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MUTAGENIC SCREENING OF DIAMINE MONOMERS

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16. Abstract

The effects of phenyl ring coupling moieties, of isomeric amine positions relative to the coupling groups, and of insertion of other coupling groups on the mutagenic response of a series of dianilines were investigated using the Ames Salmonella assay. Generally, S-9 metabolic activation from Aroclor-induced rat liver was required for mutagenic expression. The range of mutagenicity of steric isomers of several dianiline series was also investigated. No mutagenicity was found for purified samples of o,o' and m,p' isomers of methylene dianiline (MDA) and diaminobenzophenone, while varying degrees of mutagenicity were found for other isomers. The mutagenicity of "benzylogs" of MDA decreased as the degree of linear separation of the m,m' anilino groups by aromatic rings increased. Methylation and two-year storage increased mutagenic response in certain isomers of MDA. However, high performance liquid chromatography indicated there was no discernible change in m,p'-MDA samples aged under varied conditions over four months. Likewise, no change in mutagenicity was found. Retesting of p,p' and m,m' MDA isomers previously tested in 1979 by the Ames assay gave similar results for the p,p' isomer but lower mutagenicity for the m,m' isomer. The metabolic activation required for mutagenic expression obscured the bioactivity mechanisms.

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Summary

In the preceding period, correlations between the steric positions of the amino groups of dianilines and the microbial mutagenicity of a series of these compounds were investigated using the Ames Salmonella assay. The effects of phenyl ring coupling moieties, of the isomeric positions of the amino groups relative to the coupling groups, and of insertion of other coupling groups on the mutagenic response of these compounds were also investigated. In most cases, S-9 metabolic activation from Aroclor-induced rat liver was required for mutagenic expression. The range of mutagenicity of steric isomers of several dianiline compounds was also investigated. No mutagenicity was found for purified samples of o,o' and m,p' isomers of methylene dianiline (MDA) and diaminobenzophenone (DABP), while varied degrees of mutagenicity were found for the other isomers. The mutagenicity of "benzylogs" of MDA decreased as the degree of linear separation of the m,m' anilino groups by aromatic rings increased. Additional work, described in this report, showed that methylation and long-term (two-year) storage increased mutagenic response in certain isomers of methylene dianiline. However, high performance liquid chromatography indicated that there was no discernible change in m,p'-MDA samples aged under varied conditions during a four-month aging study. Likewise, no change in mutagenicity was found. Retesting of p,p' and m,m' MDA isomers by the Ames assay previously tested in 1979, gave similar results for the p,p' isomer but lower mutagenicity for the m,m' isomer.

Bioactivity mechanisms are obscured by the fact that metabolic activation is required for an expression of mutagenicity by the bioactive isomers. This indicates the ultimate mutagenic compounds are as yet unidentified metabolic products of the original isomers after treatment with S-9 fractions from Aroclor-induced rat liver.

In vitro cytoxicity assays indicate similar and moderate-to-low-acute toxicity for the DABP isomers. There appears to be no correlation between acute toxicity and mutagenicity.

CONTENTS

FIGURES

TABLES

INTRODUCTION

Aromatic amines are widely used in the preparation and use of high performance polyamides and polyimides. They are used in even larger quantities as curing agents for polyurethanes and epoxies. Certain aromatic amines have been suspected as human carcinogens (Selkirk, 1980) and certain ones have been shown to be mutagenic agents in animals; e.g., benzidine (Windholz, 1976; Lewis, 1980). Consequently, the aromatic amines as a class are receiving scrutiny to determine just how general this mutagenic behavior is. At the present time, there is no generally accepted theory that would explain the mechanism responsible for the mutagenic nature of aromatic amines.

Polymer chemists in the Materials Division of NASA's Langley Research Center have been synthesizing new and relatively rare aromatic diamines for use in thermally stable polymers for a dozen years. During this time, many different aromatic diamines have been synthesized. Only a very few of these have ever been commercially available. The preparation of these diamines was the result of the philosophy that useful and predictable chemical, physical, and mechanical properties could be tailored into the polymers if the chemicals used as starting materials contained the proper structural features. If a certain chemical feature is to be contained in a polymer, it is much easier to insert it into the polymer via the monomer than it is to modify the polymer itself.

Because of the very specific and systematic variations in the chemical structures of the diamines, significant differences in the properties of the resulting polymers were achieved. These differences, such as lower initial softening points leading to improved processability, higher final melting points and thus higher use temperatures, and better adhesive strengths, were then directly attributable to the precise variations in the diamine monomers.

The diamines used in the polymer studies thus represent a unique collection of valuable chemicals which could be used as the basis of an investigation into the steric, chemical, and electronic features occurring in aromatic diamines which cause certain ones to be potential carcinogens.

Preliminary testing done in this study using the Ames microbial mutagenicity assay (Ames et al., 1975) indicated that there was a relationship between the isomeric positions of the amine group on the diphenylmethane moiety and the mutagenicities of the diamine isomers. Therefore, an extensive study was designed to evaluate a series of aromatic diamine compounds and their corresponding steric isomers in order to gain insight into possible explanations for mechanisms of mutagenicity by the most widely accepted in vitro mutagenicity assay, the Ames Salmonella test procedure.

The Ames mutagenicity data from the entire study are summarized in a manuscript found in Appendix A of this report and in a Final Report dated April 1982. The manuscript will be submitted to the journal, "Environmental Mutagenesis."

Supplemental funds were added to this contract in 1982 for additional studies of amine degradation and oxidation products, the mechanisms of amine bioactivity, the acute toxicity of diaminobenzophenone isomers, and retesting of m,m'-MDA and p,p'-MDA to resolve inconclusive Ames bacteria assay data. This report covers data resulting from completion of each of the above tasks and resummarizes Ames data obtained from previous investigations.

AMINE DEGRADATION/OXIDATION PRODUCTS

The objective of this portion of the study was to identify and approximate the quantities of any products resulting from the aging of m,p'-diaminodiphenylmethane (m,p'-MDA). The aged samples were compared with a relatively pure sample of this isomer

[m,p'-MDA-F-2(484)] submitted earlier in this investigation and shown to have low mutagenicity by the Ames test. These samples were treated by the NASA Technical Project Officer to simulate aging under several environmental conditions. Sample m,p'-MDA-A1 (498) was aged in an inert atmosphere at ambient room temperature. Sample m,p'-MDA-A,A,RT (499) was aged in air at ambient room temperature, and sample m,p'-MDA-A,A,50 (500) was aged in air at 50°C.

High Pressure Chromatography - High pressure liquid chromatography (HPLC) was performed on these samples. The first set of instrument conditions used in this study was as follows:

Instrument: Du Pont 8800 series
Detector: Schoeffel SF 770 OV/VIS, 254 mm
Temperature: ambient
Column: Du Pont Zorbax C_8, 15 cm x 4.6 mm
Pre-column: Brownlee Spheri-5 C_8, 3 cm x 4 mm
Mobile phase gradient: 75% methanol to 100% methanol at linear rate for ten minutes then hold at 100% for 30 min.
Flow rate: 1.5 mL/min.

Using these conditions, the chromatograms shown in Figure 1 (A-D) were obtained. No significant differences among the samples are observed. However, several relatively small impurity peaks can be seen in each sample.

The samples were also analyzed using the same instrument conditions but a different solvent composition to determine more ideal separation conditions. The second solvent composition used was 60% isooctane, and 40% methanol/methylene chloride at 1 mL/min flow rate. Figure 2 (A-D) shows the four samples analyzed using the different solvent. Again, no significant differences are noted among these four samples even though the chromatograms are different from those obtained with the instrument conditions described earlier.

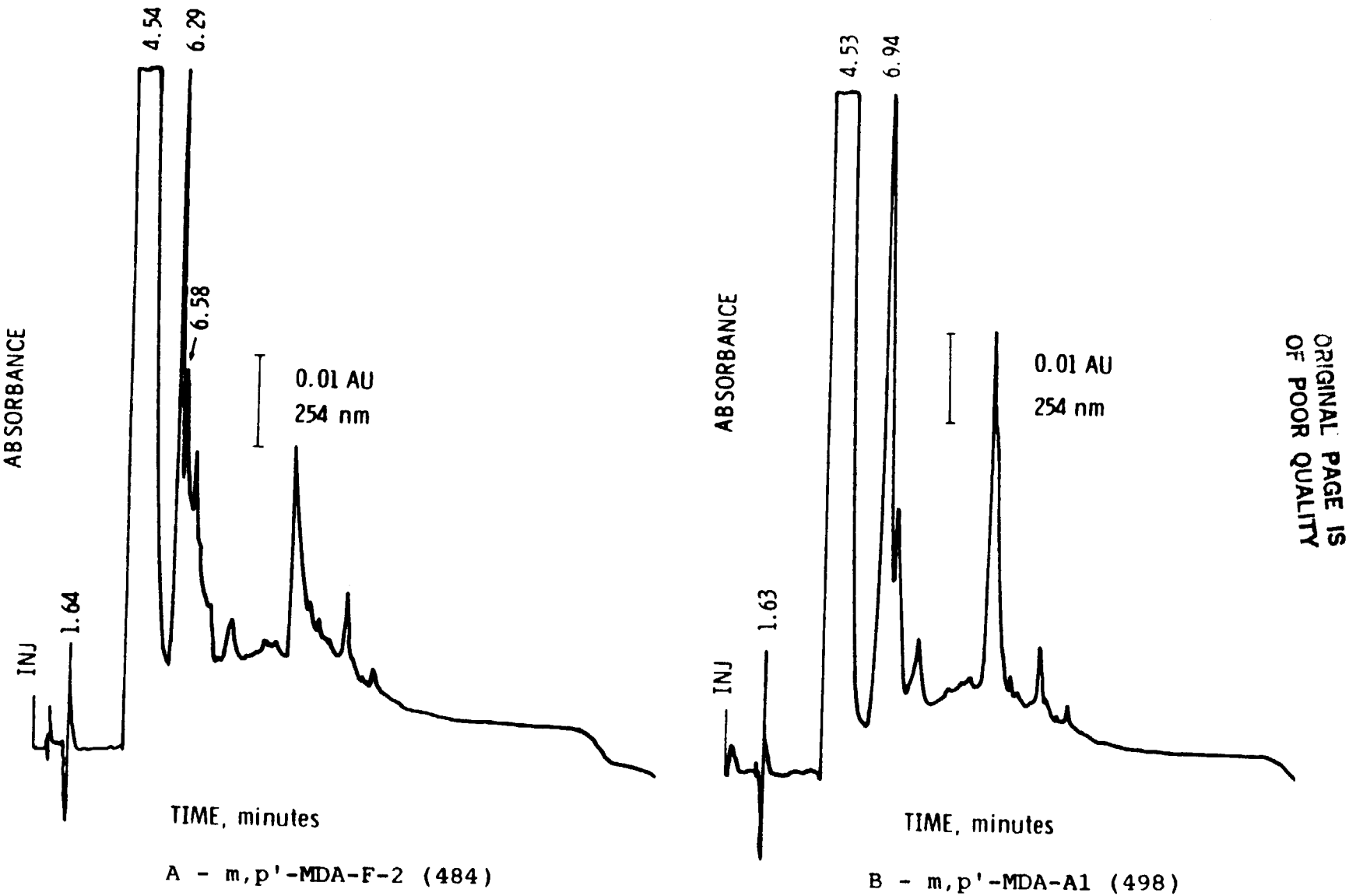
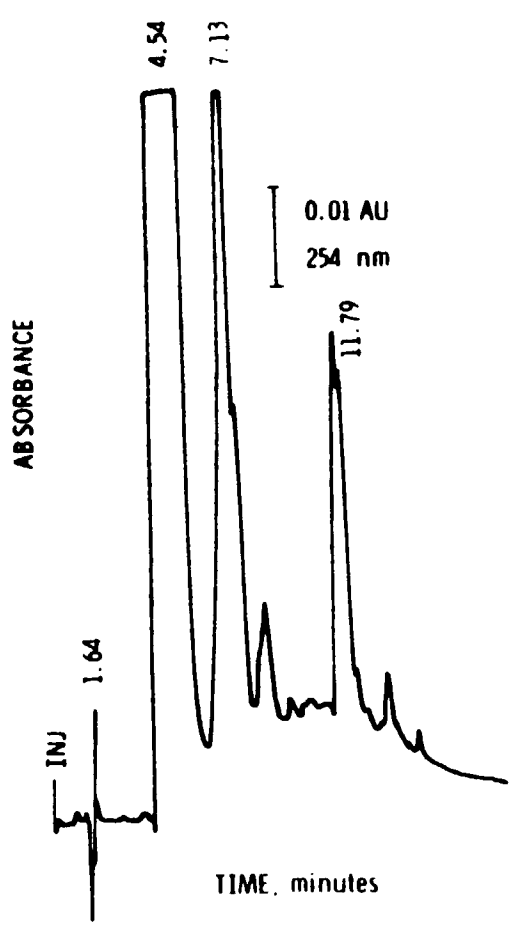
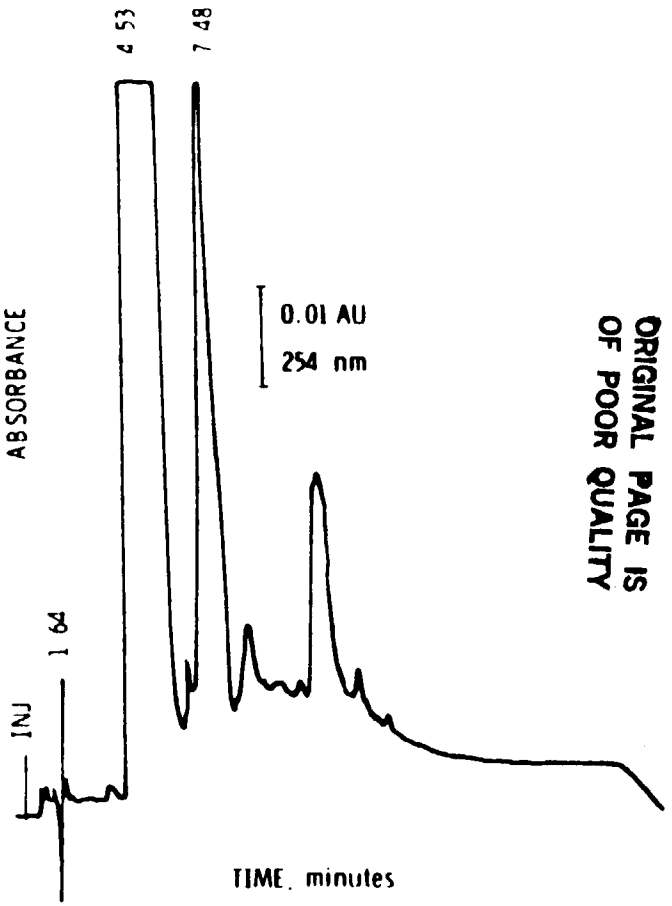


Figure 1. HPLC chromatograms of four m,p' MDA aged under different conditions (solvent:methanol).

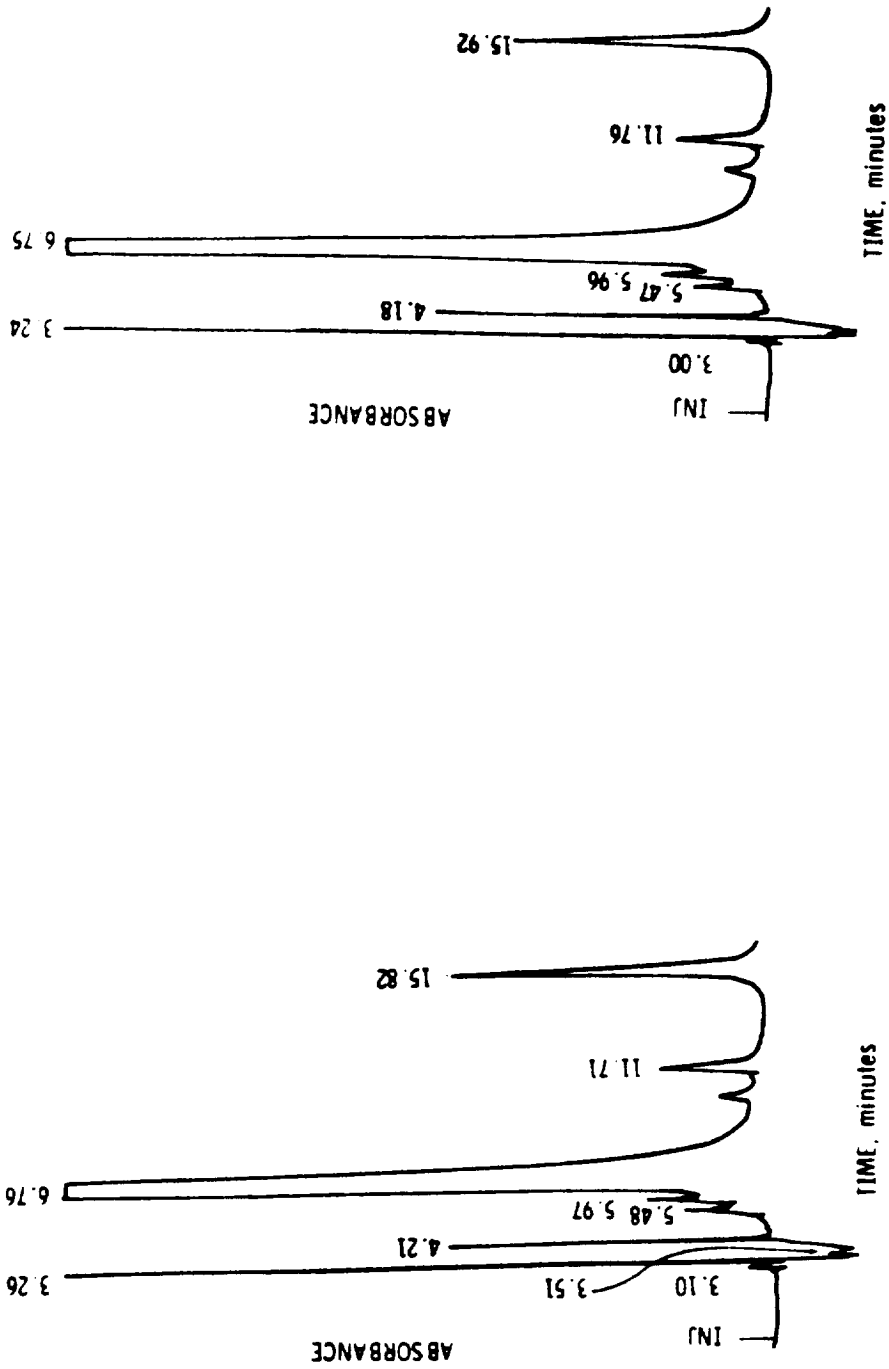


C - m,p'-MDA-A,A,RT (499)



D - m,p'-MDA-A,A50 (500)

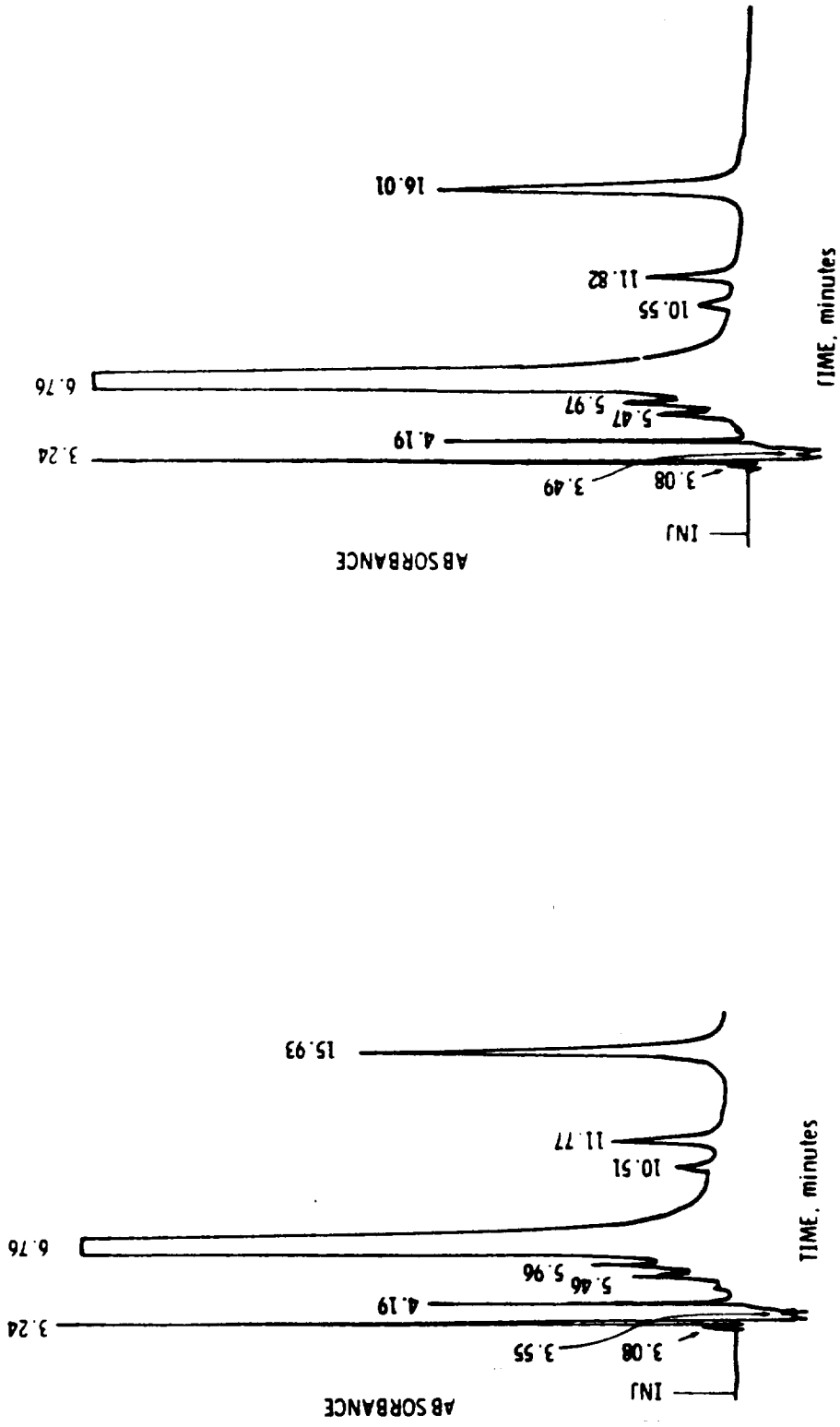
Figure 1 (continued)



B - m,p'-MDA-A1 (498)

A - m,p'-MDA-F-2 (484)

Figure 2. HPLC chromatograms of four m,p' MDA aged under different conditions (solvent:isooctane/methanol/methylene chloride).



D - m,p'-MDA-A,A50 (500)

C - m,p'-MDA-A,A,RT (499)

Figure 2 (continued)

Although the minor peaks appear to be small relative to the major MDA peak, no quantitative value can be obtained from the chromatogram because the wavelength for maximum sensitivity of each component is unknown.

Analysis for Nitrosamines - The four samples were also analyzed by HPLC using the Thermoelectron TEA detector, which is a specific detector for nitrosamines. No evidence of any nitrosamine compounds was found in any of the four samples.

Comparison of Mutagenicity - These four samples were assayed for mutagenicity (see Figures 14 and 15 in Appendix A). Minimal or no mutagenicity was found for the samples tested. No significant increase in mutagenicity was found among the samples aged under different conditions, supporting the similarity of chromatograms found by the HPLC analyses.

Because of the very small amounts of impurities in these samples, not enough material corresponding to these extraneous peaks could be recovered for identification.

Even though the samples aged in air (499, 500) had a red-brown color while the samples stored in an inert atmosphere were near white in color, no differences in composition as determined by HPLC or by the mutagenicity assay were detected. The aging time may not have been long enough. Previously, changes were noted after two years (1977-1979) of storage time of the m,p'-MDA (see Figure 13 in Appendix A).

MECHANISMS OF AMINE BIOACTIVITY

An ultimate and desirable goal of this research was to correlate chemical structure with the mutagenic potency of the aromatic diamines tested. Ideally, these compounds could provide a model for the prediction of mutagenicity by chemical structure.

The empirical data recovered from these studies show a rather dramatic difference in the mutagenic potency as determined by the Ames test system of different steric isomers in several related compounds, ranging from no mutagenic activity to highly mutagenic responses.

However, the requirement for metabolic activation indicates that an unknown compound or compounds is involved in the mutagenic behavior. Until these ultimate compounds are recovered and identified, it is very unlikely that a correlation of structure and bioactivity can be established.

This conclusion was concurred by the following academic and Monsanto authorities:

Dr. James Pyle - Professor Chemistry and Director of Graduate Research at Miami University

Dr. Joseph Cantrell - Professor of Chemistry, Miami University

Dr. George Kantor - Professor of Molecular Biology, Wright State University

Dr. D. Gerald Glasgow - Senior Research Specialist, Monsanto Research Corporation

Mr. George A. Richardson - Senior Research Chemist (organic synthesis), Monsanto Research Corporation.

Dr. George Kantor, consultant for MRC in in vitro mutagenesis testing, wrote the summary of structure/activity discussions presented in Appendix B.

ACUTE TOXICITY OF DIAMINOBENZOPHENONE (DABP) ISOMERS

The six steric isomers of diaminobenzophenone (DABP) were assayed for acute cytotoxicity by the mammalian cell clonal test. This study was performed to determine if there is correlation between *in vitro* acute toxicity and *in vitro* mutagenicity of DABP isomers, another possible key to insight into mutagenicity mechanisms. The rationale was that some compounds that exhibit acute toxicity affect the DNA synthesis or S phase of the cell replication cycle. If incorporation of such compounds in nuclear material occurs, we would expect that steric isomers that are mutagenic would also show toxicity and that the nonmutagenic isomers would be nontoxic. The data shown in Table I indicate that all isomers of DABP are moderate or low and very similar in toxicity. Therefore, mutagenicity and toxicity appear not to be correlatable.

RETEST OF m,m'- AND p,p'-MDA IN AMES TEST

Ames bacterial tests initially made on m,m'-MDA and p,p'-MDA using TA98 and TA100 *Salmonella* strains resulted in data that were statistically inconclusive according to the same computerized statistical program used in the first study under this contract. For this reason a decision was made to retest these two isomers in hopes of obtaining more conclusive results.

For the preparation of the manuscript of Appendix A, the data obtained from the Ames test of all the isomers of all the compounds were displayed graphically, plotting revertants of bacterial organisms per plate versus the dose of compound per plate. These graphs are Figures 2-14 in Appendix A. The plots were made so that all data could be more readily compared.

If the two-times-solvent-control background criteria used in the EPA criterion for revertants/plate vs. g/plate is used with the recovered data for the two isomers, moderate mutagenicity is

TABLE I. MAMMALIAN CELL CLONAL ASSAY FOR ACUTE TOXICITY OF DABP ISOMERS

DABP isomer	EC_{50}, g/mL CHO-K1 (hamster)	EC_{50}, g/mL D98S (human)	EPA toxicity rating*
m,m'	2×10^{-4}	1×10^{-4}	Low
o,p'	7.5×10^{-5}	2.5×10^{-5}	Moderate
o,o'	7.5×10^{-5}	7.5×10^{-5}	Moderate
p,p'	2.0×10^{-4}	6.0×10^{-5}	Low, moderate
m,p'	2.0×10^{-4}	1.0×10^{-4}	Moderate
o,m'	1.0×10^{-4}	7.0×10^{-5}	Low, moderate
DMSO	100% survival	100% survival	--

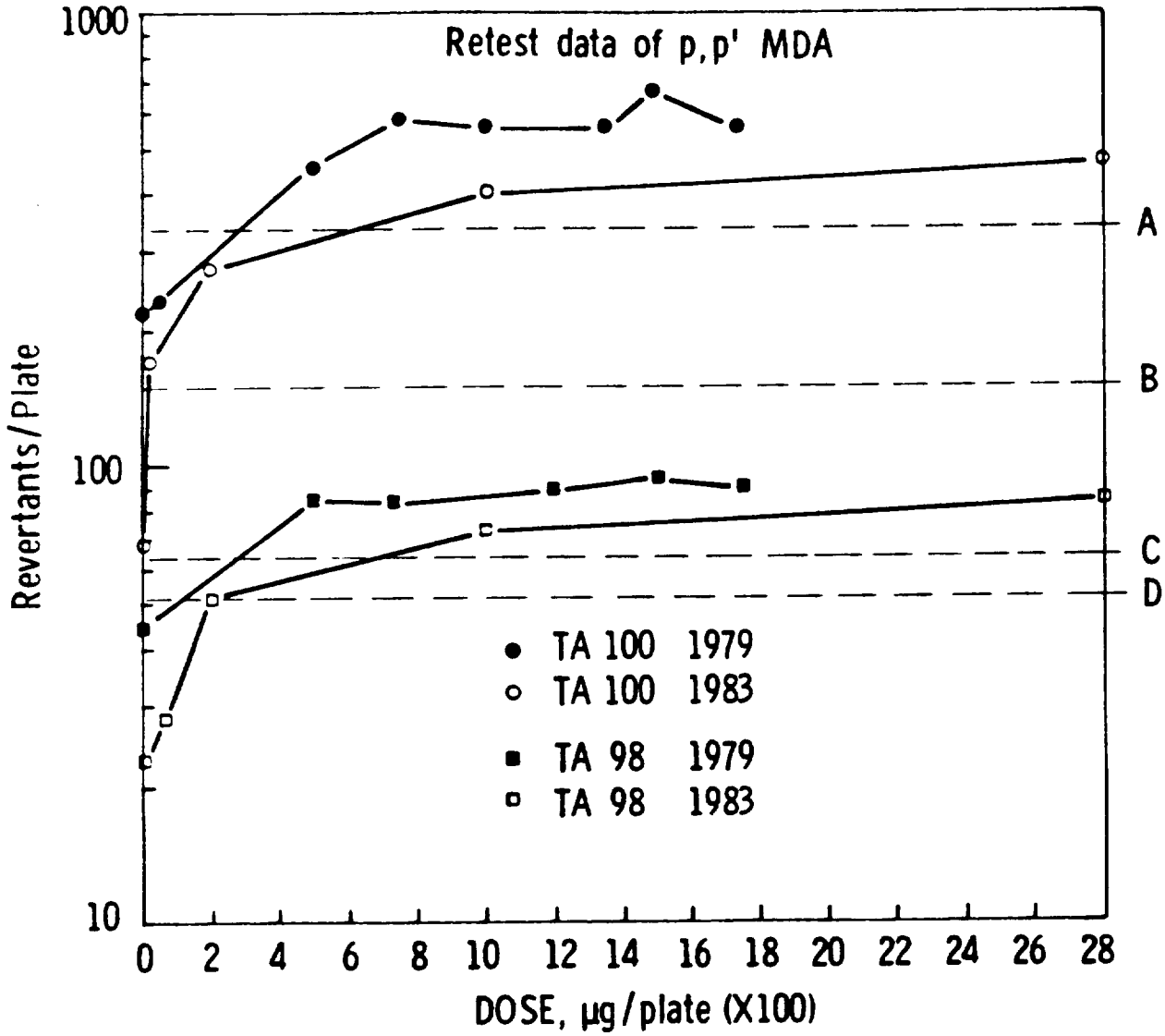
*Low: 1×10^{-4} g/mL to 1×10^{-3} g/mL
Moderate: 1×10^{-5} g/mL to 1×10^{-4} g/mL
High: $<1 \times 10^{-5}$ g/mL.

detected for both m,m'-MDA and the p,p'-MDA. However, to fulfill the requirements of this Task, these two isomers were retested. Table II lists the retest data and Figures 3 and 4 graphically display the retest data of 1983 and tests performed in 1979. Samples of these two isomers of MDA were stored from 1979 to 1983 in the dark and under refrigerator conditions but not in an inert atmosphere. The p,p' MDA samples are quite similar both in numbers of revertants and curve slopes for both strains. The m,m' MDA isomer in the retest (1983) study, produced lower numbers of revertants than the 1979 test in both strains. However, observing the graphs of both data sets there is good correlation between the 1979 and 1983 data.

TABLE II. RETEST DATA FOR m,m' MDA AND p,p' MDA

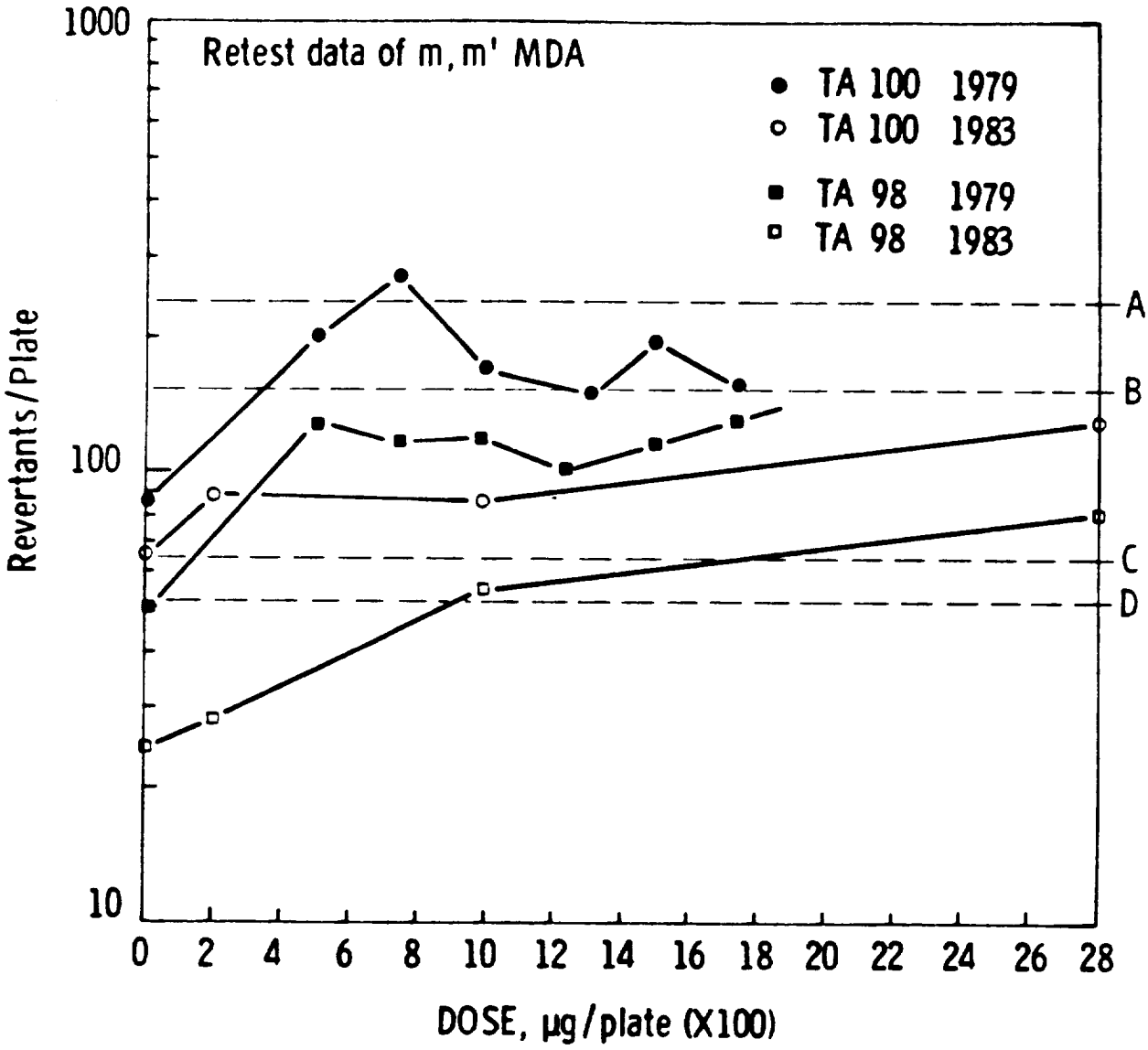
Isomer	Strain	
	TA98	TA100
m,m' MDA (1979)	Moderate	Moderate
m,m' MDA (1983)	Low	ND*
p,p' MDA (1979)	Moderate	Moderate
p,p' MDA (1983)	Moderate	Moderate

*No detectable mutagenicity.



A - $2\bar{x}$ solvent control of TA100 (1979)
B - $2\bar{x}$ solvent control of TA100 (1983)
C - $2\bar{x}$ solvent control of TA98 (1979)
D - $2\bar{x}$ solvent control of TA98 (1983)

Figure 3. Original (1979) and retest data (1983) of p,p'-MDA tested in Ames strains TA98 and TA100.



A - $2\bar{x}$ solvent control of TA100 (1979)
B - $2\bar{x}$ solvent control of TA100 (1983)
C - $2\bar{x}$ solvent control of TA98 (1979)
D - $2\bar{x}$ solvent control of TA98 (1983)

Figure 4. Original (1979) and retest data (1983) of m,m'-MDA tested in Ames strains TA98 and TA100.

APPENDIX A

MANUSCRIPT

PREFACE

The Ames data reported in Tables 3 through 6 of the Final Report issued in April 1982, were summations of the results of computerized statistical treatment procedures for objective and nonbiased assessment of test results designed for Monsanto Company products. Retests were performed when data were inconclusive. Sometimes the retest data were also inconclusive. In preparing the data for a manuscript it was observed other authors of work published in the journal "Environmental Mutagenesis", where we intend to submit the manuscript, comprising this Appendix, presented their data in graphic and/or tabular form in terms of number of revertants per plate versus quantity/plate. The graphic presentation also seemed appropriate for our work, so this method was chosen for the latest draft of our manuscript. The criterion applied for calling a compound "mutagenic" or "no mutagenicity detected" was adopted from "Level 1 Biological Testing Assessment and Data Formatting," EPA 600/7-80-079, paragraph 4b, page 30:

> If the solvent control value is within the normal range, a chemical that produces a positive dose response over three concentrations with the highest increase equal to twice the solvent control value for TA100 and two to three times the solvent control value for strain TA98 is considered to be mutagenic. For these strains, the dose-response increase should start at approximately the solvent control value.

IN VITRO MUTAGENICITY OF STERIC ISOMERS OF AROMATIC DIAMINE COMPOUNDS

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ABSTRACT

Correlations between the steric positions of the amino groups of dianilines and the microbial mutagenicity of a series of these compounds were investigated using the Ames Salmonella assay. The effects of phenyl ring coupling moieties, of the isomeric positions of the amino groups relative to the coupling groups, and of insertion of other coupling groups on the mutagenic response of these compounds were also investigated. In most cases, S-9 activation from Aroclor-induced rat liver was required for mutagenic expression. The range of mutagenicity of steric isomers of several dianiline compounds was also investigated. No mutagenicity was found for o,o' and m,p' isomers of methylene dianiline (MDA) and diaminobenzophenone (DABP), while varied degrees of mutagenicity were found for the other isomers. The mutagenicity of "benzylogs" of MDA decreased as the degree of linear separation of the m,m' anilino groups by aromatic rings increased. Methylation and long-term (two-year) storage increased mutagenic response in certain isomers of methylene dianiline.

KEYWORDS

In vitro mutagenicity, aromatic amines, dianilines, biphenyl diamines, anilino steric isomers.

INTRODUCTION

Aromatic amines are widely used in the preparation and use of high performance polyamides and polyimides. They are used in even larger quantities as curing agents for polyurethanes and epoxies. Certain aromatic amines have been suspect human carcinogens (Selkirk, 1980) and certain ones have been shown to be mutagenic agents in animals; e.g., benzidine (Windholz, 1976; Lewis, 1980). Consequently, the aromatic amines as a class are receiving scrutiny to determine just how general this mutagenic amine behavior is. At the present time, there is no generally accepted theory that would explain the mechanism responsible for the mutagenic nature of aromatic amines.

Polymer chemists in the Materials Division of NASA's Langley Research Center have been synthesizing new and relatively rare aromatic diamines for use in thermally stable polymers for a dozen years. During this time, many different aromatic diamines have been synthesized. Only a very few of these have ever been commercially available. The preparation of these diamines was the result of the philosophy that useful and predictable chemical, physical, and mechanical properties could be tailored into the polymers if the chemicals used as starting materials contained the proper structural features. If a certain chemical feature is to be contained in a polymer, it is much easier to insert it into the polymer via the monomer than it is to modify the polymer itself.

Because of the very specific and systematic variations in the chemical structures of the diamines, significant differences in the properties of the resulting polymers were achieved. These differences, such as lower initial softening points leading to improved processability, higher final melting points and thus higher use temperatures, and better adhesive strengths, were then directly attributable to the precise variations in the diamine monomers.

The diamines used in the polymer studies thus represent a unique collection of valuable chemicals which could be used as the basis of an investigation into the steric, chemical, and electronic features occurring in aromatic diamines which cause certain ones to be potential carcinogens.

Preliminary testing of this study using the Ames microbial mutagenicity assay (Ames et al., 1975) indicated that there was a relationship between the isomeric positions of the amine group on the diphenylmethane moiety and the mutagenicities of the diamine isomers. Therefore, an extensive study was designed to evaluate a series of aromatic diamine compounds and their corresponding steric isomers in order to gain insight into possible explanations for mechanisms of mutagenicity by the most widely accepted *in vitro* assay, the Ames *Salmonella* mutagenicity assay.

The specific objectives of this project were to:

- Investigate the relative mutagenicity among some or all of the six possible steric isomers of four series of dianiline compounds, namely, methylene dianiline (MDA), diaminobenzophenone (DABP), oxydianiline (ODA), and diaminodiphenyl sulfone (DDS) (see Tables I and II for structures).

- Assess relative mutagenicities among like isomers of the above series of dianiline compounds.

- Study the effects of the coupling moieties with a similar steric isomer (o,m'), of aromatic diamines, MDA, DABP, ODA, and DDS on mutagenicity.

- Investigate the effects of proximity of the two anilino groups on mutagenicity by evaluating methylene dianiline (MDA) with "benzylogs" of m,m'-MDA: m_1,m_3-terbenzyl-p_2 (m,m_3-TBz) and m_1,m_3-quaterbenzyl-p_2, p_3 (m,m_4-QBz) (see Table I).

- Investigate the effects of insertion of methyl groups in steric isomers on the mutagenicity of MDA (Table I).

- Study the effects of sample aging and storage on mutagenicity.

MATERIALS AND HANDLING METHODS

Materials

The test compounds were synthesized or otherwise obtained by Dr. Vernon Bell (NASA-Langley Research Center) and checked for purity by high pressure liquid chromatography (HPLC) and differential thermal analysis (DTA) procedures. In order to assure an extended shelf-life, the samples were stored in a refrigerator at 1-9°C, with a nitrogen head space over the materials and shielded from light.

Mutagenicity Assay

The microbial assay procedure was in general patterned after Ames et al. [1975] using histidine-dependent Salmonella typhimurium tester strains obtained from Dr. Ames' laboratory at the University of California, Berkeley. In this plate incorporation assay, strains were used to detect both frameshift and base pair substitution mutations. Tests were performed with and without* metabolic activation using Aroclor-induced rat liver S-9 fractions purchased from AMC Cancer Research Center, Lakewood, Colorado, and Litton Bionetics, Kensington, Maryland.

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*Initial tests indicated that the aromatic amines tested required S-9 activation; therefore, follow-on tests were performed with activation.

Positive and negative controls were assayed with each compound tested. The numbers of spontaneous revertants per plate of the controls were compared with the laboratory's historical averages to determine if they were within a statistically acceptable range.

The tester strains were Salmonella typhimurium histidine-dependent mutants, TA98, TA100, TA1535, TA1537, and TA1538.* These strains were verified for genetic consistency on a routine basis by phenotypic determinations of histidine and biotin auxotrophy, crystal violet sensitivity, ultraviolet light sensitivity, ampicillin sensitivity, and spontaneous reversion frequency as recommended by Ames et al. [1975]. Toxicity testing was performed to determine if test sample concentration ranges of 10, 3, 1, 0.2, 0.04, 0.01 mg/plate** could be used. Triplicate culture plates were used at all test sample concentrations and revertant colonies counted using an Artek Model 880 automatic colony counter. The sample solvent used in this study was dimethylsulfoxide (DMSO).

---

*In early studies, four and in some cases five tester strains were used. Later protocols used only TA98 and TA100; i.e., frameshift and base pair substitution mutation indicators.

**Early studies used a more narrow range; i.e., 0.01-2 mg/plate.

Evaluation Criteria

Although all data were processed using a computerized statistical treatment developed within Monsanto, we have elected to present the data graphically plotting revertants versus concentration (Figures 1-15) and then to summarize the data in Tables III-VII by the criteria of Brusick [1980]. The Ames data were evaluated as having either a positive effect or no mutagenicity detected (ND). The level of activity was defined by the lowest concentration of the test sample at which mutagenicity could be detected. In this summary of data a chemical was considered to have a positive effect if there was a mutagenic response over three concentrations and if the highest increase was, at the minimum, equal to twice the solvent control for either TA98 and TA100.

Original testing of methylenedianiline (MDA) indicated that all steric isomers required S-9 activation for positive response of the Ames strains. This held true with all other dianiline compounds tested except for 4,4'-dimethyl-3,3'-methylenedianiline, which caused a mutagenic response in TA1535 without S-9 microsomal activation factors added to the test systems. For this reason, only data from experiments where S-9 activation were used are presented. These initial experiments also indicated that the most responsive tester strains to these compounds were TA98 and TA100, representing tester strains which detect base pair (TA100) and frameshift (TA98) types of mutations. However, one MDA isomer

(m,m') illustrates an anomalous case in which TA1537 and TA1538 responded very strongly to this compound whereas TA100 and TA98 gave only marginal response.

RESULTS

Relative Mutagenicity of Steric Isomers

Table III summarizes the relative microbial mutagenicities among steric isomers of a series of five dianiline compounds differing only in ring-coupling moieties. This includes DABiP with no connecting moiety. Figures 1-9 are graphs of the mean of three replicate plates versus the dose per plate of test compounds using TA98 and TA100 tester strains. All six steric isomers were available for two of the dianiline series, MDA and DABP. Table III provides information concerning the mutagenicity testing data of each material. This table includes results from tests with the two tester strains TA98 and TA100. The relative mutagenic response was determined by the minimum effective dose (MED) which is defined as the lowest dose tested that results in revertants per plate that are twice the value (minimum) of the revertants per plate of the corresponding solvent controls. If the MED was less than 0.05 mg/plate, the mutagenic response was reported as "high." The mutagenic response was reported as "moderate" if the MED was between 0.05 and 0.5 mg per plate and "low" if the MED was greater than 0.5 mg per plate. The mutagenic response was reported as "not detected" if the number of revertants did not exceed two times the mean of the solvent controls at or below the highest dose tested.

The relative mutagenicity of the test materials can be observed from these data. The o,m', o,p', and m,m' isomers of both MDA and DABP demonstrated mutagenicity but the o,o' and m,p' isomers of MDA and DABP exhibited no mutagenic response. The p,p' isomer of MDA resulted in moderate mutagenicity, and no mutagenicity was found for the p,p' isomer of DABP. These data indicate that the steric positions of the amine groups in both the MDA and DABP series affected the mutagenicity of the isomers.

Effects of Coupling Moieties

Some similarities in relative mutagenic responses can be noted among isomers of different compounds (Table III). The o,m', o,p' and m,m' isomers all demonstrated mutagenicity in one or both strains for all compounds tested. The o,o' and m,p' isomers demonstrated no mutagenic response for all compounds, with the one exception of a moderate response to m,p' ODA in strain TA98. The test results with the p,p' isomer varied from no detectable mutagenic response to high mutagenicity depending the coupling moiety.

The intensity of mutagenicity changed relative to the coupling moieties. For example, ODA with the ether coupling moiety (-O-) had the greatest overall mutagenicity among the aromatic diamines tested. Every isomer of this compound tested demonstrated mutagenicity in one or both strains. This was the only compound

whose m,p' isomer demonstrated a mutagenic response. The DDS compound with the sulfone coupling moiety ($-SO_2$) demonstrated the least overall mutagenicity among the compounds tested.

3,5-DABP was also tested and found to have no detectable mutagenicity by the criteria stated earlier.

Effects of Proximity of Anilino Groups

The effects of linear separation of the $-NH_2$ groups in meta positions were evaluated (Table IV and Figure 10). Analogs of two-, three- and four-ring compounds of m,m'-MDA were synthesized and tested for relative microbial mutagenicity. The m,m'-MDA isomer indicated a moderate mutagenicity among the tester strains. The m_1,m_2-terbenzyl and m,m_4-quaterbenzyl benzylogs gave no detectable response in all strains. The linear separation of the anilino groups appears to "quench" the mutagenic effect of the amine groups in these compounds.

Effects of Methyl and Other Moieties on Microbial Mutagenicity

Five methylated MDA and one methylated DABP isomer were tested. The results are shown in Table V and Figures 11 and 12. In all cases but one, 2,2'-dimethyl-5,5'-MDA, the mutagenicity of the methylated isomers increased over the corresponding nonmethylated isomers. The exceptional isomer gave approximately the same

response as the nonmethylated m,m'-MDA isomer. The two methylated analogs of the p,p'-MDA isomer produced several fold more revertants than the nonmethylated isomers when tested with TA98 and TA100 strains.

In a previous study, it was found that the insertion of two isopropyl groups placed adjacent to the amino groups in both anilino moieties rendered the p,p'-MDA isomer nonmutagenic. In contrast, the insertion of chlorine atoms in each ring, as in dichlorodianiline (MOCA), greatly enhanced the mutagenicity of this same steric isomer of MDA (Gridley and Ross, unpublished).

Effects of Sample Aging and Storage on Mutagenicity of m,p'-MDA

A sample of m,p'-MDA was tested for mutagenicity in 1977 and was found to be nonmutagenic with TA98 (see Table VI and Figures 13 and 14). This same sample, retested in 1979, was found to have moderate mutagenicity. No special care had been taken to protect this sample from air or light and a discoloration was noted prior to the retest. Another sample, Fresh-1979, of this isomer was submitted for testing in 1979 and produced very low numbers of revertants. Another sample, designated Fresh-81-7-2-473, was tested and found to have high mutagenicity. This sample was analyzed by high pressure liquid chromatography and found to contain impurities. Further purification provided a sample, F2, with much reduced impurity peaks and concomitant reduction in mutagenicity.

Because of this background information, a deliberate aging study was performed with the m,p' MDA isomer. A purified sample was divided into three aliquots. Sample A was aged two months in nitrogen at ambient temperatures. Sample B was aged for two months in air at ambient temperatures. Sample C was aged in air at 50°C. These samples were then tested for mutagenicity with TA98 and TA100 tester strains. The resulting data are shown in Table VII and in Figure 15. Low to no detectable mutagenicity was found and no differences between samples were noted. The aging times was apparently too short for sample degradation to be expressed.

CONCLUSIONS

- The relative location of amine groups within the same dianiline compound affects mutagenicity, in some cases considerably.

- The chemical nature of the connecting moieties between anilino rings lead to considerable differences between the mutagenicity levels of the various compounds in this study.

- Mutagenic intensity was found to be inversely proportional to the distance between amino groups in benzylogs of m,m'-MDA.

- Methyl groups intensified the mutagenicity of five of the six isomers tested, with respect to the base nonmethylated dianilino compound.

- Sample storage methods may affect the mutagenicity of MDA compounds.

- The mutagenicity of m,p' MDA increased when the sample was stored for long periods of time; i.e., 2 years. However, changes were insignificant over shorter periods of times; i.e., 2 months.

ACKNOWLEDGMENT

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TABLE I. AROMATIC AMINES AND CORRESPONDING STRUCTURES TESTED BY AMES MUTAGENICITY ASSAY

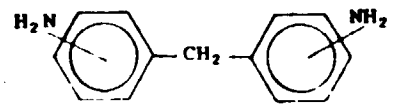
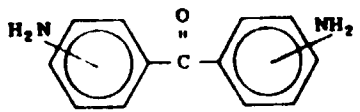
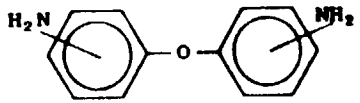
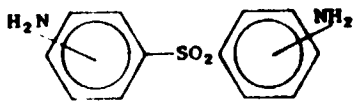
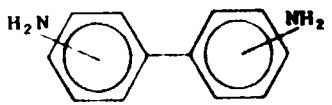
Compound	Acronym	Structure	Isomers tested
Methylenedianiline	MDA		o,o' o,m' o,p' m,p' m,m' p,p'
Diaminobenzophenone	DABP		o,o' o,m' o,p' m,p' m,m' p,p' 3,5
Oxydianiline	ODA		o,p' m,p' m,m' p,p'
Diaminodiphenylsulfone	DDS		o,m' m,p' m,m' p,p'
Diaminobiphenyl	DABiP		o,p'

TABLE I (continued)

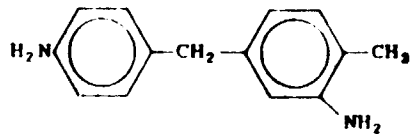
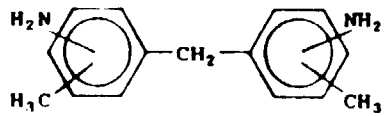
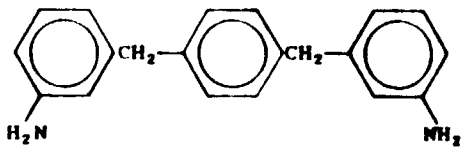
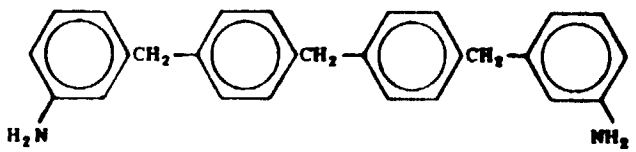
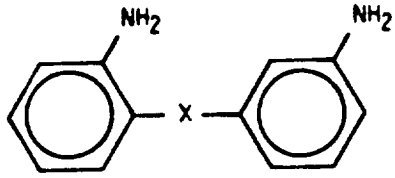
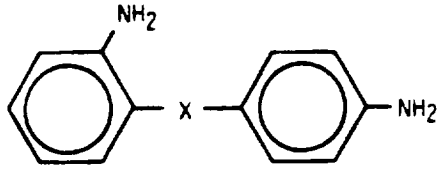
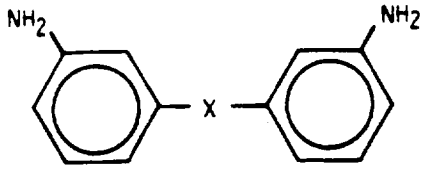
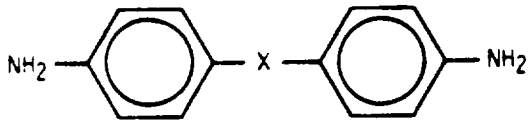
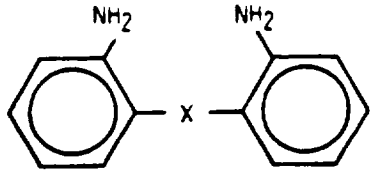
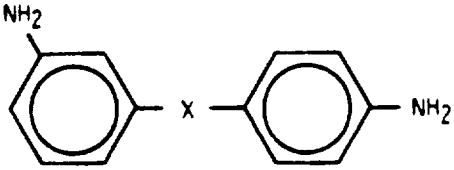
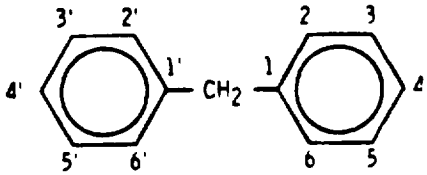
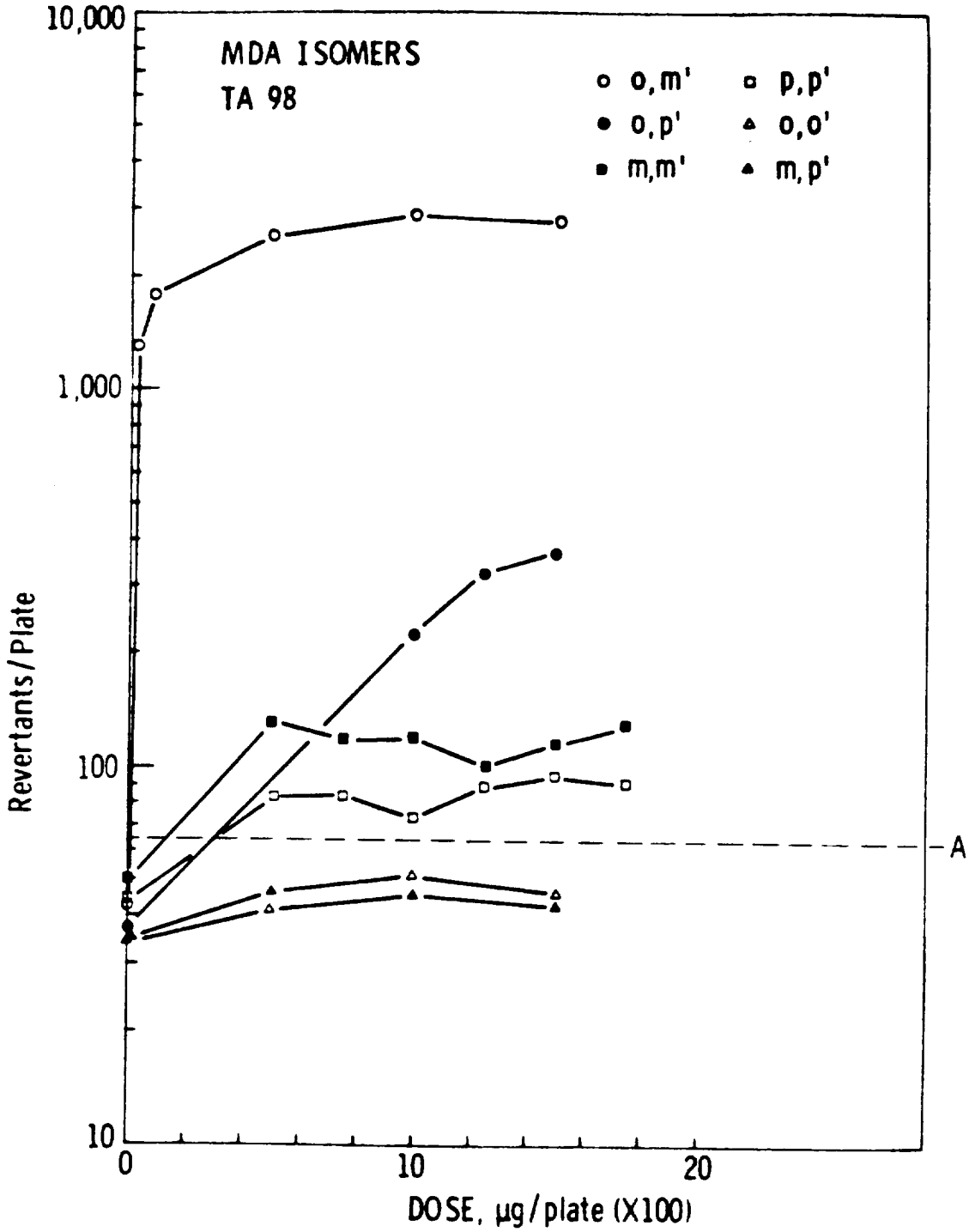
4-Methyl-3,4'-methylenedianiline	4-Me-3,4'-MDA		-
Dimethyl-methylenedianiline	DM-MDA		2,2'-DM-4,4'-MDA 3,3'-DM-4,4'-MDA 2,2'-DM-5,5'-MDA 4,4'-DM-3,3'-MDA
m_1,m_3-Terbenzyl-p_2	m_1,m_3-TBz DATBz		-
m_1,m_4-Quaterbenzyl-p_2,p_3	m_1,m_4-QBz DAQBz		-

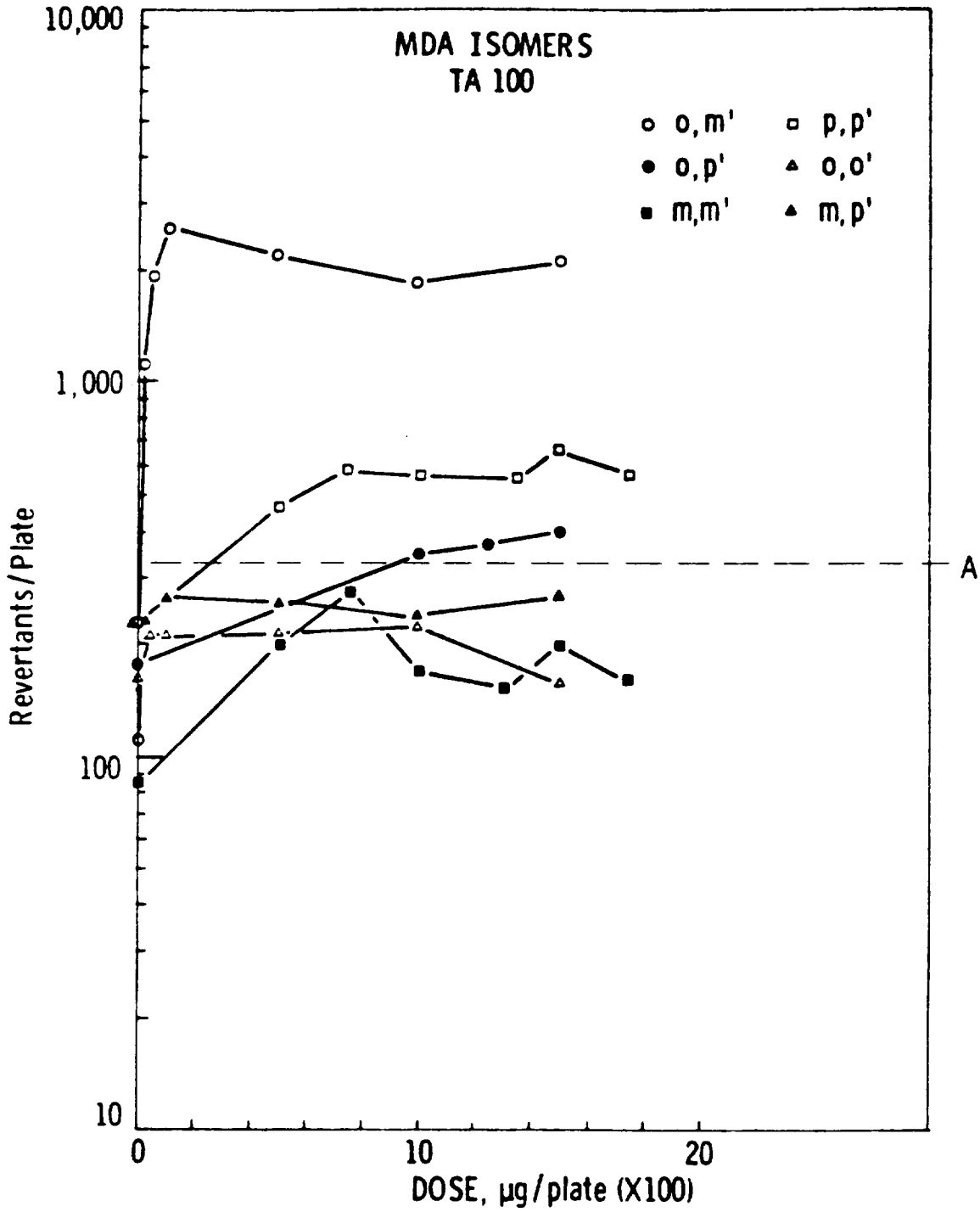
TABLE II. STRUCTURES OF STERIC ISOMERS OF AROMATIC DIAMINES

Isomer	Structure
o,m'	
o,p'	
m,m'	
p,p'	
o,o'	
m,p'	
	



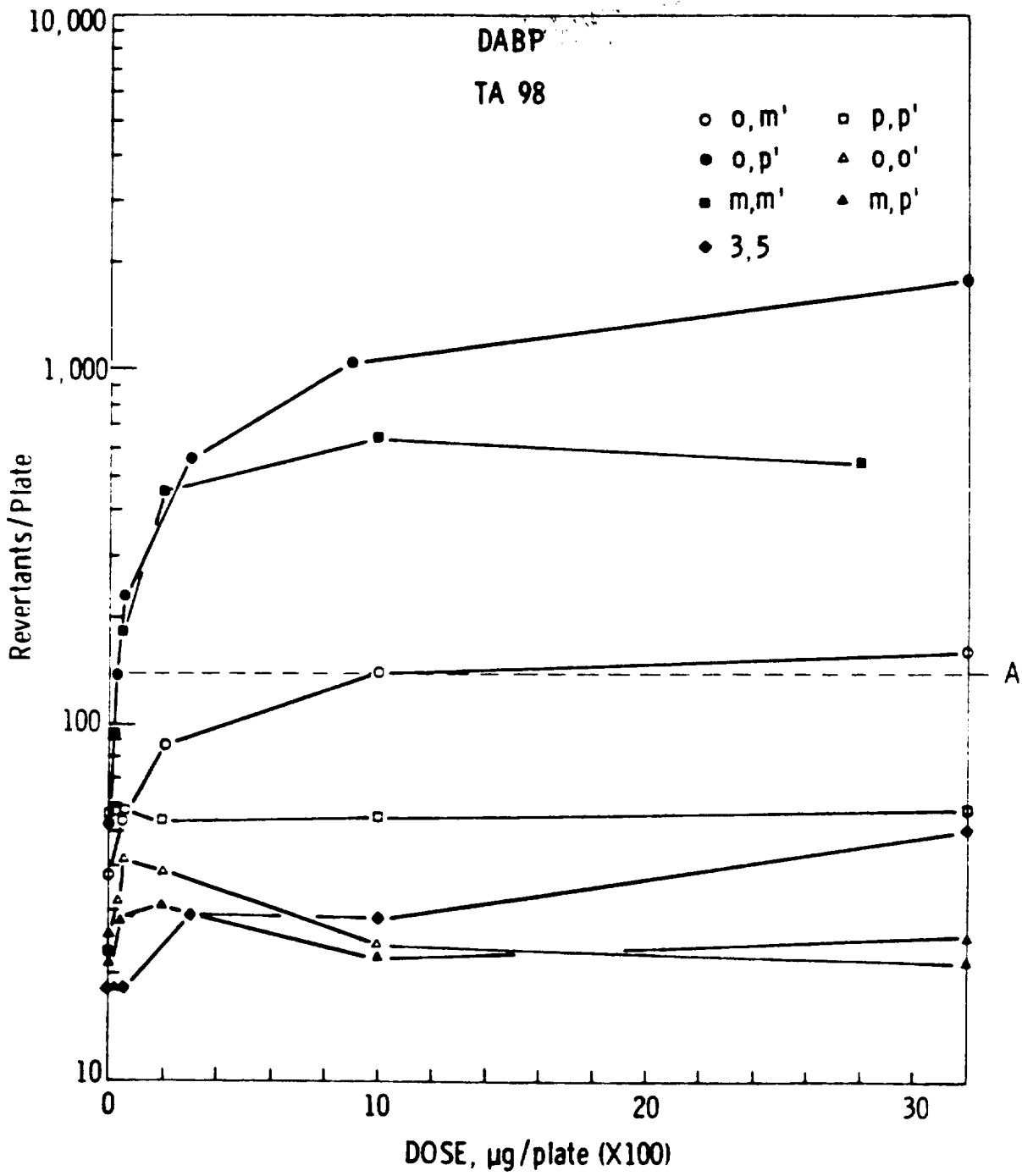
A = $2\bar{x}$ TA98 revertants by solvent controls

Figure 1. Reversion of Salmonella typhimurium strain TA98 by MDA steric isomers.



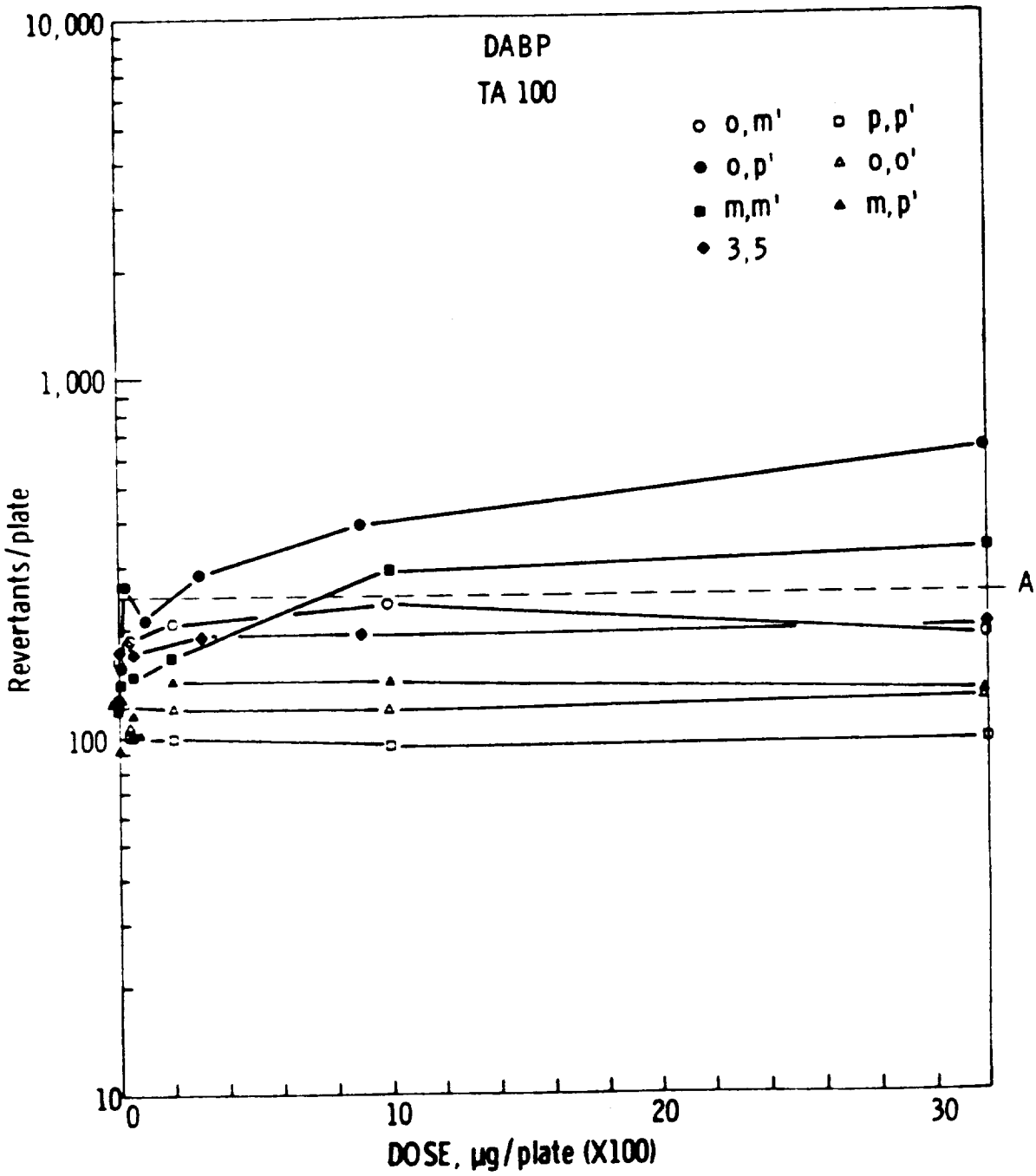
A - $2\bar{x}$ TA100 revertants by solvent controls

Figure 2. Reversion of Salmonella typhimurium strain TA100 by MDA steric isomers.



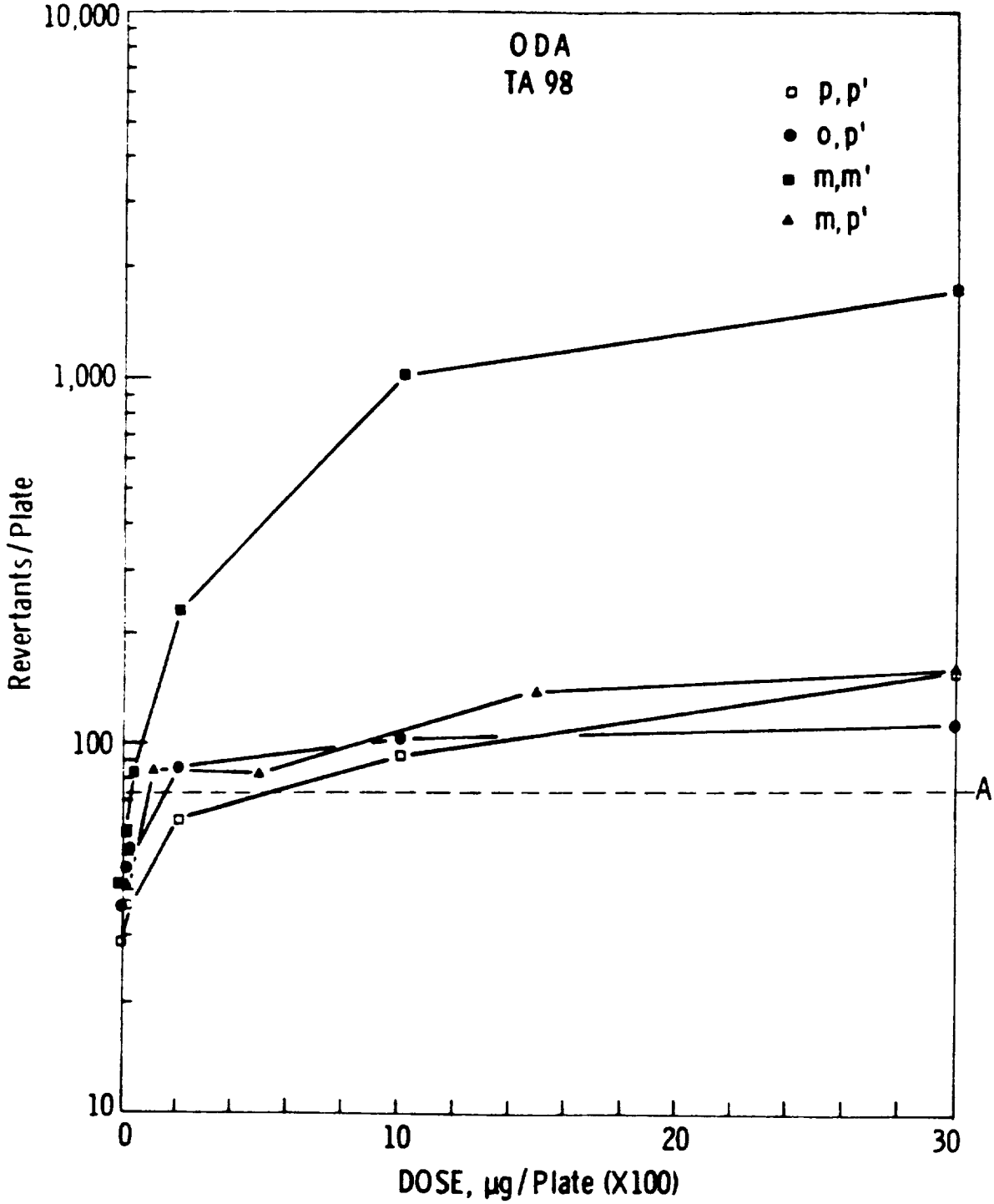
A - $2\bar{x}$ TA98 revertants by solvent controls

Figure 3. Reversion of Salmonella typhimurium TA98 by DABP steric isomers.



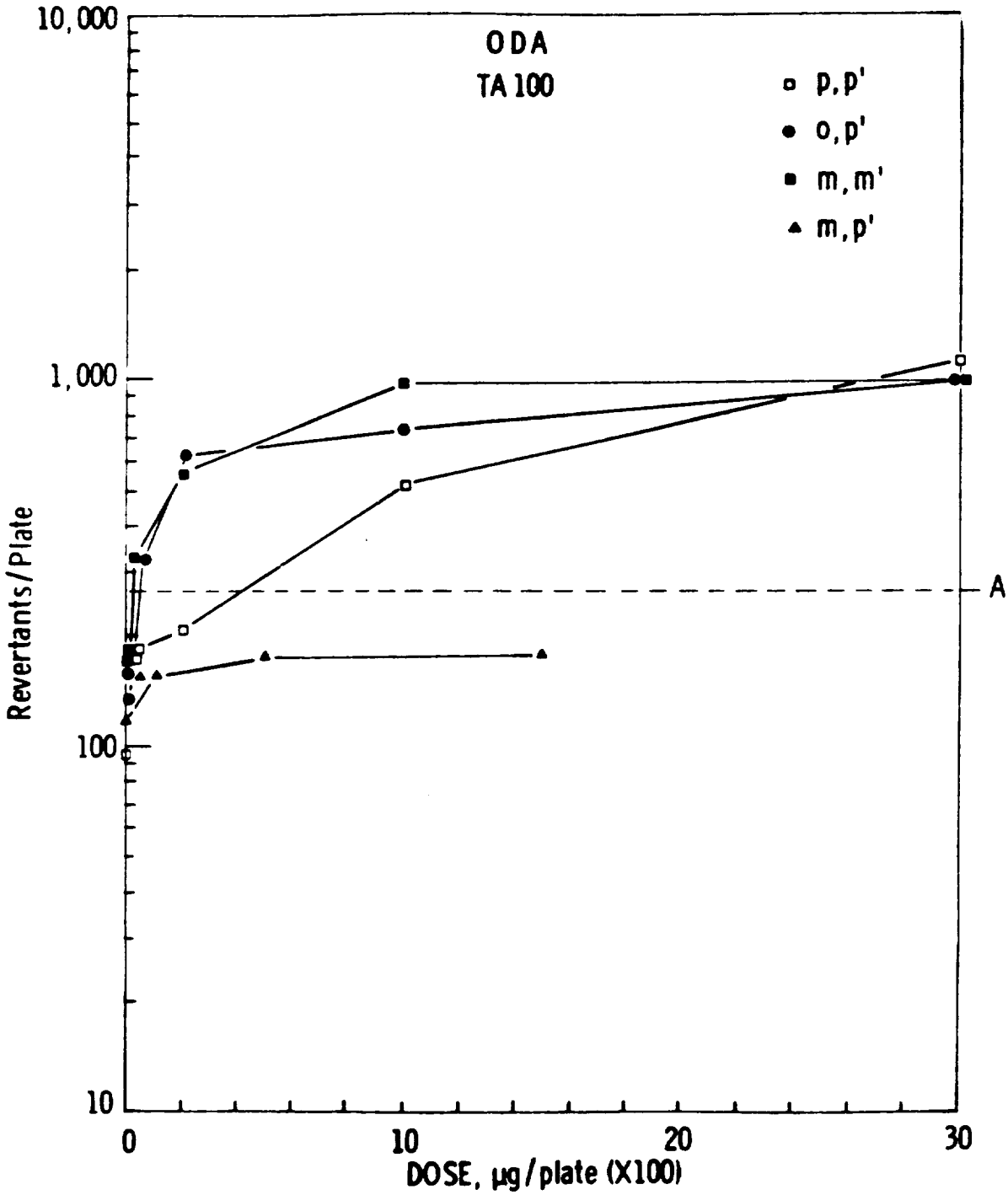
A - $2\bar{x}$ TA100 revertants by solvent controls

Figure 4. Reversion of Salmonella typhimurium TA100 by DABP steric isomers.



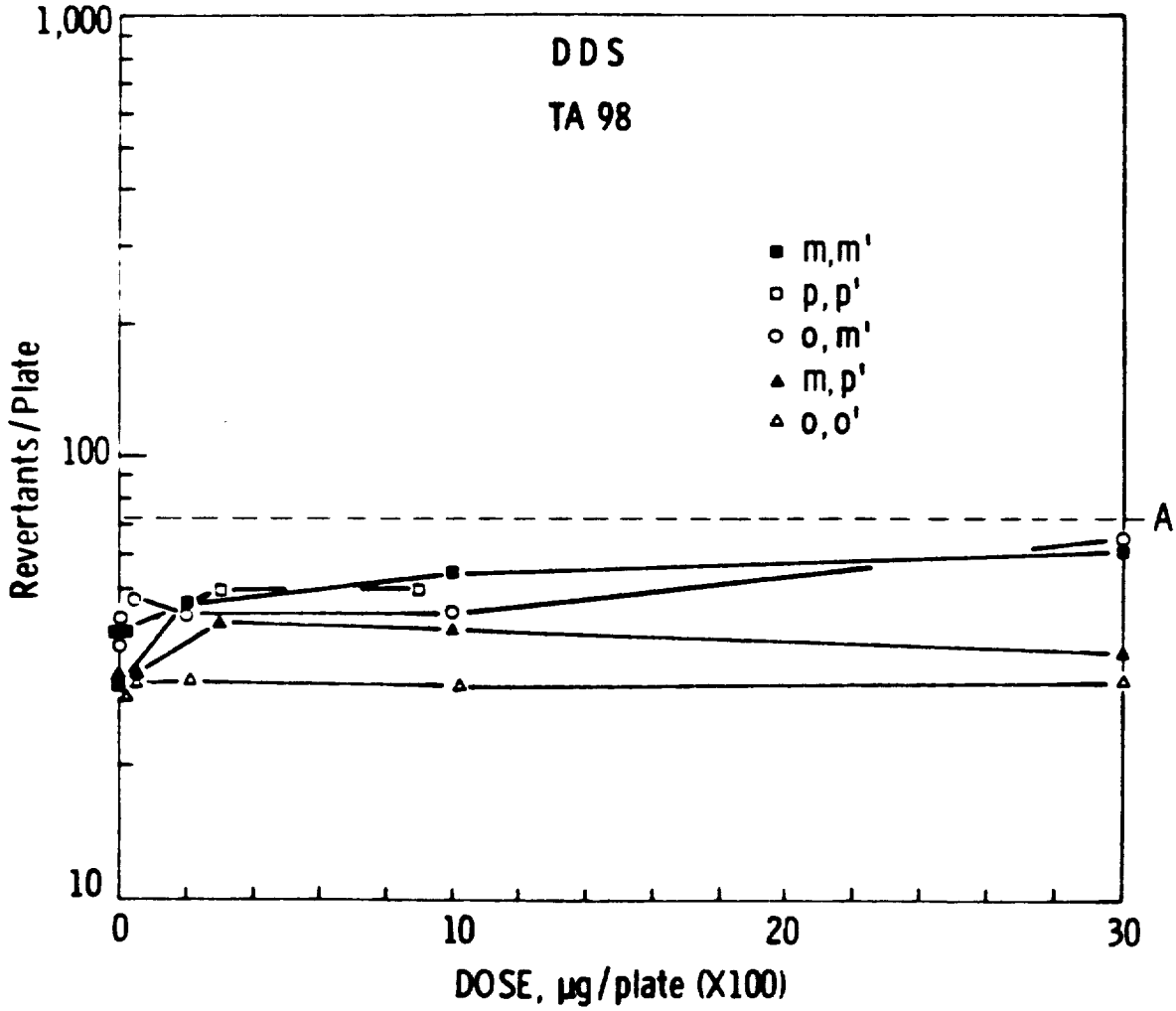
A - $2\bar{x}$ TA98 revertants by solvent controls

Figure 5. Reversion of Salmonella typhimurium TA98 by ODA steric isomers.



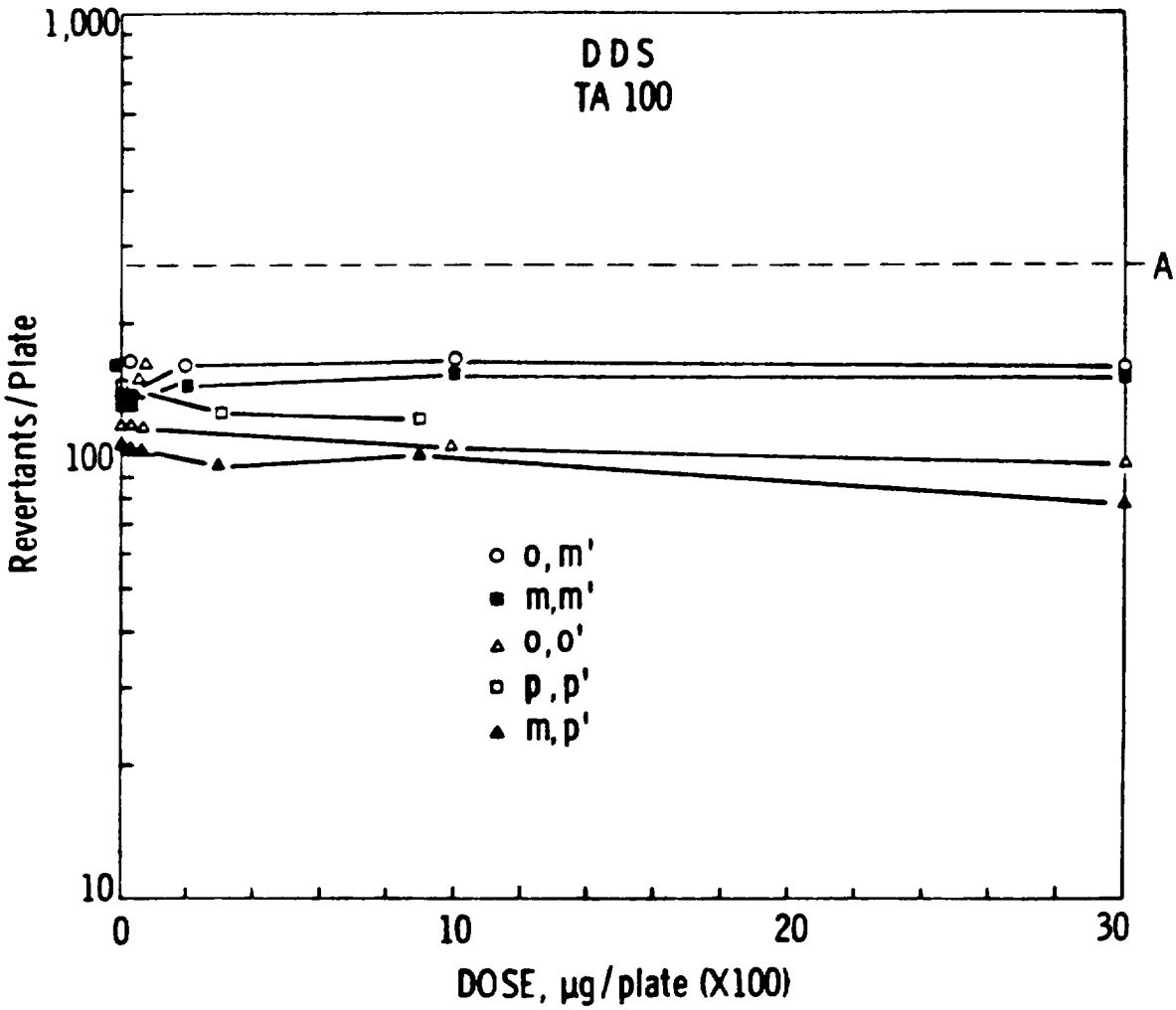
A - $2\bar{x}$ TA100 revertants by solvent controls

Figure 6. Reversion of Salmonella typhimurium TA100 by ODA steric isomers.



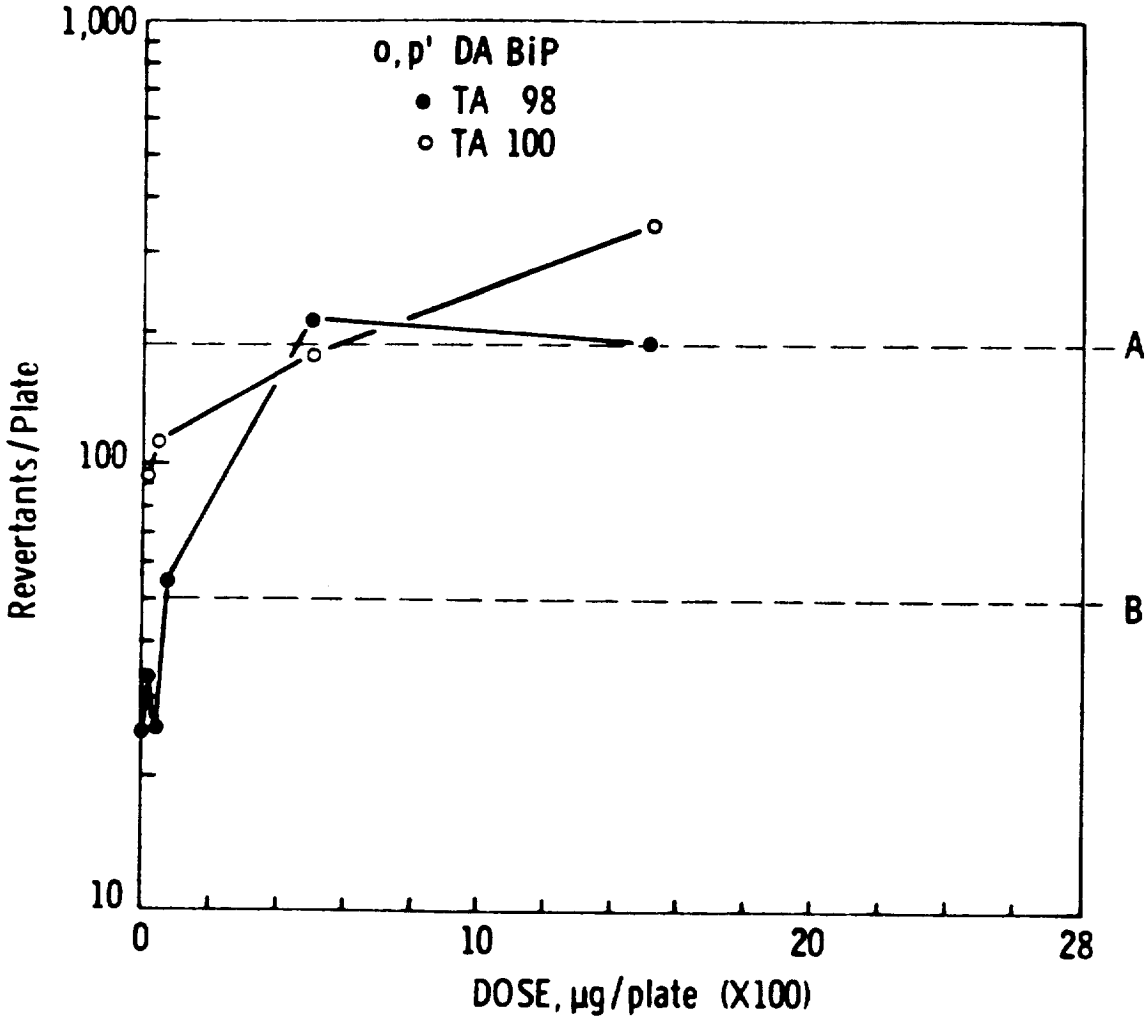
A - $2\bar{x}$ T98 revertants by solvent controls

Figure 7. Reversion of Salmonella typhimurium TA98 by DDS steric isomers.



A - $2\bar{\chi}$ TA100 revertants by solvent controls

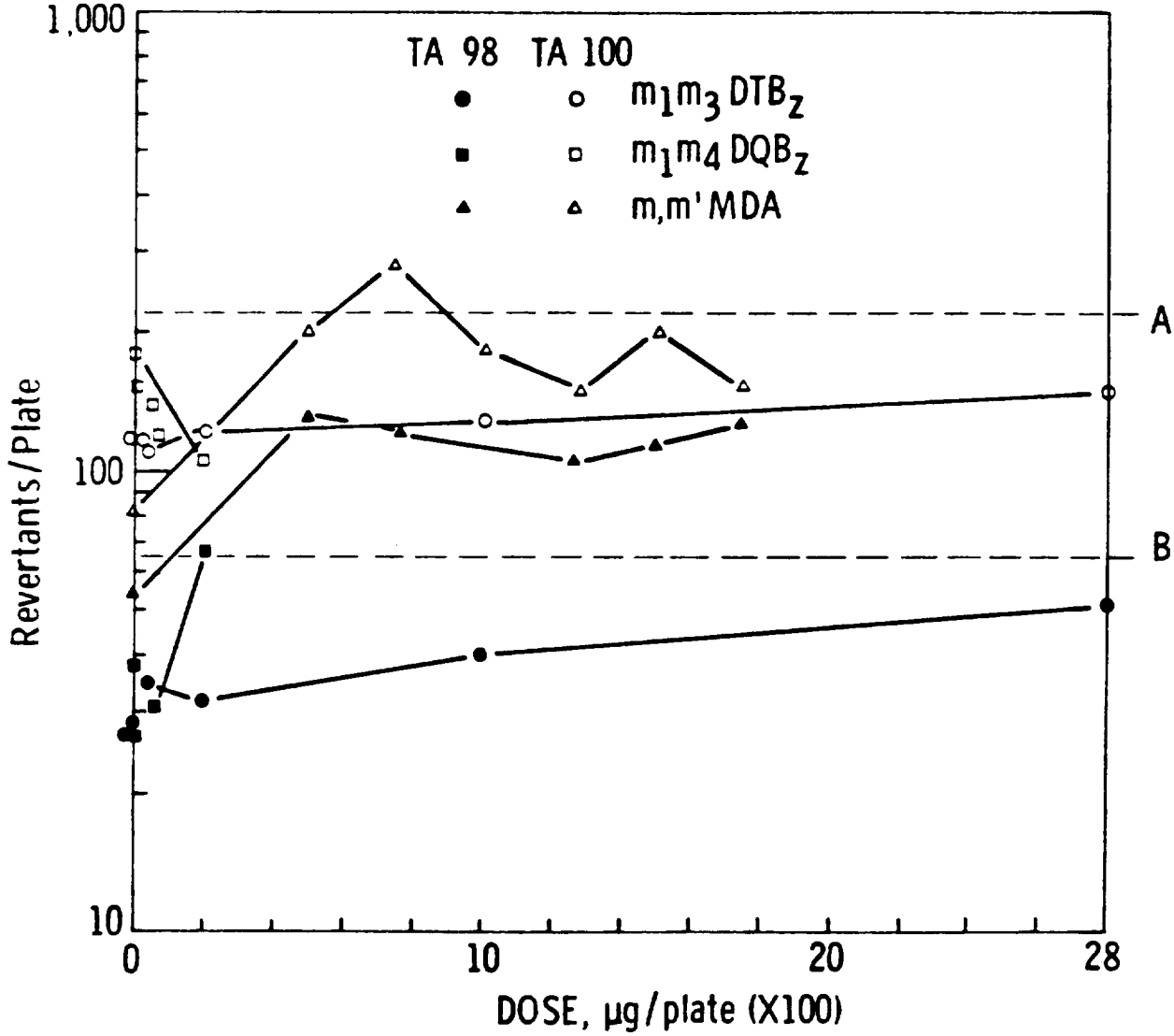
Figure 8. Reversion of Salmonella typhimurium TA100 by DDS steric isomers.



A - $2\bar{x}$ TA100 revertants by solvent controls

B - $2\bar{x}$ TA98 revertants by solvent controls

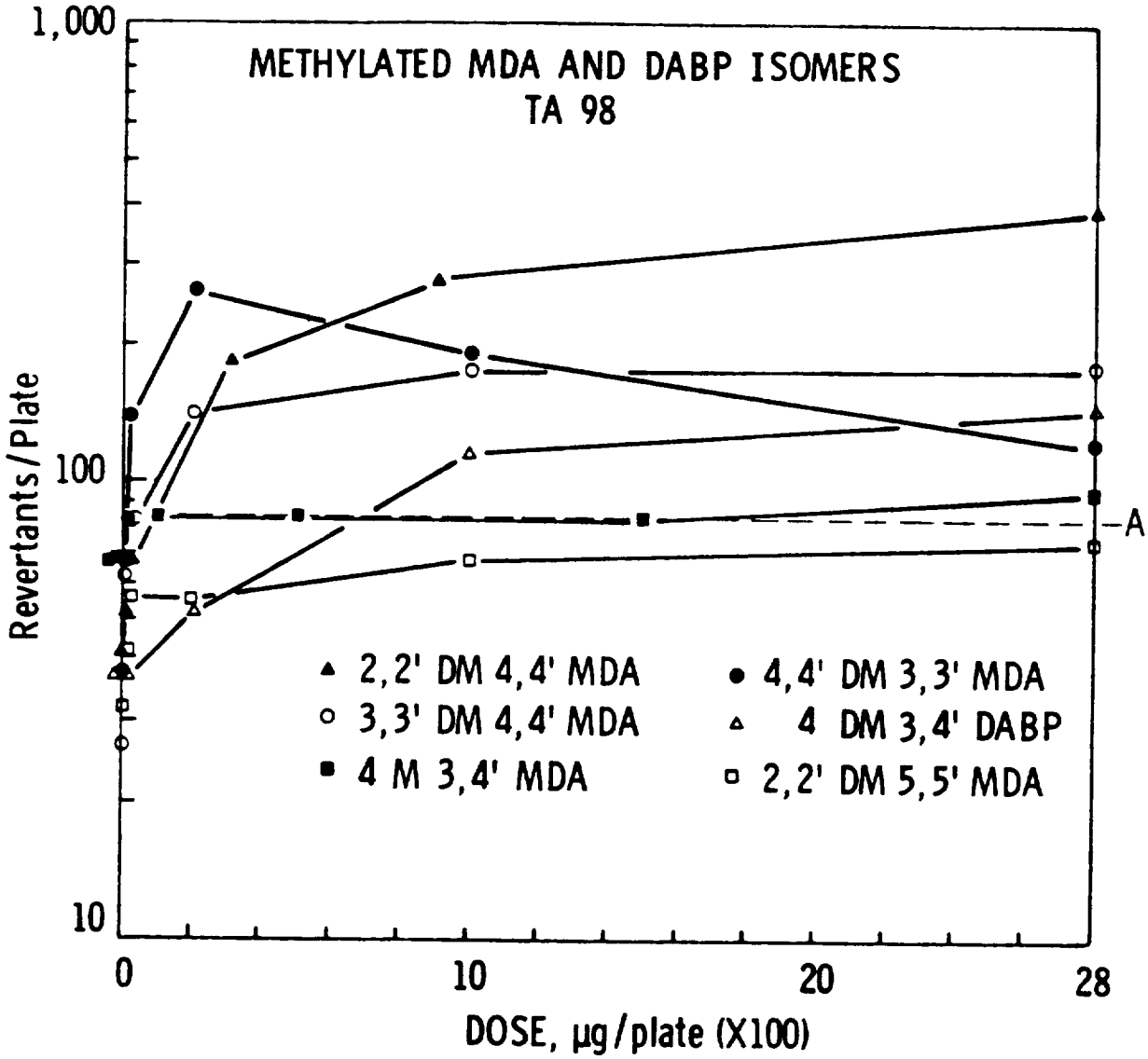
Figure 9. Reversion of Salmonella typhimurium TA 98 and TA100 by DABiP o,p' isomer.



A - $2\bar{\chi}$ TA100 revertants in solvent control

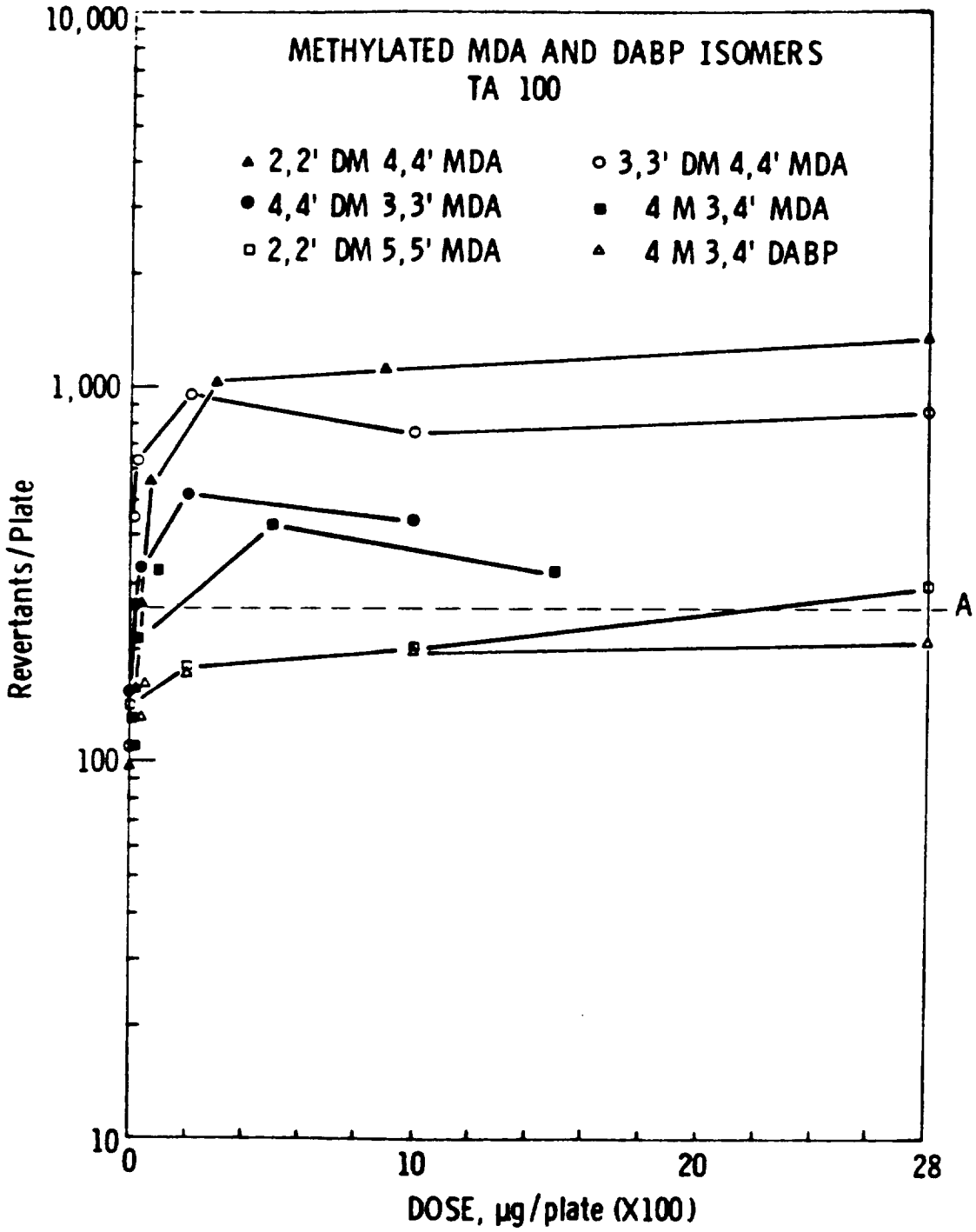
B - $2\bar{\chi}$ TA 98 revertants

Figure 10. Reversion of Salmonella typhimurium TA98 and TA100 by MDA benzologs.



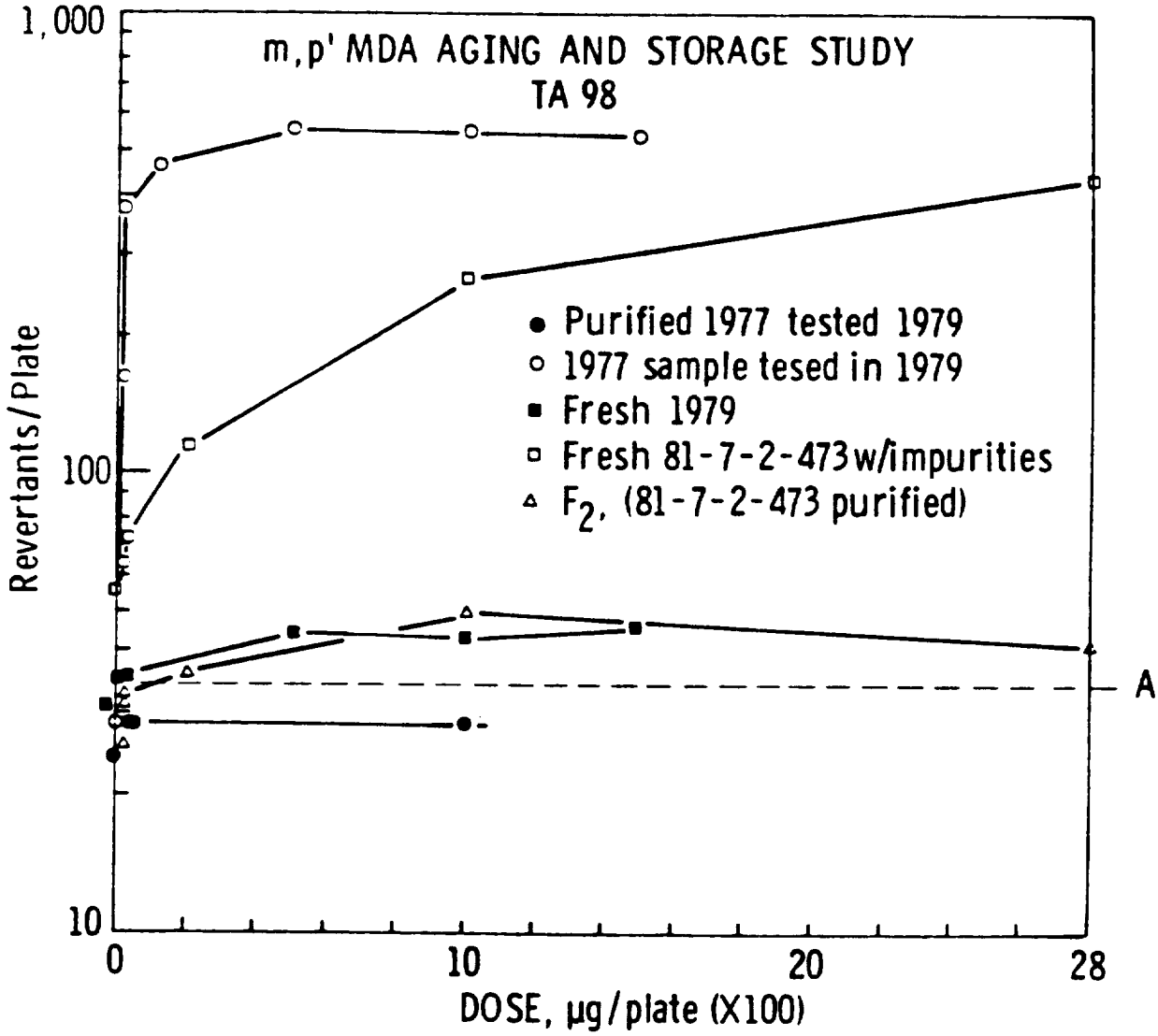
A - $2\bar{x}$ TA98 revertants by solvent controls

Figure 11. Reversion of Salmonella typhimurium TA98 by methylated MDA and DABP isomers.



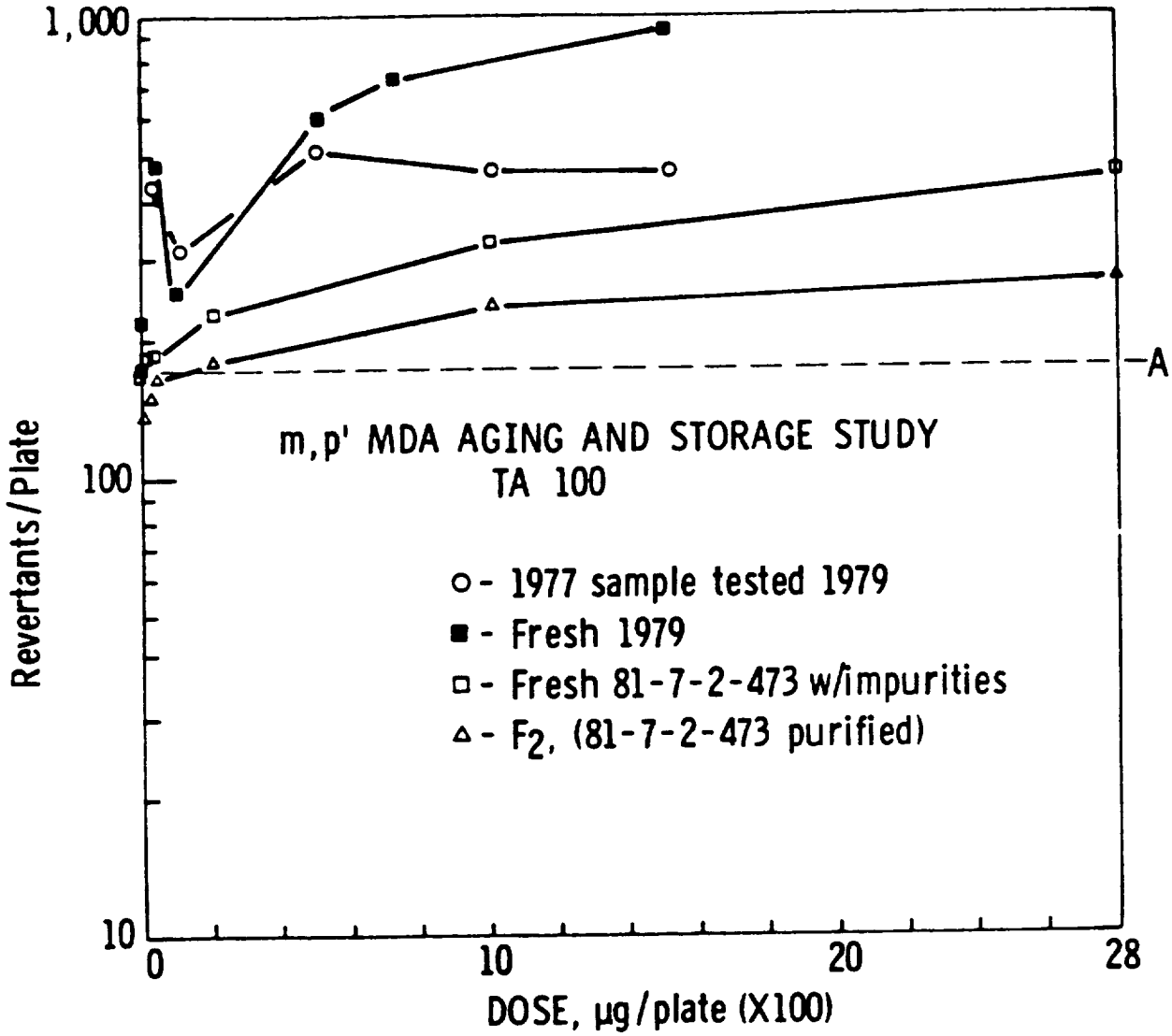
A - $2\bar{x}$TA100 revertants by solvent controls

Figure 12. Reversion of Salmonella typhimurium TA100 by methylated MDA and DABP isomers.



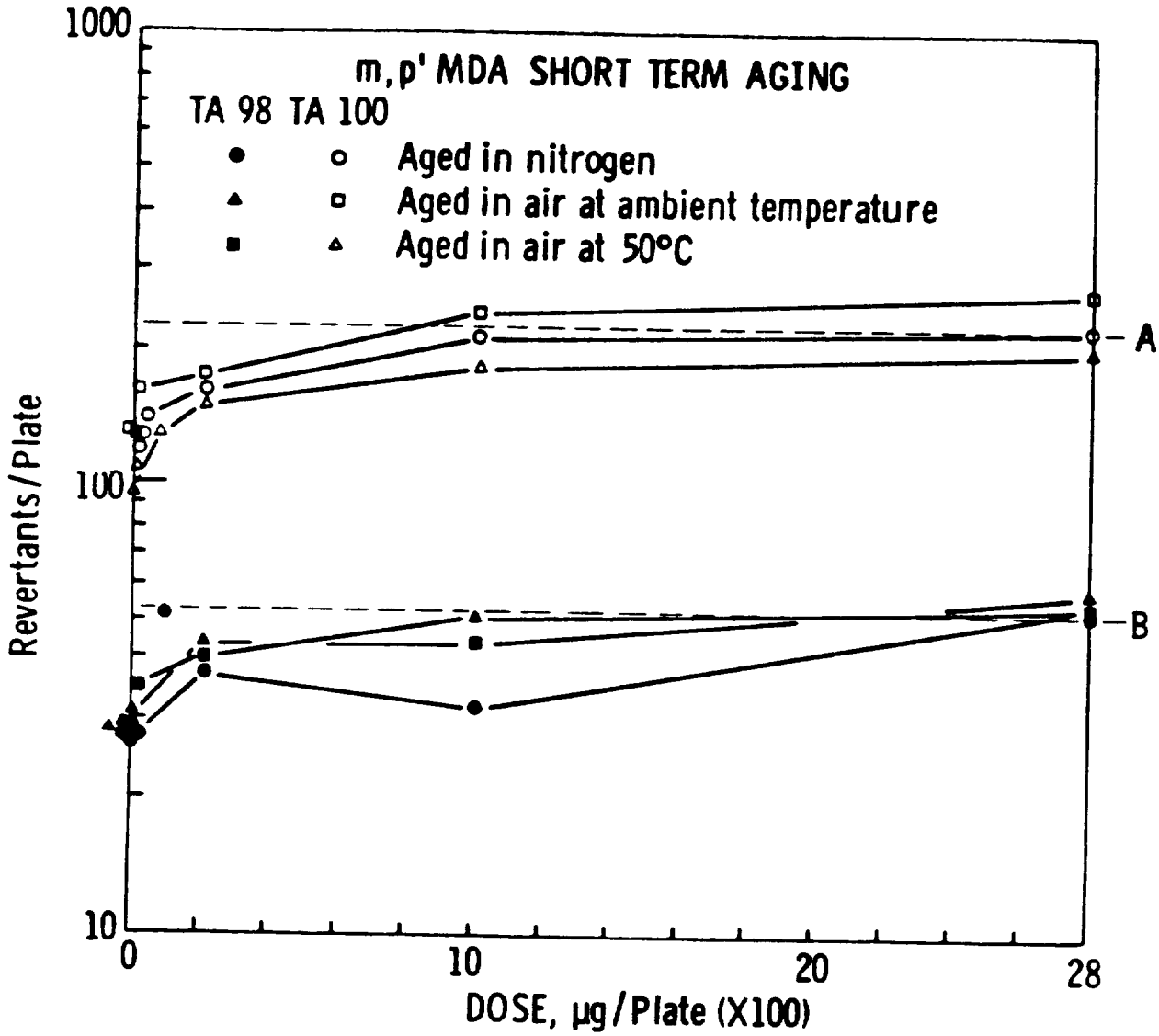
A - $2\bar{x}$ TA98 revertants by solvent control

Figure 13. Reversion of Salmonella typhimurium TA98 by various samples of m,p' MDA illustrating affects of aging and storage.



A - $2\bar{x}$ TA100 revertants by solvent controls

Figure 14. Reversion of Salmonella typhimurium TA100 by various samples of m,p' MDA illustrating effects of aging and storage.



A - $2\bar{x}$ TA100 revertants in solvent controls

B - $2\bar{x}$ TA98 revertants in solvent controls

Figure 15. Reversion of Salmonella typhimurium TA98 and TA100 by three samples to study effects of short-term aging.

TABLE III. Summary of Ames Mutagenicity Data for Dianiline Compounds and Steric Isomers

Compound	o,m'		o,p'		m,m'		p,p'	
Strain	TA98	TA100	TA98	TA100	TA98	TA100	TA98	TA100
MDA	High	High	Low[a]	Low	Moderate	Moderate	Moderate	Moderate
DABP	High	ND 10 mg[b]	High	High	High	Low	ND 10 mg	ND 10 mg
ODA	NT[c]	NT	Moderate	Moderate	Moderate	Low	Moderate	High
DDS	Low	ND 10 mg	NT	NT	Very low	ND 10 mg	ND 0.9 mg	ND 0.9 mg
DABiP	NT	NT	Moderate	Low	NT	NT	NT	NT

	o,o'		m,p'		3,5	
	TA98	TA100	TA98	TA100	TA98	TA100
MDA	ND 1.5 mg	ND 1.5 mg	ND 1.5 mg	ND 1.5 mg	NT	NT
DABP	ND 10 mg	ND 10 mg	ND 10 mg	ND 10 mg	ND 3 mg	ND 3 mg
ODA	NT	NT	Moderate	ND 5 mg	NT	NT
DDS	ND 10 mg	ND 10 mg	ND 3 mg	ND 3 mg	NT	NT
DABiP	NT	NT	NT	NT	NT	NT

[a] Did not test below 1.0 mg, revertants were 3 x background (x2).

[b] ≤1.5 mg, mutagenicity not detected at 1.5 mg/plate or less.

[c] Not tested.

TABLE IV. Summary of Ames Mutagenicity Data for MDA Benzylogs

Isomer	Strain	
	TA98	TA100
m_1, m_3-Terbenzyl-p_2	ND 10 mg[a]	ND 10 mg
m_1, m_4-Terbenzyl-p_2, p_3	ND 0.2 mg	ND 0.2 mg
m,m' MDA	Moderate[b]	Moderate[b]

[a]10 mg - mutagenicity not detected at 10 mg or less.
[b]Did not test below 0.5 mg.

TABLE V. Summary of Ames Mutagenicity Data for Methylated Compounds

Isomer	Strain	
	TA98	TA100
4,4'-DM-3,3'-MDA	High	High
3,3'-DM-4,4'-MDA	High	High
4-M-3,4'-MDA	ND ≦5 mg[a]	Moderate
2,2'-DM-5,5'-MDA	Low	Low
2,2'-DM-4,4'-MDA	Moderate	Moderate
4-M-3,4'-DABP	Low	Low

[a]≦5 mg - mutagenicity not detected at 5 mg/plate.

TABLE VI. Summary of Ames Mutagenicity Data for Assessing Effects of Aging and Storage on m,p' MDA - Long Term Study

Sample	Strain	
	TA98	TA100
A - Purified 1977, tested 1977	ND[a] 1.0 mg	NT[b]
B - 1979 Sample tested 1979	Moderate	Moderate
C - Fresh sample (1979)	ND 1.5 mg	ND 1.5 mg
D - Fresh, 81-7-2-473 with impurities	Moderate	Moderate
E - F_2,81-7-2-473 purified	ND 10 mg	Low

[a]Not detected.

[b]Not tested.

TABLE VII. Summary of Ames Mutagenicity Data for Assessing Effects of Aging and Storage on m,p' MDA - Short Term Study

Sample	Strain	
	TA98	TA100
A - m,p' AI-aged in N_2	Low	Low
B - m,p' A,A,RT-aged in air at room temperature	ND[a] 10 mg	Low
C - m,p' A,A,50-aged in air at 50°C	Low	ND 10 mg

[a]Not detected.

APPENDIX B

REPORT - MUTAGENICITY OF DIANILINE COMPOUNDS

MUTAGENICITY OF DIANILINE COMPOUNDS

Submitted to Monsanto Research Corporation by:

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I. Introduction

This report is a discussion of the results of Ross, et al. (12) presented in the report entitled "In vitro mutagenic properties of steric isomers of aromatic diamine compounds", hereafter referred to as the Ross report. The aim of my work was to determine if common structural features of aromatic diamine isomers could be identified in mutagenic compounds. The work consisted of a review of the Ross, et al. data, a literature review and the writing of this report.

The aromatic amines constitute one of the largest families of chemical structures that are known to be carcinogens. A significant effort has been made to study the mutagenicity and potential carcinogenicity of these compounds because of the known carcinogenicity of some familiar aromatic amines and because of their wide use in commercial applications. Some representative carcinogenic compounds that have been studied in detail are 2-naphthylamine, 4-aminobiphenyl and 2-acetylaminofluorene. The scientific literature is extensive. Since a complete review is beyond the scope of this report, the reader is referred to some excellent reviews of the carcinogenicity of aromatic amines (1,3,5,13,16). In this class of compounds, the dianilines have received little attention. One of the dianiline compounds used in the Ross study has been screened in some limited animal experiments for carcinogenicity. The 4,4'-methylene dianiline (o,o' MDA) isomer is toxic to rats and therefore cannot be fed at the high levels required for testing. Carcinogenicity results are consequently ambiguous. MDA may not be a carcinogen(3). Two analogs,

4,4'-methylene-bis(2-chloroaniline) and 4,4'-methylene-bis(2-methylaniline), are carcinogenic when the high levels are used (3). The best studied carcinogenic dianiline is benzidine (diaminobiphenyl), which, although known to be a carcinogen for several years, has just begun to receive detailed attention (1,10,11,18; for an excellent review of benzidine studies to 1975, see the review by T. J. Haley, ref. 4). The similarity in structure of benzidine to the dianilines studied here permits these studies to serve as models for future research into the carcinogenicity of the aromatic amines studied in the Ross report.

II. Analysis of Results

A. Previous studies using other aromatic amines that serve as model studies.

While some generalities determined in previous studies of aromatic amines are applicable to the present one, the studies most pertinent are those with benzidine. All aromatic amines, including benzidine, require metabolic activation to be mutagens. It is therefore not surprising that all dianilines in the Ross report that are mutagenic require incubation in an activation system to acquire this activity. This aspect will be discussed further in a later section. While benzidine is the most thoroughly studied related compound, studies with it are limited. In my literature review I found no studies with isomers of benzidine comparable in extent to the Ross study. Some studies address the question of mutagenic activity when ring substitutions are made. For example, 2-methylbenzidine is a more potent carcinogen than benzidine (1). In contrast, benzidine is a carcinogen and mutagen (8) while 3,3',5,5'-tetramethyl benzidine is inactive as a carcinogen and mutagen (1). Further comparisons of ring substitutions are reported in the review by Ashby (1) and the review by

Selkirk (13). A significant conclusion of results with benzidine and its derivatives is that no generalities with predictive value concerning mutagenic activity can be made.

B. Relation between chemical structure and carcinogen/mutagen activity.

1) Generalities

Ideally, one would like to identify potential carcinogens based on a knowledge of chemical structure. Many attempts at this kind of correlation have been made, with the most useful and extensive studies done with the polycyclic aromatic hydrocarbons (9,14; for reviews of this area, see ref. 1,13,16). With respect to the Ross report, some significant generalities based on literature reports can be made. These are: (1) the structure-activity pattern observed for one series of carcinogens does not apply to other series of carcinogens; (2) an unpredictability of activity for isomers of the same or related compounds exists; (3) an understanding of the activity relations between isomers will come about only after consideration of the metabolic transformations of the isomers (5,9,17). Too many exceptions to "predictability" exist in the literature to permit dependable correlations. Without knowledge of the structure of the ultimate carcinogens in isomeric series, hypotheses relating structure to activity are too much conjecture.

2) Specifics with reference to the Ross report.

The use of Salmonella strains that are reverted by different pathways, either by a frameshift mutation (TA98) or a base substitution (TA100), permits the detection of different chemical mutagenesis pathways. Unfortunately, little or no differential mutagenesis is obvious for the various isomers (Table 3). One example of a type of difference searched

for can be seen in Fig. 5 for ODA. The (m,m') isomer is classed in Table 3 as a moderate mutagen for strain TA98 and a low mutagen for TA100, consistent with the definitions of these terms. However, a look at the actual data in Fig. 5 shows that the (m,m') isomer, at higher concentrations, is highly mutagenic in the TA98 strain and low in the TA100 strain. This suggests that the activated ODA isomer is transformed to an ultimate mutagen that acts preferentially by way of frameshifts. Can similar differences be detected for related isomers? A pattern of this kind would be evidence that different isomers are activated by similar pathways. No patterns are obvious.

It is my opinion that it is too premature to search for structure-activity relations in the sets of aromatic amines studied in the Ross report. The requirement for metabolic activation for those isomers that test positive as mutagens means that the ultimate mutagens are unidentified and therefore have unknown structures. One might hope that structure-activity relations would appear because of common metabolic pathways, but no correlations are obvious. For example, the (o,m') isomer of MDA is highly mutagenic whereas the (o,m') isomer of two other compounds (DABP and DDS) are not. One can conjecture on the influence of the various groups on the electronic nature of the reactive groups but no consistent hypothesis is readily forthcoming.

C. The requirement for activation

All aromatic amines including the dianilines studied in the Ross report require metabolic activation for mutagenic activity. Structure-activity relations may become more obvious when detailed studies of metabolic pathways are completed. Activation systems are complex and

undefined. This complexity precludes the assumption of common metabolic pathways. However, considerable progress in this area has been made and several sophisticated studies can serve to offer direction in this area (for reviews, see 2,5,6,16,17).

Some generalities for activation of aromatic amines exist. For example, a common step for carcinogenic and mutagenic activation is N-hydroxylation. The ultimate carcinogen may be caused by the esterification of the N-hydroxy group (1,5,17). It has been shown in some cases that the ester derivatives bind to macromolecules such as DNA and protein. Evidently the ester derivatives are electrophilic reactants capable of binding to nucleophilic centers in DNA. For example, the well-studied aromatic amine 2-acetylaminofluorene (AAF) is converted initially to the procarcinogen N-hydroxy-AAF, which is further metabolized to the acetate of N-hydroxy-AAF by esterification of hydroxamic acid to give the ultimate carcinogen N-acetoxy-AAF. This compound has a direct action on DNA that may be responsible for its mutagenic and carcinogenic activities (5).

These generalities seem to hold true for benzidine. N-acetylated benzidine derivatives that are mutagenic are found in the urine of rats treated with benzidine (15). Benzidine can be converted in a liver extract to an N-hydroxy compound (N-hydroxy-N,N'-diacetyl-benzidine) which binds to DNA and is a direct mutagen (10). In addition, the N-hydroxy compound can be esterified to an N-acetoxy diacetylbenzidine which is proposed as the ultimate carcinogen (11). Other evidence that benzidine metabolites bind to DNA has been presented (18). These results are important as they show that the generalities for structure-activity relations detected for other aromatic amines seem to hold true for benzidine.

This suggests that activation of the dianilines may follow similar routes.

III. Conclusions

The results presented in the Ross report show that some of the steric isomers of sets of aromatic diamine compounds are mutagenic when treated with metabolic activation systems. Results of others suggest that the Salmonella/mutagenic assay conducted as described in the Ross report is a good predictive indicator of carcinogenesis (2,8). Negative results employing a single metabolic activation system do not imply that that isomer is not a mutagen. Further mutagenesis assays employing other activation systems or conditions are required.

It is suggested that one cannot predict the mutagenicity of an isomer based on its structure at this time. The ability to make this kind of prediction is an ultimate desirable goal. Several well documented reviews describe attempts of others directed toward the same goal. The goal is an achievable one but will require a significantly greater amount of information, primarily with respect to chemical transformation caused by metabolic systems.

A study of metabolic activation can lead to knowledge concerning relations between chemical structure and activity. Existing data shows that some common steps occur in the activation of aromatic amines to both pro and ultimate carcinogens. Further studies directed with this goal in mind can contribute to the eventual understanding of chemical structure and carcinogenic potential.

The Ross study is unique in that five different sets of dianiline compounds, four of which are represented by nearly all of the possible

steric isomers, were tested. To my knowledge no similar studies for the complete set of benzidine isomers or other aromatic amines have been reported.

IV. Further research

The Ross study leads to several suggestions for future research. I suggest that the most important is to concentrate on the complete set of MDA isomers and study their metabolic activation. Initially, one should work with the most mutagenic isomer, (o,m'). The pro and ultimate mutagens can be followed through fractionation of the activation mix by biological activity. One could acquire radio-labeled isomers and follow metabolic derivatives by radio-tracer methods. One can specifically search for N-hydroxylation products and N-acetoxy products. A similar survey of the remaining isomers should provide the necessary information for establishing structure-activity relations. The relations should provide useful information for an understanding of the activity of the other dianilines and for structure-activity predictions.

REFERENCES

Some of the references given are reviews. The reviews address the three areas of concern for an understanding of the mutagenicity and potential carcinogenicity of the dianiline compounds. These areas are:

A. the relation between chemical structure and carcinogenicity; (ref. 1, 13, 14)

B. the requirement for activation, mechanisms of activation, problems with activation systems, and interpretation of results using activation systems, in mutagenesis studies; (ref. 2, 5, 6, 7, 9, 13, 16, 17)

C. previous studies of mutagenicity using other aromatic amines that serve as model studies for an analysis of the dianiline mutagenesis results; (ref. 1, 3, 4, 5, 13)

Reviews were selected as references so as to keep the bibliography to a small size. The references sited in the reviews are comprehensive and serve to direct interested investigators to the relevant primary literature.

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APPENDIX C

ACUTE CYTOTOXICITY CLONAL ASSAY DATA

CYTOTOXICITY DATA FOR m,m' DABP
CELL LINE: CHO-K1

9-14-82
PAGE REF: -

CONTROL (BACKGROUND) VALUES	MEAN VALUE	STANDARD DEVIATION
444	434	27
397		
463		
448		
416		

CONCENTRATION (G/ML)	REPLICATE VALUES	MEAN VALUE	STANDARD DEVIATION	PERCENT SURVIVAL
.0003	0	0	0	0
	0			
	0			
.0001	307	310	17	71
	295			
	328			
.00005	440	434	9	100
	437			
	424			
.00001	428	418	9	100
	416			
	411			
.000005	434	465	27	100
	484			
	476			
.000001	427	430	10	100
	422			
	441			
5.00000E-07	442	439	6	100
	443			
	433			

BASIC
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CYTOTOXICITY DATA FOR m,m' DABP
CELL LINE: D98S

9-14-82
PAGE REF: -

CONTROL (BACKGROUND) VALUES	MEAN VALUE	STANDARD DEVIATION
413 424 472 406 413	426	27

CONCENTRATION (G/ML)	REPLICATE VALUES	MEAN VALUE	STANDARD DEVIATION	PERCENT SURVIVAL
.0003	0 0 0	0	0	0
.0001	224 265 228	239	23	56
.00005	356 398 372	375	21	88
.00001	433 472 447	451	20	100
.000005	479 496 495	490	10	100
.000001	484 467 483	478	10	100
5.00000E-07	497 499 499	498	1	100

BASIC
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CYTOTOXICITY DATA FOR o,p' DABP
CELL LINE: CHO-K1

9-14-82
PAGE REF: -

CONTROL (BACKGROUND) VALUES	MEAN VALUE	STANDARD DEVIATION
444	434	27
397		
463		
448		
416		

CONCENTRATION (G/ML)	REPLICATE VALUES	MEAN VALUE	STANDARD DEVIATION	PERCENT SURVIVA
.0003	0	0	0	0
	0			
	0			
.0001	163	151	11	35
	148			
	141			
.00005	373	367	16	85
	349			
	380			
.00001	420	421	6	100
	427			
	415			
.000005	450	454	11	100
	445			
	466			
.000001	346	405	51	100
	435			
	435			
5.00000E-07	421	417	15	100
	429			
	400			

BASIC
>

CYTOTOXICITY DATA FOR o,p' DABP
CELL LINE: D98S

9-14-82
PAGE REF: -

CONTROL (BACKGROUND) VALUES	MEAN VALUE	STANDARD DEVIATION
413 424 472 406 413	426	27

CONCENTRATION (G/ML)	REPLICATE VALUES	MEAN VALUE	STANDARD DEVIATION	PERCENT SURVIV
.0003	0 0 0	0	0	0
.0001	0 0 0	0	0	0
.00005	81 83 80	81	2	19
.00001	470 421 483	458	33	100
.000005	495 482 490	489	7	100
.000001	429 426 486	447	34	100
5.00000E-07	484 506 471	487	18	100

BASIC
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CYTOTOXICITY DATA FOR o,o DABP
CELL LINE: CHO-L1

9-14-82
PAGE REF: -

CONTROL (BACKGROUND) VALUES	MEAN VALUE	STANDARD DEVIATION
444 397 463 448 416	434	27

CONCENTRATION (G/ML)	REPLICATE VALUES	MEAN VALUE	STANDARD DEVIATION	PERCENT SURVIVAL
.0003	0 0 0	0	0	0
.0001	21 17 20	19	2	4
.00005	352 374 339	355	18	82
.00001	451 431 447	443	11	100
.000005	416 447 404	422	22	100
.000001	427 410 419	419	9	100
5.00000E-07	462 449 415	442	24	100

BASIC
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CYTOTOXICITY DATA FOR o,o' DABP
CELL LINE: D98S

9-14-82
PAGE REF: -

CONTROL (BACKGROUND) VALUES	MEAN VALUE	STANDARD DEVIATION
413 424 472 406 413	426	27

CONCENTRATION (G/ML)	REPLICATE VALUES	MEAN VALUE	STANDARD DEVIATION	PERCENT SURVIVAL
.0003	0 0 0	0	0	0
.0001	0 0 0	0	0	0
.00005	301 320 262	294	30	69
.00001	478 457 440	458	19	100
.000005	479 477 484	480	4	100
.000001	478 485 512	492	18	100
5.00000E-07	443 478 456	459	18	100

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CYTOTOXICITY DATA FOR p,p'-DABP
CELL LINE: CHO-K1

11-15-82
PAGE REF: -

CONTROL (BACKGROUND) VALUES	MEAN VALUE	STANDARD DEVIATION
564 515 568 516	541	29

CONCENTRATION (G/ML)	REPLICATE VALUES	MEAN VALUE	STANDARD DEVIATION	PERCENT SURVIVAL
.0003	0 0 0	0	0	0
.0001	405 485 480	457	45	84
.00007	619 605 624	616	10	100
.00004	595 586 588	590	5	100
.00001	515 520 524	520	5	100
.000005	512 553 577	547	33	100

BASIC
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CYTOTOXICITY DATA FOR P,P'-DABP
CELL LINE: D98S

11-15-82
PAGE REF: -

CONTROL (BACKGROUND) VALUES	MEAN VALUE	STANDARD DEVIATION
454 489 463 466 452	465	15

CONCENTRATION (G/ML)	REPLICATE VALUES	MEAN VALUE	STANDARD DEVIATION	PERCENT SURVIVAL
.0003	0 0 0	0	0	0
.0001	0 0 0	0	0	0
.00007	94 39 127	87	44	19
.00004	446 429 467	447	19	100
.00001	456 444 476	459	16	100
.000005	455 479 458	464	13	100

BASIC
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CYTOTOXICITY DATA FOR m,p'-DABP
CELL LINE: CHO-K1

11-15-82
PAGE REF: -

CONTROL (BACKGROUND) VALUES	MEAN VALUE	STANDARD DEVIATION
564 515 568 516	541	29

CONCENTRATION (G/ML)	REPLICATE VALUES	MEAN VALUE	STANDARD DEVIATION	PERCENT SURVIVAL
.0003	0 0 0	0	0	0
.0001	583 517 506	535	42	100
.00007	622 656 614	631	22	100
.00004	601 619 634	618	17	100
.00001	583 562 573	573	11	100
.000005	609 608 574	597	20	100

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CYTOTOXICITY DATA FOR [illegible]'-DABP
CELL LINE: D98S

11-15-82
PAGE REF: -

CONTROL (BACKGROUND) VALUES	MEAN VALUE	STANDARD DEVIATION
454 489 463 466 452	465	15

CONCENTRATION (G/ML)	REPLICATE VALUES	MEAN VALUE	STANDARD DEVIATION	PERCENT SURVIVAL
.0003	0 0 0	0	0	0
.0001	235 268 254	252	17	54
.00007	353 396 409	386	29	83
.00004	476 455 450	460	14	100
.00001	477 491 469	479	11	100
.000005	450 438 484	457	24	100

BASIC
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CYTOTOXICITY DATA FOR o,m'-DABP
CELL LINE: CHO-K1

11-15-82
PAGE REF: -

CONTROL (BACKGROUND) VALUES	MEAN VALUE	STANDARD DEVIATION
564 515 568 516	541	29

CONCENTRATION (G/ML)	REPLICATE VALUES	MEAN VALUE	STANDARD DEVIATION	PERCENT SURVIVAL
.0003	0 0 0	0	0	0
.0001	323 311 329	321	9	59
.00007	430 416 422	423	7	78
.00004	582 583 590	585	4	100
.00001	585 601 592	593	8	100
.000005	605 561 538	568	34	100

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CYTOTOXICITY DATA FOR o,m'-DABP
CELL LINE: D98S

11-15-82
PAGE REF: -

CONTROL (BACKGROUND) VALUES	MEAN VALUE	STANDARD DEVIATION
454 489 463 466 452	465	15

CONCENTRATION (G/ML)	REPLICATE VALUES	MEAN VALUE	STANDARD DEVIATION	PERCENT SURVIVAL
.0003	0 0 0	0	0	0
.0001	42 66 51	53	12	11
.00007	211 280 243	245	35	53
.00004	483 443 447	458	22	100
.00001	481 489 476	482	7	100
.000005	463 448 482	464	17	100

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:YTOTOXICITY DATA FOR DMSO
'ELL LINE: CHO-K1

11-15-82
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CONTROL (BACKGROUND) VALUES	MEAN VALUE	STANDARD DEVIATION
564 515 568 516	541	29

CONCENTRATION (UL/35 ML)	REPLICATE VALUES	MEAN VALUE	STANDARD DEVIATION	PERCENT SURVIVAL
525	607 599 670 588	616	37	100
175	666 687 634 657	661	22	100

BASIC
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CYTOTOXICITY DATA FOR DMSO
CELL LINE: D98S

11-15-82
PAGE REF: -

CONTROL (BACKGROUND) VALUES	MEAN VALUE	STANDARD DEVIATION
454 489 463 466 452	465	15

CONCENTRATION (UL/35 ML)	REPLICATE VALUES	MEAN VALUE	STANDARD DEVIATION	PERCENT SURVIVAL
525	480 471 457 518	482	26	100
175	476 490 444 455	466	21	100

BASIC
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