with S-9

Table-6-D

MUTAGENIC ACTIVITY RATIO WORKSHEET

Salmonella/Microsome Assay

Substance Assayed: Code #53 Dissolved In: DMSO

Date: 18 Nov 80 Performed By: Pulliam, Sauer

Test Compound and Concentration	TA (a)	E (b)	E act	C (c)	C act	E-C (d)	E-C act	C _{AV} (e)	C _{AV} act	MUTAR (f)	MUTA act
1.0 mg/pl	TA 98	17	25	2	36	15		23.1		0.65	
0.2 mg/pl	TA 98	15	20	2	36	13		23.1		0.56	
0.04 mg/pl	TA 98	17	18	2	36	15		23.1		0.65	
0.008 mg/pl	TA 98	17	23	2	36	15		23.1		0.65	
0.0016 mg/pl	TA 98	16	25	2	36	14		23.1		0.60	
0.00032 mg/pl	TA 98	13	25	2	36	11		23.1		0.48	
1.0 mg/pl	TA 100	66	74	76	130						
0.2 mg/pl	TA 100	89	86	76	130	13		106		0.12	
0.04 mg/pl	TA 100	75	85	76	130						
0.008 mg/pl	TA 100	84	93	76	130	8		106		0.08	
0.0016 mg/pl	TA 100	87	93	76	130	11		106		0.10	
0.00032 mg/pl	TA 100	70	90	76	130						

(a)= tester strain: (b)=no. of experimental revertant colony forming units: (c)=no. of assayed spontaneous revertants: (d)=no. revertants in excess of the assayed spontaneous revertant rate: (e)=spontaneous reversion rate calculated from historical data: (f) = E-C/C_{AV}: act = activation with S-9

Table-6-D

MUTAGENIC ACTIVITY RATIO WORKSHEET

Salmonella/Microsome Assay

Substance Assayed: Code #53 Dissolved In: DMSO

Date: 18 Nov 80 Performed By: Pulliam, Savers

Test Compound and Concentration	A (a)	E (b)	E act	C (c)	C act	E-C (d)	E-C act	CAV (e)	CAV act	MUTAR (f)	MUTA act
1.0 mg/pl	TA 1535	6	4	5	6	1		9.4	13.3	0.11	
0.2 mg/pl	TA 1535	8	7	5	6	3	1	9.4	13.3	0.32	0.08
0.04 mg/pl	TA 1535	9	9	5	6	4	3	9.4	13.3	0.43	0.23
0.008 mg/pl	TA 1535	6	9	5	6	7	7	9.4	13.3	0.76	0.08
0.0016 mg/pl	TA 1535	6	9	5	6	1	3	9.4	13.3	0.11	0.23
0.00032 mg/pl	TA 1535	13	8	5	6	8	2	9.4	13.3	0.89	0.15
1.0 mg/pl	TA 1537	3	2	3	5						
0.2 mg/pl	TA 1537	4	3	3	5	1		6.1		0.16	
0.04 mg/pl	TA 1537	2	5	3	5						
0.008 mg/pl	TA 1537	6	5	3	5	3		6.1		0.49	
0.0016 mg/pl	TA 1537	9	6	3	5	6	1	6.1	7.5	0.98	0.13
0.00032 mg/pl	TA 1537	3	6	3	5		1		7.5		0.13

(a)= tester strain; (b)=no. of experimental revertant colony forming units; (c)=no. of assayed spontaneous revertants; (d)=no. revertants in excess of the assayed spontaneous revertant rate; (e)=spontaneous reversion rate calculated from historical data; (f) = E-C/CAV act = activation with S-9

Table-6-D

MUTAGENIC ACTIVITY RATIO WORKSHEET

Salmonella/Microsome Assay

Substance Assayed: Code #53 Dissolved In: DMSO

Date: 18 Nov 80 Performed By: Pulliam, Sauers

Test Compound and Concentration	TA (a)	E (b)	E act	C (c)	C act	E-C (d)	E-C act	C _{AV} (e)	C _{AV} act	MUTAR (f)	MUT: act
1.0 mg/pl	TA 1538	14	13	2	11	12	2	8.3	17.1	1.45	0.10
0.2 mg/pl	TA 1538	9	9	2	11	7		8.3		0.84	
0.04 mg/pl	TA 1538	5	11	2	11	3		8.3		0.36	
0.008 mg/pl	TA 1538	11	16	2	11	9	5	8.3	17.1	1.08	0.26
0.0016 mg/pl	TA 1538	6	16	2	11	4	5	8.3	17.1	0.48	0.29
0.00032 mg/pl	TA 1538	9	11	2	11	7		8.3		0.84	

(a)= tester strain: (b)=no. of experimental revertant colony forming units: (c)=no. of assayed spontaneous revertants: (d)=no. revertants in excess of the assayed spontaneous revertant rate: (e)=spontaneous reversion rate calculated from historical data: (f) = E-C/C_{AV}: act = activation with S-9

Table 6-E
MUTAGENIC ACTIVITY RATIO WORKSHEET
 Salmonella/Microsome Assay

Substance Assayed: Code #91 Dissolved In: DMSO

Date: 18 Nov. 80 Performed By: Pulliam, Sauer

Test Compound and Concentration	TA (a)	E (b)	E act	C (c)	C act	E-C (d)	E-C act	CAY (e)	CAY act	MUTAR (f)	MUTAR act
0.01 mg/plate	TA 98	19	21	2	36	17		23.1		0.74	
2×10^{-3}	TA 98	20	21	2	36	18		23.1		0.78	
4×10^{-4}	TA 98	12	23	2	36	10		23.1		0.43	
8×10^{-5}	TA 98	15	24	2	36	13		23.1		0.56	
3.2×10^{-6}	TA 98	22	19	2	36	20		23.1		0.87	
0.01 mg/plate	TA 100	73	77	76	130						
2×10^{-3}	TA 100	66	69	76	130						
4×10^{-4}	TA 100	72	75	76	130						
8×10^{-5}	TA 100	36	76	76	130						
3.2×10^{-6}	TA 100	84	77	76	130						
0.01 mg/plate	TA 1535	9	9	5	6	4	3	9.4	13.3	0.43	0.20
2×10^{-3}	TA 1535	6	7	5	6	1	1	9.4	13.3	0.11	0.01
4×10^{-4}	TA 1535	9	7	5	6	4	1	9.4	13.3	0.43	0.01
8×10^{-5}	TA 1535	6	10	5	6	1	4	9.4	13.3	0.11	0.30
3.2×10^{-6}	TA 1535	10	9	5	6	5	3	9.4	13.3	0.53	0.10

(a)= tester strain: (b)=no. of experimental revertant colony forming units: (c)=no. of assayed spontaneous revertants: (d)=no. revertants in excess of the assayed spontaneous revertant rate (e)=spontaneous reversion rate calculated from historical data (f) = E-C/CAY act = activation with S-9

Table 6-E
MUTAGENIC ACTIVITY RATIO WORKSHEET
 Salmonella/Microsome Assay

Substance Assayed: Code # 91 Dissolved In: DMSO

Date: 18 Nov 80 Performed By: Pulliam, Sauer

Test Compound and Concentration	TA (a)	E (b)	E act	C (c)	C act	E-C (d)	E-C act	CAV (e)	CAV act	MUTAR (f)	MUTAR act
0.01 mg/plate	TA 1537	3	4	3	5						
2×10^{-3}	TA 1537	4	3	3	5	1		6.1		0.16	
4×10^{-4}	TA 1537	6	4	3	5	3		6.1		0.49	
8×10^{-5}	TA 1537	3	6	3	5		1		7.5		0.16
3.2×10^{-6}	TA 1537	4	7	3	5	1	2	6.1	7.5	0.16	0.23
0.01 mg/plate	TA 1538	8	10	2	11	6		8.3		0.73	
2×10^{-3}	TA 1538	6	8	2	11	4		8.3		0.48	
4×10^{-4}	TA 1538	6	9	2	11	4		8.3		0.48	
8×10^{-5}	TA 1538	6	11	2	11	4		8.3		0.48	
3.2×10^{-6}	TA 1538	9	15	2	11	7	4	8.3	17.1	0.84	0.23

(a)= tester strain: (b)=no. of experimental revertant colony forming units: (c)=no. of assayed spontaneous revertants: (d)=no. revertants in excess of the assayed spontaneous revertant rate: (e)=spontaneous reversion rate calculated from historical data: (f) = E-C/CAV: act = activation with S-9

Table-6-F
 MUTAGENIC ACTIVITY RATIO WORKSHEET
 Salmonella/Microsome Assay

Substance Assayed: Code # 37 Dissolved In: DMSO

Date: 11 Dec 80 Performed By: Pulliam, Sayers

Test Compound and Concentration	A (a)	E (b)	E act	C (c)	C act	E-C (d)	E-C act	CAV (e)	CAV act	MUTAR (f)	MUTA act
1.0 mg/pl	TA 1537	3	6	7	8						
0.2 mg/pl	TA 1537	4	4	7	8						
0.04 mg/pl	TA 1537	5	9	7	8		1		7.5		0.13
0.008 mg/pl	TA 1537	4	7	7	8						
0.0016 mg/pl	TA 1537	7	7	7	8						
0.00032 mg/pl	TA 1537	4	4	7	8						

(a)= tester strain: (b)=no. of experimental revertant colony forming units: (c)=no. of assayed spontaneous revertants: (d)=no. revertants in excess of the assayed spontaneous revertant rate (e)=spontaneous reversion rate calculated from historical data: (f) = E-C/CAV: act = activation with S-9

Table-6-G

MUTAGENIC ACTIVITY RATIO WORKSHEET

Salmonella/Microsome Assay

Substance Assayed: Code #53 Dissolved In: DMSO

Date: 11 Dec 80 Performed By: Pulliam, Sauers

Test Compound and Concentration	TA (a)	E (b)	E act	C (c)	C act	E-C (d)	E-C act	CAV (e)	CAV act	MUTAR (f)	MUTAR act
1.0 mg/pl	TA 1537	6	6	7	8						
0.2 mg/pl	TA 1537	5	7	7	8						
0.04 mg/pl	TA 1537	4	7	7	8						
0.008 mg/pl	TA 1537	4	10	7	8		2		7.5		0.26
0.0016 mg/pl	TA 1537	4	7	7	8						
0.00032 mg/pl	TA 1537	6	6	7	8						

(a)= tester strain: (b)=no. of experimental revertant colony forming units: (c)=no. of assayed spontaneous revertants: (d)=no. revertants in excess of the assayed spontaneous revertant rate: (e)=spontaneous reversion rate calculated from historical data. (f) = 1-C/CAV: act = activation with S-9

Table 6-I
MUTAGENIC ACTIVITY RATIO WORKSHEET
Salmonella/Microsome Assay

Substance Assayed: Code #91 Dissolved In: DMSO

Date: 11 Dec 80 Performed By: Sauers, Fulliam

Test Compound and Concentration	TA (a)	E (b)	E act	C (c)	C act	E-C (d)	E-C act	CAV (e)	CAV act	MUTAR (f)	MUTAR act
0.01 mg/plate	TA 1537	5	7	7	8						
2×10^{-3}	TA 1537	6	9	7	8		1		7.5		0.13
4×10^{-4}	TA 1537	5	6	7	8						
8×10^{-5}	TA 1537	7	8	7	8						
1.6×10^{-5}	TA 1537	7	6	7	8						
3.2×10^{-6}	TA 1537	5	7	7	8						

(a)= tester strain: (b)=no. of experimental revertant colony forming units: (c)=no. of assayed spontaneous revertants: (d)=no. revertants in excess of the assayed spontaneous revertant rate: (e)=spontaneous reversion rate calculated from historical data: (f) = E-C/CAV: act = activation with S-9

Table-6-k

MUTAGENIC ACTIVITY RATIO WORKSHEET

Salmonella/Microsome Assay

Substance Assayed: Code #53 Dissolved In: DMSO

Date: 16 Dec 80 Performed By: Pulliam, Sauers

Test Compound and Concentration	TA (a)	E (b)	E act	C (c)	C act	E-C (d)	E-C act	C _{AV} (e)	C _{AV} act	MUTAR (f)	MUTAR act
1.0 mg/pl	TA 98	14	18	25	30						
0.2 mg/pl	TA 98	16	17	25	30						
0.04 mg/pl	TA 98	19	17	25	30						
0.008 mg/pl	TA 98	15	10	25	30						
0.0016 mg/pl	TA 98	17	20	25	30						
0.00032 mg/pl	TA 98	11	8	25	30						
1.0 mg/pl	TA 1538	9		15	18						
0.2 mg/pl	TA 1538	14		15	18						
0.04 mg/pl	TA 1538	10		15	18						
0.008 mg/pl	TA 1538	7		15	18						
0.0016 mg/pl	TA 1538	9		15	18						
0.00032 mg/pl	TA 1538	7		15	18						

(a)= tester strain: (b)=no. of experimental revertant colony forming units: (c)=no. of assayed spontaneous revertants: (d)=no. revertants in excess of the assayed spontaneous revertant rate: (e)=spontaneous reversion rate calculated from historical data: (f) = E-C/C_{AV}: act = activation with S-9

Table-6-L

MUTAGENIC ACTIVITY RATIO WORKSHEET

Salmonella/Microsome Assay

Substance Assayed: Code #83 Dissolved In: DMSO

Date: 16 Dec 80 Performed By: Pulliam, Sauers

Test Compound and Concentration	TA (a)	E (b)	E act	C (c)	C act	E-C (d)	E-C act	CAV (e)	CAV act	MUTAR (f)	MUTAR act
1.0 mg/pl	TA 98	9	16	25	30						
0.2 mg/pl	TA 93	11	17	25	30						
0.04 mg/pl	TA 93	16	19	25	30						
0.008 mg/pl	TA 98	14	21	25	30						
0.0016 mg/pl	TA 98	17	20	25	30						
0.00032 mg/pl	TA 93	17	16	25	30						
1.0 mg/pl	TA 1538	10		15	18						
0.2 mg/pl	TA 1538	11		15	18						
0.04 mg/pl	TA 1538	14		15	18						
0.008 mg/pl	TA 1538	8		15	18						
0.00032 mg/pl	TA 1538	6		15	18						

(a)= tester strain: (b)=no. of experimental revertant colony forming units: (c)=no. of assayed spontaneous revertants: (d)=no. revertants in excess of the assayed spontaneous revertant rate: (e)=spontaneous reversion rate calculated from historical data: (f) = E-C/CAV: act = activation with S-9

Table-6-M

MUTAGENIC ACTIVITY RATIO WORKSHEET

Salmone[ll]a/Microsome Assay

Substance Assayed: Code #91 Dissolved In: DMSO

Date: 16 Dec 80 Performed By: Pulliam, Sauers

Test Compound and Concentration	TA (a)	E (b)	E act	C (c)	C act	E-C (d)	E-C act	C _{AV} (e)	C _{AV} act	MUTAR (f)	MUTAR act
0.001	TA 98	8	19	25	30						
0.002 mg/ml	TA 98	14	22	25	30						
0.0004 mg/pl	TA 98	11	18	25	30						
0.00008mg/pl	TA 98	13	19	25	30						
0.000016mg/pl	TA 98	14	23	25	30						
0.0000032mg/pl	TA 98	13	14	25	30						
0.01 mg/pl	TA 1538	6		15	18						
0.002 mg/pl	TA 1538	10		15	18						
0.0004 mg/pl	TA 1538	12		15	18						
0.00008 mg/pl	TA 1538	10		15	18						
0.000016 mg/pl	TA 1538	14		15	18						
0.0000032mg/pl	TA 1538	8		15	18						

(a)= tester strain: (b)=no. of experimental revertant colony forming units: (c)=no. of assayed spontaneous revertants: (d)=no. revertants in excess of the assayed spontaneous revertant rate: (e)=spontaneous reversion rate calculated from historical data (f) $(E-C)/C_{AV}$ act = activation with S-9

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