Comparison of the Mutagenicities of Malondialdehyde and the Side Products Formed during Its Chemical Synthesis

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ABSTRACT

Malondialdehyde, a product of polyunsaturated fatty acid metabolism and degradation, has been reported to be mutagenic and carcinogenic. The malondialdehyde used for testing was generated by the acidic hydrolysis of tetraalkoxypropanes. We have studied the production of compounds mutagenic to Salmonella typhimurium strain his D 3052 following the hydrolysis of tetraalkoxypropanes. The major mutagenic compound produced from tetraethoxypropane is β-ethoxyacrolein (90 to 100 revertants/μmol) and not malondialdehyde (3 to 5 revertants/μmol). Hydrolysis of tetramethoxypropane produces two compounds, β-methoxyacrolein (125 to 160 revertants/μmol) and 3,3-dimethoxypropionaldehyde (105 to 135 revertants/μmol), which are more mutagenic than is malondialdehyde. Using standard conditions for the hydrolysis of tetraalkoxypropanes, the yield of malondialdehyde is 25%, and the yield of β-ethoxyacrolein is 13%. Considering the differences in specific mutagenicity, the compound which accounts for the bulk of the mutagenicity of a crude hydrolysate of tetraethoxypropane is not malondialdehyde. The presence of these incomplete hydrolysis products may lead to a substantial overestimation of the actual mutagenicity and carcinogenicity of malondialdehyde.

INTRODUCTION

MDA³ is formed in animal tissue as an end product of lipid peroxidation and as a side product of prostaglandin and thromboxane biosynthesis (2, 7, 10, 13, 22). Shamberger et al. (20) have reported that MDA exhibits initiating activity in female Swiss mice using the 2-stage assay. Mukai and Goldstein (16) and Shamberger et al. (21) have reported that MDA is mutagenic in several strains of Salmonella typhimurium. The widespread occurrence of MDA in the animal kingdom coupled with its apparent initiating activity suggests that MDA may be an important causative agent of spontaneous carcinogenesis.

MDA is unstable and cannot be stored in the pure state (11). Therefore, solutions of it are usually prepared immediately prior to testing by the acidic hydrolysis of TEP or TMP (18). Previous investigators have noted that the hydrolysis of these compounds is accompanied by the production of colored by-products which are believed to be condensation polymers of MDA (9, 15). It is believed that the formation of these polymers can be avoided by using very dilute solutions of TEP or TMP for the hydrolyses (15). Since MDA is a relatively weak mutagen, high concentrations must be used to detect a mutagenic response (16, 21). This necessitates the use of concentrated solutions of TEP or TMP for the preparation of the MDA solutions.

MDA is a moderately weak acid (pKₐ, 4.46) which exists as its conjugate base at physiological pH (Ref. 17; Equation A).

The conjugate base of MDA is much less reactive than is MDA toward nucleophiles and does not participate in self-condensation reactions (4, 6). This suggests that the side products formed during the acidic hydrolysis of TEP or TMP to MDA are unlikely to be formed under physiological conditions where MDA exists exclusively as its conjugate base. If the side products are responsible for the reported initiating activity, then MDA itself may not be a mutagen and carcinogen and may play no role in spontaneous carcinogenesis.

We have recently synthesized [¹⁴C]TEP for use as a source of malondialdehyde-1,2,3,4-¹⁴C₃ in tracer studies (3). This has enabled us to quantitate the compounds produced during the acidic hydrolysis of TEP and to compare their specific mutagenicities. Our studies show that (a) the sodium salt of the conjugate base of MDA (NaMDA) is very weakly mutagenic, (b) the polymeric decomposition products formed from MDA under acidic conditions are not mutagenic, and (c) the major mutagenic compound produced from TEP using literature hydrolysis conditions is not MDA but, an intermediate hydrolysis product, BEA.

MATERIALS AND METHODS

TMP was purchased from Aldrich Chemical Co. and distilled prior to use. TEP was synthesized according to literature methods (12) or purchased from ICN Pharmaceuticals (Plainview, N. Y.). [¹⁴C]TEP was synthesized as described and demonstrated to be in excess of 95% radiochemically pure by radiogas chromatography (3). The synthesized material (25 μCi/μmol) was diluted with unlabeled TEP to produce the specific activity desired for a given experiment. Dowex 50WX-4 was purchased from J. T. Baker Chemical Co. and washed exhaustively with water to remove colored impurities. Sephadex LH-20 was purchased from Sigma Chemical Co. Water was distilled twice in glass.
**NaMDA.** TEP (1.1 g; 5 mmol) and 5 g Dowex 50 (5 mmol H\(^+\)) were suspended in 10 ml H\(_2\)O and agitated in a shaking water bath at 25° for 30 min. The slurry was titrated to pH 7.0 with 5 and 1 n NaOH. The Dowex 50 was filtered off, and the filtrate was extracted 3 times with equal volumes of water-saturated ethyl acetate. The aqueous phase was lyophilized, and the residue was dissolved in 1.5 ml H\(_2\)O. This solution was filtrate was extracted 3 times with equal volumes of water with 5 and 1 N NaOH. The Dowex 50 was filtered off, and the eluted with H\(_2\)O. Fractions (2.5 ml) were collected, and those containing the major 267-nm-absorbing material were pooled and lyophilized. NaMDA elutes from the LH-20 column at a retention volume of approximately 55 ml. The residue from the lyophilization was dissolved in a minimal volume of H\(_2\)O, and acetone was added to the cloud point. On standing overnight at -15°, fine white needles of NaMDA formed (260 mg, 40% yield). Combustion analysis indicates that these crystals contain 2 to 3 water molecules/molecule of NaMDA.

**BEA.** A modification of the procedure of Rüegg et al. (19) was used. Toluene sulfonic acid (54.3 mg; 0.3 mmol) and H\(_2\)O (1.8 g; 100 mmol) were added to TEP (22 g; 100 mmol) and stirred at 80° for 2 hr. The reflux condenser was replaced with a short-path distillation apparatus, and ethanol (4.6 g; 100 mmol) distilled off. NaHCO\(_3\) (0.5 g) was added, and the mixture was stirred at room temperature for 1 hr. Ethanol was removed by distillation at reduced pressure (41 to 45° at 140 torr). Crude BEA distilled from 130 to 153° (140 torr). Further purification was affected by preparative GC on a 6-ft column of 10% OV-17 on Chromosorb W-HP (35 ml helium per min at 110°). The isolated material exhibited a single peak when reanalyzed under these conditions. NMR (CCL): \(\delta_{1.4} t, J = 7\) Hz (3 H); \(\delta_{4.1} q, J = 7\) Hz (2 H); \(\delta_{4.5} d, J = 7, 13\) Hz (1 H); \(\delta_{7.4} d, J = 13\) Hz (1 H); and \(\delta_{9.3} d, J = 7\) Hz (1 H). Mass spectrum (70 eV), \(m/e\) (intensity): 100 (24%) and 71 (100%).

**DMP and BMA.** The above procedure was used for the hydrolysis of DMP (16.4 g; 100 mmol). Following the removal of methanol by distillation, a fraction which contained both DMP and BMA was collected at 110 to 120° (145 torr). Impurities in this fraction were removed by preparative GC on a 6-ft column of 5% SE-30 on Chromosorb W-HP (35 ml helium per min at 110°). The DMP and BMA in the collected material were separated and purified by preparative GC on a 6-ft column of 10% OV-17 on Chromosorb W-HP (35 ml helium per min at 130°). Both compounds exhibited single peaks when reanalyzed on the latter column. BMA, NMR (CCL): 83.8 s, (3 H); 5.5 dd, \(J = 8, 13\) Hz (1 H); 7.4 d, \(J = 13\) Hz (1 H); and 9.5 d, \(J = 7\) Hz (1 H). Mass spectrum (70 eV), \(m/e\) (intensity): 86 (57%), 85 (100%), 71 (10%), and 57 (20%). DMP, NMR (CCL): \(\delta_{2.7} d, J = 2.5, 6\) Hz (2 H); 3.4 s (6 H); 4.8 t, \(J = 6\) Hz (1 H); and 9.9 t, \(J = 2.5\) Hz (1 H). Mass spectrum (70 eV), \(m/e\) (intensity): 103 (15%) \(M^+\)—CH\(_3\), 75 (88%).

**Mutagenicity.** S. typhimurium tester strains were kindly provided by Professor Bruce Ames, University of California, Berkeley. The properties of the strains used have been described (1). Standard methodology for the preparation of bacterial suspensions was used. An overnight nutrient broth culture (0.1 ml) was added to molten top agar (2.0 ml; 1%) containing 50 \(\mu\)g histidine and biotin. A sterile 0.1-ml solution of the compound to be tested was added, and the mixture was vortexed and poured onto minimal-glucose plates. No enzymes for metabolic activation were included. The plates were incubated at 37°, and macroscopic colonies were counted after 58 hr.

Aqueous solutions of compounds to be tested were sterilized by passage through 0.2-\(\mu\)m Acrodiscs (Gelman Instrument Co.). Dimethyl sulfoxide, acetone, and ethanol solutions were sterile.

**Spectral Analysis of BEA and NaMDA in Hydrolyzed DMP Solutions.** Solutions of DMP incubated in neutral buffers exhibit absorbances at 245 and 267 nm due to the presence of BMA and NaMDA, respectively. Assuming that these are the only 2 absorbing species in solution and using the molar absorptivities of BMA at 245 nm (18.4/mM/cm) (19) and NaMDA at 267 nm (34.2/mM/cm), one can derive Equation B to calculate the absorbance at 267 nm.

\[ [\text{NaMDA}] = 1.09 \times (29 \Delta_{267} - 8.2 \Delta_{245}) \quad (B) \]

The absorbance at 245 nm is then calculated from Equation C.

\[ [\text{BMA}] = 55 \Delta_{245} - 0.55[\text{NaMDA}] \quad (C) \]

\(\Delta_{267}\) and \(\Delta_{245}\) are the measured absorbances at 267 and 245 nm, respectively. The concentrations of NaMDA and BMA determined from Equations B and C are in \(\mu\)M and represent the concentrations in the solutions diluted for spectrophotometric analysis. The concentrations in the stock solutions are determined by multiplying by the appropriate dilution factor.

**Analysis of TEP, BEA, and NaMDA in Hydrolyzed TEP Solutions.** The hydrolyzed TEP solutions were neutralized (pH 7) and extracted with an equal volume of CCL. An aliquot of the organic extract was injected onto a 6-ft column of 10% OV-17 on Chromosorb W-HP (2 mm i.d.) at 70°. Isothermal elution was continued for 3 min after which the temperature was programmed to 170° at 10°/min (30 ml helium per min). The amounts of BEA (8 min) and TEP (11 min) eluting from the column were quantitated by electronic integration.

The concentration of NaMDA in the aqueous phase was determined from the absorbance at 267 nm.

UV spectra were recorded on a Perkin-Elmer 124 spectrophotometer. NMR spectra were recorded on a Varian T-60. Liquid scintillation counting was performed on a Packard 2002. Preparative GC was performed on a Hewlett-Packard 720. Analytical GC was performed using a Varian 3700 equipped with a CDS-111 data system. Mass spectra were run at Schrader Analytical, Detroit, Mich.

**RESULTS**

NaMDA was tested for mutagenicity by the plate incorporation method using S. typhimurium strains his C 207, his C 3076, his D 3052, TA 1535, TA 1538, TA 1977, TA 98, and TA 100. Both a crude hydrolysate of TEP and a highly purified sample of NaMDA were tested. With both solutions, revertants to histidine independence were observed in his C 207, his C 3076, his D 3052, and TA 1977. No mutagenicity was observed in the remaining strains, but toxicity was evident in TA 98 and TA 100 at NaMDA concentrations of 1 mg/plate. The pattern observed is similar to that reported by Mukai and Goldstein (16) in that only strains containing normal excision repair are sensitive to MDA mutagenesis. Since his D 3052 yielded the highest number of revertants/plate at a given concentration of MDA, it was used for the remainder of the experiments.

Chart 1 presents a comparison of the dose-response curves in his D 3052 for a crude hydrolysate of TEP and a highly...
purified preparation of NaMDA derived from it. The conditions used for the hydrolysis were those of Protopopova and Skoldinov (18) which are standard in the literature. [¹⁴C]TEP was used, and the mutagenicities were determined using equivalent amounts of radioactivity. Since the specific activity of the starting TEP was the same for both samples, a direct comparison is possible based on μmol of TEP or MDA. It is evident that the crude hydrolysate is approximately twice as mutagenic as an equivalent amount of NaMDA. The NMR spectrum in D₂O of the NaMDA used for this experiment exhibits a d at 88.46, a t at 85.13, and a s at 84.63 (HOD impurity) (8). A s is also observed at 82.23 due to acetone remaining from the recrystallization used in the purification. Comparison of the peak intensities at 88.46 and 82.23 indicates that the NaMDA is at least 99% pure with respect to organic impurities. Since acetone is nonmutagenic, the actual purity of the NaMDA with respect to other potential mutagens must be substantially higher than 99%.

Chart 1 suggests that a mutagenic impurity is present in crude TEP hydrolysates which is removed during the purification of the NaMDA. Previous investigators have noted the presence of colored impurities in TEP hydrolysates and speculated that they represent polymers formed from MDA under the acidic conditions of the hydrolys (9, 15). We have found that columns of Sephadex LH-20 eluted with water effectively separate NaMDA from colored side products. We therefore chromatographed a crude [¹⁴C]TEP hydrolysate mixture on LH-20, estimated the MDA equivalents by liquid scintillation counting, and determined the mutagenicity of column fractions in his D 3052. The results of a typical experiment are shown in Chart 1. Comparison of the mutagenicity of a crude TEP hydrolysate with that of NaMDA, and this corresponds to a zone of mutagenicity. The major mutagenic compound elutes after the NaMDA and contains substantially less radioactivity than does the NaMDA peak. This implies that the specific mutagenicity of the second peak is substantially higher than the specific mutagenicity of NaMDA. Kwon (14) has reported that the free acid of MDA elutes after the sodium salt on Sephadex G-10. It is possible that a percentage of the material applied as the sodium salt is converted to the free acid by CO₂ dissolved in the unbuffered water and that the free acid elutes after the salt. The difference in specific mutagenicities of the 2 zones in Chart 2 suggests that this is not the case. Under the neutral conditions of the mutagenicity assay, MDA exists completely as its conjugate base. Therefore, zones eluting from the LH-20 columns which contain protonated and unprotonated MDA will be converted to the unprotonated form under the conditions of the mutagenicity assay and should exhibit identical specific mutagenicities.

Since oligomers and polymers of MDA are apparently side products of TEP hydrolysis, they may represent the mutagenic impurity (impurities) seen in Chart 2. If so, it should be possible to generate them directly by heating MDA in acid. We therefore acidified a 65 mM solution of pure NaMDA to pH 3.5 and heated it to 37°. The initially colorless solution quickly turned yellow and eventually became orange. Samples were periodically removed and neutralized to prevent further reaction. The NaMDA content and mutagenicity of the solution were assayed. Instead of increasing as expected, the mutagenicity decreases with time, and the decrease correlates with the decrease in the MDA content of the solution (Chart 3). This experiment suggests that MDA is the sole mutagen in the solution and implies that none of the polymers formed from it by self-condensation is appreciably mutagenic.

In an attempt to characterize further the mutagenic impurity present in crude TEP hydrolysates, the fractions which elute after NaMDA on LH-20 were combined and divided into 2 samples of equal volume. One sample was lyophilized to a small amount of a pale yellow powder. This residue was dissolved in water to its original volume and compared with the

![Chart 2. Chromatography of a crude TEP hydrolysate on Sephadex LH-20.](image)

—, radioactivity converted to the concentration of TEP equivalents; — — — —, his D 3052 revertants induced by 0.1-ml aliquots of column fractions.

![Chart 3. Effect of heating MDA in acid solution. MDA (65 mM) was heated to 37° at pH 3.5. Aliquots were withdrawn and neutralized. The NaMDA content was determined by the intensity of A₄₀. The mutagenicity was determined in his D 3052.](image)
unlyophilized sample. A volume of each solution containing radioactivity equivalent to 5 μmol TEP induced 85 and 0 revertants in his D 3052 when taken from the unlyophilized and lyophilized samples, respectively. Five μmol NaMDA induced 25 revertants under the same conditions. This experiment suggests that the major mutagenic impurity is volatile and is probably a small molecular species. Interestingly, although the mutagenicity decreased completely following lyophilization, the radioactivity only decreased by approximately 20%. Taken with the results in Chart 2, this is further evidence that the mutagenic impurity is substantially more mutagenic than is NaMDA.

The only compounds in a neutralized TEP hydrolysate which should be volatile are unreacted TEP and any uncharged intermediates between TEP and MDA. Since TEP is not mutagenic, the most probable candidate for the mutagenic impurity (impurities) is a hydrolytic intermediate. Chart 4 indicates the compounds which might be formed following the treatment of TEP with acid. The monohydrolysis product DEP could either undergo further hydrolysis to MDA or lose ethanol to form BEA. BEA should also undergo hydrolysis to MDA. Hydrolysis of TEP with one equivalent of H₂O according to Rüegg et al. (19) led to the isolation of only BEA. The identity of the isolated material as BEA was verified by its NMR and mass spectra. The BEA was purified by preparative GC, and its mutagenicity was assayed immediately thereafter. Analytical GC indicated that the BEA solutions underwent no hydrolysis during the time required for the plating operations. Chart 5 illustrates that BEA is approximately 18 to 20 times more mutagenic than is a sample of NaMDA assayed on the same day.

We have been unable to assay directly the mutagenicity of the other hydrolytic intermediate DEP because we have been unable to prepare it. However, we have found that hydrolysis of the structurally similar TMP under the conditions cited by Rüegg et al. leads to the formation of DMP and BMA in approximately equal amounts. These compounds were separated and purified by preparative GC and assayed in his D 3052. The results are shown in Chart 6. DMP is 22 to 27 and BMA is 25 to 32 times more mutagenic than is NaMDA. The variation in mutagenicity with different solvents is not of a magnitude to imply that a chemical reaction occurs between DMP or BMA and a given solvent to generate the actual mutagen. We also have tested aqueous solutions of NaMDA in the presence of 100 μl of acetone, ethanol, and dimethyl sulfoxide and find no increase in mutagenicity above that displayed in water alone.

The high mutagenicity of BEA and BMA relative to NaMDA was not unanticipated. These compounds possess a relatively unhindered α, β-unsaturated aldehyde functionality which

![Chart 4. Probable pathway of hydrolysis of TEP to MDA.](image-url)
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that 22% of the DMP decomposes to BMA (56%) and NaMDA (44%) in 26 hr. Essentially identical results are obtained in the presence or absence of bacteria indicating that the reactions are probably nonenzymatic.

Since BEA, BMA, and DMP are appreciably more mutagenic than is NaMDA, they appear to be candidates for the mutagenic impurity (impurities) produced during the hydrolysis of TEP or TMP. To determine if, in fact, BEA is produced during the hydrolysis of TEP, we have developed a procedure for the simultaneous analysis of TEP, BEA, and NaMDA. Chart 8 is the time course of the hydrolysis of TEP according to the method of Protopenova and Skoldinov (18). The initial [TEP] is 2 M, the [HCl] is 0.15 M, and the temperature is 60°. The TEP falls to a negligible level quite rapidly. A substantial amount of BEA is produced during the hydrolysis of TEP, and the levels decline rather slowly. Therefore, appreciable levels of BEA are present at any time during the hydrolysis of TEP. The identity of the BEA was verified by NMR spectroscopy following GC collection. Qualitatively similar observations have been made during the hydrolysis of TMP.

should be quite reactive to nucleophiles. In contrast, the mutagenicity of DMP is somewhat surprising. The possibility exists that DMP is not directly mutagenic but that it is hydrolyzed to BMA during the course of the incubation with bacteria. We therefore incubated DMP (5 mM) with his D 3052, heat-inactivated his D 3052, or phosphate-buffered saline alone at 37°. At various times, aliquots were removed, and the UV spectrum was recorded following appropriate dilutions. BMA absorbs at 245 nm (ε = 18.4 mM/cm), and NaMDA absorbs at 267 nm (ε = 34.2 mM/cm). Chart 7 displays the change in absorption spectrum as a function of time when DMP is incubated with buffer alone. The small absorbance at 245 nm at t = 0 is due to the presence of a 2% impurity of BMA in the DMP used for the experiment. Absorption bands at 245 and 267 nm can be seen growing in with time indicating the hydrolysis of DMP to BMA and NaMDA. Using the molar absorbivities of BMA and NaMDA at 245 and 267 nm, one can calculate the concentrations of both species from the absorption spectra (see "Materials and Methods"). The results of such calculations indicate...
Chart 9 is the profile of the concentrations of NaMDA and BEA which elute from a Sephadex LH-20 column following the application of a crude TEP hydrolysate. BEA elutes after NaMDA at a retention volume expected for that of the major mutagenic impurity (Chart 2). Therefore, it can be concluded that BEA is produced during the hydrolysis of TEP to MDA and that it constitutes the major mutagenic impurity in the crude hydrolysate.

DISCUSSION

Since MDA appears to be produced widely in animal tissue (2, 7, 10, 13, 22), it is important to establish whether MDA or some byproduct of its chemical synthesis is responsible for the mutagenicity and tumor-initiating activity reported for solutions of hydrolyzed TEP or TMP (16, 20, 21). The present study establishes that NaMDA purified by column chromatography and recrystallization is, in fact, very weakly mutagenic in a number of strains of S. typhimurium. The material used for testing was at least 99% pure by NMR spectroscopy and contained no detectable amounts of BEA when analyzed by GC (limit of detection, below 0.1%). Over a period of 2 years of testing different batches of NaMDA, the specific mutagenicity has been consistently 3 to 5 revertants per 10^6 bacteria per μmol of NaMDA. Since NaMDA is the form of MDA which occurs under normal physiological conditions, it appears that MDA generated in vivo is mutagenic.

Our study also establishes that the most mutagenic compound produced by the acidic hydrolysis of TEP is not MDA but rather the incomplete hydrolysis product BEA. In the case of the hydrolysis of TMP, both the intermediates DMP and BMA are more mutagenic than is MDA, and they appear to be more mutagenic than BEA as well. The greater mutagenicity of BEA and BMA than NaMDA is probably due to differences in reactivity. Although all 3 molecules possess an α,β-unsaturated aldehyde functionality, NaMDA has a full negative charge at neutral pH whereas BEA and BMA are uncharged. Model studies of the reaction of MDA with proteins and nucleic acids have shown repeatedly that the uncharged free acid of MDA is substantially more reactive than is its anionic conjugate base (4, 6). Thus, BEA and BMA are probably good models for estimating the mutagenicity of the free acid of MDA.

The mutagenicity of DMP may be due to its conversion to BMA under the conditions of the mutagenicity assay. Chart 7 shows that significant nonenzymatic conversion of DMP to BMA and MDA does occur on standing in phosphate-buffered saline. However, the extent of conversion to BMA in 26 hr (12%) does not correlate with the relative mutagenicity of DMP to BMA (85%). It may not be legitimate to compare the results of an incubation in buffer with the results of an incubation in agar. It may be that enhanced conversion of DMP to BMA occurs under the conditions of the mutagenicity assay. Alternatively, the elimination of methanol may occur after the binding of the aldehyde component of DMP to an intracellular nucleophile such as a nucleic acid base. This would effectively generate the same functionality contained in BMA at the site of attachment to a critical intracellular nucleophile.

Since BEA, BMA, and DMP are much stronger mutagens than is NaMDA, there is some concern that these compounds were responsible for a portion of the mutagenicity and carcinogenicity attributed to MDA in previous reports (16, 20, 21).

This is difficult to assess directly since no details are given regarding the concentration of TEP or TMP used to generate the MDA. We have tested the effects of acid catalyst, temperature, and acetal concentration on the formation of the incomplete hydrolysis products and find that the sole determinant of their formation is the initial concentration of the acetal (assuming the initially 2-phase system will eventually become homogeneous). When typical literature conditions are used ([TEP] = 2 M), significant amounts of BEA are formed. If, however, [TEP] = 0.4 M or less, BEA is undetectable. The acetone precipitation of NaMDA which is commonly used does not remove these impurities. Although BEA, BMA, and DMP are uncharged liquids which should be soluble in excess acetone, they apparently are occluded within the crystals of NaMDA and cannot be removed by prolonged exposure of the crystals to high vacuum.

The specific mutagenicity of MDA toward his D 3052 reported by Mukai and Goldstein (16) is probably erroneous as pointed out in the subsequent study by Shamberger et al. (21). In the latter study, one can calculate a specific mutagenicity of approximately 37 revertants/μmol MDA from the data reported for the lower concentrations of MDA. Although one must be very cautious about comparing the specific mutagenicities determined in 2 different laboratories, the value determined from the study by Shamberger et al. is substantially higher than the value which we determine for NaMDA (3 to 5 revertants/μmol). The specific mutagenicities which we have determined for BEA and BMA are 90 to 100 and 125 to 160 revertants/μmol, respectively. The presence of a small amount of BEA or BMA in the NaMDA preparations obviously could lead to an appreciable overestimation of the mutagenicity of MDA.

The presence of BEA may also have contributed to the acute toxicity of TEP administered by stomach intubation to rats (5). Under the acidic conditions of the stomach, TEP should be converted to MDA. In general, the toxicity of TEP is quite similar to that of equivalent amounts of NaMDA administered by the same route. However, differences in the slope function, time of onset of toxic action, and the symptoms observed led Crawford et al. (25) to conclude that the toxic action of TEP could not be attributed solely to NaMDA.

Although the mutagenicity of NaMDA is quite low compared...
to that of BEA or BMA, it should be pointed out that substantial amounts of NaMDA are produced under certain conditions in vivo. For example, approximately 40% of the arachidonic acid mobilized and converted to prostaglandin endoperoxides during platelet aggregation is directed to the formation of NaMDA and 12-hydroxyheptadecatrienoic acid (7). If all of the NaMDA is released from the platelet, significant blood levels of it may result. Depending upon the rate of its tissue uptake, metabolism, and nucleic acid binding, the circulating NaMDA may be responsible for cell transformation. Studies to determine the importance of these competing pathways of NaMDA utilization are in progress.

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