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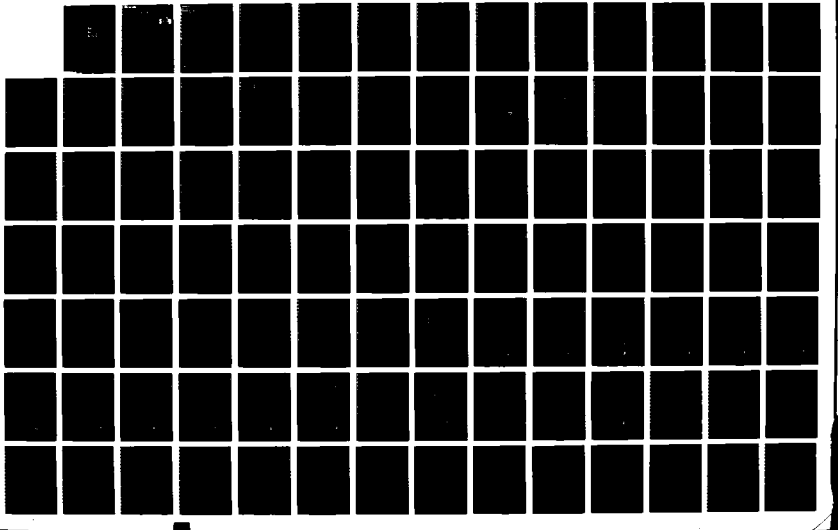

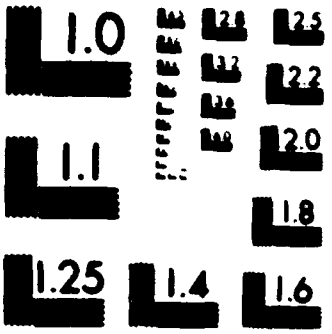

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CARCINOGENICITY OF DYES PRESENT IN COLORED SMOKE MUNITIONS

FINAL REPORT

Eugene F. Henderson, Principal Investigator
Antone L. Brooks
Ray L. Hanson

July 7, 1986

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Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
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Inhalation Toxicology Research Institute
Lovelace Biomedical and Environmental Research Institute
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The Lovelace Inhalation Toxicology Research Institute has been conducting genetic toxicology studies on organic dyes used in colored smoke munitions. This report summarizes the results of tests to determine the genotoxic potency of the dyes toward both bacteria and mammalian cells in vitro. The tests were conducted to detect mutations in bacteria and mammalian cells and to determine cell killing, alterations in cell cycle kinetics, sister chromatid exchanges, and chromosome aberration induction in mammalian cells. The dyes evaluated in the report include Solvent Red 24, Solvent Red 1, Disperse Red 15, Disperse Red 11 (~~Lot~~ 1 and ~~Lot~~ 2), and a mixture of Solvent Red 1, Disperse Red 11 and terephthalic acid. Disperse Red 11 (Lot 2) had chemical contamination that seemed to be responsible for some mutagenic activity.

In the Ames bacterial mutagenicity test, without the addition of liver microsomal fraction (S-9), there was no indication of mutagenic activity in any of the dyes evaluated. With the addition of S-9, only Disperse Red 15 and Disperse Red 11 (Lot 2) showed

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significant mutagenic activity. Mutagenic activity of Disperse Red 15 and Disperse Red 11 increased as a function of S-9 concentration. The level of mutagenic activity detected was low for all dyes tested. Solvent Red 1 was cytotoxic to mammalian cells, increased the level of SCE at a single high concentration, and showed little indication of inducing a mutagenic or clastogenic response. Disperse Red 11 (Lot 1) was not cytotoxic, mutagenic, or clastogenic toward mammalian cells but caused a slight but significant increase in the frequency of SCE induced. Disperse Red 11 (Lot 2) was not cytotoxic or clastogenic, but showed a slight but significant increase in mutations and the frequency of SCE induced. The combined results of these studies suggest that these dyes are not strong mutagenic or clastogenic agents.

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GENOTOXICITY OF DYES PRESENT IN COLORED SMOKE MUNITIONS

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The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents.

EXECUTIVE SUMMARY

The Lovelace-Inhalation Toxicology Research Institute has been conducting genetic toxicology studies on a series of organic dyes used in colored smoke munitions to provide information that will be useful in understanding the potential genetic and carcinogenic risks to workers engaged in fabrication of these smoke munitions. This report summarizes the genetic toxicology of these dyes evaluated with the Ames test using four different Salmonella tester strains and with mammalian cells from Chinese hamster ovaries. The dyes evaluated in this report include Solvent Red 24, Solvent Red 1, Disperse Red 15, Disperse Red 11 (Lot 1 and Lot 2), and a mixture of Solvent Red 1, Disperse Red 11 and Terephthalic acid.

Chemical characterization of Disperse Red 11 (Lot 2) indicated that it was contaminated with other chemicals which may have contributed to the mutagenic activity of this lot of dye. None of the dyes were mutagenic in any of the Ames Salmonella bacteria strains without the addition of S-9 or in Strain TA-1535 either with or without the addition of S-9. Disperse Red 15 and Disperse Red 11 (Lot 2) showed a weak positive mutagenic response following the addition of S-9. Solvent Red 1 was the only dye tested in mammalian cells that showed marked cytotoxicity. None of the dyes showed marked mutagenic activity or significant clastogenic activity in mammalian cells. Disperse Red 11 (Lot 2) induced a small but significant, concentration related increase in mutation frequency in mammalian cells. Disperse Red 11 and Solvent Red 1 both increased the frequency of SCE under some exposure conditions.

Taken as a whole these data suggest that, under the test conditions used none of the dyes evaluated act as strong mutagens or clastogens. The data also indicate that potential contamination of the dyes with other chemicals may influence biological activity as was observed in Disperse Red 11 (Lot 2).

FOREWORD

The authors acknowledge the contributions of all members of the staff of the Inhalation Toxicology Research Institute who helped in the completion of this work. The research was supported by the U.S. Army Medical Research and Development Command under a Memorandum of Understanding Agreement AT(29-2)-2138 with the Lovelace-Inhalation Toxicology Research Institute, which is operated for the U.S. Department of Energy under DOE Contract No DE-AC04-76EV01013.

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INTRODUCTION

The objective of this research was to determine the genotoxicity of dyes being considered for use in smoke munitions. The dyes were first characterized as to their chemical composition and purity. Next short-term cellular tests were used to rank the genotoxic potency of these dyes. This study has utilized a modified tier approach. We have measured genotoxicity, first in a bacterial mutagenesis assay, second in a mammalian cell mutation assay and third using both the induction of sister chromatid exchanges (SCE) and chromosome aberrations induced by exposure to the chemicals in vitro.

This experimental design was used to insure that tests were included that represent the different types of genetic damage. As a measure of gene mutations we have used the Salmonella mutagenesis assay, (Ames test) and the mammalian mutation assay, (Chinese hamster ovary cells with mutations detected at the hypoxanthine-guanine phosphoribosyl transferase gene locus (CHO/HGPRT)). Both of these assays were conducted with and without the addition of liver microsomal fractions (S-9) to insure adequate metabolism of the compounds being evaluated. The cytotoxicity of each of the dyes was determined in both of the mutation assays and the influence of the dyes on the cell cycle was determined in the mammalian cell mutation assay. In addition to measuring mutations it is important to evaluate the changes induced in mammalian cells at the chromosomal level. In the current studies chromosome aberrations were measured in first division cells and sister chromatid exchanges measured in second division cells.

CHEMICAL CHARACTERIZATION

A. Chemicals Tested

During the course of these studies we received 4 different dyes with two different lots of one of the dyes and terephthalic acid. For the sake of convenience we have designated the two lots of Disperse Red 11 as 'Lot 1' and 'Lot 2'. Lot 1 was manufactured by the Atlantic Chemical Co of New Jersey and Lot 2 was manufactured by Professional Chemical and Color of Georgia. The name of the dyes, the chemical name and the chemical structure are illustrated in Table 1.

B. Chemical Purity

The estimated chemical purity of the dyes is listed in Table 2. The purity of the dyes was estimated by HPLC analysis. The extinction coefficients of the components in the dyes are not known and the area percentage of the largest peak from HPLC analysis was used to provide a relative indication of the purity of the dyes. The melting points of the dyes were determined using differential scanning calorimetry.

The two samples of Disperse Red 11 were also analyzed by HPLC to detect the number of impurities present. In this experiment we used a reverse phase C-18 column with 100 percent acetonitrile as the solvent and set the detector at 254 nm. The size of the peaks provide a relative indication of the purity since the extinction coefficients of the components in the dyes are unknown. With these chromatographic conditions, peaks in the HPLC

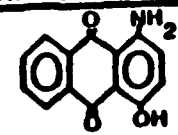
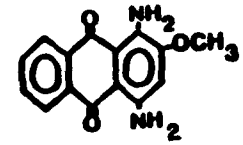
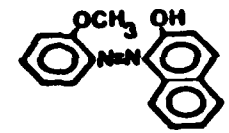
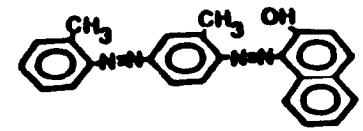
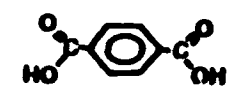
Chemical Name	Trade Name	Chemical Structure
1-Amino-1 hydroxyanthraquinone	Disperse Red 15	
1,4-Diamino-2-Methoxyanthraquinone	Disperse Red 11	
1-((2-methoxyphenyl)azo-2-Naphthol)	Solvent Red 1	
1-((4-(o-Tolylazo-o-Tolyl) Azo)-2-naphthol)	Solvent Red 24	
Terephthalic Acid	Terephthalic Acid	
--	Mixture Disperse Red 11:Solvent Red 1: Terephthalic Acid (5:25:16)	

TABLE 1: CHEMICAL NAME, TRADE NAME, AND CHEMICAL STRUCTURE OF DYES EVALUATED FOR GENOTOXICITY.

TABLE 2. THE CHEMICAL CHARACTERIZATION OF DYES USED IN SMOKE MUNITIONS

Dye Name	Estimates of Chemical Purity		
	HPLC Analysis (percent)	Melting Point Data	
		Literature	Measured
Solvent Red 24	75	181-188°C[a]	145-155°C
Disperse Red 15	59	207-208°C[b]	185-190
Disperse Red 11 (Lot 1)	99	NA[c]	234-236
Disperse Red 11 (Lot 2)	98	NA	233-235
Solvent Red 1	99	NA	181-182

[a]Aldrich "Catalog Handbook of Fine Chemicals," Aldrich Chemical Company, 1986-1987, Milwaukee, Wisconsin.

[b]Colour Index, The Society of Dyers and Colourists, Land Hamphries Printers, London, England.

[c]NA - not available.

tracings were detected eluting before the large Disperse Red 11 peak. The chromatograms are shown in Figure 1 and indicate that there are two peaks in the Disperse Red 11, Lot 2, sample suggesting a possibility of two impurities. There was some indication that one of the impurities detected in the Disperse Red 11 (Lot 2) may be 1,4-diaminoanthraquinone, a known mutagen. This compound has a similar structure to the 1,4-diamino-2-methoxyanthraquinone, which is the chemical name for Disperse Red 11. Additional characterization of the impurity was done using HPLC run with a more polar solvent, water:acetonitrile (1:1). Figure 2 shows the results of this experiment and indicates that additional impurities may be present in Disperse Red 11 (Lot 2) which were not originally identified. To help identify the impurities we conducted additional studies using the more polar solvent with 1,4-diaminoanthraquinone run as a positive control. The HPLC tracings indicated that 1,4-diaminoanthraquinone had the same mobility as one of the impurities, which tentatively identified 1,4-diaminoanthraquinone (Figure 3) as one of the impurities present. However, it was present in both lots of Disperse Red 11 (Figure 2). The data shown in Figures 2 and 3 indicate that other compounds were present in the Disperse Red 11 (Lot 2) that were not present in Disperse Red 11 (Lot 1).

EXPERIMENTAL METHODS

BACTERIAL MUTAGENICITY OF DYES

The mutagenic activity of the dyes was first tested in the Ames *Salmonella* bacterial mutagenicity assay. The tests were conducted according

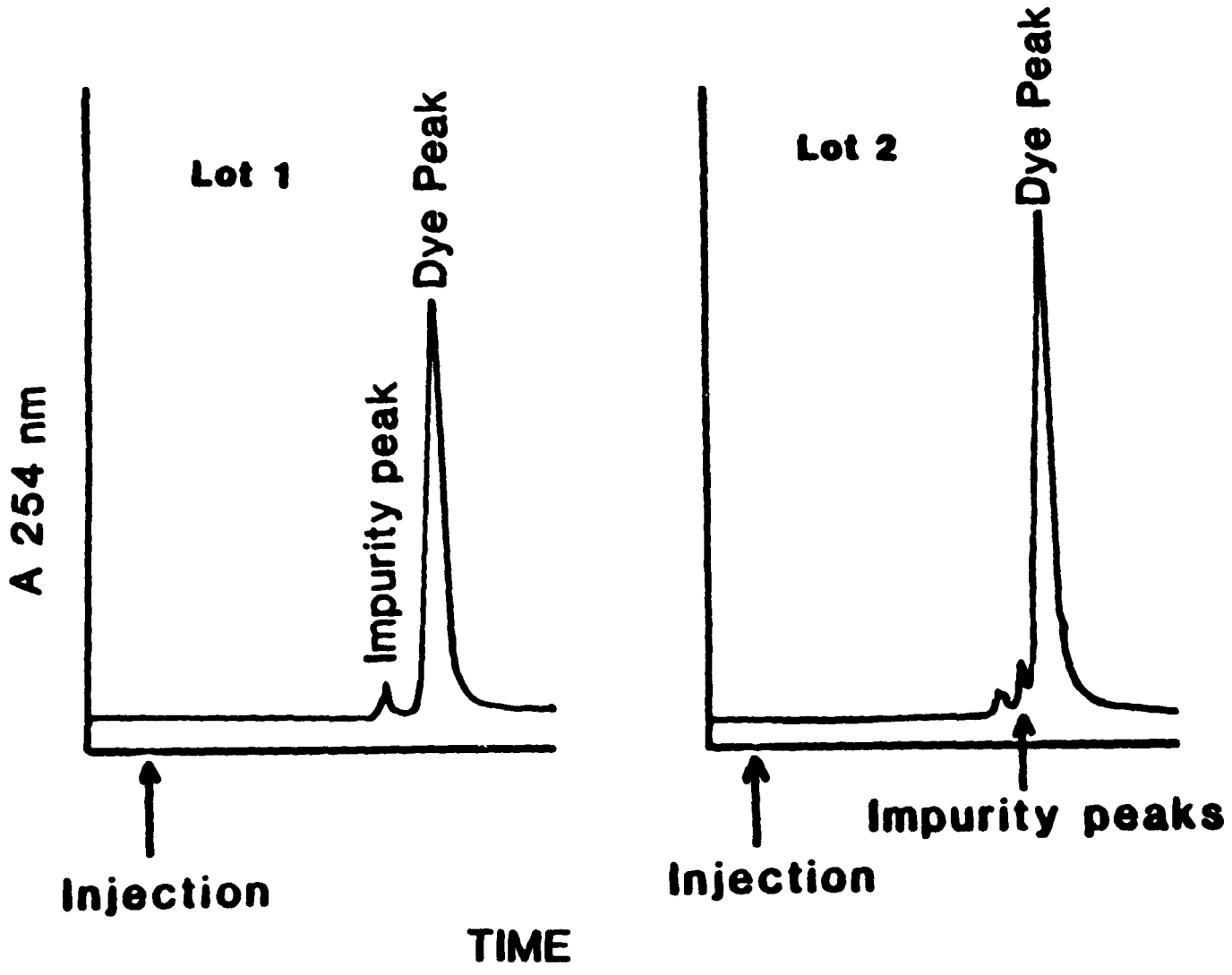


FIGURE 1: HPLC OF DISPERSE RED 11 (LOT 1 AND LOT 2) (SOLVENT 100 PERCENT ACETONITRILE).

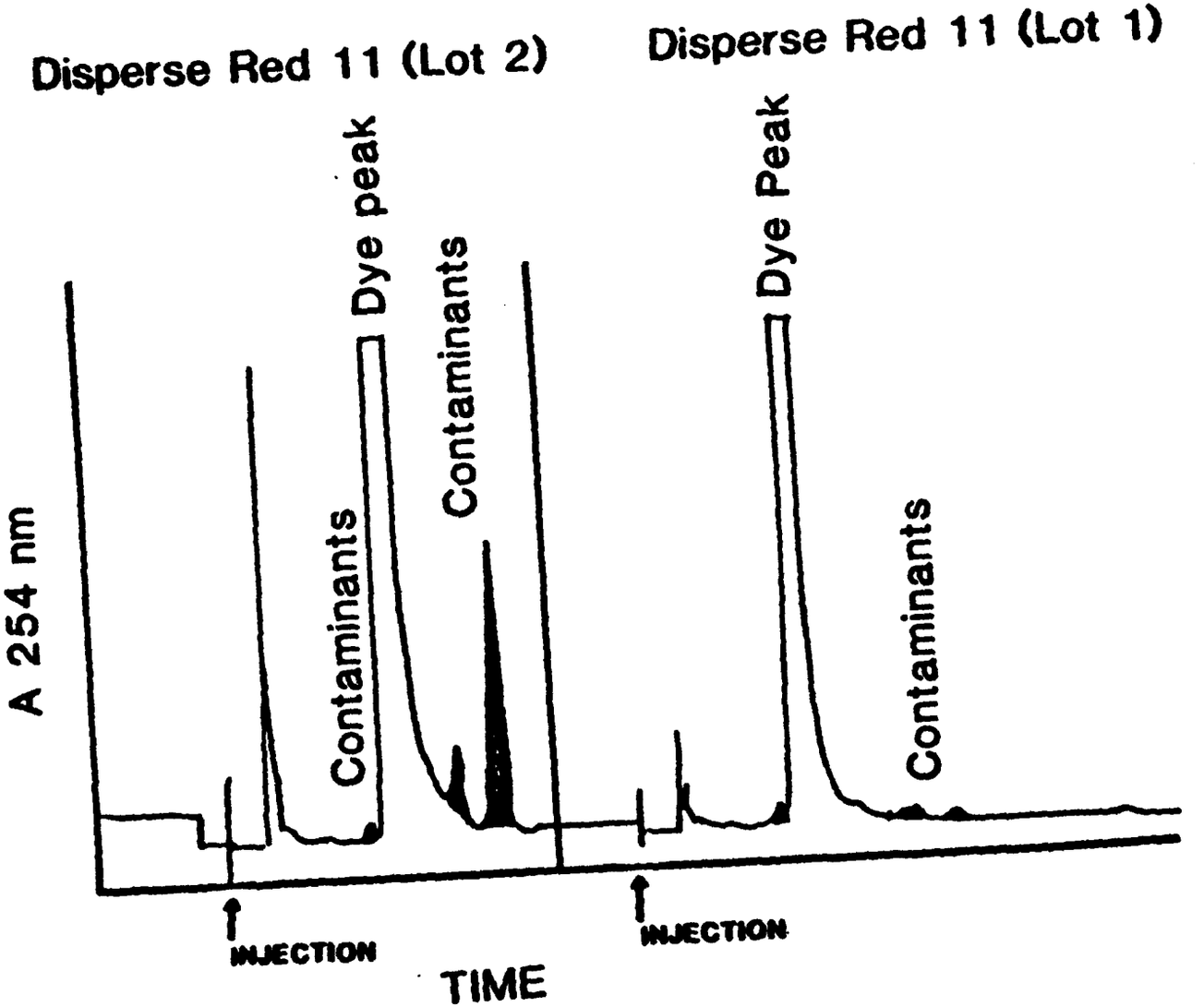
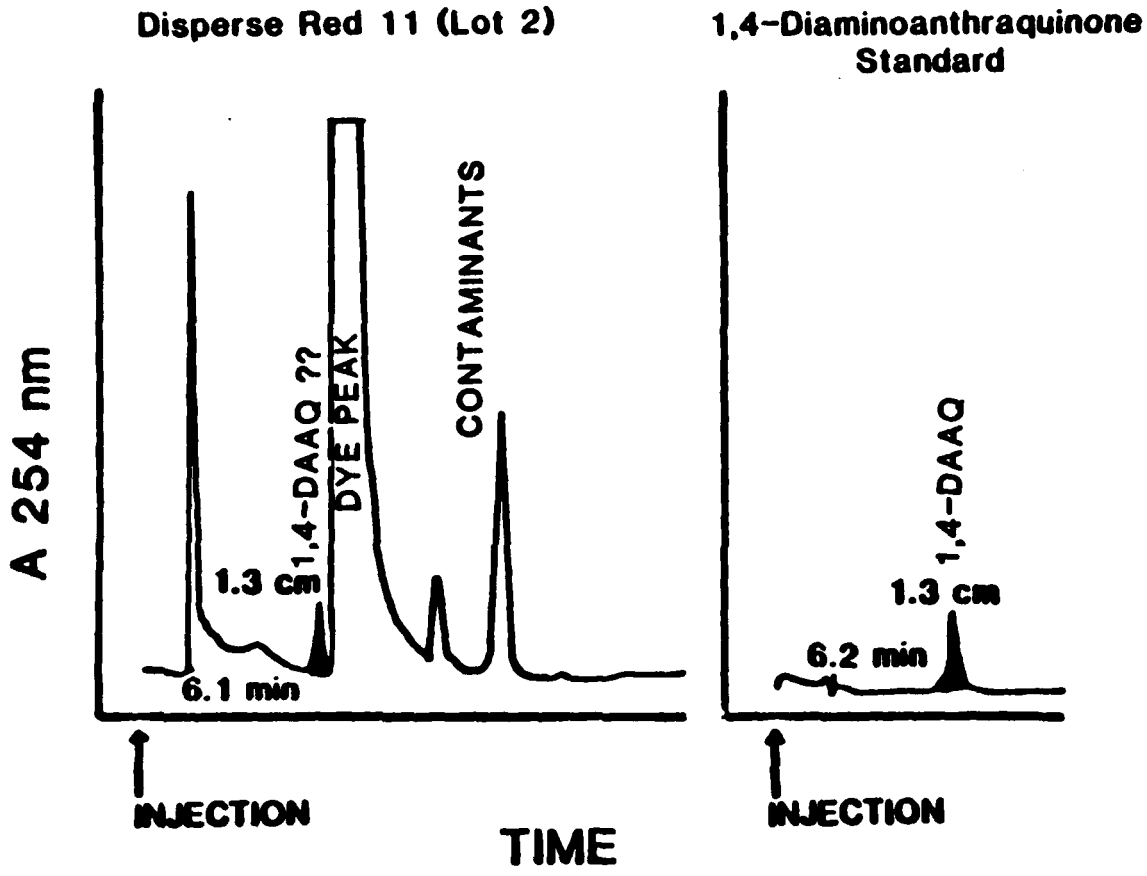


FIGURE 2: HPLC OF DISPERSE RED 11 (LOT 1 AND LOT 2) (SOLVENT 1:1 WATER:ACETONITRILE).



The peak size and its location were consistent with 1% contamination of Disperse Red 11 (Lot 2) with 1,4-diaminoanthraquinone.

FIGURE 3: TENTATIVE IDENTIFICATION OF 1,4-DIAMINOANTHRAQUINONE IN DISPERSE RED 11, LOT 2.

to the methods outlined by Ames et al. (1975) and revised by Maron and Ames (1983). Bacterial strains TA-1535, TA-1538, TA-98 and TA-100 were used to evaluate the mutagenic potency of each of the dyes. The tests were conducted both with and without the addition of Aroclor-induced rat liver microsomal preparation (S-9). The protein concentration for the S-9 was 26.6 mg protein/ml.

The dye concentration tested was limited by the amount of the dyes that could be dissolved in the media. Table 3 lists the dyes tested, the range of S-9 concentration and the dye concentrations evaluated. In addition to testing each of the dyes alone, we also evaluated a mixture of two dyes with terephthalic acid to determine if there was any interaction between the components in the induction of mutations. The mixture contained 5 parts Disperse Red 11, 25 parts Solvent Red 1 and 16 parts terephthalic acid. Two different lots of Disperse Red 11 were evaluated since there was a chemical contaminant detected in one lot (Lot 2) that was not present in the other (Lot 1). Six concentrations were tested for each dye with three plates at each concentration. In the range finding experiment a wide range of dye concentrations were tested with or without addition of a single concentration of S-9 mix (30 µl per plate). The response and apparent toxicity of the dyes was determined in the range finding experiment.

The evaluation of the data from the range finding experiments made it possible to define an appropriate dye concentration to further evaluate the mutagenic response of the dyes that showed a positive response. Since the mutagenic response was dependent on the presence of S-9, studies were conducted to determine the relationship between S-9 concentration and mutagenic response. The S-9 concentrations were varied over a range of

TABLE 3. DYE CONCENTRATIONS EVALUATED IN THE RANGE FINDING AMES TESTS

Dye Name	Dye Concentration (µg/plate)	S-9 Concentration (µl/plate)
Solvent Red 24	0-500	0, 30
Disperse Red 15	0-1000	0, 15, 30, 60, 120
Disperse Red 11	0-1000	0, 15, 30, 60, 120
Solvent Red 1	0-800	0, 15, 30, 60, 120
Terephthalic acid	0-400	0, 30
*Dye mixture	0-200	0, 15, 30, 60, 120

*Disperse Red 11:Solvent Red 1:terephthalic acid in a ratio of 5:25:11 by weight.

values (3, 6, 12 and 24% of the added cofactors or 15, 30, 60 and 120 µl/plate) of S-9 mix as shown in Table 3. These studies were conducted only with the dyes that showed a positive response as well as the mixture of dyes. The revertant frequency per plate was plotted against the concentration of the dyes and dose-response relationship determined for each dye for each S-9 concentration over the linear portion of the curve.

For a test run to be valid both the background level and the level of response to a positive control compound must fall within two standard deviations of historical control values. The positive control mutagens for each strain are 2-aminoanthracene and benzo(a)pyrene as indirect mutagens for strains TA-1538, TA-98 and TA-100. The positive control direct mutagen is 2-nitrofluorene for strains TA-1538 and TA-98 and sodium azide for strains TA-1535 and TA-100. For a mutagenic response to be considered significantly elevated it had to demonstrate a concentration related increase in mutation frequency, be twice background level for that run and be greater than the 95% confidence limit for the background rate for that tester strain. If the response from the dye did not meet these critera, it was considered to be negative. The relationship between concentration and response was evaluated by using a least squares method to fit a straight line to the data. A student's "t" test was used to test the null hypothesis that the slope was equal to zero. The goodness of fit was determined by ANOVA.

CYTOTOXICITY OF DYES IN MAMMALIAN CELLS

The methods used to evaluate the cytotoxicity of the chemicals in Chinese hamster ovary cells (CHO) has been previously published (Li 1981).

Briefly, cells were exposed for 3 hours to the dyes in Hams' F-12 media without serum. The treatment medium was removed, the cells removed from the tissue culture plate with trypsin and triplicate wells were plated with two hundred cells per well in Hams' F-12 media with 10% calf serum, incubated at 37°C in 5% CO_2 at 96% humidity for 7 days and the number of cells that formed colonies recorded. The number of colonies formed in the exposed cultures was divided by the number in the controls and a relative cloning efficiency determined.

The evaluation of the genotoxicity in mammalian cells was only conducted on Disperse Red 11 (Lot 1 and 2), Solvent Red 1 and terephthalic acid. To determine the toxicity of the dyes, two experiments were conducted. In the first experiment CHO cells were exposed for 3 hours to a range of dye concentrations, up to 400 µg/ml for terephthalic acid, 800 µg/ml for Solvent Red 1 and 1000 µg/ml of Disperse Red (Lot 2). In this experiment a visible precipitate formed for both dyes at concentrations above 100 µg/ml. This made it impossible to interpret the concentration-response relationships for cell killing. The results of this experiment are shown in Figure 4 and indicated that terephthalic acid was not cytotoxic and that at 100 µg/ml where there was no precipitate the Solvent Red 1 was more toxic (~ 2% relative cloning efficiency) than the Disperse Red 11 (Lot 2) (~ 70% relative cloning efficiency). This experiment provided information that was used to select proper doses to evaluate the cytotoxicity of the dyes in the next experiment. The concentrations selected for further evaluation of cytotoxicity were up to 100 µg/ml for both of the dyes.

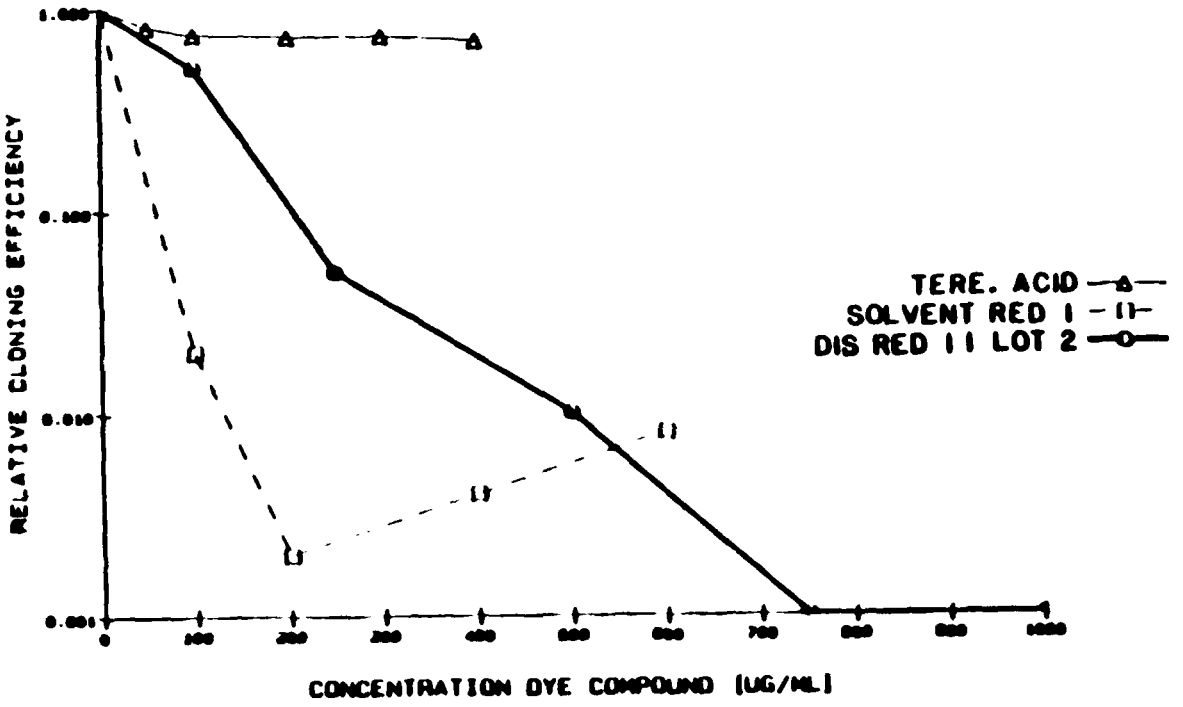


FIGURE 4: CYTOTOXICITY OF HIGH LEVELS OF RED/VIOLET DYES IN CHO CELLS.

MAMMALIAN CELL MUTAGENICITY OF DYES

The mutation assay utilizes CHO cells and measures mutations at the hypoxanthine guanine phosphoribosyl transferase gene locus (HGPRT). The test was conducted according to published procedures (Hsie et al. 1979) as modified by Li (1981). Briefly, the cells were maintained in exponential growth phase. Before each experiment 1000 cells were plated, which decreases the probability that cells which contain spontaneous mutations will be in the population. The cells were then grown for 8 days. Cultures were split and allowed to multiply for 3 days. These cells were again split and plated at 5×10^5 cells/plate and grown overnight to be used in the assay. This resulted in about 10^6 cells at the time of exposure. The cells were exposed for 3 hours to each of the dyes with or without the addition of 1.25% on 2.5% S-9 fraction. At the end of the exposure time the cells were removed from the flask with trypsin. There were 5 concentrations of dye tested per treatment. To measure the mutagenic response the cells were maintained in exponential growth by subculture for a total of 8 days. The cells were then exposed to media containing 6-thioguanine which killed all the normal cells and allowed the cells with a mutation at the HGPRT gene locus to grow. The altered cells were given an additional 8 days to form colonies. At the time of the selection additional cells were plated to determine the plating efficiency, and the plating efficiency at the time of selection was used to calculate the number of mutations per 10^6 clonable cells. Benzo(a)pyrene (BaP) was used as a positive control chemical that requires metabolic activation with S-9, and N-methyl-N'-nitro-N-nitroso-guanidine (MNNG) as a direct acting mutagen. For a mutagenic response in

this assay to be considered positive the response had to be concentration related, the peak response had to be more than 20 mutations per 10^6 clonable cells (more than twice normal background) and the response had to be repeatable. The relationship between concentration and response was evaluated by fitting a straight line to the data (least squares method). A student's "t" test was used to test the null hypothesis that the slope was equal to zero. The goodness of fit was determined by ANOVA.

SISTER CHROMATID EXCHANGES FROM DYES

Solvent Red 1 and Disperse Red 11 (Lot 1 and 2), were evaluated for the induction of cytogenetic changes. The methods for preparation of cells for the evaluation of the frequency of sister chromatid exchanges (SCE) in CHO cells has been previously published (Perry and Wolff 1974). CHO cells were exposed to graded concentrations of the dyes, 0, 5, 10 and 20 µg/ml of culture media, and harvested at 24, 30, 36, 48, and 72 hours after the start of the dye exposure. The experiments were conducted with and without the addition of 2.5% S-9 in the culture media. During and after the exposure, the cells were exposed to 10 µM bromodeoxyuridine (BrdU) to differentially label the cells for determination of the frequency of SCE induction. Because of the mitotic lag induced by the Disperse Red 11 it was necessary to determine the proper time to evaluate the SCE. Each dye and exposure concentration was evaluated at the time point where adequate numbers of second division cells could be detected. The cells from these cultures were evaluated for the frequency of SCE relative to the control cultures. To determine if a significant increase in SCE was produced, the Wilcoxon's

signed rank test was performed to determine the S-9 and batch dependence of response of the dye. The nonparametric Wilcoxon's test was used since the set of values with different batches of dye or with S-9 are not normally distributed. To determine if a significant concentrated related increase in SCE was present, a straight line was fitted to the data using a least-squares procedure. A student's t test was used to test the null hypothesis that the slope was equal to zero.

CHROMOSOME ABERRATIONS FROM DYES

The methods for preparing CHO cells to evaluate chromosome aberration induction have been previously published (Brooks et al. 1984a). Briefly, the cultures were incubated in Hams' F-12 medium with 10 percent fetal calf serum, 2.5 percent S-9 and 10 µm BrdU at 37°C and 100 percent relative humidity. The cells were exposed to 0, 5, 10 and 20 µg of dye/ml of medium for 3 hours with and without the addition of S-9. Two hours prior to harvest of the cells at 6, 9, 12 and 24 hours after the start of the culture, colchicine was added to the cultures at a final concentration of 10^{-4} M. The cells were treated with 0.075 M KCl, fixed in 3:1 acetic acid and methanol and the cells air dried on wet cold slides. The slides were evaluated for the frequency of mitotic cells and the earliest time where there was adequate number of mitotic cells for scoring was chosen (9 hrs after the end of the chemical exposure) to evaluate the frequency of chromosome aberrations induced by the treatments. In this first experiment, all the slides were coded prior to scoring and about 100 first division cells scored for the induction of chromosome aberrations. The aberration

frequency was evaluated without the scorer having a knowledge of the chemical treatment. The frequency of aberrations was recorded as has been previously reported (Brooks et al. 1972). For this group of slides there were about 100 cells scored per chemical exposure.

To increase the number of cells evaluated and because of the rather high background level of damage observed in this first experiment, an additional experiment was conducted using CHO cells exposed to 0, 5, 10 and 20 µg/ml of Disperse Red 11 (Lots 1 and 2) or 20 µg/ml of Solvent Red 1 with and without the addition of S-9. The frequency of chromosome aberrations was again measured at 9 hours after exposure on coded slides from this experiment. There were 200 cells evaluated at each concentration for each of the dyes.

RESULTS

BACTERIAL MUTAGENICITY OF DYES

The results of the range finding experiments for the bacterial mutagenicity experiments are presented in Figures 5-32. The text figure number, bacterial strain and means and standard errors of the revertants/plate used to derive these figures are included in Appendix A. In Appendix B are the statistical evaluations of the data. This includes the slope, standard error of slope, F value, and P value. Each figure represents the range finding experiment for a single dye tested in a single bacterial strain TA-98, TA-100, TA-1538 and TA-1535. The figures contain data for the test run both with and without the addition of liver microsomal

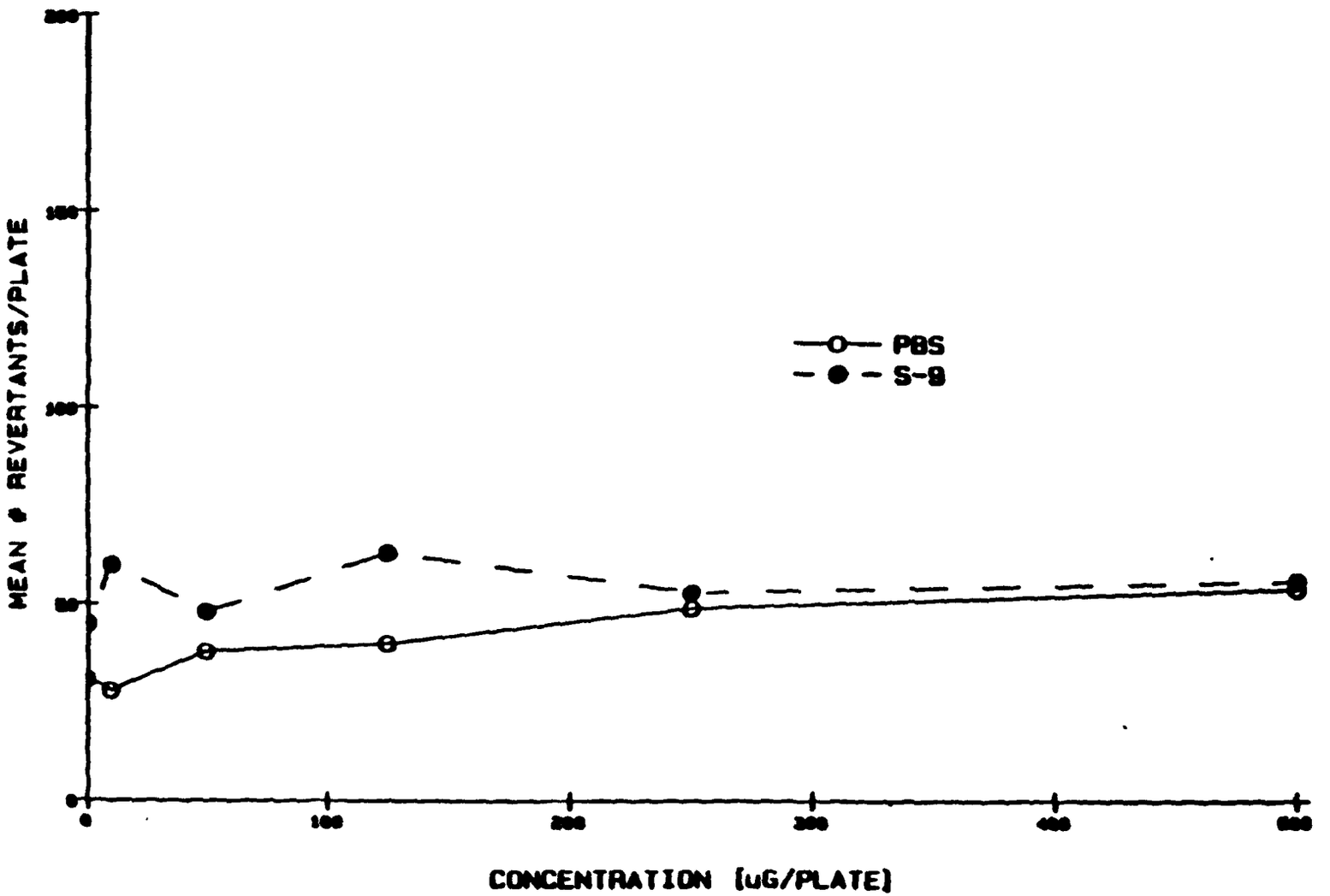


FIGURE 5: MUTAGENIC ACTIVITY OF SOLVENT RED 24 IN TA-98 STRAIN OF SALMONELLA BACTERIA WITH AND WITHOUT S-9.

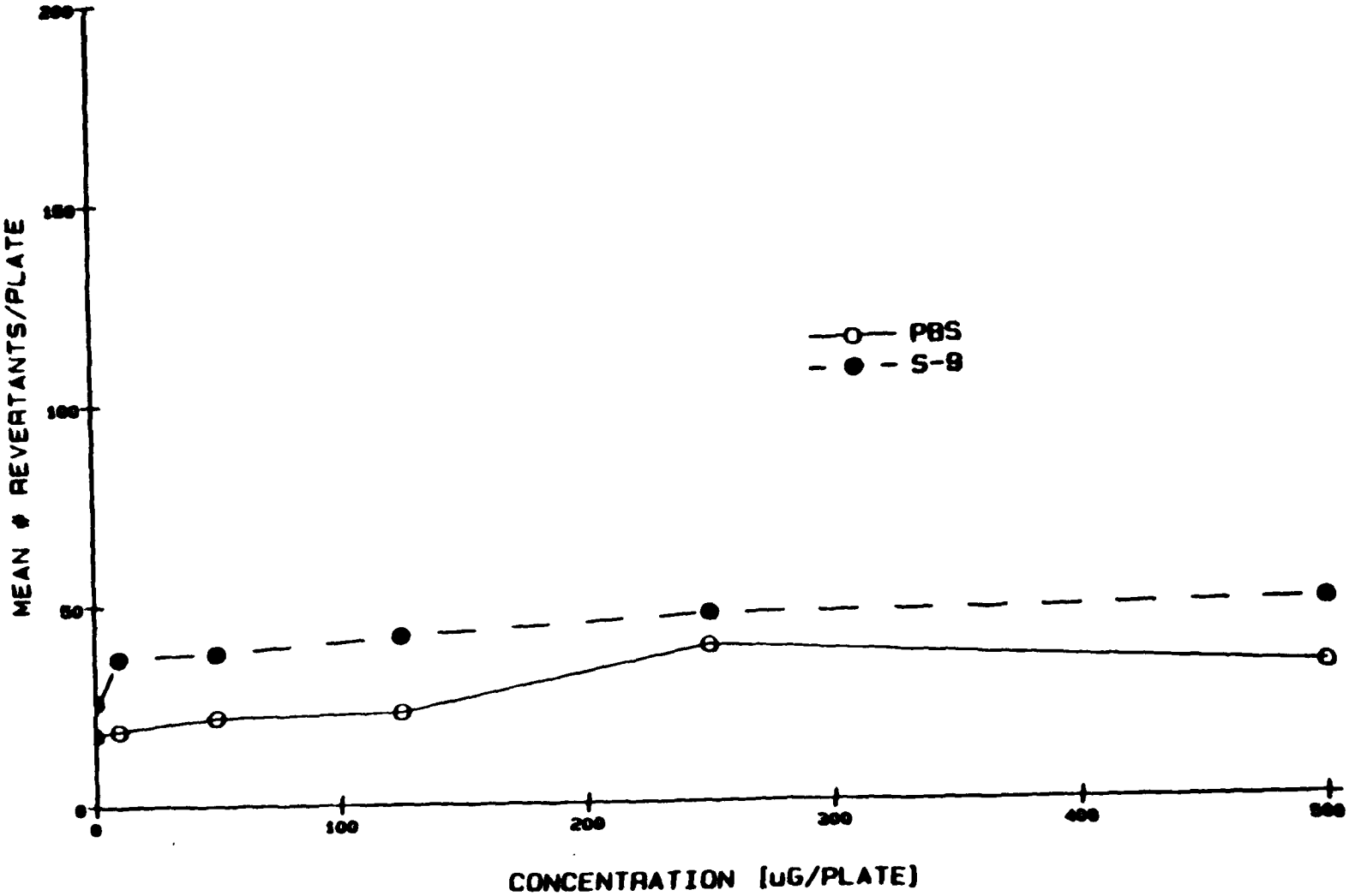


FIGURE 6: MUTAGENIC ACTIVITY OF SOLVENT RED 24 IN TA-1538 STRAIN OF SALMONELLA BACTERIA WITH AND WITHOUT S-9.

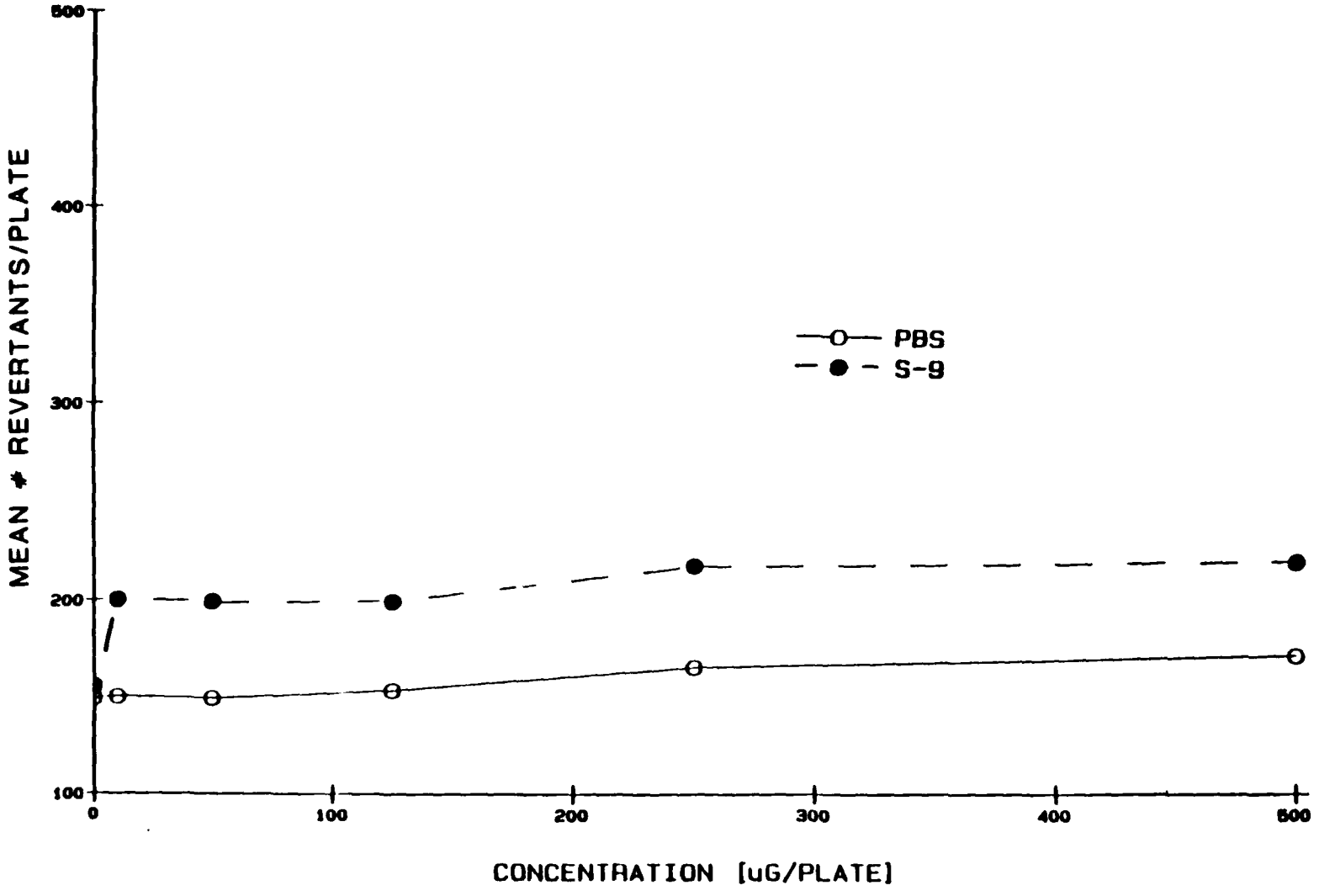


FIGURE 7: MUTAGENIC ACTIVITY OF SOLVENT RED 24 IN TA-100 STRAIN OF SALMONELLA BACTERIA WITH AND WITHOUT S-9.

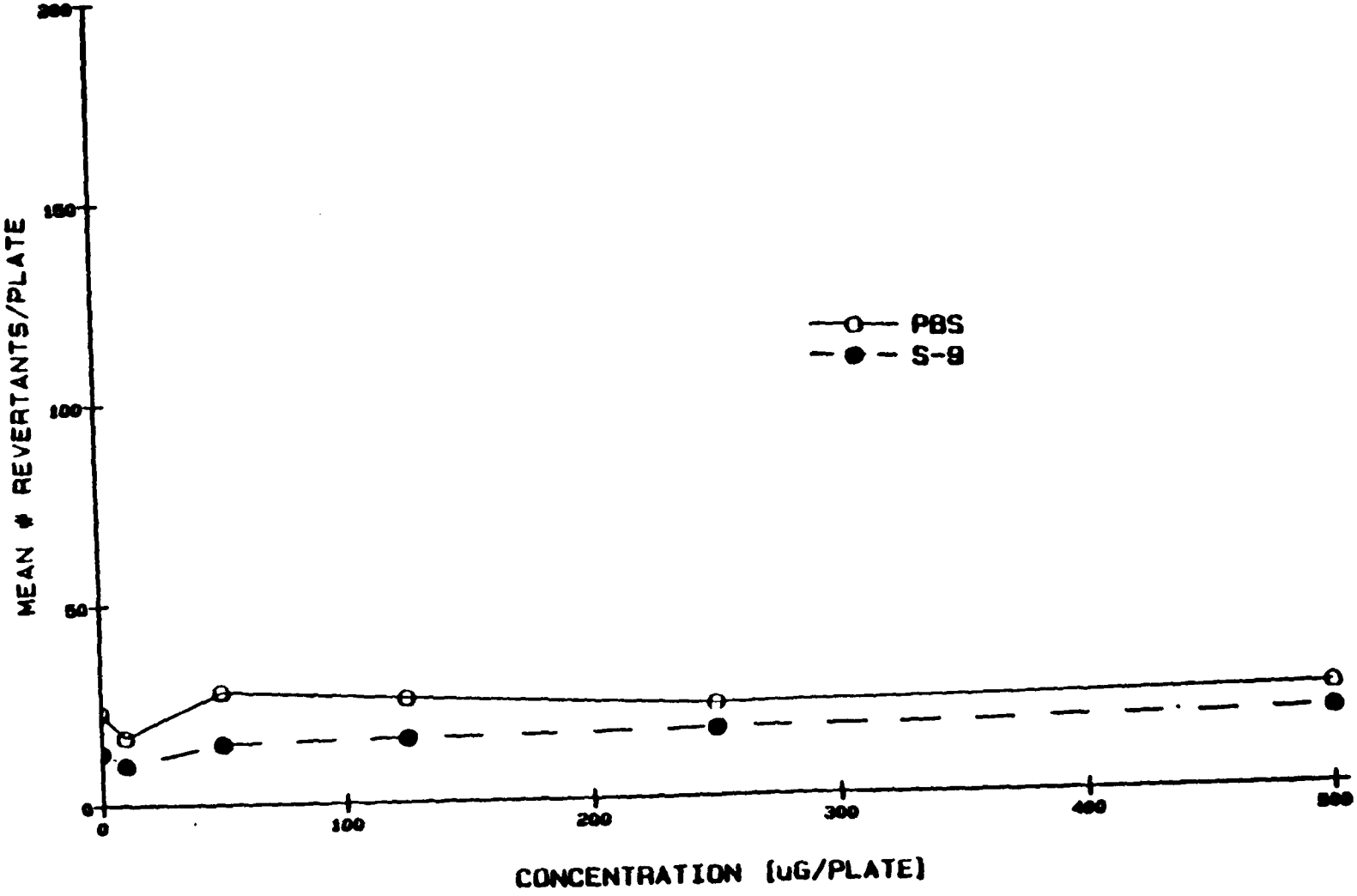


FIGURE 8: MUTAGENIC ACTIVITY OF SOLVENT RED 24 IN TA-1535 STRAIN OF SALMONELLA BACTERIA WITH AND WITHOUT S-9.

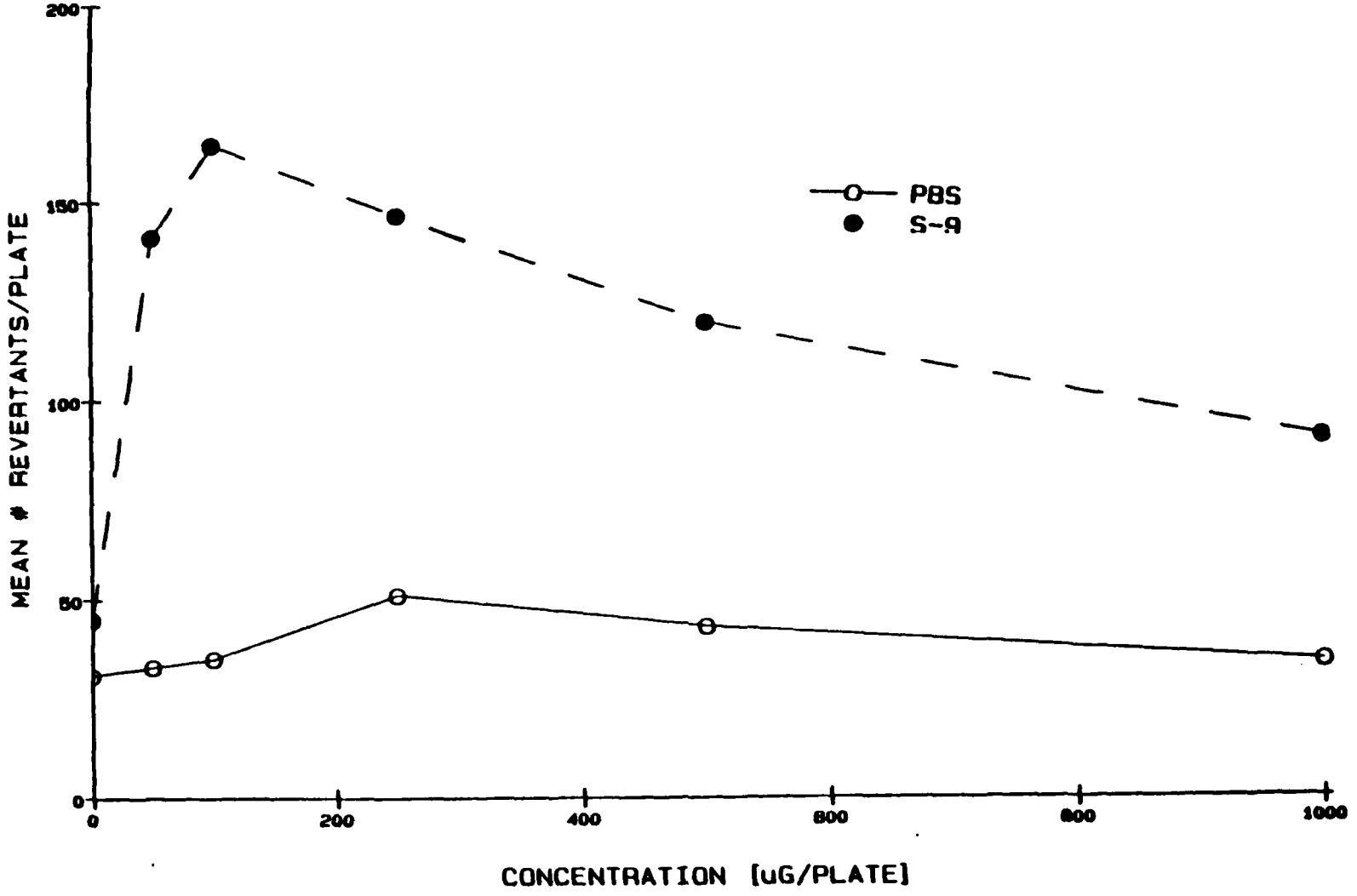


FIGURE 9: MUTAGENIC ACTIVITY OF DISPERSE RED 15 IN TA-98 STRAIN OF SALMONELLA BACTERIA WITH AND WITHOUT S-9.

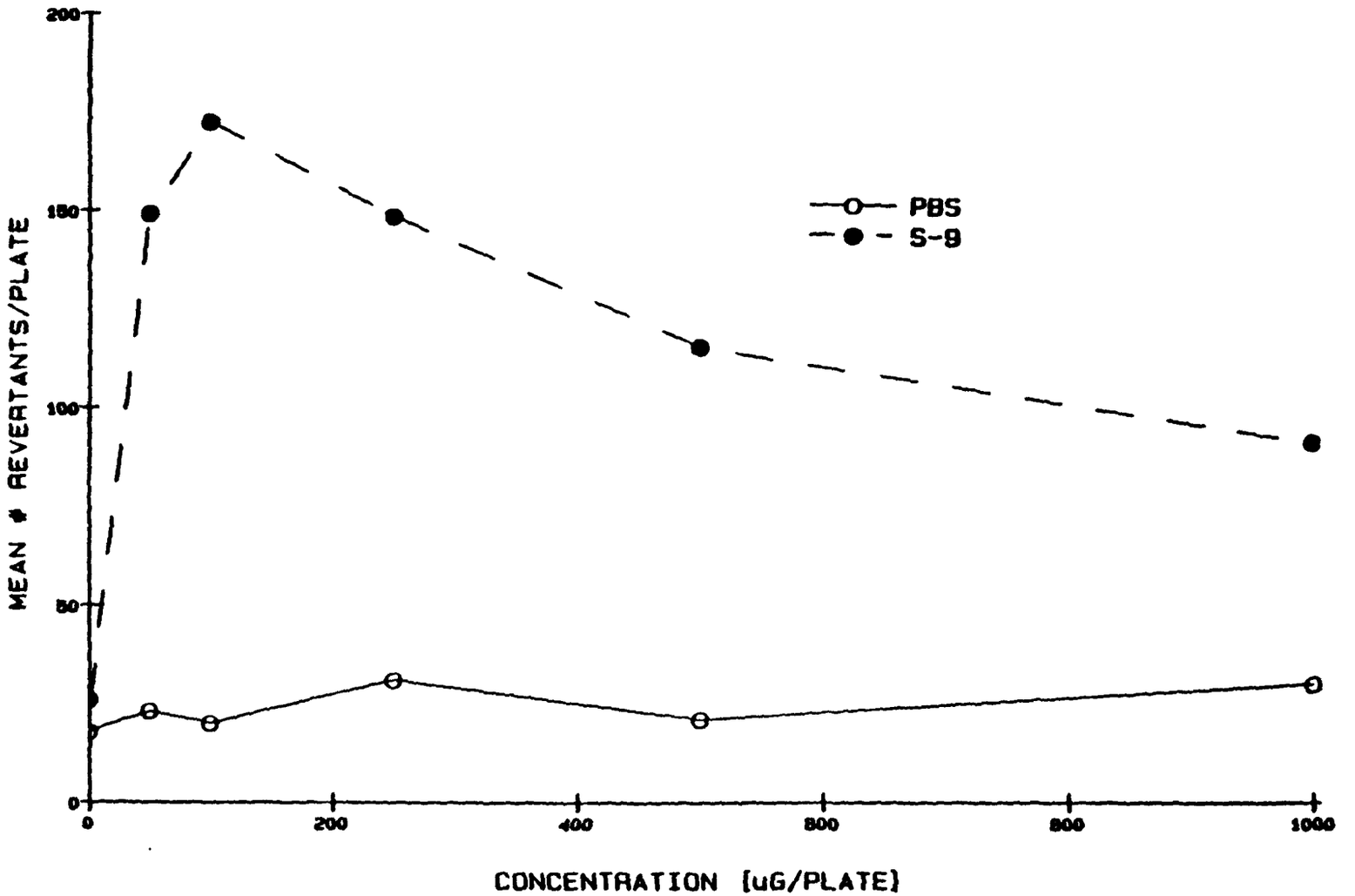


FIGURE 10: MUTAGENIC ACTIVITY OF DISPERSE RED 15 IN TA-1538 STRAIN OF SALMONELLA BACTERIA WITH AND WITHOUT S-9.

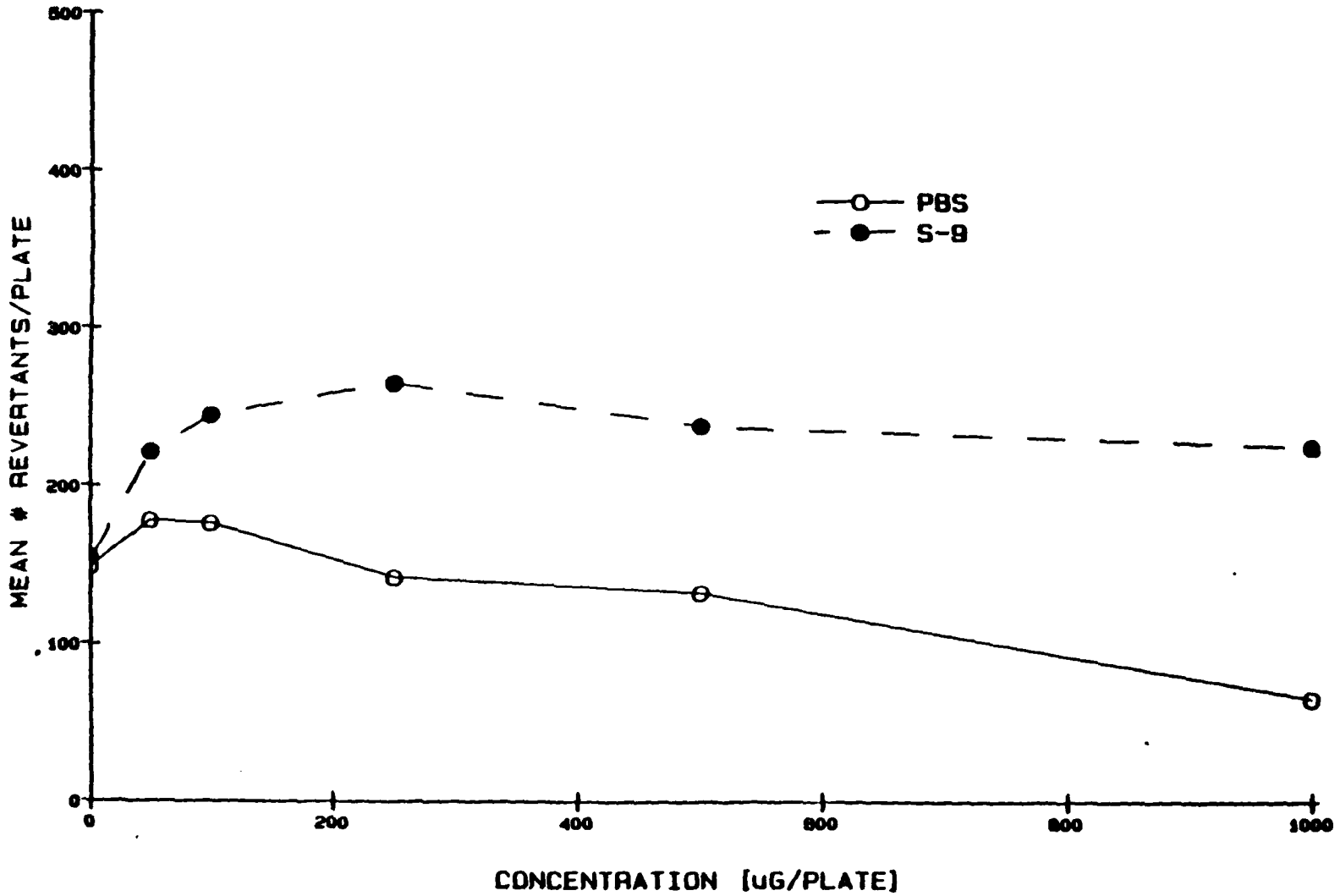


FIGURE 11: MUTAGENIC ACTIVITY OF DISPERSE RED 15 IN TA-100 STRAIN OF SALMONELLA BACTERIA WITH AND WITHOUT S-9.

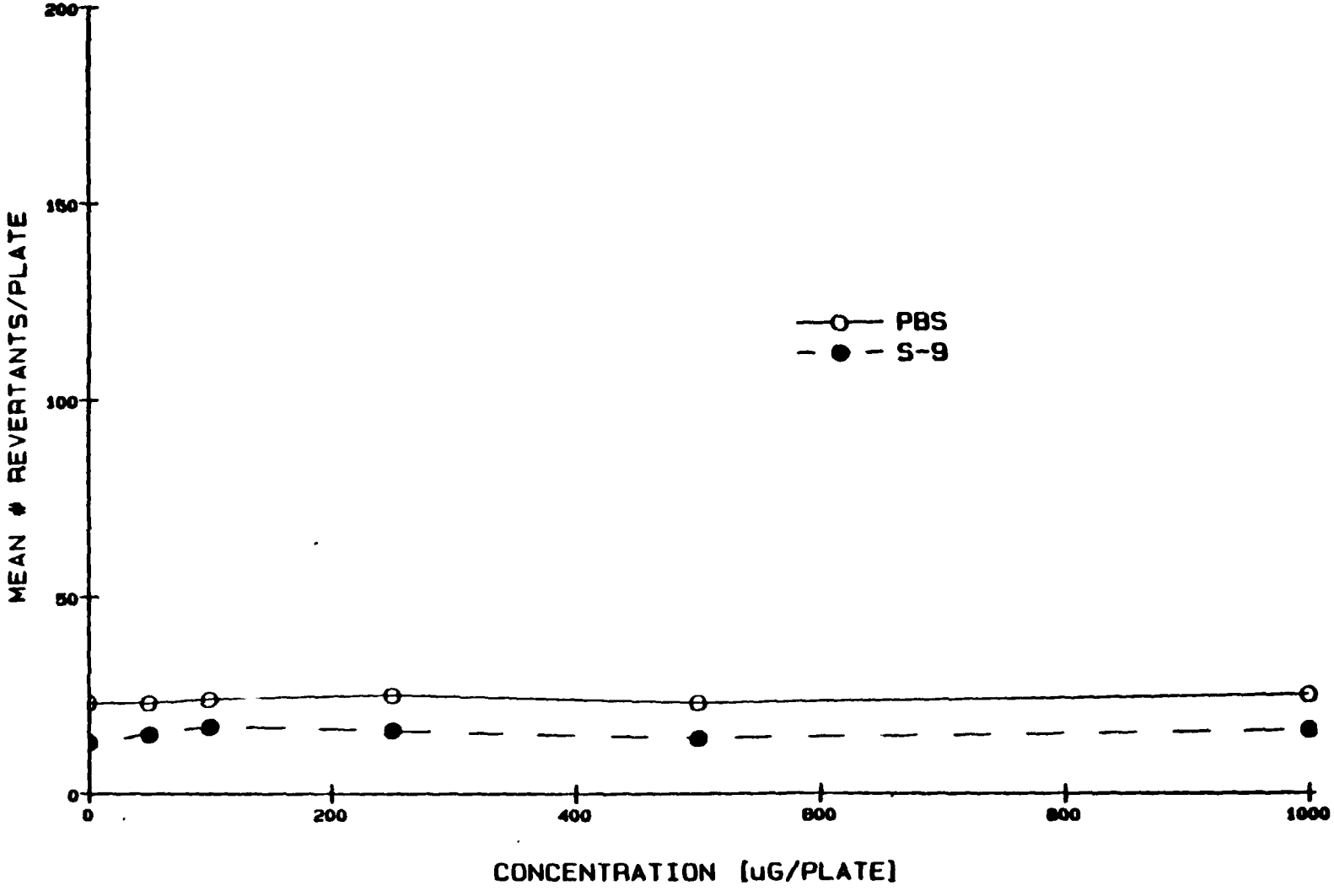
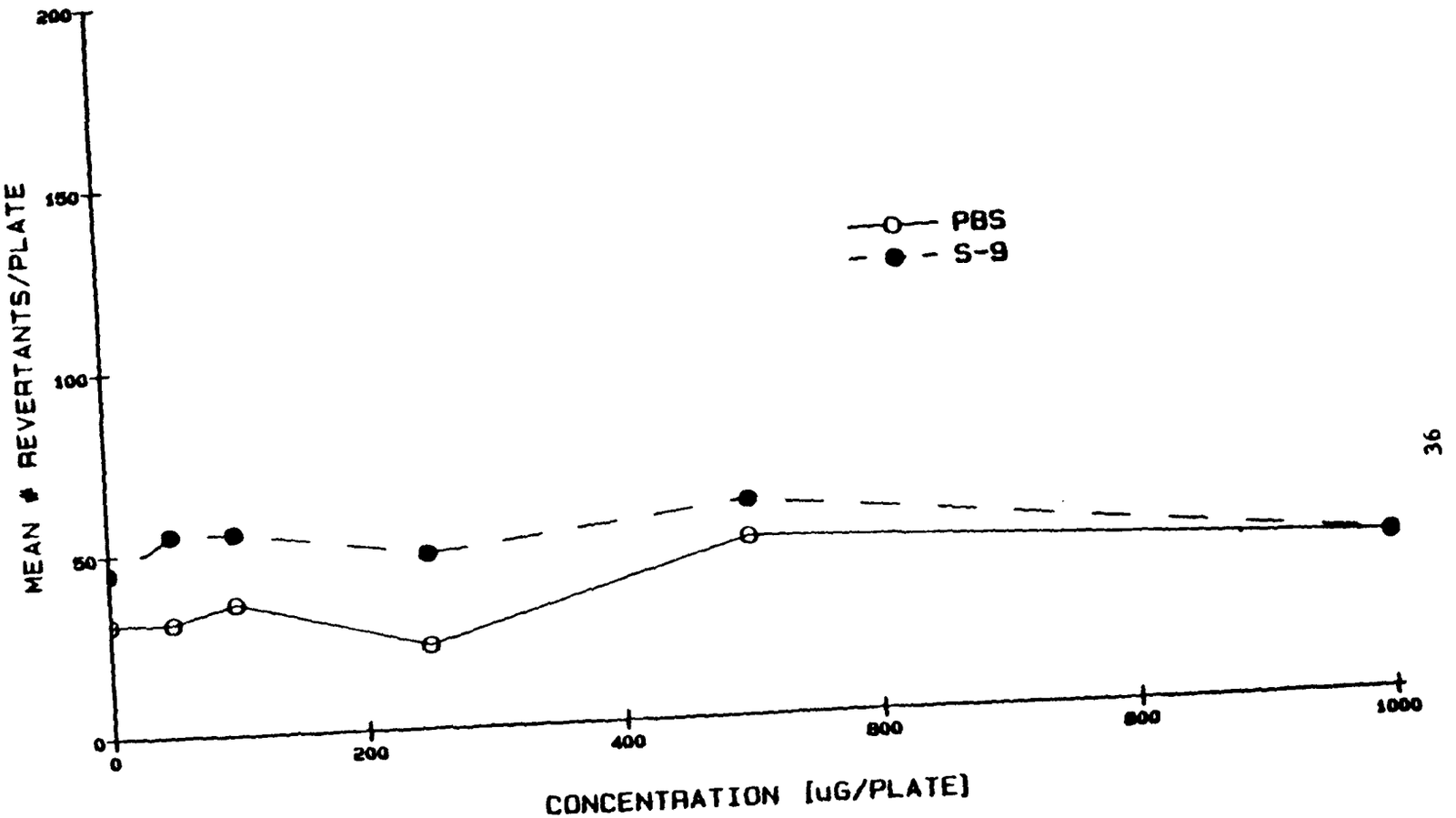
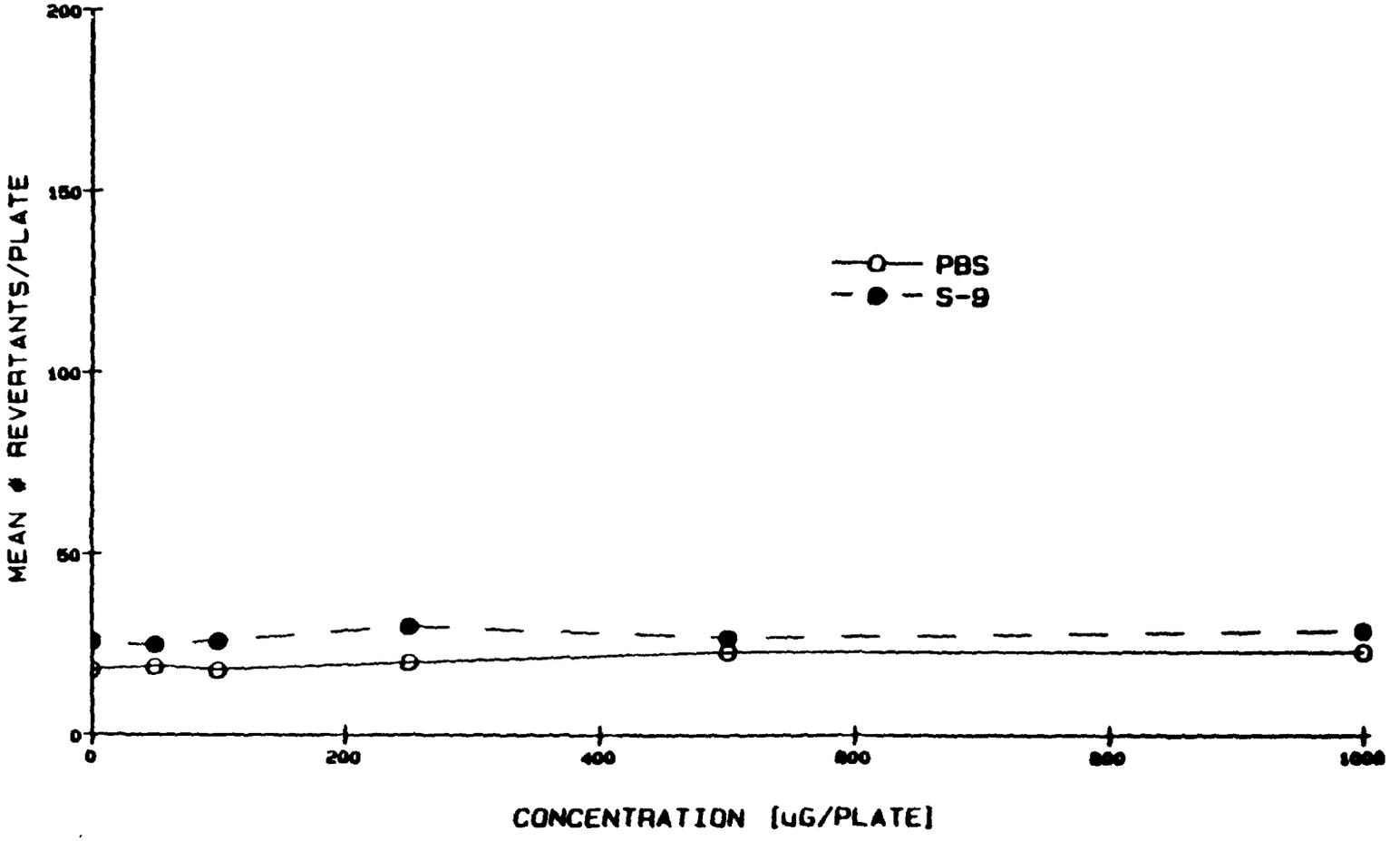


FIGURE 12: MUTAGENIC ACTIVITY OF DISPERSE RED 15 IN TA-1535 STRAIN OF SALMONELLA BACTERIA WITH AND WITHOUT S-9.



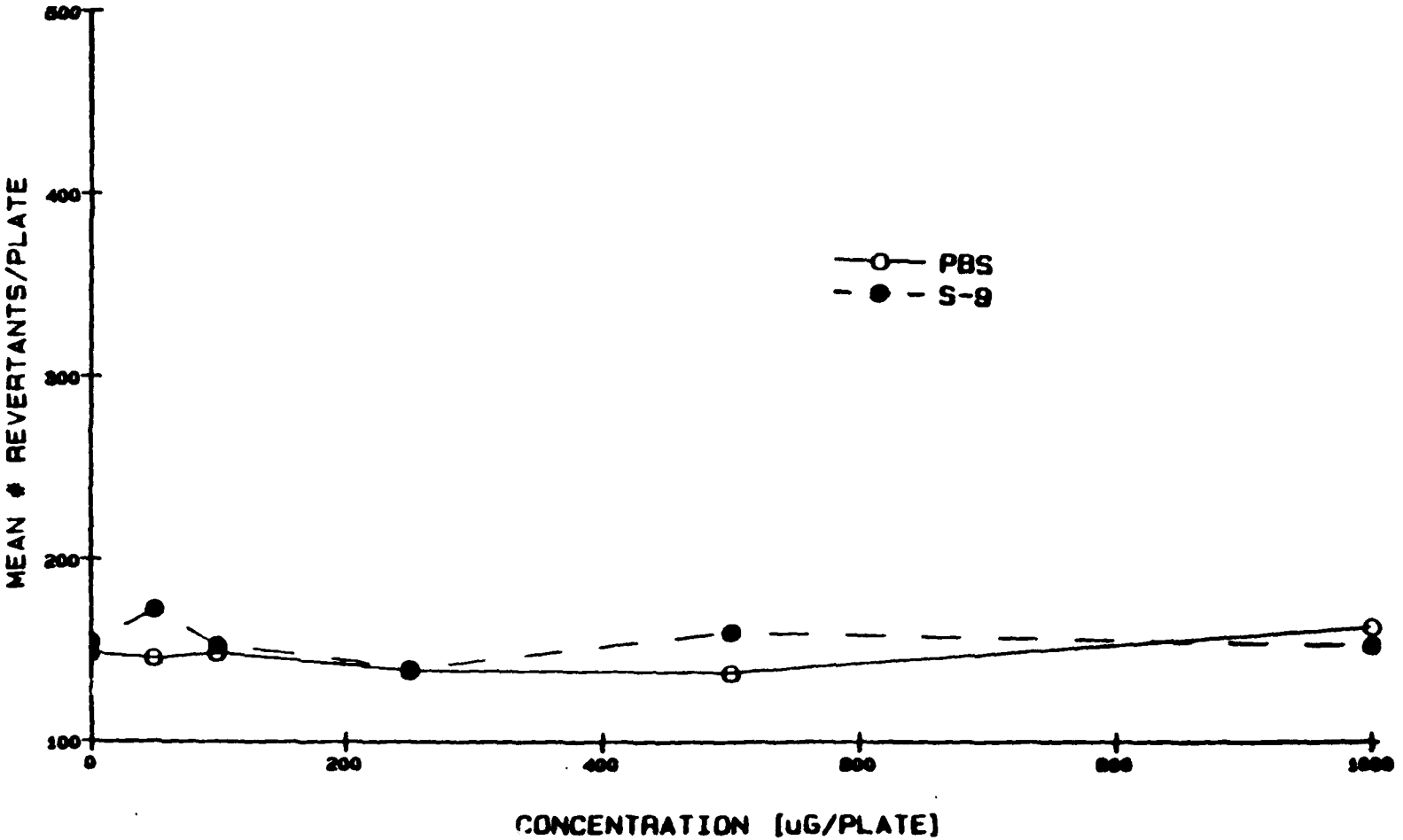
This lot of Disperse Red 11 was manufactured by the Atlantic Chemical Co. of New Jersey.

FIGURE 13: MUTAGENIC ACTIVITY OF DISPERSE RED 11 (LOT 1) IN TA-98 STRAIN OF SALMONELLA BACTERIA WITH AND WITHOUT S-9.



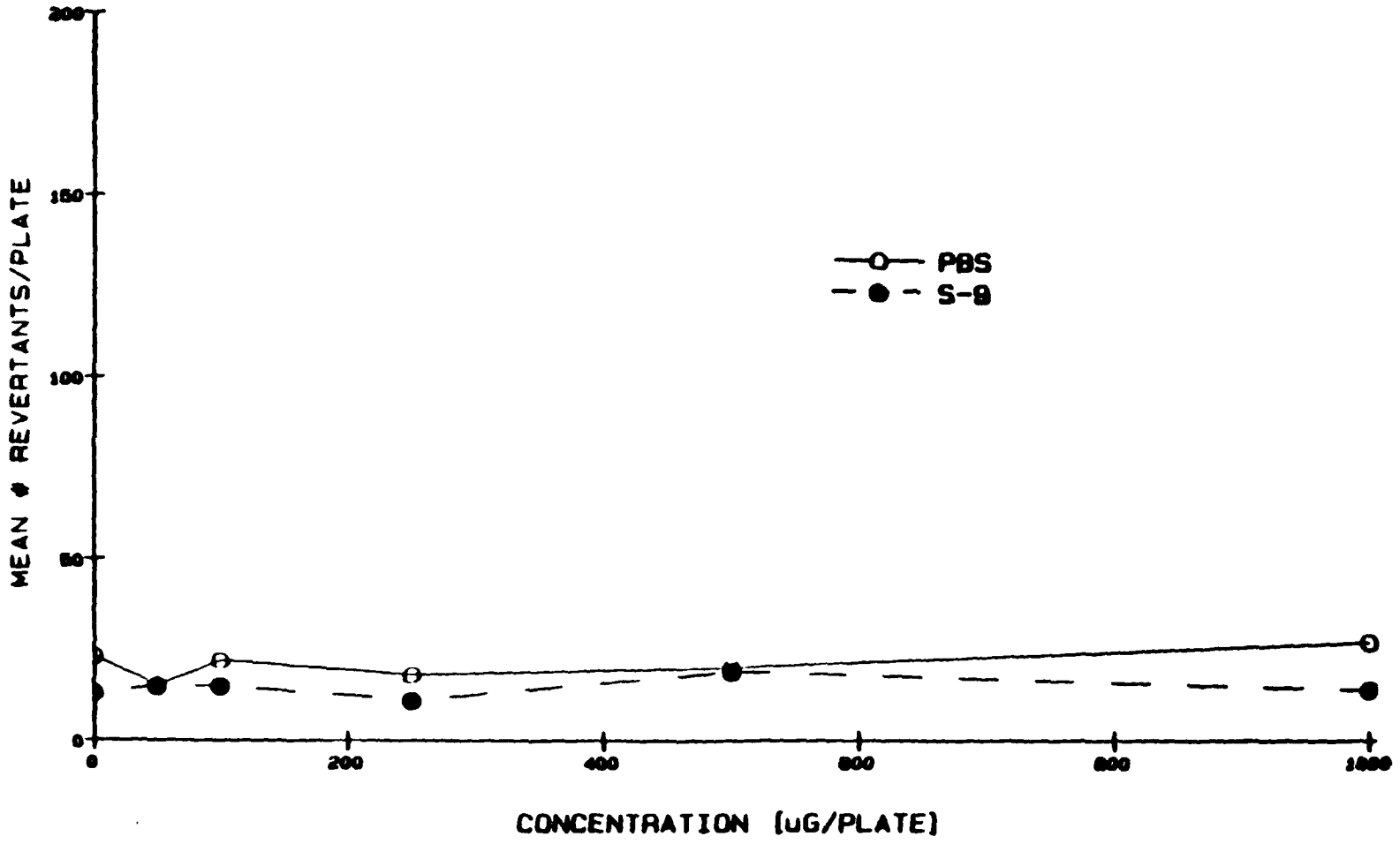
This lot of Disperse Red 11 was manufactured by the Atlantic Chemical Co. of New Jersey.

FIGURE 14: MUTAGENIC ACTIVITY OF DISPERSE RED 11 (LOT 1) IN TA-1538 STRAIN OF SALMONELLA BACTERIA WITH AND WITHOUT S-9.



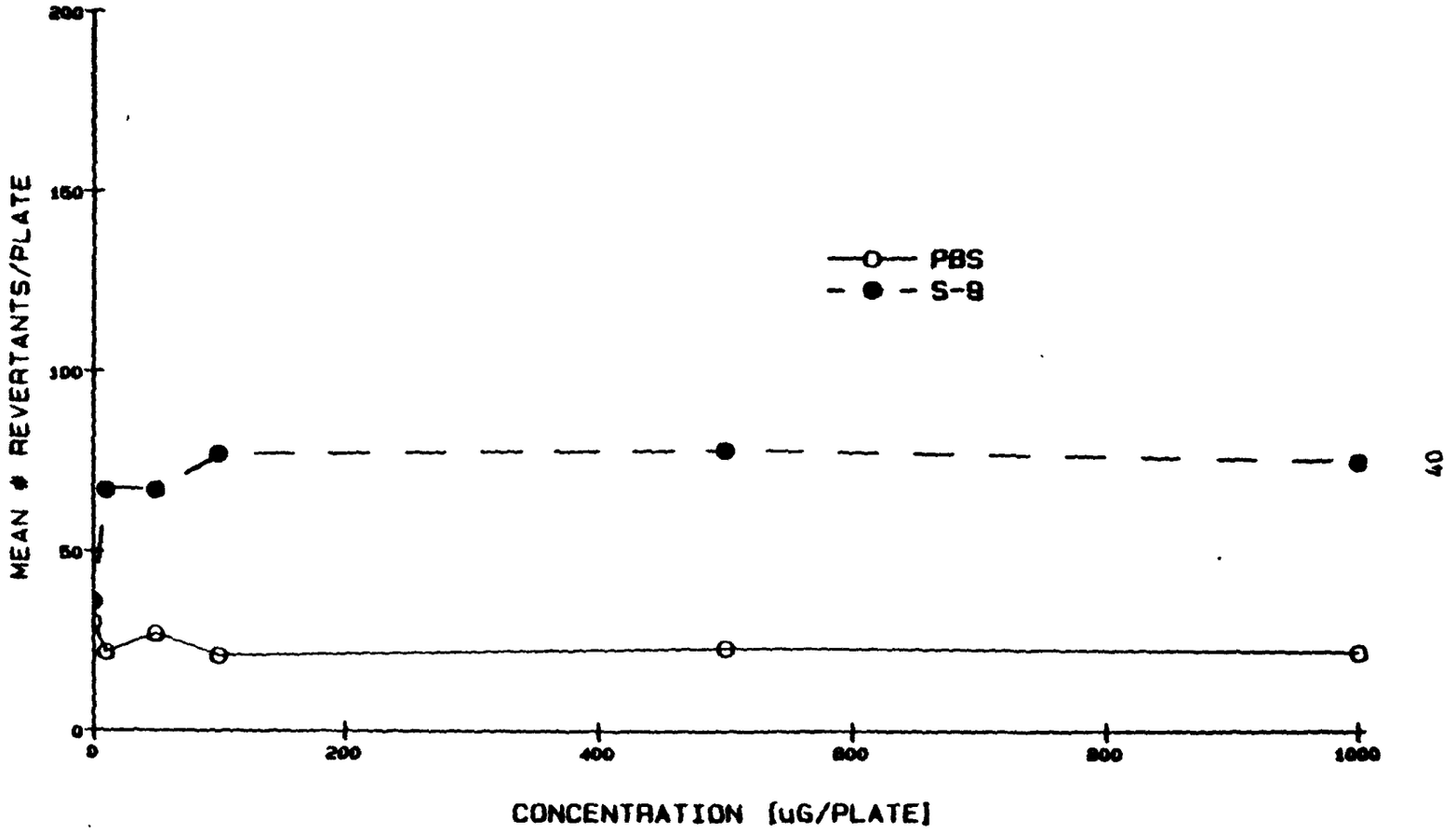
This lot of Disperse Red 11 was manufactured by the Atlantic Chemical Co. of New Jersey.

FIGURE 15: MUTAGENIC ACTIVITY OF DISPERSE RED 11 (LOT 1) IN TA-100 STRAIN OF SALMONELLA BACTERIA WITH AND WITHOUT S-9.



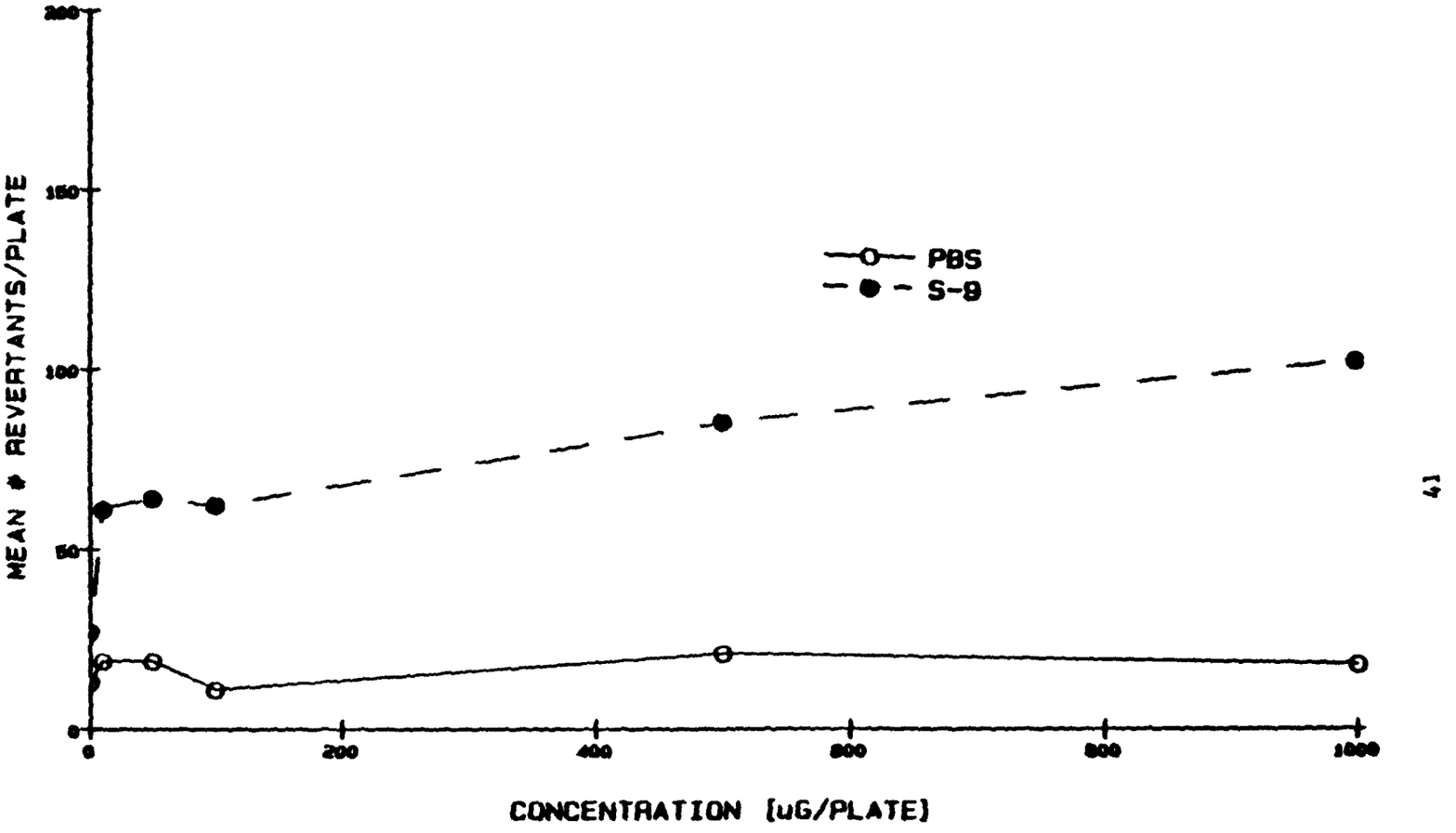
This lot of Disperse Red 11 was manufactured by the Atlantic Chemical Co. of New Jersey.

FIGURE 16: MUTAGENIC ACTIVITY OF DISPERSE RED 11 (LOT 1) IN TA-1535 STRAIN OF SALMONELLA BACTERIA WITH AND WITHOUT S-9.



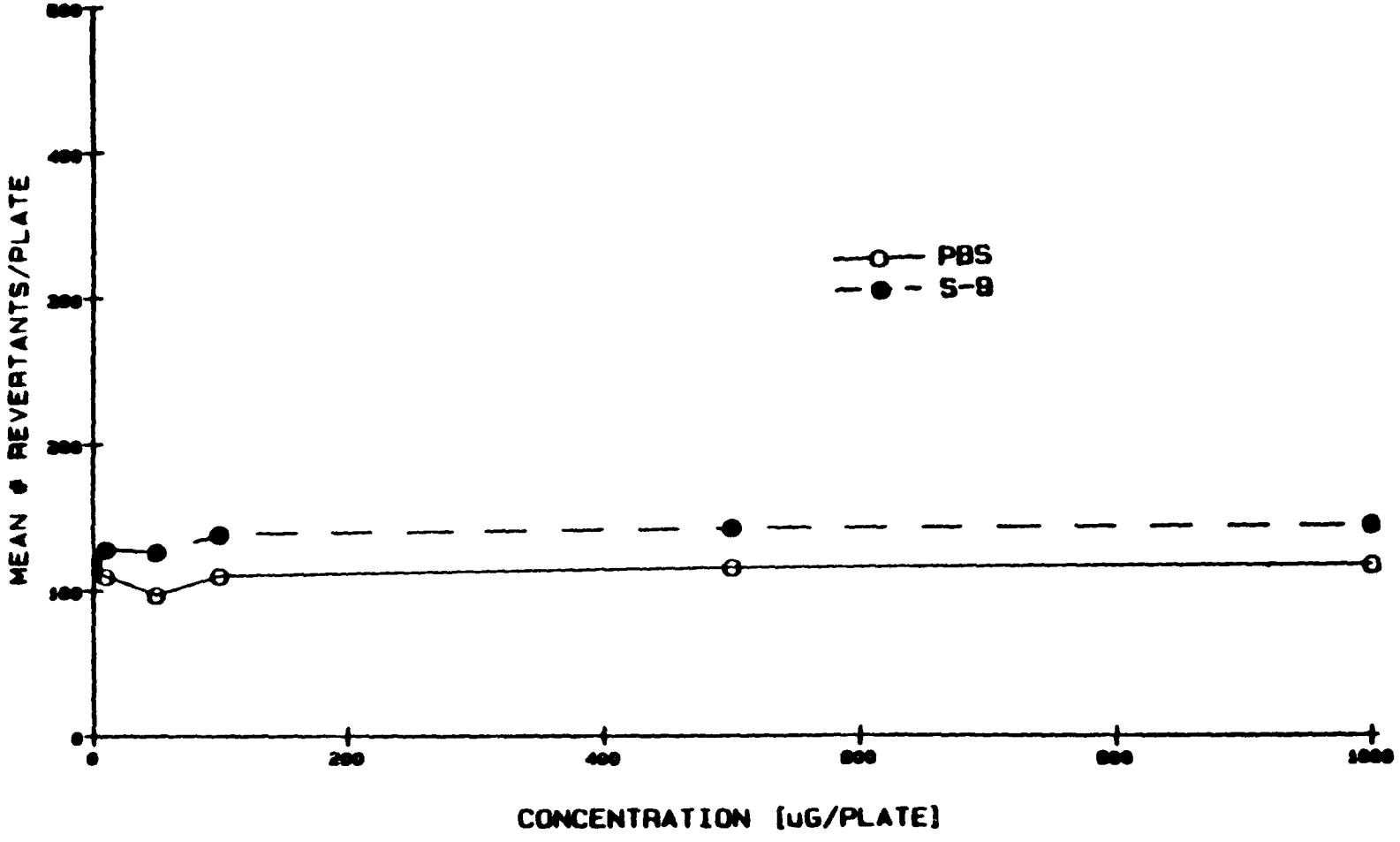
This lot of Disperse Red 11 was manufactured by Professional Chemical and Color of Georgia.

FIGURE 17: MUTAGENIC ACTIVITY OF DISPERSE RED 11 (LOT 2) IN TA-98 STRAIN OF SALMONELLA BACTERIA WITH AND WITHOUT S-9.



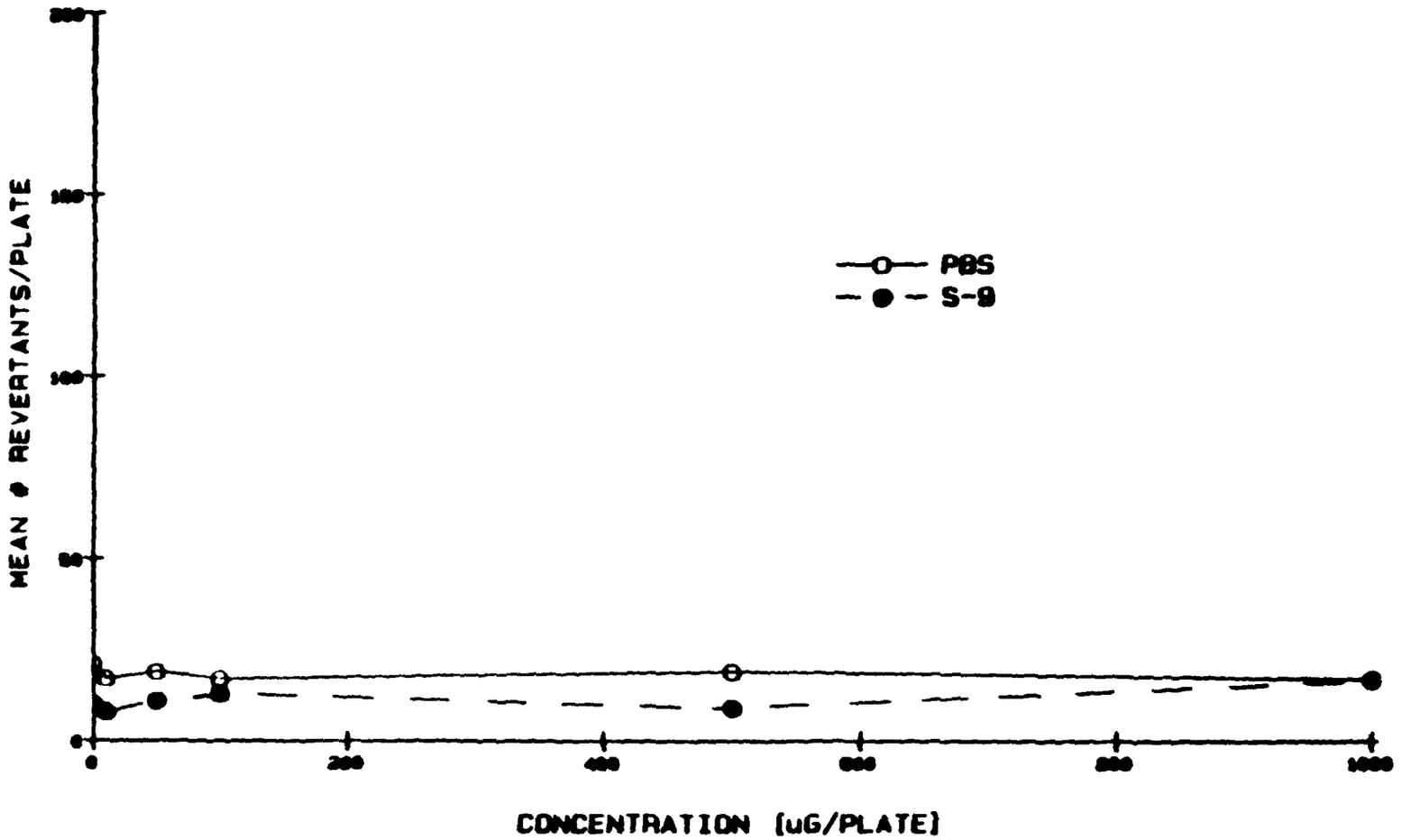
This lot of Disperse Red 11 was manufactured by Professional Chemical and Color of Georgia.

FIGURE 18: MUTAGENIC ACTIVITY OF DISPERSE RED 11 (LOT 2) IN TA-1538 STRAIN OF SALMONELLA BACTERIA WITH AND WITHOUT S-9.



This lot of Disperse Red 11 was manufactured by Professional Chemical and Color of Georgia.

FIGURE 19: MUTAGENIC ACTIVITY OF DISPERSE RED 11 (LOT 2) IN TA-100 STRAIN OF SALMONELLA BACTERIA WITH AND WITHOUT S-9.



This lot of Disperse Red 11 was manufactured by *Professional Chemical and Color of Georgia.*

FIGURE 20: MUTAGENIC ACTIVITY OF DISPERSE RED 11 (LOT 2) IN TA-1535 STRAIN OF SALMONELLA BACTERIA WITH AND WITHOUT S-9.

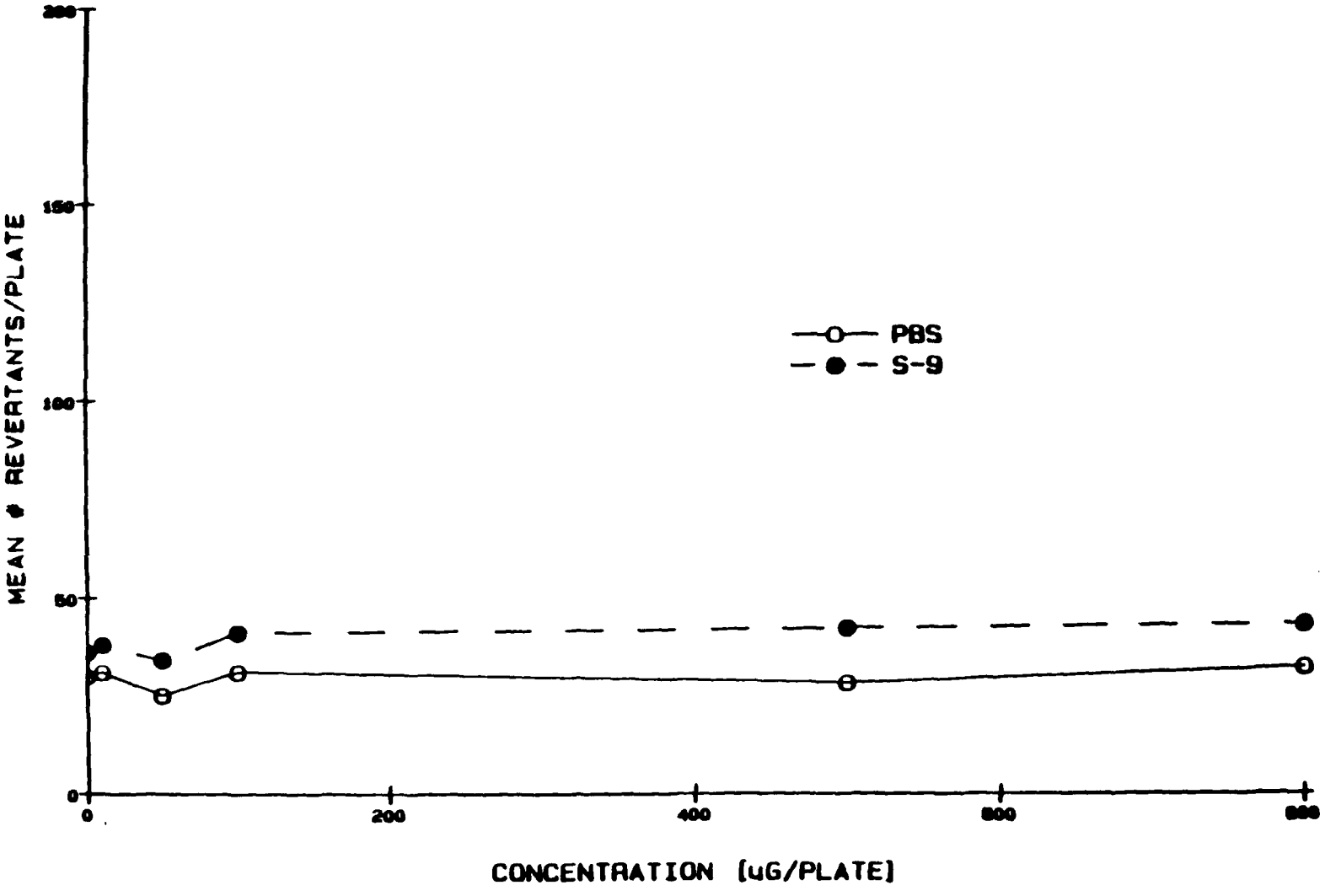


FIGURE 21: MUTAGENIC ACTIVITY OF SOLVENT RED 1 IN TA-98 STRAIN OF SALMONELLA BACTERIA WITH AND WITHOUT S-9.

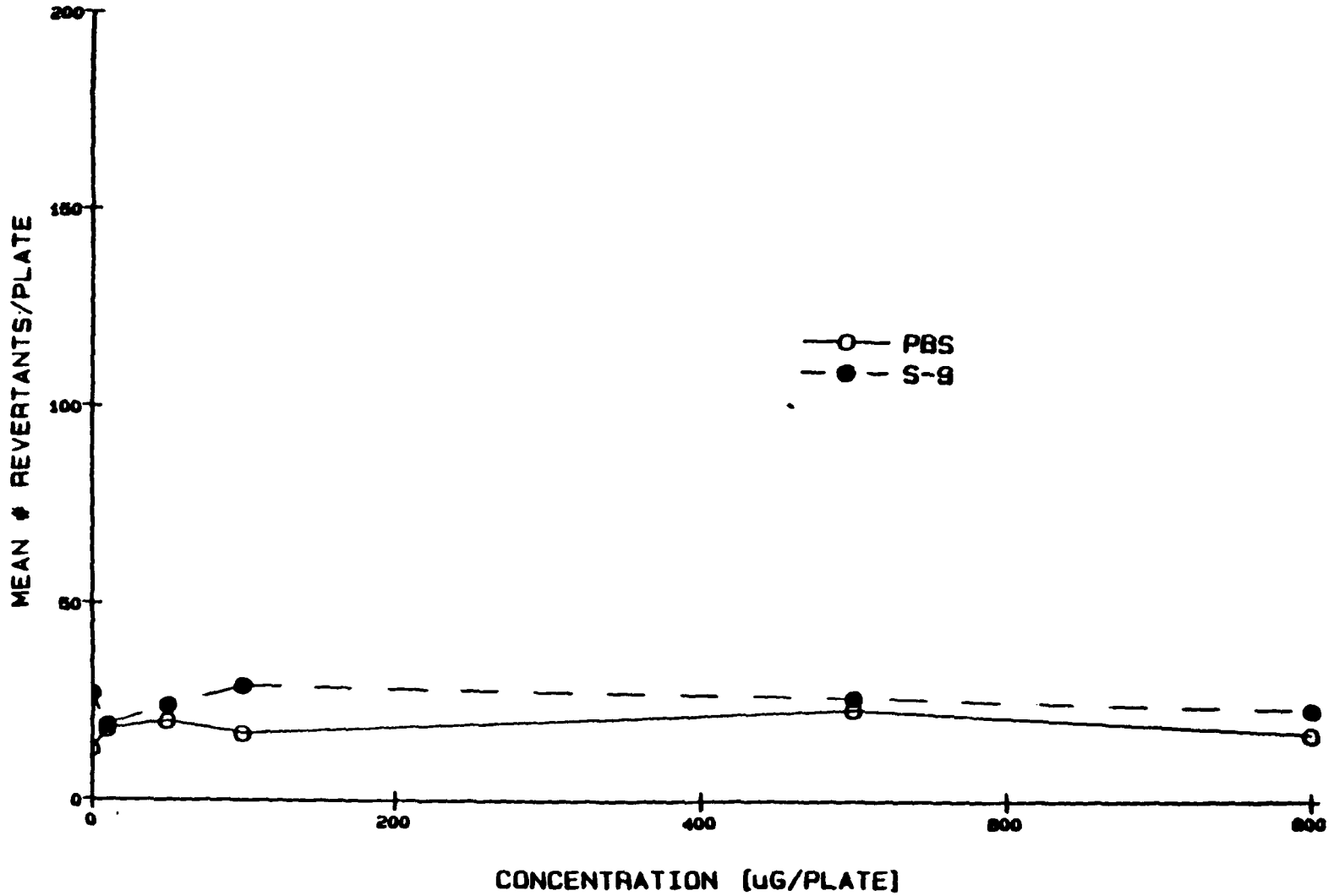


FIGURE 22: MUTAGENIC ACTIVITY OF SOLVENT RED 1 IN TA-1538 STRAIN OF SALMONELLA BACTERIA WITH AND WITHOUT S-9.

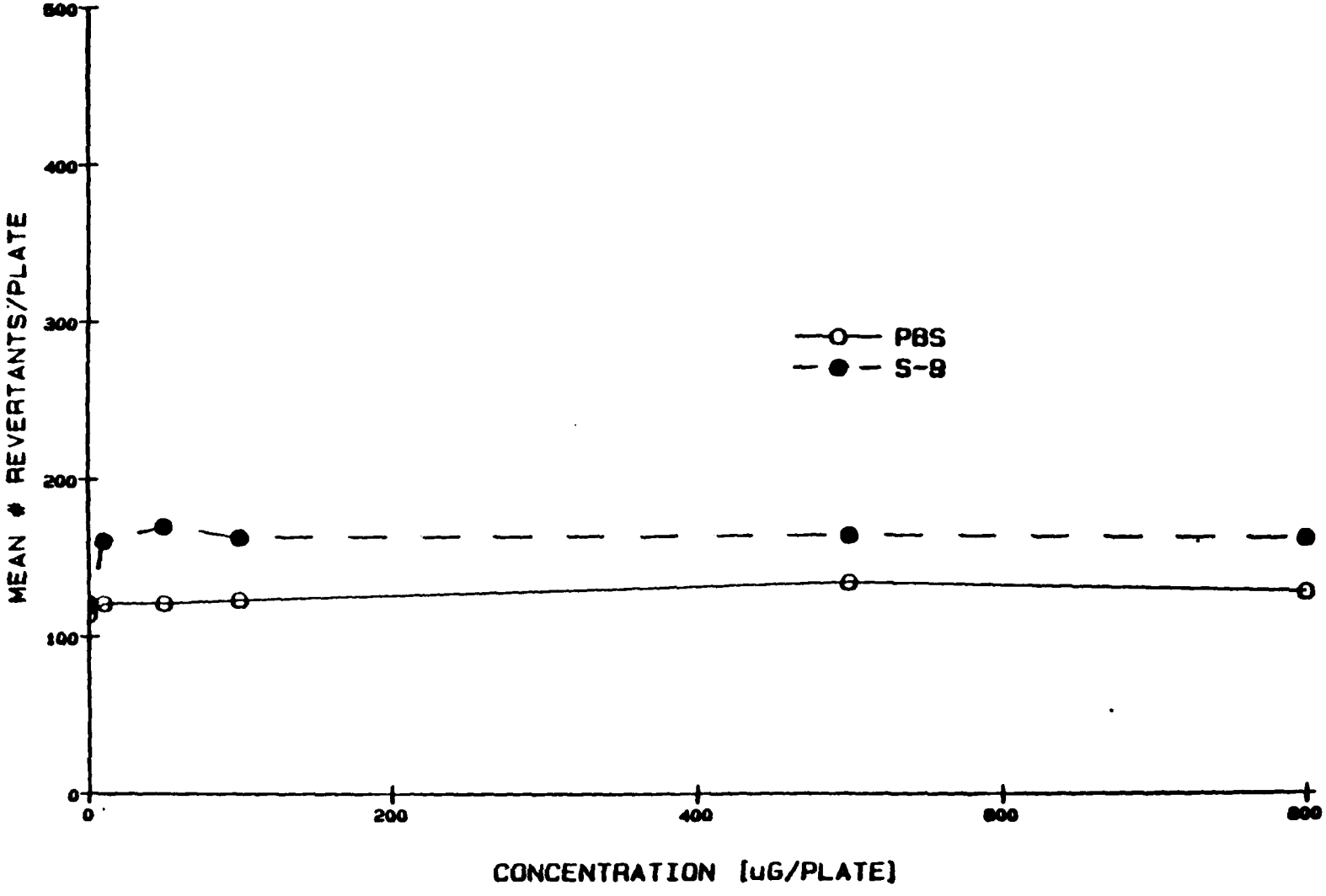


FIGURE 23: MUTAGENIC ACTIVITY OF SOLVENT RED 1 IN TA-100 STRAIN OF SALMONELLA BACTERIA WITH AND WITHOUT S-9.

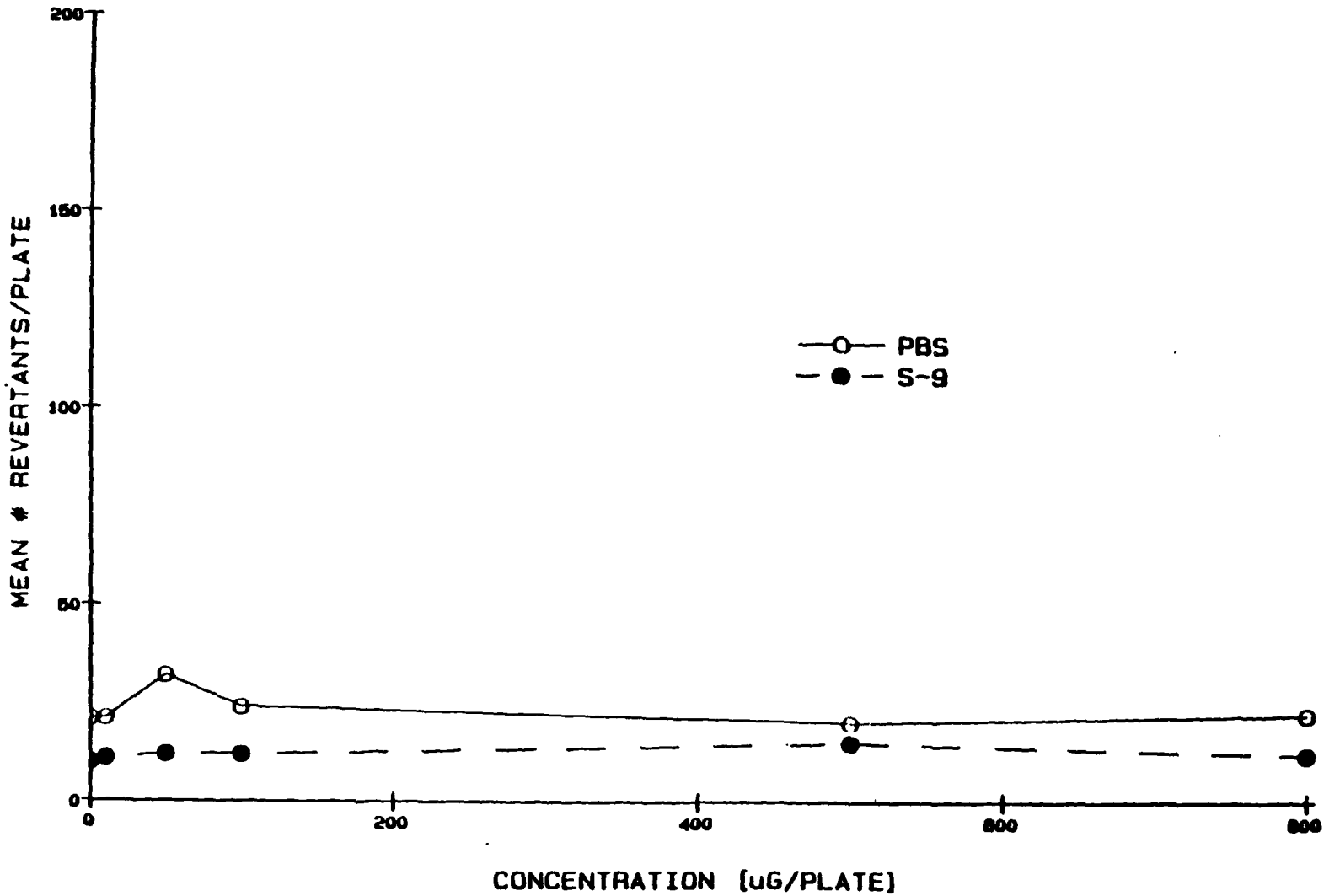


FIGURE 24: MUTAGENIC ACTIVITY OF SOLVENT RED 1 IN TA-1535 STRAIN OF SALMONELLA BACTERIA WITH AND WITHOUT S-9.

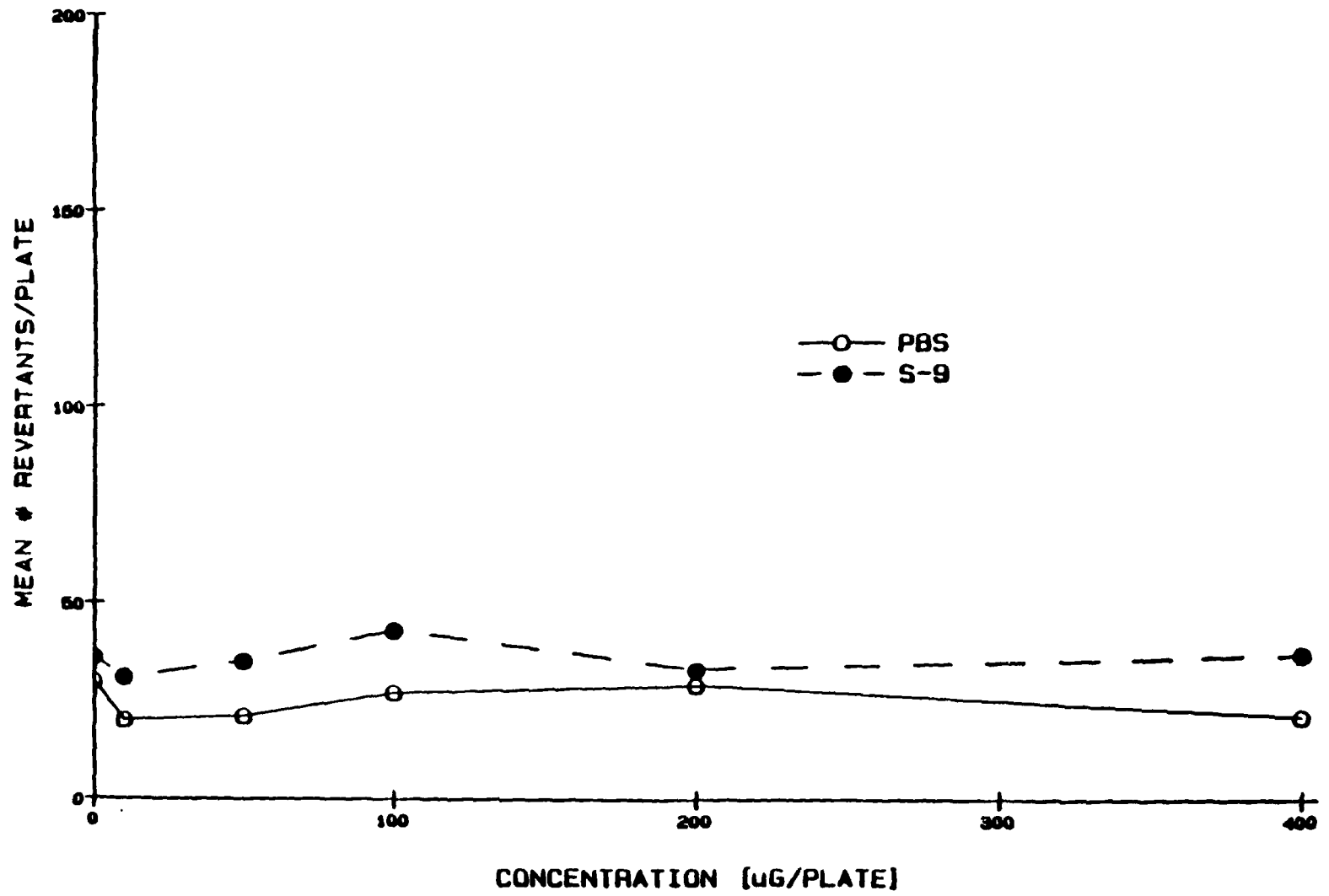


FIGURE 25: MUTAGENIC ACTIVITY OF TEREPHTHALIC ACID IN TA-98 STRAIN OF SALMONELLA BACTERIA WITH AND WITHOUT S-9.

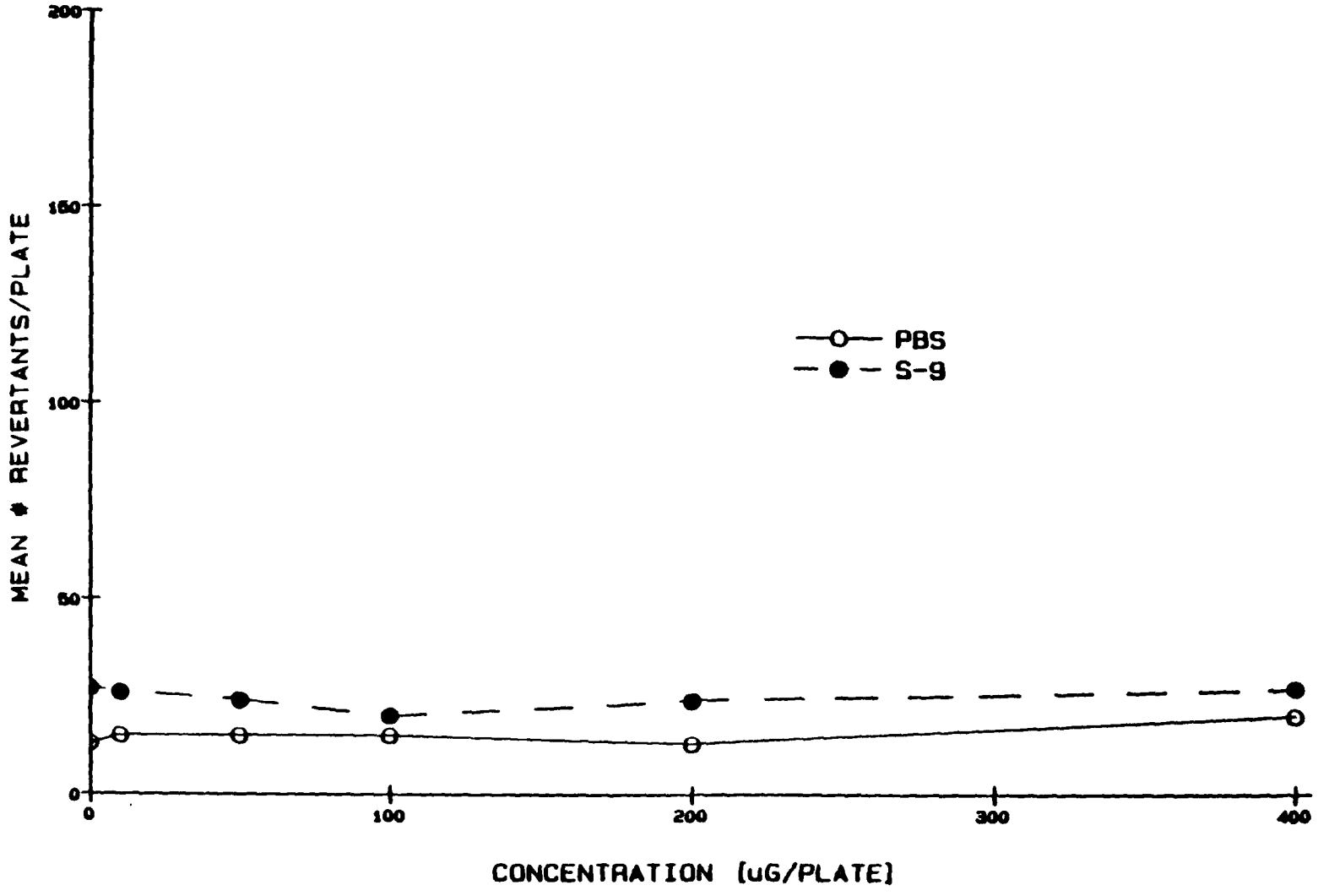


FIGURE 26: MUTAGENIC ACTIVITY OF TEREPHTHALIC ACID IN TA-1538 STRAIN OF SALMONELLA BACTERIA WITH AND WITHOUT S-9.

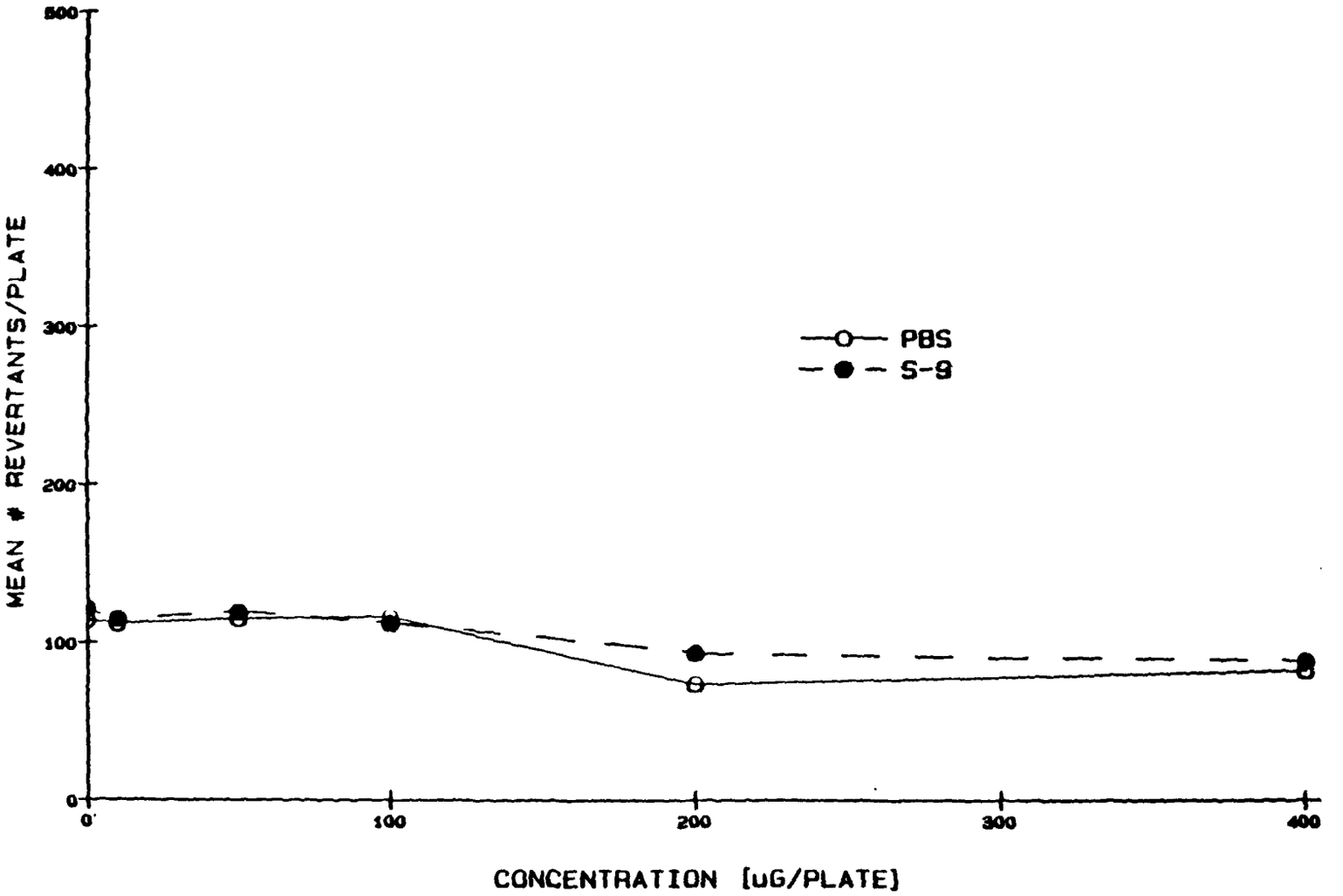


FIGURE 27: MUTAGENIC ACTIVITY OF TEREPHTHALIC ACID IN TA-100 STRAIN OF SALMONELLA BACTERIA WITH AND WITHOUT S-9.

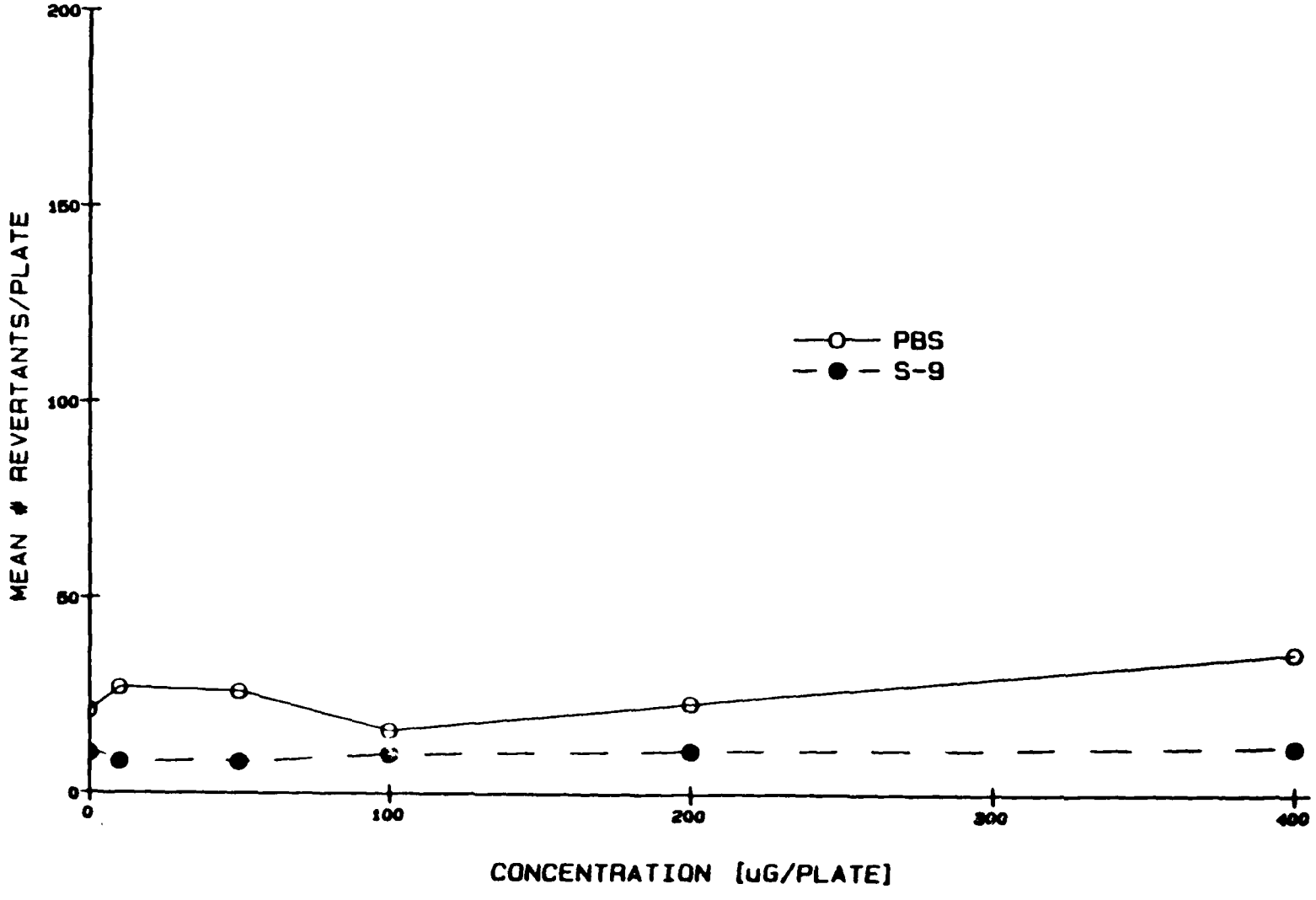


FIGURE 28: MUTAGENIC ACTIVITY OF TEREPHTHALIC ACID IN TA-1535 STRAIN OF SALMONELLA BACTERIA WITH AND WITHOUT S-9.

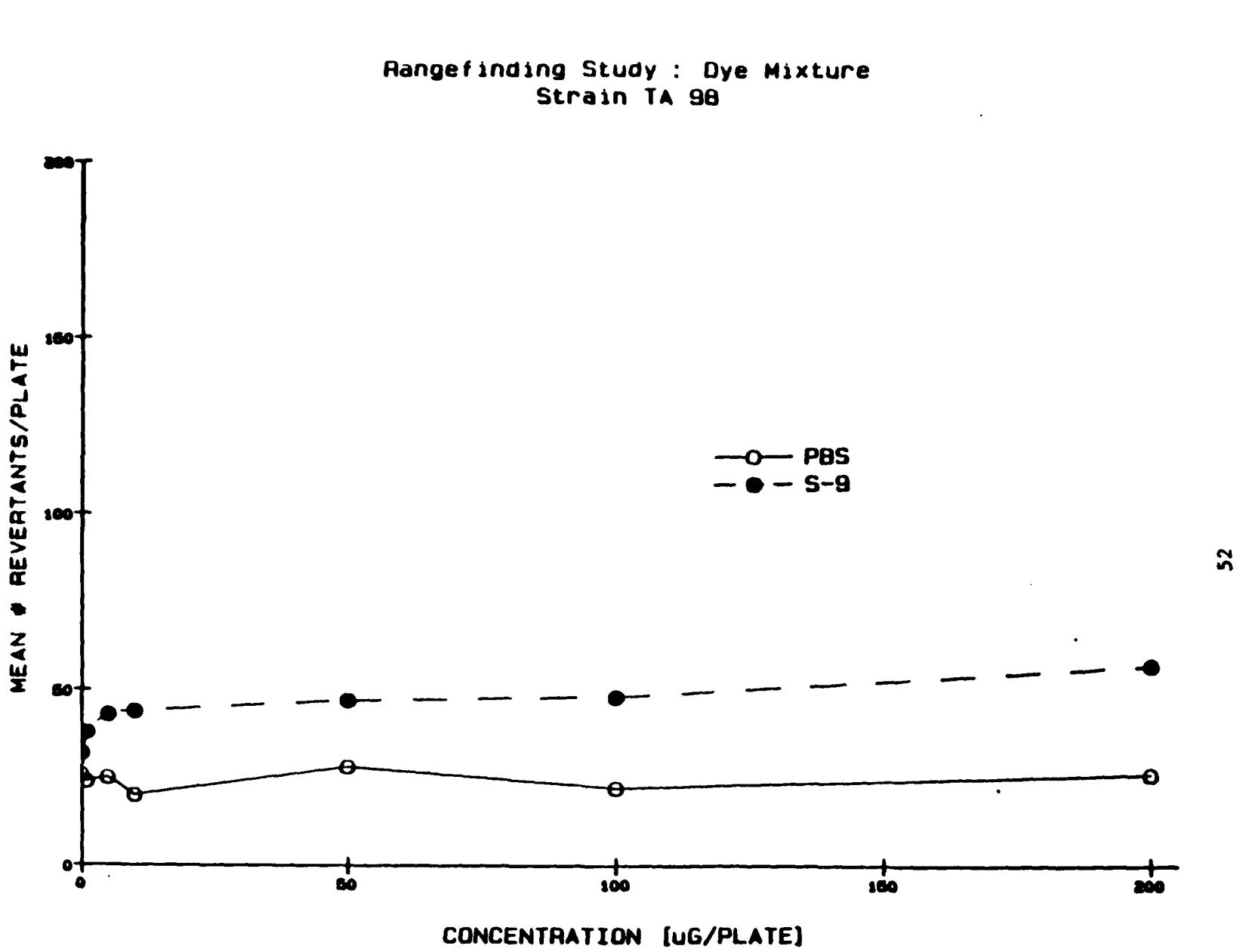


FIGURE 29: MUTAGENIC ACTIVITY OF A MIXTURE OF SOLVENT RED 1, DISPERSE RED 11 (LOT 2), AND TEREPHTHALIC ACID IN TA-98 STRAIN OF SALMONELLA BACTERIA WITH AND WITHOUT S-9.

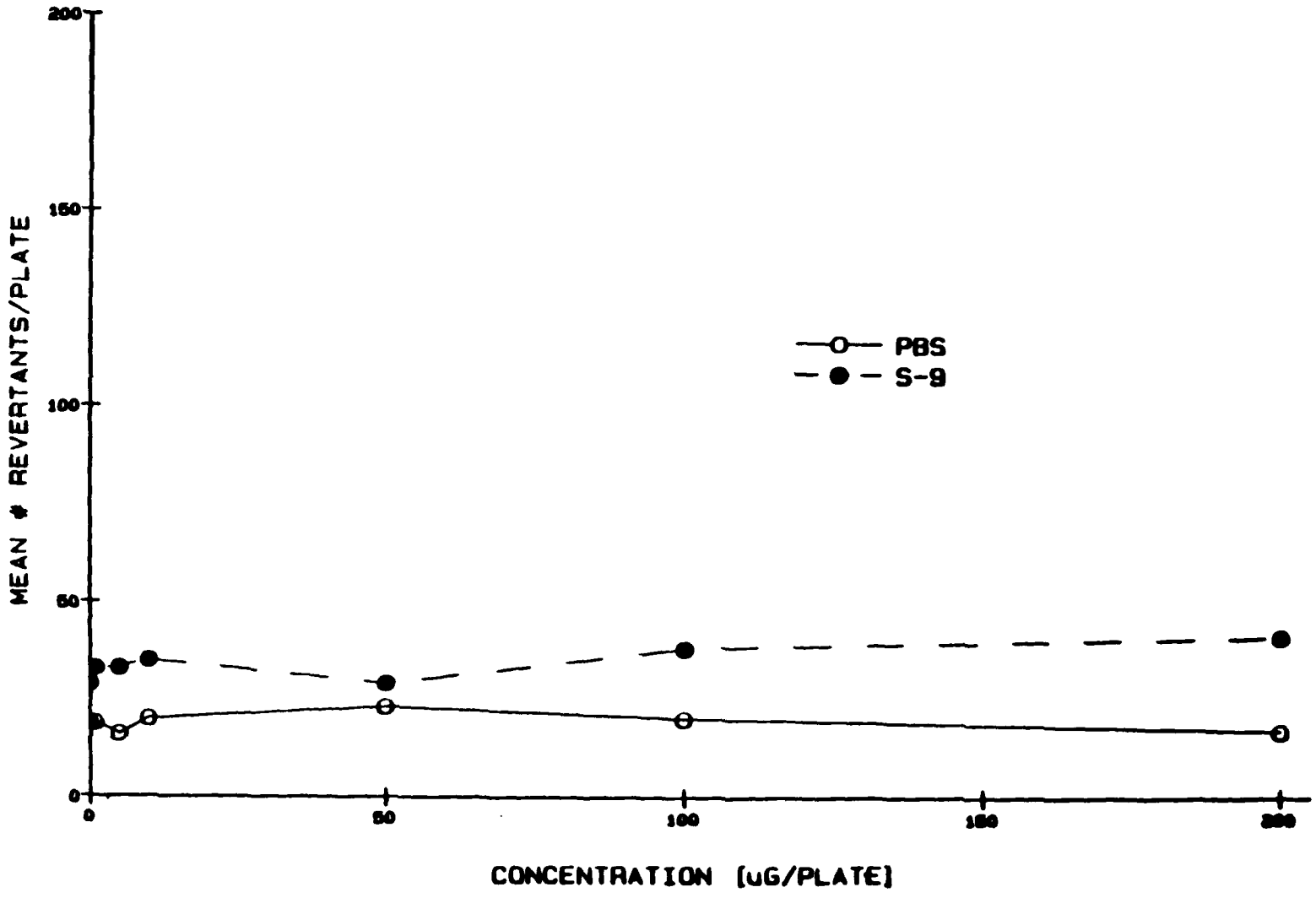
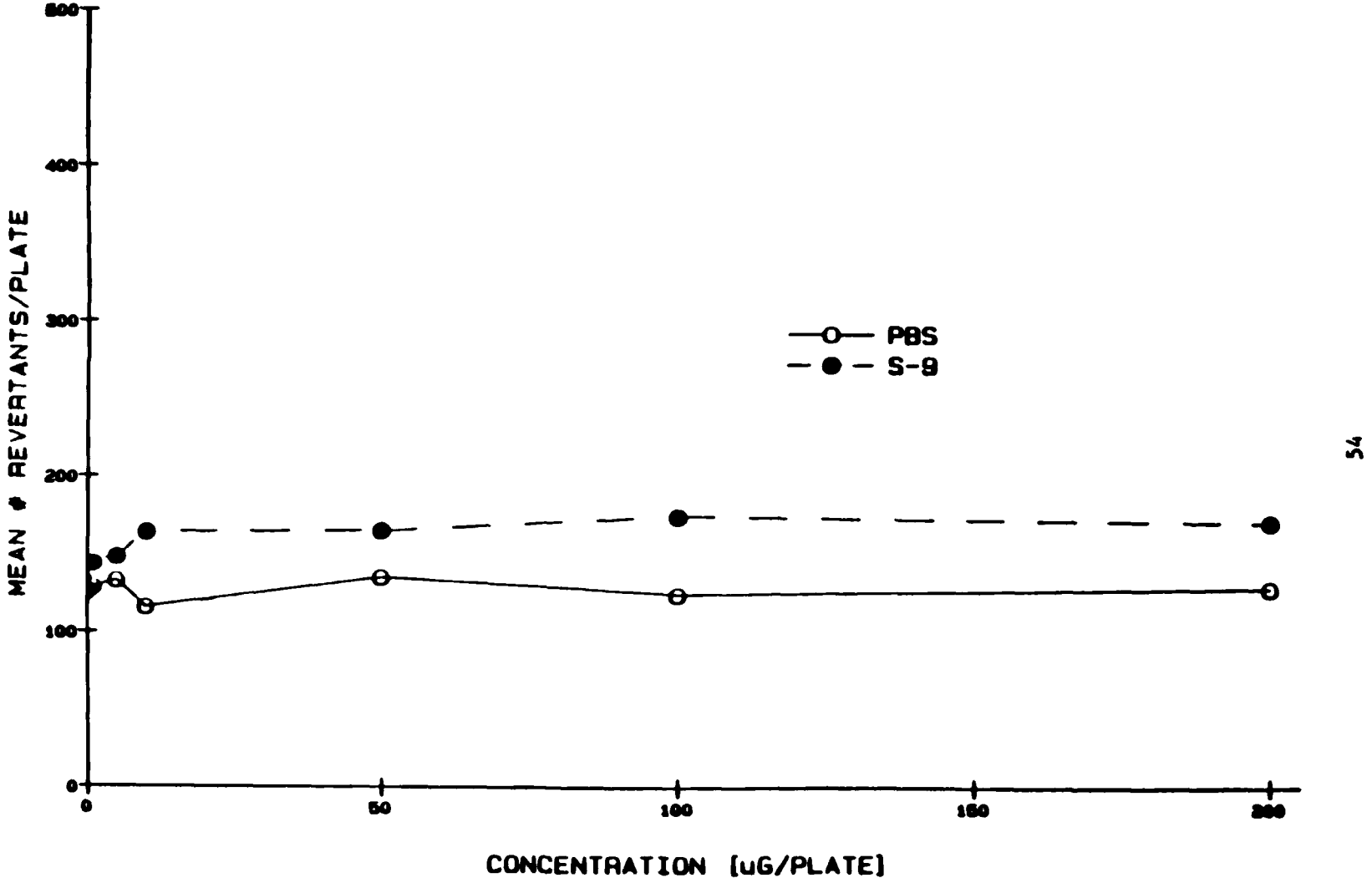


FIGURE 30: MUTAGENIC ACTIVITY OF A MIXTURE OF SOLVENT RED 1, DISPERSE RED 11 (LOT 2), AND TEREPHTHALIC ACID IN TA-1538 STRAIN OF SALMONELLA BACTERIA WITH AND WITHOUT S-9.



54

FIGURE 31: MUTAGENIC ACTIVITY OF A MIXTURE OF SOLVENT RED 1, DISPERSE RED 11 (LOT 2), AND TEREPHTHALIC ACID IN TA-100 STRAIN OF SLAMONELLA BACTERIA WITH AND WITHOUT S-9.

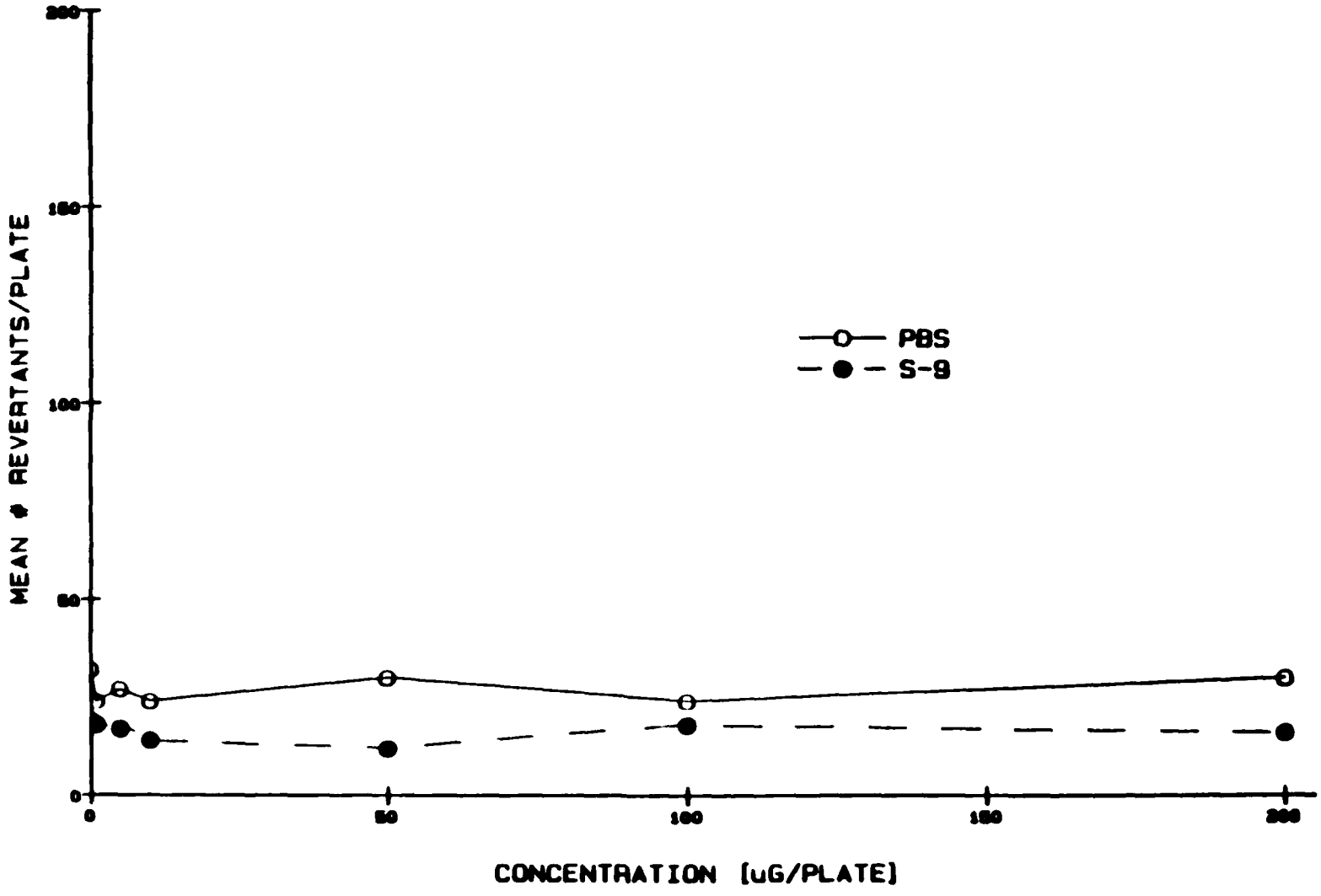


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FIGURE 32: MUTAGENIC ACTIVITY OF A MIXTURE OF SOLVENT RED 1, DISPERSE RED 11 (LOT 2), AND TEREPHTHALIC ACID IN TA-1535 STRAIN OF SALMONELLA BACTERIA WITH AND WITHOUT S-9.

fraction S-9. In the range finding experiments it was determined that without the addition of S-9 none of the dyes tested in any of the different strains had both a slope significantly different from zero and an increased mutagenic response of twice background. There were 5 samples that had slopes which were greater than zero at the P = 0.05 level of significance The highest slope for any of these samples was 0.04 revertants/mg which did not result in twice the background response over the range of dyes tested Therefore no direct mutagenic activity was detected. Strain TA-1535 showed no evidence of a significant response to any of the dyes both with and without the addition of S-9. Thus, none of the dyes produced base pair type of mutations at a frequency high enough to be detected In the range finding experiment no significant mutagenic responses, based on a slope greater than zero and a response twice background, were observed for Solvent Red 24, Disperse Red 11 (Lot 1), Solvent Red 1, terephthalic acid or the dye mixture, either with or without the addition of 30 µl S-9/plate.

The addition of 30 µl/plate S-9 resulted in a response that was greater than twice background for Disperse Red 15 in strains TA-98 and TA-1538. The response for this dye was higher than observed for any of the other dyes tested. The mutant frequency increased to a peak at 100 µg/plate, then decreased as a function of concentration. This decrease may be related to toxicity of the dye or an inactivation of the S-9 by the dye. Because of the shape of the curves, the slopes of dose response curves were not significantly different from zero if fit to the whole range of dye concentrations. If fit over the lower range of concentrations they were highly significant $p < 0.01$. For Disperse Red 11 (lot 2) there was a mutagenic response greater than twice background in both strains TA-98 and

TA-1538. These results demonstrated that both of the anthraquinone dyes tested contain some ability to produce a frame shift type of mutations in bacterial strains TA-98 and TA-1538. After the initial increase in response at low concentrations, the rather constant response seen for Disperse Red 11 (Lot 2) with TA-98 and TA-1538 (Figures 17, 18) at all concentrations tested suggest that either the dye had limited solubility or that the ability of the S-9 to metabolize the dye to an active mutagen was saturated at low concentrations of the dye.

To evaluate the possible influence of S-9 concentration on the mutagenic response of the bacteria to Disperse Red 15, Disperse Red 11 (Lot 2), Solvent Red 1 and the mixture of Solvent Red 1, Disperse Red 11 (Lot 2) and terephthalic acid dyes, additional studies were conducted at lower dye concentrations using a range of S-9 concentrations from 15-120 µl per plate. For Disperse Red 15 (Figure 33) these tests were conducted using only strain TA-98. The other dyes and the mixture were evaluated using the same S-9 concentrations (15, 30, 60 and 120 µl/plate) in all four bacterial strains TA-1538, TA-1535, TA-98 and TA-100. The results of these studies which show the influence of S-9 concentration on mutagenic activity are plotted in Figures 33-45. Each dye and each bacterial strain is plotted on a separate figure. The data used in these figures is in Appendix C. Statistical evaluation of the data is also included in the Appendix D. Again the only dyes that resulted in a significant increase in the mutation frequency, (response greater than twice background and a slope different from zero) were the anthraquinone dyes Disperse Red 15 in TA-98 (Figure 33) and Disperse Red 11 (Lot 2) tested in TA 98 and TA-1538 at S-9 concentration of 120 µl/plate $p = 0.02$ and $p = 0.005$, respectively (Figures 34, 35).

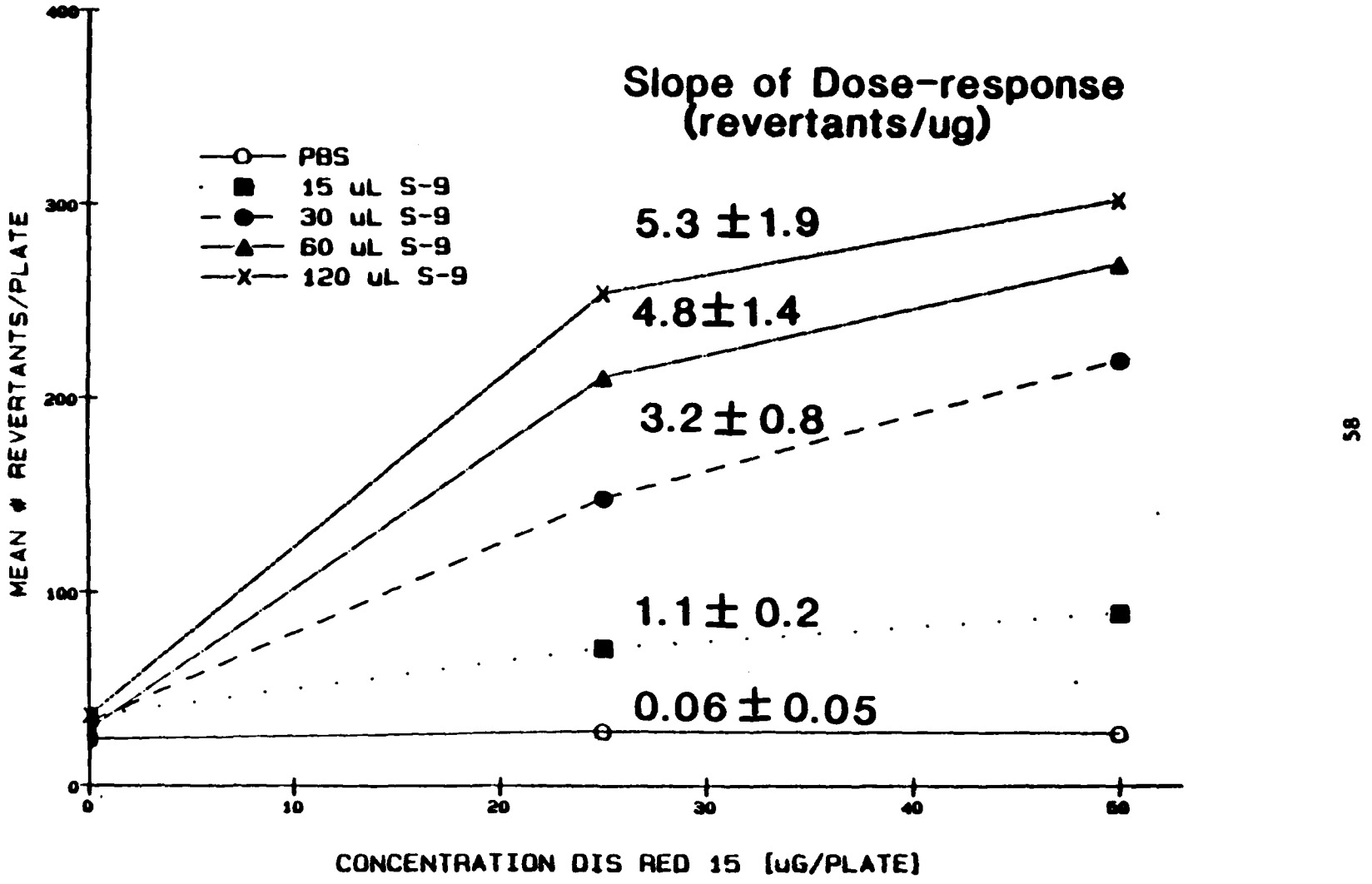

FIGURE 33: INFLUENCE OF S-9 CONCENTRATION ON MUTAGENICITY OF DISPERSE RED 15 IN TA-98.

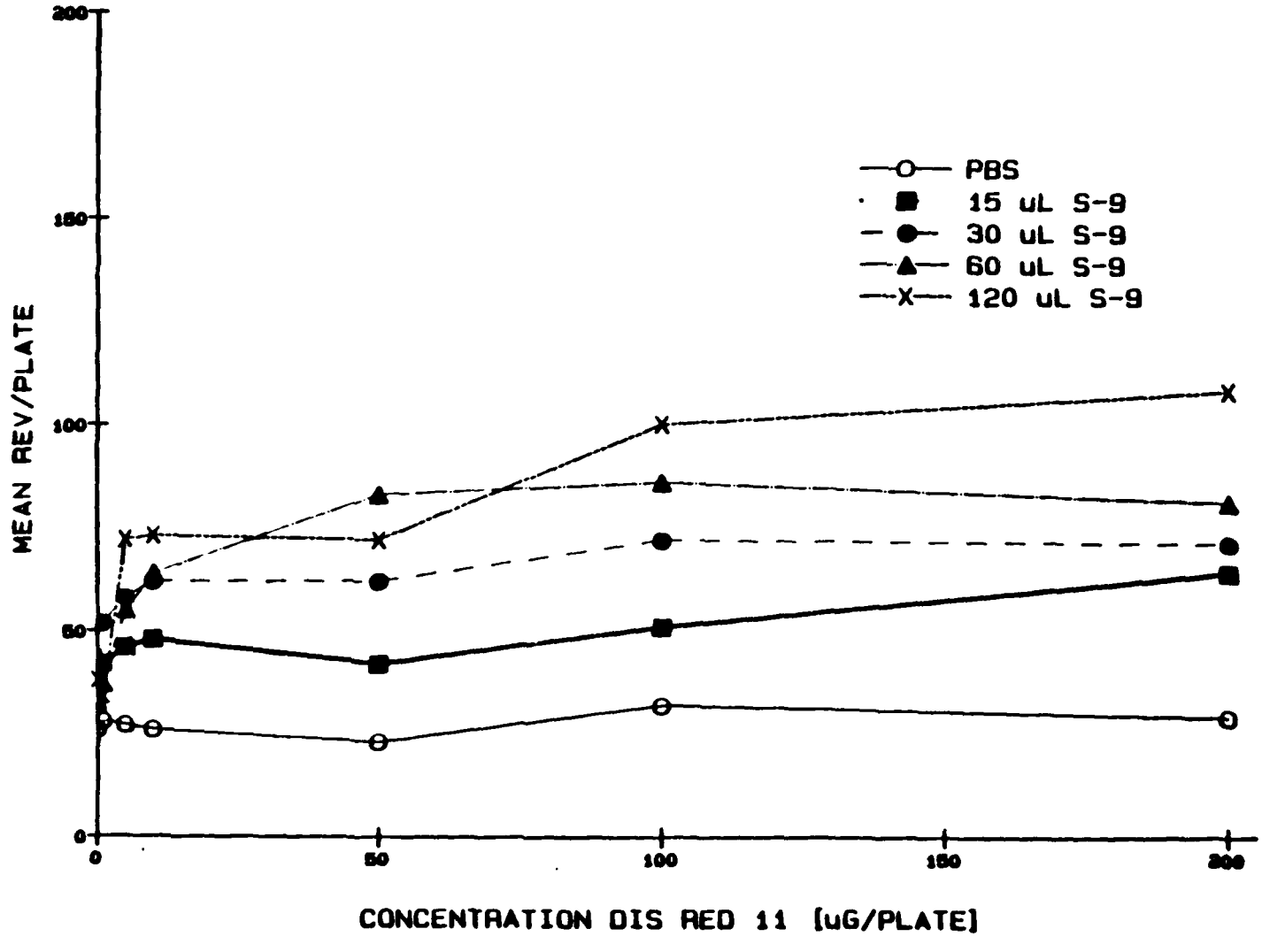

FIGURE 34: INFLUENCE OF S-9 CONCENTRATION ON MUTAGENICITY OF DISPERSE RED 11 (LOT 2) IN TA-98 STRAIN OF SALMONELLA BACTERIA.

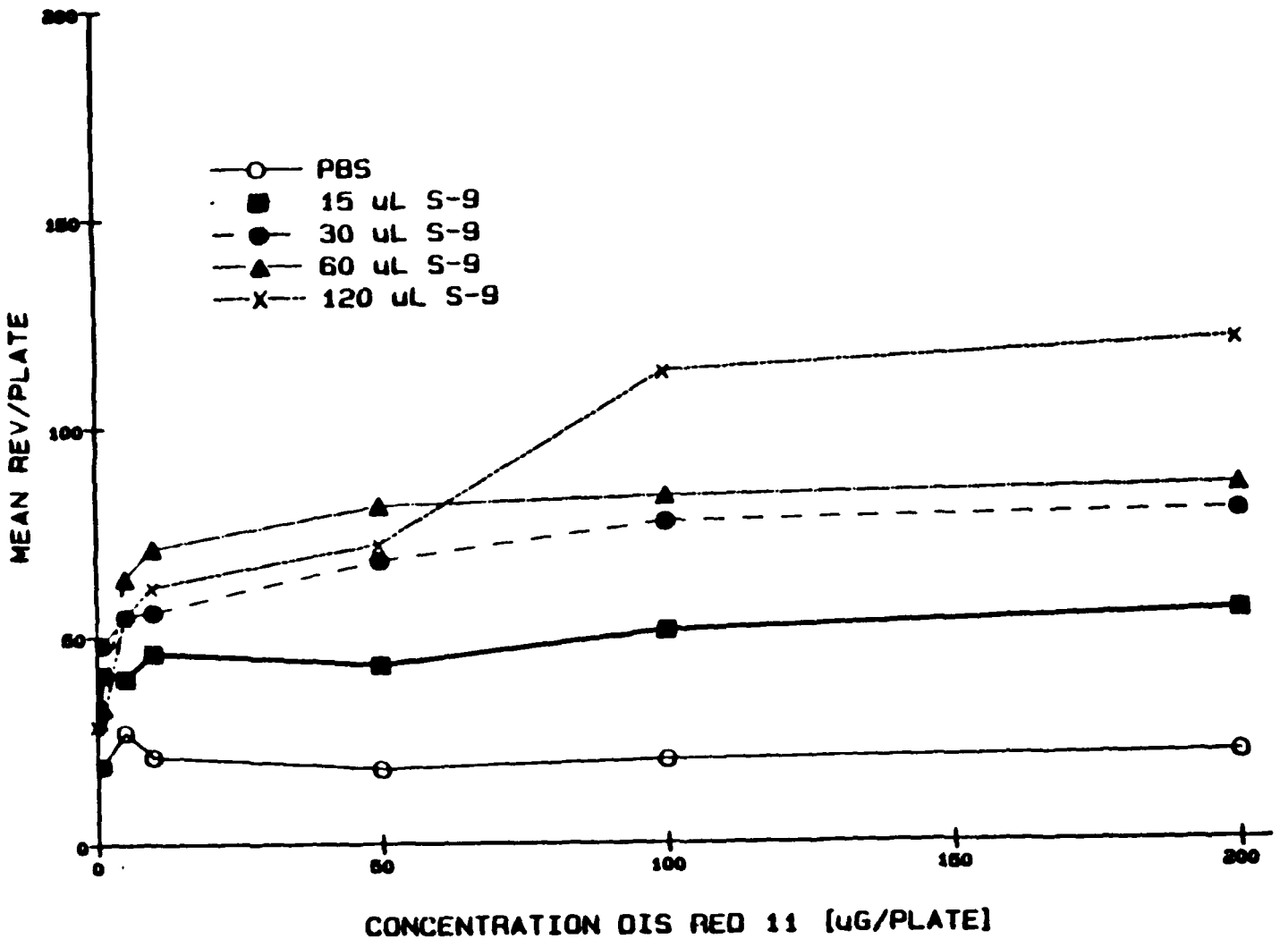

FIGURE 35: INFLUENCE OF S-9 CONCENTRATION ON MUTAGENICITY OF DISPERSE RED 11 (LOT 2) IN TA-1538 STRAIN OF SALMONELLA BACTERIA.

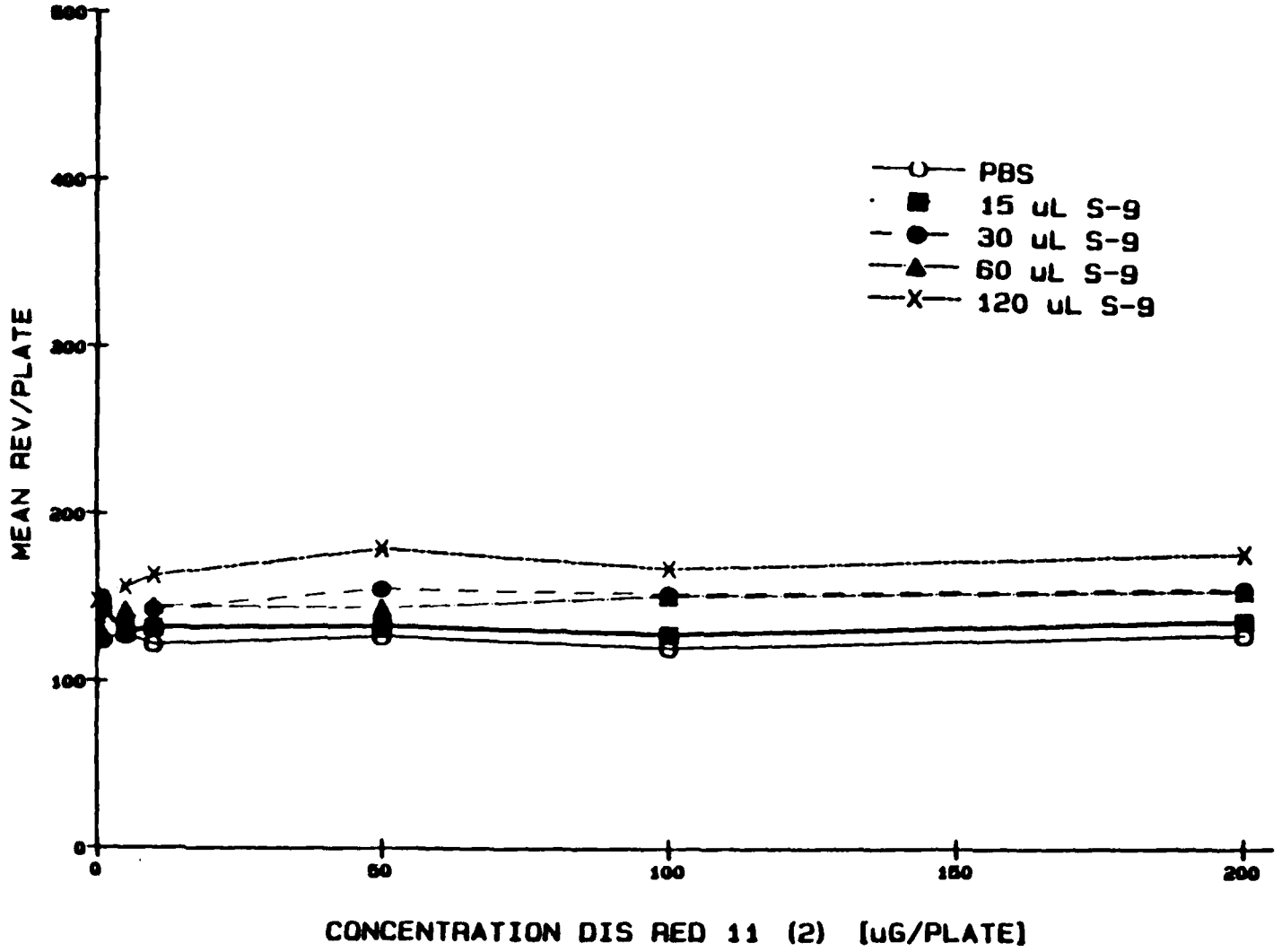

FIGURE 36: INFLUENCE OF S-9 CONCENTRATION ON MUTAGENICITY OF DISPERSE RED 11 (LOT 2) IN TA-100 STRAIN OF SALMONELLA BACTERIA.

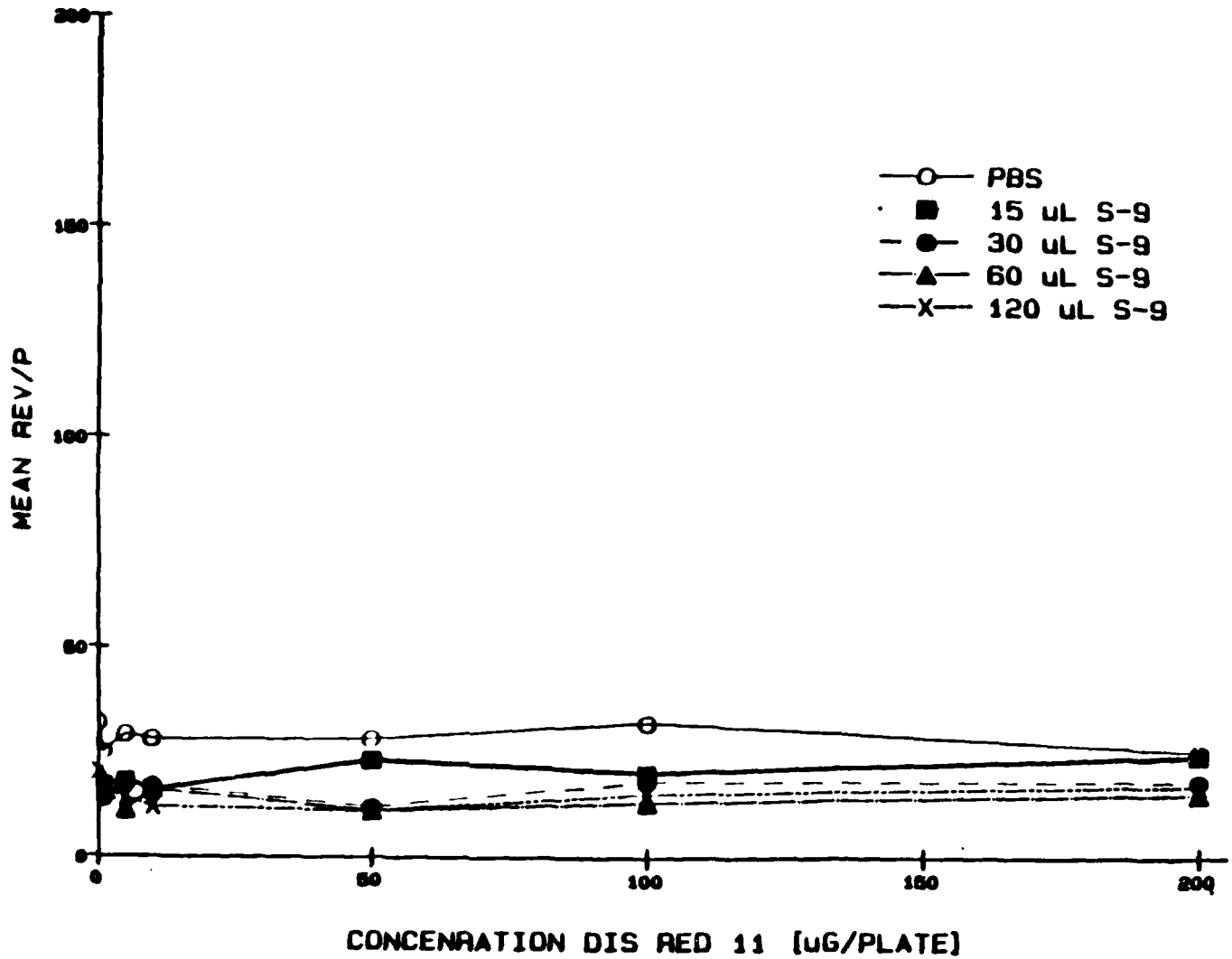

FIGURE 37: INFLUENCE OF S-9 CONCENTRATION ON MUTAGENICITY OF DISPERSE RED 11 (LOT 2) IN TA-1535 STRAIN OF SALMONELLA BACTERIA.

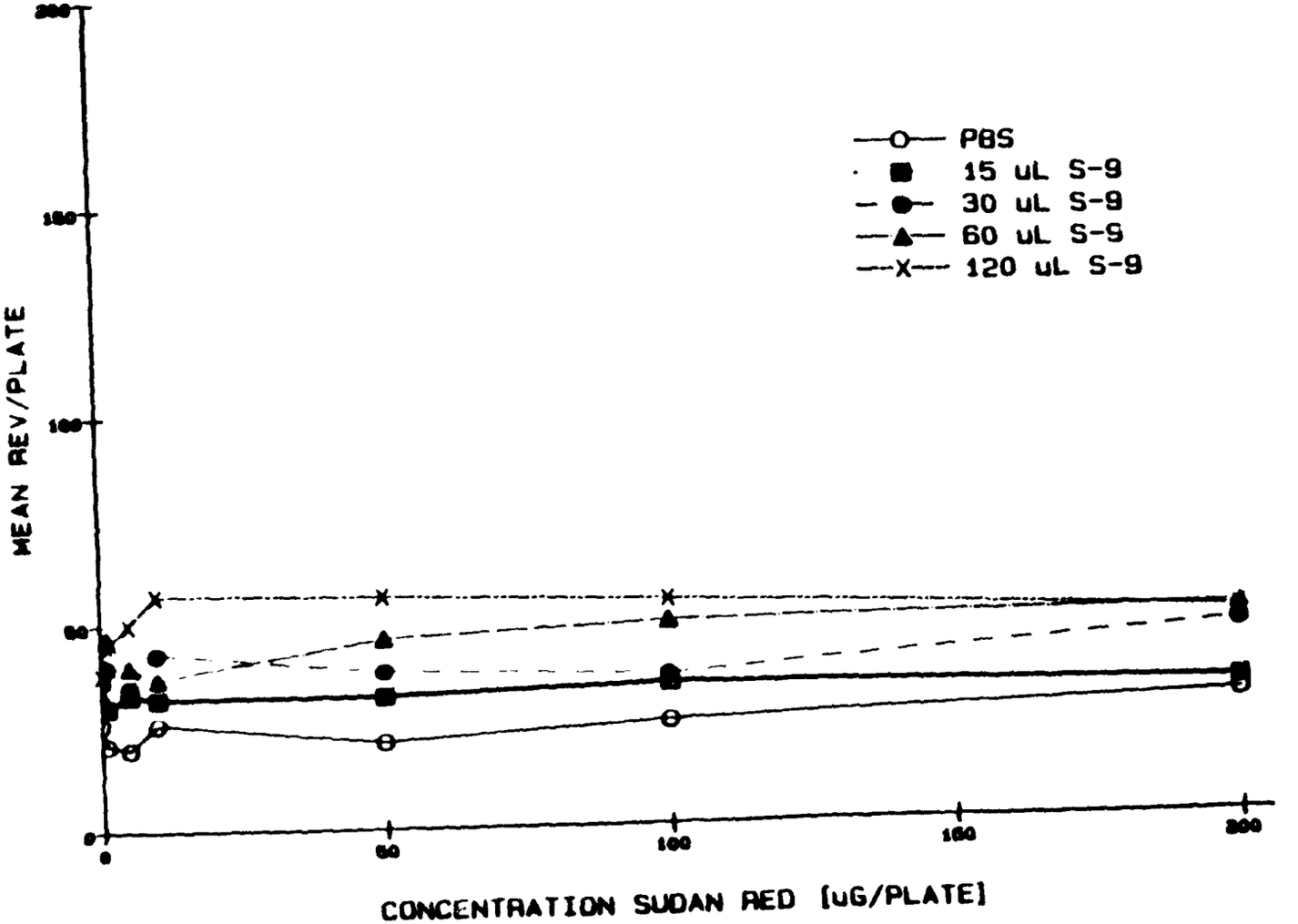

FIGURE 38: INFLUENCE OF S-9 CONCENTRATION ON MUTAGENICITY OF SOLVENT RED 1 IN TA-98 STRAIN OF SALMONELLA BACTERIA.

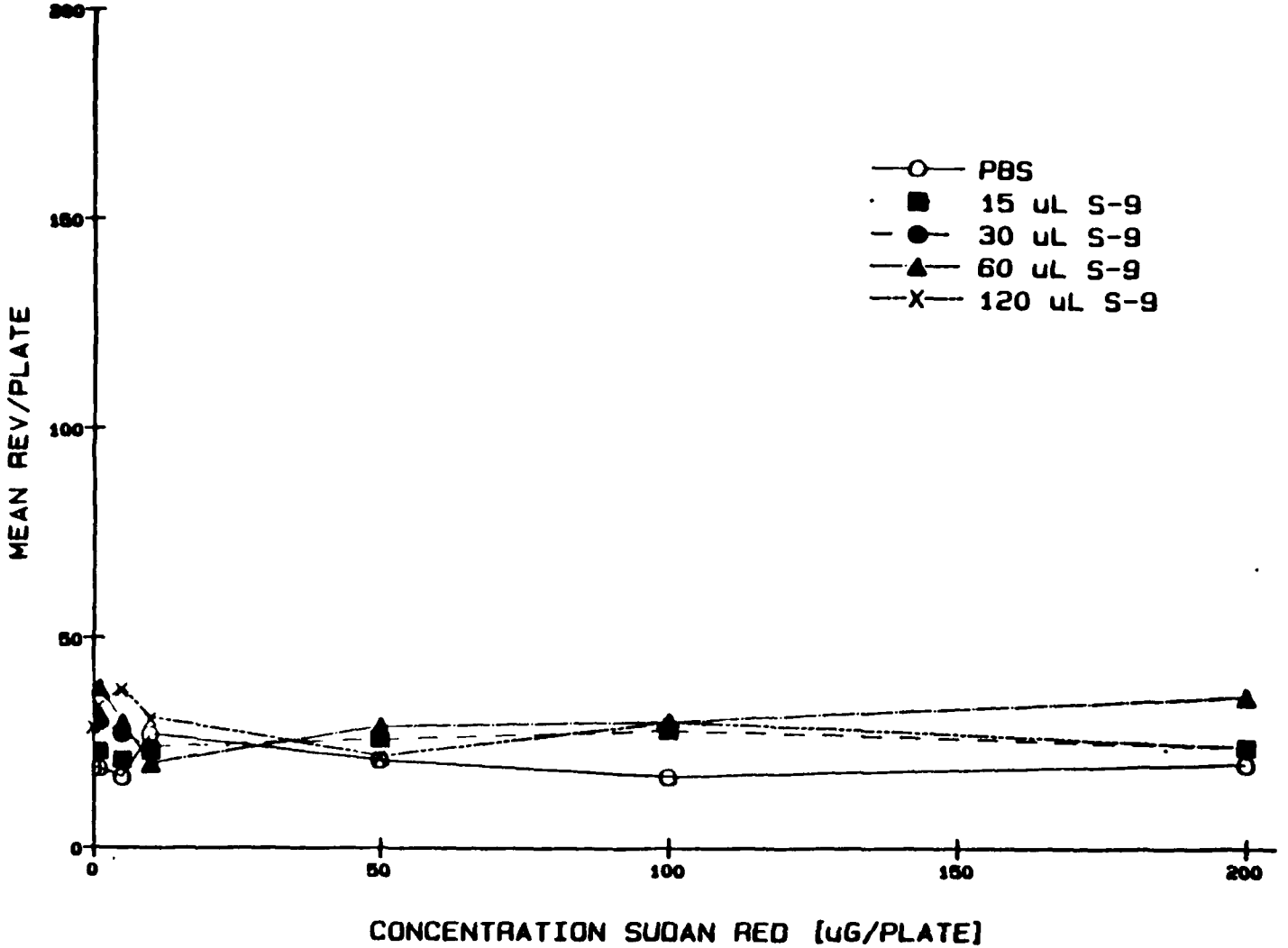

FIGURE 39: INFLUENCE OF S-9 CONCENTRATION ON MUTAGENICITY OF SOLVENT RED 1 IN TA-1538 STRAIN OF SALMONELLA BACTERIA.

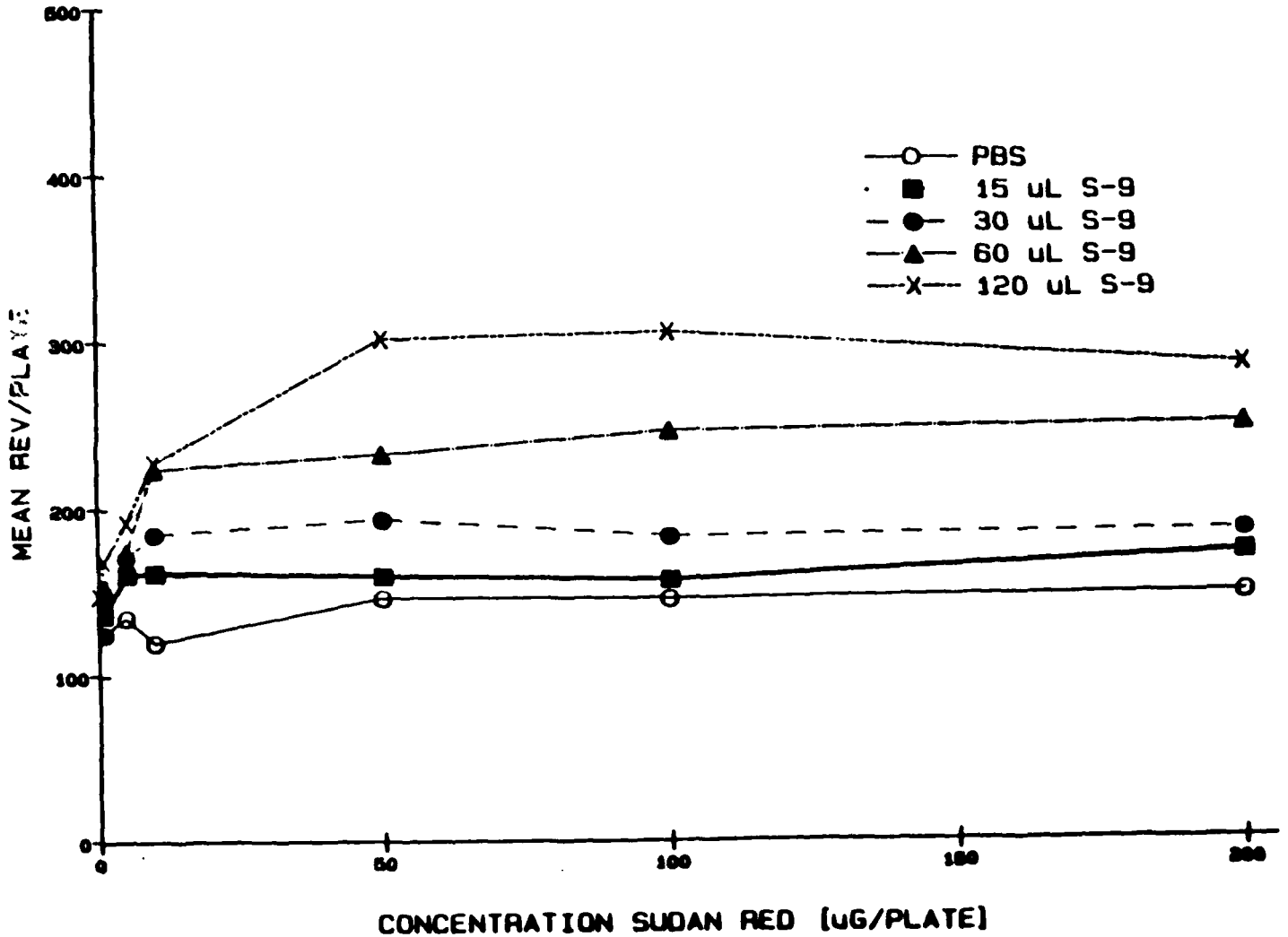

FIGURE 40: INFLUENCE OF S-9 CONCENTRATION ON MUTAGENICITY OF SOLVENT RED 1 IN TA-100 STRAIN OF SALMONELLA BACTERIA.

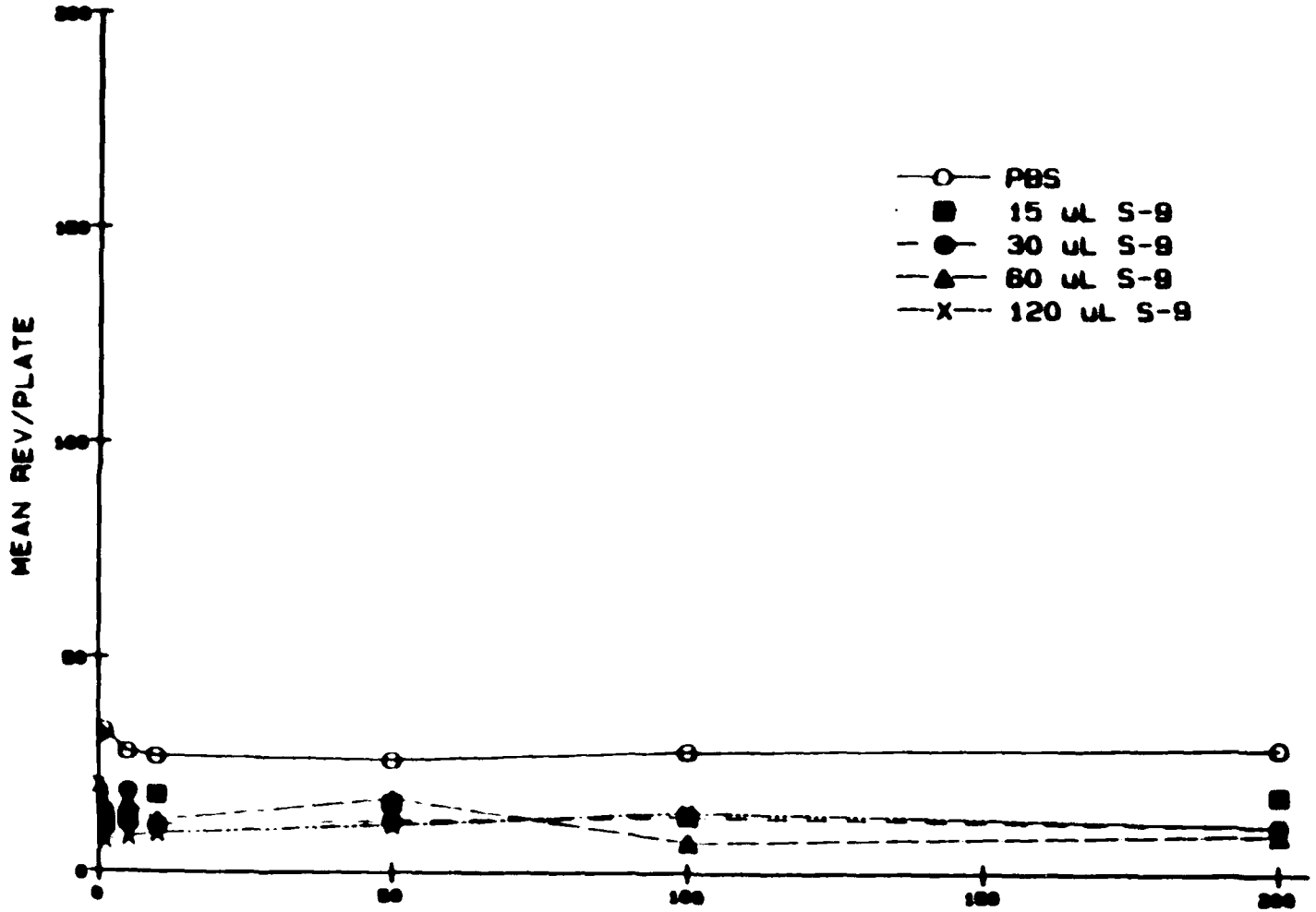

FIGURE 41: INFLUENCE OF S-9 CONCENTRATION ON MUTAGENICITY OF SOLVENT RED 1 IN TA-1535 STRAIN OF SALMONELLA BACTERIA.

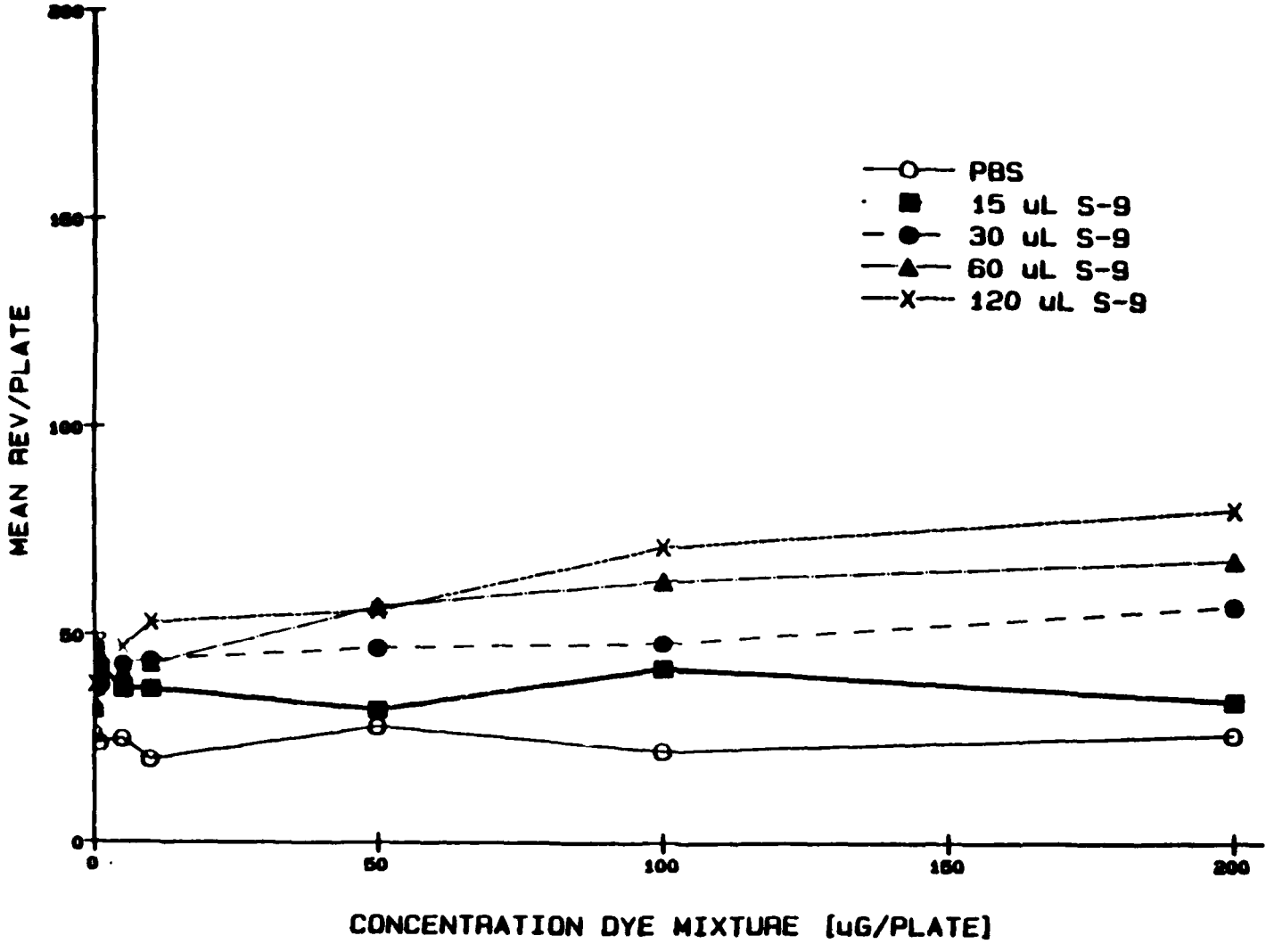

FIGURE 42: INFLUENCE OF S-9 CONCENTRATION ON MUTAGENICITY OF A MIXTURE OF SOLVENT RED 1, DISPERSE RED 11 (LOT 2), AND TEREPHTHALIC ACID IN TA-98 STRAIN OF SALMONELLA BACTERIA.

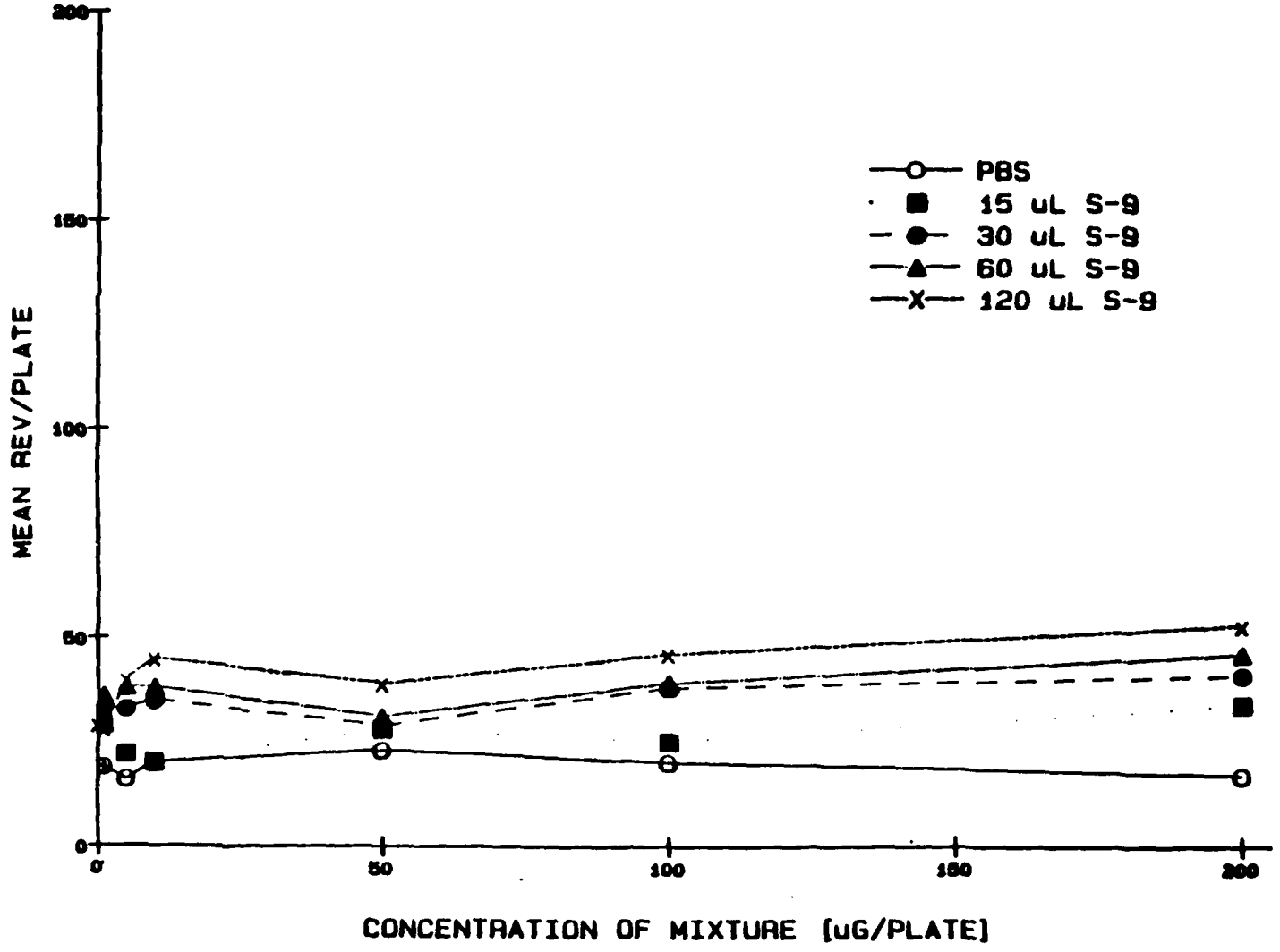

FIGURE 43: INFLUENCE OF S-9 CONCENTRATION ON MUTAGENICITY OF A MIXTURE OF SOLVENT RED 1, DISPERSE RED 11 (LOT 2), AND TEREPHTHALIC ACID IN TA-1538 STRAIN OF SALMONELLA BACTERIA.

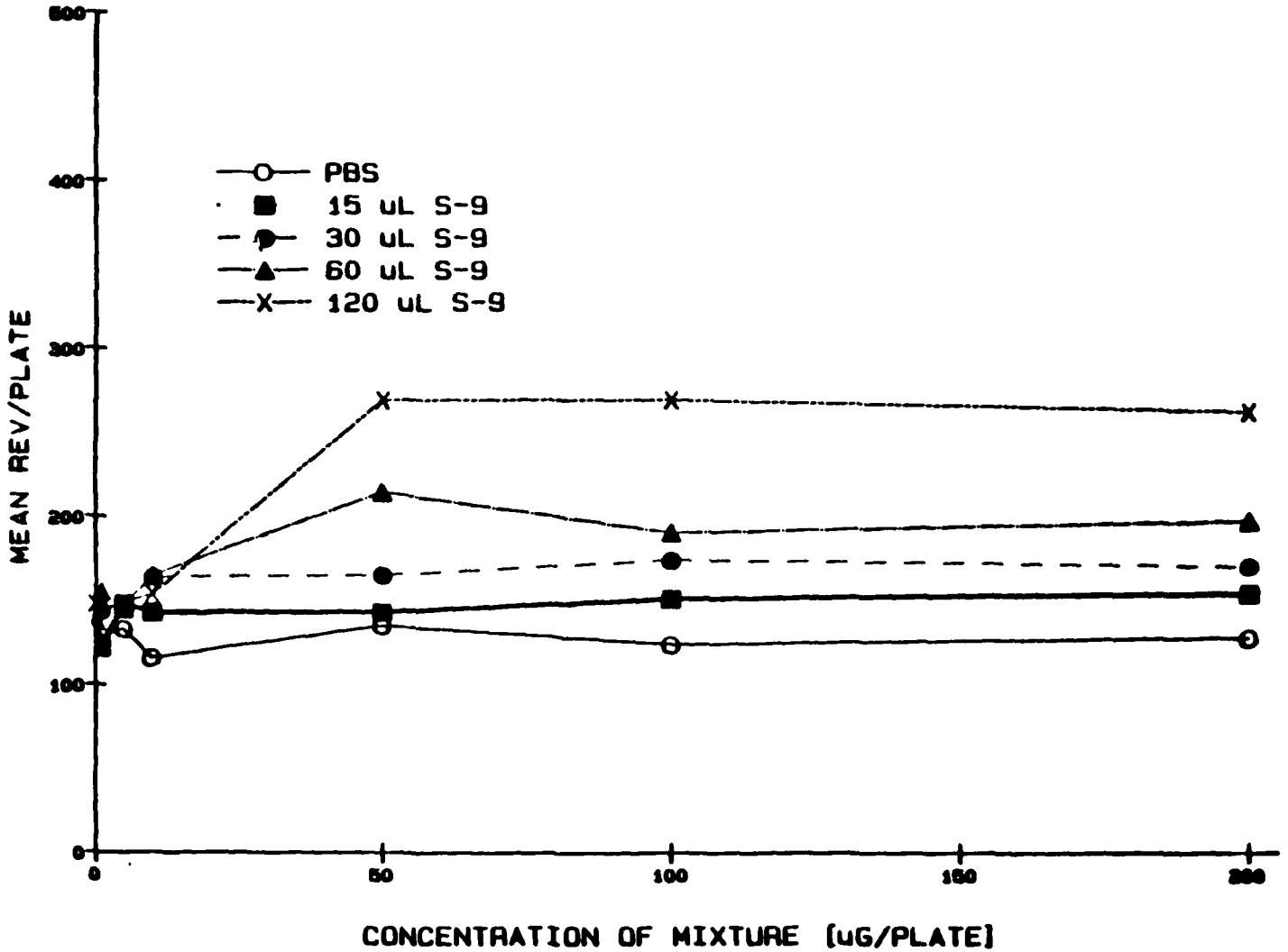

FIGURE 44: INFLUENCE OF S-9 CONCENTRATION ON MUTAGENICITY OF A MIXTURE OF SOLVENT RED 1, DISPERSE RED 11 (LOT 2), AND TERFPHTHALIC ACID IN TA-100 STRAIN OF SALMONELLA BACTERIA.

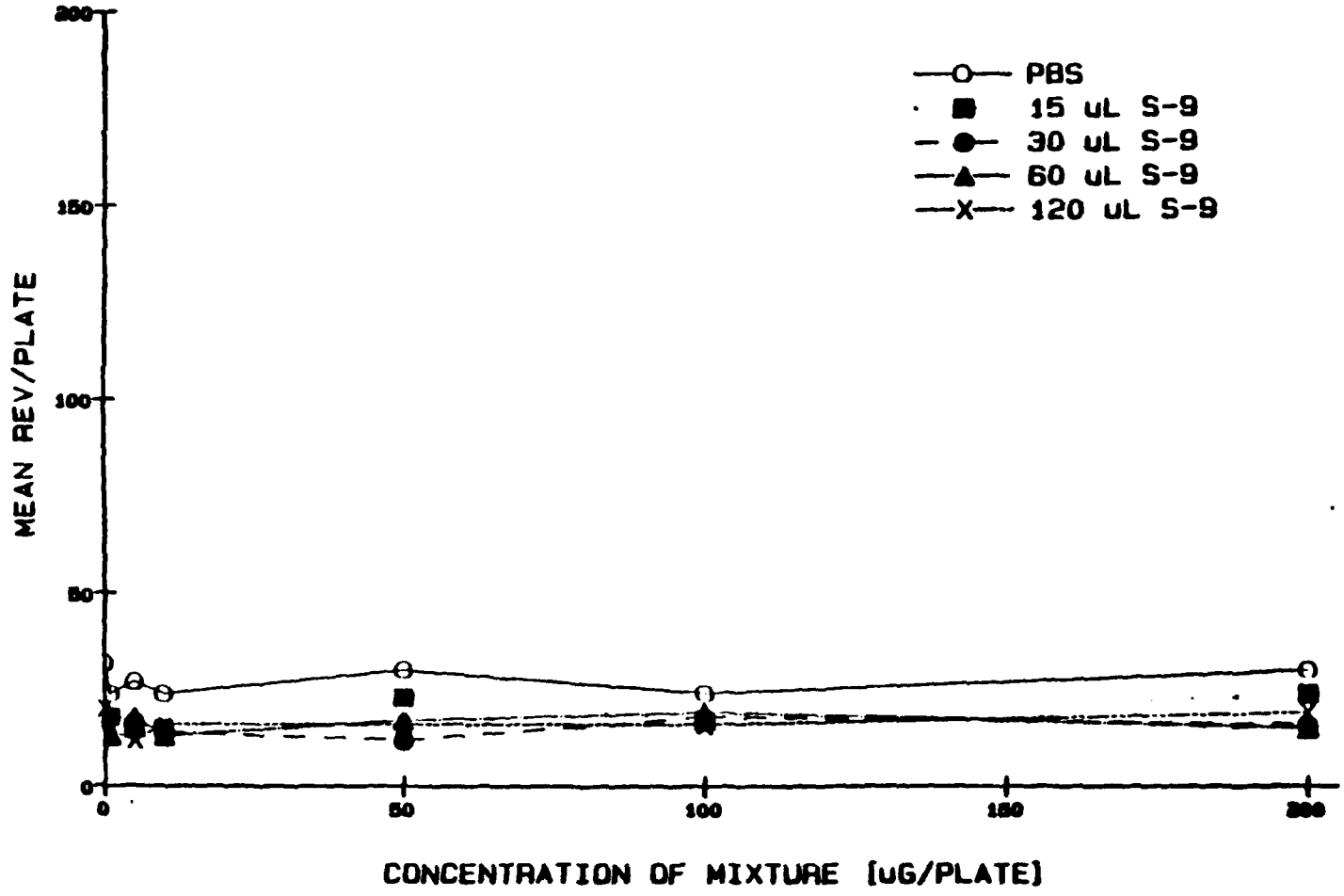

FIGURE 45: INFLUENCE OF S-9 CONCENTRATION ON MUTAGENICITY OF A MIXTURE OF SOLVENT RED 1, DISPERSE RED 11 (LOT 2), AND TEREPHTHALIC ACID IN TA-1535 STRAIN OF SALMONELLA BACTERIA.

For both Disperse Red 11 (Lot 2) and Disperse Red 15 the frequency of mutations increased as a function of S-9 concentration. For Disperse Red 15 the slopes of revertants/µg and their associated SE are shown on Figure 32. The optimum mutagenic response was observed at concentrations of S-9 between 60 and 120 µl/plate. Thus, both the anthraquinone dyes have an increased mutagenic response as a function of S-9 concentration and 60-120 µg/plate of S-9 are required to optimize their mutagenic response. The mutation frequency in the control plates increased slightly as a function of the amount of S-9 added for strains TA-98, TA-1538 and TA-100 and decreased for TA-1535. This suggests that at high concentrations of S-9 the potential increase in the amount of histidine in the S-9 mix is not causing the increase in the frequency of revertants observed.

For Solvent Red 1 tested in TA-100 (Figure 40) there was also an apparent increase in the mutagenic response as a function of the amount of S-9 added. However, the magnitude of the highest response observed after the addition of 120 µl S-9/plate was 300 revertants/plate. This value was twice the observed background for that run sample (150 revertants/plate). The slope of the concentration-response relationship, our other criteria for a positive response, was 0.61 ± 0.24 which was not significantly different from zero $p = 0.07$. The response was thus considered as equivocal.

Since there was a difference between the mutagenic response for the Disperse Red Lot 1 and Lot 2 it was necessary to retest both of these dyes at the same time. This retest would insure that there were no between run differences which were responsible for the differences in the response. The two lots were tested at the same time with and without the addition of 120 µl S-9/plate (Figure 46). Again it was demonstrated that only the

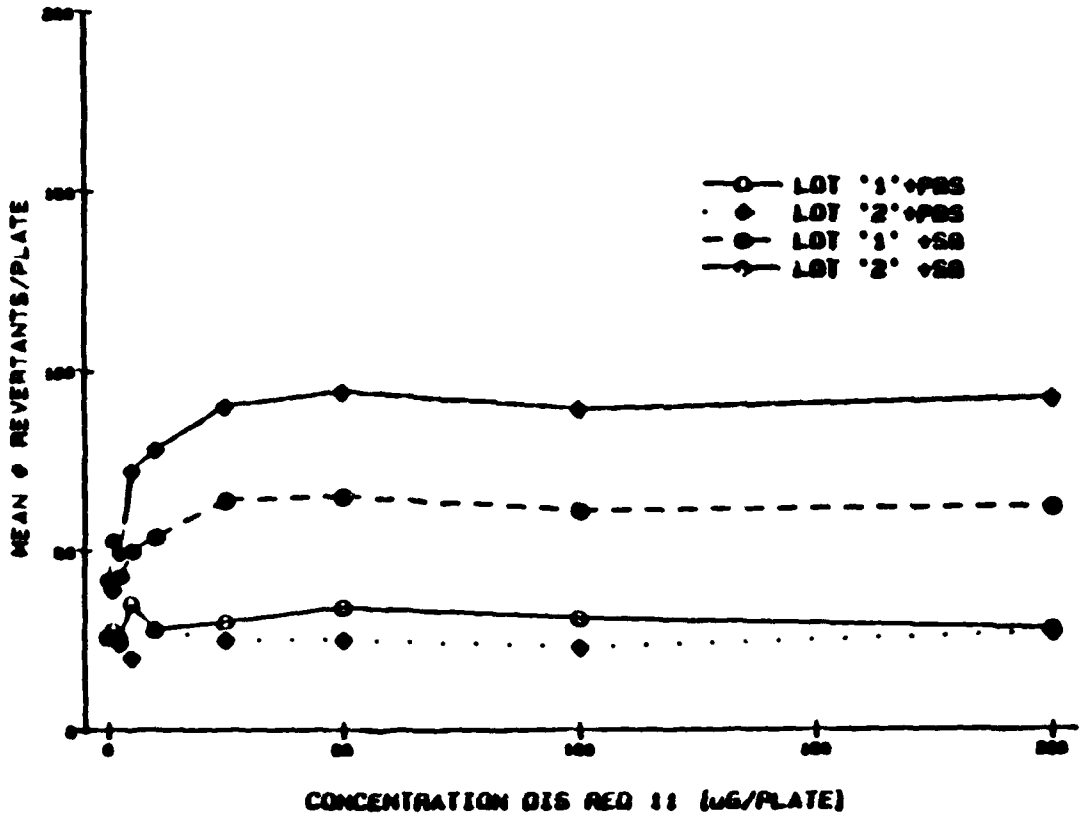

FIGURE 46: COMPARATIVE MUTAGENICITY OF DISPERSE RED 11 (LOTS 1 AND 2) AT A SINGLE S-9 CONCENTRATION (120 UL/PLATE).

Disperse Red 11 (Lot 2) resulted in more than a doubling of the mutagenic response after the addition of S-9. The shape of the concentration-response relationship was the same as previously observed, that is, the number of revertants increased as a function of concentration over a low range, then remained constant as the concentration was increased.

Since there were impurities identified in Disperse Red 11 (Lot 2) that were not present in Lot 1, research was conducted to determine if we could identify not only the chemicals which made up the impurities but also to determine if the impurities were responsible for the mutagenic activity of Disperse Red 11 (Lot 2). Using HPLC with standard compounds, one of the impurities was tentatively identified as the known bacterial mutagen 1,4-diaminoanthraquinone (Brown and Brown 1976) and Figure 3. Concentration-response relationships were derived using the Ames tests to evaluate the mutagenic activity of 1,4-diaminoanthraquinone. These tests indicated that the slope of the dose-response relationships were not different from zero suggesting that there was no mutagenic activity without the addition of S-9. The mutagenic activity measured as slopes of the concentration-response relationships increased as a function of S-9 concentration in a similar manner to Disperse Red 11 Lot 2 (Table 4). Because of these observations, a single concentration of S-9 (60 µl/plate) was used to compare the mutagenic activity of 1,4-diaminoanthraquinone with Disperse Red 11 (Lot 1 and Lot 2). The results of this study are illustrated in Figures 47 and 48. No mutagenic activity was observed without the addition of S-9 (Figure 48). The slopes of the dose response relationships for the three mutagenic anthraquinone dyes over the low concentration range, 0-10 µg/plate, had a range from 3-6 revertants/µg.

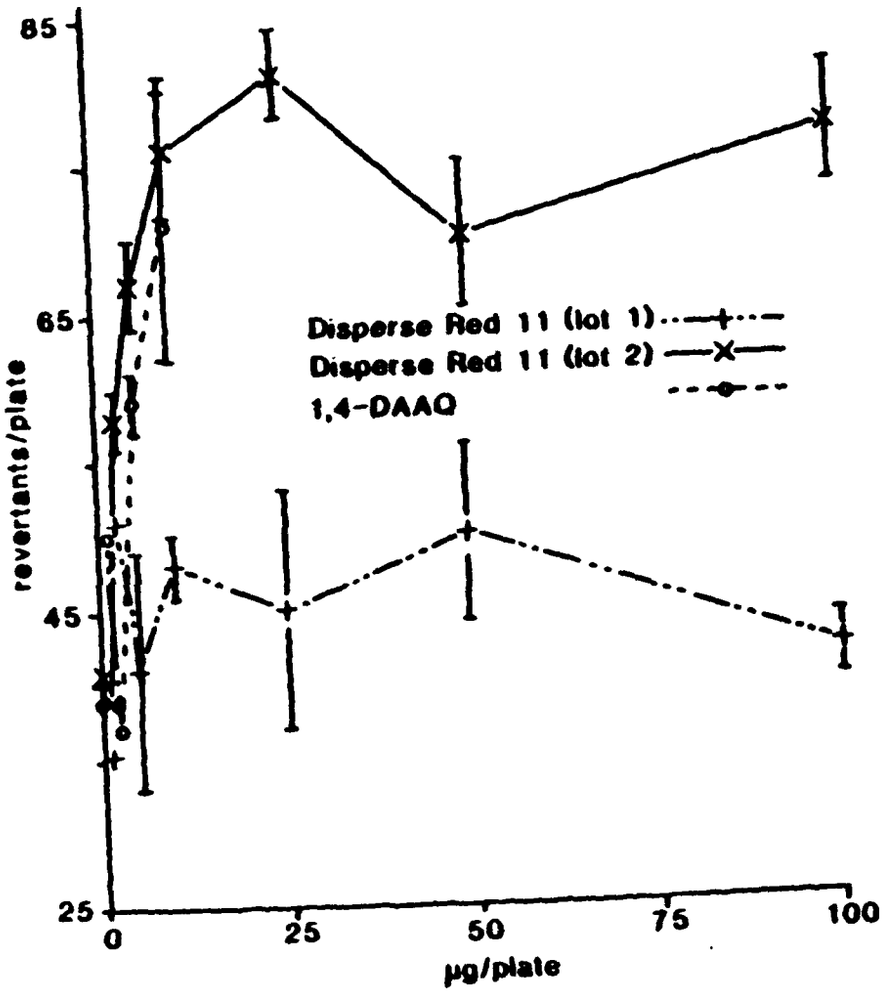

FIGURE 47: THE COMPARATIVE MUTAGENIC ACTIVITY OF DISPERSE RED (LOTS 1 AND 2) AND 1,4-DIAMINOANTHRAQUINONE WITH THE ADDITION OF S-9 (TA-98).

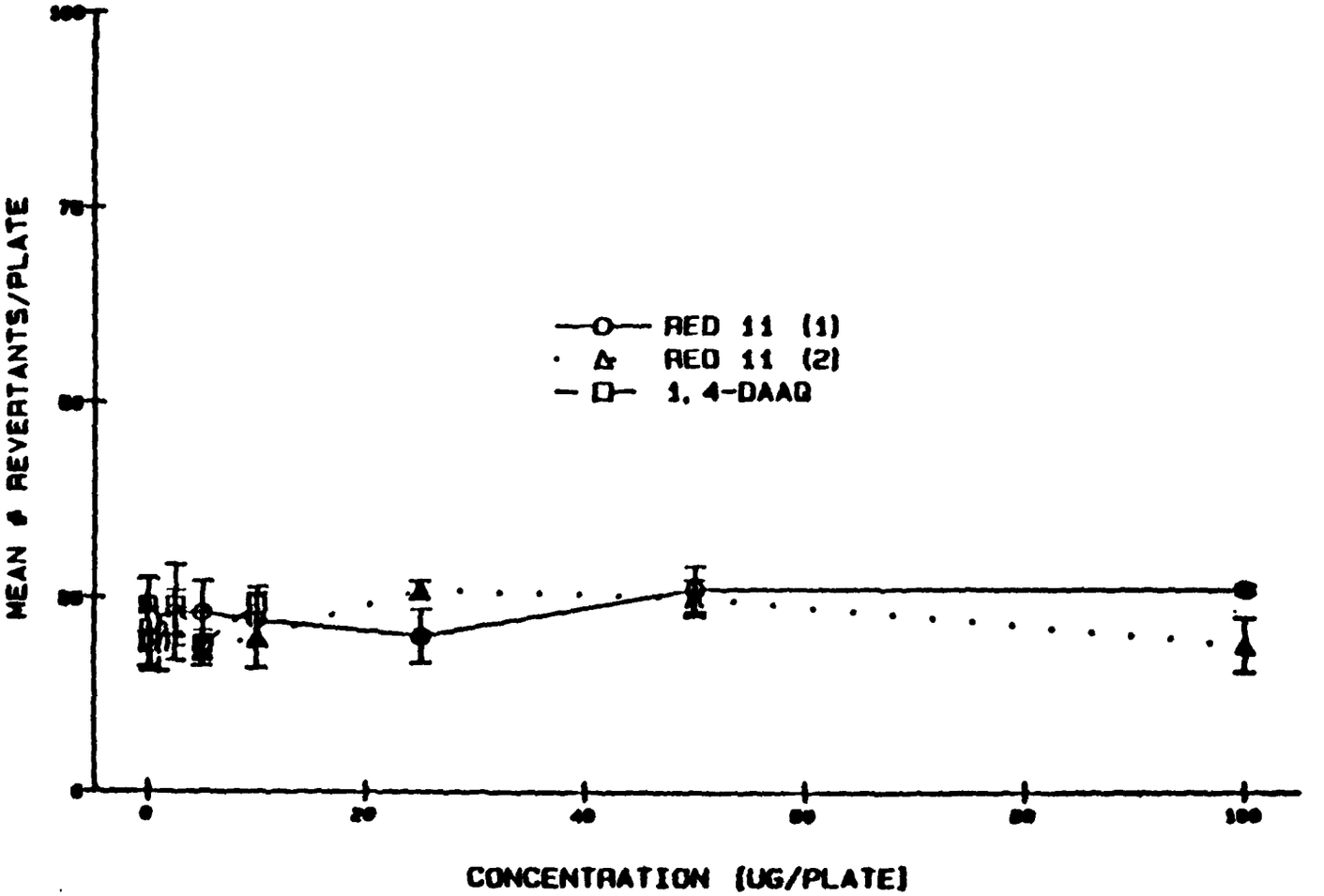

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FIGURE 48: THE COMPARATIVE MUTAGENIC ACTIVITY OF DISPERSE RED (LOTS 1 AND 2) AND 1,4-DIAMINOANTHRAQUINONE WITHOUT ADDITION OF S-9.

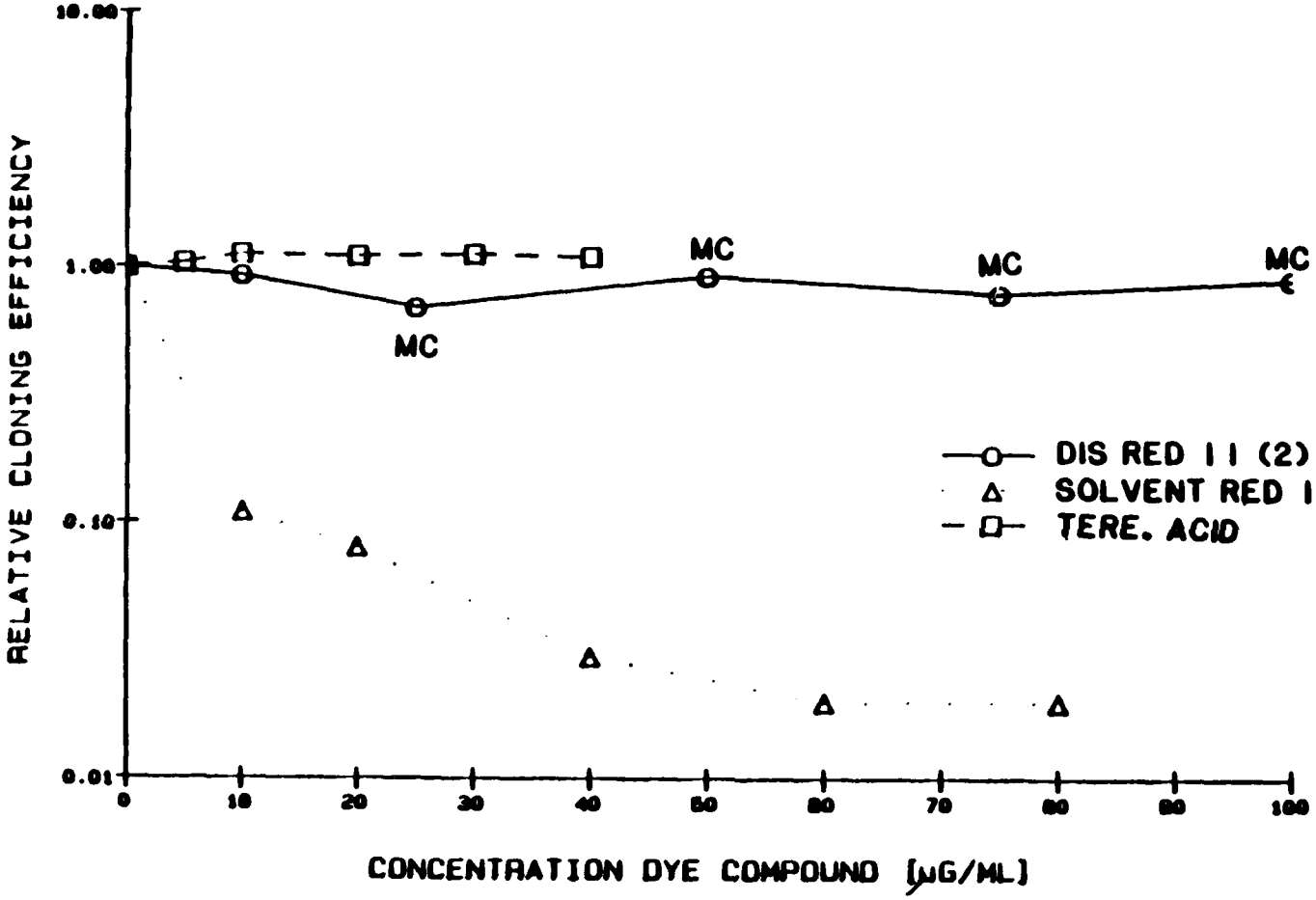
TABLE 4. SLOPES OF CONCENTRATION-RESPONSE CURVES FOR MUTAGENICITY OF DISPERSE RED 11 (LOTS 1 AND 2), DISPERSE RED 15 AND 1,4-DIAMINOANTHRAQUINONE AS A FUNCTION OF S-9 CONCENTRATION (TA-98)

Compound	Dose Range (µg/plate)	S-9 Added (µl/plate)	Slope (rev/µg)	Std. Error of Slope	Mult r^2
Dis. Red 11 (1)	0–10	0	-0.07	0.16	0.06
	0–10	60	0.76	0.81	0.22
Dis. Red 11 (2)	0–10	0	-0.33	0.25	0.37
	0–10	60	3.75	0.86	0.86
Dis. Red 15	0–50	15	1.05	0.30	0.95
	0–75	30	2.21	0.61	0.94
	0–50	60	4.78	0.98	0.97
	0–50	120	5.31	1.34	0.96
1,4-DAAQ	0–10	0	0.26	0.27	0.13
	0–10	30	3.10	0.71	0.76
	0–10	60	5.46	0.94	0.84
	0–10	120	6.81	1.29	0.82

The data from Table 4 indicate that the slopes for all the anthraquinone dyes were very similar with the exception of Disperse Red 11 Lot 1 which produced no mutagenic response Because the pure 1,4-diaminoanthraquinone had approximately the same mutagenic activity as the Disperse Red 11 (Lot 2) the presence of the small percentage of 1,4-diaminoanthraquinone in the Disperse Red 11 Lot 2 cannot account for all the mutagenic activity in this lot of dye The other unidentified contaminants must be responsible for the difference in the mutagenic activity in the two lots of Disperse Red 11 dye

CYTOTOXICITY OF DYES IN MAMMALIAN CELLS

Addition of Solvent Red 1 or Disperse Red 11 dyes to mammalian cell culture media at the level of (100 µg/ml) resulted in the formation of a precipitate as was seen in our earlier experiment Figure 4 and pages 22-23 There was no precipitate observed at concentrations of 20 µg/ml and lower The relationship between cell killing and concentration of the dye added could thus only be evaluated at concentrations below 20 µg/ml The results of this experiment are plotted in Figure 49 The figure indicates that Solvent Red 1 dye is cytotoxic at low concentrations (10 µg/ml) It also demonstrated that the Disperse Red 11 (Lot 2) was not cytotoxic over the range of concentrations tested However, it was observed that all the colonies of cells that formed were very small, microcolonies (mc), at concentrations of 25 µg of Disperse Red 11 (Lot 2)/ml of media and higher These small colonies indicate either toxicity or some other process that either stops the progression of cells at a fixed stage of the cell cycle or slows down the cell cycle but does not result in lethality to the cells



DISPERSE RED 11 CAUSED FORMATION OF MICROCOLONIES (MC)

SOLVENT RED 1 REDUCED THE NUMBER BUT NOT SIZE OF THE COLONIES

FIGURE 49: CYTOTOXICITY OF RED/VIOLET DYE COMPOUNDS IN CHINESE HAMSTER OVARY CELLS.

To evaluate this mitotic alteration, the growth of control cells was compared with that of cells exposed to 50 µg/ml of Disperse Red 11 (Lot 2). At the standard harvest time for the cytotoxicity assay (7 days after exposure) the colonies of cells exposed to the dye were much smaller than those that received no exposure. The number of cells in colonies were compared at 3 and 5 days of culture after exposure to 0 or 50 µg/ml of Disperse Red 11 (Lot 2). Ten colonies from each treatment and sacrifice time were photographed and the number of cells per colony counted. The results of this study demonstrate that three days after exposure there were 10 ± 2 cells in the colonies exposed to 50 µg/ml of Disperse Red 11 (Lot 2) compared to 42 ± 8 cells per colony in the control cultures. By 5 days the number of cells per colony was 70 ± 10 and 447 ± 52 in exposed and control cultures, respectively. This decrease was overcome in two days so that the treated cells were growing at the same rate as the control cells. There was no indication of a change in the number of cells that survive and form colonies. At the concentrations of dye tested for the induction of mutations (up to 20 µg/ml) the growth rate was back to normal at the time [illegible] of the mutant colonies.

[illegible] of Dyes

[illegible] Disperse Red 11 (Lot 1 and Lot 2), Solvent Red [illegible] a weak mutagenic effect without the [illegible] of Disperse Red 11 (Lot 1) [illegible] for mutagenic [illegible] three times

TABLE 5. THE MUTAGENICITY AND CLONING EFFICIENCY OF RED/VIOLET DYE COMPOUNDS IN CHO/HGPRT WITHOUT THE ADDITION OF S-9

Compound Tested	Conc. (µg/ml)	Mean CE (%)[a]	S. E. (CE)	Mean Mut'n. Frequency	S. E. (Mf.)	P
None	(0)	68	6	11	2	
DMSO	(0)	73	7	6	1	
MNNG	0.1	33	5	479	75	
Dis. Red 11 (1)	5.0	45	b	11	b	
	7.5	50	b	8	b	
	10.0	51	b	8	b	
	20.0	48	b	6	b	
Dis. Red 11 (2)	1.0	47	17	6	3	
	2.5	50	12	12	7	
	5.0	48	9	23	3	
	7.5	61	1	27	6	
	10.0	54	8	22	14	
	20.0	56	b	9	b	
Solvent Red 1	0.1	57	18	8	3	
	0.5	57	11	10	3	
	1.0	57	6	12	2	
	2.5	54	b	11	b	
	5.0	51	6	16	10	
	10.0	44	b	7	b	
Dye Mixture	1.0	51	15	13	1	
	2.5	47	8	5	2	
	5.0	54	6	11	4	
	7.5	49	b	10	b	
	10.0	46	6	12	5	

a. Cloning efficiency on day of exposure.

b. Single value; no standard error could be calculated.

since they showed some indication of mutagenic activity. The concentration response relationships for the means of these tests for each dye are plotted in Figures 50–53. The data demonstrate that (testing the null hypothesis that the slope was equal to zero using a student's "t" test) there was no increase in the mutation frequency as a function of dye concentration for Solvent Red 1 (slope −0.03 ± 0.4, P = .94), Disperse Red 11 (Lot 1) (slope −0.13 ± 0.1, P = 0.34), or for the mixture (slope 0.23 ± 0.3, P = 0.54). On the other hand, Disperse Red 11 (Lot 2) showed a small but significant (slope = 1.99 ± 0.6, P = 0.02) concentration related increase in mutation frequency (Figure 50) at the lower concentrations tested up to 10 µg/ml. The response then decreased at 20 µg/ml. The mutagenic activity of Disperse Red 11 (Lot 2) was significantly elevated above the control value based on a student's "t" test and the other criteria set for this assay.

The mammalian cell mutation assay was also run for the dyes with the addition of either 1.25 or 2.5 percent S-9. Adding S-9 increased the background rate and the variability of the response. Table 5 shows the results obtained without the addition of S-9 and Table 6 the results with 1.25 or 2.5% S-9. These data are plotted in Figures 54-57. In all cases there was a good response with the positive controls B(a)P and MNNG. There were a few concentrations of Solvent Red 1 that produced a response that was greater than twice background and with 2.5% S-9 there was a dose-related increase slope 2.4 ± 1.5 which was not significant P = 0.24. For the other dyes there was no concentration related increase in the mutation frequency with the P value always greater than 0.15 and the slope less than 0.25 mutations/10^6 cells/µg. There were no elevated responses in the frequency of mutations where the cells were cultured with S-9 and Disperse Red 11 (Lot 1, Figure 55 or Lot 2, Figure 54) or for the dye mixture (Figure 57).

TABLE 6. THE MUTAGENICITY AND CLONING EFFICIENCY OF RED/VIOLET DYE COMPOUNDS IN CHO/HGRPT WITH 1.25 OR 2.5 PERCENT LIVER S-9

Compound Tested	Conc. (µg/ml)	CE (%)[a] w/1.25% S-9	Mut. Freq. (1.25% S-9)	CE (%) w/2.5% S-9	Mut. Freq. (2.5% S-9)
DMSO + S-9	(0)	70	7	46	17
B(a)P only	1.0	48	0	45	20
B(a)P + S-9	1.0	39	374	53	472
Dis. Red 11 (1) + S-9	5.0	(NT)[b]	(NT)	46	24
	7.5	(NT)	(NT)	55	6
	10.0	(NT)	(NT)	62	15
	20.0	(NT)	(NT)	53	2
Dis. Red 11 (2) + S-9	1.0	49	8	(NT)	(NT)
	2.5	51	10	(NT)	(NT)
	5.0	58	22	56	11
	7.5	(NT)	(NT)	58	16
	10.0	39	3	39	13
	20.0	(NT)	(NT)	46	11
Solvent Red 1 + S-9	0.1	33	6	(NT)	(NT)
	0.5	37	27	(NT)	(NT)
	1.0	42	10	44	(NT)
	2.5	(NT)	(NT)	47	9
	5.0	44	0	41	10
	10.0	(NT)	(NT)	36	39
Dye Mixture + S-9	1.0	37	14	(NT)	(NT)
	2.5	42	14	42	31
	5.0	45	18	48	17
	7.5	(NT)	(NT)	37	16
	10.0	33	6	46	4

a Cloning efficiency from day of exposure

b (NT) - Not tested

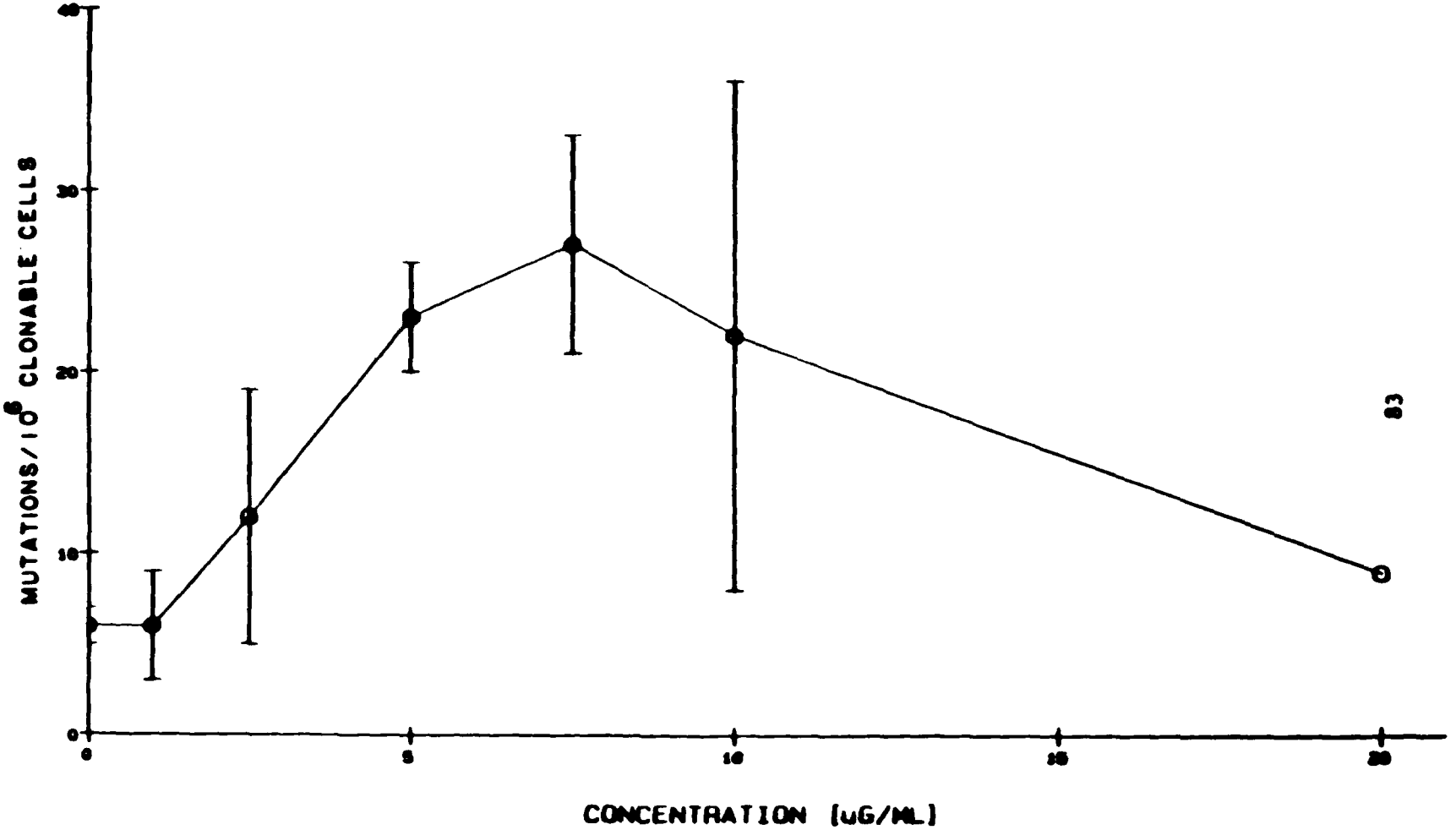


FIGURE 50: THE MUTAGENIC ACTIVITY OF DISPERSE RED 11 (LOT 2) DYE IN CHO/HGPRT GENE MUTATION SYSTEM WITHOUT THE ADDITION OF S-9.

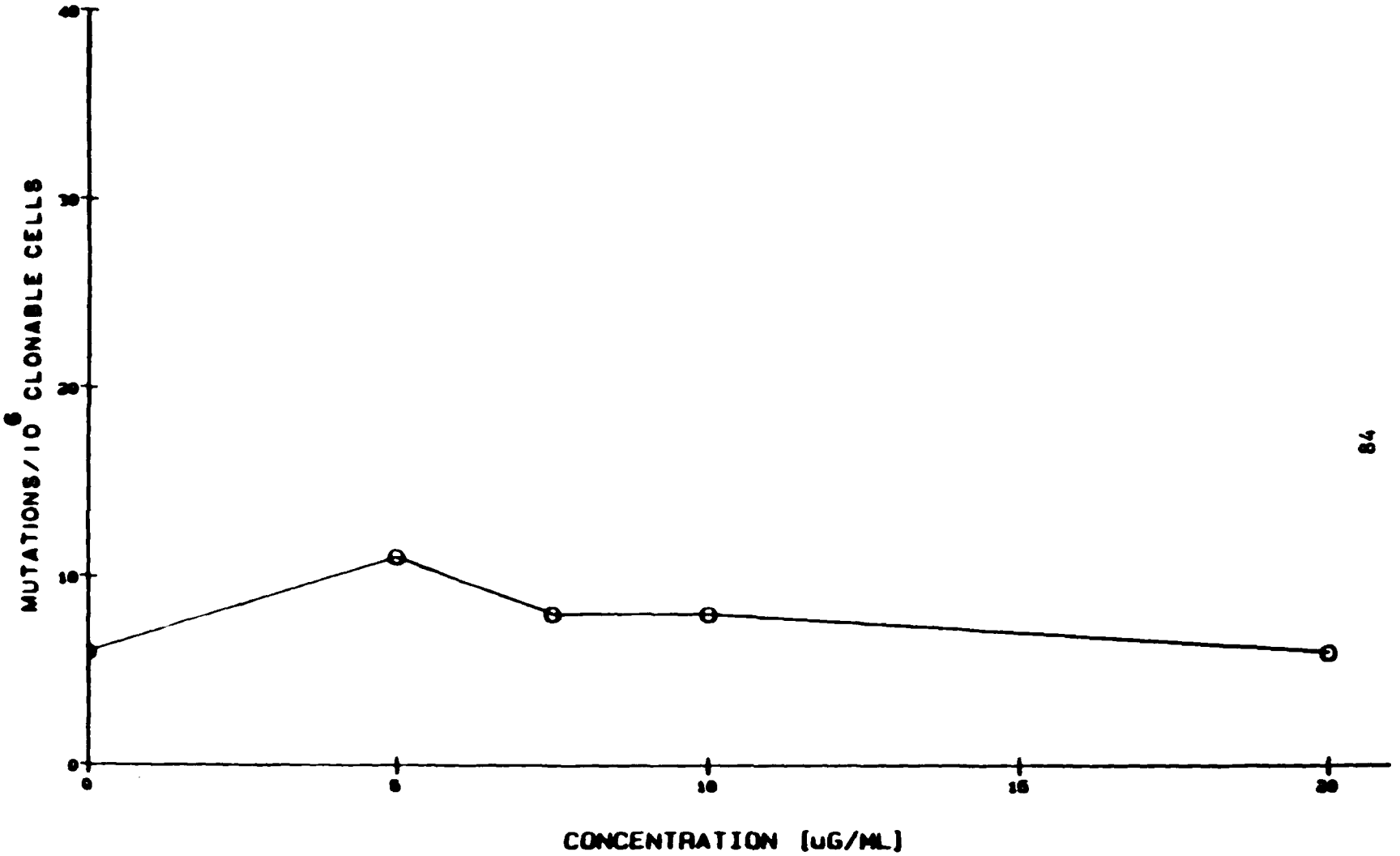
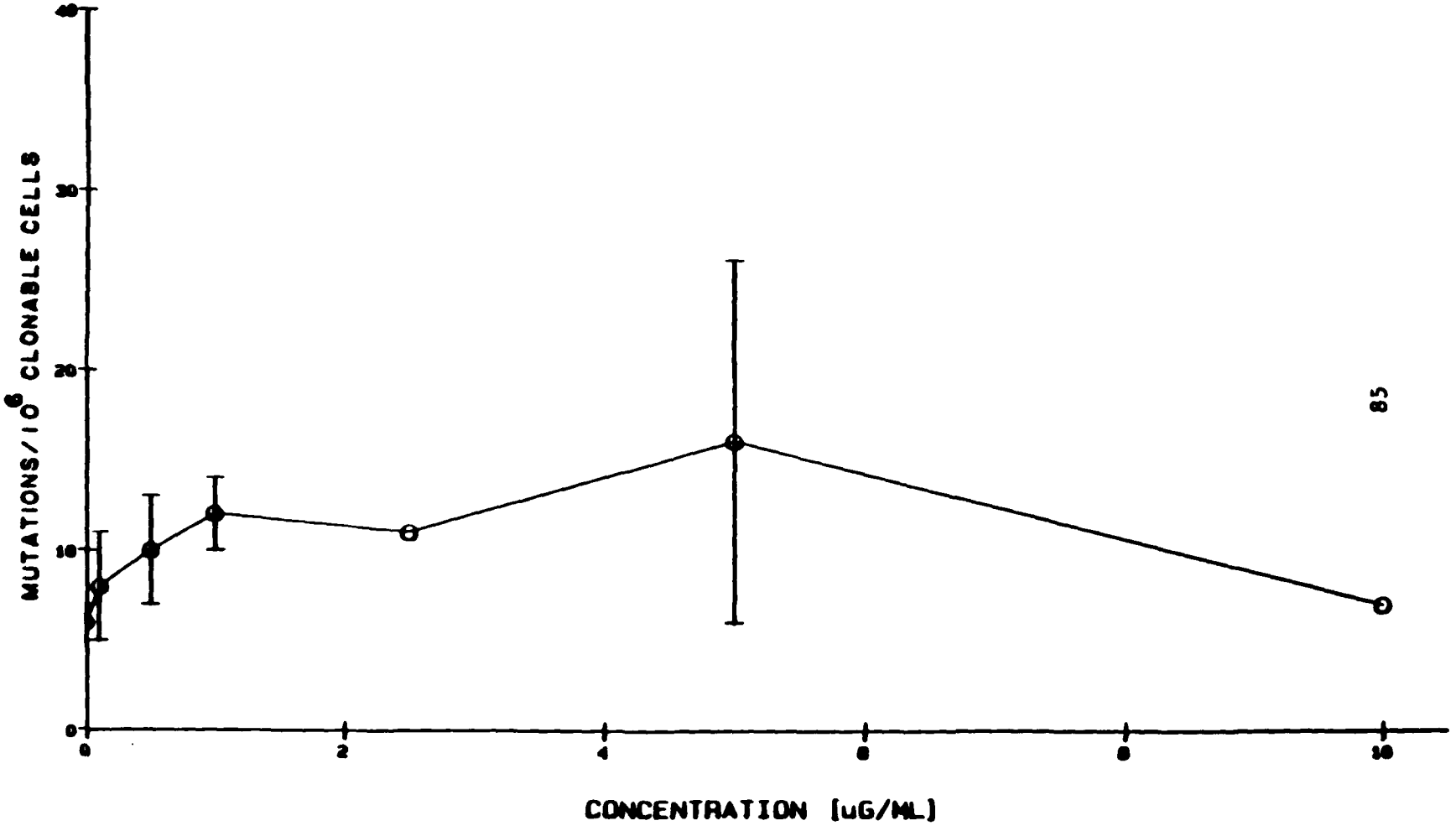
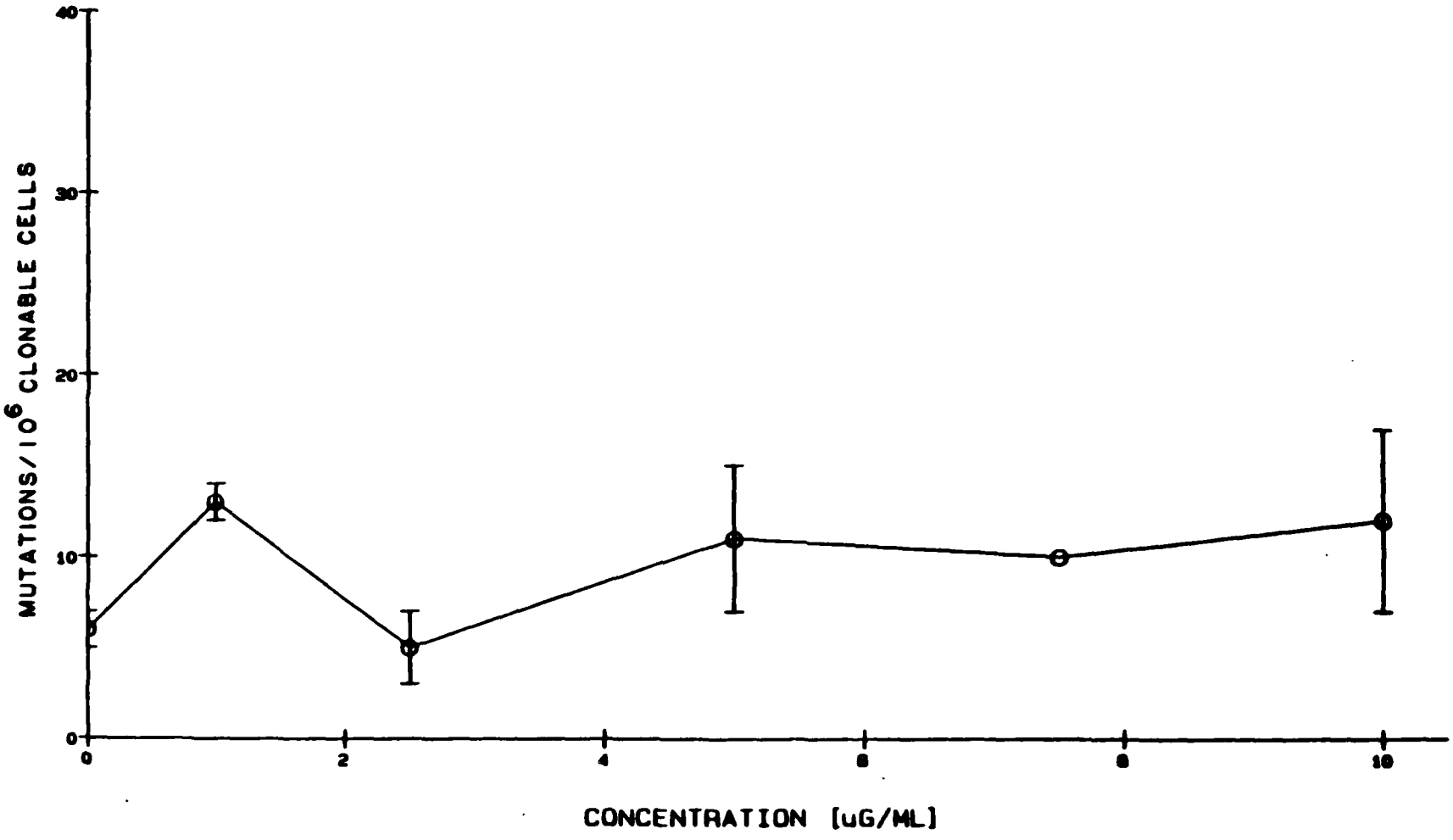


FIGURE 51: THE MUTAGENIC ACTIVITY OF DISPERSE RED 11 (LOT 1) IN CHO/HGPRT GENE MUTATION SYSTEM WITHOUT THE ADDITION OF S-9.



No standard error shown at [2.5] or [10]; single values only.

FIGURE 52: THE MUTAGENIC ACTIVITY OF SOLVENT RED 1 IN CHO/HGPRT GENE MUTATION SYSTEM WITHOUT THE ADDITION OF S-9.



No standard error shown at [7.5]; single value only.

FIGURE 53: THE MUTAGENIC ACTIVITY OF A MIXTURE OF SOLVENT RED 1, DISPERSE RED 11, AND TEREPHTHALIC ACID IN CHO/HGPRT GENE MUTATION SYSTEM WITHOUT THE ADDITION OF S-9.

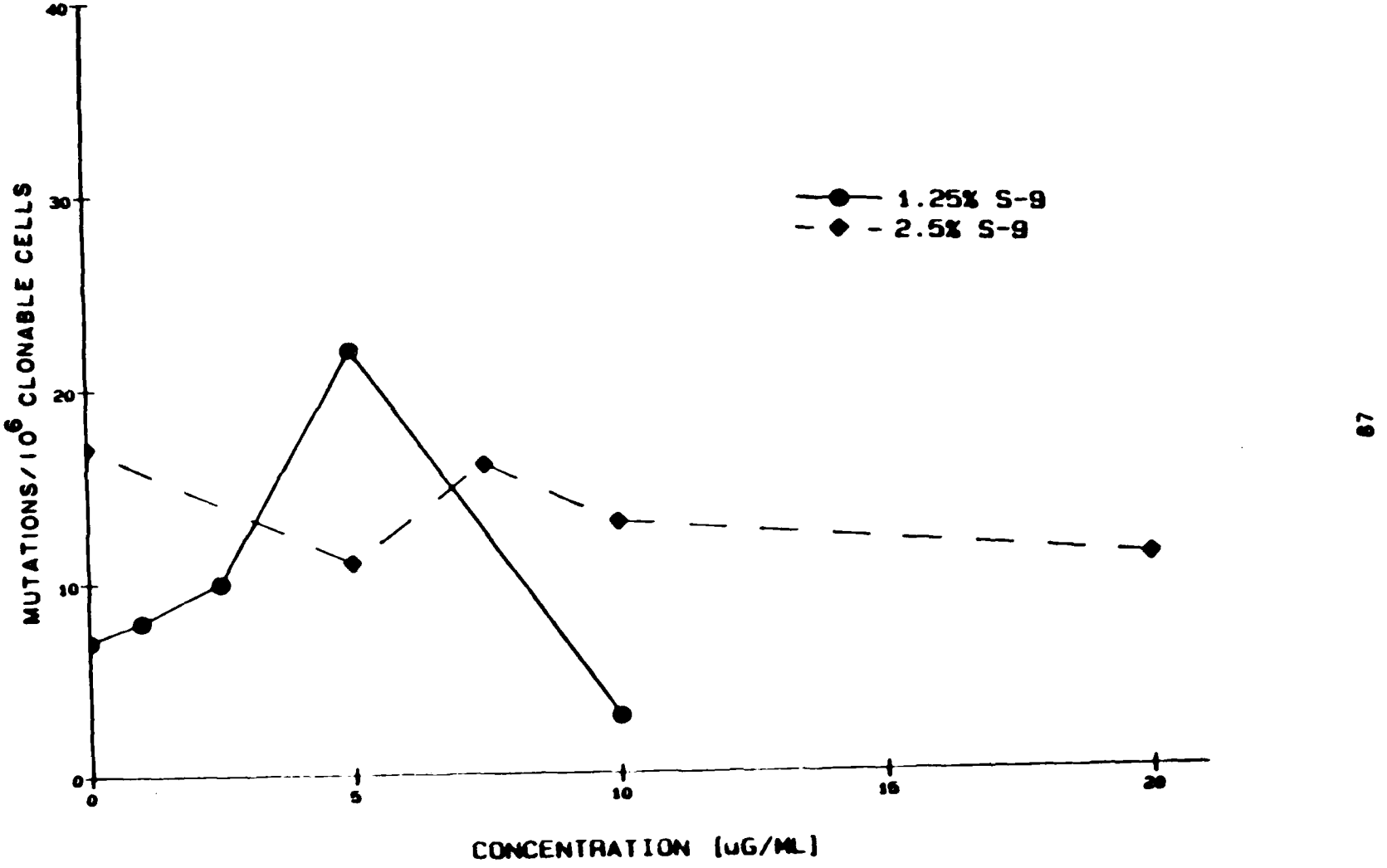


FIGURE 54: THE MUTAGENIC ACTIVITY OF DISPERSE RED (LOT 2) DYE IN CHO/HGPRT GENE MUTATION SYSTEM WITH THE ADDITION OF EITHER 1.25 OR 2.5% S-9.

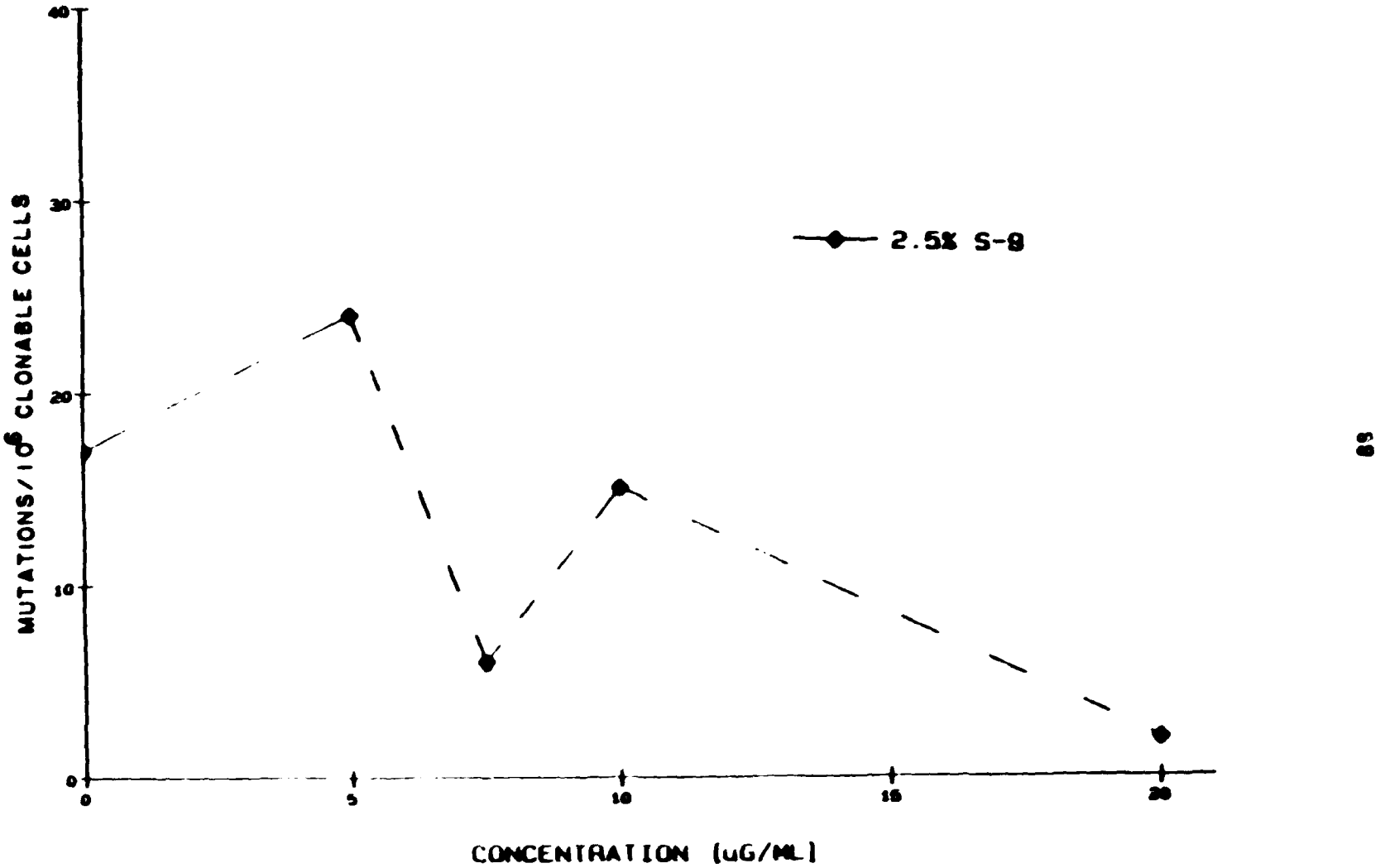


FIGURE 55: THE MUTAGENIC ACTIVITY OF DISPERSE RED 11 (LOT 1) IN CHO/HGPRT GENE MUTATION SYSTEM WITH THE ADDITION OF EITHER 1.25 OR 2.5% S-9.

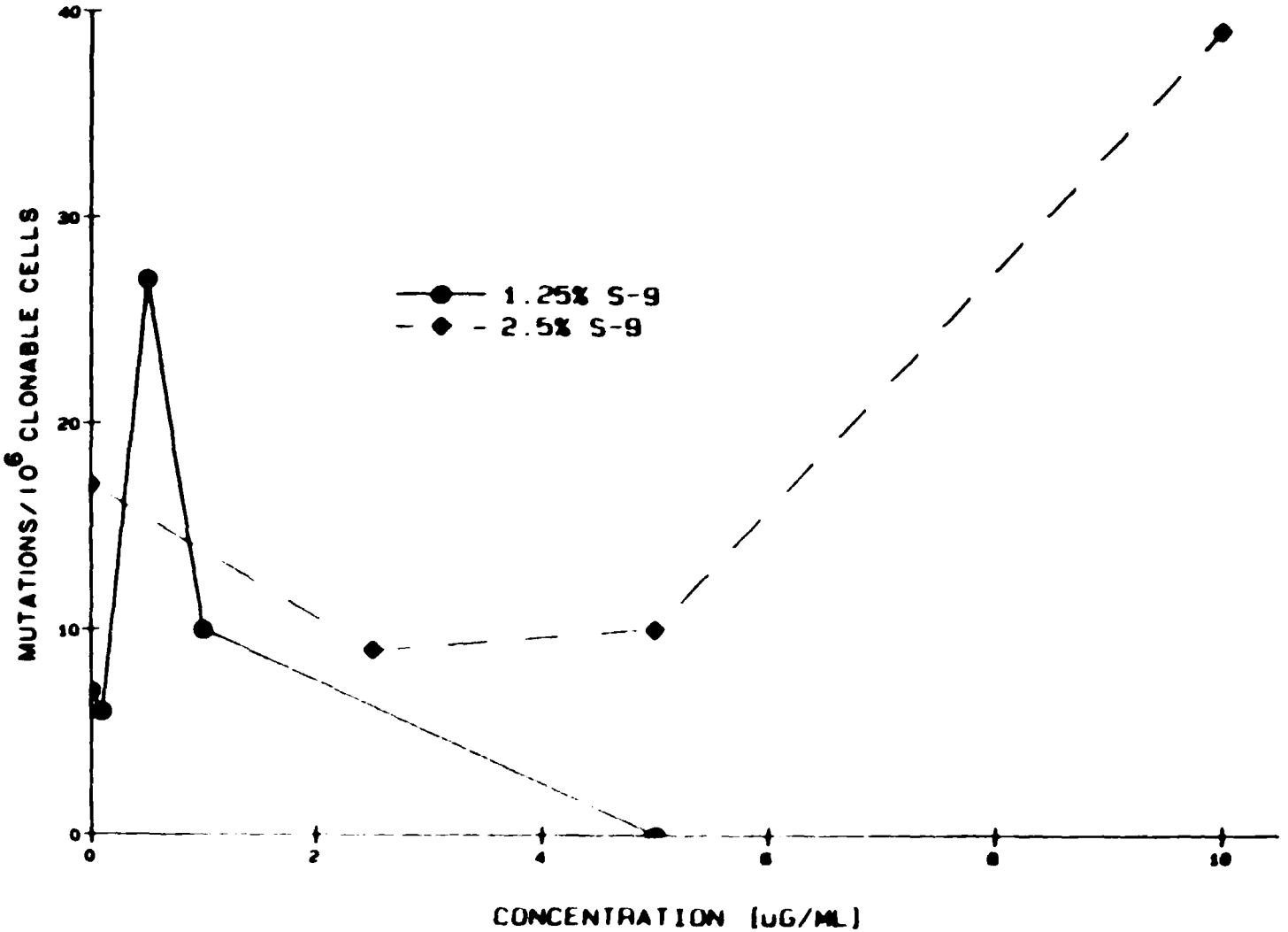


FIGURE 56: THE MUTAGENIC ACTIVITY OF SOLVENT RED 1 IN CHO/HGPRT GENE MUTATION SYSTEM WITH THE ADDITION OF EITHER 1.25 OR 2.5% S-9.

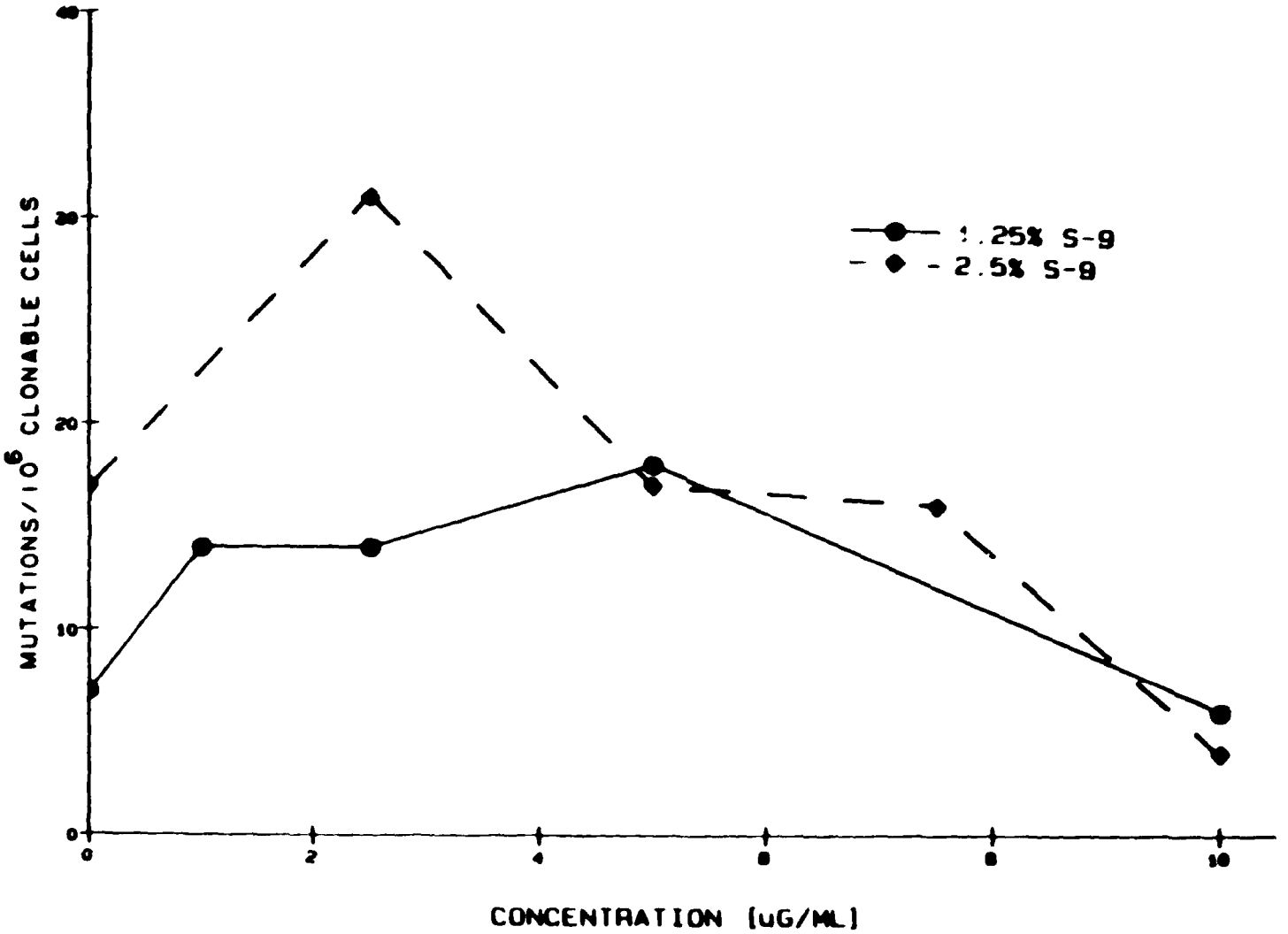


FIGURE 57: THE MUTAGENIC ACTIVITY OF A MIXTURE OF SOLVENT RED 1, DISPERSE RED 11, AND TEREPHTHALIC ACID IN CHO/HGPRT GENE MUTATION SYSTEM WITH THE ADDITION OF EITHER 1.25 OR 2.5% S-9.

SISTER CHROMATID EXCHANGES FROM DYES

A summary of the results of this study is given in Table 7. The total number of chromosomes scored, the number of SCE and the frequency of SCE per chromosome are listed. Based on statistical analyses described below, both Solvent Red 1 and Disperse Red 11 produced an increase in the frequency of SCE at the higher levels of exposure (20 µg/ml).

In Figure 58 the frequency of SCE/chromosome was related to the concentration of Solvent Red 1 dye both with and without the addition of S-9. In the presence of S-9 (2.5%) twenty µg/ml of Solvent Red 1 induced a significant increased frequency of SCE ($p < 0.05$).

For Disperse Red 11 it was important to determine which variables were important in the induction of SCE, i.e., addition of S-9, dye concentration and lot number. Because measurements from different dye lots made with and without S-9 are not necessarily normally distributed at each dye concentration, the Wilcoxon's signed rank test was used to determine the influence of S-9 and dye lot number on the frequency of SCE induced by Disperse Red 11. The influence of S-9 on the response to Disperse Red 11 (Lot 1 and 2) is plotted in Figure 59. The Wilcoxon's signed rank test indicated that there was no significant influence ($p > 0.05$) of S-9 on the frequency of SCE induced by the Disperse Red 11 (Lot 1 and 2). This made it possible to combine the data for SCE derived from tests with and without S-9 and to evaluate the influence of lot number for Disperse Red 11 on the frequency of SCE/chromosome induced. The results of this evaluation are plotted in Figure 60. Again the Wilcoxon's signed rank test indicated that there were no significant differences observed in the frequency of SCE induced as a

TABLE 7. THE SCE FREQUENCY IN CHO CELLS EXPOSED TO RED/VIOLET DYES WITH AND WITHOUT THE ADDITION OF S-9

Treatment	µg/ml	S-9	Chromosomes	SCE's	SCE/Chromosome
Control	0	+	374	105	0.28
		−	3061	953	0.31
Total			3435	1058	0.31
Dis. Red 11 (1)	5	+	447	169	0.38
		−	877	261	0.30
Dis. Red 11 (2)		+	883	341	0.39
		−	575	192	0.33
Total			2782	963	0.35
Dis. Red 11 (1)	10	+	620	284	0.46
		−	968	463	0.48
Dis. Red 11 (2)		+	518	258	0.50
		−	562	234	0.42
Total			2668	1239	0.46
Dis. Red 11 (1)	20	+	521	256	0.49
		−	538	265	0.49
Dis. Red 11 (2)		+	482	210	0.44
		−	1069	485	0.45
Total			2610	1216	0.47
Solvent Red 1	10	+	74	19	
		−	473	155	
Total			547	174	
Solvent Red 1	20	+	555	268	
		−	871	316	
Total			1426	584	
B(a)P	0.50	+	574	4	
MNNG	0.05	−	730		

AD-A181 755 GENOTOXICITY OF DYES PRESENT IN COLORED SMOKE MUNITIONS (U) LOVELACE BIOMEDICAL AND ENVIRONMENTAL RESEARCH INST ALBUQUERQ.. R F HENDERSON ET AL. 07 JUL 86 2/2

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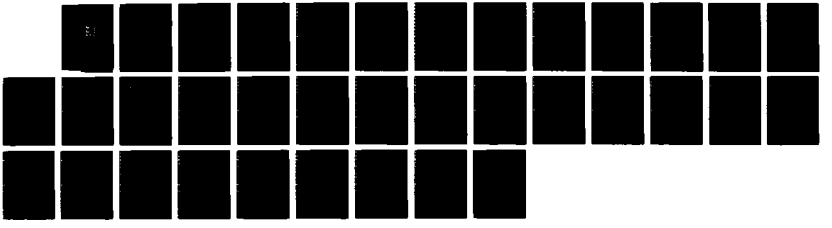

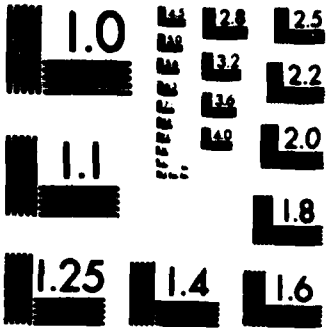

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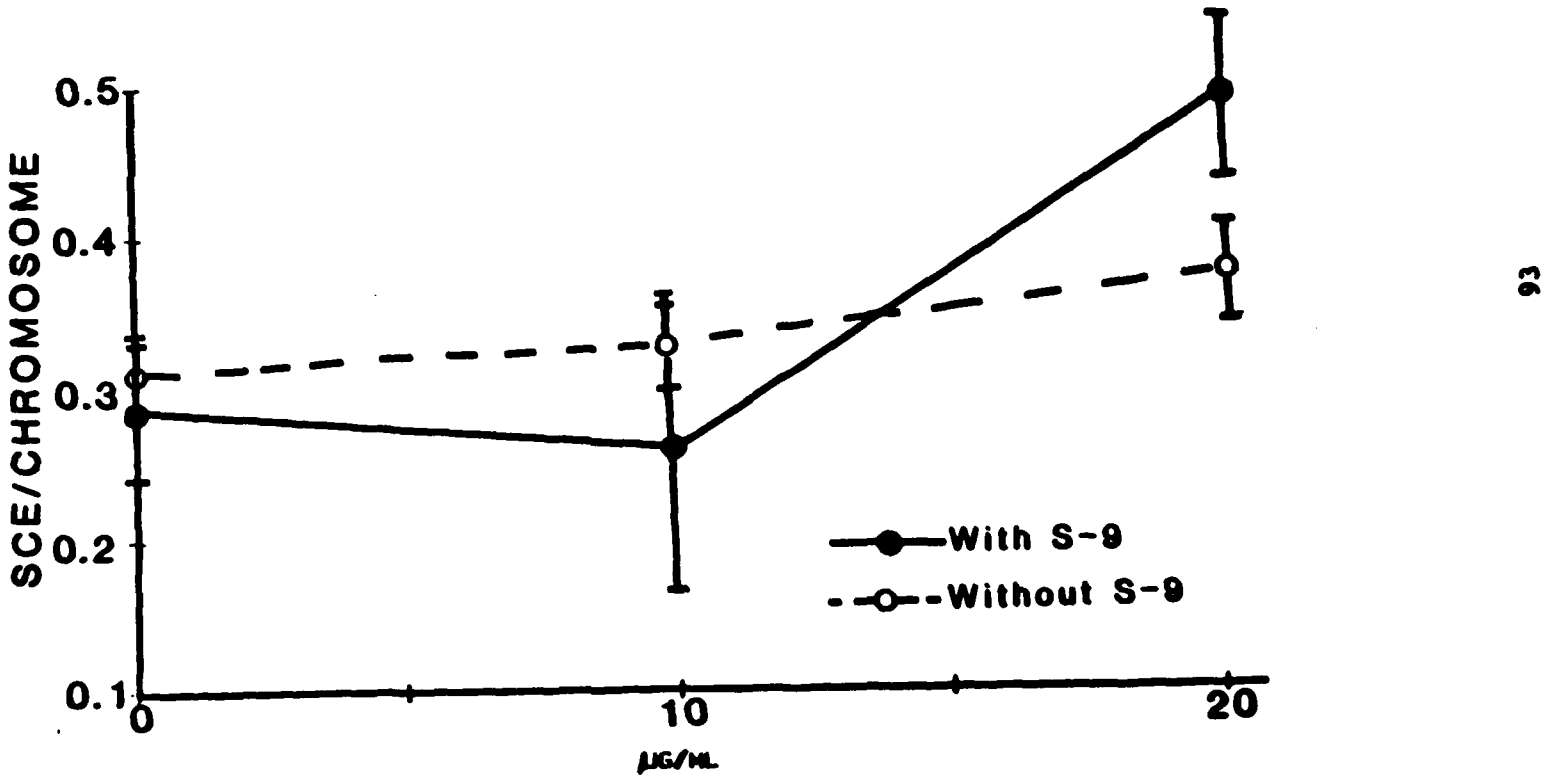

FIGURE 58: THE FREQUENCY OF SCE IN CHO CELLS EXPOSED TO SOLVENT RED 1 WITH AND WITHOUT S-9.

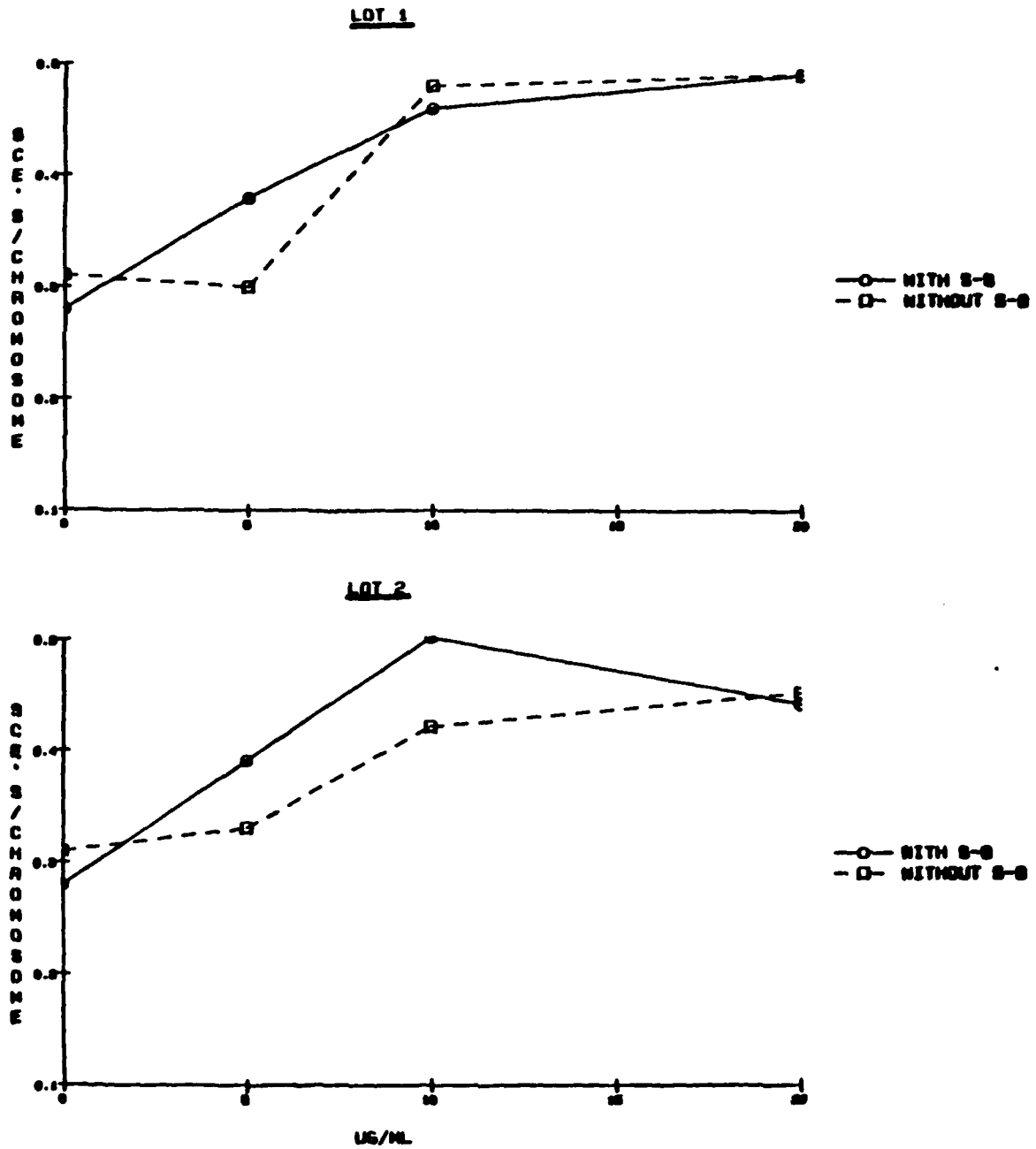

FIGURE 59: THE INFLUENCE OF S-9 ON SCE FREQUENCY IN CHO CELLS EXPOSED TO DISPERSE RED 11 (LOT 1 OR 2).

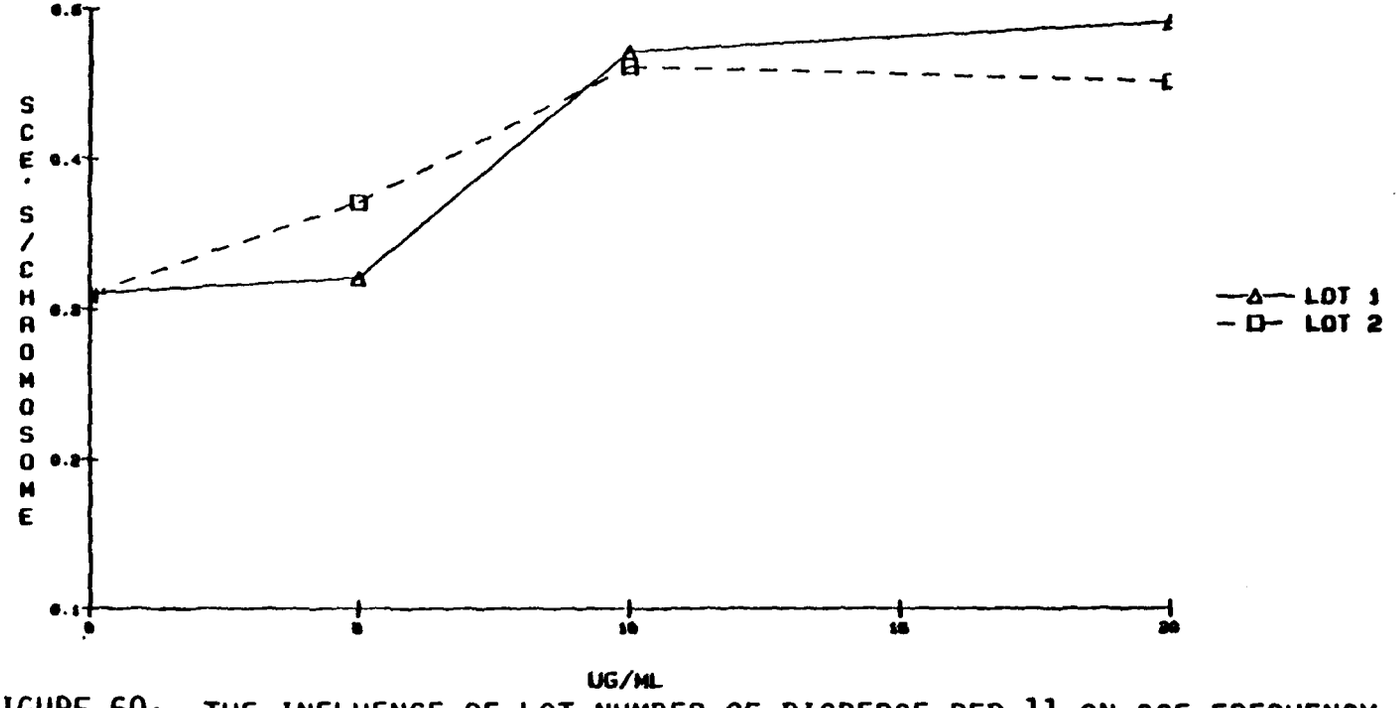

FIGURE 60: THE INFLUENCE OF LOT NUMBER OF DISPERSE RED 11 ON SCE FREQUENCY IN CHO CELLS.

function of lot number ($p > 0.05$). Since neither lot number nor S-9 concentration influenced the frequency of SCE induced by Disperse Red 11, the data were further combined and plotted as a function of Disperse Red 11 dye concentration in Figure 61. Using a Dunnett's "t" test there was a clear-cut difference ($p < 0.05$) between the SCE frequency in the controls and in the cells exposed to the Disperse Red 11. With the exception of the comparison of 10 and 20 µg/ml of dye, the concentration levels of Disperse Red 11 also produced a significant difference in SCE frequency in a multicomparison t test. Therefore, a linear concentration-response relationship was derived for the data and was shown to have a slope and standard error (0.0085 ± 0.0027 SCE/chromosome/µg/ml) that was clearly nonzero. The concentration of Disperse Red 11 was thus demonstrated to cause a significant increase in the frequency of SCE above that observed in the controls.

There have been mixed reports on the relationship between SCE frequency and the induction of cell killing. Morris et al. (1984) showed a positive correlation between cell killing and SCE induction while Carrano et al. (1978) and Brooks et al. (1984b) showed good correlation of SCE with mutations and little correlation with cell killing. The induction of increased incidence of SCE by 20 µg/ml of Solvent Red 1 with no increase in SCE at lower concentrations may be related to the cytotoxic effects of the Solvent Red 1. Since it has been reported that prolongation of the cell cycle time can increase the frequency of SCE induced and since Disperse Red 11 caused mitotic lag, especially at the higher concentrations, it is difficult to determine if the observed increase in SCE is related to the prolonged cell cycle or to genotoxicity of the Disperse Red 11. Because of

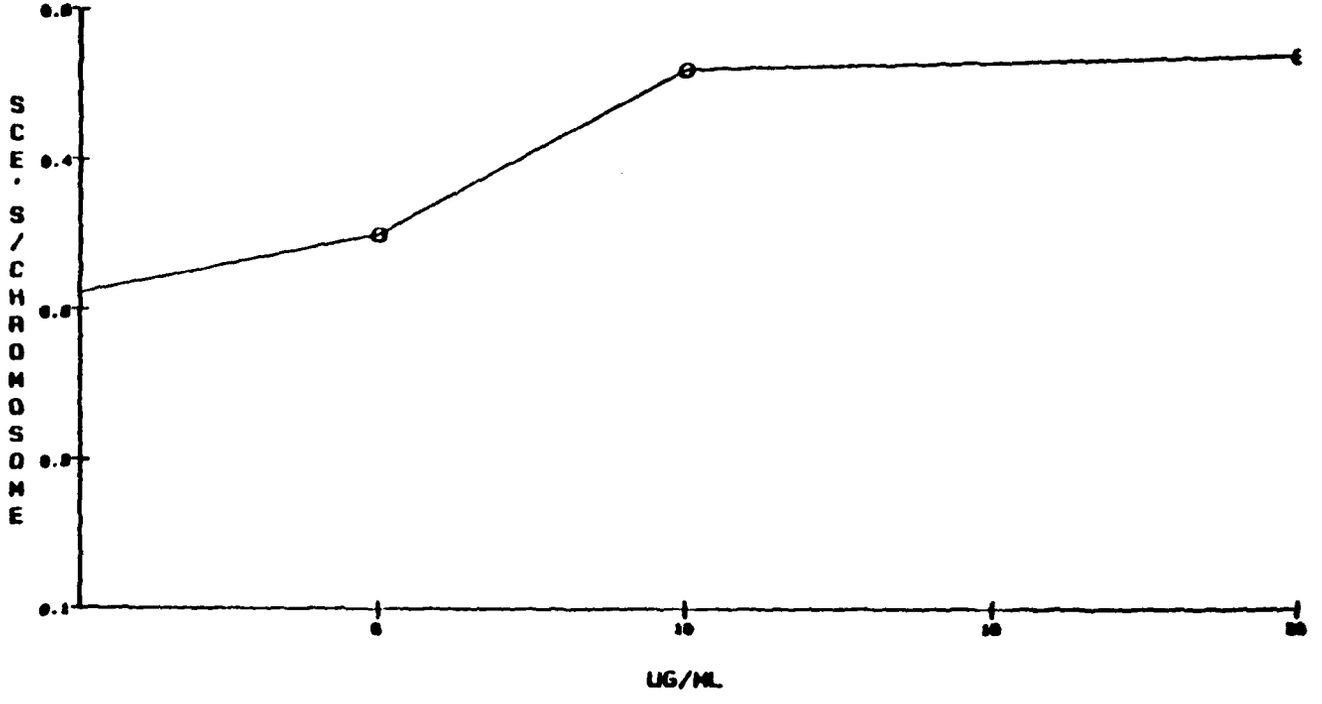

FIGURE 61: THE INFLUENCE OF CONCENTRATION OF DISPERSE RED 11 ON SCE FREQUENCY IN CHO CELLS.

the nature of the SCE assay, i.e., the requirement to score second division cells, the influence of mitotic delay on the SCE response cannot be factored out of the total observed response.

CHROMOSOME ABERRATIONS FROM DYES

The aberration frequency and the types of aberrations noted in experiment 1 are shown in Table 8. The data indicate that the control values in this experiment were between 0.03 and 0.07 aberrations/cell and the level of damage observed in the exposed cells ranged from 0.00–0.11. The 95 percent confidence limits for a binomial distribution were calculated according to the method reported by Evans and O'Riordan (1977) and is shown in Table 8. A test for significant differences between any of the exposed groups relative to the controls was conducted by calculating the normal deviate according to the methods of (Snedecor and Cochran, 1967). There were no significant increase observed between the controls and even the highest response observed in the exposed groups as demonstrated by the listed values for ($p > 0.05$) in Table 8. The only suggestion of a possible response in the cells exposed to the dyes could be seen when the total number of complex aberrations (rings, dicentrics and chromatid exchanges) was compared for the two groups, i.e., control and all the cells that received exposure to the Disperse Red. The frequency of complex exchanges in the controls (2/391 or 0.005/cell) was compared with those induced in cells exposed to the Disperse Red 11, which had 13/1022 or 0.013 exchanges per cell. The P value derived for this comparison is $P = 0.19$ which indicates that the two groups were not significantly different at the

TABLE 8. THE CHROMOSOME ABERRATION FREQUENCY IN CHO CELLS (EXPERIMENT 1) EXPOSED TO RED/VIOLET DYES WITH AND WITHOUT THE ADDITION OF S-9

Treatment	µg/ml	S-9	Total Cells	CSD	CTD	DIC	Gaps	Other	Aber.	Aber./Cell	± 95% Confidence Limits	"p" Values
Control-Media	0	+	100	4	1	0	3	2 Rings	7	0.070	± 0.050	-
Control-Media	0	-	100	3	0	0	2	0	3	0.030	± 0.033	-
Control-DMSO	0	+	100	4	1	0	8	0	5	0.050	± 0.043	-
Control-DMSO	0	-	91	4	1	0	3	0	5	0.055	± 0.047	-
Dis. Red 11 (1)	5	+	100	2	0	2	1	0	4	0.040	± 0.038	0.47
Dis. Red 11 (1)	5	-	100	1	1	0	6	0	2	0.020	± 0.027	0.54
Dis. Red 11 (1)	10	+	100	1	3	1	5	0	5	0.050	± 0.043	0.73
Dis. Red 11 (1)	20	+	100	6	2	3	13	0	11	0.110	± 0.061	0.13
Dis. Red 11 (1)	20	-	100	2	0	0	3	0	2	0.020	± 0.027	0.54
Dis. Red 11 (2)	5	+	100	5	1	1	5	0	7	0.070	± 0.050	0.73
Dis. Red 11 (2)	5	-	101	6	0	3	9	1 CtExc	10	0.099	± 0.059	0.07
Dis. Red 11 (2)	10	+	100	4	1	0	9	0	5	0.050	± 0.043	0.73
Dis. Red 11 (2)	10	-	100	0	0	1	2	0	1	0.010	± 0.020	0.13
Dis. Red 11 (2)	20	-	121	3	1	1	2	0	5	0.041	± 0.035	0.97
Solvent Red 1	5	-	100	1	0	0	3	1 CtExc	2	0.020	± 0.027	0.54
Solvent Red 1	10	+	100	1	0	0	3	0	1	0.010	± 0.020	0.05
Solvent Red 1	10	-	95	0	0	0	3	0	0	0.000	-	-
Solvent Red 1	20	+	100	1	0	2	3	0	3	0.030	± 0.033	0.27

CSD = Chromosome Deletion

CTD = Chromatid Deletion

DIC = Dicentric

CtExc = Chromatid Exchange

P = 0.05 level. No dicentrics were observed in 391 control cells and 12 dicentrics were recorded in the 1022 cells exposed to the Disperse Red 11.

Since there was such a high level of damage recorded in the control cells an additional experiment was conducted. The results of the second experiment are shown in Table 9. In this experiment the control levels were lower (0.010–0.025 aberrations/cell) than in the first experiment. The response levels for the dyes were also reduced with values ranging from (0.005 to 0.065). Again the mean value for chromosome aberrations in cells exposed to 20 µg/ml of Solvent Red 1 with or without the addition of S-9 was less than the control suggesting no genotoxic response. The individual sample that had the highest level of chromosome damage was one that was exposed to Disperse Red 11 (Lot 1) at a concentration of 20 µg/ml without the addition of S-9. This sample had an aberration frequency of 0.065 aberrations/cell which was more than twice that observed in the controls. The P value derived by the method of (Snedecor and Cochran, 1967) was P = 0.004 which suggests that this sample was the only sample which had the aberration frequency significantly elevated above the level of damage observed in the control cells for these cells exposed to media only. Exposing CHO cells to the same concentration of this lot of dye with S-9 and the Disperse Red 11 (Lot 2) at the same concentration (20 µg/ml) with or without S-9 had aberration frequency less than or equal to the controls. Since, with the single exception, there were no significant increases between any of the levels of Solvent Red 1 or Disperse Red 11 and the controls ($p > 0.05$) for either of the lots of dyes or for the S-9, the data sets were combined to test the hypothesis that, using an increased sample size it may be possible to detect a difference in the aberration

TABLE 9. THE CHROMOSOME ABERRATION FREQUENCY IN CHO CELLS (EXPERIMENT 2) EXPOSED TO RED/VIOLET DYES WITH AND WITHOUT THE ADDITION OF S-9

Treatment	µg/ml	S-9	Total Cells	CSD	CTD	DIC	Gaps	Other	Aber.	Aber./Cell	± 95% Confidence Intervals	"µ" Values
Control-DMSO	0	-	200	1	1	0	0	0	2	0.010	± 0.014	-
Control-DMSO	0	+	200	4	0	1	0	0	5	0.025	± 0.022	-
Dis. Red 11 (1)	5	-	200	2	1	0	1	0	3	0.015	± 0.017	0.65
Dis. Red 11 (1)	10	-	200	1	1	2	4	1 Ring	5	0.025	± 0.022	0.25
Dis. Red 11 (1)	20	-	200	5	3	3	12	2 CTEXC	13	0.065	± 0.034	0.004
Dis. Red 11 (1)	5	+	200	0	0	3	7	0	3	0.015	± 0.017	0.48
Dis. Red 11 (1)	10	+	200	1	1	1	1	0	3	0.015	± 0.017	0.48
Dis. Red 11 (1)	20	+	200	0	3	1	0	0	4	0.020	± 0.019	0.74
Dis. Red 11 (2)	5	-	200	3	1	1	4	2 CTEXC	7	0.035	± 0.025	0.60
Dis. Red 11 (2)	10	-	200	0	0	1	1	0	1	0.005	± 0.010	0.58
Dis. Red 11 (2)	20	-	200	1	0	1	3	0	2	0.010	± 0.014	1.0
Dis. Red 11 (2)	5	+	200	2	0	1	3	0	3	0.015	± 0.017	0.88
Dis. Red 11 (2)	10	+	200	0	0	1	0	0	1	0.005	± 0.010	0.10
Dis. Red 11 (2)	20	+	200	0	0	1	0	0	1	0.005	± 0.010	0.10
Solvent Red 1	20	-	200	0	0	1	2	0	1	0.005	± 0.010	0.58
Solvent Red 1	20	+	200	1	0	0	0	0	1	0.005	± 0.010	0.10

CSD = Chromosome Deletion DIC = Dicentric

CTD = Chromatid Deletion CTEXC = Chromatid Exchange

frequency between the control and the dye exposed cells. The aberration frequency and the associated 95% confidence level for the total cells exposed to either Solvent Red 1 and Disperse Red 11 independent of exposure concentration was related to the frequency of the aberrations in all the control cells (Figure 62). There was no significant difference between the total aberration frequency in the cells exposed to the dyes (Solvent Red 1, $P = 0.095$ or Disperse Red 11, $P = 0.84$) above that observed in controls.

The only increase observed in the first experiment on chromosome aberrations, in cells exposed to the Disperse Red 11 relative to the controls, was the small increase in the frequency of exchange type aberrations dicentrics, rings and chromatid exchanges. Because of this observation in the first experiment, the frequency of dicentrics in the cells exposed to Disperse Red 11 in the second experiment was compared to those observed in the controls. This was again possible since no influence of dye concentration, lot number or S-9 could be demonstrated on the incidence of aberrations. For this comparison there was a single dicentric observed in the 400 control cells in the second experiment (0.0025 exchanges/cell) while there were 16 dicentrics, 1 ring, and 4 chromatid type exchanges observed in the 2400 cells exposed to Disperse Red 11 dyes (0.0088 exchanges/cell). When these differences were compared with the methods of Snedecon and Cochran the exchange type aberrations observed in the cells exposed to the dyes were not significantly higher than observed in the control cells ($P = 0.19$). These data are also illustrated in Figure 62 which show that the 95% confidence intervals for the control and exposed cells show considerable overlap.

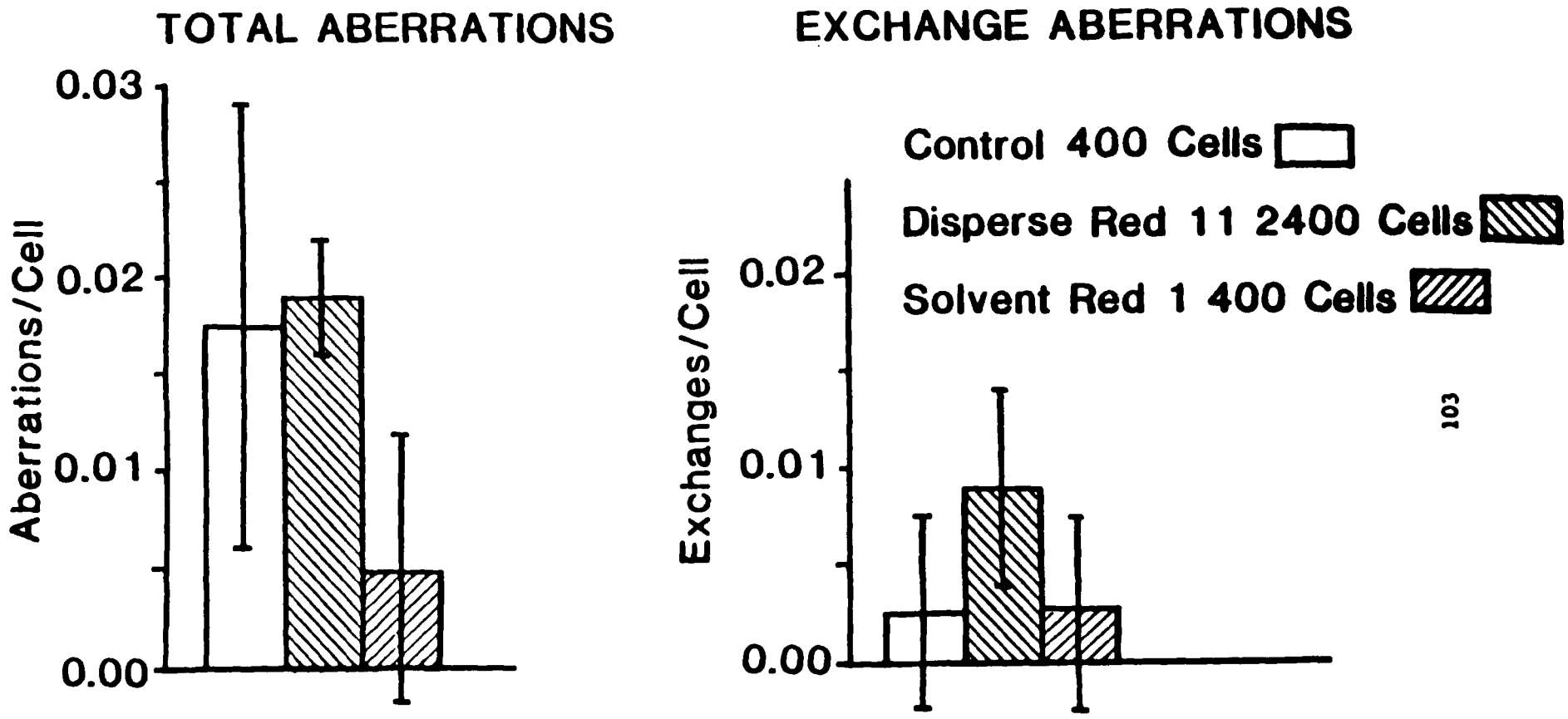

FIGURE 62: THE FREQUENCY OF TOTAL ABERRATIONS AND EXCHANGES PER CELL IN CHO CELLS EXPOSED TO RED/VIOLET DYES.

The chromosome aberration frequency was evaluated in cells at 6, 9, 12, and 24 hours after exposure to either 5 or 20 µg/ml of Disperse Red 11. This was done to insure that we did not overlook a cell cycle dependency in the production of chromosome damage by Disperse Red 11. The results of this study are in Table 10. From the table it can be seen that there were no significant elevations in responses to the dye as a function of time of sampling with the highest response 0.02 aberration/cell. At the earliest sampling time, 6 hours after the end of dye exposure there was a mitotic lag induced by the Disperse Red 11 such that we were unable to sample an adequate number of cells for evaluation. By 24 hours after the end of the exposure, most of the control cells and the cells exposed to 5 µg/ml of dye were in second division so that we again could not sample an adequate number of first division cells to evaluate chromosome damage. Cells exposed to the highest level of Disperse Red 11 (20 µg/ml) were slowed in their mitotic cell cycle to the point adequate mitotic lag that first division cells could be evaluated for aberrations. The two intermediate times had adequate numbers of first division cells in both the exposed and control cultures for scoring. The data demonstrate that there was no increase in the aberration frequency as a function of sample time.

DISCUSSION

A. CHEMICAL CHARACTERIZATION

Using HPLC with the detector at 520 nm there seemed to be major impurities in Solvent Red 24 and Disperse Red 15. HPLC analysis of Disperse

TABLE 10. THE CHROMOSOME ABERRATION FREQUENCY IN CHO CELLS EXPOSED TO RED/VIOLET DYES AS A FUNCTION OF TIME AFTER DYE EXPOSURE

Treatment	µg/ml	S-9	Hrs.	Total Cells	DIC	CSD	CTD	Gaps	Aber.	Aber./Cell	± 95% Confidence Intervals
Control	0	–	6	89			1		1	0.01	± 0.01
Control	0	+	6	90					0	0	
Dis. Red 11 (2)	5	–	6	0					0	0	
Dis. Red 11 (1)	20	+	6	9				1	0	0	
Control	0	–	9	100		2		6	2	0.02	± 0.027
Control	0	+	9	100					0	0	
Dis. Red 11 (2)	5	–	9	100		1		1	1	0.01	± 0.020
Dis. Red 11 (1)	20	+	9	173		1		1	0	0	
Control	0	–	12	100		1			1	0.01	± 0.020
Control	0	+	12	109		1	2	2	3	0.03	± 0.032
Dis. Red 11 (2)	5	–	12	100		2		4	2	0.02	± 0.027
Dis. Red 11 (1)	20	+	12	100	1				1	0.01	± 0.020
Control	0	–	24	2					0	0	
Control	0	+	24	55					0	0	
Dis. Red 11 (2)	5	–	24	1					0	0	
Dis. Red 11 (1)	20	+	24	102		1		1	1	0.01	± 0.019

DIC = Dicentric CTD = Chromatid Deletion

CSD = Chromosome Deletion

Red 11 (Lot 1 and 2) and Solvent Red 1 all indicated that they had rather high purity. However, detailed analysis of Disperse Red 11 indicated that there were impurities in Lot 2 that were not present in Lot 1. The only dyes to show a positive response in the Ames test were Disperse Red 15 and Disperse Red 11 (Lot 2). Since both of these dyes had impurities associated with them it is difficult to determine if the response was related to the dye and not to the impurity. For the Disperse Red 11 (Lot 2) we determined that the response may have been related to an impurity but were not able to identify the impurities responsible for all the mutagenic activity.

B. BACTERIAL MUTAGENICITY

A summary of the bacterial mutagenicity associated with the dye samples is shown in Table 11. The summary indicates that without the addition of S-9 none of the dyes had significant mutagenic activity. None of the dyes produced a significant positive response in TA-1535. This suggests that none of the dyes produced base pair type of mutations. Solvent Red 1, Solvent Red 24 and Disperse Red 11 (Lot 1) did not produce a significant mutagenic response in any of the bacterial strains tested at 30 µl/plate of S-9. Disperse Red 15 and Disperse Red 11 (Lot 2) (anthraquinone dyes) both produced significant mutagenic responses with bacterial strains TA-100, TA-98 and TA-1538 when tested in the presence of S-9. The magnitude of the response was increased as a function of S-9 concentration for both of these dyes in a way that was similar to that seen for 1,4-diaminoanthraquinone, a reported mutagen (Brown and Brown 1976). The concentration-response curves for these dyes increased over a low range of the dyes (0-10 µg/plate), then plateaued at higher concentrations.

STRAIN	DYE															
	Disp Red 15		Sol Red 24		Disp Red 11 (Lot 1)		Disp Red 11 (Lot 2)		Sol Red 1		Terephthalic Acid		Mixture 1		Mixture 2	
	W	W/O	W	W/O	W	W/O	W	W/O	W	W/O	W	W/O	W	W/O	W	W/O
TA-1535	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TA-100	±	-	-	-	-	-	±	-	±	-	-	-	-	-	±	-
TA-1538	+	-	-	-	-	-	+	-	-	-	-	-	-	-	±	-
TA-98	+	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-

- No indication of mutagenic activity.
+ Meets all criteria for mutagenic material.
± Response dependent on test conditions.

W:with the addition of S-9

W/O=Without S-9

Mixture 1=5:25:16 Mixture of Disperse Red 11 (Lot 1) : Solvent Red 1 : terephthalic acid

Mixture 2=5:25:16 Mixture of Disperse Red 11 (Lot 2) : Solvent Red : terephthalic acid

TABLE 11: SUMMARY OF BACTERIAL MUTAGENICITY OF RED/VIOLET DYES WITH AND WITHOUT THE ADDITION OF S-9 IN FOUR DIFFERENT STRAINS OF BACTERIA.

The magnitude of the mutagenic response at the plateau was dependent on the amount of S-9 added to the cultures. This seems to suggest that either the S-9 can activate only a fixed amount of mutagen per unit S-9, that the dyes have limited solubility, or that the dyes are inactivating the S-9 at higher dye concentrations. In any case, addition of more than 50 ug of dye per plate did not result in an increase in the mutagenic activity of the dye at any of the S-9 concentrations evaluated. Addition of higher concentrations of S-9 did cause an increase in the background level of mutations. The magnitude of this increase was small suggesting that the histidine in the S-9 is not producing the increase in the mutagenic response observed.

Studies to identify the role of the impurities present in the Disperse Red 11 (Lot 2) suggested that one of them was 1,4-diaminoanthraquinone but that the slope of the dose response relationship for this compound was similar to that for the Disperse Red 11 (Lot 2) so that it could not account for the biological activity measured in the Disperse Red 11 (Lot 2).

C. CYTOTOXICITY IN MAMMALIAN CELLS

The only dyes that were evaluated in the mammalian cell systems were Disperse Red 11 Lot 1 and Lot 2, Solvent Red 1, terephthalic acid and a mixture of (5:25:16 of Disperse Red 11:Solvent Red 1:Terephthalic acid. In the colony formation assay for cytotoxicity it was determined that terephthalic acid was not cytotoxic at concentrations up to 400 µg/ml and that Disperse Red 11 was not cytotoxic but at concentrations of 25 µg/ml caused a marked decrease in the cell colony size (microcolonies). This

observation suggested that Disperse Red 11 caused a delay in the growth of the cells. Studies determined that the growth rate of the treated cells returned to normal after about 2-3 days of culture in normal media. Solvent Red 1 was rather cytotoxic with 90% cell killing at concentration of 20 µg/ml of media.

D. INDUCTION OF MUTATIONS IN MAMMALIAN CELLS

No significant increase in the mutation frequency was observed in the CHO/HGPRT for Solvent Red 1, the dye mixture, or for Disperse Red 11 (Lot 1) when tested either with or without the addition of S-9. The only dye that resulted in a low but significant increase in the frequency of mutations measured in the CHO/HGPRT assay ($P = 0.02$) was the Disperse Red 11 (Lot 2) when tested without the addition of S-9. The mutagenic response following exposure to this dye increased from a background level of 0-10 mutants/10^6 clonable cells to a average response of 26 mutants/10^6 clonable cells at a concentration of 7.5 µg Disperse Red 11 (Lot 2) per ml of media. Addition of S-9 increased the background level to 10-20 mutants/10^6 clonable cells. There were no indications of a dye concentration-related increase in mutation frequency for any of the dyes with S-9 (P was always greater than 0.15).

E. SISTER CHROMATID EXCHANGES

Without the addition of S-9 there was no increase in the frequency of SCE following exposure of CHO cells to Solvent Red 1. In the presence of S-9, At the highest concentration of Solvent Red 1 (20 µg/ml), where

there was considerable cell killing, there was an increase in the SCE frequency. With Disperse Red 11 the frequency of SCE increased as a function of concentration with no influence of lot number or S-9. There was marked mitotic delay induced by the addition of the Disperse Red 11 which may have been related to the increase in the frequency of SCE. The SCE frequency increased as a function of concentration up to 10 µg/ml with a plateau in the response between 10 and 20 µg/ml. The genetic toxicologic significance of the observed increase is difficult to determine since the dye was shown to induce a significant delay in the cell cycle which has been reported to cause an increase in SCE frequency (Betan Court et al., 1986).

F. CHROMOSOME ABERRATIONS

Solvent Red 1 produced no increase in the frequency of chromosome aberrations at any of the concentrations tested. The highest response that was observed was for Disperse Red 11 (Lot 1) without the addition of S-9. This single response was significantly increased above the level observed in the control cells. Since this was a single observation that could not be repeated; it was not thought to be of biological significance. There was a higher frequency of exchange type aberrations induced in the cells exposed to the Disperse Red 11 but this was not significant. There was no influence of sampling time on the frequency of aberrations scored in cells exposed to Disperse Red 11.

SUMMARY

In summary, none of the dyes were mutagenic in the Ames test without the addition of S-9. Only anthraquinone dyes Disperse Red 15 and Disperse Red 11 (Lot 2) were mutagenic in TA-98 and TA-1538 with the addition of S-9. Disperse Red 15 had the highest mutagenic potency of the dyes tested. The mutagenic activity in Disperse Red 11 (Lot 2) may have been related to other compounds in the dye.

In mammalian cells Solvent Red 1 was the only dye that was cytotoxic. Solvent Red 1 and Disperse Red 11 (Lots 1 and 2) all induced significant increases in SCE frequency. None of the dyes or terephthalic acid showed marked mutagenic activity or significant clastogenic activity in mammalian cells. There was no evidence for interaction between the dyes tested and terephthalic acid to alter the genotoxic potency of the mixture. The data thus suggests that, under these test conditions, none of the dyes act as strong mutagens or clastogens.

LITERATURE CITED

Ames, B.N., J. McCann, and E. Yamasaki. 1975. Methods for detecting carcinogens as mutagens in Salmonella/mammalian microsome mutagenicity test. Mutat. Res. 31:347-363.

Betan Court, M., P. Balvanera, and R. Ortiz. 1986. Frequency of sister chromatid exchange (SCE) in bone marrow cells of severely malnourished animals during early life. Mutat. Res. 175:29-31.

Brooks, A.L., A.P. Li, J.S. Dutcher, C.R. Clark, S.J. Rothenburg, R. Kiyoura, W.E. Bechtold, and R.O. McClellan. 1984a. A comparison of genotoxicity of automotive exhaust particles from laboratory and environmental sources. Environ. Mutagen. 6:651-668.

Brooks, A.L., R.W. Shimizu, A.P. Li, J.M. Benson, and J.S. Dutcher. 1984b. The induction of sister chromatid exchanges by environmental pollutants: Relationship of SCE to other measures of genetic damage. In R.R. Tice and A. Hollaender, eds. Sister Chromatid Exchanges, 25 Years of Experimental Research. pp. 385-397.

Brooks, A.L., R.O. McClellan, and S.A. Benjamin. 1972. The effects of ^{144}Ce-^{144}Pr on the metaphase chromosomes of the Chinese hamster liver cells in vivo. Radiat. Res. 52:481-489.

Brown, J.P and R.J. Brown. 1976. Mutagenesis by 9,10-anthraquinone derivatives and related compounds in Salmonella typhimurium. Mutat. Res. 40:203-224.

Carrano, A.V., L.H. Thompson, P.L. Lindl, and J.L. Minkler. 1978. Sister chromatid exchange as an indicator of mutagenesis. Nature 271:551-553.

Evans, H.J. and M.L. O'Roirdan. 1977. Human peripheral blood lymphocytes for the analysis of chromosome aberrations in mutagen tests. In Kilbey et al., eds. Handbook of Mutagenicity Test Procedures. pp. 261-274.

Galloway, S.M., A.D. Bloom, M. Resnich, B.H. Margolin, F. Nakamura, P. Archer, and E. Zeiger. 1985. Development of a standard protocol for *in vitro* cytogenetic testing with Chinese hamster ovary cells: Comparison of results for 22 compounds in two laboratories. Environ. Mutagen. 7:1-51.

Hsie, A.W., B.D. Couch, J.P. O'Niell, J.R. San Sebastion, P.A. Brimer, R. Machanoff, J.C. Riddle, A.P. Li, J.C. Fuscoe, N. Forbes, and M.H. Hsie. 1979. Utilization of a quantitative mammalian cell mutation system, CHO/HGPRT, in experimental mutagenesis and genetic toxicology. In B.E. Butterworth, ed. Strategies for Short-term Testing for Mutagens/Carcinogens. pp. 39-54. CRC Press, West Palm Beach, FL.

Li, A.P. 1981. Simplification of the CHO/HGPRT mutation assay through the growth of Chinese hamster ovary cells as unattached cultures. Mutat. Res. 85:165-175.

Maron, D.M. and B.N. Ames. 1983. Revised methods for the Salmonella mutagenicity test. Mutat. Res. 113:173-215.

Perry, P. and S. Wolff. 1974. New giemsa method for differential staining of sister chromatids. Nature 261:156-158.

Snedecor, G.W. and W.G. Cochran. 1967. Statistical Methods, Sixth addition, pp. 22-23, Ames, Iowa.

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APPENDIX A
MUTAGENIC ACTIVITY OF RED/VIOLET DYES TESTED IN 4 STRAINS OF BACTERIA
(# REVERTANTS ± SE; N =3)

WITH OR WITHOUT (W or W/O) 30 µl/PLATE OF S-9

Solvent Red 24 (µg/plate)

Strain	0 Mean	± SE	10 Mean	± SE	50 Mean	± SE	125 Mean	± SE	250 Mean	± SE	500 Mean	± SE
TA-98 w/o	31	5	28	5	38	8	40	1	49	2	54	2
w	45	1	60	2	48	5	63	6	53	2	56	2
TA-1538 w/o	18	3	19	3	22	5	23	3	39	3	33	6
w	26	1	37	4	38	6	42	6	47	3	49	2
TA-100 w/o	149	2	150	6	149	1	153	10	165	6	171	5
w	155	5	200	3	199	8	199	6	217	9	219	8
TA-1535 w/o	23	3	17	2	28	5	26	1	23	2	25	5
w	13	1	10	3	15	2	16	2	17	3	19	3

Disperse Red 15 (µg/plate)

Strain	0 Mean	± SE	50 Mean	± SE	100 Mean	± SE	250 Mean	± SE	500 Mean	± SE	1000 Mean	± SE
TA-98 w/o	31	3	33	3	35	2	51	2	43	6	34	2
w	45	1	141	9	164	2	146	8	119	6	90	2
TA 1538 w/o	18	2	23	3	20	5	31	3	21	4	30	2
w	26	1	149	5	172	4	148	5	115	6	91	4
TA-100 w/o	149	2	178	7	176	10	142	2	132	14	65	16
w	155	5	221	7	244	17	264	2	237	21	224	10
TA 1535 w/o	23	3	23	2	24	2	25	1	23	2	25	2
w	13	1	15	1	17	3	16	5	14	2	16	1

Disperse Red 11 (Lot 1) (µg/plate)

Strain	0 Mean	± SE	50 Mean	± SE	100 Mean	± SE	250 Mean	± SE	500 Mean	± SE	1000 Mean	± SE
TA-98 w/o	31	3	31	3	36	2	23	2	49	3	43	3
w	45	1	55	3	55	5	48	3	59	8	43	5
TA-1538 w/o	18	3	19	2	18	2	20	2	23	2	23	2
w	26	1	25	5	26	2	30	3	27	2	29	2
TA-100 w/o	149	2	146	3	149	1	139	12	138	9	163	11
w	155	5	173	9	153	9	140	8	160	10	153	5
TA-1535 w/o	23	3	15	3	22	5	18	3	20	2	27	4
w	13	1	15	2	15	2	11	2	19	1	14	2

APPENDIX A (CONTINUED)

Disperse Red 11 (Lot 2) (µg/plate)

Strain	0 Mean	± SE	10 Mean	± SE	50 Mean	± SE	100 Mean	± SE	500 Mean	± SE	1000 Mean	± SE
TA-98 w/o S-9	30	3	22	3	27	1	21	4	23	1	22	3
w S-9	36	3	67	3	67	1	77	3	78	3	75	1
TA-1538 w/o S-9	13	2	19	3	19	3	21	2	21	3	18	2
w S-9	27	2	61	3	64	2	22	2	85	8	102	8
TA-100 w/o S-9	114	3	110	1	97	4	110	6	115	2	117	1
w S-9	121	5	128	2	126	2	138	10	142	9	144	2
TA-1535 w/o S-9	21	3	17	3	19	1	17	2	19	2	17	2
w S-9	10	1	8	2	11	2	13	1	9	1	17	1

Solvent Red 1 (µg/plate)

Strain	0 Mean	± SE	10 Mean	± SE	50 Mean	± SE	100 Mean	± SE	500 Mean	± SE	800 Mean	± SE
TA-98 w/o	30	3	31	3	25	2	31	6	28	2	32	1
w	36	3	38	3	34	5	41	3	42	1	43	2
TA-1538 w/o	13	2	18	2	20	3	17	2	23	2	17	2
w	27	2	19	1	24	3	29	4	26	1	23	2
TA-100 w/o	114	3	121	10	121	14	123	1	134	6	128	2
w	121	5	161	3	170	10	163	5	164	5	162	13
TA-1535 w/o	21	3	21	2	32	3	24	5	20	1	22	6
w	10	1	11	2	12	3	12	2	15	5	12	2

Terephthalic Acid (µg/plate)

Strain	0 Mean	± SE	10 Mean	± SE	50 Mean	± SE	100 Mean	± SE	200 Mean	± SE	400 Mean	± SE
TA-98 w/o S-9	30	3	20	1	21	2	27	3	29	3	21	3
w S-9	36	3	31	3	35	4	43	1	33	2	37	2
TA-1538 w/o S-9	13	2	15	2	15	1	15	1	13	2	20	3
w S-9	27	2	26	3	24	5	20	3	24	3	27	5
TA-100 w/o S-9	114	3	112	12	115	5	116	2	73	3	82	4
w S-9	121	5	115	1	119	4	112	5	93	14	88	5
TA-1535 w/o S-9	21	3	27	2	26	2	16	5	23	3	36	4
w S-9	10	1	8	1	8	1	10	3	11	3	12	2

APPENDIX A (CONTINUED)

Dye Mixture (5:25:16) (Disperse Red 11:Solvent Red 1:Terephthalic Acid)

(µg/plate)

Strain	0 Mean ± SE		1 Mean ± SE		5 Mean ± SE		10 Mean ± SE		50 Mean ± SE		100 Mean ± SE		200 Mean ± SE	
TA-98 w/o	26	2	24	4	25	3	20	5	28	1	22	4	26	3
w	32	3	38	6	43	3	44	4	47	2	48	3	57	1
TA-1538 w/o	19	2	19	1	16	2	20	4	23	4	20	4	17	2
w	29	2	33	5	33	2	35	4	29	2	38	1	41	2
TA-100 w/o	126	12	129	6	133	3	116	4	135	4	124	1	128	9
w	126	3	144	4	148	5	164	9	165	8	174	1	170	8
TA-1535 w/o	32	0	24	2	27	3	24	1	30	3	24	4	30	2
w	19	1	18	4	17	1	14	3	12	3	18	1	16	1

APPENDIX B
STATISTICAL EVALUATION OF MUTAGENIC RESPONSE OF RED/VIOLET DYES IN THE AMES SALMONELLA BACTERIA TEST

Strain	Figure No.	S-9 (30 µl/ plate)	Intercept	Slope ± SE (Revertant/mg)	"F" Value	"p" Value
Solvent Red 24						
TA-98	5	-	32.4	0.04 ± 0.009	27.0	0.006
	5	+	123	-0.04 ± 0.11	0.12	0.74
TA-1538	6	-	21.3	0.02 ± 0.01	2.1	0.22
	6	+	122	-0.03 ± 0.14	0.06	0.81
TA-100	7	-	171	-0.05 ± 0.01	57.0	0.002
	7	+	216	0.04 ± 0.09	0.27	0.62
TA-1535	8	-	23.4	0.002 ± 0.002	1.74	0.26
	8	+	14.9	0.002 ± 0.004	0.21	0.67
Disperse Red 15						
TA-98	9	-	37.2	0.002 ± 0.01	0.043	0.84
	9	+	123	-0.019 ± 0.05	0.11	0.75
TA-1538	10	-	21	0.008 ± 0.005	2.17	0.21
	10	+	122	-0.016 ± 0.07	0.056	0.82
TA-100	11	-	172	-0.10 ± 0.02	29.4	0.005
	11	+	216	0.02 ± 0.04	0.28	0.62
TA-1535	12	-	23.4	0.001 ± 0.001	1.67	0.26
	12	+	14.9	0.001 ± 0.002	0.21	0.67
Disperse Red 11 (Lot 1)						
TA-98	13	-	30.7	0.015 ± 0.009	2.46	0.19
	13	+	52.5	-0.005 ± 0.008	0.44	0.54
TA-1538	14	-	18.4	0.005 ± 0.001	18.7	0.02
	14	+	26.2	0.003 ± 0.002	2.3	0.20
TA-100	15	-	143	0.01 ± 0.01	1.51	0.28
	15	+	157	-0.005 ± .01	0.14	0.72
TA-1535	16	-	18.7	0.007 ± 0.004	2.4	0.19
	16	+	14.1	0.001 ± 0.003	0.14	0.72
Disperse Red 11 (Lot 2)						
TA-98	17	-	25.2	-0.004 ± 0.004	0.83	0.41
	17	+	61.5	0.019 ± 0.017	1.17	0.34
TA-1538	18	-	18.1	0.001 ± 0.003	0.13	0.74
	18	+	42.7	0.06 ± 0.02	7.31	0.05
TA-100	19	-	108	0.009 ± 0.007	1.80	0.25
	19	+	127	0.02 ± 0.006	7.66	0.05
TA-1535	20	-	18.7	-0.001 ± 0.002	0.52	0.51
	20	+	9.7	0.005 ± 0.002	3.96	0.12

APPENDIX B (CONTINUED)

Strain	Figure No.	S-9 (30 µl/ plate)	Intercept	Slope ± SE (Revertant/mg)	"F" Value	"P" Value
Solvent Red 1						
TA-98	21	-	28.9	0.002 ± 0.003	0.33	0.59
	21	+	36.9	0.008 ± 0.003	6.37	0.07
TA-1538	22	-	17.2	0.003 ± 0.004	0.42	0.55
	22	+	24.8	-0.001 ± 0.005	0.01	0.91
TA-100	23	-	119	0.01 ± 0.006	5.65	0.08
	23	+	149	0.01 ± 0.02	0.41	0.56
TA-1535	24	-	24.3	-0.004 ± 0.006	0.41	0.56
	24	+	11.3	0.002 ± 0.002	1.63	0.27
Terephthalic Acid						
TA-98	25	-	25.3	-0.005 ± 0.01	0.12	0.74
	25	+	35.3	0.004 ± 0.01	0.11	0.75
TA-1538	26	-	13.6	0.01 ± 0.005	5.26	0.08
	26	+	24.3	0.002 ± 0.008	0.10	0.77
TA-100	27	-	115	-0.1 ± 0.03	6.86	0.06
	27	+	118	-0.08 ± 0.02	27.6	0.006
TA-1535	28	-	21.2	0.03 ± 0.02	2.80	0.17
	28	+	8.7	0.008 ± 0.003	8.45	0.04
Dry Mixture (5:25:16) (Disperse Red 11:Solvent Red 1:Terephthalic Acid)						
TA-98	29	-	24.1	0.006 ± 0.016	0.15	0.71
	29	+	39.3	0.09 ± 0.02	15.7	0.01
TA-1538	30	-	19.4	-0.005 ± 0.01	0.12	0.74
	30	+	31.6	0.04 ± 0.02	7.85	0.04
TA-100	31	-	127	0.004 ± 0.04	0.008	0.93
	31	+	147	0.15 ± 0.08	3.98	0.10
TA-1535	32	-	26.8	0.009 ± 0.02	0.22	0.66
	32	+	16.5	-0.004 ± 0.01	0.06	0.82

APPENDIX C

THE INFLUENCE OF S-9 ON THE MUTAGENIC ACTIVITY OF RED/VIOLET DYES IN THE AMES SLAMONELLA BACTERIA TEST

Concentration (µg/plate)

Strain	S-9 %	Figure No.	µg/plate 0 Mean ± SE	1 Mean ± SE	5 Mean ± SE	10 Mean ± SE	50 Mean ± SE	100 Mean ± SE	200 Mean ± SE
Disperse Red 11 (Lot 2)									
TA-98	w/o	33	26 2	28 2	27 2	26 1	23 2	32 1	29 1
	3%		37 2	42 3	46 3	48 5	42 2	51 4	64 3
	6%		32 3	52 1	58 2	62 3	62 6	72 3	71 2
	12%		32 2	37 3	55 3	64 1	83 2	86 2	81 1
	24%		38 3	34 3	72 2	73 3	72 3	100 1	108 2
TA-1538	w/o	34	19 2	19 1	27 2	21 3	18 3	20 2	21 3
	3%		31 3	41 2	40 2	46 4	43 3	51 2	55 5
	6%		29 2	48 3	55 1	56 3	68 3	77 2	79 2
	12%		33 2	33 2	64 2	71 3	81 2	83 2	85 5
	24%		29 6	30 3	55 6	62 8	72 8	113 6	120 8
TA-100	w/o	35	126 12	125 5	128 2	122 2	127 3	120 5	128 2
	3%		147 7	143 5	129 9	132 6	133 2	128 8	136 2
	6%		126 3	149 2	136 1	143 4	155 5	152 1	155 2
	12%		139 4	128 8	143 7	145 4	144 4	151 7	154 5
	24%		148 1	153 2	156 3	163 3	179 6	167 3	176 3
TA-1535	w/o	36	32 0	25 3	29 2	28 1	28 1	32 4	25 2
	3%		16 1	17 2	18 4	16 1	23 2	20 1	24 2
	6%		19 1	14 2	17 2	17 1	12 1	18 2	18 2
	12%		17 1	16 2	11 2	16 3	11 1	13 1	15 1
	24%		20 1	16 2	14 2	12 2	11 2	15 3	17 1
Solvent Red 1									
TA-98	w/o	37	26 2	21 2	20 1	26 1	21 2	25 1	29 2
	3%		37 2	30 2	33 5	32 3	32 3	34 9	32 2
	6%		32 3	40 2	35 1	43 3	38 1	36 2	46 4
	12%		32 2	47 3	40 1	37 3	46 3	49 2	50 2
	24%		38 3	45 3	50 2	57 2	56 3	54 2	49 4
TA-1538	w/o	38	19 2	19 1	17 2	27 2	21 2	17 2	20 2
	3%		31 3	23 3	21 2	23 3	26 2	28 2	24 3
	6%		29 2	30 3	27 1	24 2	26 3	28 5	24 2
	12%		33 2	38 3	30 3	20 1	29 3	30 6	36 2
	24%		29 6	33 5	38 5	31 3	22 5	30 1	24 2

APPENDIX C (CONTINUED)

Strain	S-9 %	Figure No.	µg/plate 0 Mean ± SE		1 Mean ± SE		5 Mean ± SE		10 Mean ± SE		50 Mean ± SE		100 Mean ± SE		200 Mean ± SE	
TA-100	w/o	39	126	12	126	9	135	3	120	3	146	1	145	3	147	5
	3%		147	7	137	8	161	5	162	10	159	5	156	2	171	8
	6%		126	3	146	4	171	2	185	13	193	21	182	5	184	5
	12%		139	4	154	2	175	6	224	14	232	22	245	21	248	19
	24%		148	1	169	10	192	5	228	5	301	2	304	9	283	10
TA-1535	w/o	40	32	0	33	1	28	2	27	3	26	1	28	4	29	2
	3%		16	2	12	2	12	2	18	2	16	1	13	0	18	2
	6%		19	1	14	1	19	3	11	2	12	2	13	3	11	2
	12%		17	2	11	1	16	4	12	2	17	3	7	1	9	1
	24%		20	2	7	2	8	2	9	2	11	2	14	3	11	1
Dye Mixture (Disperse Red 11 (Lot 2):Solvent Red 1:Terephthalic Acid (5:25:16)																
TA-98	w/o	41	26	2	24	4	25	3	20	5	28	1	22	3	26	3
	3%		37	2	42	1	37	3	37	3	32	2	42	6	34	5
	6%		32	3	38	8	43	3	44	4	47	2	48	3	54	1
	12%		32	2	45	5	41	2	43	2	57	3	63	5	68	3
	24%		38	3	48	2	47	3	53	3	56	3	71	6	80	2
TA-1538	w/o	42	19	2	19	1	16	2	20	4	23	3	20	4	17	2
	3%		31	3	29	1	22	1	20	3	28	3	25	3	34	5
	6%		29	2	33	5	33	2	35	3	29	2	38	1	41	2
	12%		33	2	36	4	38	2	38	3	31	1	39	2	46	6
	24%		29	6	28	1	40	4	45	2	39	5	46	1	53	3
TA-100	w/o	43	126	12	129	6	133	3	116	3	135	3	124	1	128	3
	3%		147	7	122	4	148	7	143	3	143	5	151	3	154	5
	6%		126	3	144	3	148	5	164	9	165	8	174	1	170	8
	12%		139	4	155	3	145	1	165	8	214	10	190	2	197	6
	24%		148	1	145	6	149	5	154	7	269	17	269	16	262	4
TA-1535	w/o	44	32	0	24	2	27	3	24	1	30	3	24	4	30	2
	3%		16	1	18	4	15	2	15	2	23	3	17	1	24	4
	6%		19	1	18	4	17	1	14	3	12	3	18	1	16	1
	12%		17	1	13	5	18	2	13	1	17	1	19	3	15	2
	24%		20	1	14	1	12	3	16	2	16	2	16	1	19	2

APPENDIX D
STATISTICAL EVALUATION OF MUTAGENIC RESPONSE OF RED/VIOLET DYES
WITH GRADED LEVELS OF S-9 IN THE AMES SALMONELLA BACTERIA TEST

Strain	Figure No.	S-9 µl/plate	Intercept	Slope ± SE	"F" Value	"p" Value
Disperse Red 11 (Lot 2)						
TA-98	33	0	26.4	0.02 ± 0.01	1.38	0.29
		15	41.6	0.10 ± 0.02	19.9	0.006
		30	52.1	0.12 ± 0.06	4.05	0.10
		60	51.6	0.21 ± 0.09	4.91	0.08
		120	54.9	0.31 ± 0.09	10.8	0.02
TA-1538	34	0	20.9	−0.003 ± 0.02	0.04	0.85
		15	39.3	0.09 ± 0.03	10.6	0.02
		30	49.1	0.18 ± 0.06	8.5	0.03
		60	53.5	0.20 ± 0.10	4.3	0.09
		120	45.8	0.44 ± 0.09	21.7	0.005
TA-100	35	0	125	0.004 ± 0.02	0.05	0.84
		15	137	−0.02 ± 0.04	0.34	0.58
		30	124	0.004 ± 0.02	0.05	0.84
		60	138	0.08 ± 0.03	7.0	0.04
		120	157	0.11 ± 0.05	5.05	0.07
TA-1535	36	0	28.9	−0.01 ± 0.01	0.41	0.55
		15	17.3	0.03 ± 0.01	10.7	0.02
		30	16.0	0.007 ± 0.01	0.27	0.62
		60	14.3	−0.003 ± 0.01	0.04	0.86
		120	14.7	0.009 ± 0.02	0.07	0.80
Solvent Red 1						
TA-98	37	0	26.7	0.03 ± 0.02	3.25	0.13
		3	33.0	−0.003 ± 0.01	0.06	0.81
		6	36.6	0.04 ± 0.02	2.37	0.18
		12	39.8	0.06 ± 0.03	4.39	0.09
		24	48.9	0.02 ± 0.04	0.22	0.66
TA-1538	38	0	20.3	−0.005 ± 0.02	0.08	0.78
		3	25.1	0.002 ± 0.02	0.01	0.93
		6	27.7	−0.02 ± 0.01	1.67	0.25
		12	29.7	0.02 ± 0.03	0.44	0.54
		24	31.7	−0.04 ± 0.02	2.49	0.18

APPENDIX D (CONTINUED)

Strain	Figure No.	S-9 µl/plate	Intercept	Slope ± SE	"F" Value	"P" Value
TA-100	39	0	129	0.11 ± 0.04	6.65	0.05
		3	151	0.09 ± 0.05	3.41	0.12
		6	161	0.16 ± 0.12	1.62	0.25
		12	179	0.44 ± 0.18	5.76	0.06
		24	200	0.61 ± 0.27	4.96	0.07
TA-1535	40	0	29.4	-0.008 ± 0.01	0.29	.61
		3	14.3	0.01 ± 0.01	0.89	.39
		6	15.5	-0.02 ± 0.02	2.15	.20
		12	14.4	-0.03 ± 0.02	2.88	.15
		24	11.3	0.003 ± 0.03	0.01	.91
Dye Mixture (Disperse Red 11 (Lot 2):Solvent Red 1:Terephthalic Acid)						
TA-98	41	0	24.1	0.006 ± 0.02	0.15	0.71
		3	38.0	-0.01 ± 0.02	0.38	0.56
		6	39.6	0.07 ± 0.02	10.8	0.02
		12	41.7	0.15 ± 0.04	18.3	0.007
		24	46.0	0.18 ± 0.03	39.4	0.002
TA-1538	42	0	19.4	-0.005 ± 0.01	0.12	0.74
		3	25.2	0.03 ± 0.03	1.85	0.23
		6	31.6	0.05 ± 0.02	7.86	0.04
		12	34.8	0.05 ± 0.02	6.23	0.06
		24	35.0	0.09 ± 0.03	7.88	0.04
TA-100	43	0	127	0.003 ± 0.04	0.01	0.93
		3	139	0.07 ± 0.05	2.30	0.19
		6	147	0.15 ± 0.07	3.98	0.10
		12	159	0.25 ± 0.13	4.19	0.09
		24	164	0.66 ± 0.23	8.07	0.04
TA-1535	44	0	26.8	0.009 ± 0.01	0.22	0.66
		3	16.4	0.04 ± 0.02	5.33	0.07
		6	16.5	-0.003 ± 0.01	0.56	0.82
		12	15.8	0.004 ± 0.01	0.07	0.80
		24	15.3	0.02 ± 0.01	1.13	0.34

END

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DTIC