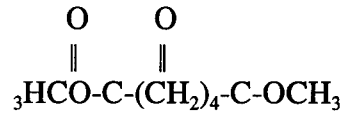
BACKGROUND AND JUSTIFICATION FOR TOXICITY TESTING OF DIMETHYL ADIPATE

Dimethyl Adipate



CAS No: 627-93-0

I. Physical and Chemical Properties

A.	Synonyms:	Dimethyl hexanedioate, hexanedioic acid dimethyl ester
B.	Molecular Formula:	$C_8H_{14}O_4$
C.	Molecular Weight:	174.22
D.	Boiling Point:	225° C at 760 mm Hg
E.	Melting Point:	10.3° C
F.	Solubility:	soluble in alcohol and ether; solubility in water limited to 2.5 to 3 percent
G.	Vapor Pressure:	<0.1 mm Hg at 20° C
H.	Reactivity:	reacts with acids, alkalies, and strong oxidants

Reference

RTECHS

II. Production and Uses

Dimethyl adipate (DMA) is synthesized by the esterification of adipic acid. DMA is part of a dibasic ester (DBE) blend that is used as a major ingredient in several paint strippers. The DMA content in DBE blends varies from about 15 to 90 percent. The other components of the DBE blends are dimethyl glutarate (DMG; $-(CH_2)_3-$) and dimethyl succinate (DMS; $-(CH_2)_2-$). The most popular DBE blends used in paint stripping

formulations contain about 90 percent DMA. The final DBE content in consumer paint strippers is usually from 20 to 50 percent.

DBE blends are also used in the coating industry to clean up polyurethane adhesives, polyurethane foams, and unsaturated polyester resins. DMA is used as a chemical intermediate and as a plasticizer in the production of paper and cellulose resins.

References

Bhooshan, B. (1989). A Preliminary Review of the Toxicity of Dimethyl Adipate. U.S. Consumer Product Safety Commission Memorandum, Washington D.C.

Jackson, H. (1991). DBE-Based Stripper Formulations In: *Proceedings of an International Conference on Reducing Risk in Paint Stripping.* Economics and Technology Division, U.S. Environmental Protection Agency, Washington, D.C.

Smith, C. (1992). Methylene Chloride: A Summary of Findings From A Consumer Use Survey, and Information on Products and Formulations. U.S. Consumer Product Safety Commission Report, Washington, D.C.

III. Toxicity

A. Human Case Reports

There are reports of blurred vision from the use of DBE-based paint strippers. Apparently, these effects occurred when the product was used under conditions of low ventilation. The DBE blends associated with the blurred vision had less than 20 percent DMA and contained higher percentages of the more volatile DMG and DMS.

B. Experimental Animal Studies

The most complete set of toxicology studies on DBE was carried out by DuPont at the Haskell Laboratory for Toxicology. They did a single dose acute study, a two week subacute study, two separate subchronic studies, a reproductive toxicity study, and a developmental toxicity study. The experiments were all done with male and female Crl:CD/BR rats exposed to DBE vapor or vapor:aerosol by inhalation. All studies used a DBE blend consisting of 66 percent DMG, 17 percent DMA, and 17 percent DMS. The staff of the U.S. Consumer Product Safety Commission (CPSC) have reviewed these studies and consider them adequate in terms of study design and data quality.

1. Single Dose and Short-term Repeated Dose Studies

This study established the lethal concentration from a 4 hour exposure to be approximately 4000 mg m^{-3}. A two week repeated dose (six hours per day, five days per week) study produced no treatment-related changes in mean body weight or histopathology of the major visceral organs at concentrations up to 1000 mg m^{-3}.

2. Subchronic Studies

The two subchronic inhalation studies investigated toxicity at different air concentrations of DBE. The first study exposed rats to DBE aerosol-vapor mixture at 160, 390, and 1000 mg m^{-3}. The second study used lower DBE vapor concentrations of 20, 76, and 390 mg m^{-3}. All animals were exposed for six hours a day, five days a week for approximately 90 days. The second study included a group of animals at each exposure level that remained unexposed to DBE for approximately six weeks post-exposure to assess recovery from any toxicity that might have occurred.

The critical toxic effect was a progressive degeneration of the nasal olfactory epithelium. Mild olfactory degeneration was found at 90 days of exposure in female and male rats exposed to 20 mg m^{-3} and above and 76 mg m^{-3} and above, respectively. The incidence, severity, and extent of the lesions increase with DBE concentration and duration of exposure. At the highest concentration (1000 mg m^{-3}), there was squamous metaplasia of over 50 percent of the olfactory epithelium in levels II and III of the nasal cavity and squamous metaplasia of the respiratory epithelium at levels I and II. Female rats were more sensitive to the nasal toxicity than males. The extent of regeneration following DBE exposure depended on the severity of the epithelial lesions. Slightly damaged epithelium underwent regeneration over a one to two week period. However, repair of severely damaged cells appeared to be minimal.

There were other adverse effects that occurred as result of subchronic DBE exposure. There was a dose-dependent decrease in liver weight beginning at 160 mg m^{-3}. Serum sodium levels were slightly decreased at 76 mg m^{-3} and above. A decrease in body weight was noted in rats exposed to the highest DBE concentration (1000 mg m^{-3}).

3. Reproductive Toxicity

A single generation reproductive effects study was conducted on male and female rats exposed to DBE concentrations up to 1000 mg m^{-3} for six hours a day, five days a week for 90 days followed by seven days a week exposure during mating, gestation, and lactation. The total study period was approximately 150 days. There were no treatment-related effects reported in the male and female fertility indices, viability index, and lactation index. There was a decrease in parental and pup weight gain and an increased incidence of delayed renal papilla development at the 1000 mg m^{-3}. A tumor (meningeal sarcoma) was found in the

olfactory bulb of the brain of a single male rat exposed to 1000 mg m^{-3}. The No Observable Adverse Effect Level (NOAEL) for maternal and reproductive toxicity was 440 mg m^{-3}.

4. Developmental Toxicity

Female rats were exposed to DBE at concentrations up to 1000 mg m^{-3} for six hours a day on days 7 to 16 of gestation. Maternal toxicity was indicated at 440 mg m^{-3} and above by significant reductions in body weight gain and food consumption. No treatment related changes were noted in fetal survival, litter size, or fetal malformations at 440 mg m^{-3} or below. There was a significant increase in the percent of litters with one or more malformed fetuses at 1000 mg m^{-3}. The NOAEL for maternal and fetal toxicity was 160 mg m^{-3} and 440 mg m^{-3}, respectively.

The developmental toxicity of DMA was examined in an earlier study of seven adipic acid esters. Groups of five pregnant Sprague Dawley rats were administered DMA by intraperitoneal injection at doses up to 0.36 ml/kg (one third the LD50) on days 5, 10, and 15 of gestation. Rats were sacrificed on day 20 of gestation. There was an increase in fetal skeletal abnormalities relative to controls at dose levels of 0.18 ml/kg and above. The no observable effect level was 0.06 ml/kg. Maternal toxicity was not assessed.

References

Keenan, C., Kelley, D., and Bogdanffy, M. (1990). Degeneration and Recovery of Rat Olfactory Epithelium Following Inhalation of Dibasic Esters *Fundam. Appl. Toxicol.* 15: 381-393.

Kelley, D., Keenan, C., and Alvarez, L. (1986). Ninety Day Inhalation Toxicity, Reproduction, and Teratology Studies with Dibasic Esters. *Toxicologist* 6: 136.

Lee, K., Valentine, R., and Bogdanffy, M. (1992). Nasal Lesion Development and Reversibility in Rats Exposed to Aerosols of Dibasic Esters. *Toxicol. Pathol.* 20: 376-393.

IV. Metabolism and Structure-Activity Relationships

The deposition and metabolism of DBE vapors in the upper respiratory tract of rats and its implications for the DBE-induced degeneration of the olfactory epithelium was extensively studied by Haskell Laboratories. DBEs are efficiently hydrolyzed by acid carboxyesterases in rodent nasal mucosa preparations to initially form the monomethyl ester and then the diacid. In vitro kinetic studies indicate that carboxyesterase activity toward DBEs increases with increasing chain length, so DMA > DMG > DMS. DMA is a much better substrate for carboxyesterase than its monomethyl adipate (MMA) and, thus, MMA is

thought to be the primary metabolite. MMA and the corresponding diacid, adipic acid (AA), are probably further metabolized to CO_2 and acetate through the usual catabolic pathways utilized for endogenous amino acids and organic acids.

Nasal olfactory mucosa has 7 to 10-fold higher carboxyesterase activity toward DBE than nasal respiratory mucosa. The olfactory mucosa from female rats has higher carboxyesterase activity than that from male rats. This may explain why female rat olfactory epithelium is particularly sensitive to DBE-induced injury. The importance of carboxyesterase for the deposition and cytotoxicity of DBE in the rodent nasal cavity was confirmed in additional studies. DMA and its monoester metabolite were shown to be cytotoxic to rat nasal explants in vitro as measured by acid phosphatase release. The cytotoxicity was dependant on carboxyesterase-mediated metabolism, which was markedly inhibited by pretreatment with a carboxyesterase inhibitor. It is hypothesized that the acid metabolites of the DBEs interfere with the tricarboxylic acid cycle in nasal tissue mitochondria. This is supported by reports that DBE significantly inhibits ATP synthesis in rat liver mitochondria.

Studies using surgically isolated upper respiratory tracts from rats show that greater than 95 percent of DMA vapor is deposited in this tissue at concentrations used in the subchronic toxicity experiments. It is likely that the efficient deposition of DMA is a result of its extensive metabolism in the rat nasal cavity. Preliminary in vitro experiments with human nasal tissue homogenates suggest that DBE metabolism in human nasal tissue is 100 to a 1000 times less active than rat nasal tissue. Comparative studies with other nasal carboxyesterase substrates and immunohistochemical analysis suggest that humans differ from rodents both in terms of enzyme substrate specificity and distribution in the nasal mucosa.

References

Bogdanffy, M., Kee, C., Hinchman, C., and Trela, B. (1991). Metabolism of Dibasic Esters by Rat Nasal Mucosal Carboxylesterase. *Drug Metab. Dispos.* 19: 124-129.

Bogdanffy, M. and Londergan, T. (1989). Inhibition of Mitochondrial ATP synthesis by Dibasic Esters in vitro. *Toxicologist* 9: 996.

Kee, C., Bogdanffy, M., Keenan, C., Keenan, K., and Resau, J. (1989). Sex and Species Differences in Metabolism of Dibasic Esters by Nasal Carboxyesterases. *Toxicologist* 9: 1139.

Morris, J., Clay, R., Trela, B., and Bogdanffy, M. (1991). Deposition of Dibasic Esters in the Upper Respiratory Tract of the Male and Female Sprague Dawley Rat. *Toxicol. Appl. Pharmacol.* 108: 538-546.

Trela, B. and Bogdanffy, M. (1991). Carboxyesterase-Dependent Cytotoxicity of Dibasic Esters in Rat Nasal Explants. *Toxicol. Appl. Pharmacol.* 107: 285-301.

Trela, B. and Bogdanffy, M. (1991). Cytotoxicity of Dibasic Esters Metabolites in Rat Nasal Explants. *Toxicol. Appl. Pharmacol.* 110: 259-267.

Trela, B., Frame, S., and Bogdanffy, M. (1992). A Microscopic and Ultrastructural Evaluation of Dibasic Esters Toxicity in Rat Nasal Explants. *Exp. Mol. Pathol.* 56: 208-218.

V. Ongoing Studies

CPSC staff is not aware of any ongoing studies on dibasic esters.

V1. Suggested Studies and Rationale for Their Recommendation

A. General Rationale

The CPSC has long been concerned about exposure to solvents during consumer use of paint strippers. In 1986, the agency determined that methylene chloride, a solvent used in the vast majority of commercial paint strippers, was a hazardous substance due its potential carcinogenicity. A labeling and enforcement policy was subsequently issued requiring warning labeling on paint strippers and other household product categories containing methylene chloride that are sold to consumers. In 1991, the CPSC staff evaluated the effectiveness of the labeling policy in reducing consumer exposure and cancer risk from use of methylene chloride-containing paint strippers. Although there was a decrease in the number of people using paint strippers and some improvements in precautionary measures that reduce exposure from that reported in the 1986 analysis, the estimated risk of cancer from the use of methylene chloride-containing paint strippers was still of concern. This finding prompted the Commission to direct its staff to acquire the data necessary to adequately assess the comparative hazard of methylene chloride-containing paint strippers with that of the major substitute formulations.

DBE-based paint strippers were identified by CPSC staff and others as a major alternative consumer formulation to the methylene chloride-containing products. There are at least five manufacturers who currently produce DBE-based paint strippers available to the consumer. Most of these are marketed as safe alternatives to the traditional methylene chloride-containing formulations despite the more limited amount of toxicity information on DBEs. DMA appears to be a reasonable surrogate compound to DBE mixtures for future toxicity studies. It is a major DBE used in paint strippers and is readily metabolized to biologically reactive metabolites by nasal carboxyesterases.

There are several areas in need of further study in order to compare the hazard of DMA (DBE), in a comprehensive manner, to that of methylene chloride and other paint stripping chemicals. These are oncogenicity/genotoxicity, sensory irritation, toxicity following subchronic dermal administration, reproductive and developmental toxicity in a

mammalian species other than the rat, neurotoxicity screening, and in vitro metabolism/toxicity studies using human upper respiratory tissue. At the urging of CPSC and the U.S. Environmental Protection Agency, the manufacturers of another paint stripping chemical, N-methylpyrrolidone, recently agreed to undertake a toxicity testing program similar to this proposal.

Reference

Nicholls, C. (1993). Briefing Package: Current Information on Methylene Chloride. U.S. Consumer Product Safety Commission Memorandum, Washington, D.C.

B. Recommended Studies

1. Short-Term Genotoxicity Tests and Oncogenicity Study

An initial battery of short-term tests should be conducted to evaluate genotoxic potential. This needs to include representative in vitro mutagenicity assays in both bacterial and mammalian cell systems (with and without the appropriate metabolic activation systems) as well as in vitro assays that detect other forms of DNA damage (e.g. unscheduled DNA synthesis, single strand breaks, etc.), and in vitro and in vivo cytogenetics tests. Clear positive results in these tests would trigger an additional battery of in vivo genotoxicity tests.

The oncogenicity study should be conducted in two mammalian species exposed to DMA by inhalation over the major portion of their life span (generally two years for rats and mice). The study design, dosing regimen, and other aspects of the protocol should be consistent with that followed by the National Toxicology Program. Because of the demonstrated cytotoxicity to nasal epithelium, it may be wise to include additional animals to evaluate cytotoxicity and cell proliferation of this tissue at various times during the course of the chronic DMA inhalation exposure. The extensive squamous metaplasia and tumor of the olfactory bulb of one animal that occurred at high doses during the subchronic inhalation study lends some experimental support to initiating the chronic inhalation study.

2. Sensory Irritation Study

The sensory irritant potential can be evaluated using a standardized test method (ASTM 981E) based on measuring the decrease in respiratory frequency in mice following short-term inhalation exposure to the chemical. The effect on respiratory rate is a reflex caused by stimulation of the trigeminal nerve system in the nasal cavity of mice. Stimulation of the trigeminal system in man is commonly associated with burning sensation in the eyes, nose and throat and, in some cases, sneezing and bradypnea. The selective uptake of DMA in the upper respiratory tract and its ability to be hydrolyzed to organic acids by nasal epithelia make this compound a candidate for sensory irritant testing.

3. Subchronic Dermal Study

A study is needed to evaluate systemic toxicity following subchronic dermal administration since one route of human exposure to DMA from the use of DBE-based paint strippers is likely to be skin contact . Testing in a species other than the rat is preferred. DBE toxicity was previously characterized in the rat following subchronic administration by inhalation. Toxicity testing in a second species usually improves the ability to extrapolate the results to humans. Water is an appropriate vehicle since DBEs emulsified in water are used in paint strippers. The rate of DMA penetration through the skin as well as its systemic bioavailability and persistence should be evaluated as part of the study protocol.

4. Reproductive and Developmental Toxicity Study

A reproductive and developmental toxicity study should be initiated in a species other than the rat. This would provide data in two species which is considered the minimum required to make a judgement that a hazard does not exist. Reproductive toxicity should be assessed in at least two generations and other aspects of the study should conform with the current EPA guidelines for reproductive and developmental toxicity testing. Dermal administration is preferred since it is a likely route of human exposure for which developmental toxicity data is lacking . However, if pilot studies indicate poor absorption or substantial skin irritancy then other routes of administration may be more appropriate. If oral administration is chosen, then the study protocol should include the necessary experiments to characterize systemic bioavailability.

5. Neurotoxicity

Experimental animal testing to screen for the existence of neurotoxicity should be performed. A repeated dose study that evaluates a battery of noninvasive neurobehavioral endpoints (functional observational battery), motor activity, and neurohistopathology is recommended. The functional observational battery and motor activity tests should be conducted at several time points (short and long term) during the exposure regimen. It may be possible to combine the neurotoxicity with the subchronic dermal toxicity experiments. Positive findings during the neurotoxicity screen may require additional testing to more fully define the toxicity.

6. Metabolism and Toxicity Studies Using Human Upper Respiratory Tissue

DMA causes nasal toxicity in rats at fairly low air concentrations. It is important to determine the concentrations that DMA might be expected to cause effects to the nose and upper respiratory tract of humans. It was shown that nasal metabolism was a critical determinant of DMA uptake and toxicity in the rat. Further studies are needed to establish the capability of human nasal and other upper respiratory tissues to absorb and metabolize DMA. Metabolism data, other physiochemical properties (e.g. air:tissue partition

coefficients) and information related to respiratory physiology/anatomy and air flow dynamics will enable predictions of the toxicologically relevant dose of DMA delivered to the human upper respiratory tract. In vitro cytotoxicity studies using human upper respiratory tissue may also be useful in extrapolating the animal data to humans.