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MUTAGENIC SCREENING OF THREE DYES FOR MARKER GRENADES IN THE
SALMONELLA REVERSION ASSAY, THE L5178Y/TK⁺/- MOUSE LYMPHOMA ASSAY,
AND IN VIVO SISTER CHROMATID EXCHANGE IN MICE

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Final Report

Prepared by

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Genetic Toxicology Division
Health Effects Research Laboratory
US Environmental Protection Agency
Research Triangle Park, North Carolina 27711

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US Army Medical Research and Development Command
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US Army Project Order No. 3804

Project Officers: Mary C. Henry, Ph.D., CPT Gary M. Bratt, P.E., CIH

Performed by
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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) Two dyes, C.I. Solvent Yellow No. 33, and a C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 mixture were tested for mutagenicity in the Salmonella Reversion Assay, the L5178Y/TK ⁺ - Mouse Lymphoma Assay, and for Sister Chromatid Exchange (SCE) in vivo in mice. The in vitro mutagenicity assays were performed both with and without exogenous activation provided by Aroclor induced rat liver S-9. A >99.9% pure sample of the yellow dye [2-(2-quinolyl)-1,3-indandione] was also tested with and without exogenous activation in the Salmonella (continued on next page)		

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Reversion Assay and the L5178Y/TK⁺/Mouse Lymphoma Assay. Neither C.I. Solvent Yellow No. 33 nor the C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 mixture were positive in inducing in vivo SCE. All three dyes were tested in the standard plate incorporation test in seven strains TA100, TA102, TA104, TA1535, TA1537, TA1538, and TA98. The dyes were negative with and without exogenous activation in TA98, TA1535 and TA1538. One test with TA1537 was positive using the >99.9% pure yellow dye. All three dyes gave weakly positive results (less than a twofold increase) with S-9 in TA100. All three dyes were clearly positive in TA102 and TA104 both with and without S-9. All three dyes were found to induce mutation at the thymidine kinase locus in mouse lymphoma cells. Preliminary experiments (not financially supported under this IAG) indicate that the three dyes are clastogenic to mouse lymphoma cells.

3. C.I. Solvent Yellow No. 33 mixture

MUTAGENIC SCREENING OF THREE DYES FOR MARKER GRENADES IN THE
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FOREWORD

All of the mutagenicity assays were performed in the Genetic Toxicology Division of the Health Effects Research Laboratory (HERL), US Environmental Protection Agency (USEPA), Research Triangle Park, NC. The Salmonella Reversion Assays were performed under the direction of Drs. Joellen Lewtas and Larry Claxton. The L5178Y/TK⁺/⁻ Mouse Lymphoma Assays were performed under the direction of Dr. Martha Moore, and the in vivo Sister Chromatid Exchange assays were performed under the direction of Dr. James Allen. The purified yellow dye (>99.9% pure 2-(2-quinolyl)-1, 3-indandione) was supplied by Drs. Rogene Henderson and Roger McClellan, Inhalation Toxicology Research Institute, Lovelace Biomedical and Environmental Research Institute, Inc., Albuquerque, New Mexico.

EXECUTIVE SUMMARY

Dyes are used by the military in M18 marker signaling grenades. A number of organic dyes are presently being evaluated for potential use in these grenades. In addition to engineering studies for their performance in the field, the US Army is concerned with evaluating any potential health hazards that might result from personal contact with the dyes in the industrial setting. A part of this testing is the analysis of potential genetic toxicity.

Three dyes, C.I. Solvent Yellow No. 33, a purified C.I. Solvent Yellow No. 33 [$>99.9\%$ 2-(2-quinolyl)-1,3-indandione], and a C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 mixture were tested for potential genotoxicity. All three dyes were tested for mutagenicity in the Salmonella Reversion Assay and the L5178Y/TK⁺ Mouse Lymphoma Assay. The C.I. Solvent Yellow No. 33 and the C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 mixture were also tested for sister chromatid exchange (SCE) induction in vivo in mice. The in vitro mutagenicity assays were performed both with and without exogenous activation provided by Aroclor induced rat liver S-9.

Neither the C.I. Solvent Yellow No. 33 nor the C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 mixture were positive in inducing in vivo SCE. All three dyes were tested in the standard plate incorporation test using Salmonella typhimurium. Seven tester strains were used (TA98, TA100, TA1535, TA1537, TA1538, TA102 and TA104). The dyes were not mutagenic either with or without exogenous activation in TA98, TA1535 and TA1538. One test with TA1537 was positive using the pure yellow dye. All three dyes gave weakly positive results (less than a twofold increase) with S-9 activation in TA100. All three dyes were clearly positive in TA102 and TA104 both with and without S-9. All three dyes were found to induce mutation at the thymidine kinase locus in mouse lymphoma cells. Preliminary experiments (not financially supported under this IAG) indicate that the dyes are also clastogenic to mouse lymphoma cells.

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INTRODUCTION

Dyes are used by the military in M18 marker signaling grenades. A number of organic dyes are presently being evaluated for potential use in these grenades. In addition to engineering studies for their performance in the field, the US Army is concerned with evaluating any potential health hazards that might result from personal contact with the dyes in the industrial setting. A part of this testing is the analysis of potential genetic toxicity.

Three dyes, a yellow dye (C.I. Solvent Yellow No. 33), a purified yellow dye (purified C.I. Solvent Yellow No. 33) and a green-yellow dye which is a mixture of C.I. Solvent Yellow No. 33 and C.I. Solvent Green No. 3 were tested in this study. C.I. Solvent Yellow No. 33 is classified chemically as a quinoline. The principle color additive of the C.I. Solvent Yellow No. 33 is 2-(2-quinolyl)-1,3-indandione. and the additive of the C.I. Solvent Green No. 3 is 1-4-di-p-toluidino anthraquinone.

In preliminary tests, (unpublished results) different production lots (from those used in this study) of the C.I. Solvent Yellow No. 33 and C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 mixture were evaluated in the Salmonella Reversion Assay (using strains TA98, TA100, TA1535, TA1537 and TA1538) and found to be non-mutagenic. The L5178Y/TK⁺/- Mouse Lymphoma Assay and in vivo Sister Chromatid exchange analysis were chosen to analyze more fully the genotoxic potential of the dyes. In the course of the study it was established that both dyes were clearly positive in the Mouse Lymphoma Assay. Since the lots used in the present studies were different from those originally tested in the Salmonella Reversion Assay, these new lots of the dyes were retested in this assay. In these studies two new strains (TA102 TA104) were utilized in addition to the five standard strains. In order to determine if the principle color additive in the yellow dye or one of the impurities was responsible for the mutagenic activity, arrangements were made to obtain a >99.9% pure sample of 2-(2-quinolyl)-1,3-indandione from the Lovelace Biomedical and Environmental Research Institute, Inc. (BRDL). This pure dye was tested both in the Mouse Lymphoma Assay and also in the Salmonella Reversion Assay (using all seven tester strains).

MATERIALS AND METHODS

Organic Dyes

The dyes tested were:

Yellow Dye - [C.I. Solvent Yellow No. 33, 2-(2-quinolyl)-1,3-indandione]

Yellow-Green Dye - [a mixture of C.I. Solvent Yellow No. 33 and C.I. Solvent Green No. 3 (1,4-di-p-toluidino anthraquinone)]

Purified Yellow Dye - [C.I. Solvent Yellow No. 33, >99.9% pure 2-(2-quinolyl)-1,3-indandione]

Chemicals

C.I. Solvent Yellow No. 33 and the C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 mixture were supplied by BRDL. Each was analyzed by high pressure liquid chromatography (HPLC; reverse phase column; gradient of 90:10 methanol:water to 100% methanol in 10 minutes; 1 ml/min flow rate; UV detection at 254 nm). C.I. Solvent Yellow No. 33 was 93.1% 2-(2-quinolyl)-1,3-indandione, <1.8% phthalic acid/anhydride and <0.4% quinaldine by weight. The C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 mixture was 95.0% 2-(2'-quinolyl)-1,3-indandione and 1,4-di-p-toluidino anthraquinone (in a 1:2 ratio), <0.6% phthalic acid/anhydride, 0.2% quinaldine, 0.1% p-toluidine and <0.1% quinazarin.

Purified yellow dye was prepared by recrystallizing C.I. Solvent Yellow No. 33 three times from ethyl acetate. HPLC analysis showed that the sum of unknown UV absorbing impurities (quantities based on peak heights relative to parent compound), and phthalic acid/anhydride and quinaldine (quantitated using standards) was <0.1% of the 2-(2'-quinolyl)-1,3-indandione present.

SALMONELLA REVERSION ASSAY

The procedures used were those of Ames, et al. (1975) with minor modifications. Modifications are included in the description that follows. For each sample, seven histidine-requiring strains were used. The strains used were TA100, TA102, TA104, TA1535, TA1537, TA1538, and TA98. The mechanisms by which each of these strains revert to prototrophy are fully discussed in other publications (Ames et al., 1975; Maron and Ames, 1983). In addition to these basic mechanisms, the reader should keep in mind the following salient points. These strains carry an rfa mutation which produces a deficiency in bacterial cell wall lipopolysaccharides and increases the cell's permeability to large molecules; the uvrB mutation which decreases genetic repair; the R-factor plasmid in strains TA98 and TA100 increases their sensitivity by participating in error-prone repair and causes a higher spontaneous mutation rate. The seven strains differ in the number of spontaneous revertants per plate generally found. Compounds which are known mutagens for the different strains, with and without activation, were included in each assay as positive controls. The retention of phenotypic characteristics were checked with each test by examining for histidine auxotrophy (lack of growth on histidine deficient medium), deep rough character (sensitivity to crystal violet on a disk), UV-repair deficiency (sensitivity to UV light), and the presence of the appropriate plasmid (resistance to ampicillin on a disk).

Frozen permanent cultures containing fresh nutrient broth cultures with dimethylsulfoxide (DMSO) were maintained at -80°C. A working source of these cultures was maintained on master plates. All strains were initially grown in nutrient (Difco) broth at 37°C for 16 hours.

Preparation of Rat Liver S-9 Mix

Male CD-1 (Fisher derived) rats weighing approximately 200 g were given a single intraperitoneal injection of Aroclor 1254 (Ar) in corn oil (200 mg/ml) at a dose of 50 mg/kg of body weight. One day prior to termination the animals were taken off food but provided water ad libitum. The livers were aseptically removed and washed in sterile cold 0.15 M KCl. All subsequent steps were performed at 0° to 4°C with cold sterile solutions and sterile glassware. The livers were minced with scissors in 0.15 M KCl (3 ml/g wet weight liver) and homogenized with a Potter-Elvehjem homogenizer. The homogenate was centrifuged for 10 min at 9,000 x g, the supernatant (S-9) decanted and stored in convenient aliquots at -80°C.

The S-9 is mixed with a cofactor solution containing 8 μ mol MgCl₂, 32 μ mol KCl, 5 μ mol glucose-6-phosphate and 4 μ mol nicotinamide adenine dinucleotide in 100 μ mol of sodium phosphate buffer, pH 7.4. The amount of S-9 used in the S-9 mix was between 0.05 and 0.1 ml S-9/ml cofactor solution.

Test Procedure

For revertant selection, minimal Vogel-Bonner medium E supplemented with 1.5 percent Difco bacto agar and 2 percent glucose was used for base agar layers. The top agar (0.6 percent Difco bacto agar, 0.5 percent NaCl) at 45°C was supplemented with minimal amounts of histidine and biotin, the bacterial broth culture ($1-2 \times 10^9$ viable cells per ml) and the test material dissolved in DMSO (supplied sterile, spectrophotometric grade). For tests without activation, 0.5 ml of buffer was added instead of the S-9 mix to the top agar. The plates were incubated in the dark at 37°C for 72 hr. The plates were examined for background growth and the number of colonies per plate were counted using an Artek 880 automatic colony counter.

Preincubation was accomplished by incubating the bacteria, the compound and/or solvent, and the activation system (S-9) (when required) at 37°C in a water bath. The culture medium was the same as the overlay agar except that the melted agar was not added until the incubation was completed. The preincubation period was 30 minutes. All other aspects of the procedure were the same as the plate incorporation test.

Statistical Analyses

Statistical tests and computer programs used were those of Stead, et al. (1981). This model assumes revertant colony formation at any dose follows a Poisson process, while the mean number of revertants per plate is a nonlinear function of up to four parameters. The resultant system of nonlinear equations is solved using a modified Gauss-Newton iterative scheme to obtain maximum likelihood estimates of the model parameters. Significance of the key parameters was tested by fitting reduced models and using likelihood ratio tests.

The determination of positives was based on the following criteria:

- The data must not vary significantly from a Poisson distribution ($p > 0.01$).
- The data must be acceptable by the test of adequacy of fit of the model ($p > 0.01$).
- The test for mutagenicity, the slope of the curve, must be significant ($p < 0.01$).
- At least a twofold increase must have occurred over spontaneous levels at one or more doses; otherwise, the response is recorded as weak and/or questionable
- All positive and negative controls must have given expected responses as compared to HERL, USEPA historical values and those published by Ames et al. (1975).
- Histidine cross-feeding and/or contamination must not have been shown to occur.

The modeling of the bioassay provides a valuable aid to the researcher; however, each curve was (and needs to be) examined individually in order to assure confidence in the apparent conclusions of the statistical process. For example, if the dose response data "fit" statistically a horizontal line (response vs. dose), the model will under some circumstances record a mutagenicity p -value less than 0.01; however, since the slope equals zero the response is negative.

The reader must also keep in mind that these particular tests were performed to maximize the chance of detecting a mutagenic response and not to provide comparative slope values. Examination of the data, therefore, shows that test doses were often adjusted due to results of a previous test. These adjustments obviously can shift results from a negative response to positive result (e.g. if a compound was initially tested at too low a dose response range) and may alter the slope value (e.g. providing more doses in the central portion of the dose-response curve).

The minimum testing requirements were as follows:

- A minimum of five doses at half-log intervals with the highest dose being highly toxic, as shown by background clearing and/or reduction in expected revertant counts per plate.
- Spontaneous and positive controls done at least in duplicate and providing the expected response as compared to HERL, USEPA historical values and those published by Ames et al. (1975).
- Positive controls (in duplicate) for the microsomal activation combination used are within normal ranges as compared to HERL, USEPA historical values and those published by Ames et al. (1975).
- These minimum criteria are carefully explained in other publications (Ames, 1975; de Serres and Shelby, 1979).

L5178Y/TK⁺/- MOUSE LYMPHOMA ASSAY

The C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 mixture, C.I. Solvent Yellow No. 33 and the purified yellow dye were evaluated for mutagenicity in the L5178Y/TK⁺/- Mouse Lymphoma Assay using the procedures of Clive and Spector, 1975, as amended by Clive et al., 1979, and Moore and Clive 1982. This in vitro mammalian system evaluates mutations affecting the thymidine kinase locus. This assay may be particularly useful in a test battery since the mutants quantitated can be divided, by colony size, into two distinct groups (small colony and large colony mutants). These two classes of mutants appear to reflect the relative clastogenic and mutagenic potential of the compound tested. Hozier et al. (1981, 1983) have shown that the majority of small colony mutants reflect chromosome damage affecting chromosome 11 (the location of the thymidine kinase gene), while large colony mutants appear to represent small scale, perhaps single gene damage.

Cell Line and Cell Maintenance

The TK⁺/-3.7.2C heterozygote of L5178Y mouse lymphoma cells (supplied by Dr. Donald Clive) was utilized. This cell line was routinely grown in supplemented Fischer's Medium for Leukemic Cells of Mice (see below). Cells were monitored daily (except for weekends)

for acceptable growth rates. For weekends, the cells were sufficiently diluted so that they would remain in log phase growth; weekend cell doubling times were always determined. Weekly, prior to use in the assay, cells were cleansed of spontaneous TK^{-/-} cells by 24 hr growth in the presence of thymidine (3µg/ml), hypoxanthine (5µg/ml), methotrexate (0.1µg/ml) and glycine (7.5µg/ml) (THMG). This was followed by 24 hr growth in THG (THMG minus methotrexate) medium. Stock cells are stored in liquid nitrogen.

Media

TK⁺/⁻ -3.7.2C cells were cultivated in Fischer's Medium for Leukemic Cells of Mice supplemented with 31 µg/ml penicillin (1650 units/mg), 50 µg/ml streptomycin sulfate, 0.1% Pluronic F68, 0.22 mg/ml sodium pyruvate(F₀P), and 10% horse serum to make F₁₀P. Medium was heat inactivated at 55° C for 45 minutes. Cells were cloned in the above described supplemented medium using 20% rather than 10% horse serum. In addition, 0.37% Noble agar was added to solidify the cloning medium for colony formation. The selective agent used for mutation at the TK locus was 1 µg/ml trifluorothymidine (TFT).

Preparation of Chemical Solutions

Concentrations were prepared on a weight per volume basis. DMSO was used as the solvent. A fresh stock of test material was used for each separate experiment.

Preparation of the Metabolic Activation System

Aroclor 1242-1254 induced rat liver S-9 was purchased from EG&G Mason Research Institute. Rats weighing 200-300 g were injected intraperitoneally with a 2:1 mixture of Aroclor 1242 and 1254 in corn oil (500 mg of total Aroclor/kg body weight). After 5 days the animals were sacrificed by CO₂ exclusion of air. They were totally immersed in a solution of Wescodyne for approximately three seconds and their heads quickly excised. The livers were removed and placed in preweighed beakers containing 0.25M sucrose. Livers were washed three times in 50-100 ml portions of cold 0.25M sucrose to yield 3 ml per gram of liver. Livers were minced and then homogenized in a teflon pestle tissue grinder. The homogenate was centrifuged at 9000 x g for

10 min at 4°C. The lipid layer was removed and discarded. The supernatant was pooled and aliquoted into sterile serum vials, and placed directly into liquid nitrogen vapor phase containers for storage prior to shipping. A sterility check and activity test for standard promutagens in the Salmonella Reversion Assay were performed prior to shipping.

Upon receipt the S-9 was stored at -70°C in a Revco freezer and tested for the ability to activate 2-acetylaminofluorene to mutagenic metabolites as based on induced mutant frequency in the standard mouse lymphoma assay.

The cofactor mix made just prior to addition was comprised of 600 mg of triphosphopyridine nucleotide (TPN) and 1125 mg of isocitric acid (trisodium salt, trihydrate) to 75 mls of F₀P (Fischer's medium supplemented but without horse serum). This solution was filter-sterilized, placed on ice and mixed with 25 ml of freshly thawed S-9 to form the S-9 mix. This mix was kept on ice until used.

Dose-Ranging Assay

The dose-ranging experiment consisted of increasing doses of the test compound to the level of highest solubility (in the DMSO solvent). One 50 ml Corning polypropylene tube seeded with 6×10^6 cells in 6.0 ml of medium with a reduced amount of serum (5% instead of 10%) was used for each dose. Four ml of serum-free Fischer's medium (F₀P) were added to each tube. The compound was dissolved in DMSO at 100 x the highest concentration to be tested. Sufficient solvent was added to each tube so that after addition of the test compound all tubes contained the same final solvent concentration. Normally 1% DMSO is the maximum used in this assay to deliver the test compound. Because of the low solubility of these dyes in DMSO the dose which could be delivered in 1% DMSO was significantly below the 1000 µg/ml normally used in a dose-ranging assay as the highest dose. Therefore, the amount of dye delivered in 2% and 3% (final concentration) DMSO and the appropriate solvent controls were also used. The test compound was added to each appropriately labelled tube, the tubes were then regassed with 5% CO₂-in-air and incubated in a roller drum at 37° C for 4 hr. Following the 4 hr exposure period the tubes are centrifuged for 10 min at 200 x g and the supernatant containing the test compound was discarded. The cells were then washed twice in 10 ml of F₁₀P (2 X 10 minute centrifugations at 200 x g), and resuspended in 20 ml of fresh F₁₀P to a final cell concentration of 3×10^5 cells/ml. The tubes were regassed with 5% CO₂-in-air and incubated in the roller drum at 37°C.

Cell counts were determined with a Coulter Counter Model ZBI at 24 hours after exposure to the compound. Relative growth (as compared to the negative control) was calculated for each culture.

Mutagenicity Assay

The doses chosen for the mutagenicity assay were based on the results of the dose-ranging study. Because the doses delivered in 3% DMSO showed no greater cell toxicity after 24 hr than the doses delivered in 2% DMSO, the 3% DMSO doses were not used. The dosing protocol is identical to that used in the dose-ranging study (i.e. cells were treated for four hours, washed and incubated at 37°C). Positive control compounds were tested with each experiment. Methyl methane-sulfonate (MMS, 15 µg/ml) was used without exogenous activation, and 2-acetylaminofluorene (2-AAF, 40 µg/ml) with S-9 activation. Cell counts were determined with a Coulter Counter Model ZBI at 24 and 48 hrs. after exposure to the compound. Each culture was diluted daily to 2×10^5 cells/ml. At the end of 48 hrs the cells were cloned. Cloning allows for the selective growth and enumeration of mutant cells in a soft agar cloning medium (CM) and for the determination of cloning efficiency. Following dilution, the cells were allowed to mix for at least 30 minutes to minimize trauma. Fifteen ml of each culture was spun at $200 \times g$ for 10 min and the supernatant decanted. Approximately 1-2 ml of F₁₀P was added to each culture for resuspension of the cell pellet. The cell pellet was vigorously resuspended to ensure a single cell suspension and placed in 100 ml of CM to give a cell concentration of 3×10^4 cells/ml. The flasks were labelled with the appropriate culture number and selective agent to be used (TFT). The cells were allowed to acclimate for 30 minutes and then a 1:50 dilution was made. (1.0 ml was transferred from each culture to prelabelled flasks containing 50 ml of CM.) After mixing for 15 minutes, 1.0 ml from each 50-ml flask was transferred to 100 ml of CM and labelled with the culture number and "VC" (cell concentration = 6 cells/ml). The selective agent, 1 µg/ml TFT, was added to the flasks containing 3×10^4 cells/ml. Three petri plates per "TFT" and "VC" flask were poured, 33 ml per 100 mm petri plate. The plates were chilled at -20° C for 12 minutes, placed in a lucite box, sealed, and gassed with 5% CO₂-in-air or placed in a 5% CO₂ incubator. The boxes were incubated for 10-11 days at 37° C.

At the end of the incubation period the plates were scored for the number of colonies per plate using an Artek Colony Counter, Model 880. TFT-resistant colonies from selected cultures showing positive mutagenicity were sized by differential counts at periodic size discriminator settings. This information was expressed as histograms showing the relative proportions of small and large colony TFT-resistant mutants. This approach is a possible means of characterizing the type of mutagenic events occurring [i.e. single gene mutations (large colonies) or chromosomal aberrations affecting the TK and other genes (small colonies)].

Calculation of Mutant Frequency

The mutant frequency was calculated by dividing the total number of mutant colonies for each culture by the number of viable cells plated for the culture (as determined by the VC plates). The spontaneous mutant frequency (solvent control) was subtracted from the total mutant frequency to give the induced mutant frequency.

Criteria for the Evaluation of the Results

The following criteria (based on the statistical methods of Clive et al., 1979) must be met to designate the test compound as a definite positive:

1. One or more doses (from at least 2 separate assays) must show a significant increase (usually at least a doubling) over the background mutant frequency at reasonable (>10%) survival.
2. There must be a multi-point dose-related response at adequate (>10% survival) cytotoxicities.

If there is no significant increase of the mutant frequency over background and if the compound has been adequately tested (with and without metabolic activation, reasonably spaced doses, adequate cytotoxicity—sufficient doses in the 10-20% survival range) then the results will be interpreted as negative.

The minimum criteria for an acceptable assay are: (1) the plating efficiency of the solvent control is between 50 and 115%, (2) the spontaneous mutant frequency of the solvent control is less than 100×10^6 and (3) the positive controls show a definite positive response.

Method for Analysis of Gross Aberrations in L5178Y/TK⁺/ Mouse Lymphoma Cells

For the analysis of gross aberrations, samples were taken from the treated cells 24 hr after the midpoint of the 4 hr treatment period. Colcemid was added and cells treated with hypotonic KCl and fixed in acetic acid: methanol (1:4). Slides were made and cells stained with Wright's Stain. Metaphase spreads showing a near normal number of chromosomes were scored for aberrations.

IN VIVO SISTER CHROMATID EXCHANGE ANALYSIS IN MICE

Male C57BL/6 mice, 3-4 mos. old, were obtained from the Jackson Laboratory, Bar Harbor, Maine, and were acclimatized for at least 10 days after receipt. Animals were housed 5 per cage in an USEPA animal facility in laminar-flow rooms, with 15 cycles/hr of biocleaned air at 60-68% relative humidity. The room temperature was maintained at 68-70° F with a 12 hr light-dark cycle. Animals were fed lab chow (non-certified Purina) and water ad libitum.

Sister Chromatid Exchange (SCE) frequencies and cell replication kinetics were analyzed in mouse bone marrow cells after DNA labelling with 5-bromodeoxyuridine (BrdU; Sigma Chemical Company, St. Louis, Mo.). In vivo labelling was achieved with BrdU tablet methodology (Allen et al., 1978; McFee et al., 1983). Fifty mg BrdU tablets were prepared with a Parr Pellet Press and 0.178 in diameter punch and die (Parr Instrument Co., Moline, Il.) and coated over approximately 85% of the surface area with melted embedded paraffin (Fisher). Each experimental animal (weighing from 22 to 30 g) was implanted subcutaneously (lateral abdominal region) with a 50 mg BrdU tablet after brief anesthetization with Metofane (Pittman-Moore) inhalation.

Dye effectiveness to induce SCEs was determined by administering the test chemical as a single intraperitoneal injection (I.P.) (<0.2 ml volume) over a 3-4 point dose range, 3-4 mice per dose. The C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 mixture was administered in 0.1 ml DMSO + 0.1 ml corn oil. The C.I. Solvent Yellow No. 33 was administered in 0.1 ml corn oil. The C.I. Solvent Yellow No. 33 was administered in 0.1 ml (per 30g) DMSO only. (Higher volumes of DMSO were determined in preliminary experiments to be toxic, as evidenced by animal death or inhibited marrow cell-cycling. While corn oil appeared to enhance the solubility of the C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 mixture, it reduced the solubility of C.I. Solvent Yellow No. 33.) The dye injections were given 1/2 hr after BrdU tablet implantation. Negative control animals

were those which received no injections, and those which were injected with the solvent only. Positive control mice were injected with 15 or 30 mg/kg cyclophosphamide (Mead-Johnson). Approximately 23 hr later, all control mice were injected I.P. with 0.6 mg/kg of colchicine (Sigma) in order to collect metaphases. Treated mice were injected with colchicine after an additional 3-4 hr since preliminary chemical injection trials had indicated that cell-cycle delays were occurring. Two hours after colchicine injection, animals were sacrificed by cervical dislocation, marrow cells were harvested and processed through hypotonic (0.075 M KCl) and fixative (3:1 methanol: glacial acetic acid) steps, and slides were prepared in accordance with standard cytogenetic methodology (Latt et al., 1981). Chromatid differential staining was achieved with the Fluorescence-plus-Giemsa (FPG) technique (Wolff and Perry, 1974; Goto et al., 1978). For each mouse SCE frequencies were analyzed in 30 randomly selected, well-differentiated second division metaphase cells which contained the diploid ± 2 chromosomal complement. Cell replication kinetics were also assessed in 200 marrow cells/ animal. The proportions of first (M_1), second (M_2) and third (M_3) division cells were determined from chromosome stain patterns.

Additional studies were performed to determine if: 1) the injected test dye was dispersing or remaining localized within the peritoneum, and 2) higher marrow cell SCE frequencies would result from giving injections over three consecutive days. Concerning the former studies, animals were examined at the time of marrow cell harvest for the appearance of internal localizations of dye particles. Peritoneal cells from control and high dose (35 mg/kg C.I. Solvent Yellow No. 33) animals were saline-washed from the peritoneum, pelleted and compared for evidence of dye crystals, and for viability (Trypan Blue Exclusion). Differential peritoneal cell counts (Wright's Stain) were also made. In the latter studies, concerned with multiple exposures to the test dye, all experimental design and cytogenetic features were the same as those described for the single exposure trials. The only protocol modification was the administration of 3 I.P. injections of the test material given 24 hr apart. BrdU tablet implantation was carried out just prior (1/2 hr) to the last injection, and cells harvested 24 - 28 hr later.

RESULTS AND DISCUSSION

SALMONELLA REVERSION ASSAY

The Salmonella bioassay is frequently used to screen substances for genotoxicity including potential carcinogenicity. The three dyes were tested in the standard plate incorporation assay using seven strains supplied by Dr. Bruce Ames. The seven strains used were TA100, TA102, TA104, TA1535, TA1537, TA1538, and TA98. In addition, the three dyes were also tested using TA100 in a preincubation assay. A summary of the results is in Tables 1A and 1B. The actual data and statistical analysis can be found in Appendix A. The results are very heterogeneous. Two of the strains that detect frame shift mutagens, namely TA98 and TA1538, gave negative responses both with and without exogenous metabolic activation for all three dyes. Although TA1537 gave a clearly positive response in only one test (with the purified yellow dye), all three dyes showed a consistent tendency for increased revertant numbers at the higher dose levels. The strain that responds almost exclusively to base pair substitution mutagens, TA1535, provided negative results both with and without exogenous metabolic activation. Even though negative results were associated with strains TA1535, TA1538, and TA98, the more non-specific strain TA100 gave a positive though weak response to all three dyes when S-9 was present. Without this mammalian metabolic activation, the TA100 results were negative. Strains TA102 and TA104 provided the clearest indication of the mutagenicity of these compounds. All three dyes were clearly positive both with and without S-9 when TA102 and TA104 were used. In contrast to the other five strains which detect mutations within GC sequences, these two strains require reversion to prototrophy within an AT rich region. These results may be typical of quinones (Maron and Ames, 1983). The use of the preincubation assay with TA100 did not provide a significant enough advantage to warrant its continued use with the other strains. Although a clear indication of mutagenicity was seen using three different strains, the three dyes were difficult to test primarily due to their solubilities. The samples began to precipitate out of solution at approximately the 100 µg per plate dose. This solubility problem not only narrowed the linear dose response range but also may have contributed to increased plate-to-plate variation. Within the bacterial assays, all three dyes gave very similar results. Since the purified yellow dye tended to yield a slope value greater than either of the other two dyes, the purified yellow dye is at least one of the major mutagenic components within the other dyes. Whether or not other mutagens are present within these dyes is not readily apparent from these data.

TABLE 1A MUTAGENICITY OF THREE ARMY DYES AS DETECTED BY
SALMONELLA TYPHIMURIUM PLATE INCORPORATION AND PREINCUBATION (*) TESTS:
 (QUALITATIVE RESULTS)

Sample (Number)	Salmonella Typhimurium Strain:						
	TA100	TA102	TA104	TA1535	TA1537	TA1538	TA98
-----WITH ACTIVATION-----							
C.I. Solvent Green No. 3-							
C.I. Solvent Yellow No. 33	+	+	+	-	? ²	-	-
Mixture	+	+	+	-	? ¹	-	-
(BMGS-84-0001)	++	+	...	...	...	-	...
C.I. Solvent Yellow No. 33	+	+	? ⁵	-	-	-	-
(BMGS-84-0002)	+	+	+	-	?	-	-
	++	+	...	...	...	-	...
Purified Yellow	+	+	? ⁵	-	?	-	-
(BMGS-84-0003)	+	+	+	-	+	-	-
	++	+	...	...	...	...	...
-----WITHOUT ACTIVATION-----							
C.I. Solvent Green No. 3 -	? ¹	+	+	-	-	- ³	-
C.I. Solvent Yellow No. 33	-	+	+	-	-	-	-
Mixture	-*	+	...	...	...	-	...
(BMGS-84-0001)							
C.I. Solvent Yellow No. 33	? ⁶	? ⁴	-	-	? ^{2,3}	-	-
(BMGS-84-0002)	-	+	+	-	?	-	-
	-*	+	...	...	...	-	...
Purified Yellow	-	? ⁴	+	-	-	-	-
(BMGS-84-0003)	-	+	+	-	?	-	-
	-*	+	...	...	...	...	...

Footnotes:

- 1 - Positive slope apparently due to single plate value.
- 2 - Positive slope apparently due to a single dose.
- 3 - Outlier was included in original calculation.
- 4 - Spontaneous control outside of normal range.
- 5 - Model did not converge adequately and results are borderline in nature.
- * - Preincubation assay.

Results are recorded as follows: -, Negative; ?, questionable +, positive.

TABLE 1B MUTAGENICITY OF THREE ARMY DYES AS DETECTED BY
SALMONELLA TYPHIMURIUM PLATE INCORPORATION AND PREINCUBATION (*) TESTS:

Sample (Number)	Salmonella Typhimurium Strain:						
	TA100	TA102	TA104	TA1535	TA1537	TA1538	TA98
-----WITH ACTIVATION-----							
C.I. Solvent Green No. 3-							
C.I. Solvent Yellow No. 33	0.2	6.9	6.1	Neg	? ²	Neg	Neg
Mixture	0.7	3.1	4.2	Neg	? ¹	Neg	Neg
(BMGS-84-0001)	1.2*	8.0	...	...	...	Neg	...
C.I. Solvent Yellow No. 33	1.8	2.6	? ⁵	Neg	Neg	Neg	Neg
(BMGS-84-0002)	1.5	5.6	2.9	Neg	?	Neg	Neg
	1.5*	5.8	...	...	...	Neg	...
Purified Yellow	1.1	4.2	? ⁵	Neg	?	Neg	Neg
(BMGS-84-0003)	1.1	9.1	7.9	Neg	0.3	Neg	Neg
	2.1*	7.6	...	...	...	...	...
-----WITHOUT ACTIVATION-----							
C.I. Solvent Green No. 3 -	? ¹	5.0	2.4	Neg	Neg	Neg ³	Neg
C.I. Solvent Yellow No. 33	Neg	3.3	2.4	Neg	Neg	Neg	Neg
Mixture	Neg*	5.1	...	...	...	Neg	...
(BMGS-84-0001)							
C.I. Solvent Yellow No. 33	(?) ⁶	? ⁴	Neg	Neg	?	Neg	Neg
(BMGS-84-0002)	Neg	1.6	3.5	Neg ^{2,3}	?	Neg	Neg
	Neg*	2.3	...	...	...	Neg	...
Purified Yellow	Neg	? ⁴	1.9	Neg	Neg	Neg	Neg
(BMGS-84-0003)	Neg	4.8	1.5	Neg	?	Neg	Neg
	Neg*	6.0	...	...	...	...	...

Footnotes:

- 1 - Positive slope apparently due to single plate value.
- 2 - Positive slope apparently due to a single dose.
- 3 - Outlier was included in original calculation.
- 4 - Spontaneous control outside of normal range.
- 5 - Model did not converge adequately and results are borderline in nature.
- 6 - Value determined (1.2) appears to be an outlier since results could not be replicated.

* - Preincubation assay.

Results are recorded as follows: Neg, Negative; ?, questionable ;
or as Revertants per µg substance per plate if positive. Each value represents
an individual independent experiment.

L5178Y/TK⁺/- Mouse Lymphoma Assay

The three dyes were tested both with and without exogenous metabolic activation in the L5178Y/TK⁺/- Mouse Lymphoma Assay. The C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 mixture and the C.I. Solvent Yellow No. 33 were determined to be only slightly soluble in DMSO making testing over the normally prescribed (doses to 1000 µg/ml.) preliminary dose range impossible. A severely truncated dose range experiment was performed. Because of the solubility in DMSO problem and the concern that the dose delivered in 1% DMSO might not cause any toxicity, doses, and the appropriate solvent controls, delivered in 2% and 3% DMSO were also tested. (Normally the highest concentration of DMSO used for this assay is 1%). The results from this preliminary dose ranging experiment are found in Table 2. The actual mutagenesis experiments were performed using doses delivered in up to 2% DMSO. The pure yellow dye was found to be slightly more soluble in DMSO than the C.I. Solvent Yellow No. 33. Consequently, it was possible to test this compound at doses up to 50 µg/ml by delivering the dose in 1% DMSO. No dose-ranging study was necessary since the dose-range had already been established for the C.I. Solvent Yellow No. 33.

Table 3 shows the first experiment testing both the C.I. Solvent Yellow No. 33 and the C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 mixture with metabolic activation. The C.I. Solvent Yellow No. 33 is clearly positive while the C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 mixture gives only a (weak) positive response at 40 µg/ml. The cultures dosed with 6 µg/ml and above of the C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 mixture showed some precipitate following the treatment and after the first centrifugation. No precipitate was observed following the final resuspension. No precipitate was observed for the C.I. Solvent Yellow No. 33. In the repeat experiment (Table 4), the C.I. Solvent Yellow No. 33 is again clearly positive. The C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 mixture gives a questionable response, with a possible positive at the high (40 µg/ml) dose. Precipitate was seen during the cell wash at doses above 16 µg/ml. Table 5 shows the C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 mixture tested over an expanded dose range. As in the previous tests, the C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 mixture shows a positive response only at the highest dose tested. Precipitate was observed during the cell wash in cultures treated at 15 µg/ml or more. The expanded dose range test for the C.I. Solvent Yellow No. 33 with metabolic activation (Table 6) confirms the positive response.

TABLE 2. DOSE RANGING EXPERIMENT FOR C.I. SOLVENT YELLOW NO. 33 AND
C.I. SOLVENT GREEN NO. 3 - C.I. SOLVENT YELLOW NO. 33 MIXTURE
IN THE MOUSE LYMPHOMA MUTAGENICITY ASSAY

<u>Concentration</u>	<u>24 hr. Relative Growth</u> (%)
negative control	100
1% DMSO	100
2% DMSO	100
3% DMSO	100
<u>C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 Mixture</u>	
2.07 µg/ml	90.1
4.14 µg/ml	92.9
8.30 µg/ml	84.4
12.4 µg/ml	70.2
16.6 µg/ml	66.2
20.7 µg/ml	75.6
41.4 µg/ml (2% DMSO)	74.0
62.1 µg/ml (3% DMSO)	85.0
<u>C.I. Solvent Yellow No. 33</u>	
1.94 µg/ml	81.2
3.90 µg/ml	83.5
7.8 µg/ml	74.9
11.6 µg/ml	74.7
15.5 µg/ml	69.8
19.4 µg/ml	76.9
38.8 µg/ml (2% DMSO)	82.8
58.2 µg/ml (3% DMSO)	72.2

TABLE 3. MOUSE LYMPHOMA ASSAY OF C.I. SOLVENT GREEN NO. 3 -
C.I. SOLVENT YELLOW NO.33 MIXTURE AND C.I. SOLVENT YELLOW NO. 33
WITH METABOLIC ACTIVATION

Concentration	Relative Suspension Growth (%)	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Freq (x10 ⁶)	Induced Mutant Freq (x10 ⁶)
Neg. Control (w/o S-9)	100.0	448	90	100.0	100.0	40.2	
Neg. Control	100.0	594	121	100.0	100.0	40.7	
Solvent Cont. (1% DMSO)	100.0	466	131	100.0	100.0	56.2	
Solvent Cont. (2% DMSO)	100.0	476	139	100.0	100.0	58.4	
Pos. Control (40 µg/ml 2 AAF)	29.5	435	815	93.3	27.5	374.7	318.5
<u>C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 Mixture</u>							
2 µg/ml	112.4	484	185	103.8	116.7	76.4	20.2
6 µg/ml*	108.8	547	116	117.4	127.7	42.4	
12 µg/ml*	112.4	420	106	90.1	101.3	50.5	
16 µg/ml*	105.8	543	189	116.5	123.3	69.6	13.4
20 µg/ml*	109.3	515	159	110.4	120.7	61.8	5.6
40 µg/ml* (2% DMSO)	74.2	475	358	99.8	74.1	150.7	92.3
<u>C.I. Solvent Yellow No.33</u>							
2 µg/ml	110.8	546	142	117.1	129.7	52.0	
6 µg/ml	110.3	570	165	122.3	134.9	57.9	1.7
12 µg/ml	97.1	436	342	93.6	90.9	156.8	100.6
16 µg/ml	66.9	416	485	89.2	59.7	233.3	177.1
20 µg/ml	36.2	339	523	72.7	26.3	308.5	252.3
40 µg/ml (2% DMSO)	toxic						

*Showed some precipitate

TABLE 4. MOUSE LYMPHOMA ASSAY OF C.I. SOLVENT GREEN NO. 3 -
C.I. SOLVENT YELLOW NO. 33 MIXTURE AND C.I. SOLVENT YELLOW NO. 33
WITH METABOLIC ACTIVATION

Concentration	Relative Suspension Growth (%)	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Freq (x10 ⁶)	Induced Mutant Freq (x10 ⁶)
Neg. Control (w/o S-9)	100.0	435	106	100.0	100.0	48.7	
Neg. Control	100.0	435	119	100.0	100.0	54.7	
Solvent Cont. (1% DMSO)	100.0	407	145	100.0	100.0	71.3	
Solvent Cont. (2% DMSO)	100.0	456	157	100.0	100.0	68.9	
Pos. Control (40 µg/ml 2 AAF)	75.8	318	401	78.2	59.3	252.2	180.9
<u>C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 Mixture</u>							
2 µg/ml	100.0	381	117	93.6	93.6	61.4	
6 µg/ml	121.9	498	143	122.4	149.2	57.4	
12 µg/ml	114.5	395	130	97.0	111.1	65.9	
16 µg/ml*	124.7	446	145	109.6	139.6	65.0	
20 µg/ml*	108.1	427	127	105.0	113.5	59.4	
40 µg/ml* (2% DMSO)	84.2	372	261	81.6	68.7	140.3	71.4
<u>C.I. Solvent Yellow No. 33</u>							
2 µg/ml	121.0	432	132	106.2	128.5	61.1	
6 µg/ml	124.2	445	145	109.4	135.9	65.1	
12 µg/ml	116.1	377	232	92.6	107.5	123.1	51.8
16 µg/ml	100.0	309	316	76.0	76.0	204.5	133.2
20 µg/ml	75.8	288	327	70.8	53.7	227.1	155.8
40 µg/ml (2% DMSO)	toxic						

*Showed some precipitate

TABLE 5. MOUSE LYMPHOMA ASSAY OF C.I. SOLVENT GREEN NO. 3 - C.I. SOLVENT YELLOW NO. 33 MIXTURE WITH METABOLIC ACTIVATION

Concen ration	Relative Suspension Growth (%)	Total Viable Clones	Total Mutant Clones	Relative Cloning Effic. (%)	Relative Total Growth (%)	Mutant Freq (x10 ⁶)	Induced Mutant Freq (x10 ⁶)
Neg. Control (w/o S-9)	100.0	378	92	100.0	100.0	48.7	
Neg. Control	100.0	301	89	100.0	100.0	59.1	
Solvent Cont. (1% DMSO)	100.0	374	93	100.0	100.0	49.8	
Solvent Cont. (2% DMSO)	100.0	344	79	100.0	100.0	46.0	
Pos. Control (40 µg/ml 2 AAF)	37.3	272	523	72.7	27.1	384.8	335.0
<u>C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 Mixture</u>							
6 µg/ml	97.9	358	100	95.8	93.8	55.8	6.0
8 µg/ml	98.3	365	108	97.6	95.9	59.2	9.4
9 µg/ml	99.3	336	109	89.9	89.3	64.9	15.1
10 µg/ml	94.2	386	135	103.2	97.2	70.0	20.2
11 µg/ml	83.0	329	96	87.9	71.9	58.4	8.6
12 µg/ml	87.3	364	105	97.4	85.1	57.7	7.9
13 µg/ml	86.8	347	117	92.8	80.6	67.5	17.7
14 µg/ml	95.1	319	103	85.4	81.2	64.5	14.7
15 µg/ml*	91.5	357	107	95.5	87.4	60.0	10.2
16 µg/ml*	89.1	320	115	85.5	76.2	71.9	22.1
17 µg/ml*	90.2	334	118	89.4	80.6	70.6	20.8
18 µg/ml*	82.7	304	97	81.4	67.3	63.8	14.0
19 µg/ml*	85.8	309	101	82.7	70.9	65.4	15.6
20 µg/ml*	84.3	319	138	85.4	72.0	86.5	36.7
40 µg/ml* (2% DMSO)	42.3	248	340	66.3	27.8	274.4	228.4

*Showed some precipitate

TABLE 6. MOUSE LYMPHOMA ASSAY OF C.I. SOLVENT YELLOW NO. 33
WITH METABOLIC ACTIVATION

Concentration	Relative Suspension Growth (%)	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Freq (x10 ⁶)	Induced Mutant Freq (x10 ⁶)
Neg. Control (w/o S-9)	100.0	326	90	100.0	100.0	55.2	
Neg. Cont.	100.0	359	110	100.0	100.0	61.3	
Solvent Cont. (1% DMSO)	100.0	304	62	100.0	100.0	40.8	
Pos. Control (40 µg/ml 2 AAF)	52.3	230	426	75.5	39.5	370.8	330.0
<u>C.I. Solvent Yellow No. 33</u>							
2 µg/ml	114.1	244	108	80.3	91.6	88.4	47.6
6 µg/ml	102.1	228	107	75.0	76.5	93.9	53.1
8 µg/ml	102.1	214	128	70.4	71.9	119.5	78.7
9 µg/ml	86.4	272	(no TFT)	89.3	77.2	-	-
10 µg/ml	90.6	281	107	92.3	83.6	76.2	35.4
11 µg/ml	85.0	249	151	81.8	69.5	121.3	80.5
12 µg/ml	78.8	225	198	74.0	58.3	176.0	135.2
13 µg/ml	66.2	261	238	85.8	56.8	182.4	141.6
14 µg/ml	70.3	281	196	92.3	64.9	139.6	98.8
15 µg/ml	66.0	179	201	58.8	38.8	224.8	184.0
16 µg/ml	52.9	255	162	83.8	44.3	127.1	86.3
17 µg/ml	57.9	209	235	68.6	39.7	225.1	184.3
18 µg/ml	45.6	169	180	55.6	25.4	212.8	172.0
19 µg/ml	48.8	235	275	77.3	37.7	233.8	193.0
20 µg/ml	39.4	220	173	72.4	28.5	157.1	116.3

The first test of the two dyes without exogenous metabolic activation is shown in Table 7. Both the C.I. Solvent Yellow No. 33 and the C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 mixture were clearly positive. Green precipitate was observed during the cell wash at the 20 and 40 $\mu\text{g/ml}$ doses of the C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 mixture. The repeat experiments (Tables 8 and 9) confirmed the positive response of both dyes without exogenous activation. Precipitate was visible in the C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 mixture treated cultures at doses above 8 $\mu\text{g/ml}$.

Both the C.I. Solvent Yellow No. 33 and the C.I. Solvent Green No. 3 C.I. Solvent Yellow No. 33 mixture give higher mutagenic activity without exogenous activation (Tables 7-9) than with activation (Tables 3-6). Both dyes are positive without metabolic activation at doses which do not show a precipitate. The C.I. Solvent Yellow No. 33 dye is also mutagenic with S-9 activation. The C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 mixture is not mutagenic at doses showing no precipitate when S-9 activation is added to the system.

The purified yellow dye was also tested both with and without activation. The results are shown in Tables 10-13. As with the C.I. Solvent Yellow No. 33, the results are clearly positive. The response with exogenous activation is much weaker (Tables 10 and 11) than the response without activation (Tables 12 and 13).

It should be noted that this mutagenicity data obtained using the mouse lymphoma assay does not in all cases show a clear increasing dose-response relationship with increasing dose. This does not negate the positive nature of the data. Compounds which have solubility problems (i.e. are tested at doses near the limit of their solubility) tend to give plateau type dose-response curves similar to those observed in these studies. In addition it is not unusual or surprising that doses as close together as those used for these studies yield mutant frequencies which do not increase with each dose. In fact the closely spaced doses can almost be considered as replicates.

An analysis of the colony size distribution of the TFT-resistant mutants indicates that all three dyes produce significant proportions of small colony mutants (Figures 1-3, data with exogeneous activation not shown). This would predict that these dyes might also be clastogenic as well as mutagenic. To evaluate this possibility, gross aberration analysis of $\text{TK}^{+/-}$ mouse lymphoma cells treated with the dyes was performed. (This aspect of the research was not a part of

TABLE 7. MOUSE LYMPHOMA ASSAY OF C.I. SOLVENT GREEN NO. 3 -
C.I. SOLVENT YELLOW NO. 33 MIXTURE AND C.I. SOLVENT YELLOW NO. 33
WITHOUT METABOLIC ACTIVATION

Concentration	Relative Suspension Growth (%)	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Freq (x10 ⁶)	Induced Mutant Freq (x10 ⁶)
Neg. Control	100.0	384	119	100.0	100.0	62.0	
Solvent Cont. (1% DMSO)	100.0	519	147	100.0	100.0	56.6	
Solvent Cont. (2% DMSO)	100.0	588	137	100.0	100.0	46.6	
Pos. Control (15 µg/ml MMS)	57.2	257	683	49.5	28.3	531.9	469.9
<u>C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 Mixture</u>							
2 µg/ml	90.1	454	177	87.5	78.8	77.9	21.3
6 µg/ml	77.5	286	422	55.0	42.6	295.5	238.9
12 µg/ml	65.7	253	561	48.8	32.1	443.1	386.5
16 µg/ml	68.6	322	658	62.1	42.6	408.4	351.8
20 µg/ml*	59.2	253	473	48.8	28.9	373.6	317.0
40 µg/ml* (2% DMSO)	65.3	83	158	14.1	9.2	381.6	335.0
<u>C.I. Solvent Yellow No. 33</u>							
2 µg/ml	80.6	275	464	52.9	42.6	337.7	281.1
6 µg/ml	70.2	250	498	48.2	33.8	398.1	341.5
12 µg/ml	67.6	241	397	46.5	31.4	329.2	272.6
16 µg/ml	66.5	235	441	45.3	30.1	375.0	318.4
20 µg/ml	61.4	244	510	47.0	28.9	417.7	361.1
40 µg/ml (2% DMSO)	57.8	244	596	41.5	24.0	488.1	441.5

*Showed some precipitate

TABLE 8. MOUSE LYMPHOMA ASSAY OF C.I. SOLVENT GREEN NO. 3 - C.I. SOLVENT YELLOW NO. 33 MIXTURE WITHOUT METABOLIC ACTIVATION

Concentration	Relative Suspension Growth (%)	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Freq (x10 ⁶)	Induced Mutant Freq (x10 ⁶)
Neg. Control	100.0	433	73	100.0	100.0	33.7	
Solvent Cont. (1% DMSO)	100.0	422	110	100.0	100.0	52.1	
Pos. Control (15 µg/ml MMS)	53.0	170	763	40.2	18.8	898.7	865.0
<u>C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 Mixture</u>							
2 µg/ml	92.9	411	129	97.4	90.5	62.7	10.6
6 µg/ml	58.0	235	408	55.8	32.3	347.0	294.9
8 µg/ml	70.4	285	(no TFT)	67.6	47.6		
9 µg/ml*	70.8	253	473	60.0	42.5	373.6	321.5
10 µg/ml*	65.7	204	542	48.4	31.8	531.4	479.3
11 µg/ml*	60.9	248	611	58.7	35.7	493.1	441.0
12 µg/ml*	61.7	321	504	76.1	47.0	314.0	261.9
13 µg/ml*	58.4	248	546	58.7	34.3	440.7	388.6
14 µg/ml*	56.5	254	582	60.2	34.0	458.6	406.5
15 µg/ml*	65.3	256	587	60.7	39.7	458.2	406.1
16 µg/ml*	62.8	227	512	53.8	33.8	451.5	399.4
17 µg/ml*	63.0	268	645	63.6	40.0	481.0	428.9
18 µg/ml*	59.8	256	575	60.7	36.3	448.9	396.8
19 µg/ml*	57.4	262	611	62.2	35.7	466.0	413.9
20 µg/ml*	62.1	245	699	58.0	36.0	571.1	519.0

*Showed some precipitate

TABLE 9. MOUSE LYMPHOMA ASSAY OF C.I. SOLVENT YELLOW NO. 33
WITHOUT METABOLIC ACTIVATION

Concentration	Relative Suspension Growth (%)	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Freq (x10 ⁶)	Induced Mutant Freq (x10 ⁶)
Neg. Control	100.0	476	116	100.0	100.0	48.8	
Solvent Cont. (1% DMSO)	100.0	540	134	100.0	100.0	49.6	
Solvent Cont. (2% DMSO)	100.0	514	132	100.0	100.0	51.3	
Pos. Control (15 µg/ml MMS)	69.8	230	942	42.5	29.7	819.8	770.2
<u>C.I. Solvent Yellow No. 33</u>							
2 µg/ml	77.6	257	545	47.6	36.9	424.4	374.8
6 µg/ml	58.4	193	739	35.8	20.9	765.0	715.4
8 µg/ml	59.6	225	773	41.7	24.3	687.1	637.5
9 µg/ml	56.6	258	725	47.8	27.0	562.0	512.4
10 µg/ml	68.6	185	674	34.2	23.5	729.4	679.8
11 µg/ml	59.1	202	792	37.4	22.1	783.4	733.8
12 µg/ml	47.7	239	654	44.2	21.1	547.7	498.1
13 µg/ml	61.0	212	767	39.2	23.9	724.3	674.7
14 µg/ml	55.7	220	737	40.8	22.7	669.4	619.8
15 µg/ml	61.7	225	697	41.7	25.7	619.6	570.0
16 µg/ml	62.0	276	796	51.1	31.7	576.8	527.2
17 µg/ml	58.0	192	674	35.6	20.6	702.1	652.5
18 µg/ml	53.5	187	624	34.7	18.6	666.7	617.1
19 µg/ml	64.4	219	657	40.6	26.1	600.0	550.4
20 µg/ml	57.7	241	636	44.7	25.8	527.4	477.8
40 µg/ml (2% DMSO)	41.8	157	812	30.6	12.8	1033.1	981.8

TABLE 10. MOUSE LYMPHOMA ASSAY OF PURIFIED YELLOW DYE WITH METABOLIC ACTIVATION

Concentration	Relative Suspension Growth (%)	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Freq (x10 ⁶)	Induced Mutant Freq (x10 ⁶)
Neg. Control	100.0	363	67	100.0	100.0	36.90	
Neg. Control (w/o S-9)	100.0	334	79	100.0	100.0	47.40	
Solvent Cont.	100.0	294	83	100.0	100.0	56.50	
Solvent Cont. (w/o S-9)	100.0	263	70	100.0	100.0	53.30	
Pos. Control (40 µg/ml 2AAF)	74.2	227	219	77.1	57.2	193.12	136.66
<u>Purified Yellow Dye</u>							
5 µg/ml	95.3	278	62	94.5	90.0	44.60	
10 µg/ml	78.3	247	123	84.1	65.8	99.50	43.0
12 µg/ml	68.9	248	180	84.3	58.1	145.30	88.8
14 µg/ml	45.5	273	232	92.9	42.2	170.00	113.5
16 µg/ml	52.6	243	186	82.6	43.5	153.10	96.6
18 µg/ml	43.2	227	197	77.1	33.3	173.70	117.2
20 µg/ml	51.8	242	142	82.2	42.6	117.40	60.9
22 µg/ml	34.8	274	203	93.1	33.4	148.40	91.9
24 µg/ml	45.7	197	188	66.9	30.6	191.10	134.6

TABLE 11. MOUSE LYMPHOMA ASSAY OF PURIFIED YELLOW DYE WITH METABOLIC ACTIVATION

Concentration	Relative Suspension Growth (%)	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Freq ($\times 10^6$)	Induced Mutant Freq ($\times 10^6$)
Neg. Control	100.0	420	113	100.0	100.0	53.8	
Neg. Control (w/out S-9)	100.0	508	178	100.0	100.0	70.1	
Solvent Cont.	100.0	457	82	100.0	100.0	35.9	
Solvent Cont. (w/out S-9)	100.0	475	205	100.0	100.0	86.3	
Pos. Control (40 $\mu\text{g/ml}$ 2AAF)	16.4	320	772	69.9	11.5	482.8	446.9
<u>Purified Yellow Dye</u>							
2.5 $\mu\text{g/ml}$	92.9	458	113	100.1	92.9	49.4	13.5
5 $\mu\text{g/ml}$	93.1	508	105	111.0	103.3	41.4	5.5
10 $\mu\text{g/ml}$	92.4	415	194	90.8	83.9	93.4	57.5
12 $\mu\text{g/ml}$	75.1	430	251	93.9	70.6	116.9	81.0
14 $\mu\text{g/ml}$	73.3	393	188	86.0	63.0	95.7	59.8
16 $\mu\text{g/ml}$	54.4	462	286	101.0	55.0	123.8	87.9
18 $\mu\text{g/ml}$	41.3	423	279	92.5	38.2	131.9	96.0
20 $\mu\text{g/ml}$	60.1	515	310	112.6	67.7	120.4	84.5
22 $\mu\text{g/ml}$	30.7	497	405	108.6	33.4	163.0	127.1
24 $\mu\text{g/ml}$	27.2	487	446	106.6	29.0	183.1	147.2

TABLE 12. MOUSE LYMPHOMA ASSAY OF PURIFIED YELLOW DYE
WITHOUT METABOLIC ACTIVATION

Concentration	Relative Suspension Growth (%)	Total Viable Clones	Total Mutant Clones	Relative Cloning Effic. (%)	Relative Total Growth (%)	Mutant Freq (x10 ⁶)	Induced Mutant Freq (x10 ⁶)
Neg. Control	100.0	447	90	100.0	100.0	40.3	
Solvent Cont.	100.0	471	104	100.0	100.0	44.2	
Pos. Control (15 µg/ml MMS)	61.1	237	645	53.0	32.4	544.3	504.0
<u>Purified Yellow Dye</u>							
0.1 µg/ml	110.9	503	103	106.8	118.4	41.0	
0.5 µg/ml	107.9	372	144	79.0	85.2	77.4	33.2
2.5 µg/ml	60.7	262	683	55.5	33.6	525.9	461.7
5 µg/ml	50.2	200	493	42.4	21.3	493.5	449.3
10 µg/ml	33.3	130	542	27.5	9.2	836.4	792.2
20 µg/ml	30.9	120	566	25.5	7.9	943.3	899.1
30 µg/ml	53.3	224	476	47.5	25.3	425.4	381.2
40 µg/ml	57.5	239	506	50.7	29.2	423.8	379.6
50 µg/ml	53.8	181	353	38.3	20.6	390.9	346.7

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TABLE 13. MOUSE LYMPHOMA ASSAY OF PURIFIED YELLOW DYE
WITHOUT METABOLIC ACTIVATION

Concentration	Relative Suspension Growth (%)	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Freq (x10 ⁶)	Induced Mutant Freq (x10 ⁶)
Neg. Control	100.0	299	116	100.0	100.0	77.6	
Solvent Cont.	100.0	326	150	100.0	100.0	92.1	
Positive Control (15 µg/ml MMS)	74.4	183	289	61.2	45.6	315.8	238.2
<u>Purified Yellow Dye</u>							
0.1 µg/ml	120.1	323	116	98.9	118.8	72.0	
0.5 µg/ml	87.4	250	148	76.8	67.1	118.3	26.2
1.0 µg/ml	54.7	168	198	51.6	28.2	235.7	143.6
2.5 µg/ml	71.5	203	239	62.2	44.5	235.7	143.6
5 µg/ml	62.9	153	212	46.9	29.5	277.1	185.0
10 µg/ml	61.4	151	263	46.4	28.5	347.9	255.8
20 µg/ml	52.9	158	258	48.4	25.6	326.9	234.8
30 µg/ml	49.6	134	212	41.1	20.4	316.9	224.8
40 µg/ml	57.3	142	203	43.6	25.0	385.5	293.4
50 µg/ml	43.8	127	222	39.0	17.8	349.1	257.0

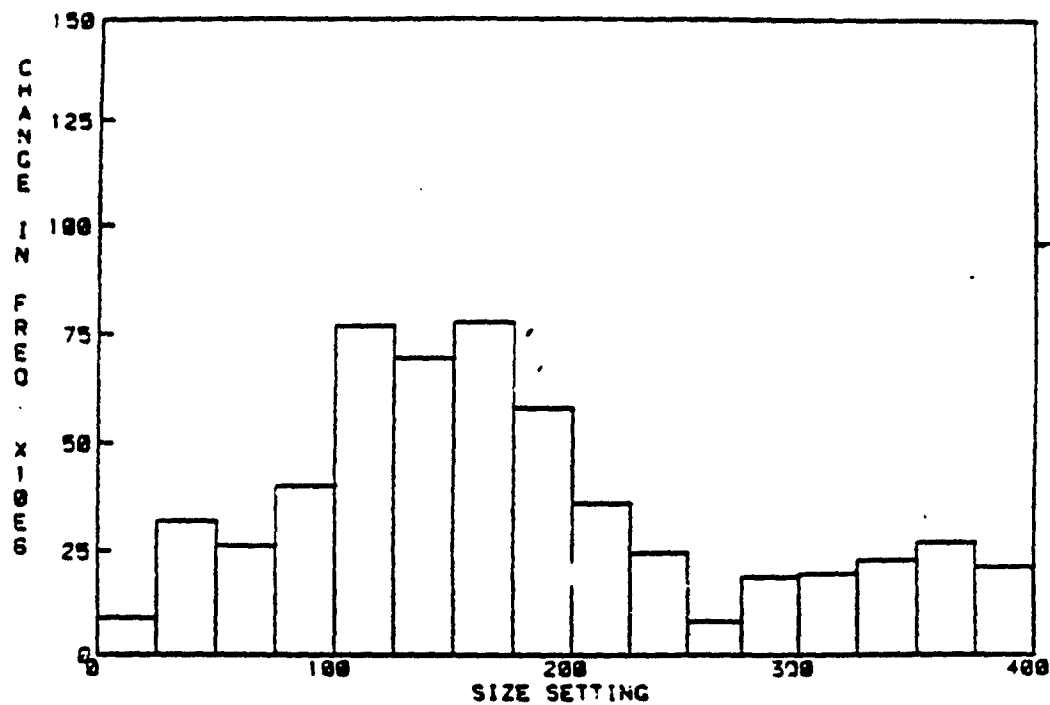


Figure 1: Relative size distribution of TFT-resistant mutants following treatment with 20 μ g/ml of C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33, without exogenous metabolic activation. The small colonies are shown in the left peak; the large colonies in the right peak.

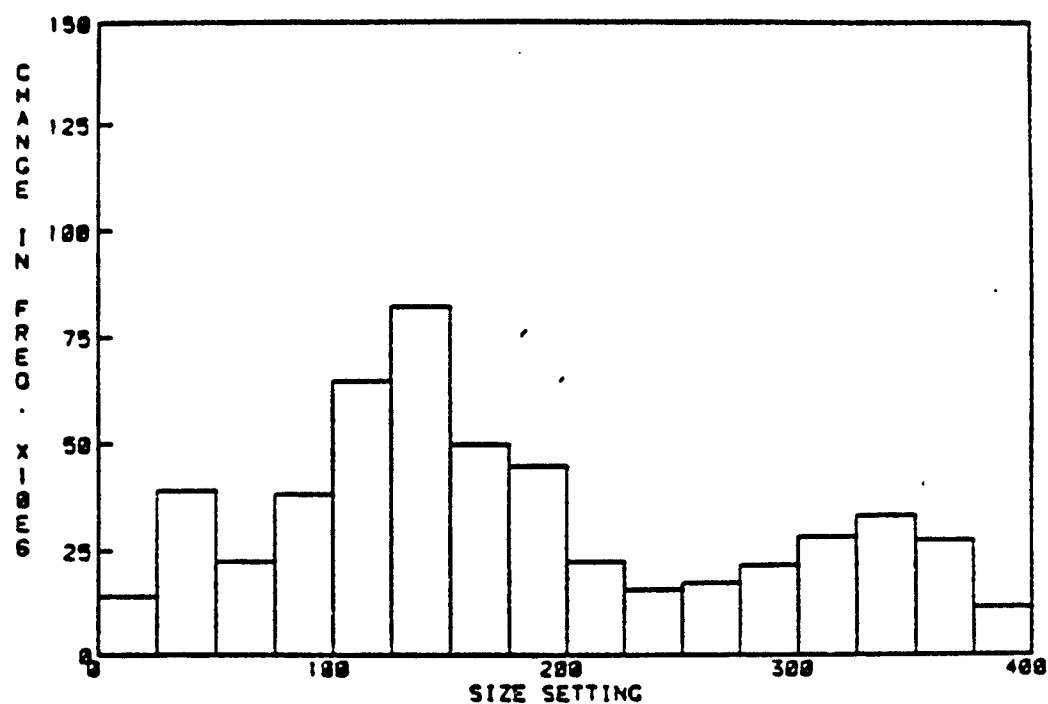


Figure 2: Relative size distribution of TFT-resistant mutants following treatment with 20 $\mu\text{g/ml}$ of C.I. Solvent Yellow No. 33, without exogenous metabolic activation. The small colonies are shown in the left peak; the large colonies in the right peak.

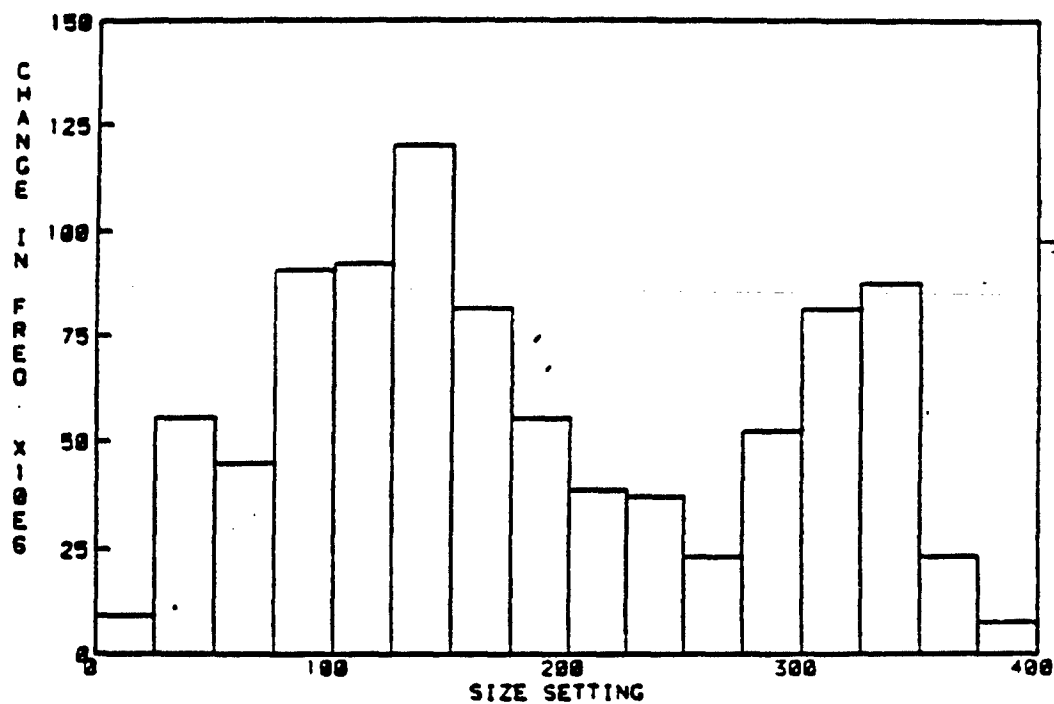


Figure 3: Relative size distribution of TFT-resistant mutants following treatment with 10 $\mu\text{g/ml}$ of >99.9% pure yellow dye, without exogenous metabolic activation. The small colonies are shown in the left peak; the large colonies in the right peak.

the work requested by the US Army. It is included because of its significance and usefulness in the evaluation of these dyes for potential human health hazard.) All three dyes were found to be clastogenic (Figures 4-6) to these mouse lymphoma cells. It should be noted that 100 cells/dose were analyzed in Figures 4 and 5 while 50 cells/dose were analyzed for Figure 6. Chromosome breaks, translocations and chromosome deletions were induced by the dyes.

In Vivo Sister Chromatid Exchange Analysis in Mice

The results from analyses of marrow cell SCEs and cell kinetics are presented in Tables 14 thru 19. Individual animal mean SCE values and relative proportions of metaphase cells at the first, second and third division after BrdU and a single C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 mixture exposure are provided in Table 14. Data for each exposure group are summarized in Table 15. Cyclophosphamide was clearly effective as a positive control. SCE frequencies were 7-8 times higher than negative control values, and significantly higher numbers of first division cells (with lower numbers of second and third division cells) evidenced a cytotoxic effect. However, there was no increase in SCE frequency at any dose due to exposure to the C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 mixture. A greater prevalence of third division cells sometimes noted in the dye treatment group was likely caused by the later times of cell harvest. The C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 mixture was observed to be dissolved in solution at the 10 and 20 mg/kg doses, and precipitated out of solution at the 40 mg/kg dose.

Data similarly tabulated for single intraperitoneal exposure trials with the C.I. Solvent Yellow No. 33 are presented in Tables 16 and 17. This dye was also ineffective in inducing SCEs. Cyclophosphamide clearly induced SCEs and slowed cell-cycling; however, no such effects were observed after exposure to the C.I. Solvent Yellow No. 33. SCE levels were not significantly different from control levels, and higher numbers of third division cells (lower numbers of first division cells) probably were a reflection of later cell harvest times. The C.I. Solvent Yellow No. 33 was observed to be in solution at the 5 and 15 mg/kg doses and precipitated out of solution at the 25 and 35 mg/kg doses.

Similar negative results were obtained after exposure to multiple injections of the C.I. Solvent Yellow No. 33 (Tables 18 and 19). Cyclophosphamide was effective; however, the dye-treated animals generally showed no greater SCE frequencies or cytotoxicity. One

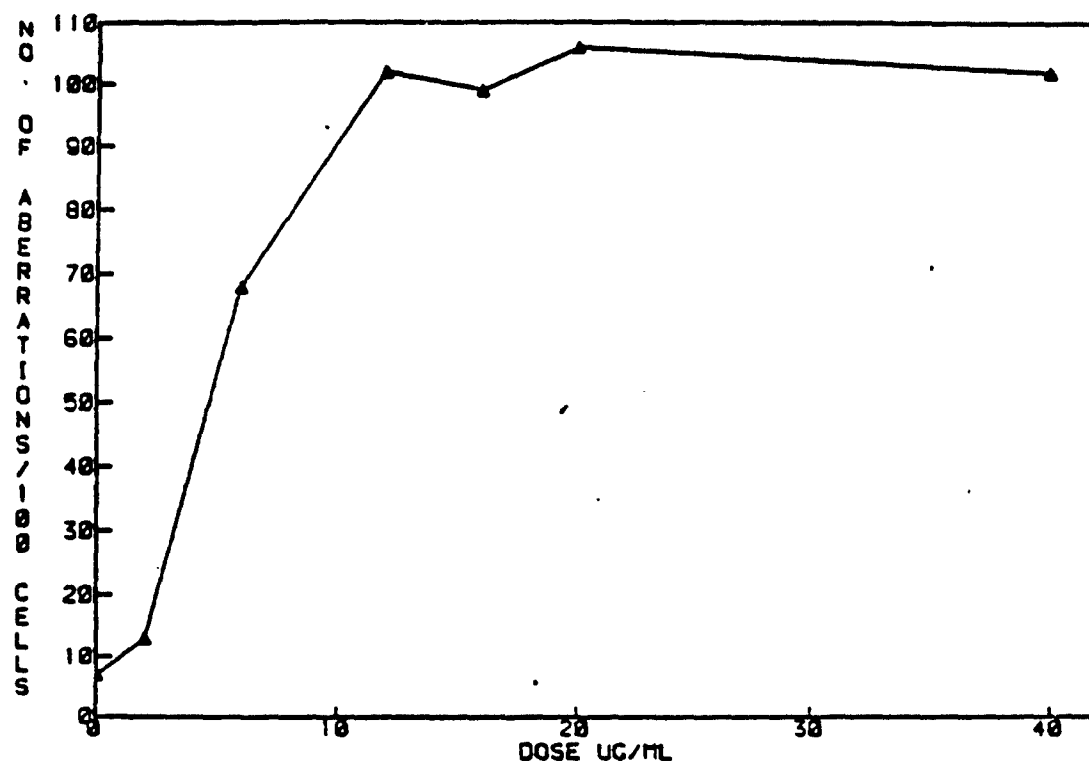


Figure 4: Gross aberration frequency in L5178Y/TK⁺/⁻ Mouse Lymphoma cells following treatment with C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 mixture, without exogenous metabolic activation.

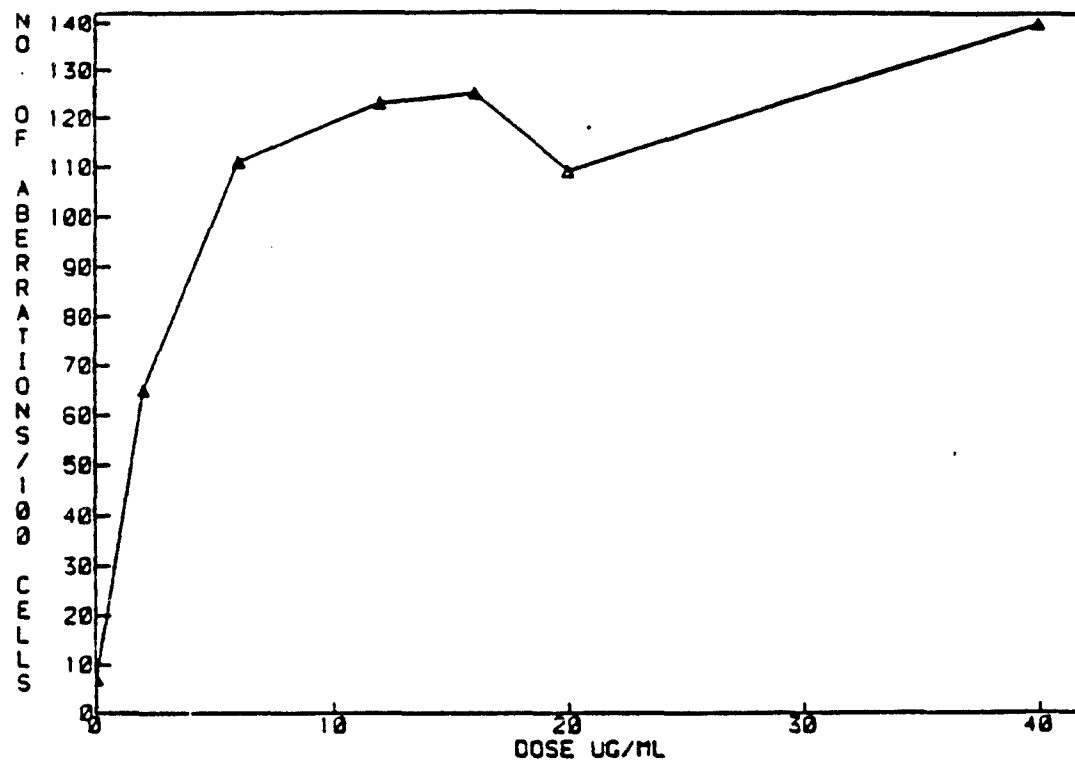


Figure 5: Gross aberration frequency in L5178Y/Tk⁺/⁻ Mouse Lymphoma cells following treatment with C.I. Solvent Yellow No. 33, without exogenous metabolic activation.

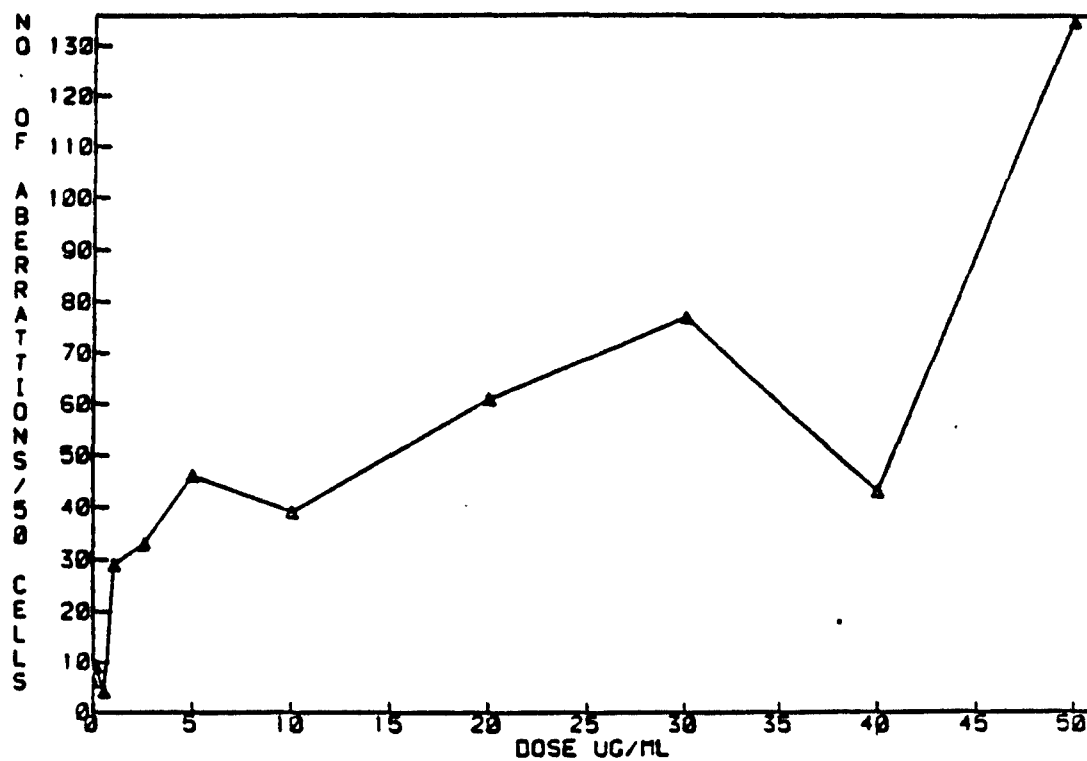


Figure 6: Gross aberration frequency in L5178Y/TK⁺/₋ Mouse Lymphoma cells following treatment with the >99.9% pure yellow dye, without exogenous metabolic activation.

TABLE 14. SCE AND CELL REPLICATION KINETICS ANALYSES OF MOUSE
BONE MARROW CELLS AFTER IN VIVO SINGLE EXPOSURE (I.P.) TO
C.I. SOLVENT GREEN NO. 3 - C.I. SOLVENT YELLOW NO. 33 MIXTURE:
SUMMARY/INDIVIDUAL ANIMAL

Treatment	Animal	SCE/Cell ^a		Cell Kinetics (%) ^b		
		Mean $\pm$ (S.D.)		M1	M2	M3
Negative Control	1	4	(1.7)	26.5	63.5	10.0
	2	5	(2.2)	20.0	71.5	8.5
	3	4	(1.8)	34.0	64.0	2.0
	4	No SCD ^c				
Solvent Control (DMSO + Corn Oil)	1	4	(2.0)	31.0	68.0	1.0
	2	3	(1.5)	28.0	66.0	6.0
	3	4	(1.7)	12.5	79.5	8.0
	4	4	(1.9)	12.0	82.0	6.0
Positive Control (Cyclophosphamide 15 mg/kg)	1	26	(6.8)	48.0	45.0	7.0
	2	33	(7.3)	50.0	47.0	3.0
	3	29	(8.1)	35.5	58.0	6.5
	4	29	(6.7)	57.0	42.0	1.0
C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 Mixture						
10 mg/kg	1	4	(1.9)	20.5	74.5	5.0
	2	3	(1.4)	14.5	74.0	11.5
	3	4	(2.2)	11.6	78.4	10.0
	4	3	(2.0)	17.5	81.0	1.5
20 mg/kg	1	3	(1.5)	5.0	55.0	40.0
	2	4	(2.1)	13.5	64.5	22.0
	3	3	(1.5)	5.5	37.0	57.5
	4	No SCD				
40 mg/kg	1	3	(1.8)	12.0	29.5	58.5
	2	3	(1.6)	42.0	58.0	0.0
	3	3	(1.3)	23.5	60.9	15.6
	4	4	(2.0)	31.5	59.0	9.5

a - Mean of 30 cells/animal

b - Based on 200 spreads/animal

c - SCD= Sister Chromatid Differentiation

TABLE 15. SCE AND CELL REPLICATION KINETICS ANALYSES OF MOUSE BONE MARROW CELLS AFTER IN VIVO SINGLE EXPOSURE (I.P.) TO DYE C.I. SOLVENT GREEN NO. 3 - C.I. SOLVENT YELLOW NO. 33 MIXTURE: SUMMARY/TREATMENT GROUP

<u>Treatment</u>	<u>Number of Animals</u>	<u>SCE/Cell^a</u>		<u>Cell Kinetics (%)^a</u>		
		<u>Mean ± (S.E.)</u>		<u>M1</u>	<u>M2</u>	<u>M3</u>
Negative Control	3	4	(0.8)	27	66	7
Solvent Control (DMSO + Corn Oil)	4	4	(0.6)	21	74	5
Positive Control (Cyclophosphamide 15 mg/kg)	4	29	(2.6)	48	48	4
<u>C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 Mixture</u>						
10 mg/kg	4	3	(0.4)	16	77	7
20 mg/kg	3	3	(0.6)	8	52	40
40 mg/kg	4	3	(0.4)	27	52	21

^a Mean of 3-4 animals/group

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TABLE 16. SCE AND CELL REPLICATION KINETICS ANALYSES OF MOUSE BONE MARROW CELLS AFTER IN VIVO SINGLE EXPOSURE (I.P.) TO C.I. SOLVENT YELLOW NO. 33: SUMMARY/INDIVIDUAL ANIMAL

Treatment	Animal	SCE/Cell ^a		Cell Kinetics (%) ^b		
		Mean	± (S.D.)	M1	M2	M3
Negative Control	1	4	(1.9)	33.5	54.0	12.5
	2	4	(2.1)	15.5	69.5	15.0
	3	4	(2.2)	17.5	77.5	5.0
	4	4	(1.9)	12.5	77.5	10.0
Solvent Control (DMSO)	1	4	(1.9)	6.0	83.0	11.0
	2	5	(2.1)	25.0	65.0	10.0
	3	5	(1.9)	12.5	80.0	7.5
	4	4	(1.3)	10.0	63.0	27.0
Positive Control (Cyclophosphamide 30 mg/kg)	1	57	(11.2)	79.0	21.0	0.0
	2	53	(9.6)	23.5	75.0	1.5
	3	50	(11.1)	29.0	65.0	6.0
	4	50	(10.6)	45.0	53.0	2.0
<u>C.I. Solvent Yellow No 33</u>						
5 mg/kg	1	5	(2.2)	14.0	70.0	16.0
	2	4	(2.1)	12.5	49.0	38.5
	3	5	(2.9)	8.0	60.0	32.0
	4	5	(2.6)	14.0	70.0	16.0
15 mg/kg	1	4	(1.9)	7.5	62.5	30.0
	2	5	(2.8)	6.0	70.0	24.0
	3	3	(1.1)	6.0	34.0	60.0
	4	No SCD ^c		-	-	-
25 mg/kg	1	2	(1.5)	14.5	46.5	39.0
	2	4	(2.3)	19.5	58.0	22.5
	3	3	(1.9)	13.5	26.5	60.0
	4	No SCD		-	-	-
35 mg/kg	1	2	(1.2)	2.5	14.5	83.0
	2	3	(1.6)	4.5	29.0	66.5
	3	4	(2.5)	14.0	56.5	29.5
	4	4	(2.6)	6.0	32.0	62.0

a Mean of 30 cells/animal

b Based on 200 spreads/animal

c SCD - Sister Chromatid Differentiation

TABLE 17. SCE AND CELL REPLICATION KINETICS ANALYSES OF MOUSE BONE MARROW CELLS AFTER IN VIVO SINGLE EXPOSURE (I.P.) TO C.I. SOLVENT YELLOW NO. 33: SUMMARY/TREATMENT GROUP

Treatment	Number of Animals	SCE/Cell ^a Mean $\pm$ (S.E.)	Cell Kinetics (%) ^a		
			M1	M2	M3
Negative Control	4	4 (0.3)	19.8	69.6	10.6
Solvent Control (DMSO)	4	5 (0.7)	13.4	72.8	13.8
Positive Control (Cyclophosphamide 30 mg/kg)	4	52 (3.5)	44.1	53.5	2.4
<u>C.I. Solvent Yellow No. 33</u>					
5 mg/kg	4	5 (0.5)	12.2	62.3	25.5
15 mg/kg	3	4 (0.8)	6.5	55.5	38.0
25 mg/kg	3	3 (0.7)	5.8	43.7	40.5
35 mg/kg	4	3 (0.7)	6.8	33.0	60.2

^a mean of 3-4 animals/group

TABLE 18. SCE AND CELL REPLICATION KINETICS ANALYSES OF MOUSE BONE MARROW CELLS
AFTER IN VIVO REPEATED (OVER 3 DAYS) EXPOSURES (I.P.) TO
C.I. SOLVENT YELLOW NO. 33: SUMMARY/INDIVIDUAL ANIMAL

Treatment	Animal	SCE/Cell ^a Mean $\pm$ (S.D.)		Cell Kinetics (%) ^b		
				M1	M2	M3
Negative Control	1	3	(1.8)	12.0	73.0	15.0
	2	3	(1.9)	10.0	84.0	6.0
	3	3	(1.5)	5.0	85.0	10.0
	4	3	(1.2)	10.0	76.0	14.0
Solvent Control (DMSO)	1	4	(1.7)	4.0	66.0	30.0
	2	4	(2.6)	1.0	27.0	72.0
	3	4	(2.1)	4.0	64.0	32.0
	4	4	(1.6)	2.0	46.0	52.0
Positive Control (Cyclophosphamide 15 mg/kg)	1	33	(8.5)	6.0	82.0	12.0
	2	35	(8.7)	15.0	71.0	14.0
	3	33	(5.0)	4.0	84.0	12.0
	4	31	(7.3)	7.0	86.0	7.0
<u>C.I. Solvent Yellow No. 33</u>						
5 mg/kg/day	1	5	(2.0)	2.0	40.0	58.0
	2	4	(2.1)	0.0	49.0	51.0
	3	3	(1.2)	0.0	19.0	81.0
	4	4	(2.0)	0.0	32.0	68.0
15 mg/kg/day	1	4	(2.1)	4.0	59.0	37.0
	2	4	(1.9)	5.0	75.0	20.0
	3	3	(1.5)	4.0	57.0	39.0
	4	4	(1.7)	3.0	31.0	66.0
25 mg/kg/day	1	3	(1.5)	1.0	53.0	41.0
	2	4	(1.5)	2.0	36.0	62.0
	3	3	(1.9)	3.0	53.0	44.0
	4	3	(1.3)	0.0	13.0	87.0
35 mg/kg/day	1	3	(1.6)	0.0	6.0	94.0
	2	7	(2.5)	21.0	75.0	4.0
	3	4	(2.1)	0.0	54.0	46.0
	4	3	(1.4)	3.0	31.0	66.0

^a Mean of 30 cells/animal

^b Mean of 200 spreads/animal

TABLE 19. SCE AND CELL REPLICATION KINETICS ANALYSES OF MOUSE BONE MARROW CELLS AFTER IN VIVO REPEATED (OVER 3 DAYS) EXPOSURES (I.P.) TO C.I. SOLVENT YELLOW NO. 33: SUMMARY/TREATMENT GROUP

<u>Treatment</u>	Number of Animals	SCE/Cell ^a		Cell Kinetics (%) ^a		
		Mean	± (S.E.)	M1	M2	M3
Negative Control	4	3	(0.1)	9	80	11
Solvent Control (DMSO)	4	4	(0.2)	3	51	46
Positive Control (Cyclophosphamide 15 mg/kg)	4	33	(1.3)	8	81	11
<u>C.I. Solvent Yellow No. 33</u>						
5 mg/kg/day	4	4	(0.7)	1	35	64
15 mg/kg/day	4	4	(0.2)	4	55	41
25 mg/kg/day	4	3	(0.4)	1	40	59
35 mg/kg/day	4	4	(1.7)	6	42	52

^a Mean of 4 animals/treatment

exceptional animal at the highest dose did reveal a small but significant SCE increase, and slowed cell-cycling as well. The exposure group as a whole did not reveal SCE induction or cytotoxicity effects.

Dye coloration or crystal evidence was not apparent in the peritoneum of animals dissected at the time of marrow cell harvest. There were also no crystals of dye evident in peritoneal cell pellets examined under a microscope. Peritoneal cell viabilities from 2 mice treated with 35 mg/kg C.I. Solvent Yellow No. 33 were similar to those of control (96.9% and 97.7% for treated animals vs. 95.2% and 97.5% for control animals). The percentage of cells represented as macrophages was also comparable between negative control and treated mice (83-84%). No traces of the dyes were observed in the marrow cell preparations.

Higher dose testing of both the C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 mixture and C.I. Solvent Yellow No. 33 was constrained by DMSO toxic effects and dye solubility in DMSO. Regardless of dye concentration, DMSO was determined in preliminary trials to inhibit cell cycling at injection volumes approximating 0.15 ml, and to cause animal death at higher doses. Doses considered to be at, or near, the limits of solubility in 0.1 ml of DMSO (20 mg/kg C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 mixture; 15 mg/kg C.I. Solvent Yellow No. 33), and higher doses clearly as particulate suspensions, failed to give any clear evidence of SCE induction or cytotoxicity. Preliminary experiments suggesting cell-cycle delay effects from the dyes were not confirmed. It is now felt that these effects probably stemmed from the DMSO solvent initially used at higher concentrations. With the exception of one mouse revealing an approximate doubling of the control SCE values after multiple C.I. Solvent Yellow No. 33 injections at the highest dose, the data for both dyes were uniformly negative.

Although it is possible that the dyes may not have been distributed to the bone marrow, there was no evidence of dye localization in or around the peritoneal cavity. Further, there was no indication that peritoneal cells were stimulated by the dye. In the context of the present study, it is presumed that the I.P. injected dyes are distributed to marrow cells, and are inactive for SCE induction. Additional studies to evaluate effects after alternative routes of exposure (i.e. oral), in different cell-types (i.e. lung-from primary cell cultures established after inhalation exposure), and/or in cell cultures after in vitro exposure (so as to circumvent possible liver detoxification effects) are suggested if further confirmation of

negative activity for this genotoxic end-point is desired. In conclusion, cytogenetic evaluations, specifically SCE analysis in bone marrow cells of mice exposed in vivo, have not revealed any evidence of genotoxic potential associated with these dyes.

Conclusions

Two dyes, C.I. Solvent Yellow No. 33 and a C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 mixture were tested for mutagenicity in seven strains of *Salmonella typhimurium*, the L5178Y/TK⁺/- mouse lymphoma assay and in vivo in mice for sister chromatid exchange analysis. A purified C.I. Solvent Yellow No. 33 was tested in the seven strains of *Salmonella typhimurium*, and the L5178Y/TK⁺/- mouse lymphoma assay. In vivo, the two dyes were incapable of inducing SCEs. In vitro all three dyes gave a positive response for gene mutation in *Salmonella* strains TA102 and TA104. In vitro, all three dyes induced gene mutation at the TK locus in the mouse lymphoma assay. A large proportion of the mutants were small colonies predicting that the dyes might be clastogenic. (Preliminary studies for gross aberrations using mouse lymphoma cells confirm that the dyes can induce chromosome breaks, translocations and chromosome deletions.)

In evaluating the significance of the results from this test battery it is important to consider the differences between the in vivo and the in vitro results, as well as the different sensitivities of the endpoints. The negative in vivo results could have resulted from the non-genotoxic nature of the dyes, the failure of the dyes to reach the target tissue, or the specific inability of the dyes to induce SCEs. In performing the tests, care was taken to observe that dye crystals were not apparent in the peritoneum of animals dissected at the time of marrow cell harvest.

The ability of the dyes to induce SCEs was also questioned. In preliminary studies (Doerr and Moore, unpublished data, U.S. EPA, 1984) the pure yellow dye was evaluated for its ability to induce SCEs in mouse lymphoma cells in vitro. The results were negative. It appears therefore that the negative in vivo results may be due to the insensitivity of the endpoint to the dyes rather than a true in vivo non-genotoxicity of the dyes. If a further evaluation of these dyes is desired, it would be interesting to test them in vivo for the induction of gross aberrations and in a different cell line (possibly human lymphocytes) in vitro for gross aberrations.

The purified C.I. Solvent Yellow No. 33 was tested to determine if the dye itself or the impurities were responsible for the observed mutagenic activity. This purified dye was found to be mutagenic in Salmonella, and at the TK locus of mouse lymphoma cells. It was also found to be clastogenic to mouse lymphoma cells. From these studies it is clear that the dye itself, not an impurity, is mutagenic. This dye is present both in the yellow dye and the green-yellow mixture.

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Appendix A

Data and statistical analysis for the Salmonella typhimurium bioassays of 3 Army dyes. The data is ordered in the following manner. Data sheets for individual experiments which include testing of a single compound both with and without activation are followed by two pages showing the statistical analysis of the data with and without activation. Cultures with S-9 activation are indicated as RLA026 or RLA027. BMGS-84-0001 is the code for the C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 Mixture; BMGS-84-0002 is C.I. Solvent Yellow No. 33; and BMGS-84-0003 is the purified yellow dye.

**MUTAGENICITY TESTING OF C.I. SOLVENT GREEN NO. 3 -
C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM**

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE GREEN
RESEARCH LAB: G88A ON 03/30/84

08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION

STRAIN: TA1CC

COMPOUND	A C T	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE					MEAN	STD
			A	B	C	D	E		
POS CONTROL									
NAAZIDE	-	3.00	1179	1205	1180			1188.00	14.73
2-AA	RLA026	0.50	363	360	278			340.33	36.69
NEG CONTROL									
DIMETHYLSULF	RLA026	100.00U	105	101	103			103.00	2.00
	-	100.00U	103	134	97			111.33	19.86
BMGS-34-UC01									
	RLA026	1.00	108	103				105.50	3.54
	RLA026	5.00	126	132				129.00	4.24
	RLA026	10.00	142	109				125.50	23.33
	RLA026	30.00	133	104				118.50	20.51
	RLA026	50.00	138	106				122.00	22.63
	RLA026	100.00	173	165				169.00	5.66
	RLA026	300.00	153	144				148.50	6.36
	RLA026	500.00	161	156				159.50	2.12
	RLA026	1000.00	142	134				138.00	5.66
	-	1.00	114	126				121.00	9.90
	-	5.00	102	144				123.00	29.70
	-	10.00	108	72				90.00	25.46
	-	30.00	132	127				129.50	3.54
	-	50.00	116	125				120.50	6.36
	-	100.00	131	113				122.00	12.73
	-	300.00	110	138				124.00	19.80
	-	500.00	165	145				155.00	14.14
	-	1000.00	131	117				124.00	9.90

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY S-9 : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIX/PLATE : 500UGS

T=-TOXIC
TNTC-TCO NUMEROUS TO COUNT
NATC-NOT ABLE TO COUNT

G-PGS T-PPT
N-NGS P-PPM
M-MGS B-PPB
L-NLS I-MM
U-ULS C-UM

Best Available Copy

MUTAGENICITY TESTING OF C.I. SOLVENT GREEN NO. 3 -
C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE GREEN
RESEARCH LAB: GB&A ON 03/30/84

08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION

STRAIN: TA100

•RLA026

ABOVE 100 UG/PLATE, THE SAMPLE APPEARS TO PRECIPITATE OUT OF SOLUTION.

STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT GREEN NO. 3 -
C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0001 LAB: CBBA ACTIVATION: + RLA026
STRAIN: TA100 DATE: 03/30/84 TECHNICIAN: MJK

DOSE UNITS	PLATE COUNTS	MEAN	S.D.
.00 UGS	105 101 103	103.00	2.00
.50* UGS	363 360 298	340.33	36.60
1.00 UGS	108 103	105.50	3.54
5.00 UGS	126 132	129.00	4.24
10.00 UGS	142 109	125.50	23.33
30.00 UGS	133 104	116.50	20.51
50.00 UGS	138 106	122.00	22.63
100.00 UGS	173 165	169.00	5.66
300.00 UGS	153 144	148.50	6.36

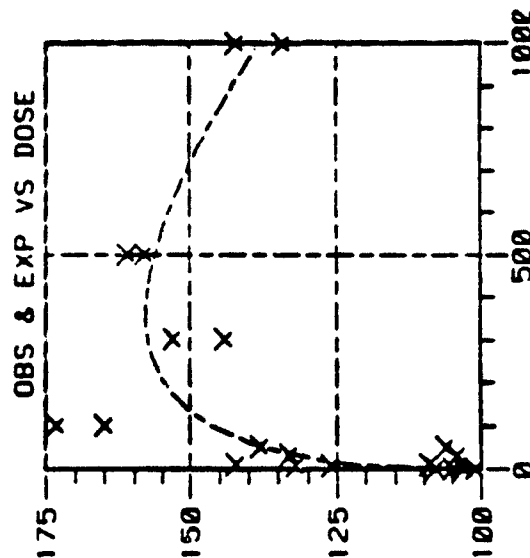
MORE THAN 9 DOSE LEVELS USED IN COMPUTATION BUT NOT DISPLAYED

ESTS. 103.565 1.8926 .4446 .00058
B(0) B(1) B(2) B(3)

TEST CHI-SQUARE DF P LOGL
POISSON 13 14 11 .2842 -76.8837
ADEQUACY 17.22 6 .0085 -85.4925
TOXICITY 9.36 1 .0022 -90.1714
MUTAGENICITY 54.67 2 .0000 -112.8263

AVERAGE SLOPE (NONLIN. MODEL) = .210
95% CONF. LIMITS = (.096, .461)

AVERAGE SLOPE (LINEAR REGR.) = .095
95% CONF. LIMITS = (.040, .150)



STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT GREEN NO. 3 -
C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0001 LAB: GB8A ACTIVATION: -
STRAIN: TA100 DATE: 03/30/84 TECHNICIAN: MJK

DOSE UNITS	PLATE COUNTS	MEAN	S.D.
0.00 UGS	103 134 97	111.33	19.86
3.00* UGS	1179 1205 1180	1188.00	14.73
1.00 UGS	114 128	121.00	9.00
5.00 UGS	102 144	123.00	20.70
10.00 UGS	108 72	90.00	25.46
30.00 UGS	132 127	129.50	3.54
50.00 UGS	116 125	120.50	6.36
100.00 UGS	131 113	122.00	12.73
300.00 UGS	110 138	124.00	19.80

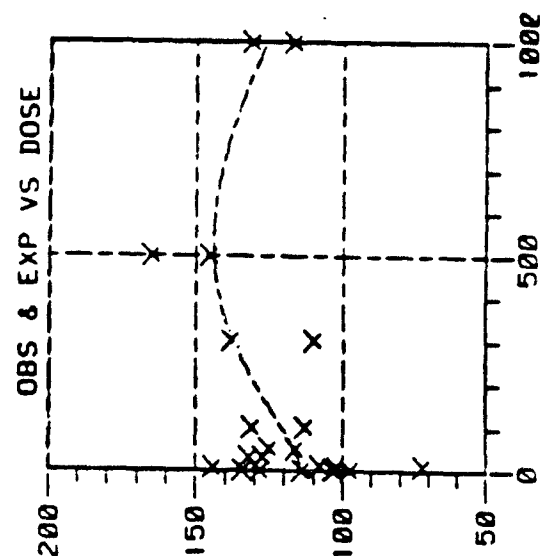
MORE THAN 9 DOSE LEVELS USED IN COMPUTATION BUT NOT DISPLAYED

ESTS. 114.862 -2.4707 1.2399 .00149
B(0) B(1) B(2) B(3)

TEST CHI-SQUARE DF P LOGL
POISSON 29.27 11 .0021 -84.1549
ADEQUACY 21.64 6 .0014 -94.9766
TOXICITY 5.91 1 .0151 -97.9301
MUTAGENICITY 17.72 2 .0001 -103.8347

AVERAGE SLOPE (NONLIN. MODEL) = .375
95% CONF. LIMITS = (.133, 1.059)

AVERAGE SLOPE (LINEAR REGR.) = .071
95% CONF. LIMITS = (.020, .121)



**MUTAGENICITY TESTING OF C.I. SOLVENT GREEN NO. 3 -
C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM**

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE GREEN
RESEARCH LAB: GBDA OM 04/06/84

08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION

STRAIN: TA100

COMPOUND	A C T	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE					MEAN	STD
			A	B	C	D	E		
POS CONTROL									
NAAZIDE	-	3.00	1253	1350	1320			1307.67	49.66
2-NF	RLA026	0.50	953	814	842			869.00	74.28
NEG CONTROL									
DIMETHYLSULF	RLA026	100.00U	115	125	114			118.00	6.08
	-	100.00U	109	102	110			107.00	4.36
BNGS-84-0001									
	RLA026	1.00	129	127				128.00	1.41
	RLA026	5.00	143	141				142.00	1.41
	RLA026	10.00	158	173				165.50	10.61
	RLA026	30.00	146	154				150.00	5.66
	RLA026	50.00	151	164				157.50	9.19
	RLA026	100.00	185	166				176.50	12.02
	RLA026	300.00	173	153				163.00	14.14
	-	1.00	113	129				121.00	11.31
	-	5.00	125	161				143.00	25.46
	-	10.00	115	114				114.50	0.71
	-	30.00	130	131				130.50	0.71
	-	50.00	137	117				127.00	14.14
	-	100.00	109	111				110.00	1.41
	-	300.00	129	126				127.50	0.71

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY S-9 : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIX/PLATE : SCCUGS

T*-TOXIC
TNTC-TOO NUMEROUS TO COUNT
NATC-NOT ABLE TO COUNT

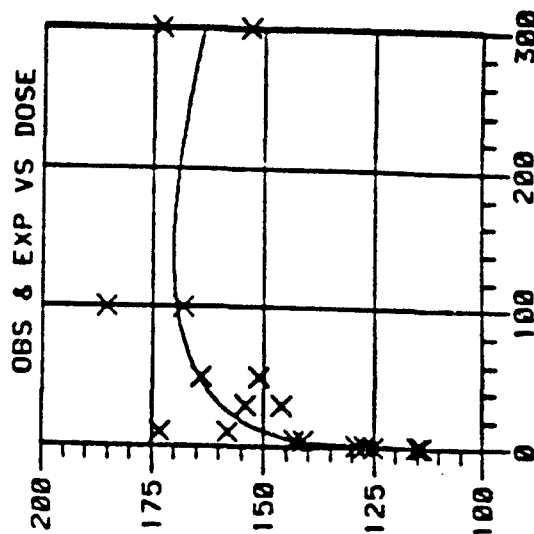
G-PGS T-PPT
N-NGS P-PPH
M-MGS B-PPB
L-NLS I-MM
U-ULS C-UM

STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT GREEN NO. 3 -
C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0001 LAB: CB8A ACTIVATION: + RL8026
STRAIN: TA100 DATE: 04/06/84 TECHNICIAN: MJK

DOSE UNITS	PLATE COUNTS	MEAN	S.D.
.00 UGS	115 125 114	118.00	6.08
.50* UGS	953 812 842	869.00	74.28
1.00 UGS	129 127	128.00	1.41
5.00 UGS	143 141	142.00	1.41
10.00 UGS	158 173	165.50	10.61
30.00 UGS	146 154	150.00	5.66
50.00 UGS	151 164	157.50	9.19
100.00 UGS	185 168	176.50	12.02
300.00 UGS	173 153	163.00	14.14

ESTS.	B(0)	B(1)	B(2)	B(3)
	117.484	2.7833	.3095	.00086
TEST	CHI-SQUARE	DF	P	LOGL
POISSON	4.13	9	.9025	-60.0989
ADEQUACY	6.33	4	.1761	-63.2616
TOXICITY	2.67	1	.1024	-64.5952
MUTAGENICITY	38.20	2	.0000	-82.3596
AVERAGE SLOPE (NONLIN. MODEL)	= .673			
95% CONF. LIMITS	= (.324, 1.396)			
AVERAGE SLOPE (LINEAR REGR.)	= .460			
95% CONF. LIMITS	= (.231, .688)			



STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT GREEN NO. 3 -
C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0001 LAB: CBBA ACTIVATION: -
STRAIN: TA100 DATE: 04/06/84 TECHNICIAN: MJK

DOSE UNITS PLATE COUNTS				MEAN	S.D.
00	UGS	109	102	110	107.00
3.00*	UGS	1253	1350	1320	1307.67
1.20	UGS	113	129		121.00
5.00	UGS	125	161		143.00
10.00	UGS	115	114		114.50
30.00	UGS	130	131		130.50
50.00	UGS	137	117		127.00
100.00	UGS	109	111		110.00
300.00	UGS	129	128		128.50

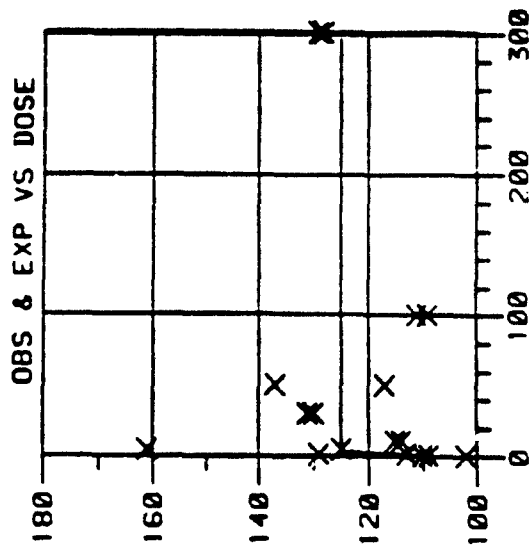
ESTS. 107.000 2.8846 .0000
B(0) B(1) B(2)

NO EVIDENCE OF TOXICITY

TEST	CHI-SQUARE	DF	P	LOCL
POISSON	7.55	9	.5801	-60.1832
ADEQUACY	11.51	5	.0421	-65.9395
MUTAGENICITY	6.74	2	.0343	-69.3111

AVERAGE SLOPE (NONLIN. MODEL) = .060
95% CONF. LIMITS = (.008, .473)

AVERAGE SLOPE (LINEAR REGR.) = .019
95% CONF. LIMITS = (-.057, .094)



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**MUTAGENICITY TESTING OF C.I. SOLVENT GREEN NO. 3 -
C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM**

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE GREEN
RESEARCH LAB: G88A ON 04/06/84

08/27/84

TEST TYPE: PLATE TEST - PREINCUBATION

STRAIN: TA100

COMPOUND	A C T	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE					MEAN	STD
			A	B	C	D	E		
POS CONTROL									
NAAZIDE	-	3.00	1227	1278	1267			1257.33	26.84
2-AA	RLA026	0.50	354	307	337			332.67	23.80
NEG CONTROL									
DIMETHYLSULF	RLA026	100.000	108	117	115			113.33	4.73
	-	100.000	144	132	127			134.33	8.74
BMGS-34-1001									
	RLA026	1.00	115	137				126.00	15.56
	RLA026	5.00	137	141				139.00	2.83
	RLA026	10.00	105	149				127.00	31.11
	RLA026	30.00	139	135				138.50	0.71
	RLA026	50.00	165	170				167.50	3.54
	RLA026	100.00	166	184				175.00	12.73
	RLA026	300.00	163	152				157.50	7.78
	-	1.00	105	142				123.50	26.16
	-	5.00	108	131				119.50	16.26
	-	10.00	137	115				126.00	15.56
	-	30.00	145	145				145.00	0.00
	-	50.00	161	129				145.00	22.63
	-	100.00	117	120				118.50	2.12
	-	300.00	117	115				117.50	0.71

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY 3-9 : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIX/PLATE : 50UGS

T*-TOXIC
TNTC-TOO NUMEROUS TO COUNT
NATC-NOT ABLE TO COUNT

G-PGS T-PPT
N-NGS P-PPH
M-MGS B-PPB
L-NLS I-MM
U-ULS C-CM

STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT GREEN NO. 3 -
C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0001 LAB: CBBA ACTIVATION: + RL026
STRAIN: TA100 DATE: 04/06/84 TECHNICIAN: MJK

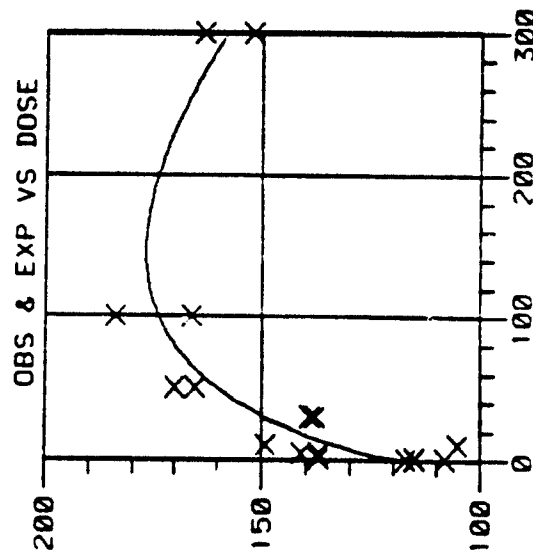
DOSE UNITS	PLATE COUNTS	MEAN	S.D.
.00 UCS	108 117 115	113.33	4.73
.50* UCS	354 307 337	332.67	23.80
1.00 UCS	115 137	126.00	15.56
5.00 UCS	137 141	139.00	2.83
10.00 UCS	105 149	127.00	31.11
30.00 UCS	139 138	138.50	.71
50.00 UCS	165 170	167.50	3.54
100.00 UCS	166 184	175.00	12.73
300.00 UCS	163 152	157.50	7.78

B(0) B(1) B(2) B(3)
ESTS. 118.077 1.1529 7883 .00311

TEST CHI-SQUARE DF P LGCL
POISSON 11.38 9 .2504 -63.3098
ADEQUACY 6.06 4 .1949 -66.3387
TOXICITY 6.57 1 .0104 -69.6224
MUTAGENICITY 46.50 2 .0000 -89.5874

AVERAGE SLOPE (NONLIN. MODEL) = 1.195
95% CONF. LIMITS = (.471, 3.029)

AVERAGE SLOPE (LINEAR REGR.) = .579
95% CONF. LIMITS = (.359, .798)



STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT GREEN NO. 3 -
C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0001 LAB: CBBA ACTIVATION: -
STRAIN: TA100 DATE: 04/06/84 TECHNICIAN: MJK

DOSE UNITS	PLATE COUNTS	MEAN	S.D.
00 UGS	144 132 127	134.33	8.74
3.00* UGS	1227 1278 1267	1257.33	26.84
1.00 UGS	105 142	123.50	26.16
5.00 UGS	108 131	119.50	16.26
10.00 UGS	137 115	126.00	15.56
30.00 UGS	145 145	145.00	.00
50.00 UGS	161 129	145.00	22.63
100.00 UGS	117 120	118.50	2.12
300.00 UGS	117 118	117.50	.71

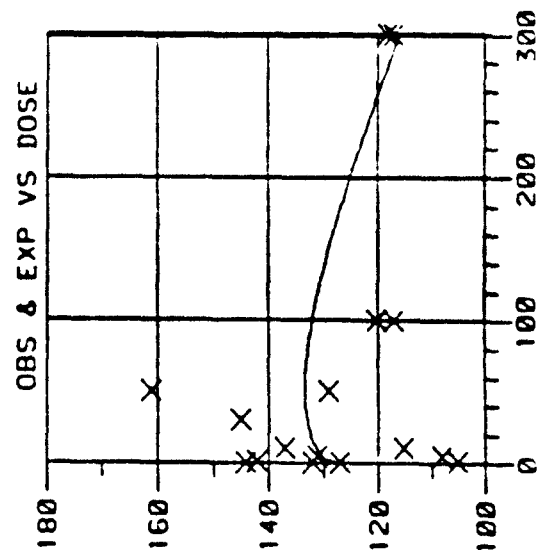
ESTS. 127.988 B(0) --- B(1) B(2) B(3)
--- .0841 .6859 .00151

TEST CHI-SQUARE DF P LOGL

POISSON 14.39 9 .1092 -64.0920
ADEQUACY 10.61 4 .0313 -69.3993
TOXICITY 3.59 1 .0582 -71.1938
MUTAGENICITY 1.27 2 .5292 -70.0357

AVERAGE SLOPE (NONLIN. MODEL) = .374
95% CONF. LIMITS = (.008, 18.518)

AVERAGE SLOPE (LINEAR REGR.) = .541
95% CONF. LIMITS = (-.252, 1.333)



**MUTAGENICITY TESTING OF C.I. SOLVENT GREEN NO. 3 -
C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM**

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE GREEN

RESEARCH LAB: GDBA ON 06/05/84

08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION

STRAIN: TA102

COMPOUND	A C T	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE					MEAN	STD
			A	B	C	D	E		
POS CONTROL									
OTHER PGS	RLA027	30.00	1253	1203	1096			1184.00	80.21
	-	0.50	1451	1509	1507			1489.00	32.92
NEG CONTROL									
DIMETHYLSULF	RLA027	100.00u	269	325	302			298.67	28.15
	-	100.00u	215	213	203			210.33	6.43
BMGS-34-0001									
	RLA027	10.00	311	336				324.50	19.09
	RLA027	30.00	395	448				421.50	37.49
	RLA027	50.00	480	526				503.00	32.53
	RLA027	100.00	586	631				608.50	31.82
	RLA027	300.00	654	694				674.00	26.23
	-	10.00	301	290				295.50	7.78
	-	30.00	380	413				396.50	23.33
	-	50.00	456	407				431.50	34.65
	-	100.00	477	389				433.00	62.23
	-	300.00	500	383				441.50	82.73

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY S-9 : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIX/PLATE : 500UGS

T--TOXIC
TNTC-TCO NUMEROUS TO COUNT
NATC-NOT ABLE TO COUNT

G-PGS T-PPT
N-NGS P-PPH
M-MGS B-PPB
L-NLS I--M
U-ULS C-UP

Best Available Copy

MUTAGENICITY TESTING OF C.I. SOLVENT GREEN NO. 3 -
C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE GREEN
RESEARCH LAB: GBBA ON 06/05/84
TEST TYPE: STANDARD PLATE INCORPORATION

08/27/84

STRAIN: TA102

•RLA627

POSITIVE CONTROL USED WAS DANTRON.

MITCHMYCIN-C WAS USED AS THE POSITIVE CONTRL.

STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT GREEN NO. 3 -
C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

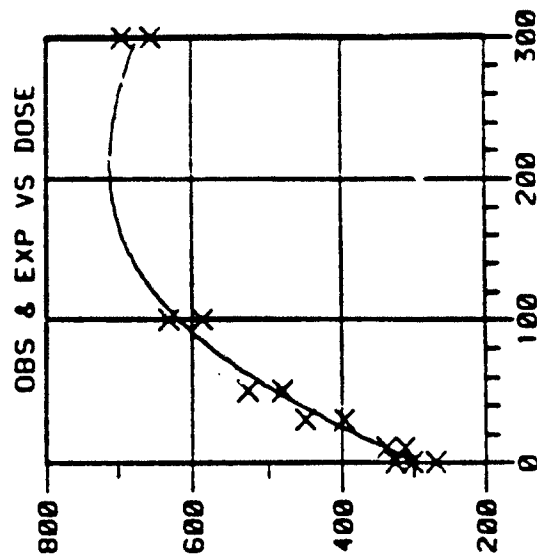
SAMPLE ID: BMCS-84-0001 LAB: G88A ACTIVATION: + RLA027
STRAIN: TA102 DATE: 06/05/84 TECHNICIAN: NJK

DOSE UNITS PLATE COUNTS			MEAN S.D.	
00	UCS	269 325 302	298.67	28.15
30.00*	UCS	1253 1203 1096	1184.00	80.21
10.00	UCS	311 338	324.50	19.09
30.00	UCS	395 448	421.50	37.48
50.00	UCS	480 526	503.00	32.53
100.00	UCS	586 631	608.50	31.82
300.00	UCS	654 694	674.00	28.28

ESTS. 295.096 1.5877 1.0599 .00418
B(0) B(1) B(2) B(3)

TEST CHI-SQUARE DF P LOGL

POISSON 14.72 7 .0398 -58.8369
ADEQUACY 2.06 2 .3572 -59.8663
TOXICITY 37.55 1 .0000 -78.6427
MUTAGENICITY 570.44 2 .0000 -345.0842
AVERAGE SLOPE (NONLIN. MODEL) = 6.883
95% CONF. LIMITS = (4.256, 11.130)
AVERAGE SLOPE (LINEAR REGR.) = 1.194
95% CONF. LIMITS = (.746, 1.642)



STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT GREEN NO. 3 -
C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

SAMPLE ID, BMGS-84-0001 LAB: G8BA ACTIVATION: -
STRAIN, TA102 DATE: 06/05/84 TECHNICIAN, MJA

DOSE UNITS PLATE COUNTS			MEAN	S.D.
.00	UGS	215 213 203	210.33	6.43
.50*	UGS	1451 1509 1507	1489.00	32.92
10.00	UGS	301 290	295.50	7.78
30.00	UGS	380 413	396.50	23.33
50.00	UGS	456 407	431.50	34.65
100.00	UGS	477 389	433.00	62.23
300.00	UGS	500 383	441.50	82.73

2-14

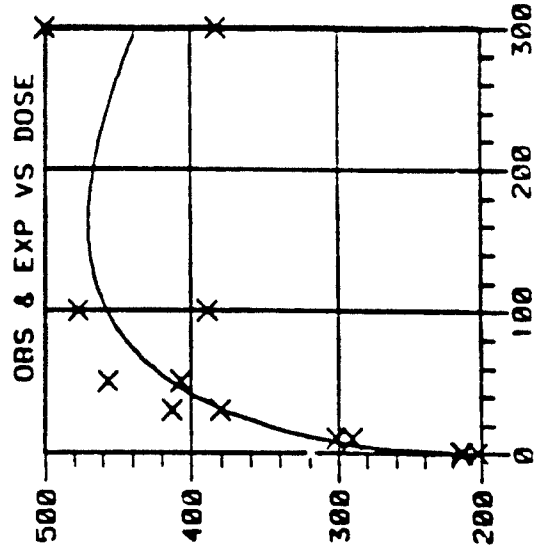
ESTS. 208.958 3.5406 B(1) B(2) B(3)

TEST CHI-SQUARE DF P LOGL

POISSON 29.20 7 .0001 -64.4863
ADEQUACY 8.06 2 .0178 -68.5167
TOXICITY 19.97 1 .0000 -78.5011
MUTAGENICITY 340.27 2 .0000 -238.6500

AVERAGE SLOPE (NONLIN. MODEL) = 5.041
95% CONF. LIMITS = (4.279, 5.940)

AVERAGE SLOPE (LINEAR REGR) = 4.497
95% CONF. LIMITS = (3.414, 5.580)



MUTAGENICITY TESTING OF C.I. SOLVENT GREEN NO. 3 -
C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
 OF ARMY DYE GREEN

RESEARCH LAB: GDBA ON 06/08/84

08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION

STRAIN: TA102

COMPOUND	A C T	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE					MEAN	STD
			A	B	C	D	E		
POS CONTROL									
OTHER POS	RLAQ27	30.00	(4) 1176	1203				1192.00	22.63
	-	0.50	1182	1166	1170			1173.33	7.57
NEG CONTROL									
DIMETHYLSULF	RLAQ27	100.000	257	234	239			243.33	12.10
	-	100.000	154	(4) 145				149.50	6.36
BMGS-84-0001									
	RLAQ27	10.00	(4) 270					270.00	0.00
	RLAQ27	30.00	412	400				406.00	8.49
	RLAQ27	50.00	453	417				445.00	11.31
	RLAQ27	100.00	547	507				527.00	26.28
	RLAQ27	300.00	684	660				672.00	16.97
	-	10.00	214	203				208.50	7.78
	-	30.00	375	330				352.50	31.82
	-	50.00	346	392				369.00	32.53
	-	100.00	432	449				440.50	12.02
	-	300.00	462	490				486.00	5.66

PHENOCOPY CHECK : TRUE MUTANTS
 STERILITY S-9 : NOT CONTAMINATED
 SAMPLE STERILITY: NOT CONTAMINATED
 ACT MIX/PLATE : 500UGS

T*-TOXIC
 INTC-TCO NUMEROUS TO COUNT
 NATC-NOT ABLE TO COUNT

G-PGS T-OPY
 N-NGS P-PPM
 M-MGS B-PPB
 L-NLS :-MM
 U-ULS C-UM

MUTAGENICITY TESTING OF C.I. SOLVENT GREEN NO. 3 -
C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAY: WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE GREEN
RESEARCH LAB: GBBA ON 06/08/84

08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION

STRAIN: TA102

BACKGROUNDS:

(4) CONTAMINATED

MUTAGENICITY TESTING OF C.I. SOLVENT GREEN NO. 3 -
C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE GREEN
RESEARCH LAB: 688A ON 06/08/84
TEST TYPE: STANDARD PLATE INCORPORATION

08/27/84

STRAIN: TA102

•RLA27

DANTHRON WAS USED AS A POSITIVE CONTROL.

MITOMYCIN C WAS USED AS A POSITIVE CONTROL.

STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT GREEN NO. 3 -
C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

SAMPLE ID, BMCS-84-0001 LAB, CBBA ACTIVATION, + RLA027
STRAIN, TA102 DATE, 06/08/84 TECHNICIAN, MJK

DOSE UNITS	PLATE COUNTS	MEAN	S.D.
00 UGS	257 234 239	243.33	12.10
30.00* UGS	1176 1208	1192.00	22.63
10.00 UGS	270	270.00	.00
30.00 UGS	412 400	406.00	8.49
50.00 UGS	453 437	445.00	11.31
100.00 UGS	547 507	527.00	28.28
300.00 UGS	684 660	672.00	16.97

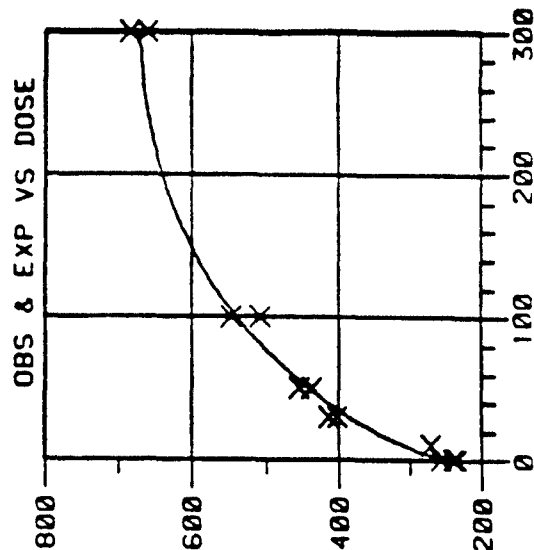
ESTS. 240.569 2.5598 B(1) B(2) B(3)

TEST CHI-SQUARE DF P LOGL

POISSON 3.61 6 .7287 -48.7529
ADEQUACY 7.92 2 .0191 -52.7105
TOXICITY 7.93 1 .0049 -56.6762
MUTAGENICITY 646.84 2 .0000 -376.1290

AVERAGE SLOPE (NONLIN. MODEL) = 3.123
95% CONF. LIMITS = (1.896, 5.145)

AVERAGE SLOPE (LINEAR REGR.) = 1.307
95% CONF. LIMITS = (.914, 1.699)

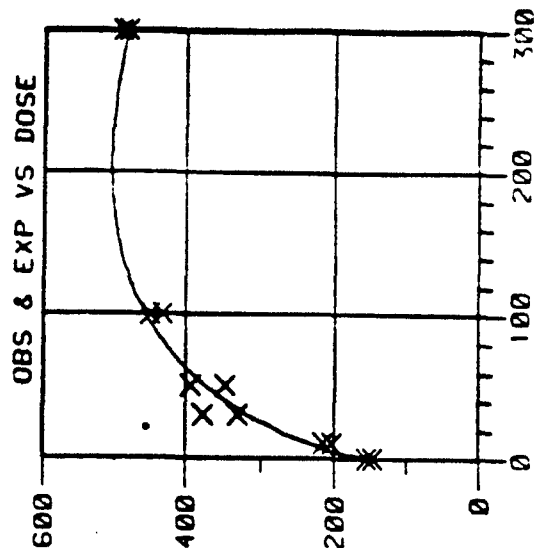


STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT GREEN NO. 3 -
C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0001 LAB: CB8A ACTIVATION: -
STRAIN: TA102 DATE: 06/08/84 TECHNICIAN: MJK

DOSE UNITS PLATE COUNTS		MEAN S.D.	
00	UGS 154 145	140.50	6.36
50*	UGS 1182 1170	1173.33	7.57
100	UGS 214 203	208.50	7.78
300	UGS 375 330	352.50	31.82
500	UGS 346 392	369.00	32.53
1000	UGS 432 449	440.50	12.02
3000	UGS 482 490	486.00	5.66

ESTS. B(0) 146.049 B(1) 2.9088 B(2) 7012 B(3) 00288
TEST CHI-SQUARE DF P LOCL
POISSON 6.69 6 3500 -48.7717
ADEQUACY 14.02 2 0000 -55.7794
TOXICITY 31.93 1 0000 -71.7453
MUTAGENICITY 569.27 2 0000 -340.4157
AVERAGE SLOPE (NONLIN. MODEL) = 3.335
95% CONF. LIMITS = (2.056, 5.409)
AVERAGE SLOPE (LINEAR REGR.) = 900
95% CONF. LIMITS = (427, 1373)



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MUTAGENICITY TESTING OF C.I. SOLVENT GREEN NO. 3 -
C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
 OF ARMY DYE GREEN

RESEARCH LAB: GBBA ON 06/15/84

08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION

STRAIN: TA102

COMPOUND	A C T	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE					MEAN	STD
			A	B	C	D	E		
POS CONTROL									
OTHER POS	RLA027	0.50	1252	1377	1392	1370		1347.75	64.49
	-	30.00	1382	1320	1363			1361.00	35.54
NEG CONTROL									
DIMETHYLSULF	RLA027	100.000	264	264	266			264.67	1.15
	-	100.000	302	182	213			201.00	12.53
EMGS-34-0001									
	RLA027	1.00	281	268				274.50	9.19
	RLA027	5.00	286	252				269.00	24.04
	RLA027	10.00	293	281				287.00	8.49
	RLA027	30.00	332	363				360.50	31.22
	RLA027	50.00	426	380				403.00	32.53
	RLA027	100.00	601	530				565.50	50.20
	RLA027	300.00	712	710				714.00	2.83
	-	1.00	203	190				196.50	9.19
	-	5.00	216	255				235.50	27.58
	-	10.00	241	230				239.50	2.12
	-	30.00	322	312				320.00	11.31
	-	50.00	348	420				388.00	56.57
	-	100.00	437	447				443.00	8.49
	-	300.00	457	430				446.50	14.65

PHENOCOPY CHECK : TRUE MUTANTS
 STERILITY S-9 : NOT CONTAMINATED
 SAMPLE STERILITY: NOT CONTAMINATED
 ACT MIX/PLATE : 500UGS

T*-TOXIC
 TNTC-TOO NUMEROUS TO COUNT
 NATC-NOT ABLE TO COUNT

G-PGS T-PPT
 N-NGS P-PPM
 M-MGS B-PPB
 L-NLS I-MM
 U-ULS C-CM

MUTAGENICITY TESTING OF C.I. SOLVENT GREEN NO. 3 -
C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE GREEN
RESEARCH LAB: GBDA CN 06/15/84
TEST TYPE: STANDARD PLATE INCORPORATION

08/27/84

STRAIN: TA102

•RLAG27

MITOMYCIN C WAS USED AS THE POSITIVE CONTROL.

CANTHON WAS USED AS THE POSITIVE CONTROL.

STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT GREEN NO. 3 -
C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

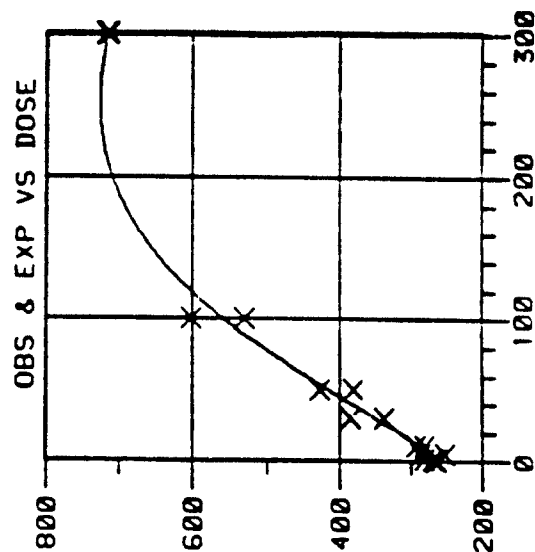
SAMPLE ID: BMGS-84-0001 LAB: GBBA ACTIVATION: + RLA027
STRAIN: TA102 DATE: 06/15/84 TECHNICIAN: MJK

DOSE UNITS	PLATE COUNTS	MEAN	S.D.
.00 UGS	264 264 266	264.67	1.16
.50* UGS	1252 1377 1392 1370	1347.75	64.49
1.00 UGS	281 268	274.50	9.19
5.00 UGS	286 252	269.00	24.04
10.00 UGS	293 281	287.00	8.49
30.00 UGS	338 383	360.50	31.82
50.00 UGS	426 380	403.00	32.53
100.00 UGS	601 530	565.50	50.20
300.00 UGS	712 716	714.00	2.83

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	12.62	9	.1806	-71.9629
ADEQUACY	2.26	4	.6889	-73.0906
TOXICITY	26.94	1	.0000	-86.5600
MUTAGENICITY	957.75	2	.0000	-551.9666

AVERAGE SLOPE (NONLIN. MODEL) = 7.993
95% CONF. LIMITS = (2.377, 26.875)

AVERAGE SLOPE (LINEAR REGR.) = 1.547
95% CONF. LIMITS = (1.260, 1.833)



STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT GREEN NO. 3 -
C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMCS-84-0001 LAB: GB8A ACTIVATION: -
STRAIN: TA102 DATE: 06/15/84 TECHNICIAN: MJK

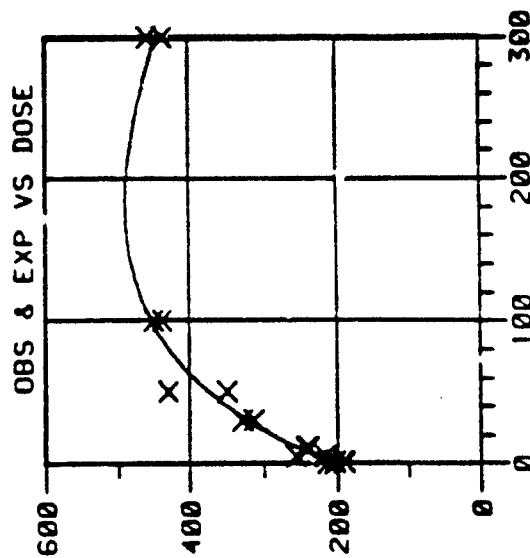
DOSE UNITS	PLATE COUNTS	MEAN	S.D.
.00 UGS	202 188 213	201.00	12.53
30.00* UGS	1380 1320 1383	1361.00	35.54
1.00 UGS	203 190	196.50	9.10
5.00 UGS	216 255	235.50	27.58
10.00 UGS	241 238	239.50	2.12
30.00 UGS	328 312	320.00	11.31
50.00 UGS	348 428	388.00	56.57
100.00 UGS	437 449	443.00	8.49
300.00 UGS	457 436	446.50	14.85

ESTS. B(0) 197.003 B(1) 2.1277 B(2) .8743 B(3) .00388

TEST CHI-SQUARE DF P LOGL
POISSON 14.54 9 .1042 -72.9985
ADEQUACY 3.96 4 .4108 -72.9809
TOXICITY 50.55 1 .0000 -98.2539
MUTAGENICITY 552.01 2 .0000 -348.9849

AVERAGE SLOPE (NONLIN. MODEL) = 5.133
95% CONF. LIMITS = (4.358, 6.046)

AVERAGE SLOPE (LINEAR REGR.) = 3.767
95% CONF. LIMITS = (3.101, 4.433)



MUTAGENICITY TESTING OF C.I. SOLVENT GREEN NO. 3 -
C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE GREEN
RESEARCH LAB: 688A ON 06/05/84

08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION

STRAIN: TA104

COMPOUND	A C T	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE					MEAN	STD
			A	B	C	D	E		
POS CONTROL									
2-AA	RLA027	3.00	2424	2346	2444			2405.33	50.65
OTHER POS	-	50.00	1736	1657	1766			1726.33	65.04
NEG CONTROL									
DIMETHYLSULF	RLA027	100.00U	350	316	290			318.67	30.09
	-	100.00U	241	222	252			238.33	15.18
BMGS-34-J001									
	RLA027	1.00	314	354				334.00	28.28
	RLA027	5.00	314	354				334.00	28.28
	RLA027	10.00	361	360				360.50	0.71
	RLA027	30.00	450	455				452.50	3.54
	RLA027	50.00	532	505				518.50	19.09
	RLA027	100.00	544	512				531.00	18.38
	RLA027	300.00	512	396				454.00	84.02
	-	1.00	300	242				271.00	41.01
	-	5.00	263	203				234.00	41.01
	-	10.00	266	262				264.00	2.63
	-	30.00	283	287				285.00	2.63
	-	50.00	328	280				304.00	33.94
	-	100.00	330	325				327.50	3.54
	-	300.00	305	289				297.00	11.31

PHENOCOPIY CHECK : TRUE MUTANTS
STERILITY S-9 : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIX/PLATE : 500UGS

T--TOXIC
TNTC-TOO NUMEROUS TO COUNT
NATC-NOT ABLE TO COUNT

G-PGS T-PPT
N-NGS P-PPH
M-MGS B-PPB
L-NLS I--H
U-ULS C-UM

MUTAGENICITY TESTING OF C.I. SOLVENT GREEN NO. 3 -
C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE GREEN
RESEARCH LAB: GBDA ON 06/03/84
TEST TYPE: STANDARD PLATE INCORPORATION

08/27/84

STRAIN: TA104

METHYL GLYOXAL WAS USED AS THE POSITIVE CONTROL.

STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT GREEN NO. 3 -
C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMCS-84-0001 LAB: C88A ACTIVATION: + RLA027
STRAIN: TA104 DATE: 06/05/84 TECHNICIAN: MJK

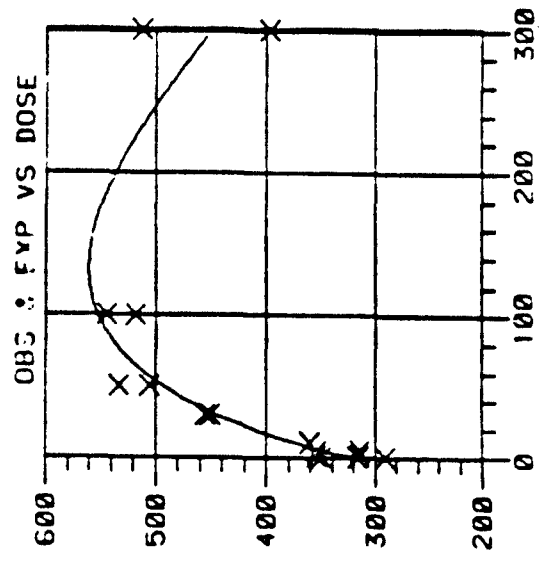
DOSE UNITS	PLATE COUNTS	MEAN	S.D.
.00 UGS	350 316 290	318.67	30.09
3.00* UGS	2424 2348 2444	2405.33	50.65
1.00 UGS	314 354	334.00	28.28
5.00 UGS	314 354	334.00	28.28
10.00 UGS	361 360	360.50	71
30.00 UGS	450 455	452.50	3.54
50.00 UGS	532 505	518.50	19.09
100.00 UGS	544 518	531.00	18.38
300.00 UGS	512 396	454.00	82.02

B(0) B(1) B(2) B(3)
ESTS. 315.927 2.3098 .8702 .00454

TEST CHI-SQUARE DF P LOGL
POISSON 26.66 9 .0016 -79.8677
ADEQUACY 7.30 4 .1208 -83.5187
TOXICITY 78.24 1 .0000 -122.6363
MUTAGENICITY 270.32 2 .0000 -218.6802

AVERAGE SLOPE (NONLIN. MODEL) = 6.062
95% CONF. LIMITS = (5.258, 6.988)

AVERAGE SLOPE (LINEAR REGR.) = 4.041
95% CONF. LIMITS = (3.430, 4.651)



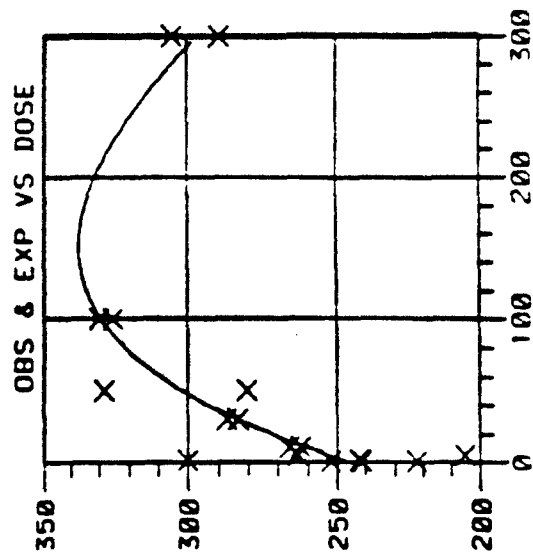
STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT GREEN NO. 3 -
C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMCS-84-0001 LAB: C88A ACTIVATION: -
STRAIN: TA104 DATE: 06/05/84 TECHNICIAN: MJK

DOSE UNITS	PLATE COUNTS	MEAN	S.D.
00 UGS	241 222 252	238.33	15.18
50.00* UGS	1736 1657 1786	1726.33	65.04
1.00 UGS	300 242	271.00	41.01
5.00 UGS	263 205	234.00	41.01
10.00 UGS	266 262	264.00	2.83
30.00 UGS	283 287	285.00	2.83
50.00 UGS	328 280	304.00	33.94
100.00 UGS	330 325	327.50	3.54
300.00 UGS	305 289	297.00	11.31

B(0) B(1) B(2) B(3)
ESTS. 245.663 .0130 .9951 .00396
TEST CHI-SQUARE DF P LOGL
POISSON 19.64 9 .0202 -73.1519
ADEQUACY 8.24 4 .0833 -77.2695
TOXICITY 11.81 1 .0006 -83.1734
MUTAGENICITY 50.29 2 .0000 -102.4142

AVERAGE SLOPE (NONLIN. MODEL) = 2.444
95% CONF. LIMITS = (1.176, 5.080)
AVERAGE SLOPE (LINEAR REGR.) = 1.183
95% CONF. LIMITS = (.404, 1.963)



MUTAGENICITY TESTING OF C.I. SOLVENT GREEN NO. 3 -
C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
 OF ARMY DYE GREEN

RESEARCH LAB: 688A ON 06/08/84

08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION

STRAIN: TA104

COMPOUND	A C T	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE					MEAN	STD
			A	B	C	D	E		
POS CONTRL									
2-AA	RLA027	3.00	2053	2002	2006			2047.00	42.32
OTHER POS	-	50.00	1810	1718	1700			1769.33	46.92
NEG CONTROL									
DIMETHYLSULF	RLA027	100.00U	363	402	375			380.00	19.97
	-	100.00U	277	260	279			274.00	7.00
BMGS-84-0001									
	RLA027	1.00	353	351				352.00	1.41
	RLA027	5.00	383	381				382.00	1.41
	RLA027	10.00	405	370				387.50	24.75
	RLA027	30.00	416	469				442.50	37.48
	RLA027	50.00	505	460				485.50	27.58
	RLA027	100.00	532	532				532.00	0.00
	RLA027	300.00	532	524				528.00	5.66
	-	1.00	298	250				274.00	33.94
	-	5.00	290	260				279.00	15.56
	-	10.00	305	310				311.50	9.19
	-	30.00	317	319				318.00	1.41
	-	50.00	308	317				312.50	6.36
	-	100.00	376	370				376.00	0.00
	-	300.00	330	352				341.00	15.56

PHENOCOPY CHECK : TRUE MUTANTS
 STERILITY S-9 : NOT CONTAMINATED
 SAMPLE STERILITY: NOT CONTAMINATED
 ACT MIX/PLATE : 500UGS

T--TOXIC
 TNC-TOO NUMEROUS TO COUNT
 NATC-NOT ABLE TO COUNT

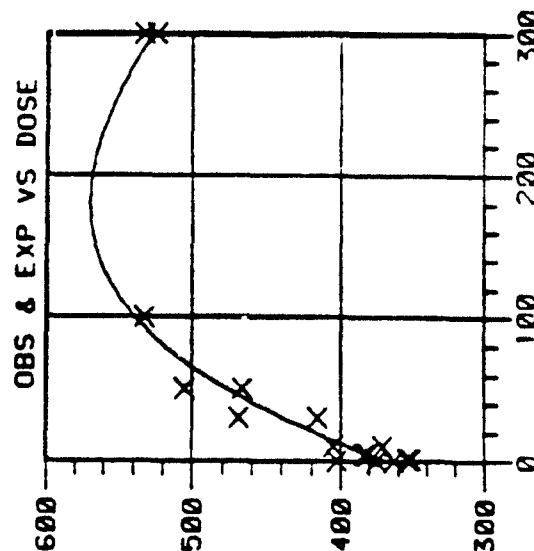
G-PGS T-PPT
 N-NGS P-PPH
 M-MGS B-PPB
 L-NLS I-MM
 U-ULS C-UM

STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT GREEN NO. 3 -
C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMCS-84-0001 LAB: CBBA ACTIVATION: + RLA027
STRAIN: TA104 DATE: 06/08/84 TECHNICIAN: MJK

DOSE UNITS	PLATE COUNTS	MEAN	S.D.
0.00	UCS 363 402 375	380.00	19.07
3.00*	UCS 2053 2002 2086	2047.00	42.32
1.00	UCS 353 351	352.00	1.41
5.00	UCS 383 381	382.00	1.41
10.00	UCS 405 370	387.50	24.75
30.00	UCS 416 469	442.50	37.48
50.00	UCS 505 466	485.50	27.58
100.00	UCS 532 532	532.00	.00
300.00	UCS 532 524	528.00	5.66

ESTS 367.656 1.4110 1.0030 00374
TEST CHI-SQUARE DF P LOGL
POISSON 8 49 9 .4854 -71.3638
ADEQUACY 3.81 4 .4317 -73.2709
TOXICITY 22.50 1 .0000 -84.5231
MUTAGENICITY 166.13 2 .0000 -156.3335
AVERAGE SLOPE (NONLIN. MODEL) = 4.157
95% CONF. LIMITS = (1.543, 11.203)
AVERAGE SLOPE (LINEAR REGR.) = 1.729
95% CONF. LIMITS = (1.369, 2.089)



STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT GREEN NO. 3 -
C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

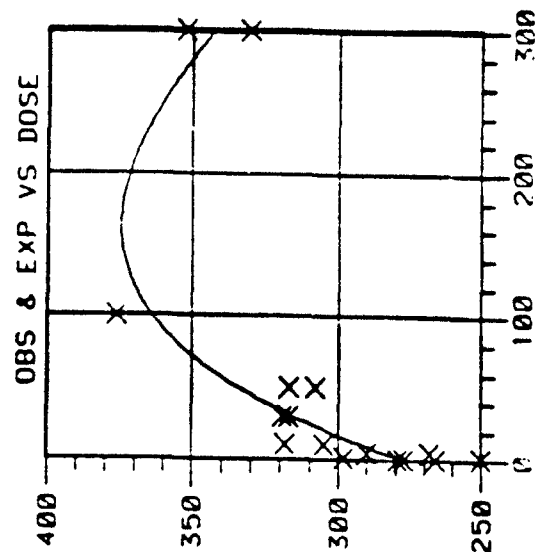
SAMPLE ID: BMCS-84-0001 LAB: GBBA ACTIVATION: -
STRAIN: TA104 DATE: 06/08/84 TECHNICIAN: MJK

DOSE UNITS	PLATE COUNTS	MEAN	S.T.
00	UGS 277 266 279	274.00	7.00
50.00*	UGS 1810 1718 1780	1769.33	46.92
1.00	UGS 298 250	274.00	33.94
5.00	UGS 290 268	279.00	15.56
10.00	UGS 305 318	311.50	9.19
30.00	UGS 317 319	318.00	1.41
50.00	UGS 308 317	312.50	6.36
100.00	UGS 376 376	376.00	.00
300.00	UGS 330 52	341.00	15.56

ESTS.	275.931	1.0077	9704	00347
TEST	CHI-SQUARE	DF	P	LOGL
POISSON	6.55	9	.6842	-67.5713
ADEQUACY	6.88	4	.1426	-71.0095
TOXICITY	9.43	1	.0021	-75.7233
MUTAGENICITY	54.17	2	.0000	-98.0952

AVERAGE SLOPE (NONLIN. MODEL) = 2.391
95% CONF. LIMITS = (.859, 6.653)

AVERAGE SLOPE (LINEAR REGR.) = .942
95% CONF. LIMITS = (.689, 1.194)



**MUTAGENICITY TESTING OF C.I. SOLVENT GREEN NO. 3 -
C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM**

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE GREEN
RESEARCH LAB: G80A ON 06/05/84

08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION

STRAIN: TA1535

COMPOUND	A C T	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE					MEAN	STD
			A	B	C	D	E		
POS CONTROL									
MAAZIDE	-	3.00	1068	1057	1018			1047.67	26.27
2-AA	HLA027	3.00	154	154	157			155.00	1.73
NEG CONTROL									
DIMETHYLSULF	HLA027	100.00U	32	33	18			27.67	6.39
	-	100.00U	55	50	40			48.33	7.64
BMGS-34-0001									
	RLAC27	10.00	25	15				20.50	6.36
	HLA027	30.00	24	29				26.50	3.54
	HLA027	50.00	28	29				28.50	0.71
	RLAC27	100.00	17	20				18.50	2.12
	HLA027	300.00	14	21				17.50	4.95
	-	10.00	61	52				56.50	6.36
	-	30.00	28	32				30.00	2.83
	-	50.00	40	32				36.00	5.66
	-	100.00	51	41				46.00	7.07
	-	300.00	36	42				39.00	4.24

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY S-9 : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIX/PLATE : SCCUGS

T+--TOXIC
TNTC-TOO NUMEROUS TO COUNT
NATC-NOT ABLE TO COUNT

G-PGS T-PPT
N-NGS P-PPM
M-MGS 9-PP9
L-NLS I-MM
U-ULS C-UM

STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT GREEN NO. 3 -
C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0001 LAB: CBBA ACTIVATION: + RLAD27
STRAIN: TA1535 DATE: 06/05/84 TECHNICIAN: MJK

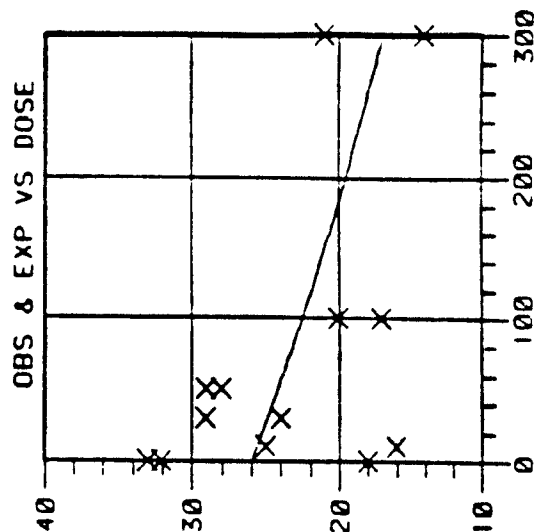
DOSE UNITS	PLATE COUNTS	MEAN	S.D.
.00 UGS	32 33 18	27.67	8.30
3.00* UGS	154 154 157	155.00	1.73
10.00 UGS	25 16	20.50	6.36
30.00 UGS	24 29	26.50	3.54
50.00 UGS	28 29	28.50	.71
100.00 UGS	17 20	18.50	2.12
300.00 UGS	14 21	17.0	4.95

ESTS. B(0) B(1) B(2) B(3)
25.973***** .0000 .00144

TEST CHI-SQUARE DF P LOGL
POISSON 9.19 7 .2391 -37.0998
ADEQUACY 5.74 2 .0568 -39.9689
TOXICITY 5.54 1 .0185 -42.7413
MUTAGENICITY .00 21.0000 -39.9689

AVERAGE SLOPE (NONLIN. MODEL) = .000
95% CONF. LIMITS = (.000, .000)

AVERAGE SLOPE (LINEAR REG.) = .050
95% CONF. LIMITS = (-.162, .262)



STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT GREEN NO. 3 -
C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0001 LAB: CBBA ACTIVATION: -
STRAIN: TA1535 DATE: 06/05/84 TECHNICIAN: MJK

DOSE UNITS	PLATE COUNTS	MEAN	S.D.
.00	UGS 55 50 40	48.33	7.64
3.00*	UGS 1068 1057 1018	1047.67	26.27
10.00	UGS 61 52	56.50	6.36
30.00	UGS 28 32	30.00	2.83
50.00	UGS 40 32	36.00	5.66
100.00	UGS 51 41	46.00	7.07
300.00	UGS 36 42	39.00	4.24

ESTS. B(0) B(1) B(2)
48.333 2.0168 .0000

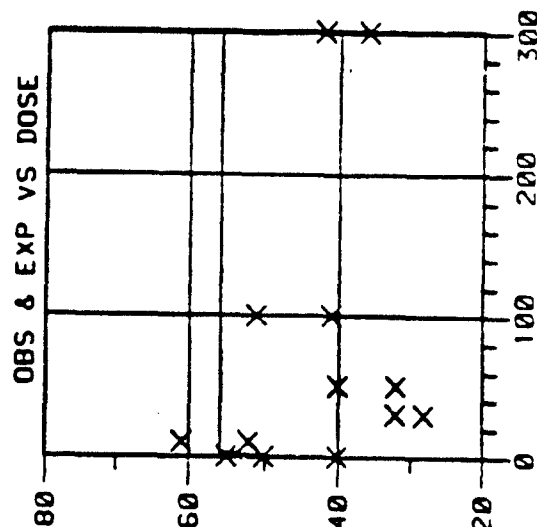
NO EVIDENCE OF TOXICITY

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	5.83	7	.5592	-39.2126
ADEQUACY	60.07	3	.0000	-69.2456
MUTAGENICITY	.01	2	.9950	-50.2144

AVERAGE SLOPE (NONLIN. MODEL) = .075
95% CONF. LIMITS = (.022, .258)

AVERAGE SLOPE (LINEAR REGR.) = .066
95% CONF. LIMITS = (.120, .253)

WARNING: 4 PARAMETER MODEL DID NOT CONVERGE



Best Available Copy

**MUTAGENICITY TESTING OF C.I. SOLVENT GREEN NO. 3 -
C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM**

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE GREEN

RESEARCH LAB: G86A

ON 06/08/84

08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION

STRAIN: TA1535

COMPOUND	A C T	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE					MEAN	STD
			A	B	C	D	E		
POS CONTROL									
MAAIDE	-	3.00	1003	979	1014			998.67	17.90
Z-AA	RLA027	3.00	89	103	109			100.33	10.26
NEG CONTROL									
DIMETHYLSULF	RLA027	100.000	25	24	18			22.33	5.79
	-	100.000	43	26	28			32.33	9.29
BMGS-34-0001									
	RLA027	10.00	14	14				14.00	0.00
	RLA027	30.00	19	15				17.00	2.83
	RLA027	50.00	17	16				17.50	0.71
	RLA027	100.00	21	21				21.00	0.00
	RLA027	300.00	11	9				10.00	1.41
	-	10.00	36	27				31.50	6.36
	-	30.00	36	17				26.50	13.44
	-	50.00	24	33				28.50	6.36
	-	100.00	41	27				34.00	9.90
	-	300.00	21	17				19.00	2.83

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY S-S : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIA/PLATE : 500UGS

T--TOXIC
INTC-TGO NUMEROUS TO COUNT
NATC-NOT ABLE TO COUNT

G-PGS T-PPT
N-NGS P-PPM
M-MGS E-PPB
L-NLS I-MM
U-ULS C-UM

STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT GREEN NO. 3 -
C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0001 LAB: CBBA ACTIVATION: + RLA027
STRAIN: TA1535 DATE: 06/08/84 TECHNICIAN: MJK

DOSE UNITS	PLATE COUNTS	MEAN	S.D.
.00 UGS	25 24 18	22.33	3.79
3.00* UGS	89 103 109	100.33	10.26
10.00 UGS	14 14	14.00	.00
30.00 UGS	19 15	17.00	2.83
50.00 UGS	17 18	17.50	.71
100.00 UGS	21 21	21.00	.00
300.00 UGS	11 9	10.00	1.41

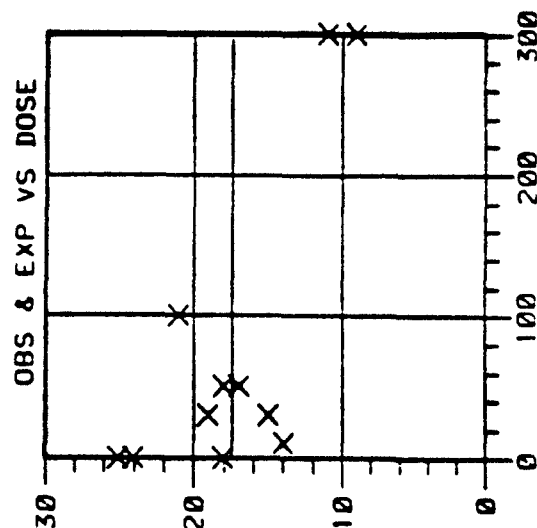
B(0) B(1) B(2)
ESTS. 17.386-663.7156 .0000

NO EVIDENCE OF TOXICITY

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	1.98	7	.9608	-31.3414
ADEQUACY	14.13	3	.0027	-38.4079
MUTAGENICITY	.00	21	.0000	-38.4079

AVERAGE SLOPE (NONLIN. MODEL) = .000
95% CONF. LIMITS = (.000)

AVERAGE SLOPE (LINEAR REGR.) = .010
95% CONF. LIMITS = (-.057, .076)
WARNING: 4 PARAMETER MODEL DID NOT CONVERGE

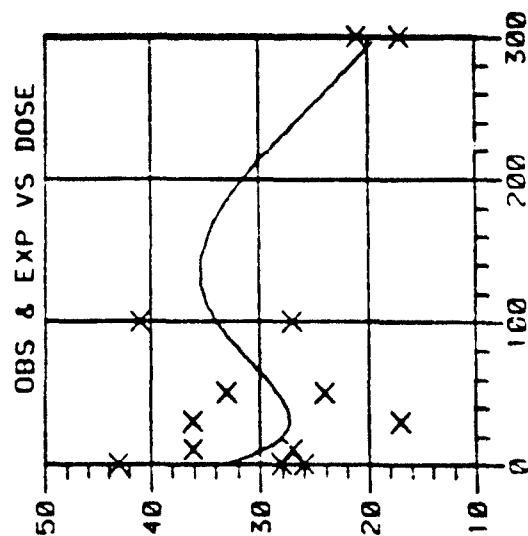


STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT GREEN NO. 3 -
C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0001 LAB: CBBA ACTIVATION: -
STRAIN: TA1535 DATE: 06/08/84 TECHNICIAN: MJK

DOSE UNITS	PLATE COUNTS	MEAN	S.D.
00 UGS	43 26 28	32.33	8.20
3.00* UGS	1003 979 1014	988.67	17.00
10.00 UGS	36 27	31.50	6.36
30.00 UGS	36 17	26.50	13.44
50.00 UGS	24 33	28.50	6.36
100.00 UGS	41 27	34.00	9.90
300.00 UGS	21 17	19.00	2.83

ESTS. 33.140 -5.1746 2.1060 01309
TEST CHI-SQUARE DF P LOGL
POISSON 18.16 7 .0113 -42.6575
ADEQUACY .32 2 .8511 -42.8187
TOXICITY 14.73 1 .0001 -50.1831
MUTAGENICITY 3.94 2 .1393 -44.7902
AVERAGE SLOPE (NONLIN. MODFL) = .428
95% CONF. LIMITS = (.203, .904)
AVERAGE SLOPE (LINEAR REGR.) = -.097
95% CONF. LIMITS = (-.373, .179)



**MUTAGENICITY TESTING OF C.I. SOLVENT GREEN NO. 3 -
C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM**

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE GREEN
RESEARCH LAB: G88A ON 06/05/84

G8/27/84

TEST TYPE: STANDARD PLATE INCORPORATION

STRAIN: TA1537

COMPOUND	A C T	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE					MEAN	STD
			A	B	C	D	E		
POS CONTROL									
9-AA	-	100.00	1258	1335	1432			1348.33	97.68
2-AA	RLA027	3.00	561	472	408			480.33	76.84
NEG CONTROL									
DIMETHYLSULF	RLA027	100.000	20	16	17			17.67	2.08
	-	100.000	9	5	9			8.33	0.58
BMGS-34-0001									
	RLA027	10.00	14	28				21.00	9.90
	RLA027	30.00	25	20				22.50	3.54
	RLA027	50.00	39	21				30.00	12.73
	RLA027	100.00	30	24				27.00	4.24
	RLA027	300.00	27	20				26.50	0.71
	-	10.00	16	12				14.00	2.83
	-	30.00	14	17				15.50	2.12
	-	50.00	14	20				17.00	4.24
	-	100.00	16	14				15.00	1.41
	-	300.00	16	13				14.50	2.12

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY S-9 : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIX/PLATE : 500UGS

T*-TOXIC
TNTC-TOO NUMEROUS TO COUNT
NATC-NOT ABLE TO COUNT

G-PGS T-PPT
N-NGS P-PPM
M-MGS B-PPB
L-NLS I-IM
U-ULS C-UM

STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT GREEN NO. 3 -
C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0001 LAB: C88A ACTIVATION: + RLA027
STRAIN: TA1537 DATE: 06/05/84 TECHNICIAN: MJK

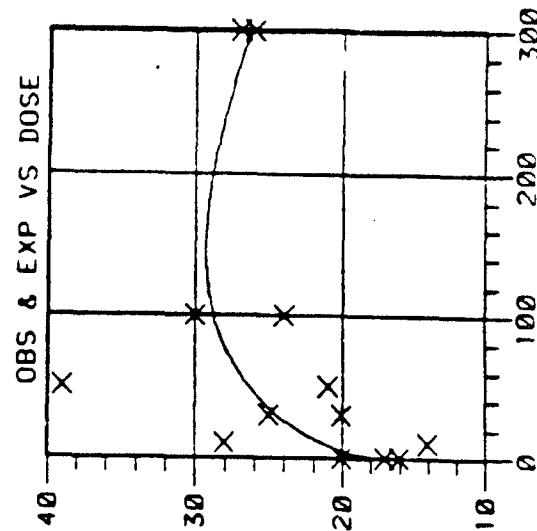
DOSE UNITS	PLATE COUNTS	MEAN	S.D.
00 UCS	20 16 17	17.67	2.08
3.00* UCS	561 472 408	480.33	76.84
10.00 UCS	14 28	21.00	9.90
30.00 UCS	25 20	22.50	3.54
50.00 UCS	39 21	30.00	12.73
100.00 UCS	30 24	27.00	4.24
300.00 UCS	27 26	26.50	.71

ESTS. B(0) 17.567 B(1) 0.0943 B(2) 0.6743 B(3) 0.00276

TEST CHI-SQUARE DF P LOGL
POISSON 11.80 7 .1074 -38.2940
ADEQUACY 1.50 2 .4723 -39.0441
TOXICITY 1.25 1 .2627 -39.6713
MUTAGENICITY 9.33 2 .0094 -43.7073

AVERAGE SLOPE (NONLIN. MODEL) = .255
95% CONF. LIMITS = (.034, 1.924)

AVERAGE SLOPE (LINEAR REGR.) = .226
95% CONF. LIMITS = (.004, .448)



STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT GREEN NO. 3 -
C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMCS-84-0001 LAB: GBBA ACTIVATION: -
STRAIN: TA1537 DATE: 06/05/84 TECHNICIAN: HJK

DOSE UNITS PLATE COUNTS			MEAN S.D.	
00	UGS	8	8	33
100.00*	UGS	1258	1335	1452
10.00	UGS	16	12	
30.00	UGS	14	17	
50.00	UGS	14	20	
100.00	UGS	16	14	
300.00	UGS	16	13	
			1348	33
			14.00	97.60
			15.50	2.83
			17.00	2.12
			15.00	4.24
			15.00	1.41
			14.50	2.12

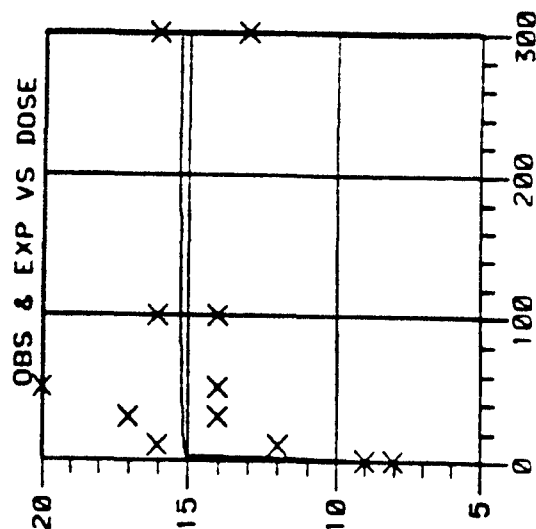
ESTS. B(0) 8.333 B(1) 1.8945 B(2) .0081

NO EVIDENCE OF TOXICITY

TEST CHI-SQUARE DF P LOGL
POISSON 2.44 7 .9312 -29.9930
ADEQUACY .68 3 .8768 -30.3354
MUTAGENICITY 8.92 2 .0115 -34.7979

AVERAGE SLOPE (NONLIN. MODEL) = .137
95% CONF. LIMITS = (.007, 2.882)

AVERAGE SLOPE (LINEAR REGR.) = .162
95% CONF. LIMITS = (.067, .257)



Best Available Copy

MUTAGENICITY TESTING OF C.I. SOLVENT GREEN NO. 3 -
C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE GREEN

RESEARCH LAB: G88A ON 06/06/84

08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION

STRAIN: TA1537

COMPOUND	A C T	UGS PER PLATE	MISTIDINE REVERTANTS PER PLATE					MEAN	STD
			A	B	C	D	E		
POS CONTROL									
9-AA	-	100.00	512	605	453			523.33	76.63
2-AA	RLA027	3.00	314	316	311			313.67	2.52
NEG CONTROL									
DIMETHYLSULF	RLA027	100.000	20	16	16			18.00	2.00
	-	100.000	12	7	17			12.00	5.00
BMGS-34-0001									
	RLA027	10.00	26	15				20.50	7.78
	RLA027	30.00	20	31				25.50	7.78
	RLA027	50.00	21	24				22.50	2.12
	RLA027	100.00	(4)	34				39.00	0.00
	RLA027	300.00	24	29				26.50	3.54
	-	10.00	25	21				23.00	2.53
	-	30.00	17	16				17.50	0.71
	-	50.00	13	16				14.50	2.12
	-	100.00	18	16				17.00	1.41
	-	300.00	24	27				25.50	2.12

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY S-9 : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIX/PLATE : 500UGS

T*-TOXIC
INTC-TOO NUMEROUS TO COUNT
NATC-NOT ABLE TO COUNT
G-PGS T-PPT
N-MGS P-PPN
M-MGS B-PPB
L-NLS I-MM
U-ULS C-UM

MUTAGENICITY TESTING OF C.I. SOLVENT GREEN NO. 3 -
C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE GREEN
RESEARCH LAB: G88A ON 06/08/84
TEST TYPE: STANDARD PLATE INCORPORATION

08/27/84

STRAIN: TA1537

BACKGROUNDS:

(4) CONTAMINATED

STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT GREEN NO. 3 -
C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0001 LAB: GB8A ACTIVATION: + RLA027
STRAIN: TA1537 DATE: 06/08/84 TECHNICIAN: NJK

DOSE UNITS PLATE COUNTS				MEAN S.D.	
.00	UCS	20	18	18.00	2.00
3.00*	UCS	314	311	313.67	2.52
10.00	UCS	26	15	20.50	7.78
30.00	UCS	20	31	25.50	7.78
50.00	UCS	21	24	22.50	2.12
100.00	UCS	24	39	39.00	.00
300.00	UCS	24	29	26.50	3.54

ESTS. B(0) B(1) B(2) B(3)

18.817 -2.6207 1.4612 .00829

TEST CHI-SQUARE DF P LOGL

POISSON 6.44 6 .3758 -33.0643

ADEQUACY 2.97 2 .2262 -34.5505

TOXICITY 3.84 1 .0501 -36.4701

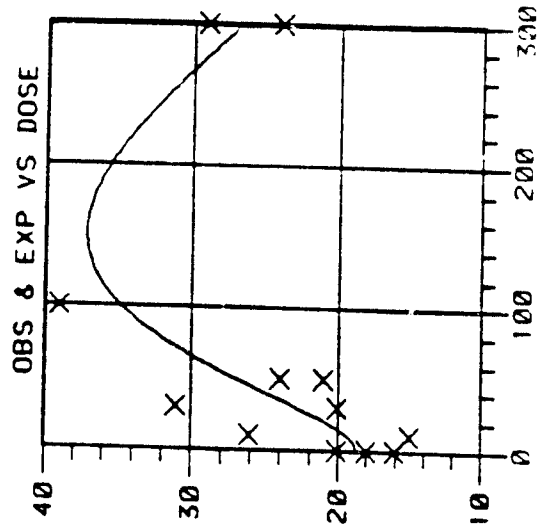
MUTAGENICITY 12.07 2 .0024 -40.5855

AVERAGE SLOPE (NONLIN. MODEL) = .609

95% CONF. LIMITS = (.098, 3.761)

AVERAGE SLOPE (LINEAR REG.) = .180

95% CONF. LIMITS = (.078, .283)



STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT GREEN NO. 3 -
C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0001 LAB: CBBA ACTIVATION: -
STRAIN: TA1537 DATE: 06/08/84 TECHNICIAN: MJK

DOSE UNITS PLATE COUNTS			MEAN S.D.	
.00	UCS	12 7 17	12.00	5.00
100.00*	UCS	512 605 453	523.33	76.63
10.00	UCS	25 21	23.00	2.83
30.00	UCS	17 18	17.50	.71
50.00	UCS	13 16	14.50	2.12
100.00	UCS	18 16	17.00	1.41
300.00	UCS	24 27	25.50	2.12

A-43

ESTS. 12.093 1.4476 .1353
B(0) B(1) B(2)

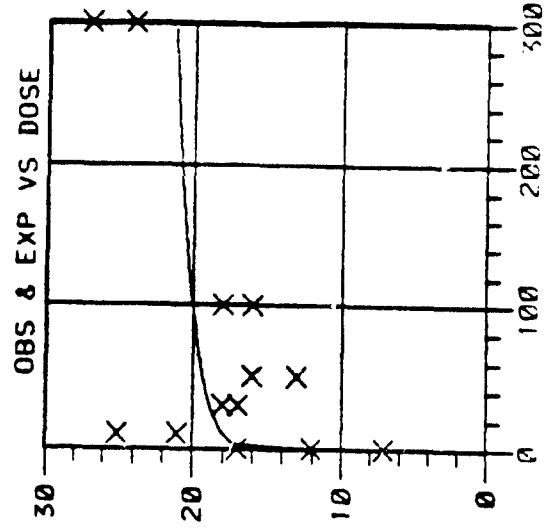
NO EVIDENCE OF TOXICITY

TEST CHI-SQUARE DF P LOGL
POISSON 5.15 7 .6420 -33.0167
ADEQUACY 8.01 3 .0458 -37.0219
MUTAGENICITY 8.44 2 .0147 -41.2401

AVERAGE SLOPE (NONLIN. MODEL) = .031
95% CONF. LIMITS = (.015, .064)

AVERAGE SLOPE (LINEAR REGR.) = .032
95% CONF. LIMITS = (.007, .057)

WARNING: 4 PARAMETER MODEL DID NOT CONVERGE



**MUTAGENICITY TESTING OF C.I. SOLVENT GREEN NO. 3 -
C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM**

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE GREEN

RESEARCH LAB: GBBA ON 06/05/84

08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION

STRAIN: TA1538

COMPOUND	A C T	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE					MEAN	STD
			A	B	C	D	E		
POS CONTROL									
2-NF	-	3.00	615	652	628			631.67	18.77
2-AA	RLA027	0.50	1051	1063	1052			1058.67	5.36
NEG CONTROL									
DIMETHYLSULF	RLA027	100.000	41	19	36			32.00	11.53
	-	100.000	18	13	15			15.33	2.52
BMGS-34-UCU1									
	RLA027	10.00	36	33				34.50	2.12
	RLA027	30.00	42	33				37.50	6.36
	RLA027	50.00	32	40				36.00	5.66
	RLA027	100.00	40	26				33.00	9.90
	RLA027	300.00	19	19				19.00	0.00
	-	10.00	19	16				17.50	2.12
	-	30.00	17	17				17.00	0.00
	-	50.00	20	15				17.50	3.54
	-	100.00	150	19				59.50	57.28
	-	300.00	15	12				13.50	2.12

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY S-9 : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIX/PLATE : 500UGS

T+ - TOXIC
TNTC - TOO NUMEROUS TO COUNT
NATC - NOT ABLE TO COUNT

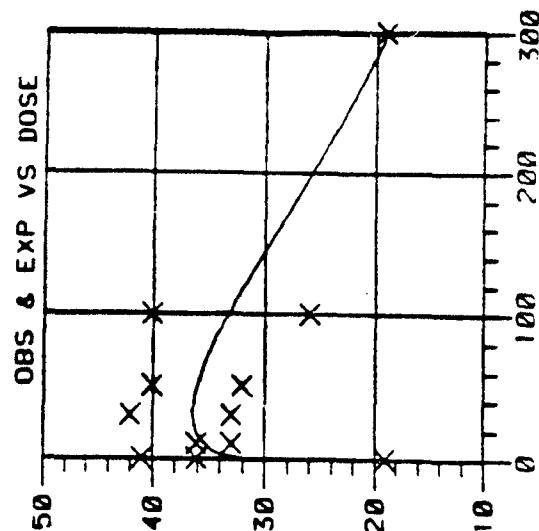
G-PGS T-DPT
N-NGS P-PPM
M-MGS B-PPB
L-NLS I-MM
U-ULS C-UM

STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT GREEN NO. 3 -
C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0001 LAB: CBBA ACTIVATION: + RLA027
STRAIN: TA1538 DATE: 06/05/84 TECHNICIAN: MJK

DOSE UNITS PLATE COUNTS				MEAN S.D.	
.00	UCS	41	19	32.00	11.53
.50*	UCS	1061	1063	1058.67	5.86
10.00	UCS	36	33	34.50	2.12
30.00	UCS	42	33	37.50	6.36
50.00	UCS	32	40	36.00	5.66
100.00	UCS	40	26	33.00	9.90
300.00	UCS	19	19	19.00	.00

ESTS.	B(0)	B(1)	B(2)	B(3)
	31.963	.2290	.5908	.00429
TEST	CHI-SQUARE	DF	P	LOGL
POISSON	13.38	7	.0633	-.413010
ADEQUACY	.11	2	.9443	-.413584
TOXICITY	19.40	1	.0000	-.510603
MUTAGENICITY	3.46	2	.1774	-.430876
AVERAGE SLOPE (NONLIN. MODEL) = .254				
95% CONF. LIMITS = (.000, *****)				
AVERAGE SLOPE (LINEAR REG.) = .088				
95% CONF. LIMITS = (-.157, .333)				



STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT GREEN NO. 3 -
C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

SAMPLE ID, BMGS-84-0001 LAB, CB8A ACTIVATION: -
STRAIN, TA1538 DATE, 06/05/84 TECHNICIAN, MJK

DOSE UNITS PLATE COUNTS			MEAN S.D.	
.00	UCS	18 13 15	15.33	2.52
3.00*	UCS	615 652 628	631.67	18.77
10.00	UCS	19 16	17.50	2.12
30.00	UCS	17 17	17.00	.00
50.00	UCS	20 15	17.50	3.54
100.00	UCS	100 19	59.50	57.28
200.00	UCS	15 12	13.50	2.12

ESTS. B(0) B(1) B(2) B(3)
18.419 -9.7494 3.5567 02628

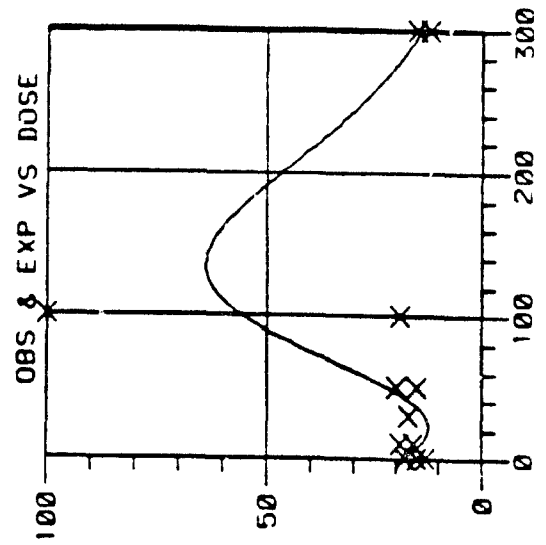
TEST CHI-SQUARE DF P LOCL

POISSON 57.27 7.0000 -62.3009
ADEQUACY 7.73 2.0210 -66.1655
TOXICITY 86.01 1.0000 -109.1693
MUTAGENICITY 110.96 2.0000 -121.6466

AVERAGE SLOPE (NONLIN. MODEL) = 7.574

AVERAGE SLOPE (LINEAR REGR.) = .413
95% CONF. LIMITS = (.057, .769)

WARNING: 4 PARAMETER MODEL DID NOT CONVERGE



**MUTAGENICITY TESTING OF C.I. SOLVENT GREEN NO. 3 -
C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM**

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE GREEN

RESEARCH LAB: G98A ON 06/08/84

08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION

STRAIN: TA1538

COMPOUND	A C T	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE					MEAN	STD
			A	B	C	D	E		
POS CONTROL									
2-YF	-	3.00	502	604	528			544.67	53.00
2-AA	RLA027	3.30	816	807	828			817.00	10.54
NEG CONTROL									
DIMETHYLSULF	RLA027	100.000	60	62	73			71.67	9.07
	-	100.000	21	16	19			19.33	1.53
BMGS-34-0001									
	FLA027	10.00	52	50				51.00	1.41
	RLA027	30.00	60	67				73.50	9.19
	RLA027	50.00	57	62				59.50	3.54
	RLA027	100.00	47	63				56.00	12.73
	RLA027	300.00	39	23				30.50	10.51
	-	10.00	15	12				13.50	2.12
	-	30.00	16	16				17.00	1.41
	-	50.00	17	12				14.50	3.54
	-	100.00	13	13				14.00	1.41
	-	300.00	11	10				13.50	3.54

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY S-9 : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIN/PLATE : 500UGS

T=TOXIC
TNTC=TOO NUMEROUS TO COUNT
NATC=NOT ABLE TO COUNT

G-PGS T-PPT
N-MGS P-PPH
M-MGS B-PPG
L-NLS I-OM
U-ULS C-UM

MUTAGENICITY TESTING OF C.I. SOLVENT GREEN NO. 3 -
C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE GREEN

RESEARCH LAB: 688A CN 06/08/84

08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION

STRAIN: TA1538

*RLA027

SPONTANEOUS COUNT IS HIGH DUE TO SMALL SALMONELLA COLONIES ON THE PLATE.

STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT GREEN NO. 3 -
C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

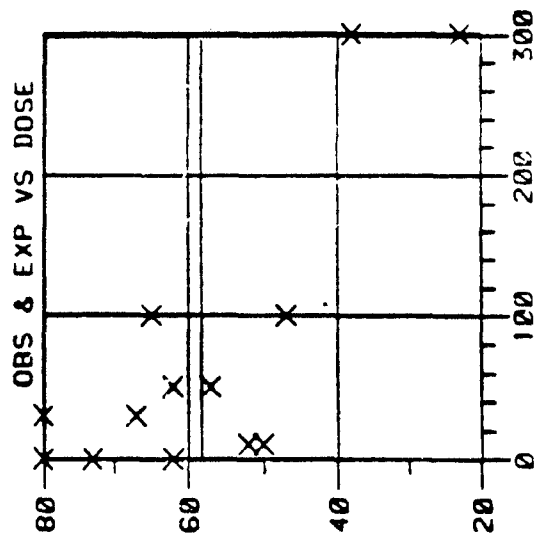
SAMPLE ID: BMGS-84-0001 LAB: CBBA ACTIVATION: + RLA027
STRAIN: TA1538 DATE: 06/08/84 TECHNICIAN: HJK

DOSE UNITS PLATE COUNTS			MEAN S.D.	
.00	UGS	80 62 73	71.67	9.07
.50*	UGS	816 807 828	817.00	10.54
10.00	UGS	52 50	51.00	1.41
30.00	UGS	80 67	73.50	9.10
50.00	UGS	57 62	59.50	3.54
100.00	UGS	47 65	56.00	12.73
300.00	UGS	38 23	30.50	10.61

B(0) B(1) B(2)

ESTS. 58.174*****
NO EVIDENCE OF TOXICITY
TEST CHI-SQUARE DF P LOGL

POISSON 10.28 7 .1734 -43.2544
ADEQUACY 50.17 3 .0000 -68.3413
MUTAGENICITY .00 2 .9999 -68.3414
AVERAGE SLOPE (NONLIN. MODEL) = .000
95% CONF. LIMITS = (.000, .000)
AVERAGE SLOPE (LINEAR REGR.) = -.082
95% CONF. LIMITS = (-.486, .322)



STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT GREEN NO. 3 -
C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0001 LAB: G8BA ACTIVATION: -
STRAIN: TA1538 DATE: 06/08/84 TECHNICIAN: MJK

DOSE UNITS PLATE COUNTS			MEAN S.D.	
.00	UGS	21 18 19	19.33	1.53
3.00*	UGS	502 604 528	544.67	53.00
10.00	UGS	15 12	13.50	2.12
30.00	UGS	16 18	17.00	1.41
50.00	UGS	17 12	14.50	3.54
100.00	UGS	13 15	14.00	1.41
300.00	UGS	11 16	13.50	3.54

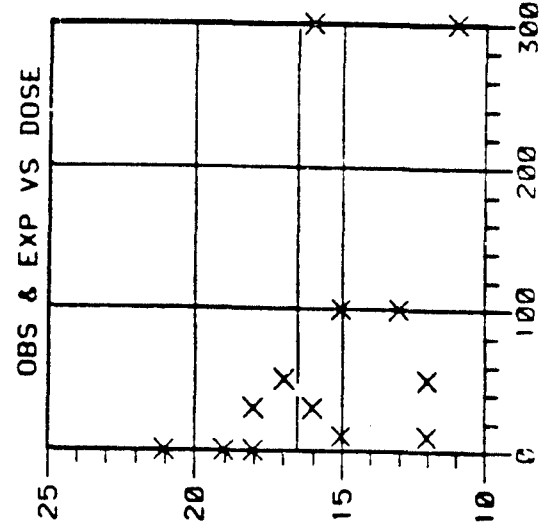
ESTS. B(0) B(1) B(2)
16.515*****659.4364

NO EVIDENCE OF TOXICITY

TEST	CHI-SQUARE	DF	P	LOCL
POISSON	2.62	7	.9175	---
ADEQUACY	5.44	3	.1421	-31.0826
MUTAGENICITY	.01	2	.9950	-33.8042
				-33.2910

AVERAGE SLOPE (NONLIN. MODEL) = .000
95% CONF. LIMITS = (.000)

AVERAGE SLOPE (LINEAR REGR.) = -.065
95% CONF. LIMITS = (-.165, .036)



**MUTAGENICITY TESTING OF C.I. SOLVENT GREEN NO. 3 -
C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM**

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE GREEN
RESEARCH LAB: G88A ON Q6/20/84

08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION

STRAIN: TA1538

COMPOUND	A C T	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE					MEAN	STD
			A	B	C	D	E		
POS CONTROL									
2-NF	-	3.00	492	504	551			515.67	31.18
2-AA	RLA027	0.50	668	714	737			706.33	35.13
NEG CONTROL									
DIMETHYLSULF	RLA027	100.000	45	39	36			40.00	4.58
	-	100.000	8	12	16			12.00	4.00
BMGS-34-3001									
	RLA027	10.00	41	31				36.00	7.07
	RLA027	30.00	50	41				45.50	6.36
	RLA027	50.00	38	39				38.50	6.71
	RLA027	100.00	51	46				49.50	2.12
	RLA027	300.00	25	27				26.00	1.41
	-	10.00	9	13				12.00	4.24
	-	30.00	13	16				14.50	2.12
	-	50.00	17	16				17.50	0.71
	-	100.00	19	17				18.00	1.41
	-	300.00	21	16				18.50	3.54

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY S-9 : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIX/PLATE : 500UGS

T*-TOXIC
TNC-TGO NUMEROUS TO COUNT
NATC-NUT ABLE TO COUNT

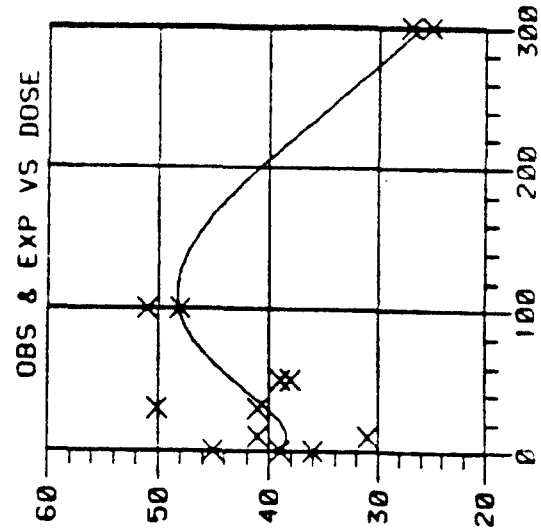
G-PGS T-PPT
N-MGS P-PPM
M-MGS B-PPB
L-NLS I-MM
U-ULS C-UM

STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT GREEN NO. 3 -
C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMCS-84-0001 LAB: CBBA ACTIVATION: + RLA027
STRAIN: TA1538 DATE: 06/20/84 TECHNICIAN: MJK

DOSE UNITS PLATE COUNTS			MEAN S.D.	
.00	UCS	45 30 36	40.00	4.58
.50*	UCS	668 714 737	706.33	35.13
10.00	UCS	41 31	36.00	7.07
30.00	UCS	50 41	45.50	6.36
50.00	UCS	38 39	38.50	7.1
100.00	UCS	51 48	49.50	2.12
300.00	UCS	25 27	26.00	1.41

TEST	CHI-SQUARE	DF	P	LOCL
POISSON	3.51	7	.8342	-37.4507
ADEQUACY	2.84	2	.2416	-38.8712
TOXICITY	15.96	1	.0001	-46.8525
MUTAGENICITY	8.13	2	.0172	-42.9354
AVERAGE SLOPE (NONLIN. MODEL) = .909				
95% CONF. LIMITS = (.063, 13.066)				
AVERAGE SLOPE (LINEAR REGR.) = .097				
95% CONF. LIMITS = (.007, .187)				



STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT GREEN NO. 3 -
C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMCS-84-0001 LAB: CB8A ACTIVATION: -
STRAIN: TA1538 DATE: 06/20/84 TECHNICIAN: MJK

DOSE UNITS	PLATE COUNTS	MEAN	S.D.
.00 UGS	8 12 16	12.00	4.00
3.00* UGS	492 504 551	515.67	31.18
10.00 UGS	9 15	12.00	4.24
30.00 UGS	13 16	14.50	2.12
50.00 UGS	17 18	17.50	.71
100.00 UGS	19 17	18.00	1.41
300.00 UGS	21 16	18.50	3.54

A-53

ESTS. B(0) B(1) B(2) B(3)
11.743 -1.8610 1.0086 .00398

TEST CHI-SQUARE DF P LOGL

POISSON 5.29 7.6243 -32.1536

ADEQUACY .39 2.8241 -32.3471

TOXICITY .86 1.3539 -32.7768

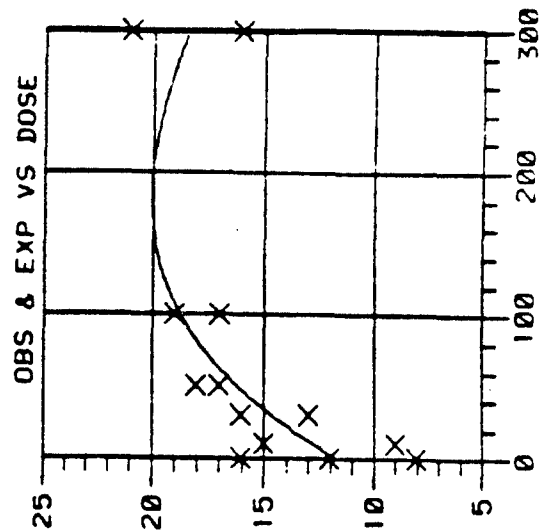
MUTAGENICITY 6.51 2.0386 -35.6013

AVERAGE SLOPE (NONLIN. MODEL) = .161

95% CONF. LIMITS = (.005, 5.3471)

AVERAGE SLOPE (LINEAR REGR.) = .112

95% CONF. LIMITS = (.014, .210)



**MUTAGENICITY TESTING OF C.I. SOLVENT GREEN NO. 3 -
C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM**

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE GREEN
RESEARCH LAB: G88A ON 03/30/84

08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION

STRAIN: TA98

COMPOUND	A C T	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE					MEAN	STD
			A	B	C	D	E		
POS CONTROL									
2-NF	-	3.00	300	312	315			309.00	7.94
2-AA	RLA026	0.50	875	837	853			855.00	17.08
NEG CONTROL									
DIMETHYLSULF	RLA026	100.000	60	41	48			49.67	9.61
	-	100.000	28	33	30			30.33	2.52
BMGS-84-0001									
	RLA026	1.00	51	50				50.50	0.71
	RLA026	5.00	50	40				45.00	7.07
	RLA026	10.00	53	42				47.50	7.78
	RLA026	30.00	53	46				50.50	3.54
	RLA026	50.00	49	56				53.50	6.36
	RLA026	100.00	54	57				55.50	2.12
	RLA026	300.00	49	61				55.00	8.49
	RLA026	500.00	63	47				55.00	11.31
	RLA026	1000.00	52	26				39.00	18.38
	-	1.00	24	27				25.50	2.12
	-	5.00	27	25				26.00	1.41
	-	10.00	32	54				43.00	15.56
	-	30.00	26	30				28.00	2.83
	-	50.00	25	25				25.00	0.00
	-	100.00	29	20				24.50	6.36
	-	300.00	19	26				22.50	4.95
	-	500.00	21	25				23.00	2.83
	-	1000.00	25	15				21.50	4.95

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY 3-9 : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIX/PLATE : 500UGS

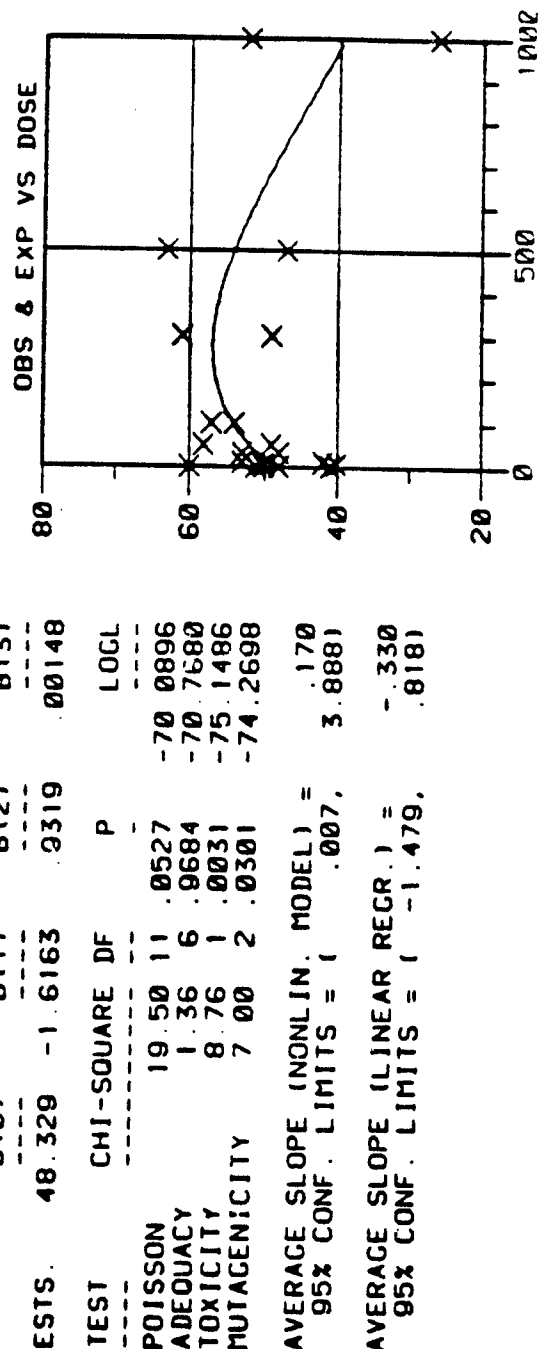
T=-TOXIC
TNTC-TOO NUMEROUS TO COUNT
NATC-NOT ABLE TO COUNT

G-PGS T-PPT
N-MGS P-PPM
M-MGS B-PPB
L-NLS I-PP
U-ULS C-UM

STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT GREEN NO. 3 -
C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0001 LAB: C88A ACTIVATION: + RLA026
STRAIN: TA98 DATE: 03/30/84 TECHNICIAN: MJK

DOSE UNITS PLATE COUNTS			MEAN	S.D.
00	UCS	60	41	48
50*	UCS	875	837	853
1.00	UCS	51	50	
5.00	UCS	50	40	
10.00	UCS	53	42	
30.00	UCS	53	48	
50.00	UCS	49	58	
100.00	UCS	54	57	
300.00	UCS	49	61	
MORE THAN 9 DOSE LEVELS USED IN COMPUTATION BUT NOT DISPLAYED				
B(0)	B(1)	B(2)	B(3)	
48.329	-1.6163	.9319	.00148	



STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT GREEN NO. 3 -
C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0001 LAB: GBBA ACTIVATION: -
STRAIN: TA98 DATE: 03/30/84 TECHNICIAN: MJK

DOSE UNITS	PLATE COUNTS	MEAN	S.D.
0.00 UGS	28 33 30	30.33	2.52
3.00* UGS	300 312 315	309.00	7.94
1.00 UGS	24 27	25.50	2.12
5.00 UGS	27 25	26.00	1.41
10.00 UGS	32 54	43.00	15.56
30.00 UGS	26 30	28.00	2.83
50.00 UGS	25 25	25.00	.00
100.00 UGS	29 20	24.50	6.36
300.00 UGS	19 26	22.50	4.95

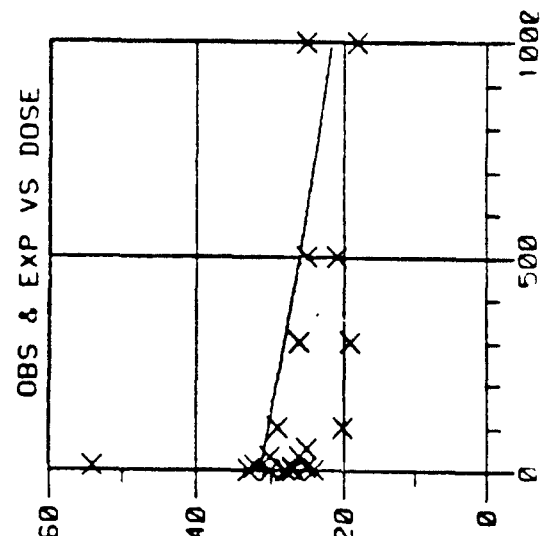
MORE THAN 9 DOSE LEVELS USED IN COMPUTATION BUT NOT DISPLAYED

ESTS. 30.469 - 0631 .0000 .00038
B(1) B(2) B(3)

TEST CHI-SQUARE DF P LOGL
POISSON 10.81 11 .4590 -59.1673
ADEQUACY 20.57 6 .0022 -69.4521
TOXICITY 15.89 1 .0001 -77.3966
MUTAGENICITY .01 2 .9950 -67.5145

AVERAGE SLOPE (NONLIN. MODEL) = .094
95% CONF. LIMITS = (.000, *****)

AVERAGE SLOPE (LINEAR REGR.) = 1.269
95% CONF. LIMITS = (-.077, 2.615)



MUTAGENICITY TESTING OF C.I. SOLVENT GREEN NO. 3 -
C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
 OF ARMY DYE GREEN
 RESEARCH LAB: GBBA ON 04/06/84

08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION

STRAIN: TA98

COMPOUND	A C T	UGS PER PLATE	MISTIDINE REVERTANTS PER PLATE					MEAN	STD
			A	B	C	D	E		
POS CONTROL									
2-NF	-	3.00	250	270	255			258.33	10.41
2-AA	RLA026	0.50	740	825	817			794.00	46.94
NEG CONTROL									
DIMETHYLSULF	RLA026	100.000	42	31	43			38.67	6.66
	-	100.000	23	29	40			24.00	4.58
BMGS-34-JC01									
	RLA026	1.00	37	40				38.50	2.12
	RLA026	5.00	61	45				53.00	11.31
	RLA026	10.00	29	32				30.50	2.12
	RLA026	30.00	56	46				52.00	5.66
	RLA026	50.00	45	53				49.00	5.66
	RLA026	100.00	50	46				49.00	1.41
	RLA026	300.00	50	56				53.00	4.24
	-	1.00	26	27				26.50	0.71
	-	5.00	21	29				25.00	5.66
	-	10.00	25	31				28.00	4.24
	-	30.00	26	27				26.50	0.71
	-	50.00	30	29				29.50	0.71
	-	100.00	41	16				29.50	16.26
	-	300.00	20	31				25.50	7.78

PHENOCOPY CHECK : TRUE MUTANTS
 STERILITY S-9 : NOT CONTAMINATED
 SAMPLE STERILITY: NOT CONTAMINATED
 ACT MIX/PLATE : 500UGS

T*-TOXIC
 INTC-T00 NUMEROUS TO COUNT
 NATC-NGT ABLE TO COUNT

G-PGS T-PPT
 N-NGS P-PPM
 M-MGS B-PPB
 L-NLS I-MM
 U-ULS C-UM

Best Available Copy

MUTAGENICITY TESTING OF C.I. SOLVENT GREEN NO. 3 -
C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE GREEN

RESEARCH LAB: GBHA ON 04/06/84

08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION

STRAIN: TA98

•RLAG20

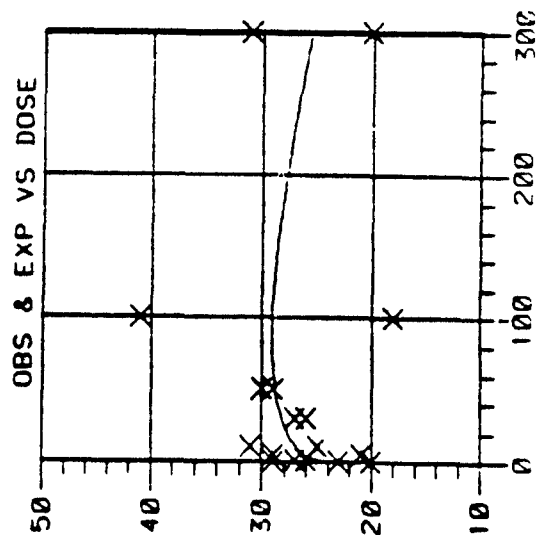
ABOVE 100 UG/PLATE, THE SAMPLE APPEARS TO PRECIPITATE OUT OF SOLUTION.

STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT GREEN NO. 3 -
C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0001 LAB: CBBA ACTIVATION: -
STRAIN: TA98 DATE: 04/06/84 TECHNICIAN: MJK

DOSE UNITS	PLATE COUNTS	MEAN	S.D.
.00 UGS	23 29 20	24.00	4.58
3.00* UGS	250 270 255	258.33	10.41
1.00 UGS	26 27	26.50	.71
5.00 UGS	21 29	25.00	5.66
10.00 UGS	25 31	28.00	4.24
30.00 UGS	26 27	26.50	.71
50.00 UGS	30 29	29.50	16.26
100.00 UGS	41 18	25.50	7.78
300.00 UGS	20 31		

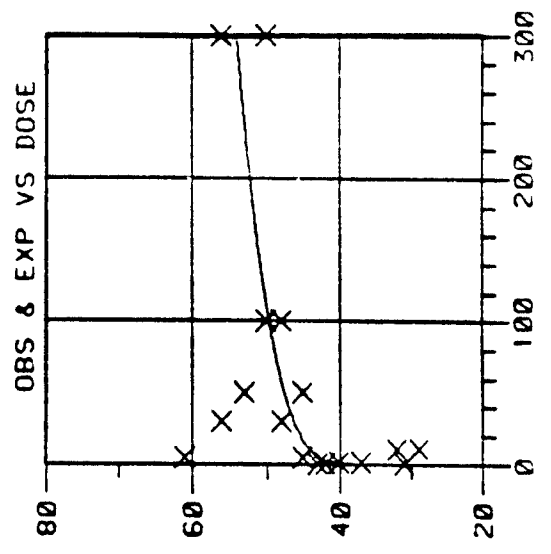
ESTS. B(0) 24.352 B(1) 1211 B(2) 5262 B(3) 00166
CHI-SQUARE DF P LOGL
POISSON 15.07 9.0891 -51.0585
ADEQUACY .61 4.9618 -51.3741
TOXICITY .76 1.3825 -51.7555
MUTAGENICITY 2.00 2.3672 -52.3760
AVERAGE SLOPE (NONLIN. MODEL) = 139
95% CONF. LIMITS = (034, 574)
AVERAGE SLOPE (LINEAR REGR.) = 080
95% CONF. LIMITS = (-.018, 178)



STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT GREEN NO. 3 -
C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0001 LAB: G88A ACTIVATION: + RLA026
STRAIN: TA98 DATE: 04/06/84 TECHNICIAN: MJK

DOSE UNITS	PLATE COUNTS	MEAN	S.D.
.00 UGS	42 31 43	38.67	6.66
.50* UGS	740 825 817	794.00	46.94
1.00 UGS	37 40	38.50	2.12
5.00 UGS	61 45	53.00	11.31
10.00 UGS	29 32	30.50	2.12
30.00 UGS	56 48	52.00	5.66
50.00 UGS	45 53	49.00	5.66
100.00 UGS	50 48	49.00	1.41
300.00 UGS	50 56	53.00	4.24



ESTS.	B(0)	B(1)	B(2)
	38.301	1.0715	.2941
NO EVIDENCE OF TOXICITY			
TEST	CHI-SQUARE	DF	P
POISSON	6.62	9	.6765
ADEQUACY	15.58	5	.0081
MUTAGENICITY	8.58	2	.0137
AVERAGE SLOPE (NONLIN. MODEL) = .052			
95% CONF. LIMITS = (.014, .194)			
AVERAGE SLOPE (LINEAR REGR.) = .041			
95% CONF. LIMITS = (-.003, .086)			

MUTAGENICITY TESTING OF C.I. SOLVENT YELLOW NO. 33
IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE YELLOW
RESEARCH LAB: 688A ON 03/30/84

08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION

STRAIN: TA100

COMPOUND	A C T	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE					MEAN	STD
			A	B	C	D	E		
POS CONTROL									
NAAZIDE	-	3.00	1179	1205	1180			1188.00	14.73
2-AA	RLA026	0.50	363	360	298			340.33	36.69
NEG CONTROL									
DIMETHYLSULF	RLA026	100.00u	105	101	103			103.00	2.00
	-	100.00u	103	134	97			111.33	19.86
BMGS-34-0001									
	RLA026	1.00	133	131				132.00	1.41
	RLA026	3.00	115	132				123.50	12.02
	RLA026	10.00	137	156				146.50	13.44
	RLA026	30.00	172	200				186.00	19.80
	RLA026	50.00	170	162				166.00	5.66
	RLA026	100.00	146	161				153.50	10.61
	RLA026	300.00	149	138				143.50	7.78
	RLA026	500.00	132	130				131.00	1.41
	RLA026	1000.00	125	135				130.00	7.07
	-	1.00	129	113				121.00	11.31
	-	3.00	141	141				141.00	0.00
	-	10.00	120	144				132.00	16.97
	-	30.00	154	166				160.00	8.49
	-	50.00	151	154				152.50	2.12
	-	100.00	142	141				141.50	0.71
	-	300.00	129	135				132.00	4.24
	-	500.00	139	108				123.50	21.92
	-	1000.00	93	114				102.50	13.44

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY S-9 : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIA/PLATE : 500UGS

T*-TOXIC
TNTC-TWO NUMEROUS TO COUNT
NATC-NOT ABLE TO COUNT

G-PGS T-PPT
N-NGS P-PPM
M-NGS B-PPB
L-NLS I-MM
U-ULS C-UM

STATISTICAL ANALYSIS: MUTAGENICITY OF
C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0002 LAB: CBBA ACTIVATION: + RLA026
STRAIN: TA100 DATE: 03/30/84 TECHNICIAN: MJK

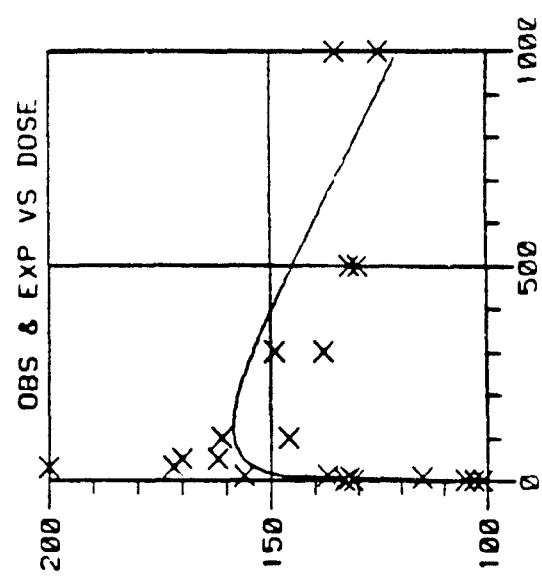
DOSE UNITS	PLATE COUNTS	MEAN	S.D.
0.00 UGS	105 101 103	103.00	2.00
0.50* UGS	363 360 298	340.33	36.69
1.00 UGS	133 131	132.00	1.41
5.00 UGS	115 132	123.50	12.02
10.00 UGS	137 156	142.50	13.44
30.00 UGS	172 200	186.00	19.80
50.00 UGS	170 162	166.00	5.66
100.00 UGS	146 161	153.50	10.61
300.00 UGS	149 138	143.50	7.78

MORE THAN 9 DOSE LEVELS USED IN COMPUTATION BUT NOT DISPLAYED

ESTS. 102.328 3.4880 1439 00046
B(10) B(11) B(12) B(13)

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	6.35	11	.8490	-74.1956
ADEQUACY	25.88	6	.0002	-87.1344
TOXICITY	17.29	1	.0000	-95.7803
MUTAGENICITY	52.69	2	.0000	-113.4797

AVERAGE SLOPE (NONLIN. MODEL) = 1.779
95% CONF. LIMITS = (.516, 6.129)
AVERAGE SLOPE (LINEAR REGR.) = 2.471
95% CONF. LIMITS = (1.699, 3.242)



STATISTICAL ANALYSIS: MUTAGENICITY OF
C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMCS-84-0002 LAB: CBBA ACTIVATION: -
STRAIN: TA100 DATE: 03/30/84 TECHNICIAN: MJK

DOSE UNITS PLATE COUNTS			MEAN S.D.	
DOSE UNITS	PLATE COUNTS		MEAN	S.D.
0.00 UGS	103 134 97		111.33	19.86
3.00* UGS	1179 1205 1180		1188.00	14.73
1.00 UGS	129 113		121.00	11.31
5.00 UGS	141 141		141.00	.00
10.00 UGS	120 144		132.00	16.97
30.00 UGS	154 166		160.00	8.49
50.00 UGS	151 154		152.50	2.12
100.00 UGS	142 141		141.50	.71
300.00 UGS	129 135		132.00	4.24

MORE THAN 9 DOSE LEVELS USED IN COMPUTATION BUT NOT DISPLAYED

ESTS. 110.469 2.9785 1807 .00059

TEST CHI-SQUARE DF P LOGL

POISSON 15.59 11 .1205 -78.5701

ADQUACY 7.81 6 .2525 -82.4743

TOXICITY 29.90 1 .0000 -97.4251

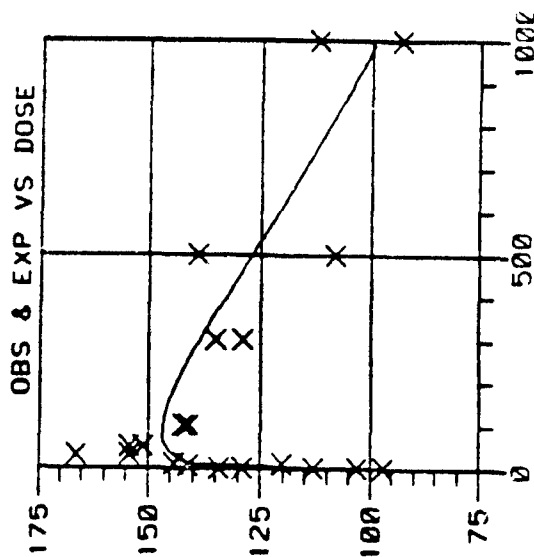
MUTAGENICITY 25.98 2 .0000 -95.4635

AVERAGE SLOPE (NONLIN. MODEL) = 1.212

95% CONF. LIMITS = (.377, 1.674)

AVERAGE SLOPE (LINEAR REGR.) = 1.428

95% CONF. LIMITS = (.616, 2.241)



MUTAGENICITY TESTING OF C.I. SOLVENT YELLOW NO. 33
IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
 OF ARMY DYE YELLOW
 RESEARCH LAB: GBBB ON 04/06/84

08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION

STRAIN: TA100

COMPOUND	A C T	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE					MEAN	STD
			A	B	C	D	E		
POS CONTROL									
NAAZIDE	-	3.00	1253	1350	1320			1307.67	49.66
Z-AA	RLA026	3.50	953	816	842			869.00	74.28
NEG CONTROL									
DIMETHYLSULF	RLA026	100.00U	115	125	114			118.00	6.08
	-	100.00U	109	102	110			107.00	4.36
BMGS-34-0002									
	RLA026	1.00	132	151				141.50	13.44
	RLA026	5.00	144	132				138.00	8.49
	RLA026	10.00	153	149				151.00	2.83
	RLA026	30.00	174	153				163.50	14.65
	RLA026	50.00	146	174				160.00	19.80
	RLA026	100.00	167	151				159.00	11.31
	RLA026	300.00	156	135				145.50	14.85
	-	1.00	130	130				130.00	0.00
	-	5.00	112	129				120.50	12.02
	-	10.00	128	112				120.00	11.31
	-	30.00	98	117				107.50	13.44
	-	50.00	126	132				129.00	4.24
	-	100.00	140	126				133.00	9.90
	-	300.00	111	116				114.50	4.95

PHENOCOPY CHECK : TRUE MUTANTS
 STERILITY S-9 : NOT CONTAMINATED
 SAMPLE STERILITY: NOT CONTAMINATED
 ACT MIX/PLATE : 500UGS

T*-TOXIC
 TNTC-TOO NUMEROUS TO COUNT
 NATC-NOT ABLE TO COUNT

G-PGS T-PPT
 N-NGS P-PPH
 M-MGS B-PPB
 L-NLS I-PM
 U-ULS C-UM

STATISTICAL ANALYSIS: MUTAGENICITY OF
C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMCS-84-0002 LAB: G88A ACTIVATION: + RLA026
STRAIN: TA100 DATE: 04/06/84 TECHNICIAN: MJK

DOSE UNITS	PLATE COUNTS	MEAN	S.D.
.00 UGS	115 125 114	118.00	6.08
.50* UGS	953 812 842	869.00	74.28
1.00 UGS	132 151	141.50	13.44
5.00 UGS	144 132	138.00	8.49
10.00 UGS	153 149	151.00	2.83
30.00 UGS	174 153	163.50	14.85
50.00 UGS	146 174	160.00	19.80
100.00 UGS	167 151	159.00	11.31
300.00 UGS	156 135	145.50	14.85

ESTS. B(0) 118.182 B(1) 2.9511 B(2) 2489 B(3) 00102

TEST CHI-SQUARE DF P LOCL

POISSON 8.60 9 .4753 -62.1904

ADEQUACY 1.62 4 .8061 -62.9979

TOXICITY 4.45 1 .0350 -65.2215

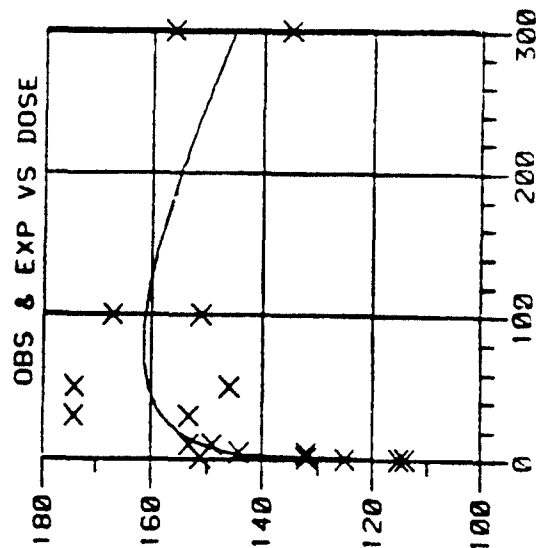
MUTAGENICITY 26.13 2 .0000 -76.0619

AVERAGE SLOPE (NONLIN. MODEL) = 1.486

95% CONF. LIMITS = (.760, 2.909)

AVERAGE SLOPE (LINEAR REGR.) = 1.245

95% CONF. LIMITS = (.553, 1.936)



STATISTICAL ANALYSIS: MUTAGENICITY OF
C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

SAMPLE ID, BMGS-84-0002 LAB, CB8A ACTIVATION: -
STRAIN: TA100 DATE: 04/06/84 TECHNICIAN, MJK

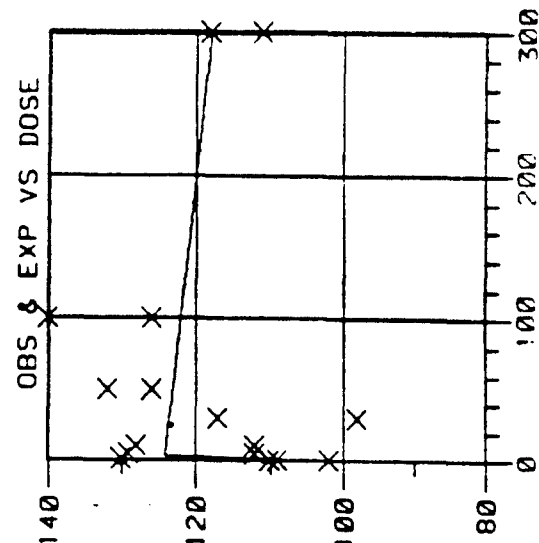
DOSE UNITS	PLATE COUNTS	MEAN	S.D.
.00 UCS	109 102 110	107.00	4.36
3.00* UCS	1253 1350 1320	1307.67	49.66
1.00 UCS	130 130	130.00	.00
5.00 UCS	112 129	120.50	12.02
10.00 UCS	128 112	120.00	11.31
30.00 UCS	98 117	107.50	13.44
50.00 UCS	126 132	129.00	4.24
100.00 UCS	140 126	133.00	9.90
300.00 UCS	111 118	114.50	4.95

ESTS. 106.793 2.8538 B(1) B(2) B(3)

TEST CHI-SQUARE DF P LOGL

POISSON 5.39 9 .7990 -58.9452
ADEQUACY 8.02 4 .0908 -62.9552
TOXICITY .52 1 .4719 -63.2139
MUTAGENICITY 5.24 2 .0730 -65.5729

AVERAGE SLOPE (NONLIN MODEL) = .347
95% CONF. LIMITS = (.041, 2.972)
AVERAGE SLOPE (LINEAR REGR.) = .129
95% CONF. LIMITS = (-.239, .497)
WARNING: 3 PARAMETER MODEL DID NOT CONVERGE



**MUTAGENICITY TESTING OF C.I. SOLVENT YELLOW NO. 33
IN SALMONELLA TYPHIMURIUM**

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE YELLOW
RESEARCH LAB: G88A ON 04/06/84

08/27/84

TEST TYPE: PLATE TEST - PREINCUBATION

STRAIN: TA100

COMPOUND	A C T	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE					MEAN	STD
			A	B	C	D	E		
POS CONTROL									
NAAZIDE	-	0.00	1227	1278	1267			1257.33	26.84
2-AA	RLA026	0.50	354	307	337			332.67	23.80
NEG CONTROL									
DIMETHYLSULF	RLA026	100.00u	108	117	115			113.33	4.73
	-	100.00u	144	132	127			134.33	6.74
5MGS-84-0002									
	RLA026	1.00	129	133				131.00	2.83
	RLA026	5.00	132					132.00	0.00
	RLA026	10.00	151	140				145.50	7.78
	RLA026	30.00	156	180				168.00	16.97
	RLA026	50.00	194	203				198.50	6.36
	RLA026	100.00	155	154				154.50	0.71
	RLA026	300.00	172	137				154.50	24.75
	-	1.00	111	129				120.00	12.73
	-	5.00	111	137				124.00	18.39
	-	10.00	100	133				116.50	25.33
	-	30.00	139	101				120.00	26.87
	-	50.00	194	125				159.50	48.79
	-	100.00	103	110				106.50	4.95
	-	300.00	123	115				119.00	5.66

PHENOCOPIY CHECK : TRUE MUTANTS
STERILITY S-9 : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIX/PLATE : 500UGS

T=TOXIC
TNTC=TOO NUMEROUS TO COUNT
NATC=NOT ABLE TO COUNT

G-PGS T-PPT
N-NGS P-PPM
M-MGS B-PPB
L-NLS I-MM
U-ULS C-UM

STATISTICAL ANALYSIS: MUTAGENICITY OF
C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMCS-84-0002 LAB: CBBA ACTIVATION: + RL0026
STRAIN: TA100 DATE: 04/06/84 TECHNICIAN: MJK

DOSE UNITS	PLATE COUNTS	MEAN	S.D.
.00 UGS	108 117 115	113.33	4.73
.50* UGS	354 307 337	332.67	23.80
1.00 UGS	129 133	131.00	2.83
5.00 UGS	132	132.00	.00
10.00 UGS	151 140	145.50	7.78
30.00 UGS	156 180	168.00	16.97
50.00 UGS	194 203	198.50	6.36
100.00 UGS	155 154	154.50	.71
300.00 UGS	172 137	154.50	24.75

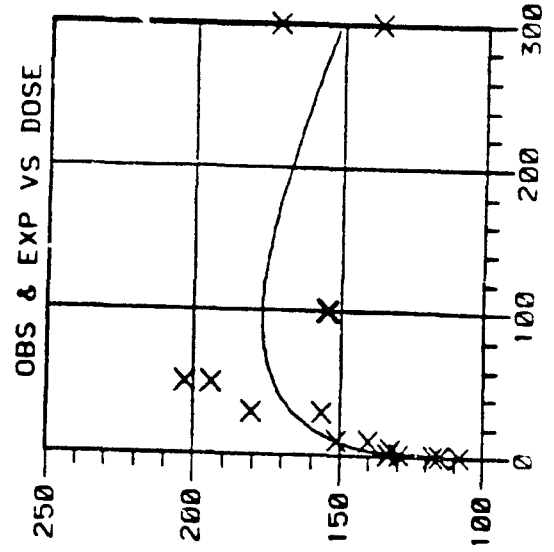
ESTS. 112.794 2.8208 B(1) B(2) B(3)

TEST CHI-SQUARE DF P LOGL

POISSON 6.76 8 .5631 -57.9736
ADEQUACY 14.72 4 .0053 -65.3314
TOXICITY 12.75 1 .0004 -71.7061
MUTAGENICITY 55.73 2 .0000 -93.1984

AVERAGE SLOPE (NONLIN. MODEL) = 1.543
95% CONF. LIMITS = (1.022, 2.329)

AVERAGE SLOPE (LINEAR REGR.) = 1.548
95% CONF. LIMITS = (1.250, 1.846)



STATISTICAL ANALYSIS: MUTAGENICITY OF
C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMCS-84-0002 LAB: CB8A ACTIVATION: -
STRAIN: TA100 DATE: 04/06/84 TECHNICIAN: MJK

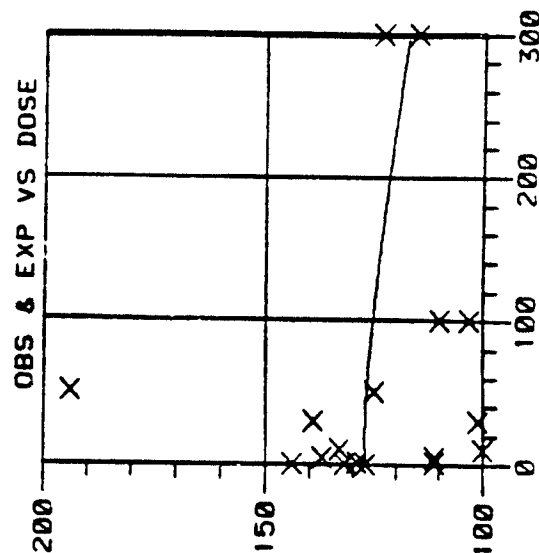
DOSE UNITS	PLATE COUNTS	MEAN	S.D.
0.00 UGS	144 132 127	134.33	8.74
3.00* UGS	1227 1278 1267	1257.33	26.84
1.00 UGS	111 129	120.00	12.73
5.00 UGS	111 137	124.00	18.38
10.00 UGS	100 133	116.50	23.33
30.00 UGS	139 101	120.00	26.87
50.00 UGS	194 125	159.50	48.79
100.00 UGS	103 110	106.50	4.95
300.00 UGS	123 115	119.00	5.66

ESTS. 126.876 -1.4883 .7835 .00074
B(0) B(1) B(2) B(3)

TEST CHI-SQUARE DF P LOGL
POISSON 31.33 9 .0003 -72.3402
ADEQUACY 26.56 4 .0000 -85.6211
TOXICITY 1.30 1 .2534 -86.2732
MUTAGENICITY .02 2 .9905 -85.6306

AVERAGE SLOPE (NONLIN. MODEL) = .066
95% CONF. LIMITS = (.000, *****)

AVERAGE SLOPE (LINEAR REGR.) = -.033
95% CONF. LIMITS = (-.151, .086)
WARNING: 3 PARAMETER MODEL DID NOT CONVERGE



MUTAGENICITY TESTING OF C.I. SOLVENT YELLOW NO. 33
IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
 OF ARMY DYE YELLOW
 RESEARCH LAB: GBBA ON 06/01/84

08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION

STRAIN: TA102

COMPOUND	A C T	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE					MEAN	STD
			A	B	C	D	E		
POS CONTROL									
OTHER POS	RLA027	30.00	1111	984	1009			1034.67	67.28
	-	0.50	349	335	302			355.33	24.13
NEG CONTROL									
DIMETHYLSULF	RLA027	100.000	108	109	100			105.67	4.93
	-	100.000	57	40	42			46.33	9.29
EMGS-34-0002									
	RLA027	10.00	100	100				100.00	0.00
	RLA027	30.00	144	162				153.00	12.73
	RLA027	50.00	186	187				186.50	0.71
	RLA027	100.00	213	190				201.50	16.26
	RLA027	300.00	188	206				197.00	12.73
	-	10.00	65	101				93.00	11.31
	-	30.00	113	91				102.00	15.56
	-	50.00	113	110				111.50	2.12
	-	100.00	74	100				87.00	16.38
	-	300.00	96	115				105.50	13.44

PHENOCOPY CHECK : TRUE MUTANTS
 STERILITY S-4 : NOT CONTAMINATED
 SAMPLE STERILITY: NOT CONTAMINATED
 ACT MIX/PLATE : 500UGS

T--TOXIC
 TNC-TCC NUMEROUS TO COUNT
 NATC-NOT ABLE TO COUNT

G-PGS T-OPT
 N-MGS P-PPM
 M-MGS B-PPB
 L-NLS I--M
 U-ULS C-UM

MUTAGENICITY TESTING OF C.I. SOLVENT YELLOW NO. 33
IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE YELLOW
RESEARCH LAB: G88A ON 06/01/84

08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION

STRAIN: TA1C2

•RLAC27

POSITIVE CONTROL USED WAS DANTHRON. DYES START TO PRECIPITATE OUT OF SOLUTION AT THE 300UG DOSE.

-
POSITIVE CONTROL USED WAS MITOMYCIN C.

STATISTICAL ANALYSIS: MUTAGENICITY OF
C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMCS-84-0002 LAB: GBBA ACTIVATION: + RLA027
STRAIN: TA102 DATE: 06/01/84 TECHNICIAN: MJK

DOSE UNITS	PLATE COUNTS	MEAN	S.D.
00	UGS 108 109 100	105.67	4.93
30.00*	UGS 111 984 1009	1034.67	67.28
10.00	UGS 100 100	100.00	.00
30.00	UGS 144 162	153.00	12.73
50.00	UGS 186 187	186.50	.71
100.00	UGS 213 190	201.50	16.26
300.00	UGS 188 206	197.00	12.73

ESTS. B(0) 101.044 B(1) 5856 B(2) 1.0826 B(3) 00532

TEST CHI-SQUARE DF P LOGL

POISSON 3.66 7 .8183 -46.2231

ADEQUACY 9.62 2 .0081 -51.0330

TOXICITY 27.39 1 .0000 -64.7274

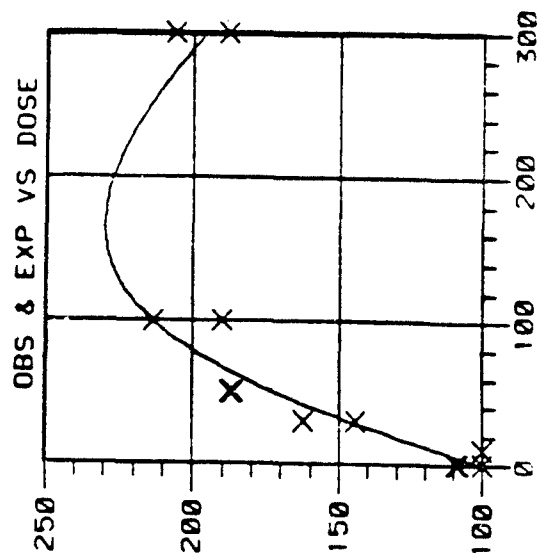
MUTAGENICITY 149.87 2 .0000 -125.9691

AVERAGE SLOPE (NONLIN. MODEL) = 2.628

95% CONF. LIMITS = (1.746, 3.955)

AVERAGE SLOPE (LINEAR REGR.) = 1.073

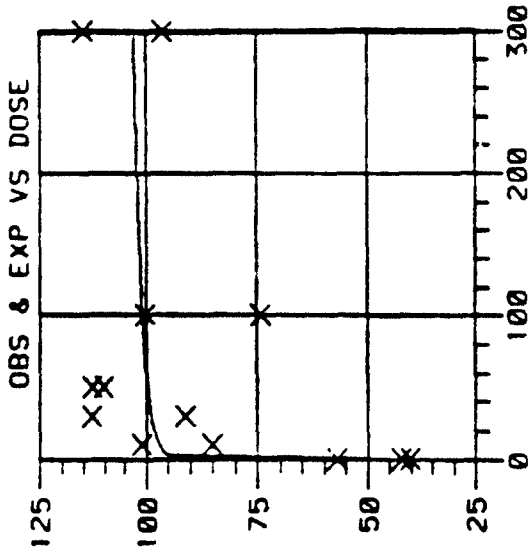
95% CONF. LIMITS = (.756, 1.390)



STATISTICAL ANALYSIS: MUTAGENICITY OF
C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0002 LAB: CB8A ACTIVATION: -
STRAIN: TA102 DATE: 06/01/84 TECHNICIAN: MJK

DOSE UNITS		PLATE COUNTS		MEAN		S.D.	
.00	UCS	57	40	42	46.33	9.29	
.50*	UCS	349	335	382	355.33	24.13	
10.00	UCS	85	101		93.00	11.31	
30.00	UCS	113	91		102.00	15.56	
50.00	UCS	113	110		111.50	2.12	
100.00	UCS	74	100		87.00	18.38	
300.00	UCS	96	115		105.50	13.44	



ESTS.	46.332	3.8521	.0316	
NO EVIDENCE OF TOXICITY				
TEST	CHI-SQUARE	DF	P	LOGL
POISSON	13.11	7	.0694	-47.1769
ADEQUACY	7.38	3	.0607	-50.8666
MUTAGENICITY	87.17	2	.0000	-94.4495
AVERAGE SLOPE (NONLIN. MODEL) = .188				
95% CONF. LIMITS = (.151, .234)				
AVERAGE SLOPE (LINEAR REGR.) = .106				
95% CONF. LIMITS = (-.032, .244)				

MUTAGENICITY TESTING OF C.I. SOLVENT YELLOW NO. 33
IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE YELLOW
RESEARCH LAB: GBBA ON 06/06/84

08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION

STRAIN: TA102

COMPOUND	A C T	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE					MEAN	STD
			A	B	C	D	E		
POS CONTROL									
OTHER POS	RLAQ27	30.00	(4) 1176	1208				1192.00	22.63
	-	0.50	1182	1169	1170			1173.33	7.57
NEG CONTROL									
DIMETHYLSULF	RLAQ27	100.000	257	234	239			243.33	12.10
	-	100.000	154	(4) 145				149.50	6.36
6MGS-34-J002									
	RLAQ27	10.00	403	441				422.00	26.87
	RLAQ27	30.00	538	464				501.00	52.33
	RLAQ27	50.00	603	603				603.50	3.54
	RLAQ27	100.00	640	665				652.50	17.69
	RLAQ27	300.00	678	662				670.00	11.31
	-	10.00	341	350				345.50	6.36
	-	30.00	474	440				457.00	24.04
	-	50.00	506	424				465.00	57.98
	-	100.00	441	463				452.00	15.56
	-	300.00	485	517				501.00	22.63

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY S-9 : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIX/PLATE : 500UGS

T+-TOXIC
TNTC-TOO NUMEROUS TO COUNT
NATC-NOT ABLE TO COUNT

G-PGS Y-PPT
H-NGS P-PPH
M-MGS B-PPB
L-NLS I-MM
U-ULS C-UM

MUTAGENICITY TESTING OF C.I. SOLVENT YELLOW NO. 33
IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE YELLOW
RESEARCH LAB: GB&A ON 06/06/84

08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION

STRAIN: TA102

BACKGROUNDS:

(4) CONTAMINATED

MUTAGENICITY TESTING OF C.I. SOLVENT YELLOW NO. 33
IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE YELLOW
RESEARCH LAB: 688A ON 06/08/84

08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION

STRAIN: TA102

•RLAC27

DANTHRON WAS USED AS THE POSITIVE CONTROL.

-
MITOMYCIN C WAS USED AS THE POSITIVE CONTROL.

STATISTICAL ANALYSIS: MUTAGENICITY OF
C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMCS-84-0002 LAB: G8BA ACTIVATION: + RLA027
STRAIN: TA102 DATE: 06/08/84 TECHNICIAN: MJK

DOSE UNITS PLATE COUNTS			MEAN S.D.	
.00 UCS	257	234	243.33	12.10
30.00* UCS		1176	1192.00	22.63
10.00 UCS	403	441	422.00	26.87
30.00 UCS	538	464	501.00	52.33
50.00 UCS	603	608	605.50	3.54
100.00 UCS	640	665	652.50	17.68
300.00 UCS	678	662	670.00	11.31

ESTS. B(0) 243.447 B(1) 4.0857 B(2) 4853 B(3) 00192

TEST CHI-SQUARE DF P LOGL

POISSON 9.07 7 .2477 -56.3735

ADEQUACY 3.93 2 .1404 -58.3371

TOXICITY 24.06 1 .0000 -70.3655

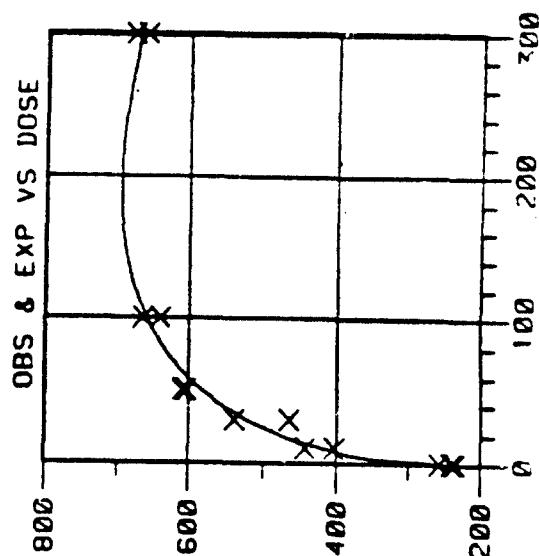
MUTAGENICITY 768.54 2 .0000 -442.0078

AVERAGE SLOPE (NONLIN. MODEL) = 5.559

95% CONF. LIMITS = (4.325, 7.145)

AVERAGE SLOPE (LINEAR REGR.) = 3.867

95% CONF. LIMITS = (2.480, 5.254)

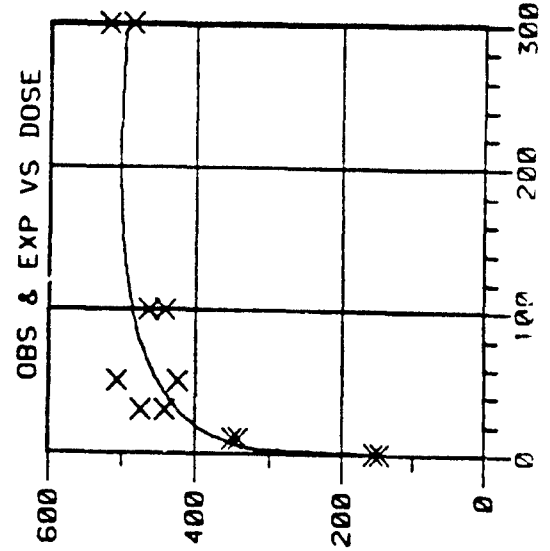


STATISTICAL ANALYSIS: MUTAGENICITY OF
C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0002 LAB: GBBA ACTIVATION: -
STRAIN: TAI02 DATE: 06/08/84 TECHNICIAN: MJK

DOSE UNITS	PLATE COUNTS	MEAN	S.D.
.00 UGS	154	149.50	6.36
.50* UGS	1182	1173.33	7.57
10.00 UGS	341	345.50	6.36
30.00 UGS	474	457.00	24.04
50.00 UGS	506	465.00	57.98
100.00 UGS	441	452.00	15.56
300.00 UGS	485	501.00	22.63

ESTS. 149.142 4.8437 .2383 .00067
B(0) B(1) B(2) B(3)
TEST CHI-SQUARE DF P LOGL
POISSON 10.44 6 .1073 -51.6990
ADEQUACY 13.07 2 .0015 -58.2338
TOXICITY 5.90 1 .0152 -61.1818
MUTAGENICITY 534.73 2 .0000 -325.5995
AVERAGE SLOPE (NONLIN. MODEL) = 1.647
95% CONF. LIMITS = (1.329, 2.042)
AVERAGE SLOPE (LINEAR REGR.) = .667
95% CONF. LIMITS = (.052, 1.283)



**MUTAGENICITY TESTING OF C.I. SOLVENT YELLOW NO. 33
IN SALMONELLA TYPHIMURIUM**

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE YELLOW
RESEARCH LAB: GBHA ON 06/15/84

08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION

STRAIN: TA102

COMPOUND	A C T	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE					MEAN	STD
			A	B	C	D	E		
POS CONTROL									
OTHER POS	RLA027	0.50	1252	1377	1392	1370		1347.75	64.49
	-	30.00	1380	1320	1363			1361.00	35.54
NEG CONTROL									
DIMETHYLSULF	RLA027	100.00U	264	264	266			264.67	1.15
	-	100.00U	202	188	213			201.00	12.53
BMGS-34-0002									
	RLA027	1.00	283	292				287.50	6.36
	RLA027	5.00	317	377				347.00	42.43
	RLA027	10.00	403	341				372.00	43.84
	RLA027	30.00	549	522				535.50	19.09
	RLA027	50.00	698	649				673.50	34.65
	RLA027	100.00	711	707				709.00	2.83
	RLA027	300.00	777	701				739.00	53.74
	-	1.00	217	191				204.00	16.33
	-	5.00	300	280				290.00	14.14
	-	10.00	363	359				371.00	16.97
	-	30.00	417	413				415.00	2.83
	-	50.00	448	443				445.50	3.54
	-	100.00	463	461				462.00	1.41
	-	300.00	435	500				467.50	45.96

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY S-9 : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIX/PLATE : 500UGS

T*-TOXIC
INTC-T00 NUMEROUS TO COUNT
NATC-NOT ABLE TO COUNT

G-PGS T-PPT
N-NGS P-PPM
M-MGS B-PPB
L-NLS I-MM
U-ULS C-UM

STATISTICAL ANALYSIS: MUTAGENICITY OF
C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0002 LAB: CBBA ACTIVATION: + RLA027
STRAIN: TA102 DATE: 06/15/84 TECHNICIAN: MJK

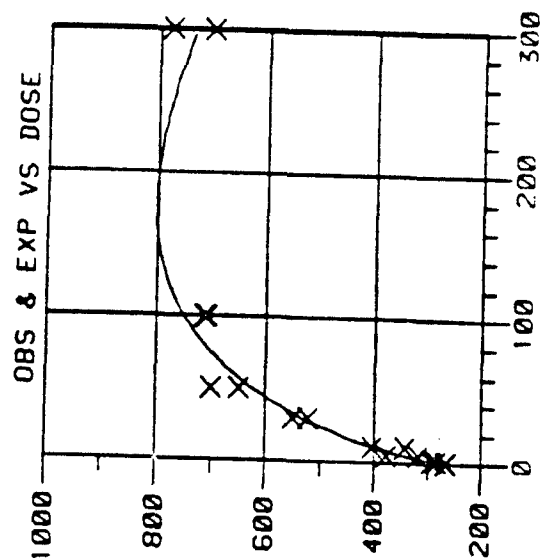
DOSE UNITS PLATE COUNTS			MEAN	S.D.
.00	UGS	264 264 266	264.67	1.16
.50*	UGS	1252 1377 1392 1370	1347.75	64.49
1.00	UGS	283 292	287.50	6.36
5.00	UGS	317 377	347.00	42.43
10.00	UGS	403 341	372.00	43.84
30.00	UGS	549 522	535.50	19.09
50.00	UGS	698 649	673.50	34.65
100.00	UGS	711 707	709.00	2.83
300.00	UGS	777 701	739.00	53.74

ESTS. 261.427 3.3232 7267 .00337
B(0) B(1) B(2) B(3)

TEST CHI-SQUARE DF P LOGL
POISSON 16.89 9 .0505 -75.8276
ADEQUACY 15.48 4 .0038 -83.5691
TOXICITY 100.28 1 .0000 -133.7075
MUTAGENICITY 1277.73 2 .0000 -722.4327

AVERAGE SLOPE (NONLIN. MODEL) = 5.838
95% CONF. LIMITS = (4.328, 7.873)

AVERAGE SLOPE (LINEAR REGR.) = 1.490
95% CONF. LIMITS = (.797, 2.184)

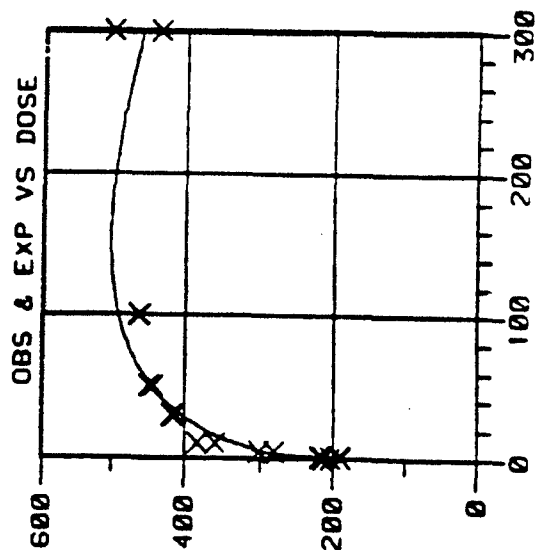


STATISTICAL ANALYSIS: MUTAGENICITY OF
C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0002 LAB: GBBA ACTIVATION: -
STRAIN: TA102 DATE: 06/15/84 TECHNICIAN: MJK

DOSE UNITS	PLATE COUNTS	MEAN	S.D.
00	UGS 202 188 213	201.00	12.53
30.00*	UGS 1380 1320 1383	1361.00	35.54
1.00	UGS 217 191	204.00	18.38
5.00	UGS 300 280	290.00	14.14
10.00	UGS 383 359	371.00	16.97
30.00	UGS 417 413	415.00	2.83
50.00	UGS 448 443	445.50	3.54
100.00	UGS 463 461	462.00	1.41
300.00	UGS 435 500	467.50	45.96

ESTS. 192.567 3.9137 .4608 .00219
B(0) B(1) B(2) B(3)
TEST CHI-SQUARE DF P LOGL
POISSON 9.26 9 .4140 -69.5208
ADEQUACY 27.87 4 .0000 -83.4568
TOXICITY 50.29 1 .0000 -108.6019
MUTAGENICITY 590.24 2 .0000 -378.5747
AVERAGE SLOPE (NONLIN. MODEL) = 2.312
95% CONF. LIMITS = (1.888, 2.830)
AVERAGE SLOPE (LINEAR REGR.) = .724
95% CONF. LIMITS = (.258, 1.191)



MUTAGENICITY TESTING OF C.I. SOLVENT YELLOW NO. 33
IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
 OF ARMY DYE YELLOW
 RESEARCH LAB: GBBA ON 06/01/84

08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION

STRAIN: TA104

COMPOUND	A C T	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE					MEAN	STD
			A	B	C	D	E		
POS CONTROL									
2-AA	HLA027	3.00	2393	2440	2407			2433.33	37.45
OTHER POS	-	17.00	705	851	777			797.67	46.52
NEG CONTROL									
DIMETHYLSULF	HLA027	100.000	312	311	340			321.00	16.46
	-	100.000	262	323	271			285.33	32.93
BMGS-84-0002									
	HLA027	10.00	485	467				476.00	12.73
	HLA027	30.00	466	491				478.50	17.68
	HLA027	50.00	483	465				474.00	12.73
	HLA027	100.00	474	492				483.00	12.73
	HLA027	300.00	341	340				343.50	3.54
	-	10.00	343	303				323.00	26.28
	-	30.00	290	295				292.50	3.54
	-	50.00	305	325				315.00	14.14
	-	100.00	323	319				321.00	2.63
	-	300.00	293	319				306.00	15.39

PHENOCOPY CHECK : TRUE MUTANTS
 STERILITY S-Y : NOT CONTAMINATED
 SAMPLE STERILITY: NOT CONTAMINATED
 ACT MIX/PLATE : 500UGS

T*-TOXIC
 TNC-TCO NUMEROUS TO COUNT
 NATC-NCT ABLE TO COUNT

G-PGS T-PPT
 N-NGS P-PPM
 M-MGS B-PPB
 L-NLS I-NNM
 U-ULS C-UM

MUTAGENICITY TESTING OF C.I. SOLVENT YELLOW NO. 33
IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE YELLOW
RESEARCH LAB: GBBA ON 06/01/84

08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION

STRAIN: TA104

POSITIVE CONTROL USED WAS METHYL GLYOXAL.

STATISTICAL ANALYSIS: MUTAGENICITY OF
C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0002 LAB: GBBA ACTIVATION: + RLA027
STRAIN: TA104 DATE: 06/01/84 TECHNICIAN: MJK

DOSE UNITS	PLATE COUNTS	MEAN	S.D.
00	UGS 312 311 340	321.00	16.46
3.00*	UGS 2393 2440 2467	2433.33	37.45
10.00	UGS 485 467	476.00	12.73
30.00	UGS 466 491	478.50	17.68
50.00	UGS 483 455	474.00	12.73
100.00	UGS 474 492	483.00	12.73
300.00	UGS 341 346	343.50	3.54

B(0) B(1) B(2)
ESTS. 329.883 3.5885 .4055

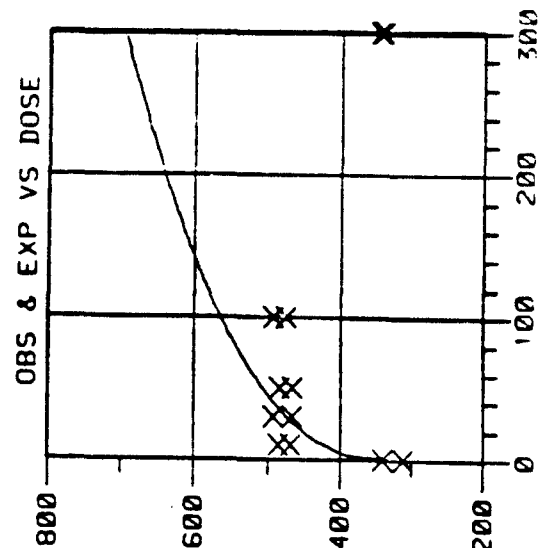
NO EVIDENCE OF TOXICITY

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	3.40	7	.8462	-52.8098
ADEQUACY	483.88	3	.0000	-294.7511
MUTAGENICITY	.01	2	.9950	-136.4160

AVERAGE SLOPE (NONLIN. MODEL) = 3.535

AVERAGE SLOPE (LINEAR REGR.) = 2.715
95% CONF. LIMITS = (.694, 4.735)

WARNING: 4 PARAMETER MODEL DID NOT CONVERGE
WARNING: 3 PARAMETER MODEL DID NOT CONVERGE

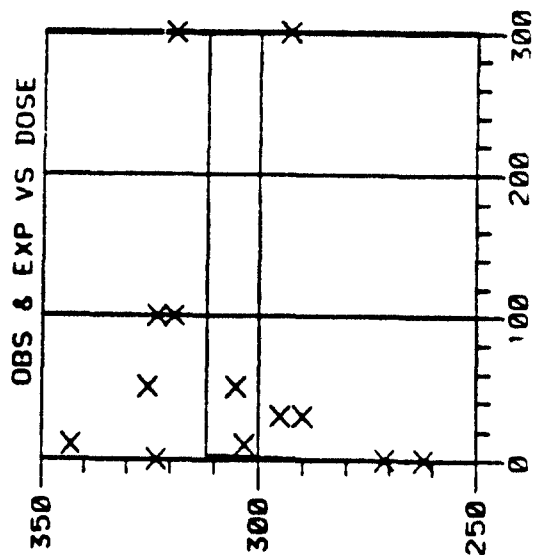


STATISTICAL ANALYSIS: MUTAGENICITY OF
C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0002 LAB: CBBA ACTIVATION: -
STRAIN: TA104 DATE: 06/01/84 TECHNICIAN: MJK

DOSE UNITS	PLATE COUNTS	MEAN	S.D.
.00	UCS 262 323 271	285.33	32.93
17.00*	UCS 765 851 777	797.67	46.58
10.00	UCS 343 303	323.00	28.28
30.00	UCS 290 295	292.50	3.54
50.00	UCS 305 325	315.00	14.14
100.00	UCS 323 319	321.00	2.83
300.00	UCS 293 319	306.00	18.38

ESTS. 285.333 3.2723 0000
B(0) B(1) B(2)
NO EVIDENCE OF TOXICITY
TEST CHI-SQUARE DF P LOCL
POISSON 11.88 7 1044 -54.9980
ADEQUACY 4.05 3 .2559 -57.0243
MUTAGENICITY 5.25 2 .0723 -59.6507
AVERAGE SLOPE (NONLIN. MODEL) = .527
95% CONF. LIMITS = (.267, 1.042)
AVERAGE SLOPE (LINEAR REGR.) = .344
95% CONF. LIMITS = (-.574, 1.262)



MUTAGENICITY TESTING OF C.I. SOLVENT YELLOW NO. 33
IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE YELLOW

RESEARCH LAB: GBBA ON 06/02/84

08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION

STRAIN: TA104

COMPOUND	A C T	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE					MEAN	STD
			A	B	C	D	E		
POS CONTROL									
2-AA	HLAC27	3.00	2053	2002	2086			2047.00	42.32
OTHER POS	-	50.00	1810	1715	1780			1769.33	46.92
NEG CONTROL									
DIMETHYLSULF	HLAC27	100.000	363	402	375			380.00	19.97
	-	100.000	277	266	279			274.00	7.00
BMGS-34-0002									
	HLAC27	1.00	329	301				315.00	19.80
	HLAC27	3.00	361	358				359.50	2.12
	HLAC27	10.00	442	483				462.50	28.99
	HLAC27	30.00	533	502				517.50	21.92
	HLAC27	50.00	500	536				518.00	25.46
	HLAC27	100.00	476	500				488.00	16.97
	HLAC27	300.00	452	451				451.50	0.71
	-	1.00	258	281				269.50	16.26
	-	3.00	317	271				294.00	32.53
	-	10.00	324	314				319.00	7.07
	-	30.00	324	317				320.50	4.95
	-	50.00	347	299				323.00	33.94
	-	100.00	343	326				334.50	12.02
	-	300.00	336	340				338.00	2.83

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY S-9 : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIA/PLATE : 500UGS

T--TOXIC
INTC-TOO NUMEROUS TO COUNT
NATC-NOT ABLE TO COUNT

G-PGS T-PPT
N-NGS P-PPM
M-NGS B-PPB
L-NLS I-MM
U-ULS C-UM

MUTAGENICITY TESTING OF C.I. SOLVENT YELLOW NO. 33
IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE YELLOW
RESEARCH LAB: GB9A ON 06/08/84
TEST TYPE: STANDARD PLATE INCORPORATION

08/27/84

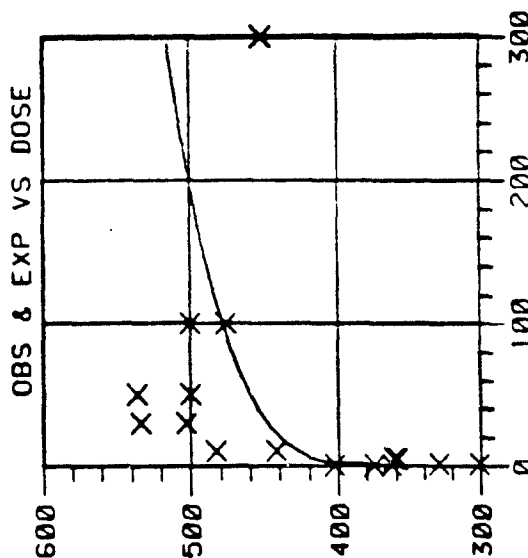
STRAIN: TA104

METHYL GLYOXAL WAS USED AS A POSITIVE CONTROL.

STATISTICAL ANALYSIS: MUTAGENICITY OF
C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0002 LAB: CBBA ACTIVATION: + RL0027
STRAIN: TA104 DATE: 06/08/84 TECHNICIAN: MJK

DOSE UNITS	PLATE COUNTS	MEAN	S.D.
00	UCS 363 402 375	380.00	19.97
3.00*	UCS 2053 2002 2086	2047.00	42.32
1.00	UCS 329 301	315.00	19.80
5.00	UCS 361 358	359.50	2.12
10.00	UCS 442 483	462.50	28.09
30.00	UCS 533 502	517.50	21.92
50.00	UCS 500 536	518.00	25.46
100.00	UCS 476 500	488.00	16.97
300.00	UCS 452 451	451.50	.71



ESTS. 360 889 3.6116 .2501

NO EVIDENCE OF TOXICITY

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	7.94	9	.5397	-71.0742
ADEQUACY	114.24	5	.0000	-128.1922
MUTAGENICITY	84.98	2	.0000	-170.6841

AVERAGE SLOPE (NONLIN. MODEL) = 2.890
95% CONF. LIMITS = (2.367, 3.527)

AVERAGE SLOPE (LINEAR REGR.) = 5.704
95% CONF. LIMITS = (3.429, 7.978)

WARNING: 4 PARAMETER MODEL DID NOT CONVERGE

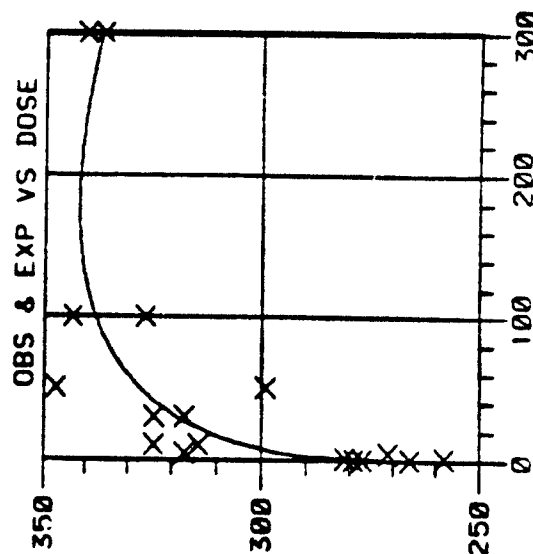
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STATISTICAL ANALYSIS: MUTAGENICITY OF
C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0002 LAB: CB8A ACTIVATION: -
 STRAIN: TA104 DATE: 06/08/84 TECHNICIAN: MJK

DOSE UNITS PLATE COUNTS				MEAN S.D.	
.00	UCS	277	266	279	7.00
50.00*	UCS	1810	1718	1780	1769.33
1.00	UCS	258	281		46.92
5.00	UCS	317	271		269.50
10.00	UCS	324	314		16.26
30.00	UCS	324	317		294.00
50.00	UCS	347	299		32.53
100.00	UCS	343	326		319.00
300.00	UCS	336	340		7.07
					320.50
					4.95
					323.00
					33.94
					334.50
					12.02
					338.00
					2.83

ESTS. 270.935 2.5639 .4248 .00072
 TEST CHI-SQUARE DF P LOGL
 POISSON 9.19 9 .4196 -68.8684
 ADEQUACY 3.37 4 .4978 -70.5536
 TOXICITY 2.19 1 .1385 -71.6508
 MUTAGENICITY 33.03 2 .0000 -87.0686
 AVERAGE SLOPE (NONLIN. MODEL) = 3.453
 95% CONF. LIMITS = (.908, 13.129)
 AVERAGE SLOPE (LINEAR REGR.) = 4.781
 95% CONF. LIMITS = (2.220, 7.333)



MUTAGENICITY TESTING OF C.I. SOLVENT YELLOW NO. 33
IN SALMONELLA TYPHIMURIUM

VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF AR-1 DYE YELLOW
RESEARCH LAB: GBSA ON 06/01/84

08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION

STRAIN: TA1535

COMPOUND	A C T	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE					MEAN	STD
			A	B	C	D	E		
POS CONTROL									
NAAZIDE	-	3.00	1021	1031	939			1003.67	39.00
2-AA	RLAC27	3.00	153	164	132			149.67	16.26
NEG CONTROL									
DIMETHYLSULF	RLAC27	100.000	26	24	29			26.33	2.52
	-	100.000	37	44	33			38.00	5.57
BMGS-34-0002									
	RLAC27	10.00	27	28				27.50	0.71
	RLAC27	30.00	18	25				21.50	4.95
	RLAC27	50.00	17	21				19.00	2.83
	RLAC27	100.00	19	15				17.00	2.83
	RLAC27	300.00	25	10				21.50	4.95
	-	10.00	48	30				42.00	8.49
	-	30.00	31	26				29.50	2.12
	-	50.00	24	32				28.00	5.66
	-	100.00	36	30				36.00	0.00
	-	300.00	55	37				46.00	12.73

PHENOCOPI CHECK : TRUE MUTANTS
STERILITY S-9 : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIX/PLATE : 500UGS

T*-TOXIC
TNTC-TOO NUMEROUS TO COUNT
NATC-NOT ABLE TO COUNT

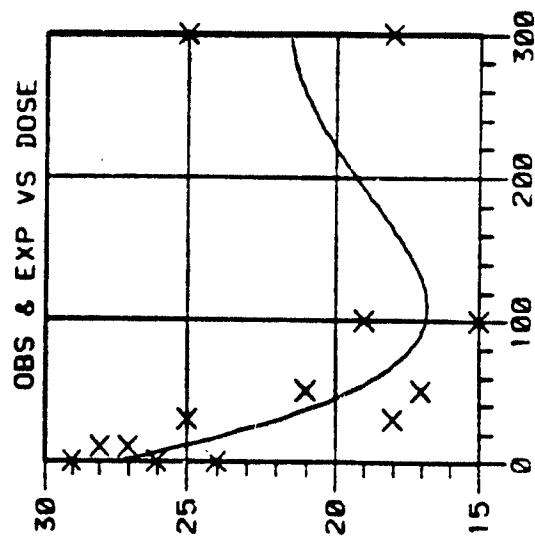
G-PGS T-PPT
N-RGS P-PPH
M-RGS E-PPB
L-NLS I-MM
U-ULS C-UM

STATISTICAL ANALYSIS: MUTAGENICITY OF
C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0002 LAB: G8BA ACTIVATION: + RL027
STRAIN: TA1535 DATE: 06/01/84 TECHNICIAN: MJK

DOSE UNITS PLATE COUNTS			MEAN S.D.	
.00	UGS	26 24 29	26.33	2.52
3.00*	UGS	153 164 132	149.67	16.26
10.00	UGS	27 28	27.50	.71
30.00	UGS	18 25	21.50	4.95
50.00	UGS	17 21	19.00	2.83
100.00	UGS	19 15	17.00	2.83
300.00	UGS	25 18	21.50	4.95

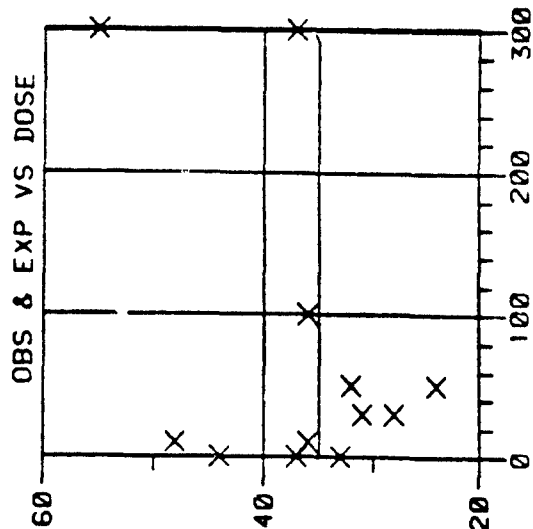
ESTS. 27.364 -10.4407 2.7614 00789
TEST CHI-SQUARE DF P LOGL
POISSON 3.67 7 .8169 -33.9265
ADEQUACY .52 2 .7705 -34.1873
TOXICITY 7.68 1 .0056 -38.0263
MUTAGENICITY 6.33 2 .0422 -37.3520
AVERAGE SLOPE (NONLIN. MODEL) = .674
95% CONF. LIMITS = (.001, 823.050)
AVERAGE SLOPE (LINEAR REGR.) = -.015
95% CONF. LIMITS = (-.040, .010)



STATISTICAL ANALYSIS: MUTAGENICITY OF
C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0002 LAB: GBBA ACTIVATION: -
STRAIN: TA1535 DATE: 06/01/84 TECHNICIAN: MJK

DOSE UNITS	PLATE COUNTS	MEAN	S.D.
0.00 UGS	37 44 33	38.00	5.57
3.00* UGS	1021 1031 959	1003.67	30.00
10.00 UGS	48 36	42.00	8.49
30.00 UGS	31 28	29.50	2.12
50.00 UGS	24 32	28.00	5.66
100.00 UGS	36 36	36.00	.00
300.00 UGS	55 37	46.00	12.73



B(0) B(1) B(2)
ESTS. 35.013*****322.3196

NO EVIDENCE OF TOXICITY

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	8.16	7	.3184	-39.3381
ADEQUACY	8.27	3	.0407	-43.4740
MUTAGENICITY	5.24	2	.0728	-46.0935

AVERAGE SLOPE (NONLIN. MODEL) = .037
95% CONF. LIMITS = (.013, .099)

AVERAGE SLOPE (LINEAR REGR.) = .032
95% CONF. LIMITS = (.011, .075)

**MUTAGENICITY TESTING OF C.I. SOLVENT YELLOW NO. 33
IN SALMONELLA TYPHIMURIUM**

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE YELLOW
RESEARCH LAB: GBBA ON 06/08/84

08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION

STRAIN: TA1535

COMPOUND	A C T	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE					MEAN	STD
			A	B	C	D	E		
POS CONTROL									
NAAZIDE	-	3.00	1003	979	1014			998.67	17.90
2-AA	RLA027	3.00	89	103	109			100.33	10.26
NEG CONTROL									
DIMETHYLSULF	RLA027	100.00	25	24	18			22.33	3.79
	-	100.00	43	26	48			32.33	9.29
BMGS-34-0002									
	RLA027	10.00	20	20				20.00	0.00
	RLA027	30.00	9	14				13.50	6.39
	RLA027	50.00	15	14				14.50	0.71
	RLA027	100.00	20	17				18.50	2.12
	RLA027	300.00	10	16				13.00	4.24
	-	10.00	25	31				28.00	4.24
	-	30.00	32	36				34.00	2.83
	-	50.00	36	33				34.50	2.12
	-	100.00	41	50				45.50	5.36
	-	300.00	29	34				31.50	3.54

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY S-S : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIX/PLATE : 500UGS

T--TOXIC
INTC-TOO NUMEROUS TO COUNT
NATC-NOT ABLE TO COUNT

G-PGS T-PPT
N-NGS P-PPM
M-MGS B-PPB
L-NLS :-MM
U-LLS C-UM

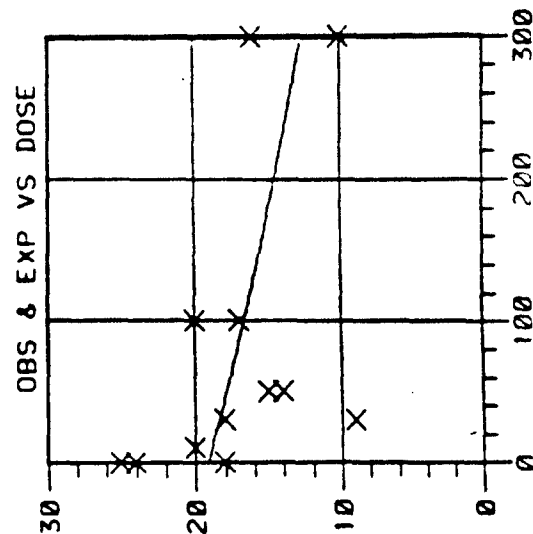
STATISTICAL ANALYSIS: MUTAGENICITY OF
C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0002 LAB: CBBA ACTIVATION: + RLA027
STRAIN: TA1535 DATE: 06/08/84 TECHNICIAN: HJK

DOSE UNITS	PLATE COUNTS	MEAN	S.D.
.00 UGS	25 24 18	22.33	3.70
3.00* UGS	89 103 109	100.33	10.26
10.00 UGS	20 20	20.00	.00
30.00 UGS	9 18	13.50	6.36
50.00 UGS	15 14	14.50	.71
100.00 UGS	20 17	18.50	2.12
300.00 UGS	10 16	13.00	4.24

A-94

ESTS. 19.124*****
TEST CHI-SQUARE DF P LOGL
POISSON 5.95 7 .5461 -33.3539
ADEQUACY 6.25 2 .0440 -36.4774
TOXICITY 3.84 1 .0500 -38.3974
MUTAGENICITY .00 21.0000 -36.4774
AVERAGE SLOPE (NONLIN. MODEL) = .000
95% CONF. LIMITS = (.000, .000)
AVERAGE SLOPE (LINEAR REG.) = -.040
95% CONF. LIMITS = (-.116, .037)



STATISTICAL ANALYSIS: MUTAGENICITY OF
C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

SAMPLE ID, BMGS-84-0002 LAB, CBBA ACTIVATION, -
STRAIN, TA1535 DATE, 06/08/84 TECHNICIAN, MJK

DOSE UNITS	PLATE COUNTS	MEAN	S.D.
00	UGS 43 26 28	32.33	9.29
3.00*	UGS 1003 979 1014	998.67	17.90
10.00	UGS 25 31	28.00	4.24
30.00	UGS 32 36	34.00	2.83
50.00	UGS 36 33	34.50	2.12
100.00	UGS 41 50	45.50	6.36
300.00	UGS 29 34	31.50	3.54

ESTS. 31.624 -3.4714 1.7390 .01016

TEST CHI-SQUARE DF P LOGL

POISSON 7.64 7 .3658 -38.5172

ADEQUACY .75 2 .6858 -38.8944

TOXICITY 8.15 1 .0043 -42.9689

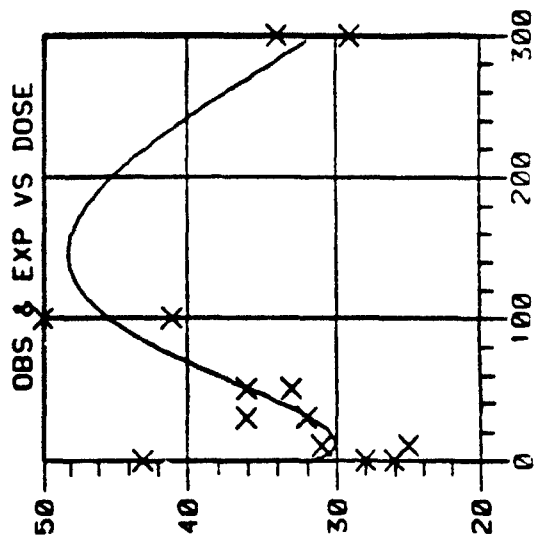
MUTAGENICITY 9.32 2 .0095 -43.5539

AVERAGE SLOPE (NONLIN. MODEL) = .934

95% CONF. LIMITS = (.187, 4.659)

AVERAGE SLOPE (LINEAR REGR.) = .143

95% CONF. LIMITS = (.045, .241)



MUTAGENICITY TESTING OF C.I. SOLVENT YELLOW NO. 33
IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE YELLOW

RESEARCH LAB: G8dA GN 06/01/84

08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION

STRAIN: TA1537

COMPOUND	A C T	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE					MEAN	STD
			A	B	C	D	E		
POS CONTROL									
9-AA	-	100.00	1129	1111	820			1040.00	132.86
2-AA	RLA027	3.00	304	380	343			342.33	32.00
NEG CONTROL									
DIMETHYLSULF	RLA027	100.000	17	15	27			19.67	6.43
	-	100.000	14	10	9			13.33	5.03
BMGS-84-0002									
	RLA027	10.00	19	29				23.50	7.78
	RLA027	30.00	25	25				25.00	0.00
	RLA027	50.00	22	33				30.50	3.54
	RLA027	100.00	32	25				28.50	4.95
	-	10.00	15	12				13.50	2.12
	-	30.00	13	13				13.00	0.00
	-	50.00	13	10				17.00	1.~1
	-	100.00	25	17				21.00	5.66
	-	300.00	25	23				24.00	1.41

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY S-7 : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIA/PLATE : 500UGS

T*-TOXIC
TNTC-TWO NUMEROUS TO COUNT
NATC-NOT ABLE TO COUNT

G-PGS T-PPT
N-RGS P-PPM
M-RGS B-PPB
L-NLS I-MM
U-ULS C-UM

STATISTICAL ANALYSIS: MUTAGENICITY OF
C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0002 LAB: CBBA ACTIVATION: + RLA027
 STRAIN: TA1537 DATE: 06/01/84 TECHNICIAN: MJK

DOSE UNITS PLATE COUNTS			MEAN S.D.	
.00	UGS	17 15 27	19 67	6 43
3.00*	UGS	304 380 343	342.33	38.00
10.00	UGS	18 29	23.50	7.78
30.00	UGS	25 25	25.00	.00
50.00	UGS	28 33	30.50	3.54
100.00	UGS	32 25	28.50	4.95

B(0) B(1) B(2)

 ESTS. 19.603 6530 3628

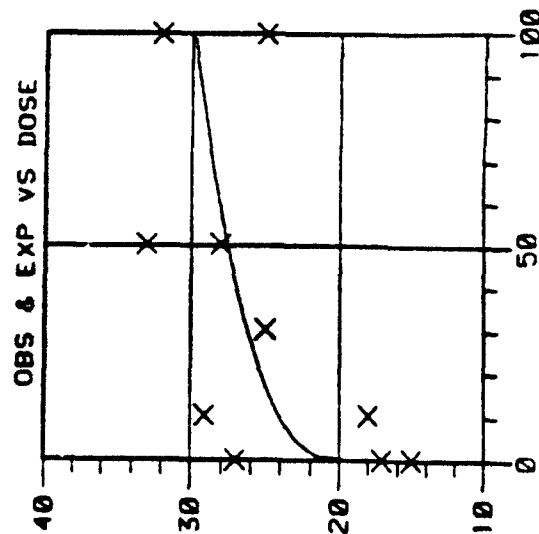
NO EVIDENCE OF TOXICITY

TEST CHI-SQUARE DF P LOGL

 POISSON 8.05 6 .2347 -31.6221
 ADEQUACY .87 2 .6484 -32.0554
 MUTAGENICITY 6.20 2 .0451 -35.1536

AVERAGE SLOPE (NONLIN. MODEL) = .159
 95% CONF. LIMITS = (.064, .397)

AVERAGE SLOPE (LINEAR REGR.) = .200
 95% CONF. LIMITS = (.035, .365)



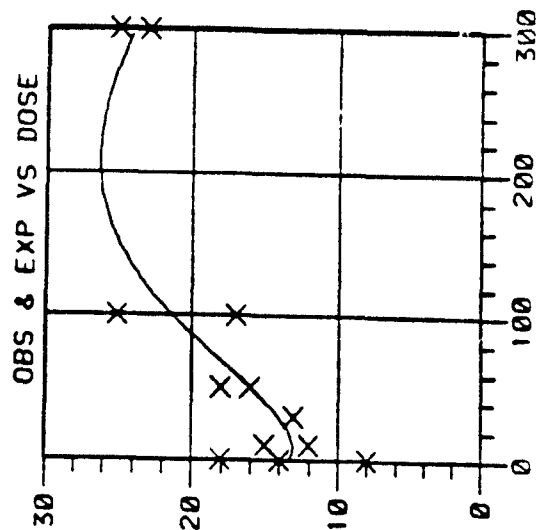
STATISTICAL ANALYSIS: MUTAGENICITY OF
C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMCS-84-0002 LAB: CBBA ACTIVATION: -
STRAIN: TA1537 DATE: 06/01/84 TECHNICIAN: MJR

DOSE UNITS	PLATE COUNTS	MEAN	S.D.
00 UGS	14 18 8	13.33	5.03
100.00* UGS	1129 1111 880	1040.00	138.86
10.00 UGS	15 12	13.50	2.12
30.00 UGS	13 13	13.00	.00
50.00 UGS	18 16	17.00	1.41
100.00 UGS	25 17	21.00	5.66
300.00 UGS	25 23	24.00	1.41

A-98

ESTS. B(0) 13.378 B(1) -4.2615 B(2) 1.6609 B(3) .00702
TEST CHI-SQUARE DF P LOGL
POISSON 5.86 7 .5564 -33.0506
ADEQUACY .33 2 .8486 -33.2147
TOXICITY 1.26 1 .2615 -33.8451
MUTAGENICITY 13.06 2 .0015 -39.7471
AVERAGE SLOPE (NONLIN. MODEL) = .296
95% CONF. LIMITS = (.009, 9.754)
AVERAGE SLOPE (LINEAR REGR.) = .080
95% CONF. LIMITS = (.024, .137)



MUTAGENICITY TESTING OF C.I. SOLVENT YELLOW NO. 33
IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
 OF ARMY DYE YELLOW
 RESEARCH LAB: G88A ON 06/06/84

08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION

STRAIN: TA1537

COMPOUND	A C T	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE					MEAN	STD
			A	B	C	D	E		
POS CONTROL									
9-AA	-	100.00	512	605	453			523.33	76.63
2-AA	RLA027	3.00	314	316	311			313.67	2.52
NEG CONTROL									
DIMETHYLSULF	RLA027	100.000	20	16	16			16.00	2.00
	-	100.000	12	7	17			12.00	5.00
BMGS-34-0002									
	RLA027	10.00	26	17				21.50	6.36
	RLA027	30.00	27	33				30.00	4.24
	RLA027	50.00	36	40				38.00	2.83
	RLA027	100.00	24	31				27.50	4.95
	RLA027	300.00	31	26				28.50	3.54
	-	10.00	12	12				12.00	0.00
	-	30.00	29	19				24.00	7.07
	-	50.00	28	26				27.00	1.41
	-	100.00	13	19				18.50	0.71
	-	300.00	20	15				17.50	3.54

PHENOCOPY CHECK : TRUE MUTANTS
 STERILITY S-9 : NOT CONTAMINATED
 SAMPLE STERILITY: NOT CONTAMINATED
 ACT MIX/PLATE : 500UGS

T=TOXIC
 INTC-TWO NUMEROUS TC COUNT
 NATC-NOT ABLE TO COUNT

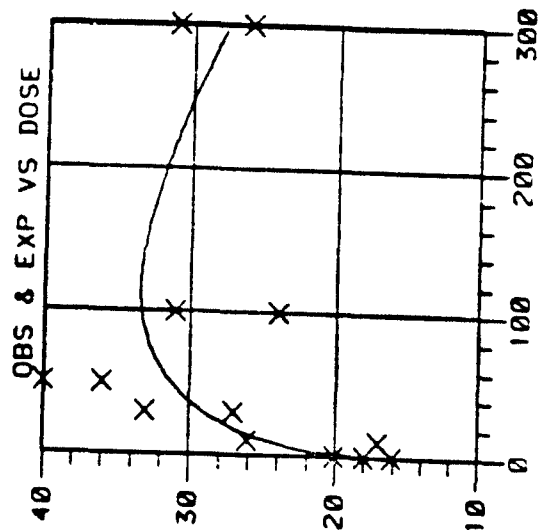
G-PGS T-PPT
 N-NGS P-PPM
 M-MGS B-PPB
 L-NLS I-MM
 U-ULS C-UM

STATISTICAL ANALYSIS: MUTAGENICITY OF
C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMCS-84-0002 LAB: CBBA ACTIVATION: + RLA027
STRAIN: TA1537 DATE: 06/08/84 TECHNICIAN: MJK

DOSE UNITS PLATE COUNTS				MEAN S.D.	
00	UGS	20	18	18.00	2.00
3.00*	UGS	314	311	313.67	2.52
10.00	UGS	26	17	21.50	6.36
30.00	UGS	27	33	30.00	4.24
50.00	UGS	36	40	38.00	2.83
100.00	UGS	24	31	27.50	4.95
300.00	UGS	31	26	28.50	3.54

ESTS.	B(0)	B(1)	B(2)	B(3)
	17.685	7996	5412	.00292
TEST	CHI-SQUARE DF P			
POISSON	4.47	7	.7245	LOCL
ADEQUACY	5.86	2	.0535	
TOXICITY	2.93	1	.0869	
MUTAGENICITY	15.94	2	.0003	
AVERAGE SLOPE (NONLIN. MODEL) = .370				
95% CONF. LIMITS = (.164, .833)				
AVERAGE SLOPE (LINEAR REGR.) = .403				
95% CONF. LIMITS = (.291, .515)				

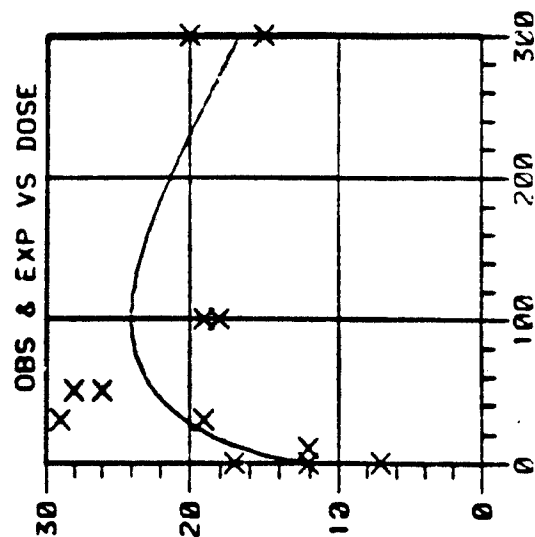


STATISTICAL ANALYSIS: MUTAGENICITY OF
C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0002 LAB: CBBA ACTIVATION: -
 STRAIN: TA1537 DATE: 06/08/84 TECHNICIAN: MJK

DOSE UNITS PLATE COUNTS			MEAN	S.D.
00	UCS	12	7	17
100.00*	UCS	512	605	453
10.00	UCS	12	12	12
30.00	UCS	29	19	19
50.00	UCS	28	26	26
100.00	UCS	18	19	19
300.00	UCS	20	15	15
			12.00	5.00
			523.33	76.63
			12.00	00
			24.00	7.07
			27.00	1.41
			18.50	.71
			17.50	3.54

ESTS.	11.475	B(1)	2026	B(2)	6690	B(3)	00464
TEST	CHI-SQUARE	DF	P	LOGL			
POISSON	7.07	7	.4221				
ADEQUACY	8.50	2	.0143				
TOXICITY	5.42	1	.0199				
MUTAGENICITY	15.40	2	.0005				
AVERAGE SLOPE (NONLIN. MODEL) =							
95% CONF. LIMITS =							
AVERAGE SLOPE (LINEAR REGR.) =							
95% CONF. LIMITS =							



**MUTAGENICITY TESTING OF C.I. SOLVENT YELLOW NO. 33
IN SALMONELLA TYPHIMURIUM**

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE YELLOW
RESEARCH LAB: GBBA CN G6/Q1/84

08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION

STRAIN: TA1538

COMPOUND	A C T	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE					MEAN	STD
			A	B	C	D	E		
FOS CONTROL									
2-NF	-	5.00	476	432	512			473.33	40.07
2-AA	RLAC27	5.50	704	729	692			708.33	16.88
NEG CONTROL									
DIMETHYLSULF	RLAC27	100.000	26	25	24			25.00	1.00
	-	100.000	17	13	18			16.00	2.65
BMGS-84-0002									
	RLAC27	10.00	44	36				40.00	5.66
	RLAC27	30.00	28	27				28.50	0.71
	RLAC27	50.00	31	27				29.00	2.83
	RLAC27	100.00	36	29				32.50	4.95
	RLAC27	300.00	28	33				33.00	7.07
	-	10.00	28	21				24.50	4.95
	-	30.00	13	2				10.50	3.54
	-	50.00	15	21				18.00	4.24
	-	100.00	18	17				17.50	0.71
	-	300.00	19	29				24.00	7.07

PHENOCOPIY CHECK : TRUE MUTANTS
STERILITY S-Y : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIX/PLATE : SCOUTS

T--TOXIC
TNTC-TOO NUMEROUS TO COUNT
NATC-NOT ABLE TO COUNT

G-PGS T-PPT
H-PGS P-PPH
M-PGS B-PPB
L-NLS I-EM
U-ULS C-UM

STATISTICAL ANALYSIS: MUTAGENICITY OF
C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0002 LAB: CBBA ACTIVATION: + RLA027
STRAIN: TA1538 DATE: 06/01/84 TECHNICIAN: MJK

DOSE UNITS	PLATE COUNTS	MEAN	S.D.
00 UGS	26 25 24	25.00	1.00
50* UGS	704 729 692	708.33	18.88
100 UGS	44 36	40.00	5.66
300 UGS	28 29	28.50	.71
500 UGS	31 27	29.00	2.83
1000 UGS	36 29	32.50	4.95
3000 UGS	28 38	33.00	7.07

ESTS. 25.000 2.4592 .0000

NO EVIDENCE OF TOXICITY

TEST	CHI-SQUARE	DF	P	LOG L
POISSON	3.44	7	.8413	-35.8944
ADEQUACY	9.79	3	.0204	-40.7917
MUTAGENICITY	.01	2	.9950	-40.6844

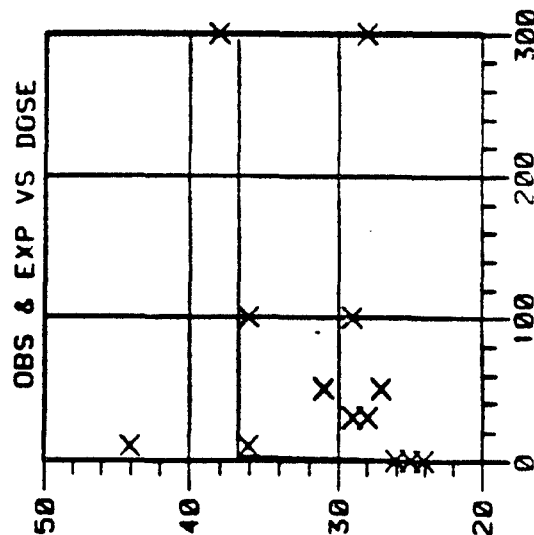
AVERAGE SLOPE (NONLIN. MODEL) = .117

AVERAGE SLOPE (LINEAR REGR.) = .024

95% CONF. LIMITS = (-.082, .130)

WARNING: 4 PARAMETER MODEL DID NOT CONVERGE

WARNING: 3 PARAMETER MODEL DID NOT CONVERGE



STATISTICAL ANALYSIS: MUTAGENICITY OF
C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0002 LAB: GBBA ACTIVATION: -
STRAIN: TA1538 DATE: 06/01/84 TECHNICIAN: MJK

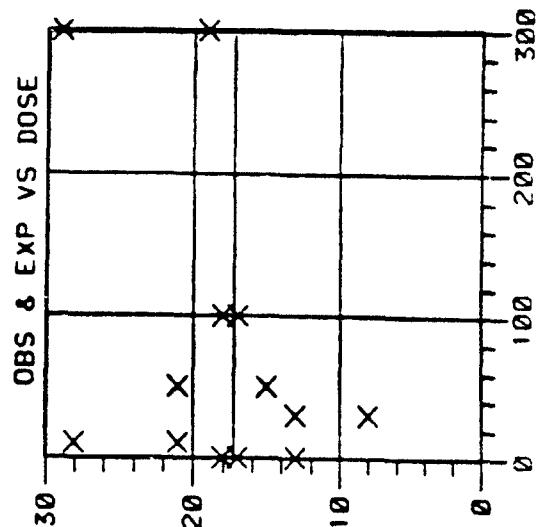
DOSE UNITS			PLATE COUNTS		MEAN		S.D.	
.00	UCS	17	13	18	16.00	2.65		
3.00*	UCS	476	432	512	473.33	40.07		
10.00	UCS	28	21		24.50	4.95		
30.00	UCS	13	8		10.50	3.54		
50.00	UCS	15	21		18.00	4.24		
100.00	UCS	18	17		17.50	.71		
300.00	UCS	19	29		24.00	7.07		

B(0) B(1) B(2)
ESTS. 17.187*****

NO EVIDENCE OF TOXICITY

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	6.18	7	.5192	-33.6811
ADEQUACY	11.88	3	.0078	-39.6216
MUTAGENICITY	3.99	2	.1357	-41.6189

AVERAGE SLOPE (NONLIN. MODEL) = .023
AVERAGE SLOPE (LINEAR REGR.) = .022
95% CONF. LIMITS = (-.008, .052)
WARNING: 3 PARAMETER MODEL DID NOT CONVERGE



**MUTAGENICITY TESTING OF C.I. SOLVENT YELLOW NO. 33
IN SALMONELLA TYPHIMURIUM**

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE YELLOW
RESEARCH LAB: GBRA ON 06/06/84

08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION

STRAIN: TA1538

COMPOUND	A C T	UGS PER PLATE	MISTIDINE REVERTANTS PER PLATE					MEAN	STD
			A	B	C	D	E		
POS CONTROL									
2-NF	-	5.00	502	604	528			544.67	53.00
2-AA	RLA027	0.50	816	807	828			817.00	10.54
NEG CONTROL									
DIMETHYLSULF	RLA027	100.000	60	62	73			71.67	9.07
	-	100.000	21	12	19			19.33	1.53
6PGS-84-0002									
	RLA027	10.00	31	31				31.00	0.00
	RLA027	30.00	37	27				32.00	7.07
	RLA027	50.00	36	39				38.50	3.71
	RLA027	100.00	37	28				32.50	6.36
	RLA027	300.00	25	26				28.00	0.00
	-	10.00	8	13				10.50	3.54
	-	30.00	9	14				11.50	3.54
	-	50.00	14	27				21.50	10.61
	-	100.00	17	19				18.00	1.41
	-	300.00	12	12				15.00	4.24

PHENOCOPI CHECK : TRUE MUTANTS
STERILITY S-V : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT PIX/PLATE : 500UGS

T*-TOXIC
TNTC-TOO NUMEROUS TO COUNT
NATC-NOT ABLE TO COUNT

G-PGS T-PPT
N-NGS B-PPH
M-MGS B-PPB
L-NLS I-MM
U-ULS C-UM

MUTAGENICITY TESTING OF C.I. SOLVENT YELLOW NO. 33
IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE YELLOW
RESEARCH LAB: GBBA ON 06/08/64
TEST TYPE: STANDARD PLATE INCORPORATION

08/27/84

STRAIN: TA1538

•RLAC27

SPONTANEOUS COUNT FOR 1538 IS HIGH. SMALL COLONIES ON THE PLATE ACCOUNTED FOR THE HIGH COUNT. SMALL COLONIES WERE SALMONELLA.

STATISTICAL ANALYSIS: MUTAGENICITY OF
C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

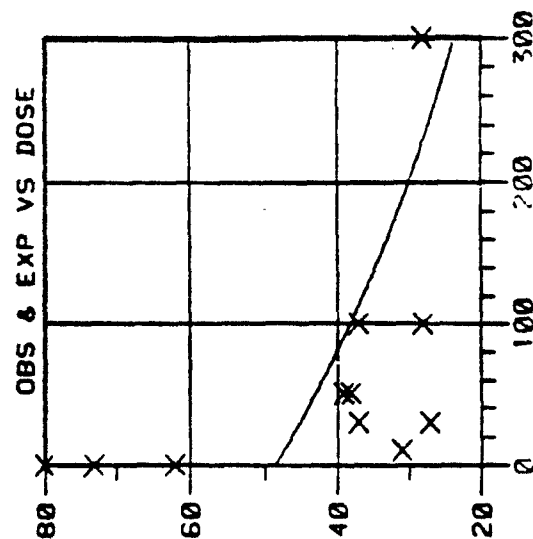
SAMPLE ID: BMGS-84-0002 LAB: GBBA ACTIVATION: + RLA027
STRAIN: TA1538 DATE: 06/08/84 TECHNICIAN: HJK

DOSE UNITS	PLATE COUNTS	MEAN	S.D.
00 UCS	80 62 73	71.67	9.07
50* UCS	816 807 828	817.00	10.54
10.00 UCS	31 31	31.00	.00
30.00 UCS	37 27	32.00	7.07
50.00 UCS	38 39	38.50	.71
100.00 UCS	37 28	32.50	6.36
300.00 UCS	28 28	28.00	.00

ESTS. B(0) B(1) B(2) B(3)
48.430 -82.0591 .0000 .00240

TEST CHI-SQUARE DF P LOGL
POISSON 5.12 7 .6454 -38.2959
ADEQUACY 54.57 2 .0000 -65.5830
TOXICITY 167.22 1 .0000 -149.1949
MUTAGENICITY .01 2 .9950 -65.5829

AVERAGE SLOPE (NONLIN. MODEL) = .000
95% CONF. LIMITS = (.000)
AVERAGE SLOPE (LINEAR REGR.) = -.579
95% CONF. LIMITS = (-1.160, .001)



STATISTICAL ANALYSIS: MUTAGENICITY OF
C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMCS-84-0002 LAB: GB8A ACTIVATION: -
STRAIN, TA1538 DATE: 06/08/84 TECHNICIAN: MJK

DOSE UNITS	PLATE COUNTS	MEAN	S.D.
00 UCS	21 18 19	19.33	1.53
3.00* UCS	502 604 528	544.67	53.00
10.00 UCS	8 13	10.50	3.54
30.00 UCS	9 14	11.50	3.54
50.00 UCS	14 29	21.50	10.61
100.00 UCS	17 19	18.00	1.41
300.00 UCS	12 18	15.00	4.24

ESTS. 16.328 -5.2186 1.9665 .01119 B(0) B(1) B(2) B(3)

TEST CHI-SQUARE DF P LOGL

POISSON 9.06 7 .2482 -34.3809

ADEQUACY 10.12 2 .0063 -39.4434

TOXICITY 2.92 1 .0875 -40.9034

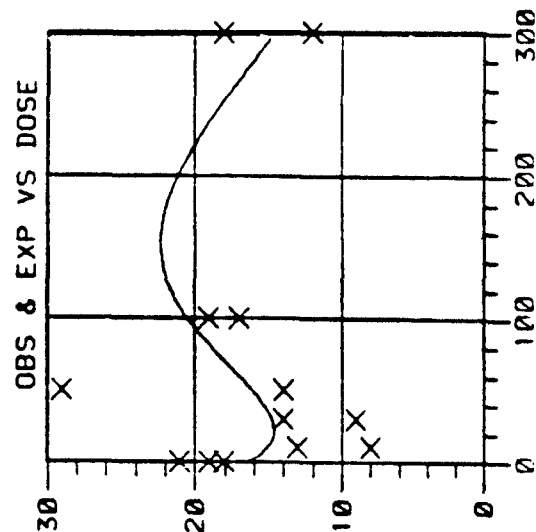
MUTAGENICITY 2.89 2 .2358 -40.8881

AVERAGE SLOPE (NONLIN. MODEL) = .237

95% CONF. LIMITS = (.067, .837)

AVERAGE SLOPE (LINEAR REGR.) = .044

95% CONF. LIMITS = (-.191, .280)



**MUTAGENICITY TESTING OF C.I. SOLVENT YELLOW NO. 33
IN SALMONELLA TYPHIMURIUM**

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE YELLOW
RESEARCH LAB: 688A ON 06/20/84

08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION

STRAIN: TA1538

COMPOUND	A C T	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE					MEAN	STD
			A	B	C	D	E		
POS CONTROL									
2-4F	-	3.00	492	504	551			515.67	31.18
2-AA	RLA027	0.50	668	714	737			706.33	35.13
NEG CONTROL									
DIMETHYLSULF	RLA027	100.00U	45	39	36			40.00	4.58
	-	100.00U	8	12	16			12.00	4.00
6*GS-84-0002									
	RLA027	10.00	31	28				29.50	2.12
	RLA027	30.00	31	28				29.50	2.12
	RLA027	50.00	39	43				41.00	2.63
	RLA027	100.00	36	32				34.00	2.83
	RLA027	300.00	25	23				24.00	1.41
	-	10.00	19	20				19.50	0.71
	-	30.00	17	7				12.00	7.07
	-	50.00	14	13				14.50	0.71
	-	100.00	16	14				17.50	2.12
	-	300.00	17	13				16.00	1.41

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY S-9 : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIA/PLATE : 500UGS

T*-TOXIC
TNTC-TOO NUMEROUS TO COUNT
NATC-NOT ABLE TO COUNT

G-PGS T-PPT
N-NGS P-PPM
M-MGS B-PPB
L-NLS I-MM
U-ULS C-MM

STATISTICAL ANALYSIS: MUTAGENICITY OF
C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMCS-84-0002 LAB: G88A ACTIVATION: + RLA027
STRAIN: TA1538 DATE: 06/20/84 TECHNICIAN: MJK

DOSE UNITS	PLATE COUNTS	MEAN	S.D.
.00 UGS	45 39 36	40.00	4.58
.50* UGS	668 714 737	706.33	35.13
10.00 UGS	31 28	29.50	2.12
30.00 UGS	31 28	29.50	2.12
50.00 UGS	39 43	41.00	2.83
100.00 UGS	36 32	34.00	2.83
300.00 UGS	25 23	24.00	1.41

ESTS. B(0) B(1) B(2) B(3)
36.675***** .0000 .00129

TEST CHI-SQUARE DF P LOGL

POISSON 1.87 7 .9667 -35.6165

ADEQUACY 8.19 2 .0166 -39.7126

TOXICITY 6.49 1 .0108 -42.9579

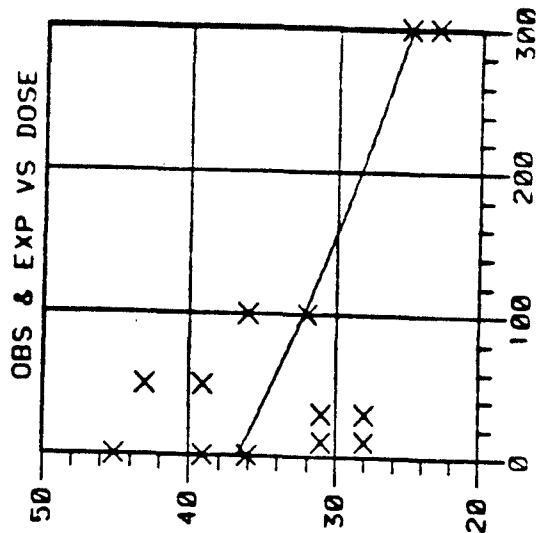
MUTAGENICITY .01 2 .9950 -39.7126

AVERAGE SLOPE (NONLIN. MODEL) = .000

95% CONF. LIMITS = (.000)

AVERAGE SLOPE (LINEAR REGR.) = .018

95% CONF. LIMITS = (-.215, .251)



STATISTICAL ANALYSIS: MUTAGENICITY OF
C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMCS-84-0002 LAB: CB8A ACTIVATION: -
STRAIN: TA1538 DATE: 06/20/84 TECHNICIAN: MJK

DOSE UNITS PLATE COUNTS				MEAN S.D.	
.00	UCS	8	12	12.00	4.00
3.00*	UCS	492	504	515.67	31.18
10.00	UCS	19	20	19.50	7.1
30.00	UCS	17	7	12.00	7.07
50.00	UCS	14	15	14.50	7.1
100.00	UCS	16	19	17.50	2.12
300.00	UCS	17	15	16.00	1.41

ESTS. B(0) B(1) B(2)
12.000 2.0748 .0000

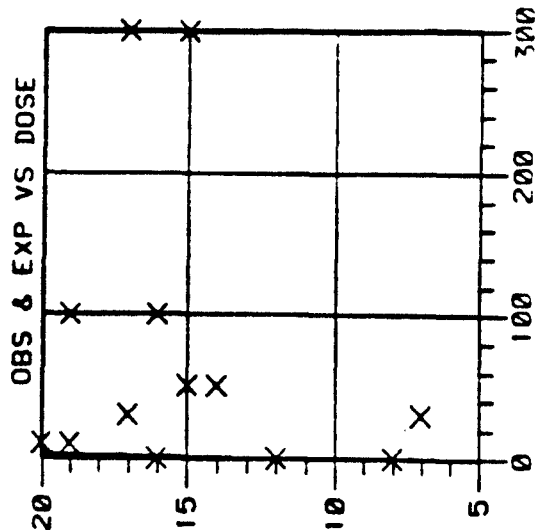
NO EVIDENCE OF TOXICITY

TEST CHI-SQUARE DF P LOGL
POISSON 7.28 7 .4008 -33.0852
ADEQUACY 13.07 3 .0045 -39.6227
MUTAGENICITY .01 2 .9950 -36.4062

AVERAGE SLOPE (NONLIN. MODEL) = .080

AVERAGE SLOPE (LINEAR REGR.) = .031
95% CONF. LIMITS = (-.043, .105)

WARNING: 4 PARAMETER MODEL DID NOT CONVERGE
WARNING: 3 PARAMETER MODEL DID NOT CONVERGE



Best Available Copy

MUTAGENICITY TESTING OF C.I. SOLVENT YELLOW NO. 33
IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE YELLOW

RESEARCH LAB: G88A ON 03/30/84

08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION

STRAIN: TA98

COMPOUND	A C T	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE					MEAN	STD
			A	B	C	D	E		
POS CONTROL									
2-NF	-	3.00	300	312	315			309.00	7.94
2-AA	RLA026	0.50	575	837	553			655.00	19.38
NEG CONTROL									
DIMETHYLSULF	RLA026	100.00U	60	41	48			49.67	9.61
	-	100.00U	28	33	30			30.33	2.52
BMGS-34-0002									
	RLA026	1.00	57	51				54.00	4.24
	RLA026	5.00	55	50				52.50	3.54
	RLA026	10.00	45	52				48.50	4.95
	RLA026	30.00	55	57				56.00	1.41
	RLA026	50.00	67	54				60.50	9.19
	RLA026	100.00	61	45				53.00	11.31
	RLA026	300.00	48	51				49.50	2.12
	RLA026	500.00	48	43				45.50	3.54
	RLA026	1000.00	29	40				34.50	7.78
	-	1.00	29	26				27.50	2.12
	-	5.00	30	30				30.00	0.00
	-	10.00	48	33				40.50	10.61
	-	30.00	36	16				27.00	12.73
	-	50.00	21	29				25.00	5.66
	-	100.00	29	20				24.50	6.36
	-	300.00	29	30				29.50	0.71
	-	500.00	37	26				32.50	6.36
	-	1000.00	32	23				27.50	6.36

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY S-Y : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIX/PLATE : 500UGS

T+--TOXIC
INTC-TWO NUMEROUS TO COUNT
NATC-NOT ABLE TO COUNT

G-PGS T-PPT
N-NGS P-PPM
M-MGS B-PPB
L-NLS I-MM
U-ULS C-UM

STATISTICAL ANALYSIS: MUTAGENICITY OF
C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMCS-84-0002 LAB: CBBA ACTIVATION: + RLA026
STRAIN: TA98 DATE: 03/30/84 TECHNICIAN: HJK

DOSE UNITS	PLATE COUNTS	MEAN	S.D.
.00 UGS	60 41 48	49.67	9.61
.50* UGS	875 837 853	855.00	19.08
1.00 UGS	57 51	54.00	4.24
5.00 UGS	55 50	52.50	3.54
10.00 UGS	45 52	48.50	4.95
30.00 UGS	55 57	56.00	1.41
50.00 UGS	67 54	60.50	9.19
100.00 UGS	61 45	53.00	11.31
300.00 UGS	48 51	49.50	2.12

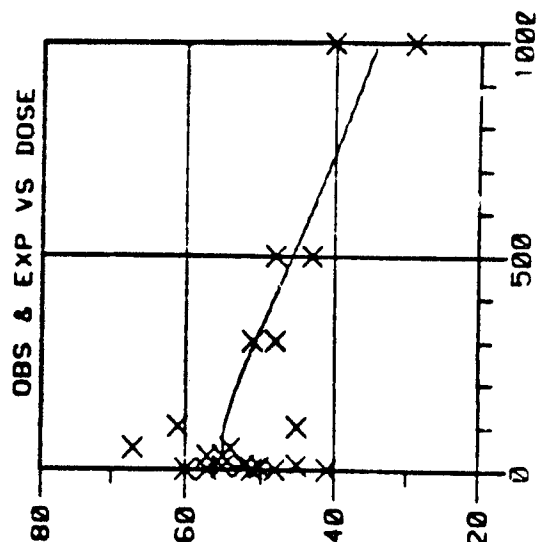
MORE THAN 9 DOSE LEVELS USED IN COMPUTATION BUT NOT DISPLAYED

ESTS. 49.708 .8167 .2968 .00068
B(0) B(1) B(2) B(3)

TEST CHI-SQUARE DF P LOGL
POISSON 10.76 11 .4635 -65.6976
ADEQUACY 2.54 6 .8640 -66.9672
TOXICITY 19.48 1 .0000 -76.7056
MUTAGENICITY 2.39 2 .3020 -68.1645

AVERAGE SLOPE (NONLIN. MODEL) = .145
95% CONF. LIMITS = (.036, .575)

AVERAGE SLOPE (LINEAR REGR.) = .188
95% CONF. LIMITS = (.006, .370)



STATISTICAL ANALYSIS: MUTAGENICITY OF
C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0002 LAB: GBBA ACTIVATION: -
STRAIN: TA98 DATE: 03/30/84 TECHNICIAN: MJK

DOSE UNITS PLATE COUNTS

DOSE UNITS	PLATE COUNTS	MEAN	S.D.
00 UGS	28 33 30	30.33	2.52
3.00* UGS	300 312 315	309.00	7.94
1.00 UGS	29 26	27.50	2.12
5.00 UGS	30 30	30.00	.00
10.00 UGS	48 33	40.50	10.61
30.00 UGS	36 18	27.00	12.73
50.00 UGS	21 29	25.00	5.66
100.00 UGS	29 20	24.50	6.36
300.00 UGS	29 30	29.50	.71

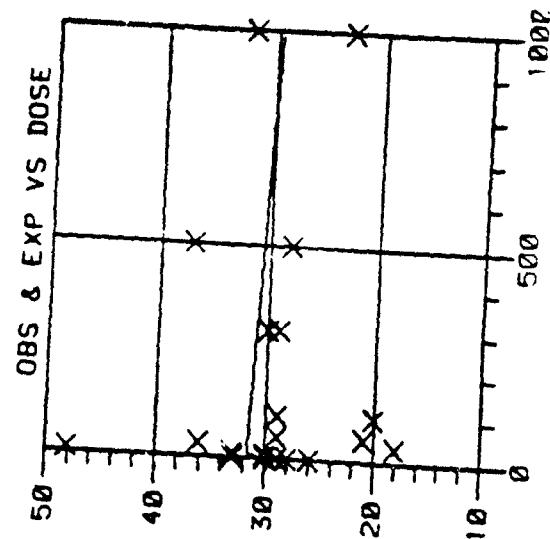
MORE THAN 9 DOSE LEVELS USED IN COMPUTATION BUT NOT DISPLAYED

ESTS. 30.346 .2573 .0000 .00007
B(10) B(11) B(12) B(13)

TEST CHI-SQUARE DF P LOCL
POISSON 15.03 11 .1812
ADEQUACY 14.21 6 .0274
TOXICITY 2.15 1 .1424
MUTAGENICITY .01 2 .9950

AVERAGE SLOPE (NONLIN. MODEL) = .003
95% CONF. LIMITS = (.000, .4736, .7261)

AVERAGE SLOPE (LINEAR REGR.) = .003
95% CONF. LIMITS = (-.017, .022)



MUTAGENICITY TESTING OF C.I. SOLVENT YELLOW NO. 33
IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
 OF ARMY DYE YELLOW
 RESEARCH LAB: GBBA ON 04/06/84

08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION

STRAIN: TA98

COMPOUND	A C T	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE					MEAN	STD
			A	B	C	D	E		
FOS CONTROL									
2-NF	-	3.00	250	270	255			258.33	10.41
2-AA	RLA026	0.50	740	825	817			794.00	46.94
NEG CONTROL									
DIMETHYLSULF	RLA026	100.00U	42	31	43			38.67	6.66
	-	100.00U	23	29	20			24.00	4.58
BMGS-84-0002									
	RLA026	1.00	45	45				45.00	0.00
	RLA026	5.00	45	54				49.50	6.36
	RLA026	10.00	40	56				48.00	11.31
	RLA026	30.00	62	67				65.50	4.95
	RLA026	50.00	49	51				50.00	1.41
	RLA026	100.00	49	40				44.50	6.36
	RLA026	300.00	56	52				54.00	2.63
	-	1.00	31	20				25.50	7.78
	-	5.00	33	25				29.00	5.66
	-	10.00	27	21				24.00	4.24
	-	30.00	28	31				29.50	2.12
	-	50.00	24	26				27.00	1.41
	-	100.00	17	30				23.50	7.19
	-	300.00	20	35				27.50	10.61

PHENOCOPY CHECK : TRUE MUTANTS
 STERILITY S-Y : NOT CONTAMINATED
 SAMPLE STERILITY: NOT CONTAMINATED
 ACT MIX/PLATE : 500UGS

T*-TOXIC
 INTC-TOO NUMEROUS TO COUNT
 NATC-NOT ABLE TO COUNT

G-PGS T-PPT
 N-NGS P-PPM
 M-MGS B-PPB
 L-NLS I-MM
 U-ULS C-UM

STATISTICAL ANALYSIS: MUTAGENICITY OF
C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0002 LAB: GBBA ACTIVATION: + RLA026
STRAIN: TA98 DATE: 04/06/84 TECHNICIAN: MJK

DOSE UNITS	PLATE COUNTS	MEAN	S.D.
.00 UGS	42 31 43	38.67	6.66
.50* UGS	740 825 817	794.00	46.04
1.00 UGS	45 45	45.00	.00
5.00 UGS	45 54	49.50	6.36
10.00 UGS	40 56	48.00	11.31
30.00 UGS	62 69	65.50	4.95
50.00 UGS	49 51	50.00	1.41
100.00 UGS	49 40	44.50	6.36
300.00 UGS	56 52	54.00	2.83

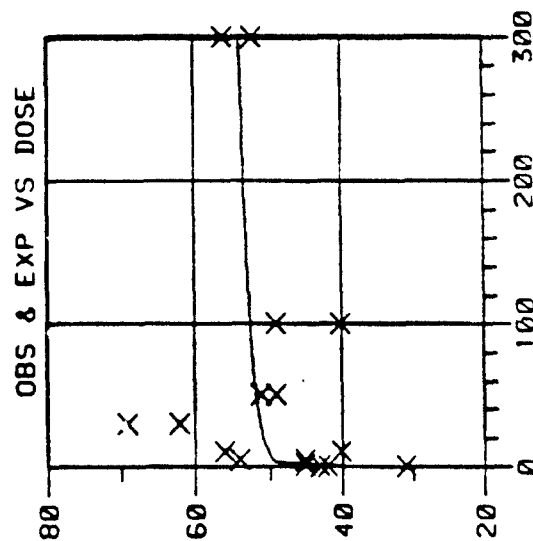
ESTS. 38.591 2.2519 .0817

NO EVIDENCE OF TOXICITY

TEST CHI-SQUARE DF P LOGL
POISSON 7.25 9 .6111 -52.2272
ADEQUACY 10.63 5 .0593 -57.5407
MUTAGENICITY 9.05 2 .0108 -62.0658

AVERAGE SLOPE (NONLIN. MODEL) = .051
95% CONF. LIMITS = (.020, .126)

AVERAGE SLOPE (LINEAR REGR.) = .023
95% CONF. LIMITS = (-.024, .070)



STATISTICAL ANALYSIS: MUTAGENICITY OF
C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

SAMPLE ID, BMCS-84-0002 LAB, CBBA ACTIVATION, -
STRAIN, TA98 DATE, 04/06/84 TECHNICIAN, MJK

DOSE UNITS PLATE COUNTS			MEAN S.D.	
.00	UGS	23 20 20	24.00	4.58
3.00*	UGS	250 270 255	258.33	10.41
1.00	UGS	31 20	25.50	7.78
5.00	UGS	33 25	29.00	5.66
10.00	UGS	27 21	24.00	4.24
30.00	UGS	28 31	29.50	2.12
50.00	UGS	26 28	27.00	1.41
100.00	UGS	17 30	23.50	9.19
300.00	UGS	20 35	27.50	10.61

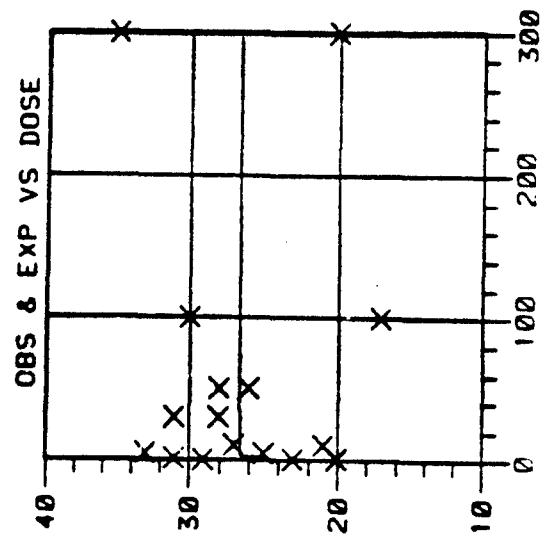
B(0) B(1) B(2)
ESTS. 24.000 9060 0125

NO EVIDENCE OF TOXICITY

TEST CHI-SQUARE DF P LOGL
POISSON 13.89 9 .1263 -50.2357
ADEQUACY 2.47 5 .7808 -51.4714
MUTAGENICITY .64 2 .7257 -51.7920

AVERAGE SLOPE (NONLIN. MODEL) = .086
95% CONF. LIMITS = (.004, 1.836)

AVERAGE SLOPE (LINEAR REGR.) = .142
95% CONF. LIMITS = (-.117, .401)



MUTAGENICITY TESTING OF PURIFIED YELLOW DYE
IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE PURIFIED YELLOW
RESEARCH LAB: GB8A ON 03/30/84

08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION

STRAIN: TA1CO

COMPOUND	A C T	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE					MEAN	STD
			A	B	C	D	E		
POS CONTROL									
NAAZIDE	-	3.00	1179	1205	1160			1188.00	14.73
2-AA	RLA026	0.50	363	360	298			340.33	36.69
NEG CONTROL									
DIMETHYLSULF	RLA026	100.000	105	101	103			103.00	2.00
	-	100.000	103	134	97			111.33	19.86
BMGS-34-0003									
	RLA026	1.00	112	104				108.00	5.86
	RLA026	5.00	138	134				136.00	2.83
	RLA026	10.00	111	112				111.50	0.71
	RLA026	30.00	153	144				148.50	6.36
	RLA026	50.00	149	143				146.00	4.24
	RLA026	100.00	131	142				136.50	7.78
	RLA026	300.00	127	122				124.50	3.54
	RLA026	500.00	113	106				109.50	4.95
	RLA026	1000.00	101	117				109.00	11.31
	-	1.00	126	86				107.00	26.87
	-	5.00	101	127				114.00	16.38
	-	10.00	98	113				105.50	10.61
	-	30.00	102	133				117.50	21.92
	-	50.00	127	117				122.00	7.07
	-	100.00	125	109				117.00	11.31
	-	300.00	98	120				109.00	15.56
	-	500.00	113	104				108.50	6.36
	-	1000.00	88					88.00	0.00

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY S-9 : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIX/PLATE : 500UGS

T--TOXIC
TNTC-TOO NUMEROUS TO COUNT
NATC-NOT ABLE TO COUNT

G-PGS T-BPT
N-NGS P-BPM
M-MGS P-BPB
L-ALS I-BM
U-ULS C-UM

STATISTICAL ANALYSIS: MUTAGENICITY OF PURIFIED YELLOW
DYE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMCS-84-0003 LAB: CB8A ACTIVATION: + RLA026
STRAIN: TA100 DATE: 03/30/84 TECHNICIAN: HJK

DOSE UNITS		PLATE COUNTS		MEAN	S.D.
0.00	UGS	105	101	103.00	2.00
0.50*	UGS	363	360	340.33	36.60
1.00	UGS	112	104	108.00	5.66
5.00	UGS	138	134	136.00	2.83
10.00	UGS	111	112	111.50	.71
30.00	UGS	153	144	148.50	6.36
50.00	UGS	149	143	146.00	4.24
100.00	UGS	131	142	136.50	7.78
300.00	UGS	127	122	124.50	3.54

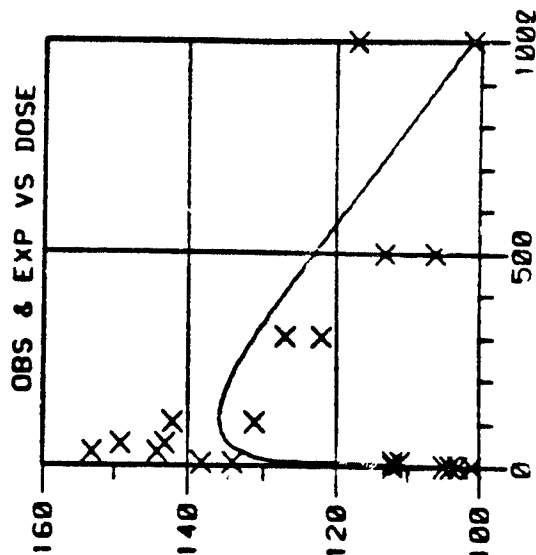
MORE THAN 8 DOSE LEVELS USED IN COMPUTATION BUT NOT DISPLAYED

ESTS. 101.857 2.8822 .1777 .00047
B(0) B(1) B(2) B(3)

TEST CHI-SQUARE DF P LOGL
POISSON 2.77 11 .9934 -71.0675
ADEQUACY 19.00 6 .0040 -80.6108
TOXICITY 16.01 1 .0001 -88.6153
MUTAGENICITY 22.94 2 .0000 -92.0788

AVERAGE SLOPE (NONLIN. MODEL) = 1.089
95% CONF. LIMITS = 1 .198, 6.0021

AVERAGE SLOPE (LINEAR REGR.) = 1.326
95% CONF. LIMITS = 1 .663, 1.9891



STATISTICAL ANALYSIS: MUTAGENICITY OF PURIFIED YELLOW
DYE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMCS-84-0003 LAB: G88A ACTIVATION: -
STRAIN: TA100 DATE: 03/30/84 TECHNICIAN: HJK

DOSE UNITS	PLATE COUNTS	MEAN	S.D.
.00 UGS	103 134 97	111.33	19.86
3.00* UGS	1179 1205 1180	1188.00	14.73
1.00 UGS	126 88	107.00	26.87
5.00 UGS	101 127	114.00	18.38
10.00 UGS	98 113	105.50	10.61
30.00 UGS	102 133	117.50	21.92
50.00 UGS	127 117	122.00	7.07
100.00 UGS	125 109	117.00	11.31
300.00 UGS	98 120	109.00	15.56

MORE THAN 9 DOSE LEVELS USED IN COMPUTATION BUT NOT DISPLAYED

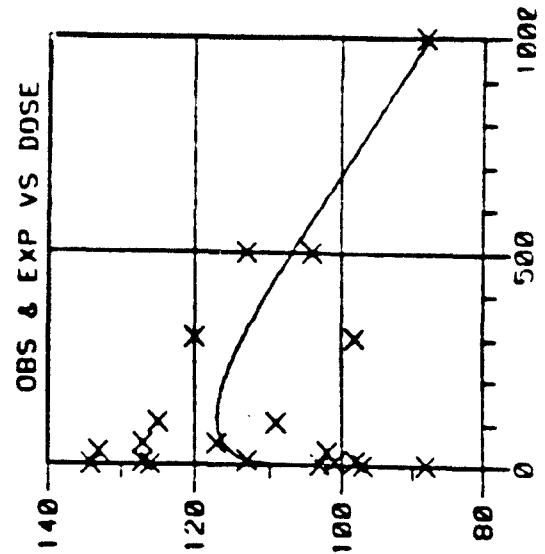
B(0) B(1) B(2) B(3)

ESTS. 109.252 3003 5198 .00059

TEST	CHI-SQUARE	DF	P	LOCL
POISSON	26.05	10	.0037	-78.3845
ADEQUACY	2.55	6	.8629	-79.6594
TOXICITY	7.80	1	.0052	-83.5003
MUTAGENICITY	2.82	2	.2435	-81.0718

AVERAGE SLOPE (NONLIN. MODEL) = .264
95% CONF. LIMITS = (.049, 1.425)

AVERAGE SLOPE (LINEAR REGR.) = .229
95% CONF. LIMITS = (-.715, 1.173)



**MUTAGENICITY TESTING OF PURIFIED YELLOW DYE
IN SALMONELLA TYPHIMURIUM**

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE PURIFIED YELLOW
RESEARCH LAB: G88A ON 04/06/84

08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION

STRAIN: TA100

COMPOUND	A C T	UGS PER PLATE	MISTIDINE REVERTANTS PER PLATE					MEAN	STD
			A	B	C	D	E		
POS CONTROL									
NAAZIDE	-	5.00	1253	1350	1320			1307.67	49.65
2-AA	RLA026	0.50	953	812	842			869.00	74.28
NEG CONTROL									
DIMETHYLSULF	RLA026	100.00u	115	125	114			118.00	6.08
	-	100.00u	109	102	110			107.00	4.36
BMGS-34-0003									
	RLA026	1.00	108	127				117.50	13.44
	RLA026	5.00	150	113				131.50	26.16
	RLA026	10.00	152	133				142.50	13.44
	RLA026	30.00	180	175				177.50	3.54
	RLA026	50.00	125	142				133.50	12.02
	RLA026	100.00	152	134				143.00	12.73
	RLA026	300.00	144	160				152.00	11.31
	-	1.00	115	156				135.50	28.95
	-	5.00	114	137				125.50	16.26
	-	10.00	98	132				115.00	24.04
	-	30.00	115	139				127.00	16.97
	-	50.00	132	110				121.00	15.56
	-	100.00	151	102				126.50	34.61
	-	300.00	116	116				117.00	1.41

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY S-v : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIX/PLATE : 500UGS

T*-TOXIC
TNTC-TWO NUMEROUS TO COUNT
NATC-NOT ABLE TO COUNT

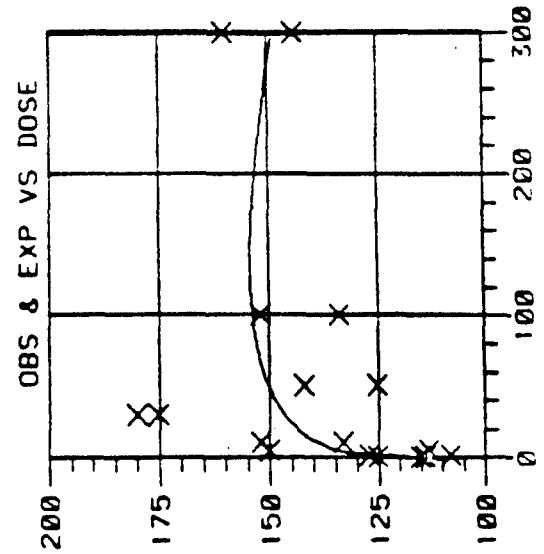
G-PGS T-PP
N-NGS P-PP
M-NGS B-PP
L-NLS I-MM
U-ULS C-MM

STATISTICAL ANALYSIS: MUTAGENICITY OF PURIFIED YELLOW
DYE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0003 LAB: G88A ACTIVATION: + RLA026
STRAIN: TA100 DATE: 04/06/84 TECHNICIAN: MJK

DOSE UNITS	PLATE COUNTS	MEAN	S.D.
00 UGS	115 125 114	118.00	6.08
50* UGS	953 812 842	869.00	74.28
100 UGS	108 127	117.50	13.44
500 UGS	150 113	131.50	26.16
1000 UGS	152 133	142.50	13.44
3000 UGS	180 175	177.50	3.54
5000 UGS	125 142	133.50	12.02
10000 UGS	152 134	143.00	12.73
30000 UGS	144 160	152.00	11.31

ESTS.	116.104	2.4457	3.172	00075
TEST	CHI-SQUARE	DF	P	LOCL
POISSON	11.76	9	.2270	-63.3202
ADEQUACY	19.74	4	.0006	-73.1919
TOXICITY	2.00	1	.1576	-74.1904
MUTAGENICITY	21.14	2	.0000	-83.7619
AVERAGE SLOPE (NONLIN. MODEL)	= 1.131			
95% CONF. LIMITS	= (.569, 2.248)			
AVERAGE SLOPE (LINEAR REG.)	= 2.005			
95% CONF. LIMITS	= (1.360, 2.650)			



STATISTICAL ANALYSIS: MUTAGENICITY OF PURIFIED YELLOW
DYE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0003 LAB: GBBA ACTIVATION: -
STRAIN: TA100 DATE: 04/06/84 TECHNICIAN: MJK

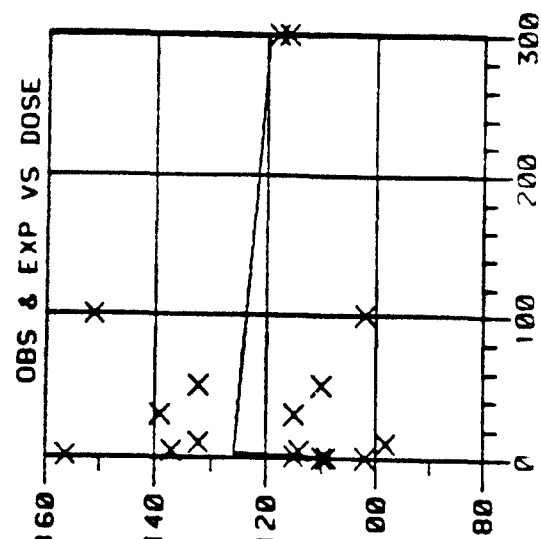
DOSE UNITS	PLATE COUNTS	MEAN	S.D.
00	UGS 109 102 110	107.00	4.36
3.00*	UGS 1253 1350 1320	1307.67	49.66
1.00	UGS 115 156	135.50	28.90
5.00	UGS 114 137	125.50	16.26
10.00	UGS 98 132	115.00	24.04
30.00	UGS 115 139	127.00	16.97
50.00	UGS 132 110	121.00	15.56
100.00	UGS 151 102	126.50	34.65
300.00	UGS 116 118	117.00	1.41

ESTS. 107.000 2.9337 0000 00017
B(0) B(1) B(2) B(3)

TEST CHI-SQUARE DF P LOGL
POISSON 27.47 9 .0012 -70.1066
ADEQUACY 3.82 4 .4312 -72.0157
TOXICITY .88 1 .3487 -72.4547
MUTAGENICITY 6.82 2 .0331 -75.4241

AVERAGE SLOPE (NONLIN. MODEL) = 1.880
95% CONF. LIMITS = (.284, 12.429)

AVERAGE SLOPE (LINEAR REGR.) = .079
95% CONF. LIMITS = (-3.331, 3.489)
WARNING: 3 PARAMETER MODEL DID NOT CONVERGE



MUTAGENICITY TESTING OF PURIFIED YELLOW DYE
IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE PURIFIED YELLOW
RESEARCH LAB: GBBA ON 04/06/84

08/27/84

TEST TYPE: PLATE TEST - PREINCUBATION

STRAIN: TA100

COMPOUND	A C T	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE					MEAN	STD
			A	B	C	D	E		
POS CONTROL									
NAAZIDE	-	3.00	1227	1278	1267			1257.33	26.24
2-AA	RLA026	0.50	354	307	337			332.67	23.80
NEG CONTROL									
DIMETHYLSULF	RLA026	100.00U	108	117	115			113.33	4.73
	-	100.00U	144	132	127			134.33	6.74
BMGS-84-U003									
	RLA026	1.00	126	146				136.00	14.14
	RLA026	5.00	141	155				148.00	9.90
	RLA026	10.00	153	145				149.00	5.66
	RLA026	30.00	167	177				172.00	7.07
	RLA026	50.00	190	159				174.50	21.92
	RLA026	100.00	173	180				176.50	4.95
	RLA026	300.00	173	144				158.50	20.51
	-	1.00	137	129				133.00	5.66
	-	5.00	130	131				130.50	0.71
	-	10.00	131	150				140.50	13.44
	-	30.00	91	119				105.00	19.80
	-	50.00	107	126				116.50	13.44
	-	100.00	113	118				115.50	3.54
	-	300.00	89	92				90.50	2.12

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY S-9 : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIX/PLATE : SCCUGS

T*-TOXIC
TNTC-TOO NUMEROUS TO COUNT
NATC-NOT ABLE TO COUNT

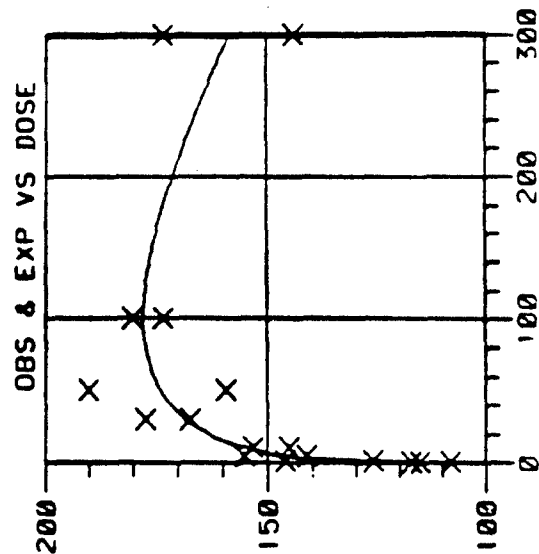
G-PGS T-PPT
N-MGS P-PPM
M-MGS B-PPB
L-NLS I-MM
U-ULS C-UM

STATISTICAL ANALYSIS: MUTAGENICITY OF PURIFIED YELLOW
DYE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0003 LAB: CB8A ACTIVATION: + RLA026
STRAIN: TA100 DATE: 04/06/84 TECHNICIAN: HJK

DOSE UNITS PLATE COUNTS			MEAN	S. D.
.00	UCS	108	117	115
.50*	UCS	354	307	337
1.00	UCS	126	146	
5.00	UCS	141	155	
10.00	UCS	153	145	
30.00	UCS	167	177	
50.00	UCS	190	159	
100.00	UCS	173	180	
300.00	UCS	173	144	
			113.33	4.73
			332.67	23.80
			136.00	14.14
			148.00	9.90
			149.00	5.66
			172.00	7.07
			174.50	21.92
			176.50	4.95
			158.50	20.51

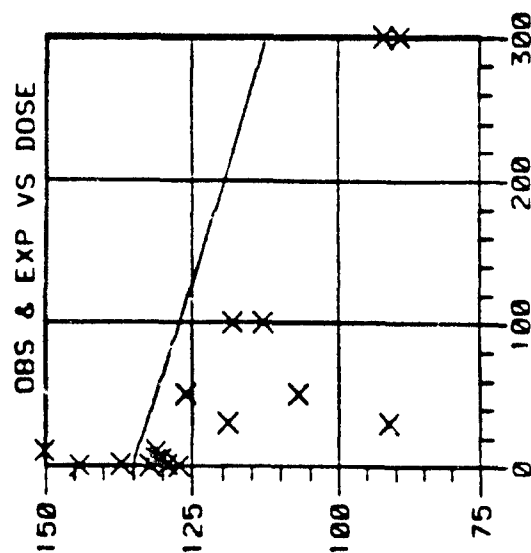
ESIS	113.551	3.0270	B(1)	B(2)	B(3)
TEST	CHI-SQUARE	DF	P	LOGL	
POISSON	8.58	9	.4771	-62.4701	
ADEQUACY	.70	4	.9514	-62.8197	
TOXICITY	8.26	1	.0040	-66.9522	
MUTAGENICITY	55.31	2	.0000	-90.4723	
AVERAGE SLOPE (NONLIN. MODEL) = 2.071					
95% CONF. LIMITS = (1.176, 3.647)					
AVERAGE SLOPE (LINEAR REGR.) = 1.628					
95% CONF. LIMITS = (.903, 2.354)					



A-126

DOSE UNITS	PLATE COUNTS	MEAN	S. D.
.00	UGS 144 132 127	134.33	8.74
3.00*	UGS 1227 1278 1267	1257.33	26.84
1.00	UGS 137 129	133.00	5.66
5.00	UGS 130 131	130.50	.71
10.00	UGS 131 150	140.50	13.44
30.00	UGS 91 119	105.00	19.80
50.00	UGS 107 126	116.50	13.44
100.00	UGS 113 118	115.50	3.54
300.00	UGS 89 92	90.50	2.12

B(0)	B(1)	B(2)	B(3)
-----	-----	-----	-----
ESTS. 134.370	- .3883	.0000	.00061
TEST	CHI-SQUARE	DF	P
-----	-----	-----	-----
POISSON	8.11	9	.5235
ADEQUACY	27.91	4	.0000
TOXICITY	11.82	1	.0006
MUTAGENICITY	.01	2	.9950
			-60.3959
			-74.3498
			-80.2595
			-65.5337
AVERAGE SLOPE (NONLIN. MODEL) =			.068
95% CONF. LIMITS = (			.000,*****)
AVERAGE SLOPE (LINEAR REG.) =			.544
95% CONF. LIMITS = (			-.794, 1.883)



**MUTAGENICITY TESTING OF PURIFIED YELLOW DYE
IN SALMONELLA TYPHIMURIUM**

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE PURIFIED YELLOW
RESEARCH LAB: G88A ON 06/01/84

08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION

STRAIN: TA102

COMPOUND	A C T	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE					MEAN	STD
			A	B	C	D	E		
POS CONTROL									
OTHER PCS	RLAC27	30.00	1111	964	1069			1034.67	67.21
	-	0.50	349	335	362			355.33	24.11
NEG CONTROL									
DIMETHYLSULF	RLAC27	100.00u	108	109	100			105.67	4.93
	-	100.00u	57	40	42			46.33	9.29
BMGS-34-UGG3									
	RLAC27	10.00	103	87				95.00	11.31
	RLAC27	30.00	177	177				177.00	0.00
	RLAC27	50.00	197	200				198.50	2.12
	RLAC27	100.00	221	202				211.50	13.44
	RLAC27	300.00	136	126				132.00	5.66
	-	10.00	99	111				105.00	8.49
	-	30.00	111	127				119.00	11.31
	-	50.00	96	86				91.00	7.07
	-	100.00	100	161				130.50	43.13
	-	300.00	138	131				134.50	4.95

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY S-9 : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIX/PLATE : 500UGS

T--TOXIC
TNTC-TOO NUMEROUS TO COUNT
NATC-NOT ABLE TO COUNT

G-PGS T-PPT
N-MGS P-PPM
H-MGS B-PPB
L-NLS I-MM
U-ULS C-UM

Best Available Copy

MUTAGENICITY TESTING OF PURIFIED YELLOW DYE
IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE PURIFIED YELLOW
RESEARCH LAB: 688A ON 06/01/84
TEST TYPE: STANDARD PLATE INCORPORATION

08/27/84

STRAIN: TA102

•RLAG27

POSITIVE CONTROL USED WAS DANTRON.

-
POSITIVE CONTROL USED WAS MITOMYCIN C.

STATISTICAL ANALYSIS: MUTAGENICITY OF PURIFIED YELLOW
DYE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0003 LAB: CBBA ACTIVATION: + RLA027
STRAIN: TA102 DATE: 06/01/84 TECHNICIAN: MJK

DOSE UNITS PLATE COUNTS			MEAN S.D.	
.00	UCS	108 109 100	105.67	4.03
30.00*	UCS	1111 984 1009	1034.67	67.28
10.00	UCS	103 87	95.00	11.31
30.00	UCS	177 177	177.00	.00
50.00	UCS	197 200	198.50	2.12
100.00	UCS	221 202	211.50	13.44
300.00	UCS	136 128	132.00	5.66

ESTS. B(0) 99.494 B(1) .7251 B(2) 1.1552 B(3) .00838

TEST CHI-SQUARE DF P LOGL

POISSON 2.93 7 .8917 -45.6625

ADEQUACY 17.78 2 .0001 -54.5501

TOXICITY 96.48 1 .0000 -102.7911

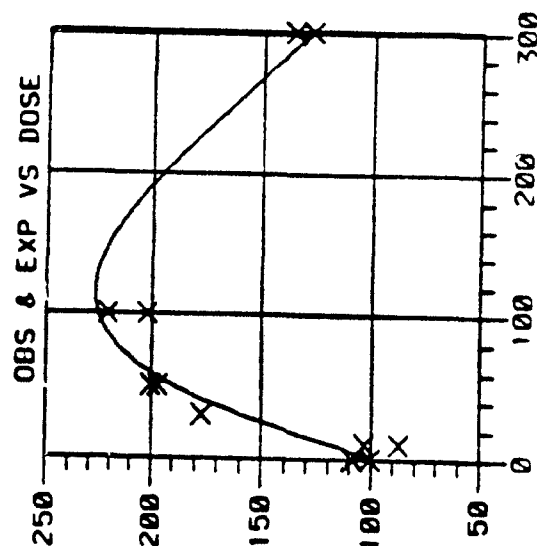
MUTAGENICITY 163.70 2 .0000 -136.3977

AVERAGE SLOPE (NONLIN. MODEL) = 4.220

95% CONF. LIMITS = (2.928, 6.083)

AVERAGE SLOPE (LINEAR REGR.) = 1.197

95% CONF. LIMITS = (.755, 1.638)



STATISTICAL ANALYSIS: MUTAGENICITY OF PURIFIED YELLOW
DYE IN SALMONELLA TYPHIMURIUM

SAMPLE ID, BMCS-84-0003 LAB, CBBA ACTIVATION, -
STRAIN, TA102 DATE, 06/01/84 TECHNICIAN, MJK

DOSE UNITS PLATE COUNTS				MEAN S.D.	
.00	UGS	57	40	46.33	9.20
.50*	UGS	349	335	355.33	24.13
10.00	UGS	89	111	105.00	8.49
30.00	UGS	111	127	119.00	11.31
50.00	UGS	96	86	91.00	7.07
100.00	UGS	100	161	130.50	43.13
300.00	UGS	138	131	134.50	4.95

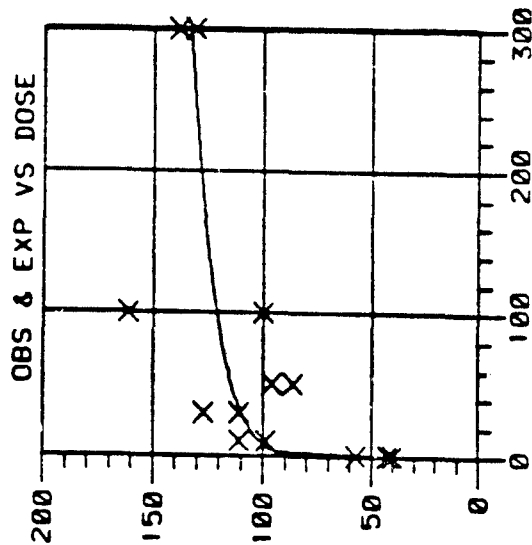
ESTS. B(0) 46.385 B(1) 3.6986 B(2) .1335

NO EVIDENCE OF TOXICITY

TEST CHI-SQUARE DF P LOGL
POISSON 20.48 .0046 -51.6254
ADEQUACY 13.54 3 .0036 -58.3937
MUTAGENICITY 141.98 2 .0000 -129.3820

AVERAGE SLOPE (NONLIN. MODEL) = .747
95% CONF. LIMITS = (.666, .838)

AVERAGE SLOPE (LINEAR REGR.) = .639
95% CONF. LIMITS = (.145, 1.123)



MUTAGENICITY TESTING OF PURIFIED YELLOW DYE
IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
 OF ARMY DYE PURIFIED YELLOW
 RESEARCH LAB: GBBA ON 06/05/84

08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION

STRAIN: TA102

COMPOUND	A C T	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE					MEAN	STD
			A	B	C	D	E		
POS CONTROL									
OTHER POS	RLA027	30.00	1253	1203	1096			1194.00	80.21
	-	0.50	1451	1509	1507			1489.00	32.92
NEG CONTROL									
DIMETHYLSULF	RLA027	100.000	269	325	302			298.67	28.15
	-	100.000	215	213	203			210.33	6.43
BMGS-34-0003									
	RLA027	10.00	478	407				442.50	50.20
	RLA027	30.00	575	551				563.00	16.97
	RLA027	50.00	642	592				617.00	35.36
	RLA027	100.00	656	589				622.50	47.38
	RLA027	300.00	429	455				441.50	19.09
	-	10.00	400	503				451.50	72.63
	-	30.00	456	441				448.50	10.61
	-	50.00	468	429				448.50	27.58
	-	100.00	402	427				444.50	24.75
	-	300.00	447	424				435.50	16.26

PHENOCOPY CHECK : TRUE MUTANTS
 STERILITY S-9 : NOT CONTAMINATED
 SAMPLE STERILITY: NOT CONTAMINATED
 ACT MIX/PLATE : 500UGS

T=TOXIC
 TNC=TOO NUMEROUS TO COUNT
 NATC=NOT ABLE TO COUNT

G-PGS T-RPT
 N-RGS B-RPM
 M-RGS B-RPG
 L-NLS I-MM
 U-ULS C-UM

MUTAGENICITY TESTING OF PURIFIED YELLOW DYE
IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE PURIFIED YELLOW
RESEARCH LAB: G88A ON 06/05/84
TEST TYPE: STANDARD PLATE INCORPORATION

08/27/84

STRAIN: TA102

•RLAG27

POSITIVE CONTROL USED WAS DANTHRON.

POSITIVE CONTROL USED WAS MITOMYCIN C.

STATISTICAL ANALYSIS: MUTAGENICITY OF PURIFIED YELLOW
DYE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMCS-84-0003 LAB: C88A ACTIVATION: + RLA027
STRAIN: TA102 DATE: 06/05/84 TECHNICIAN: MJK

DOSE UNITS	PLATE COUNTS	MEAN	S.D.
00 UGS	269 325 302	288.67	28.15
30.00* UGS	1253 1203 1086	1184.00	80.21
10.00 UGS	478 407	442.50	50.20
30.00 UGS	575 551	563.00	16.97
50.00 UGS	642 592	617.00	35.36
100.00 UGS	656 589	622.50	47.38
300.00 UGS	428 455	441.50	19.00

ESTS. 297.756 3.7944 5934 .00433

TEST CHI-SQUARE DF P LOGL

POISSON 17.97 7 .0121 -60.8696

ADEQUACY 1.77 2 .4132 -61.7534

TOXICITY 120.57 1 .0000 -122.0388

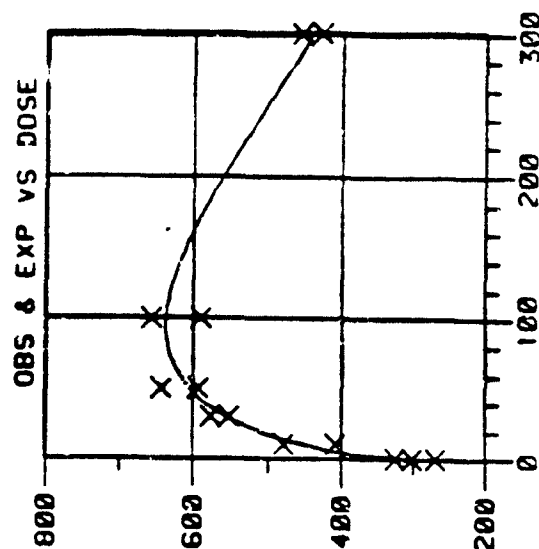
MUTAGENICITY 433.32 2 .0000 -278.4109

AVERAGE SLOPE (NONLIN. MODEL) = 9.059

95% CONF. LIMITS = (8.120, 10.097)

AVERAGE SLOPE (LINEAR REGR) = 6.326

95% CONF. LIMITS = (4.550, 8.103)



STATISTICAL ANALYSIS: MUTAGENICITY OF PURIFIED YELLOW
DYE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0003 LAB: G88A ACTIVATION: -
STRAIN: TA102 DATE: 06/05/84 TECHNICIAN: MJK

DOSE UNITS	PLATE COUNTS	MEAN	S.D.
.00 UCS	215 213 203	210.33	6.43
.50* UCS	1451 1509 1507	1480.00	32.92
10.00 UCS	400 503	451.50	72.83
30.00 UCS	456 441	448.50	10.61
50.00 UCS	468 429	448.50	27.58
100.00 UCS	462 427	444.50	24.75
300.00 UCS	447 424	435.50	16.26

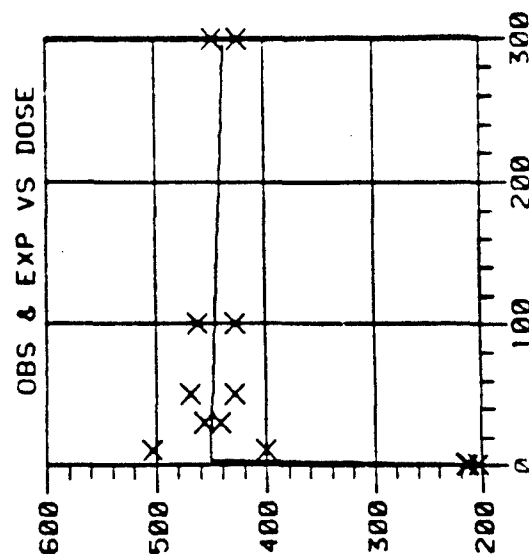
ESTS. 210.329 5.4833 B(1) B(2) B(3)

TEST CHI-SQUARE DF P LOGL

POISSON 16.07 7 .0245 -58.5117
ADEQUACY .02 2 .9906 -58.5211
TOXICITY 3.87 1 .0492 -60.4549
MUTAGENICITY 385.47 2 .0000 -251.2576

AVERAGE SLOPE (NONLIN. MODEL) = 4.813
95% CONF. LIMITS = (3.739, 6.194)

AVERAGE SLOPE (LINEAR REGR.) = 4.185
95% CONF. LIMITS = (.980, 7.390)
WARNING: 3 PARAMETER MODEL DID NOT CONVERGE



**MUTAGENICITY TESTING OF PURIFIED YELLOW DYE
IN SALMONELLA TYPHIMURIUM**

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE PURIFIED YELLOW
RESEARCH LAB: 688A ON 06/15/84

08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION

STRAIN: TA102

COMPOUND	A C T	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE					MEAN	STD
			A	B	C	D	E		
POS CONTROL									
OTHER PGS	RLA027	0.50	1252	1377	1392	1370		1347.75	64.49
	-	30.00	1380	1320	1363			1361.00	35.54
NEG CONTROL									
DIMETHYLSULF	RLA027	100.000	264	264	266			264.67	1.15
	-	100.000	202	188	213			201.00	12.53
6MGS-34-JC03									
	RLA027	1.00	279	239				259.00	28.28
	RLA027	5.00	329	334				331.50	3.54
	RLA027	10.00	364	392				378.00	19.80
	RLA027	30.00	538	532				535.00	4.24
	RLA027	50.00	626	638				632.00	8.49
	RLA027	100.00	656	643				649.50	9.19
	RLA027	300.00	639	617				629.00	14.14
	-	1.00	211	217				214.00	4.24
	-	5.00	313	304				308.50	8.36
	-	10.00	341	342				341.50	0.71
	-	30.00	404	426				415.00	15.56
	-	50.00	475	407				441.00	48.08
	-	100.00	441	413				427.00	19.80
	-	300.00	368	406				384.00	22.63

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY S-9 : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIX/PLATE : 500UGS

T*-TOXIC
INTC-TOO NUMEROUS TO COUNT
NATC-NOT ABLE TO COUNT

G-PGS T-PPT
N-PGS P-PPH
M-PGS B-PPB
L-NLS I-MM
U-ULS C-UM

STATISTICAL ANALYSIS: MUTAGENICITY OF PURIFIED YELLOW
DYE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0003 LAB: G8BA ACTIVATION: + RLA027
STRAIN: TA102 DATE: 06/15/84 TECHNICIAN: MJK

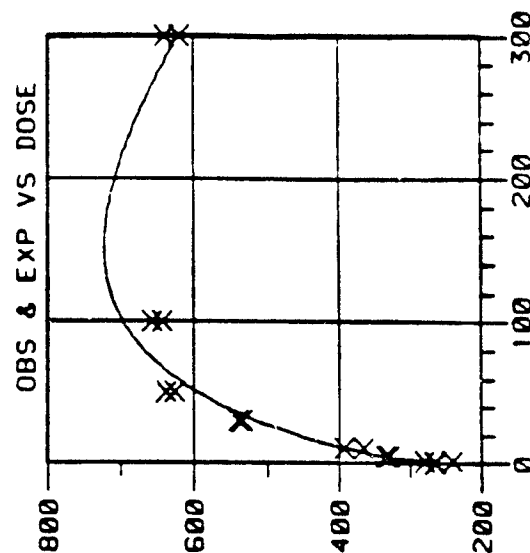
DOSE UNITS	PLATE COUNTS	MEAN	S.D.
.00 UGS	264 264 266	264.67	1.16
.50* UGS	1252 1377 1392 1370	1347.75	64.49
1.00 UGS	279 239	259.00	28.28
5.00 UGS	329 334	331.50	3.54
10.00 UGS	364 392	378.00	19.80
30.00 UGS	538 532	535.00	4.24
50.00 UGS	626 638	632.00	8.49
100.00 UGS	656 643	649.50	9.19
300.00 UGS	639 619	629.00	14.14

ESTS. 252.524 3.3777 7060 00372
B(0) B(1) B(2) B(3)

TEST CHI-SQUARE DF P LOCL
POISSON 4.77 9 .8539 -69.3218
ADEQUACY 18.35 4 .0011 -78.4985
TOXICITY 131.22 1 .0000 -144.1083
MUTAGENICITY 1013.78 2 .0000 -585.3907

AVERAGE SLOPE (NONLIN. MODEL) = 7.567
95% CONF. LIMITS = (6.535, 8.762)

AVERAGE SLOPE (LINEAR REG.) = 4.181
95% CONF. LIMITS = (3.031, 5.332)

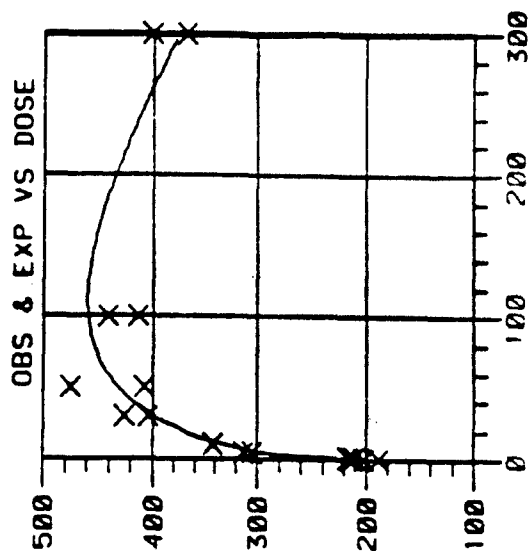


STATISTICAL ANALYSIS: MUTAGENICITY OF PURIFIED YELLOW
DYE IN *SALMONELLA TYPHIMURIUM*

SAMPLE ID: BMCS-84-0003 LAB: GBBA ACTIVATION: -
STRAIN: TA102 DATE: 06/15/84 TECHNICIAN: MJK

DOSE UNITS	PLATE COUNTS	MEAN	S. D.
0.00	UGS 202 188 213	201.00	12.53
30.00*	UGS 1380 1320 1383	1361.00	35.54
1.00	UGS 211 217	214.00	4.24
5.00	UGS 313 304	308.50	6.36
10.00	UGS 341 342	341.50	7.1
30.00	UGS 404 426	415.00	15.56
50.00	UGS 475 407	441.00	48.08
100.00	UGS 441 413	427.00	19.80
300.00	UGS 368 400	384.00	22.63

ESTS. 194.489 3.9460 4507 .00279
B(0) B(1) B(2) B(3)
TEST CHI-SQUARE DF P LOCL
POISSON 9.86 9 3623 -69.5632
ADEQUACY 17.08 4 .0019 -78.1015
TOXICITY 79.42 1 .0000 -117.8105
MUTAGENICITY 442.87 2 .0000 -299.5369
AVERAGE SLOPE (NONLIN. MODEL) = 6.032
95% CONF. LIMITS = (5.524, 6.587)
AVERAGE SLOPE (LINEAR REGR.) = 4.656
95% CONF. LIMITS = (3.219, 6.093)



MUTAGENICITY TESTING OF PURIFIED YELLOW DYE
IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE PURIFIED YELLOW
RESEARCH LAB: GBBA ON 06/01/84

08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION

STRAIN: TA104

COMPOUND	A C T	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE					MEAN	STD
			A	B	C	D	E		
POS CONTROL									
2-AA	RLA027	3.00	2393	2440	2457			2433.33	37.45
OTHER PLS	-	17.00	765	851	777			797.67	46.58
NEG CONTROL									
DIMETHYLSULF	RLA027	100.00U	312	311	340			321.00	16.46
	-	100.00U	262	323	271			285.33	32.93
BMGS-34-0003									
	RLA027	10.00	460	515				487.50	38.89
	RLA027	30.00	476	510				496.00	26.28
	RLA027	50.00	485	412				448.50	51.62
	RLA027	100.00	273	301				287.00	19.80
	RLA027	300.00	439	377				408.00	43.64
	-	10.00	381	370				379.50	2.12
	-	30.00	347	341				344.00	4.24
	-	50.00	330	340				338.00	11.31
	-	100.00	359	310				338.50	26.99
	-	300.00	309	297				303.00	8.49

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY S-9 : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIX/PLATE : 500UGS

T*-TOXIC
INTC-TOO NUMEROUS TO COUNT
NATC-NOT ABLE TO COUNT

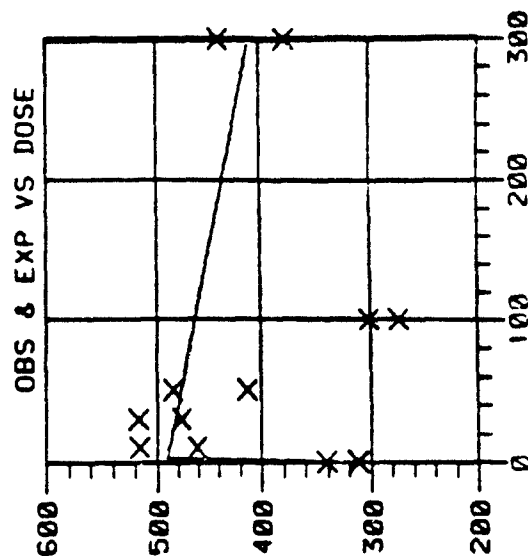
G-PGS T-PPT
N-NGS P-PPM
M-MGS B-PPH
L-NLS I-MM
U-ULS C-UM

STATISTICAL ANALYSIS: MUTAGENICITY OF PURIFIED YELLOW
DYE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0003 LAB: C88A ACTIVATION: + RLA027
STRAIN: TA104 DATE: 06/01/84 TECHNICIAN: MJK

DOSE UNITS	PLATE COUNTS	MEAN	S.D.
00	UGS 312 311 340	321.00	16.46
3.00*	UGS 2393 2440 2467	2433.33	37.45
10.00	UGS 460 515	487.50	38.80
30.00	UGS 476 516	496.00	28.28
50.00	UGS 485 412	448.5	51.62
100.00	UGS 273 301	287.00	19.80
300.00	UGS 439 377	408.00	43.84

ESTS.	321.000	5.1383	.0000	.00061
TEST	CHI-SQUARE	DF	P	LOGL
POISSON	18.42	7	.0102	-59.9766
ADEQUACY	158.35	2	.0000	-139.1502
TOXICITY	61.28	1	.0000	-169.7917
MUTAGENICITY	52.03	2	.0000	-165.1676
AVERAGE SLOPE (NONLIN. MODEL) =				.568
95% CONF. LIMITS = (			.443,	.729)
AVERAGE SLOPE (LINEAR REGR.) =				-.050
95% CONF. LIMITS = (			-.547,	.446)

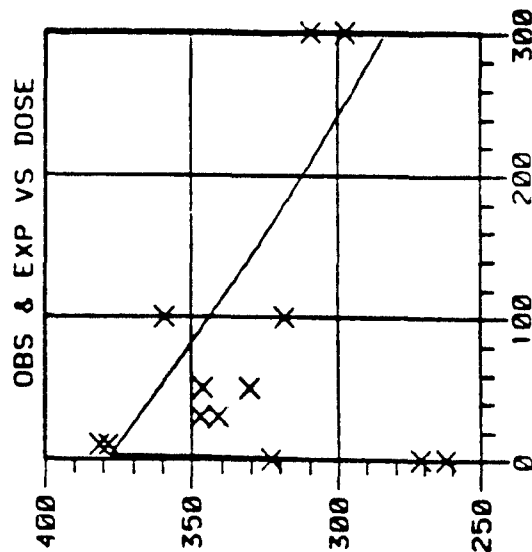


STATISTICAL ANALYSIS: MUTAGENICITY OF PURIFIED YELLOW
DYE IN SALMONELLA TYPHIMURIUM

SAMPLE ID, BMGS-84-0003 LAB: CBBA ACTIVATION: -
STRAIN, TA104 DATE, 06/01/84 TECHNICIAN: MJK

DOSE UNITS	PLATE COUNTS	MEAN	S.D.
00 UGS	262 323 271	285.33	32.93
17.00* UGS	765 851 777	797.57	46.58
10.00 UGS	381 378	379.50	2.12
30.00 UGS	347 341	344.00	4.24
50.00 UGS	330 346	338.00	11.31
100.00 UGS	359 318	338.50	28.99
300.00 UGS	309 297	303.00	8.49

ESTS. 285.337 4.5354 0000 00097
B(0) B(1) B(2) B(3)
TEST CHI-SQUARE DF P LOGL
POISSON 10.76 7.1493 -54.8752
ADEQUACY 9.07 2.0107 -59.4089
TOXICITY 13.62 1.0002 -66.2179
MUTAGENICITY 28.25 2.0000 -73.5332
AVERAGE SLOPE (NONLIN. MODEL) = 1.865
95% CONF. LIMITS = (1.349, 2.579)
AVERAGE SLOPE (LINEAR REGR.) = .721
95% CONF. LIMITS = (-.697, 2.138)



MUTAGENICITY TESTING OF PURIFIED YELLOW DYE
IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
 OF ARMY DYE PURIFIED YELLOW
 RESEARCH LAB: GBBA ON 06/03/84

08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION

STRAIN: TA104

COMPOUND	A C T	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE					MEAN	STD
			A	B	C	D	E		
FOS CONTROL									
2-AA	RLA027	3.00	2424	2346	2444			2405.33	50.65
OTHER POS	-	50.00	1657	1786	1736			1726.33	65.04
NEG CONTROL									
DIMETHYLSULF	RLA027	100.00U	350	316	290			318.67	30.09
	-	100.00U	241	252	242			238.33	15.18
BMGS-84-0003									
	RLA027	1.00	352	334				343.00	12.73
	RLA027	5.00	424	366				395.00	41.01
	RLA027	10.00	439	477				458.00	26.87
	RLA027	30.00	526	503				514.50	16.26
	RLA027	50.00	491	540				515.50	34.65
	RLA027	100.00	447	440				443.50	4.95
	RLA027	300.00	410	402				406.00	5.66
	-	1.00	263	254				258.50	6.36
	-	5.00	258	286				273.00	21.21
	-	10.00	291	301				296.00	7.07
	-	30.00	294	290				292.00	2.83
	-	50.00	315	307				311.00	5.66
	-	100.00	293	291				292.00	1.41
	-	300.00	294	284				299.00	7.07

PHENOCOPY CHECK : TRUE MUTANTS
 STERILITY S-9 : NOT CONTAMINATED
 SAMPLE STERILITY: NOT CONTAMINATED
 ACT MIX/PLATE : 500UGS

T*-TOXIC
 TNTC-TOO NUMEROUS TO COUNT
 NATC-NOT ABLE TO COUNT

G-PGS T-PPT
 N-NGS P-PPM
 M-MGS B-PPB
 L-NLS I-MM
 U-ULS C-MM

MUTAGENICITY TESTING OF PURIFIED YELLOW DYE
IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE PURIFIED YELLOW
RESEARCH LAB: GBBA, ON 06/05/84

08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION

STRAIN: TA104

POSITIVE CONTROL USED WAS METHYL GLYOXAL.

STATISTICAL ANALYSIS: MUTAGENICITY OF PURIFIED YELLOW
DYE IN *SALMONELLA* TYPHIMURIUM

SAMPLE ID: BMCS-84-0003 LAB: GBBA ACTIVATION: + RLA027
STRAIN: TA104 DATE: 06/05/84 TECHNICIAN: MJK

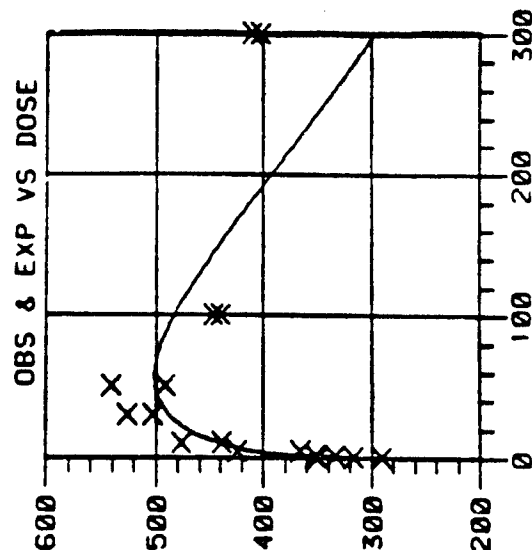
DOSE UNITS	PLATE COUNTS	MEAN	S.D.
00	UGS 350 316 290	318.67	30.00
3.00*	UGS 2424 2348 2444	2405.33	50.65
1.00	UGS 352 334	343.00	12.73
5.00	UGS 424 366	395.00	41.01
10.00	UGS 439 477	458.00	26.87
30.00	UGS 526 503	514.50	16.26
50.00	UGS 491 540	515.50	34.65
100.00	UGS 447 440	443.50	4.95
300.00	UGS 410 402	406.00	5.66

ESTS. 312.210 3 9616 4431 00394
B(0) B(1) B(2) B(3)

TEST CHI-SQUARE DF P LOGL
POISSON 14.97 9 .0919 -74.2715
ADEQUACY 88.65 4 .0000 -118.5981
TOXICITY 2.50 1 .1136 -119.8498
MUTAGENICITY 116.51 2 .0000 -176.8549

AVERAGE SLOPE (NONLIN. MODEL) = 7.905

AVERAGE SLOPE (LINEAR REGR.) = 6.283
95% CONF. LIMITS = (4.136, 8.430)
WARNING: 4 PARAMETER MODEL DID NOT CONVERGE



MUTAGENICITY TESTING OF PURIFIED YELLOW DYE
IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE PURIFIED YELLOW
RESEARCH LAB: G8BA, ON 06/05/84
TEST TYPE: STANDARD PLATE INCORPORATION

08/27/84

STRAIN: TA104

POSITIVE CONTROL USED WAS METHYL GLYOXAL.

STATISTICAL ANALYSIS: MUTAGENICITY OF PURIFIED YELLOW
DYE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0003 LAB. CBBA ACTIVATION: - HJK
STRAIN: TA104 DATE: 06/05/84 TECHNICIAN:

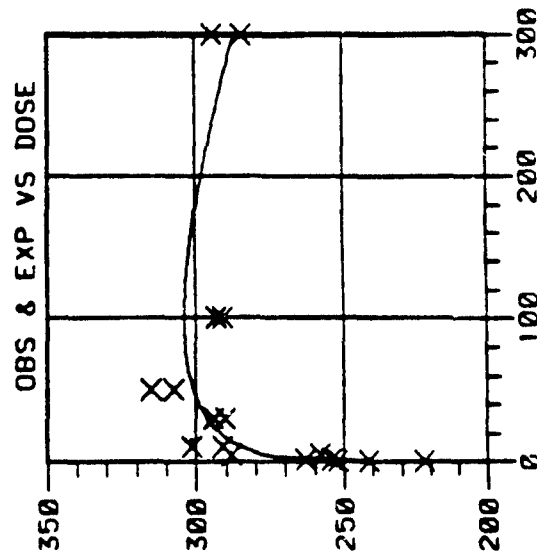
DOSE UNITS	PLATE COUNTS	MEAN	S.D.
00 UCS	241 252 222	238.33	15.18
50.00* UCS	1657 1786 1736	1726.33	65.04
1.00 UCS	263 254	258.50	6.36
5.00 UCS	258 288	273.00	21.21
10.00 UCS	291 301	296.00	7.37
30.00 UCS	294 290	292.00	2.83
50.00 UCS	315 307	311.00	5.56
100.00 UCS	293 291	292.00	1.41
300.00 UCS	294 284	289.00	7.07

A-144

ESTS. 237.591 3.2852 .2606 .00072
B(0) B(1) B(2) B(3)

TEST CHI-SQUARE DF P LOGL
POISSON 4.22 9 .8966 -65.5625
ADEQUACY 3.10 4 .5420 -67.1101
TOXICITY 4.01 1 .0452 -69.1151
MUTAGENICITY 31.25 2 .0000 -82.7328

AVERAGE SLOPE (NONLIN. MODEL) = 1.481
95% CONF. LIMITS = (.711, 3.084)
AVERAGE SLOPE (LINEAR REGR.) = 1.179
95% CONF. LIMITS = (.606, 1.753)



MUTAGENICITY TESTING OF PURIFIED YELLOW DYE
IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE PURIFIED YELLOW
RESEARCH LAB: 6B8A ON 06/01/84

08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION

STRAIN: TA1535

COMPOUND	A C T	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE					MEAN	STD
			A	B	C	D	E		
POS CONTROL									
MAAZIDE	-	3.00	1021	1031	959			1003.67	39.00
2-AA	RLA027	3.00	153	164	132			149.67	16.26
NEG CONTROL									
DIMETHYLSULF	RLA027	100.00U	26	24	29			26.33	2.52
	-	100.00U	37	44	33			38.00	5.57
BMGS-34-0003									
	RLA027	10.00	18	13				15.50	3.54
	RLA027	30.00	14	10				15.00	1.41
	RLA027	50.00	27	19				23.00	5.66
	RLA027	100.00	19	17				18.00	1.41
	RLA027	300.00	22	20				21.00	1.41
	-	10.00	29	42				35.50	9.19
	-	30.00	44	40				42.00	2.83
	-	50.00	36	32				34.00	2.83
	-	100.00	36	42				39.00	4.24
	-	300.00	42					42.00	0.00

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY S-9 : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIX/PLATE : 500UGS

T+--TOXIC
TNTC-TWO NUMEROUS TO COUNT
NATC-NOT ABLE TO COUNT

G-PGS T-PPT
N-NGS P-PPH
M-MGS 9-PPG
L-NLS :-OR
U-ULS C-UM

STATISTICAL ANALYSIS: MUTAGENICITY OF PURIFIED YELLOW
DYE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMCS-84-0003 LAB: GBBA ACTIVATION: + RL027
STRAIN: TA1535 DATE: 06/01/84 TECHNICIAN: MJK

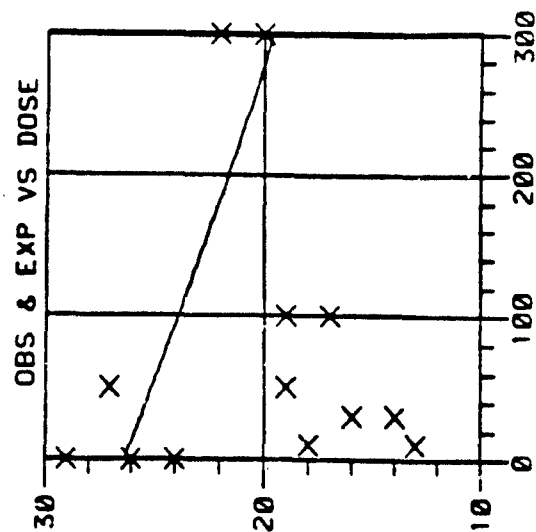
DOSE UNITS	PLATE COUNTS	MEAN	S.D.
00 UGS	26 24 29	26.33	2.52
3.00* UGS	153 164 132	149.67	16.26
10.00 UGS	18 13	15.50	3.54
30.00 UGS	14 16	15.00	1.41
50.00 UGS	27 19	23.00	5.66
100.00 UGS	19 17	18.00	1.41
300.00 UGS	22 20	21.00	1.41

ESTS. B(0) B(1) B(2) B(3)
26.272 -2.7822 .0002 .00099

TEST CHI-SQUARE DF P LOGL
POISSON 3.02 7 .8833 -32.9037
ADEQUACY 24.02 2 .0000 -44.9131
TOXICITY 7.01 1 .0081 -48.4184
MUTAGENICITY .01 2 .9950 -38.7526

AVERAGE SLOPE (NONLIN. MODEL) = .000
95% CONF. LIMITS = (.000, *****)

AVERAGE SLOPE (LINEAR REGR.) = -.001
95% CONF. LIMITS = (-.030, .027)



**MUTAGENICITY TESTING OF PURIFIED YELLOW DYE
IN SALMONELLA TYPHIMURIUM**

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE PURIFIED YELLOW
RESEARCH LAB: G88A ON 06/05/84

08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION

STRAIN: TA1535

COMPOUND	A C T	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE					MEAN	STD
			A	B	C	D	E		
POS CONTROL									
NAAZIDE	-	3.00	1068	1057	1018			1047.67	26.27
2-AA	RLA027	3.00	154	154	157			155.00	1.73
NEG CONTROL									
DIMETHYLSULF	RLA027	100.00U	32	33	18			27.67	6.39
	-	100.00U	55	50	40			48.33	7.64
EMGS-94-0003									
	RLA027	10.00	14	18				16.00	2.83
	RLA027	30.00	9	17				13.00	5.66
	RLA027	50.00	24	25				26.00	2.83
	RLA027	100.00	19	20				19.50	0.71
	RLA027	300.00	21	22				21.50	0.71
	-	10.00	42	55				48.50	9.19
	-	30.00	39	39				39.00	0.00
	-	50.00	50	37				43.50	9.19
	-	100.00	48	43				45.50	3.54
	-	300.00	51	50				50.50	0.71

PHENOCOPIY CHECK : TRUE MUTANTS
STERILITY S-9 : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIX/PLATE : 500UGS

T*-TOXIC
TNTC-TOO NUMEROUS TO COUNT
NATC-NOT ABLE TO COUNT

G-PGS T-PPT
N-MGS P-PPM
M-MGS B-PPB
L-NLS I-MM
U-ULS C-UM

STATISTICAL ANALYSIS: MUTAGENICITY OF PURIFIED YELLOW
DYE IN SALMONELLA TYPHIMURIUM

SAMPLE ID, BMCS-84-0003 LAB: CBBA ACTIVATION: + RLA027
STRAIN: TA1535 DATE: 06/05/84 TECHNICIAN: MJK

DOSE UNITS	PLATE COUNTS	MEAN	S.D.
00 UGS	32 33 18	27.67	8.30
3.00* UGS	154 154 157	155.00	1.73
10.00 UGS	14 18	16.00	2.83
30.00 UGS	9 17	13.00	5.66
50.00 UGS	24 28	26.00	2.83
100.00 UGS	19 20	19.50	.71
300.00 UGS	21 22	21.50	.71

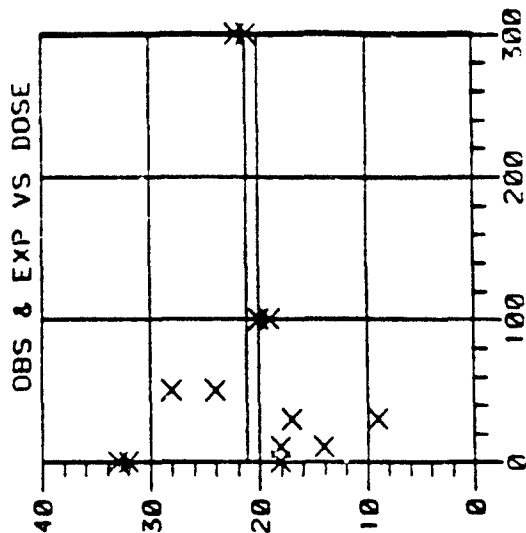
B(0) B(1) B(2)
ESTS. 21.160-320.3121 .0000

NO EVIDENCE OF TOXICITY

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	8.40	7	.2984	-35.9188
ADEQUACY	17.87	3	.0005	-44.8517
MUTAGENICITY	.00	21	.0000	-44.8517

AVERAGE SLOPE (NONLIN. MODEL) = .000
95% CONF. LIMITS = (.000, .000)

AVERAGE SLOPE (LINEAR REGR.) = -.002
95% CONF. LIMITS = (-.041, .037)



STATISTICAL ANALYSIS: MUTAGENICITY OF PURIFIED YELLOW
DYE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0003 LAB: CBBA ACTIVATION: -
STRAIN: TA1535 DATE: 06/05/84 TECHNICIAN: NJK

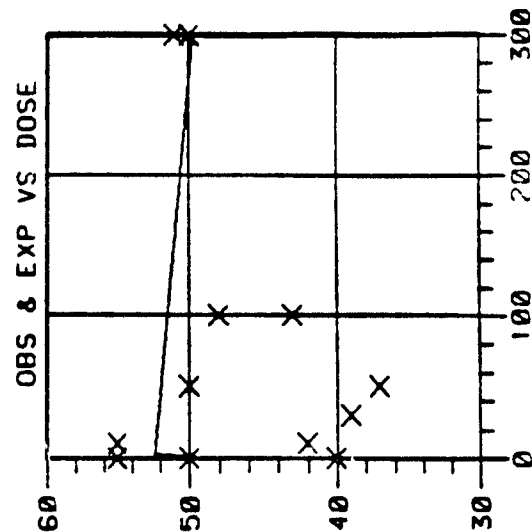
DOSE UNITS	PLATE COUNTS	MEAN	S.D.
00 UGS	55	48.33	7.64
3.00* UGS	1068	1047.67	26.27
10.00 UGS	42	48.50	9.19
30.00 UGS	39	39.00	.00
50.00 UGS	50	43.50	9.19
100.00 UGS	48	45.50	3.54
300.00 UGS	51	50.50	.71

ESTS. B(0) 48.332 B(1) 1.3938 B(2) .0000 B(3) .00017

TEST CHI-SQUARE DF P LOGL
POISSON 6.38 7 .4958 -40.0330
ADEQUACY 12.11 2 .0023 -46.0889
TOXICITY 6.92 1 .0085 -49.5500
MUTAGENICITY .01 2 .9950 -42.0575

AVERAGE SLOPE (NONLIN. MODEL) = .013
95% CONF. LIMITS = (.000, 226.752)

AVERAGE SLOPE (LINEAR REG.) = .014
95% CONF. LIMITS = (-.021, .049)
WARNING: 3 PARAMETER MODEL DID NOT CONVERGE



MUTAGENICITY TESTING OF PURIFIED YELLOW DYE
IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
 OF ARMY DYE PURIFIED YELLOW
 RESEARCH LAB: GBBA ON 06/01/84

08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION

STRAIN: TA1537

COMPOUND	A L T	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE					MEAN	STD
			A	B	C	D	E		
POS CONTROL									
9-AA	-	100.00	1129	1111	860			1040.00	138.86
2-AA	RLA027	5.00	304	380	343			342.33	36.00
NEG CONTROL									
DIMETHYLSULF	RLA027	100.00U	17	15	27			19.67	6.43
	-	100.00U	14	18	8			13.33	5.03
BMGS-34-UGG3									
	RLA027	10.00	18	19				18.50	0.71
	RLA027	30.00	36	33				34.50	2.12
	RLA027	50.00	39	36				38.50	0.71
	RLA027	100.00	24	22				23.00	1.41
	RLA027	300.00	21	19				20.00	1.41
	-	10.00	19	12				15.50	4.95
	-	30.00	14	17				15.50	2.12
	-	50.00	20	7				13.50	9.19
	-	100.00	26	14				20.00	8.49
	-	300.00	12	10				11.00	1.41

PHENOCOPY CHECK : TRUE MUTANTS
 STERILITY S-9 : NOT CONTAMINATED
 SAMPLE STERILITY: NOT CONTAMINATED
 ACT MIX/PLATE : SUGGS

T*-TOXIC
 INTC-TCO NUMEROUS TO COUNT
 NATC-NOT ABLE TO COUNT

G-PGS T-PPT
 N-PGS P-PPM
 M-PGS S-PPB
 L-NLS I-MM
 U-ULS C-UM

STATISTICAL ANALYSIS: MUTAGENICITY OF PURIFIED YELLOW
DYE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMCS-84-0003 LAB: GBBA ACTIVATION: + RLA027
STRAIN: TA1537 DATE: 06/01/84 TECHNICIAN: HJK

DOSE UNITS PLATE COUNTS			MEAN S.D.	
.00	UCS	17 15 27	19.67	6.43
3.00*	UCS	304 380 343	342.33	38.00
10.00	UCS	18 19	18.50	.71
30.00	UCS	36 33	34.50	2.12
50.00	UCS	39 38	38.50	.71
100.00	UCS	24 22	23.00	1.41
300.00	UCS	21 19	20.00	1.41

ESTS. B(0) 18.882 B(1) 5697 B(2) .6336 B(3) .00501

TEST CHI-SQUARE DF P LOGL

POISSON 4.56 7 .7134 -34.8443

ADEQUACY 13.94 2 .0009 -41.8119

TOXICITY 12.85 1 .0003 -48.2346

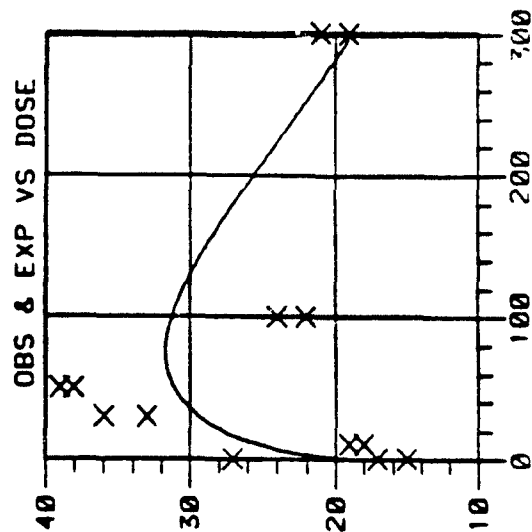
MUTAGENICITY 13.83 2 .0010 -48.7252

AVERAGE SLOPE (NONLIN. MODEL) = .422

95% CONF. LIMITS = .191, .929

AVERAGE SLOPE (LINEAR REGR.) = .426

95% CONF. LIMITS = .266, .587

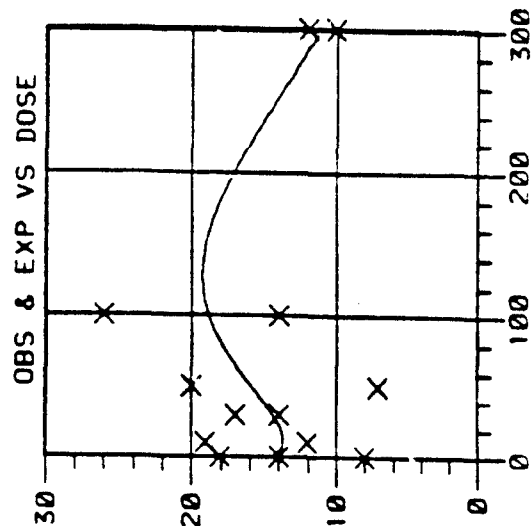


STATISTICAL ANALYSIS: MUTAGENICITY OF PURIFIED YELLOW
DYE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMCS-84-0003 LAB: CBBA ACTIVATION: -
STRAIN: TA1537 DATE: 06/01/84 TECHNICIAN: MJK

DOSE UNITS	PLATE COUNTS	MEAN	S.D.
00 UCS	14 18 8	13.33	5.03
100.00* UCS	1129 1111 880	1040.00	138.86
10.00 UCS	19 12	15.50	4.95
30.00 UCS	14 17	15.50	2.12
50.00 UCS	20 7	13.50	9.19
100.00 UCS	26 14	20.00	8.49
300.00 UCS	12 10	11.00	1.41

ESTS.	14.348	-3.9506	1.6535	.01042
TEST	CHI-SQUARE	DF	P	LOGL
POISSON	15.71	7	.0279	-37.2216
ADEQUACY	1.74	2	.4189	-38.0917
TOXICITY	5.16	1	.0231	-40.6723
MUTAGENICITY	3.73	2	.1552	-39.9550
AVERAGE SLOPE (NONLIN. MODEL)	= .248			
95% CONF. LIMITS	= (.069, .887)			
AVERAGE SLOPE (LINEAR REGR.)	= .003			
95% CONF. LIMITS	= (-.168, .174)			



MUTAGENICITY TESTING OF PURIFIED YELLOW DYE
IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
 OF ARMY DYE PURIFIED YELLOW
 RESEARCH LAB: 688A ON 06/05/84

08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION

STRAIN: TA1537

COMPOUND	A C T	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE					MEAN	STD
			A	B	C	D	E		
POS CONTROL									
9-AA	-	100.00	1258	1335	1452			1348.33	97.68
2-AA	RLA027	5.00	561	472	408			480.33	76.84
NEG CONTROL									
DIMETHYLSULF	RLA027	100.00u	20	16	17			17.67	2.08
	-	100.00u	8	6	9			8.33	0.58
6MGS-34-UC03									
	RLA027	10.00	28	26				27.00	1.41
	RLA027	30.00	42	36				39.00	4.24
	RLA027	50.00	30	30				30.00	0.00
	RLA027	100.00	37	31				34.00	4.24
	RLA027	300.00	19	30				24.50	7.79
	-	10.00	20	20				20.00	0.00
	-	30.00	17	19				18.00	1.41
	-	50.00	14	12				13.00	1.41
	-	100.00	13	19				16.00	4.24
	-	300.00	21	12				16.50	6.36

PHENOCOPY CHECK : TRUE MUTANTS
 STERILITY S-9 : NOT CONTAMINATED
 SAMPLE STERILITY: NOT CONTAMINATED
 ACT MIX/PLATE : 500UGS

T+ - TOXIC
 TNTC - TOO NUMEROUS TO COUNT
 NATC - NOT ABLE TO COUNT

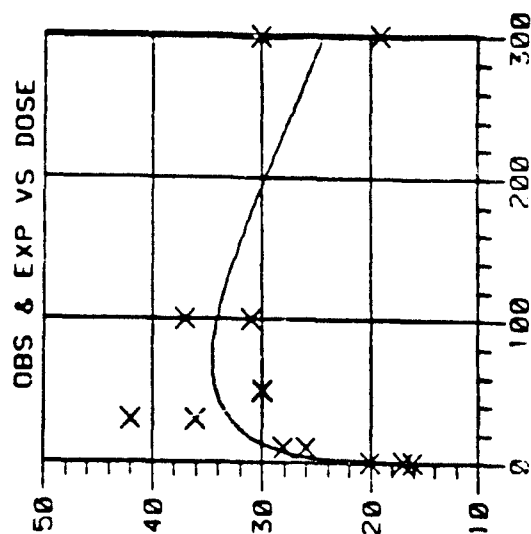
G-PGS T-PPT
 N-PGS P-PPH
 M-PGS B-PPB
 L-NLS I-PH
 U-ULS C-UP

STATISTICAL ANALYSIS: MUTAGENICITY OF PURIFIED YELLOW
DYE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0003 LAB: GB8A ACTIVATION: + RLA027
STRAIN: TA1537 DATE: 06/05/84 TECHNICIAN: MJK

DOSE UNITS	PLATE COUNTS	MEAN	S.D.
00	UGS 20 16 17	17.67	2.08
3.00*	UGS 561 472 408	480.33	76.84
10.00	UGS 28 26	27.00	1.41
30.00	UGS 42 36	39.00	4.24
50.00	UGS 30 30	30.00	.00
100.00	UGS 37 31	34.00	4.24
300.00	UGS 19 30	24.50	7.78

ESTS.	B(0)	B(1)	B(2)	B(3)
17.613	1.6844	3629	00300	
TEST	CHI-SQUARE	DF	P	LOGL
POISSON	4.02	7	.7769	-35.3616
ADEQUACY	3.52	2	.1723	-37.1201
TOXICITY	5.81	1	.0159	-40.0245
MUTAGENICITY	21.64	2	.0000	-47.9387
AVERAGE SLOPE (NONLIN. MODEL) = .284				
95% CONF. LIMITS = (.062, 1.292)				
AVERAGE SLOPE (LINEAR REGR.) = .135				
95% CONF. LIMITS = (.016, .253)				



STATISTICAL ANALYSIS: MUTAGENICITY OF PURIFIED YELLOW
DYE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0003 LAB: CBBA ACTIVATION: -
STRAIN: TA1537 DATE: 06/05/84 TECHNICIAN: MJK

DOSE UNITS	PLATE COUNTS	MEAN	S.D.
00 UCS	8 8 9	8.33	.58
100.00* UCS	1258 1335 1452	1348.33	97.69
10.00 UCS	20 20	20.00	.00
30.00 UCS	17 19	18.00	1.41
50.00 UCS	14 12	13.00	1.41
100.00 UCS	13 19	16.00	4.24
300.00 UCS	21 12	16.50	6.36

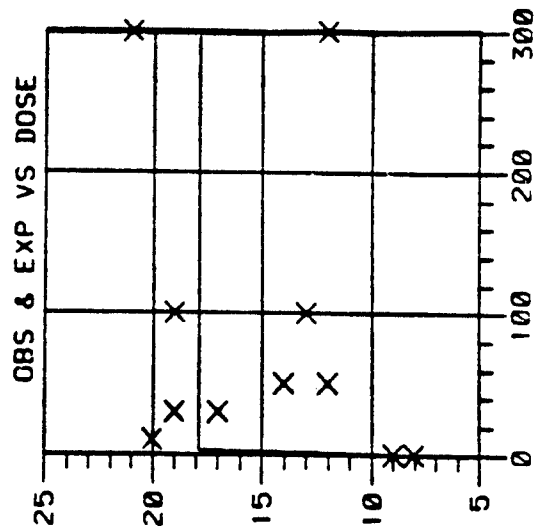
ESTS. B(0) 8.251 B(1) 2.2653 B(2) .0000

NO EVIDENCE OF TOXICITY

TEST CHI-SQUARE DF P LOGL
POISSON 3.92 7 .7884 -31.1534
ADEQUACY 4.07 3 .2542 -33.1877
MUTAGENICITY 11.62 2 .0030 -38.9962

AVERAGE SLOPE (NONLIN. MODEL) = .096
95% CONF. LIMITS = (.012, .781)

AVERAGE SLOPE (LINEAR REG.) = .035
95% CONF. LIMITS = (-.048, .118)



MUTAGENICITY TESTING OF PURIFIED YELLOW DYE
IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
 OF ARMY DYE PURIFIED YELLOW
 RESEARCH LAB: 688A ON 06/01/84

08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION

STRAIN: TA1538

COMPOUND	A C T	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE					MEAN	STD
			A	B	C	D	E		
POS CONTROL									
2-NF	-	3.00	476	432	512			473.33	40.07
2-AA	RLA027	0.50	704	729	692			708.33	16.88
NEG CONTROL									
DIMETHYLSULF	RLA027	100.00U	26	25	24			25.00	1.00
	-	100.00U	17	13	18			16.00	2.65
BMGS-34-C003									
	RLA027	10.00	25	32				31.50	9.19
	RLA027	30.00	29	28				28.50	0.71
	RLA027	50.00	21	37				29.00	11.31
	RLA027	100.00	17	42				29.50	17.68
	RLA027	300.00	28	20				24.00	5.66
	-	10.00	26	19				22.50	4.95
	-	30.00	19	18				18.50	0.71
	-	50.00	20	17				18.50	2.12
	-	100.00	15	19				17.00	2.83
	-	300.00	21	17				19.00	2.83

PHENOCOPY CHECK : TRUE MUTANTS
 STERILITY S-9 : NOT CONTAMINATED
 SAMPLE STERILITY: NOT CONTAMINATED
 ACT MIX/PLATE : 500UGS

T*-TOXIC
 TNC-TOO NUMEROUS TO COUNT
 NATC-NOT ABLE TO COUNT

G-PGS T-PPT
 N-NGS P-PPM
 M-MGS B-PPB
 L-NLS I-MM
 U-ULS C-UM

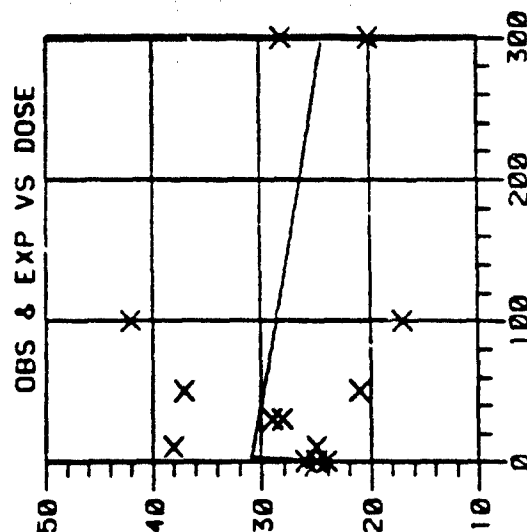
STATISTICAL ANALYSIS: MUTAGENICITY OF PURIFIED YELLOW
DYE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMCS-84-0003 LAB: CBBA ACTIVATION: + RLA027
STRAIN: TA1538 DATE: 06/01/84 TECHNICIAN: MJK

DOSE UNITS	PLATE COUNTS	MEAN	S.D.
.00 UCS	26 25 24	25.00	1.00
.50* UCS	704 729 692	708.33	18.88
10.00 UCS	25 38	31.50	9.19
30.00 UCS	29 28	28.50	.71
50.00 UCS	21 37	29.00	11.31
100.00 UCS	17 42	29.50	17.68
300.00 UCS	28 20	24.00	5.66

ESTS.	25.000	1.7852	.0000	.00080
TEST	CHI-SQUARE	DF	P	LOGL
POISSON	19.12	7	.0078	-43.1435
ADEQUACY	.35	2	.8409	-43.3168
TOXICITY	6.48	1	.0109	-46.5550
MUTAGENICITY	2.19	2	.3347	-44.4113

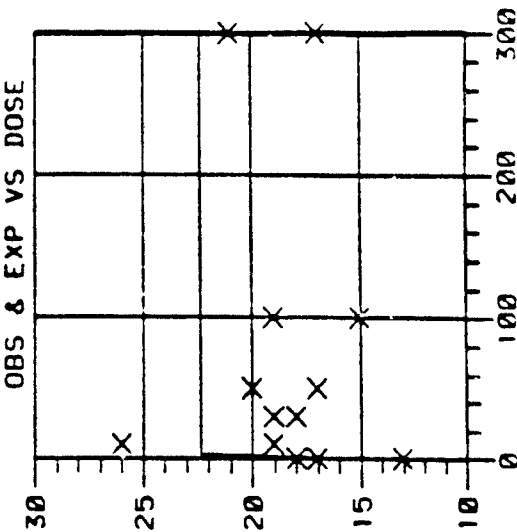
AVERAGE SLOPE (NONLIN. MODEL) = .119
95% CONF. LIMITS = (.000, 48.867)
AVERAGE SLOPE (LINEAR REGR.) = .053
95% CONF. LIMITS = (-.156, .262)



STATISTICAL ANALYSIS: MUTAGENICITY OF PURIFIED YELLOW
DYE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0003 LAB: CBBA ACTIVATION: -
STRAIN: TA1538 DATE: 06/01/84 TECHNICIAN: HJK

DOSE UNITS	PLATE COUNTS	MEAN	S.D.
00 UCS	17 13 18	16.00	2.65
3.00* UCS	476 432 512	473.33	40.07
10.00 UCS	26 19	22.50	4.95
30.00 UCS	19 18	18.50	.71
50.00 UCS	20 17	18.50	2.12
100.00 UCS	15 19	17.00	2.83
300.00 UCS	21 17	19.00	2.83



ESTS. 16.000 1.8443 .0000
B(0) B(1) B(2)

NO EVIDENCE OF TOXICITY

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	3.13	7	.8731	-32.4292
ADEQUACY	6.60	3	.0858	-35.7287
MUTAGENICITY	.01	2	.9950	-33.0027

AVERAGE SLOPE (NONLIN. MODEL) = .126
95% CONF. LIMITS = (.030, .542)

AVERAGE SLOPE (LINEAR REGR.) = .021
95% CONF. LIMITS = (-.104, .145)

MUTAGENICITY TESTING OF PURIFIED YELLOW DYE
IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE PURIFIED YELLOW
RESEARCH LAB: GBSA ON 06/05/84

08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION

STRAIN: TA1538

COMPOUND	A C T	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE					MEAN	STD
			A	B	C	D	E		
POS CONTROL									
2-NF	-	3.00	615	652	628			631.67	18.77
2-AA	RLA027	0.50	1061	1063	1052			1058.67	5.86
NEG CONTROL									
DIMETHYLSULF	RLA027	100.00U	41	19	36			32.00	11.53
	-	100.00U	18	13	15			15.33	2.52
3MGS-84-0003									
	RLA027	10.00	32	30				31.00	1.41
	RLA027	30.00	44	45				44.50	0.71
	RLA027	50.00	27	33				30.00	4.24
	RLA027	100.00	30	32				31.00	1.41
	RLA027	300.00	29	32				30.50	2.12
	-	10.00	14	14				14.00	0.00
	-	30.00	20	16				19.00	1.41
	-	50.00	9	10				12.50	4.95
	-	100.00	19	14				16.50	3.54
	-	300.00	9	17				13.00	5.66

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY S-9 : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIX/PLATE : 50UGS

T*-TOXIC
TNTC-TOO NUMEROUS TO COUNT
NATC-NOT ABLE TO COUNT

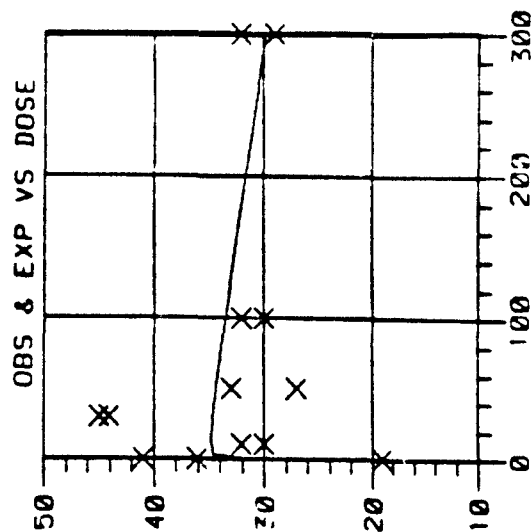
G-PGS T-PPT
N-NGS P-PPM
M-MGS B-PPB
L-NLS I-IRM
U-ULS C-UM

STATISTICAL ANALYSIS: MUTAGENICITY OF PURIFIED YELLOW
DYE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0003 LAB: CSBA ACTIVATION: + RLA027
STRAIN: TA1538 DATE: 06/05/84 TECHNICIAN: MJK

DOSE UNITS	PLATE COUNTS	MEAN	S.D.
.00 UGS	41 19 36	32.00	11.53
.50* UGS	1061 1063 1052	1058.67	5.86
10.00 UGS	32 30	31.00	1.41
30.00 UGS	44 45	44.50	.71
50.00 UGS	27 33	30.00	4.24
100.00 UGS	30 32	31.00	1.41
300.00 UGS	29 32	30.50	2.12

ESTS.	31.906	B(1)	B(11)	B(12)	B(13)
TEST	CHI-SQUARE	DF	P	LOGL	
POISSON	9.20	7	.2386	-39.5176	
ADEQUACY	7.56	2	.0228	-43.2975	
TOXICITY	6.87	1	.0088	-46.7338	
MUTAGENICITY	.58	2	.7471	-43.5890	
AVERAGE SLOPE (NONLIN. MODEL) =				.070	
95% CONF. LIMITS = (			.001,	5.188)	
AVERAGE SLOPE (LINEAR REGR.) =				.044	
95% CONF. LIMITS = (				-.263,	

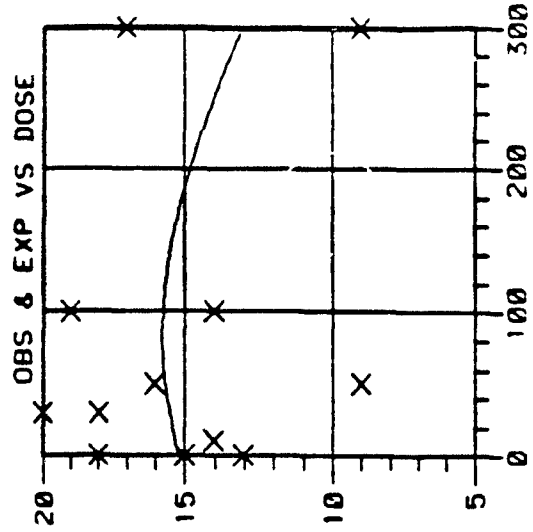


STATISTICAL ANALYSIS: MUTAGENICITY OF PURIFIED YELLOW
DYE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0003 LAB: G8BA ACTIVATION: -
STRAIN: TA1538 DATE: 06/05/84 TECHNICIAN: MJK

DOSE UNITS PLATE COUNTS			MEAN	S.D.
.00	UGS	18 13 15	15.33	2.52
3.00*	UGS	615 652 628	631.67	18.77
10.00	UGS	14 14	14.00	.00
30.00	UGS	20 18	19.00	1.41
50.00	UGS	9 16	12.50	4.95
100.00	UGS	19 14	16.50	3.54
300.00	UGS	9 17	13.00	5.66

ESTS. B(0) B(1) B(2) B(3) ---
 15.205 -2.8680 1.0597 .00366
 TEST CHI-SQUARE DF P LOGL ---
 POISSON 6.11 7 5269 -32.5657
 ADEQUACY 3.14 2 2079 -34.1366
 TOXICITY 1.00 1 3176 -34.6360
 MUTAGENICITY .19 2 9081 -34.2330
 AVERAGE SLOPE (NONLIN. MODEL) = .072
 95% CONF. LIMITS = (-.300, .209.011)
 AVERAGE SLOPE (LINEAR REGR.) = -.021
 95% CONF. LIMITS = (-.139, .098)



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MUTAGENICITY TESTING OF PURIFIED YELLOW DYE IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE PURIFIED YELLOW
RESEARCH LAB: G88A ON 03/30/84

08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION

STRAIN: TA98

COMPOUND	A C T	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE					MEAN	STD
			A	B	C	D	E		
POS CONTROL									
2-NF	-	3.00	300	312	315			309.00	7.94
2-AA	RLA026	0.50	575	837	853			855.00	19.08
NEG CONTROL									
DIMETHYLSULF	RLA026	100.00u	60	41	48			49.67	9.61
	-	100.00u	28	33	30			30.33	2.52
BMGS-94-0003									
	RLA026	1.00	49	32				40.50	12.02
	RLA026	5.00	48	46				48.00	0.00
	RLA026	10.00	45	37				41.00	5.66
	RLA026	30.00	37	50				43.50	9.19
	RLA026	50.00	43	61				52.00	12.73
	RLA026	100.00	45	43				44.00	1.41
	RLA026	300.00	37	22				29.50	10.61
	RLA026	500.00	32	35				33.50	2.12
	RLA026	1000.00	36	35				35.50	0.71
	-	1.00	21	26				23.50	3.54
	-	5.00	26	25				25.50	0.71
	-	10.00	25	26				26.50	2.12
	-	30.00	21	24				22.50	2.12
	-	50.00	22	30				26.00	5.66
	-	100.00	17	24				20.50	4.95
	-	300.00	43	27				35.00	11.31
	-	500.00	20	26				23.00	4.24
	-	1000.00	23	25				24.00	1.41

PHENOCOPIY CHECK : TRUE MUTANTS
STERILITY S-9 : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIX/PLATE : 50UGS

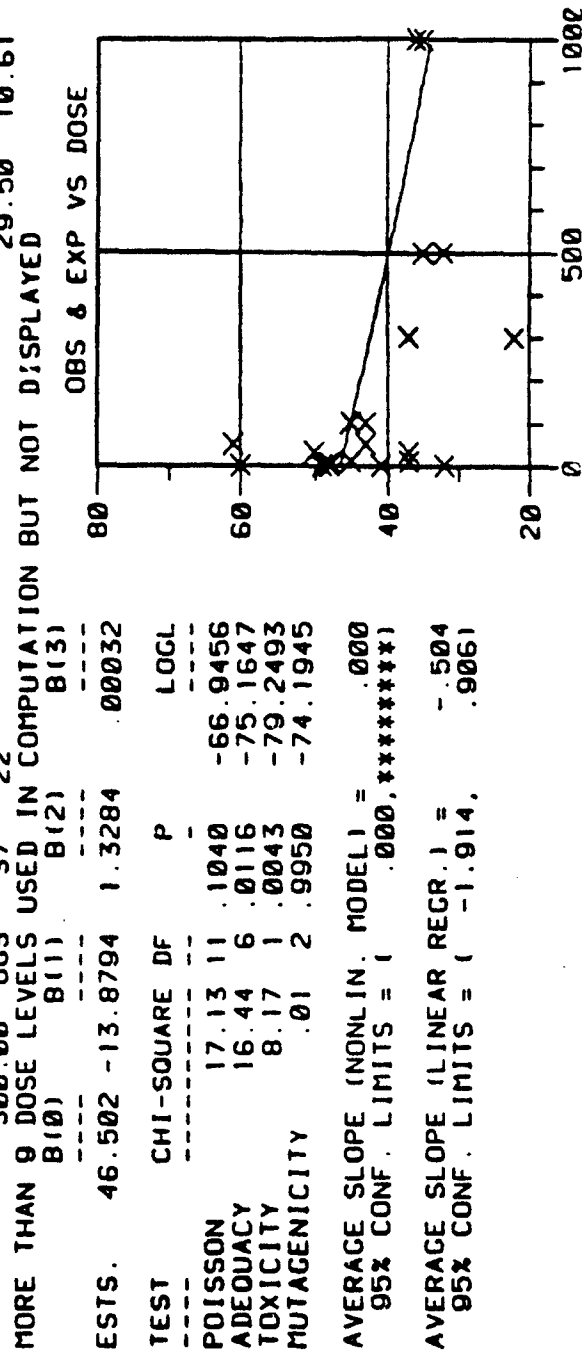
T*-TOXIC
TNTC-TWO NUMEROUS TO COUNT
NATC-NOT ABLE TO COUNT

G-PGS T-PPT
N-NGS P-PPM
M-MGS B-PPB
L-NLS I-MM
U-ULS C-UM

STATISTICAL ANALYSIS: MUTAGENICITY OF PURIFIED YELLOW
DYE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMCS-84-0003 LAB: G8BA ACTIVATION: + RLA026
STRAIN: TA98 DATE: 03/30/84 TECHNICIAN: MJK

DOSE UNITS PLATE COUNTS				MEAN	S.D.
.00	UGS	60	41	48	49.67
.50*	UGS	875	837	853	9.61
1.00	UGS	49	32		855.00
5.00	UGS	48	48		19.08
10.00	UGS	45	37		40.50
30.00	UGS	37	50		12.02
50.00	UGS	43	61		48.00
100.00	UGS	45	43		.00
300.00	UGS	37	22		41.00
MORE THAN 9 DOSE LEVELS USED IN COMPUTATION BUT NOT DISPLAYED					5.66
B(10)	B(11)	B(12)	B(13)		43.50
46.502	-13.8794	1.3284	.00032		9.19
ESTS.	CHI-SQUARE	DF	P	LOGL	52.00
	POISSON	17.13	11	1040	12.73
	ADEQUACY	16.44	6	.0116	44.00
	TOXICITY	8.17	1	.0043	1.41
	MUTAGENICITY	.01	2	.9950	10.61



AVERAGE SLOPE (NONLIN. MODEL) = .000
95% CONF. LIMITS = (.000, *****)
AVERAGE SLOPE (LINEAR REGR.) = -.504
95% CONF. LIMITS = (-1.914, .906)

STATISTICAL ANALYSIS: MUTAGENICITY OF PURIFIED YELLOW
DYE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMCS-84-0003 LAB: CB8A ACTIVATION: -
STRAIN: TA98 DATE: 03/30/84 TECHNICIAN: MJK

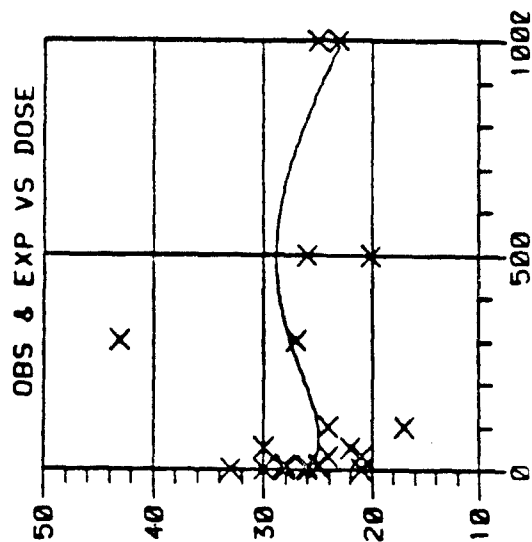
DOSE UNITS	PLATE COUNTS	MEAN	S.D.
00 UGS	28 33 30	30.33	2.52
3.00* UGS	300 312 315	309.00	7.94
1.00 UGS	21 26	23.50	3.54
5.00 UGS	26 25	25.50	.71
10.00 UGS	25 28	26.50	2.12
30.00 UGS	21 24	22.50	2.12
50.00 UGS	22 30	26.00	5.66
100.00 UGS	17 24	20.50	4.95
300.00 UGS	43 27	35.00	11.31

MORE THAN 9 DOSE LEVELS USED IN COMPUTATION BUT NOT DISPLAYED

ESTS. 26.262 -5.6194 1.5624 .00218
B(0) B(1) B(2) B(3)

TEST CHI-SQUARE DF P LOGL
POISSON 8.29 11 .6873 -57.5101
ADEQUACY 11.20 6 .0823 -63.1109
TOXICITY 1.73 1 .1890 -63.9737
MUTAGENICITY 1.40 2 .4956 -63.8130

AVERAGE SLOPE (NONLIN. MODEL) = .090
95% CONF. LIMITS = (.013, .620)
AVERAGE SLOPE (LINEAR REGR.) = .024
95% CONF. LIMITS = (-.004, .052)



MUTAGENICITY TESTING OF PURIFIED YELLOW DYE
IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
 OF ARMY DYE PURIFIED YELLOW
 RESEARCH LAB: G88A ON 04/06/84

08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION

STRAIN: TA98

COMPOUND	A C T	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE					MEAN	STD
			A	B	C	D	E		
POS CONTROL									
2-NF	RLA026	0.50	740	825	817			794.00	46.94
	-	3.00	250	270	255			258.33	10.41
NEG CONTROL									
DIMETHYLSULF	RLA026	100.00U	42	31	43			38.67	6.66
	-	100.00U	23	29	20			24.00	4.58
BMGS-34-0003									
	RLA026	1.00	24	39				31.50	10.61
	RLA026	5.00	42	42				42.00	0.00
	RLA026	10.00	52	49				50.50	2.12
	RLA026	30.00	50	74				62.00	16.97
	RLA026	50.00	52	54				53.00	1.41
	RLA026	100.00	63	62				62.50	0.71
	RLA026	300.00	36	32				34.00	2.83
	-	1.00	37	28				32.50	6.36
	-	5.00	29	20				24.50	6.36
	-	10.00	28	24				26.00	2.83
	-	30.00	39	16				28.50	14.85
	-	50.00	27	40				33.50	9.19
	-	100.00	20	21				20.50	0.71
	-	300.00	29	32				30.00	2.83

PHENOCOPY CHECK : TRUE MUTANTS
 STERILITY S-9 : NOT CONTAMINATED
 SAMPLE STERILITY: NOT CONTAMINATED
 ACT MIX/PLATE : 500UGS

T--TOXIC
 TNC-TWO NUMEROUS TO COUNT
 NATC-NOT ABLE TO COUNT

G-PGS T-PPT
 N-NGS P-PPH
 M-MGS B-PPB
 L-NLS I-IRH
 U-ULS C-UM

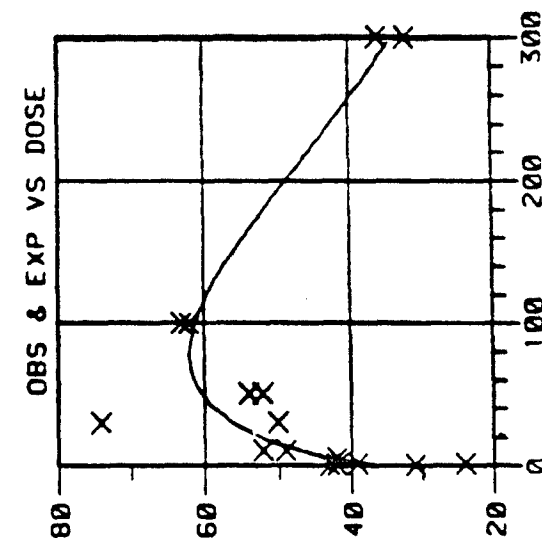
STATISTICAL ANALYSIS: MUTAGENICITY OF PURIFIED YELLOW
DYE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0003 LAB: CBBA ACTIVATION: + RL0026
STRAIN: TA98 DATE: 04/06/84 TECHNICIAN: MJK

DOSE UNITS	PLATE COUNTS	MEAN	S.D.
00 UGS	42 31 43	38.67	6.66
50* UGS	740 825 817	794.00	46.94
1.00 UGS	24 39	31.50	10.61
5.00 UGS	42 42	42.00	.00
10.00 UGS	52 40	50.50	2.12
30.00 UGS	50 74	62.00	16.97
50.00 UGS	52 54	53.00	1.41
100.00 UGS	63 62	62.50	.71
300.00 UGS	36 32	34.00	2.83

A-167

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	10.88	9	.2840	-53.4546
ADEQUACY	6.82	4	.1459	-56.8628
TOXICITY	30.69	1	.0000	-72.2075
MUTAGENICITY	37.36	2	.0000	-75.5451
AVERAGE SLOPE (NONLIN. MODEL) = .756				
95% CONF. LIMITS = (.240, 2.381)				
AVERAGE SLOPE (LINEAR REGR.) = .251				
95% CONF. LIMITS = (.104, .399)				

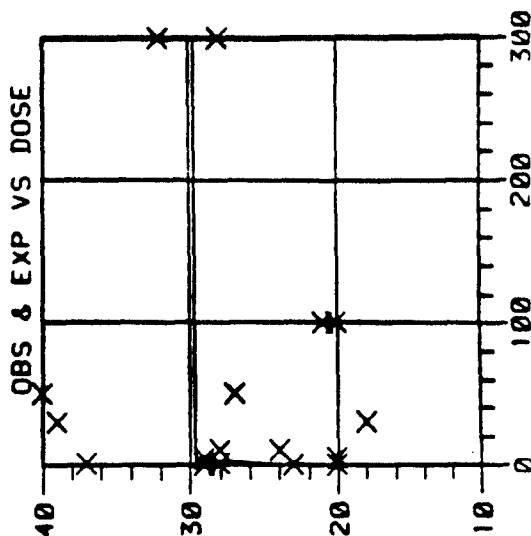


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STATISTICAL ANALYSIS: MUTAGENICITY OF PURIFIED YELLOW
DYE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMCS-84-0003 LAB: GB5A ACTIVATION: -
STRAIN: TA98 DATE: 04/06/84 TECHNICIAN: MJK

DOSE UNITS	PLATE COUNTS	MEAN	S.D.
.00 UCS	23 29 20	24.00	4.58
3.00* UCS	250 270 255	258.33	10.41
1.00 UCS	37 28	32.50	6.36
5.00 UCS	29 20	24.50	6.36
10.00 UCS	28 24	26.00	2.83
30.00 UCS	39 18	28.50	14.85
50.00 UCS	27 40	33.50	9.19
100.00 UCS	20 21	20.50	.71
300.00 UCS	28 32	30.00	2.83



ESTS. B(0) B(1) B(2)
24.000 1.7260 .0000

NO EVIDENCE OF TOXICITY

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	15.51	9	.0779	-51.3718
ADEQUACY	10.72	5	.0573	-56.7301
MUTAGENICITY	.07	2	.9650	-56.7657

AVERAGE SLOPE (NONLIN. MODEL) = .019
95% CONF. LIMITS = (.004, .089)

AVERAGE SLOPE (LINEAR REGR.) = .007
95% CONF. LIMITS = (-.030, .043)