

Justification of Dibasic Esters (DBE) Category and Overview of DBE Robust Summaries

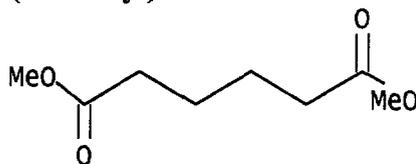
Introduction

The Dibasic Esters (DBE) Category represents three refined dibasic ester solvents: Dimethyl Adipate (DMA), Dimethyl Glutarate (DMG), Dimethyl Succinate (DMS) and the mixture of these three compounds (DBEs)¹. DBE², the primary product, is distilled to produce six DBE fractions for specialty applications (DBE-2, DBE-3, DBE-4, DBE-5, DBE-6, DBE-9). Fractions DBE-4, DBE-5, and DBE-6 are the pure dimethyl esters DMS, DMG, and DMA, respectively. Fractions DBE-2, DBE-3, and DBE-9 are atypical mixtures of the three dimethyl esters used in specialty applications. DBEs are clear, colorless liquids, having a mild, agreeable odor. They are readily soluble in alcohols, ketones, ethers, and many hydrocarbons, but are only slightly soluble in water and higher paraffins. They are used as solvents (e.g., industrial coatings, industrial cleaners, paint removers, inks), plasticizers, polymer intermediates and specialty chemical intermediates. Exposure to DBEs from these uses, specifically paint stripping, has been evaluated by the US EPA (1994).

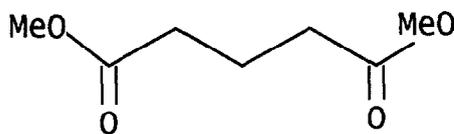
DBE is presented as a Category based upon the similarities of DMS, DMG, and DMA in structures (see structures below), physicochemical properties (Table 1), and consistent responses in ecotoxicology (Tables 3 and 4) and human health toxicology (Table 5 and 6) studies. The Category includes four Robust Summaries, the three individual dimethyl esters, DMS (CAS# 106-65-0), DMG (CAS# 1119-40-0), and DMA (CAS# 627-93-0) and the mixture DBE (CAS# 95481-62-2).

Structures of Three Dibasic (Dimethyl) Esters

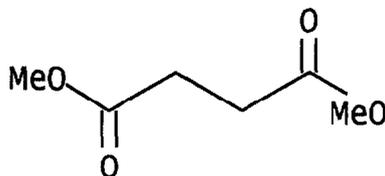
Dimethyl Adipate



Dimethyl Glutarate



Dimethyl Succinate



¹ DBEs are also referred to as DMEs (Dimethyl Esters), but DBE is designation in this document.

² A mixture of three dimethyl esters, DMA, DMG, and DMS at proportions ranging from 10-25, 55-65, and 15-25 percent, respectively.

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DMS, DMG, and DMA are highly similar in structure differing by only one alkyl carbon. These compounds are the dimethyl esters of four, five and six carbon dicarboxylic acids, succinic, glutaric and adipic acids, respectively. The close structural similarity, combined with similar test results, is the basis for the DBE Category.

Availability of Data for SIDS Data Elements

Four separate robust summaries are provided in support of the DBE Category. Table 2, below, shows that most of the SIDS data elements are addressed for one or more of the individual DBEs or the mixture. For many of the data elements, acceptable data are available for all four members of the category. Data are available for at least one chemical in the category in all 20 data elements. Data are available for all four DBEs for 14 of 20 data elements. As shown in Table 2 the robust summaries for DBE, DMS, DMG, and DMA are nearly complete.

Correlation of Physicochemical properties

Reported values for physicochemical properties are presented in Table 1 with data for DBE (column 2) and the three dibasic esters (DMS, DMG, and DMA). With the exception of melting point and partition coefficient, the values for physicochemical properties demonstrate a progressive pattern (positively or negatively correlated) related to molecular weight of the three dimethyl esters (DMS, DMG, and DMA). The DBE values are generally intermediate in the range of values for DMS, DMG, and DMA. The ranges of values observed for individual properties are small. The consistency of the relationships among these four chemicals is strong and supports the appropriate development of the DBE Category.

Correlation of Ecotoxicity Data

Ecotoxicology data are presented in Table 3. Comparison of available data for ecotoxicity shows DBEs are “slightly” to “practically non-toxic” to fish and aquatic invertebrates. LC_{50} or EC_{50} values for fish, invertebrates, and algae range from: 50-100 mg/L to 25.7 mg/L, 497 mg/L to 3,317 mg/L, and 4.4 mg/L to 11.9 mg/L, respectively. These values appear to be correlated with a gradient in solubility for the three, dimethyl esters (Table 2) and inversely correlated with the molecular weights. The data for all three ecotoxicology data elements further support the development of a DBE category.

**Table 1: Comparison of Physicochemical Properties
For DBE and Three Dibasic Esters^a**

Physicochemical Properties	DBE ^b (Mixture)	DMS (DBE-4)	DMG (DBE-5)	DMA (DBE-6)
	95481-62-2	106-65-0	1119-40-0	627-93-0
Molecular Weight (g/M)	159	146	160	174
Melting Point (°C)	-20	19	-37	8.5
Boiling Point (°C)	196-225	196	213.5- 214	230.9
Density	1.092	1.11	1.0876	1.062
Vapor Pressure (Torr @ 20°C)	0.2	0.9	0.1	<0.05
Partition Coefficient	0.19 (m)	0.19 (m)	--	1.03 (c)
Water Solubility (wt. %)	5.3	7.5	4.3	2.4
Flash point (°C)	100 (212)	94 (200)	107 (225)	119 (235)

^aAdapted from Dupont Co. (1994). Technical Information: Dibasic Esters (DBE) with the exception of Partition Coefficient that was taken from the robust summaries for the respective DBEs.

^b A mixture of three dimethyl esters, DMA, DMG, and DMS at proportions ranging from 10-25, 55-65, and 15-25 percent, respectively.

Correlation of Acute Toxicity Data

Acute toxicity studies (oral toxicity, dermal toxicity, skin irritation and eye irritation) for four test materials (DBE, DMA, DMG and DMS) were conducted by the DBE Group. These studies were conducted in the same laboratory under the same test conditions, and results are compared in Table 4, below. The results are essentially the same for dermal toxicity, skin irritation and eye irritation (BioDynamics 1992a,b,c,d,e,f,g,h,i,j,k,l,m,n,o,p). Acute oral toxicity values vary above and below the highest exposure level of 5000 mg/kg. Reported LD₅₀ values for rats from previous studies with DBE (8191 mg/kg) (Dupont Co. 1981) and DMS (>5,000 and 6892 mg/kg) (IUCLID 2000) were >5,000 mg/kg consistent with the most recent studies with the other DBE materials. The results of these studies show a high level of consistency for acute toxicity between the four chemicals in the DBE Category.

Correlation of Results from Repeated-Dose Studies

As shown in Table 5, subchronic (90-day) inhalation studies have been conducted at varying levels of exposure for all four chemicals in the DBE Category. DMG and DBE were evaluated at a range of exposure concentrations and show a similar dose response. All four DBEs have been evaluated at the nominal exposure level of 400 mg/m³ and effect levels are compared where a dose response is available (Table 6). The major effect observed for all four materials is an increase in degeneration of rat nasal epithelium. Where other effects were observed, similar patterns are observed for DMS, DMG, and DMA. The consistent pattern of response in these subchronic exposures (Table 6) indicates that a valid chemical category exists for these materials.

Table 2: Availability of Data for Each SIDS Data Element for the DBE Category Including DBE and Three Individual Dimethyl Esters

No.	SIDS Data Elements	DBE	DMA	DMG	DMS
		95481-62-2	627-93-0	1119-40-0	106-65-0
Physicochemical Properties					
1	Melting Point	√	√	√	√
2	Boiling Point	√	√	√	√
3	Vapor Pressure	√	√	√	√
4	Partition Coefficient	√	√	√	√
5	Water Solubility	√	√	√	√
6	Flashpoint	√	√	√	√
Environmental Fate					
7	Photodegradation	√	√	--	√
8	Stability in Water	--	√	--	--
9	Transport (Fugacity)	--	√	√	√
10	Biodegradation	√	√	√	√
11	Bioconcentration	--	√	√	√
Ecotoxicology					
12	Acute Toxicity to Fish	√	√	√	√
13	Acute Toxicity to Daphnia	√	√	√	√
14	Acute Toxicity to Aquatic Plants	--	√	√	√
Mammalian Toxicology					
15	Acute Toxicity				
a	○ Acute Oral Toxicity	√	√	√	√
b	○ Acute Dermal Toxicity	√	√	√	√
c	○ Acute Inhalation Toxicity	√	--	--	--
d	○ Skin Irritation	√	√	√	√
e	○ Eye Irritation	√	√	√	√
16	Repeated Dose Toxicity				
a	○ Oral	√	--	--	--
b	○ Inhalation	√	√	√	√
17	Developmental Toxicity	√	√	--	--
18	Reproductive Toxicity	√	√	√	√
19	<i>in vivo</i> Genotoxicity	√	--	√	√
20	<i>in vitro</i> Genotoxicity	√	√	√	√

Conclusions

The conclusion of this data analysis for four DBE materials (DBE, DMS, DMG, and DMA) is that a DBE Category is justified. The very consistent pattern of structural and physicochemical properties and results of biological and toxicological studies support this conclusion. The use of a DBE Category, combined with the relatively complete matrix of SIDS data elements, indicates that there is no additional testing required (See Test Plan).

Table 3: Comparison of Ecotoxicity Data for DBE and Component Dibasic Esters

Test organism	DBE	DMS	DMG	DMA
Fish (96-h LC ₅₀) (mg/L, ppm)	>18 and <24 (slightly toxic)	50-100 (slightly to practically non-toxic)	30.9 (slightly toxic)	25.7 (calc.) (slightly toxic)
<i>Daphnia magna</i> ^a (48-h EC ₅₀) (mg/L, ppm)	136; >112 and <150 (practically non-toxic)	3,317	1,275	497
Green algae (96-h EC ₅₀) (mg/L, ppm)	--	11.9	7.2	4.4

^a Two separate *D. magna* studies are available for DBE and both show highly consistent results.

Table 4: Comparison of Acute Toxicity Data for DBE and Three Individual Component Dimethyl Esters^a

Test	DBE	DMS	DMG	DMA
Rat Oral Toxicity (mg/kg)	>500 and <5,000	>500 and <5,000	>5,000	>5,000
Rabbit Dermal Toxicity (mg/kg)	>5,000	>5,000	>5,000	>5,000
Rabbit Skin Irritation (ADIS)	0.0	0.0	0.0	0.0
Rabbit Eye Irritation	Mild to Moderate	Mild to Moderate	Mild to Moderate	Moderate

^a BioDynamics (1992a,b,c,d,e,f,g,h,i,j,k,l,m,n,o,p)

Table 5: Dose Response from 90-day Inhalation Studies with Four DBEs

Exposure level (mg/m ³)	Test Materials			
	DMA ^a	DMG ^a	DMS ^a	DBE ^b
Control				
10	--	NOEC	--	--
20	--	--	--	NOAEC ^c
50	--	Significant effects	--	--
80	--	--	--	Significant effects
400	Significant effects	Significant effects	Significant effects	Significant effects

^a Dupont (2000)

^b Dupont Co. (1987), Keenan, C.M. et al. (1988), Keenan, C.M. et al. (1990)

Table 6: Response of Rats Following Exposure to 400 mg/m³ of Four DBE's for 90-days by Inhalation.

Test materials	DMA ^a	DMG ^a	DMS ^a	DBE ^b
Nominal Concentrations	390 mg/m ³	410 mg/m ³	400 mg/m ³	390 mg/m ³
Effects/endpoints				
Mortality	N	N	N	N
Food consumption	↓	↓	N	N
Food Efficiency	↓	N	N	
Mean body weight	↓	↓	N	
Mean bodyweight gain	↓	↓	N	
Clinical signs of toxicity	N	N	N	N
Clinical pathology	N	N	N	--
<i>Cell proliferation (CP)</i>				
○ Liver	↑	N	↑	↑ ^c
○ Lungs	↑	N	N	--
○ Nose Level II	↑	↑	N	--
○ Nose Level III	N	↑	↑	--
Neurobehavioral battery	N	N	N	--
Neuropathology	N	N	N	--
<i>Reproductive Endpoints</i>				
○ Sperm motility & morphology	N	N	N	--
○ Sperm count	N (increase not significantly different).	↑	↑	--
○ LH	N	↑	N	
○ FSH	N	N	N	
○ Testosterone	N	↓	N	--
○ Estradiol	N	N	↓	--
○ Progesterone	N	N	N	
○ Estrous cycle	N	N	N	--
<i>Effects on olfactory mucosa</i>				
○ Degradation & atrophy	↑	↑	↑	↑
○ Focal respiratory metaplasia	↑	↑	↑	--

^a Dupont (2000)

^b Dupont Co. (1987), Keenan, C.M. et al. (1988), Keenan, C.M. et al. (1990)

^c Observed effect based on increased liver weight without confirming cell proliferation.

References

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Bio/dynamics Inc. (1992o). Primary Dermal Irritation Study in Rabbits (DMS, Dimethyl Succinate). Submitted to Monsanto Company Reference Numbers: 92-6322, BD-92-245, October 22, 1992.

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Summary of DBE Category Robust Summaries

Dibasic esters (DBEs) are a solvent mixture of dimethyl succinate (DMS), dimethyl glutarate (DMG), and dimethyl adipate (DMA) or refined fractions of individual dimethyl esters. Physicochemical properties have been summarized as part of the Category Justification (above). DBEs are readily biodegradable with biodegradation half-lives of a few days. Model calculations indicate that DBEs photodegrade with half-lives of a few days to a few weeks. In water DBEs are predicted to be hydrolytically stable (half-life of > 2 years), but have a low potential for bioconcentration in aquatic organisms.

DBEs have very low acute oral toxicities with LD₅₀s in rats generally > 5,000 mg/kg (Category IV classification) with two exceptions reported as >500 and <5,000 mg/kg b.wt. for DBE (the mixture) and DMS, indicating possible Category III classification. By skin absorption, DBEs have a low order of acute toxicity to rabbits with dermal LD₅₀s of >5,000 mg/kg (Category IV). Based upon the most recent GLP studies DBEs are not considered to produce primary dermal irritation as defined in EPA Guidelines and are classified as Category IV. Earlier studies did show moderate irritation in one of six rabbits, but these results were not repeated in later studies. All four DBE materials are considered to produce eye irritation as defined by EPA Guidelines. Mild to moderate irritation involving the cornea was observed in rabbits with recovery by 7 days. This is consistent with Category III classification. DBEs are not skin sensitizers, and are not Class B poisons via skin or inhalation exposures. DBE is slightly toxic by inhalation with 1- and 4-hour LC₅₀s in rats of > 10.7 and > 11 mg/L, respectively. In subchronic inhalation studies with all four DBEs, degeneration of the olfactory epithelium of the nose was observed. This change in the nasal tissues is related to enzymatic hydrolysis of DBE within the nasal cavity. However, risk to human nasal tissue due to DBE toxicity is likely to be reduced when compared to rats since DBEs are hydrolyzed more slowly in humans. No information is available on the carcinogenic potential of DBEs. A range of studies with DMS, DMG, DMA and DBE did not produce genetic damage in animals or bacterial cell cultures. DBE was positive in one study with cultured mammalian cells, but the positive findings were not apparent when the assay was repeated. Testing in rats indicates DBEs are not developmental or reproductive toxicants. In aquatic organisms, DBEs are "slightly" to "practically non-toxic" in fish and aquatic invertebrates.

Test Plan for the Dibasic Esters Category

No.	SIDS Data Elements	Data Available	Data Acceptable	Testing Required
		Y/N	Y/N	Y/N
Physicochemical Properties				
1	Melting Point	Y	Y	N
2	Boiling Point	Y	Y	N
3	Vapor Pressure	Y	Y	N
4	Partition Coefficient	Y	Y	N
5	Water Solubility	Y	Y	N
6	Flash Point	Y	Y	N
Environmental Fate				
7	Photodegradation	Y	Y	N
8	Stability in Water	Y	Y	N
9	Transport (Fugacity)	Y	Y	N
10	Biodegradation	Y	Y	N
11	Bioconcentration	Y	Y	N
Ecotoxicology				
12	Acute Toxicity to Fish	Y	Y	N
13	Acute Toxicity to Daphnia	Y	Y	N
14	Acute Toxicity to Aquatic Plants	Y	Y	N
Mammalian Toxicology				
15	Acute Toxicity	Y	Y	N
a	Acute Oral Toxicity	Y	Y	N
b	Acute Dermal Toxicity	Y	Y	N
c	Acute Inhalation Toxicity	Y	Y	N
d	Skin Irritation	Y	Y	N
e	Eye Irritation	Y	Y	N
16	Repeated Dose Toxicity	Y	Y	N
a	Oral	Y	Y	N
b	Inhalation	Y	Y	N
17	Developmental Toxicity	Y	Y	N
18	Reproductive Toxicity	Y	Y	N
19	<i>in vivo</i> Genotoxicity	Y	Y	N
20	<i>in vitro</i> Genotoxicity	Y	Y	N

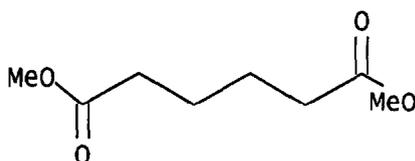
Robust Summaries for Dibasic Ester Solvents: Dibasic Ester Mixture (DBE)

1. Substance Information

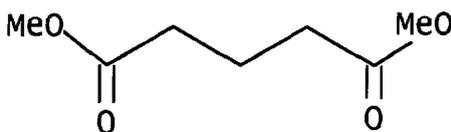
- 1.1. Chemical Name:** Dibasic Esters (DBE)¹
1.2. CAS Registry No: 95481-62-2
1.3. Component CAS Nos.: Dimethyl adipate: 627-93-0
Dimethyl glutarate: 1119-40-0
Dimethyl succinate: 106-65-0

1.4. Structural Formula:

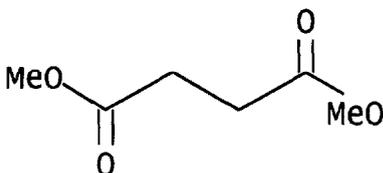
Dimethyl Adipate



Dimethyl Glutarate



Dimethyl Succinate



1.5. Other Names:

Estrasol, mixture of dimethyl adipate, dimethyl succinate, and dimethyl glutarate hexanedioic acid, dimethyl ester, mixture with dimethyl butanedioate and dimethyl pentanedioate, butanedioic acid, dimethyl ester mixture with dimethyl hexanedioate and dimethyl pentanedioate, dibasic acid ester (dimethyl succinate/glutarate/adipate), dimethyl succinate/glutarate/adipate, pentanedioic acid, dimethyl ester, mixture with dimethyl butanedioate and dimethyl hexanedioate, dibasic dimethyl esters

¹ DBE is a mixture of the dimethyl esters of adipic (10-25%), glutaric (55-75%), and succinic (19-26%) acids. There are also traces of methanol, water, and <10 ppm of hydrogen cyanide.

of adipic acid, succinic acid, and glutaric acid.

2. Physical-Chemical Properties

2.1. Melting Point

Value: ~ - 20°C
Decomposition: No Data
Sublimation: No Data
Method: No Data
GLP: No Data
Reliability: Not assignable because limited study information was available
Reference: Dupont Co (2001). Material Safety Data Sheet FE000011

2.2. Boiling Point

Value: 196-225°C
Decomposition: No Data
Pressure: 760 mm Hg
Method: No Data
GLP: No Data
Reliability: Not assignable because limited study information was available.
Reference: Dupont Co (2001). Material Safety Data Sheet FE000011

2.3. Density

Value: 1.092
Temperature: 20°C
Method: No Data
GLP: No Data
Reliability: Not assignable because limited study information was available
Reference: Dupont Co (2001). Material Safety Data Sheet FE000011

2.4. Vapor Pressure

Value: 0.2 mmHg
Temperature: 20°C
Decomposition: No Data
Method: No Data
GLP: No Data
Reliability: Not assignable because limited study information was available
Reference: Dupont Co. (2001). Material Safety Data Sheet FE000011

2.5. Partition Coefficient (log K_{ow})

Value:	0.19 (measured)
Temperature:	25°C
Decomposition:	No Data
Method:	No Data
GLP:	No Data
Reliability:	Not assignable because limited study information was available.
Reference:	IUCLID (2000). IUCLID Dataset. European Chemicals Bureau, European Commission. Datasheet for dibasic esters, 2/18/00 [Subsequently referenced as IUCLID (2000)]

Additional References for Partition Coefficient

0.37 @ 25°C (calculated using CLOGP version 3.42). IUCLID (2000)

2.6. Water Solubility

Value:	5.3 wt% in water
Temperature:	20°
PH/Pka:	No Data
Method:	No Data
GLP:	No Data
Reliability:	Not assignable because limited study information was available
Reference:	Dupont Co (2001). Material Safety Data Sheet FE000011

Additional References for Water Solubility

121-131 g/L @ 25°C. IUCLID (2000)

3. Environmental Fate

3.1. Photodegradation

Degradation:	Ca. 50% after 24.8 days
Rate Constant:	4.32×10^{-13} cm ³ /molecule/sec
Sensitizer:	OH
Method:	Calculated using ATMOSPHERIC OXIDATION PROGRAM (AOP), version 1.51 Syracuse Research Corporation.
GLP:	No Data

Reliability: Not assignable because limited study information was available
Reference: IUCLID (2000)

3.2. Stability in Water

Concentration: No Data
Half-life: No Data
Percent Hydrolyzed: No Data
Method: No Data
GLP: No Data
Reliability: No Data
Reference: No Data

3.3. Transport (Fugacity)

Media: No Data
Distributions: No Data
Adsorption: No Data
Coefficient: No Data
Volatility: No Data
Method: No Data
GLP: No Data
Reference: No Data
Reliability: No Data

3.4. Biodegradation

Type: Shake Flask Method
Value: Half-life ranged from 2.5 to 3.2 days
Method: DBE was subjected to microbial biodegradation testing using the shake-flask method.
GLP: No
Test Substance: 99% total dibasic esters (DBE)
Results: The half-life for the high concentration of DBE (10 mg C/L) was 2.5 days while at the low DBE concentration (5 mg C/L), the half-life was 3.2 days. In both cases, 36% of the theoretical cumulative percent CO₂ was respired by the mixed microbial system.
Reliability: Moderate (scientifically defensible or guideline method, non-GLP)
Reference: Dupont Co. (1982). Unpublished Data, Haskell Laboratory Report No. 698-82.

Additional References for Biodegradation:

IUCLID (2000)

3.5. Bioconcentration

Value: No Data
Method:
GLP:
Reference:
Reliability:

4. Ecotoxicity

4.1. Acute Toxicity to Fish

Type: 96-h LC₅₀
Species: *Pimephales promelas* (Fathead Minnow)
Value: >18 ppm (v/v) and <24 ppm (v/v)
Method: DBE toxicity to fathead minnows was evaluated using all-glass exposure chambers with 10 liters of water and 10 fish per chamber under static conditions. Exposures were monitored for temperature, pH, dissolved oxygen and other water quality parameters. The system was maintained as 16 hrs. light/ 8 hrs. dark photoperiod without aeration. Exposure levels were reported as nominal. Mortality counts and observations every 24 hours.
GLP: No
Reliability: Moderate (Scientifically defensible or guideline method, nominal concentrations, non GLP).
Reference: Dupont Co. (1982). Unpublished Data, Haskell Laboratory Report No. 282-82.

Additional References for Acute Toxicity to Fish:

96-hr LC₅₀ 50-100 ppm (*Brachydanio rerio*). IUCLID (2000)

4.2. Acute Toxicity to Invertebrates

Type: 48-h EC₅₀ Study
Guideline: EPA. (1987). User's Guide: procedures for conducting *Daphnia magna* toxicity bioassays. EPA/600/8-87/011.
Species: *Daphnia magna* (Water Flea).
Value: 48-h EC₅₀ is 136 ppm DBE (DME)
Method: *Daphnia* were exposed to DBE (DME) for 48 hours under static conditions in replicate glass chambers with 250 ml volume with exposure concentrations ranging from 0 to 300 ppm. Exposures were monitored for temperature, pH, dissolved oxygen and other water quality parameters. The system was

maintained at 20°C and 24 hours light. as Exposure levels were measured at initiation and at 48 hours.

GLP: Yes

Results: 48-h EC₅₀ is 136 ppm DBE (DME), based on non-linear interpolation of the data. All measured concentrations exceeded nominal values for all exposure levels. Environmental and water quality parameters were within acceptable ranges.

Reliability: High (Scientifically defensible or guideline method, measured concentrations, GLP).

Reference: Monsanto. (1992). Determination of acute toxicity of mixed dimethyl esters (DME) to *Daphnia magna*. MO-92-9961.

Type: **48-h EC₅₀**

Species: *Daphnia magna* (Water Flea).

Value: >112 ppm (v/v) and <150 ppm (v/v)

Method: *Daphnia* were exposed to DBE under static conditions in replicate glass chambers with 250 ml volume for 48 hours. Exposures were monitored for temperature, pH, dissolved oxygen and other water quality parameters. The system was maintained as 16 hrs. light/ 8 hrs. dark photoperiod without aeration. Exposure levels were reported as nominal.

GLP: No

Reliability: Moderate (Scientifically defensible or guideline method, nominal concentrations, non GLP).

Reference: Dupont Co. (1982). Unpublished Data, Haskell Laboratory Report No. 308-82.

4.3. Acute Toxicity to Aquatic Plants

Type: No Data

Species: No Data

Value: No Data

Method: No Data

GLP: No Data

Reliability: No Data

Reference: No Data

5. Mammalian Toxicity

5.1. Acute Toxicity

Type: **Acute Oral LD50**

Guideline: EPA (40 CFR) 798.1175
Species/strain: Rats/Crl:CD®
Sex: Male
Value: LD50 (rats) = 8191 mg/kg
Method: No Data
GLP: No Data
Reliability: Moderate (Scientifically defensible or guideline method, non-GLP)
Reference: Dupont Co. (1981). Unpublished Data, Haskell Laboratory Report No. 646-81.

Type: Oral LD₅₀
Guideline: EPA (40 CFR) 798.1175
Species/Strain: Rats/Crl:CD®(CD)BR
Value: > 500 mg/kg b. wt. and < 5000 mg/kg b. wt.
Method: DBE was administered a single dose via oral gavage at two dose levels (500 and 5000 mg/kg b.wt.) with a 14 day observation period. Test material was administered as received. 5 females and 5 males were used for each dose level. Necropsies were performed on animals that died and that survived to 14 days post-administration.
GLP: Yes
Test Substance: 99.5% total dibasic esters (DBE, DME) (12% DMA, 62% DMG, and 26% DMS)
Results: At 500 and 5,000 mg/kg b. wt. mortality rates were 0/10 and 8/10, respectively. The mortalities occurred in the first two days. In the 500 mg/kg treatment level the only abnormality was decreased food consumption and fecal volume for one of ten animals on days 2 and 3. In the 5,000 mg/kg treatment group observations included: yellow-cervical staining; decreased activity and lethargy, hunched appearance and labored breathing. Surviving animals showed no abnormalities and necropsies of dead animals revealed discoloration in the lungs (edema in one individual). Other observations in single animals were reddened pancreas and yellow fluid in the stomach.
Reliability: High (Scientifically defensible or guideline method, GLP)
Reference: Bio/dynamics Inc. (1992). Acute Oral Toxicity in Rats (DME, Dimethyl Ester). Submitted to Monsanto Company Reference Numbers: 92-6316, BD-92-242, October 22, 1992.

Remarks: This study was designed as one in a series of four acute studies conducted with each of four different dibasic ester test materials: DBE (mixture), DMA, DMG, and DMS.

Additional References for Acute Oral Toxicity:

ALD² (rat) = 11,000 mg/kg (Dupont Co, 1965).

ALD (rat) = 17,000 mg/kg (Dupont Co, 1978).

Dupont Co. (1965). Unpublished Data, Haskell Laboratory Report No. 88-65.

Dupont Co. (1978). Unpublished Data, Haskell Laboratory Report No. 219-78.

Dupont Co. (1981). Unpublished Data, Haskell Laboratory Report No. 646-81.

DBE is not a Class B poison under Dept. of Trans. Regulations

Dupont Co. (1977). Unpublished Data, Haskell Laboratory Report No. 686-77.

A method was developed for measuring the effects of toxicants on visual function. The method employed visual evoked potentials (VEPs) elicited with patterned and flashed visual stimuli as measures of the effects of toxicants on visual function. DBE when administered orally by gavage at doses of 20, 200, or 2000 mg/kg failed to alter the pattern of VEPs of rats. A similar response was seen in rats administered DBE by inhalation and by nasal drops with and without a carboxylesterase inhibitor.

Boyes, W.K., and H.K. Hudnell (1993). U.S. EPA Report dated 12-21-93 (C-2592).

Type:	Dermal LD50
Guideline:	EPA (40 CFR) 798.1100
Species/Strain	New Zealand White Rabbits
Value:	> 5000 mg/kg b. wt.
Method:	DBE was administered a single dose applied directly to the skin over a 12 x 14 cm area approximating 10% of the body surface. Contact with excess material was maintained for 24 hours and animals were observed for 14 days after initiation of dose. A total of 5 females and 5 males were used for this study. Test material was administered as received. Study was

² Approximate Lethal Dose

designed as one in a series of four acute studies conducted with each of four different dibasic ester test materials: DBE (mixture), DMA, DMG, and DMS.

GLP: Yes

Test Substance: 99.5% total esters (DME) (12% DMA, 62% DMG, and 26% DMS)

Results: All animals survived after dermal treatment at 5,000 mg/kg b. wt. Animals gained weight during 7 days post-treatment, but all animals lost weight or remained stable from day 7 to 14 post-treatment. All animals were free of systemic toxicity throughout the study and no abnormalities were observed during post-mortem macroscopic observation. However, one animal exhibited fecal staining (days 2 and 3), and one animal exhibited reddening at the site of application.

Reliability: High (Scientifically defensible or guideline method, GLP)

Reference: Bio/dynamics Inc. (1992). Acute Dermal Toxicity in Rabbits (DME, Dimethyl Ester). Submitted to Monsanto Company Reference Numbers: 92-6317, BD-92-242, October 22, 1992.

Remarks: This study was designed as one in a series of four acute studies conducted with each of four different dibasic ester test materials: DBE (mixture), DMA, DMG, and DMS.

Additional References for Acute Dermal Toxicity:

LD50 (rabbits) = > 2250 mg/kg. b.wt.

Dupont Co. (1981). Unpublished Data, Haskell Laboratory Report No. 634-81.

LD50 (rabbits) = >5000 mg/kg b.wt. IUCLID (2000)

DBE did not produce deaths in rabbits at doses up to 3400 mg/kg. No toxic or pathologic signs were observed.

Dupont Co. (1965). Unpublished Data, Haskell Laboratory Report No. 88-65.

Type: Inhalation 4-hour LC50
Species/strain: Rats/Crl:CD®(CD)BR
Value: 4-hour LC50 (rat) = > 11 mg/L.

Method: Groups of five male and five female rats were exposed nose-only for a single 4-hour period to aerosol/vapor mixtures of DBE in air. Possible ocular effects were evaluated in one group of four male rats exposed whole-body. Indirect ophthalmoscopy was used at 30 minutes and at 1 day following exposure. Animals were observed for clinical signs during a 14-d observation period.

GLP No

Test Substance: 99% total dibasic esters (DBE, DME) (17% DMA, 66% DMG, and 16.5% DMS)

Results: No deaths occurred following exposure to DBE at concentrations as high as 11 mg/L, the highest concentration tested. Clinical signs of toxicity noted either during or immediately after exposure to DME concentrations of 5.6 mg/L or greater included red ocular or nasal discharges, lethargy, labored breathing, or hunched posture. One day after exposure, rats exhibited slight to severe weight loss and some rats had red nasal, ocular, or oral discharges, wet, yellow-stained perineum, and hunched posture. With the exception of sporadic, slight to moderate weight losses in some rats during the first or second weeks of the recovery period, all clinical signs were transient and had resolved by study day four. Ophthalmologic examinations revealed only mild chemosis (swelling or edema) in the bulbar conjunctiva of the rats from the 5.6 mg/L of DBE .

Reliability: Moderate (Scientifically defensible or guideline method, non-GLP).

Reference: Dupont Co. (1990). Unpublished Data, Haskell Laboratory Report No. 453-90.

Remarks: This study was designed as one in a series of four acute studies conducted with each of four different dibasic ester test materials: DBE (mixture), DMA, DMG, and DMS.

Additional References for Acute Inhalation Studies:

4-hour ALC³ (rat) = 4.1 mg/L (Dupont Co, 1977)
 4-hour ALC (rat) = 4.5 mg/L (Dupont Co 1978)
 4-hour ALC (rat) = 6.1 mg/L (Dupont Co, 1965)

³ Approximate Lethal Concentration

In these studies, clinical signs of toxicity during exposure included labored respiration, eye irritation, and a red-tinged nasal discharge. The rats recovered within two days after exposure.

Dupont Co. (1977). Unpublished Data, Haskell Laboratory Report No. 535-77.

Dupont Co. (1978). Unpublished Data, Haskell Laboratory Report No. 471-78.

Dupont Co. (1965). Unpublished Data, Haskell Laboratory Report No. 88-65.

1-hour LC50 (rat) = > 10.7 mg/L. No deaths occurred in a group of 10 rats exposed for one hour to 10.7 mg/L of an aerosol of DBE. The mass medium diameter of the aerosol was 5.4 mm. No signs of toxicity were observed.

Dupont Co. (1980). Unpublished Data, Haskell Laboratory Report No. 436-80.

Dupont Co. (1990). Unpublished Data, Haskell Laboratory Report No. 453-90.

Eye Irritation and Ocular effects:

Mild Ocular irritation was observed one hour post-exposure following a four-hour inhalation exposure to 15 or 60 ppm of DBE. No Changes in Anterior Chamber Depth (ADC) were observed.

Dupont Co. (1992). Unpublished Data, Haskell Laboratory Report No. 362-92.

Inhalation exposure at 60 ppm for up to one hour failed to alter visual evoked potentials (VEPs) in rats.

Boyes, W.K., and H.K. Hudnell (1993). U.S. EPA Report dated 12-21-93 (C-2592).

Olfactory Effects:

Lee, K.P. et al. (1992a). The Toxicologist, 21(1):398 (Abstract 1571).

Lee, K.P. et al. (1992b). Toxicol. Pathol., 20(3, Part 1):376-393.

Morris, J.B. et al. (1991) Toxicol. Appl. Pharmacol., 108(3):538-546.

Type:	Primary Dermal Irritation
Guideline:	EPA (40 CFR) 798.4470
Species/Strain	New Zealand White Rabbits
Value:	Average Dermal Irritation Score (ADIS) was 0.0

Method: DME (DBE) was administered to six animals (3 female/3 male) as a single dose applied directly to two 1 x 1 inch areas of skin on the back and held in place with semi-occlusive dressings for 4 hours. Animals were observed for subsequent 3 days and treated areas were observed at 30 minutes and 24, 48, and 72 hours. Test material was administered as received.

GLP: Yes

Test Substance: 99.5% total dibasic esters (DBE, DME) (12% DMA, 62% DMG, and 26% DMS)

Results: The ADIS for DME is 0.0. No irritation was observed with the exception of "barely perceptible" erythema in one of two treated areas in one animal at 0.5 hours after removal. This material would probably not be considered to produce dermal irritation as defined in EPA Guidelines.

Reliability: High (Scientifically defensible or guideline method, GLP).

Reference: Bio/dynamics Inc. (1992). Primary Dermal Irritation Study in Rabbits (DME, Dimethyl Ester). Submitted to Monsanto Company Reference Numbers: 92-6318, BD-92-242, October 22, 1992.

Remarks: This study was designed as one in a series of four acute studies conducted with each of four different dibasic ester test materials: DBE (mixture), DMA, DMG, and DMS.

Additional References for Dermal Irritation:

Pure DBE (100%) and a 10% emulsion in water were tested on the skin of male and female guinea pigs. During the primary irritation phase, no dermal irritation was observed. DBE did not produce delayed hypersensitivity or allergic reactions (Dupont Co, 1989a). DBE was evaluated for acute skin irritation in three male and three female rabbits. One rabbit exhibited mild erythema by 24 hours following treatment. Two rabbits exhibited moderate erythema within 48 hours of treatment and one of these had slight edema. By 72 hours, one rabbit exhibited severe erythema with fissuring of the skin and slight edema. No moderate to mild erythema and no edema were observed in the other five rabbits at 72 hours after treatment (Dupont Co, 1989b).

Dupont Co. (1989a). Unpublished Data, Haskell Laboratory Report No. 71-89.

Dupont Co. (1989b). Unpublished Data, Haskell Laboratory Report No. 77-89.

To determine if dermal exposure to DBE could cause eye irritation or other ocular effects, a group of four rabbits was dermally administered 50 ml (20 mg/kg) of DBE. No DBE-related abnormalities were observed in the cornea, lens, or retina by indirect ophthalmoscopy. A slight, but not statistically significant, increase in the anterior chamber depth (ACD) in the eye (3-6%) was observed. No changes in corneal thickness were observed. In an attempt to reproduce this effect on ACD, two other groups of four rabbits were dermally administered 200 ml (80 mg/kg) of DBE. No changes in ACD were noted in one study, while a minimal (2.3%) increase was noted in the other (Dupont Co, 1992).

Dupont Co. (1992). Unpublished Data, Haskell Laboratory Report No. 362-92.

IUCLID (2000)

DBE is not a Class B poison under Dept. of Trans. Regulations when tested by the skin absorption route.

Dupont Co. (1977). Unpublished Data, Haskell Laboratory Report No. 834-77.

Dupont Co. (1978). Unpublished Data, Haskell Laboratory Report No. 127-78.

Type:	Eye Irritation
Guideline:	EPA (40 CFR) 798.4500
Species/Strain	New Zealand White Rabbits
Value:	DME (DBE) produced mild to moderate, transient ocular irritation.
Method:	A single ocular administration of DBE (0.1 ml) was applied to 3 male and 3 female adult rabbits followed by observations at 1, 24, 48, 72 hours and 7 and 10 days. The observation period continued up to 10 days or until no signs of irritation were present. The cornea, iris and conjunctivae were observed and lesions were graded. Test material was administered as received.
GLP:	Yes
Test Substance:	99.5% total esters (DBE) (12% DMA, 62% DMG, and 26% DMS)
Results:	DBE produced mild to moderate, transient ocular irritation. This material would be considered to produce eye irritation as defined in the EPA Guidelines. All six animals exhibited slight to moderate

irritation of the conjunctivae (redness, chemosis, and discharge) and three exhibited iridial changes. Three animals exhibited slight changes in opacity or ulceration of the cornea. One animal had no positive scores throughout the study, four of six animals were clear of irritation within 48 hours, and the remaining animal was clear by 7 days.

Reliability: High (Scientifically defensible or guideline method, GLP).

Reference: Bio/dynamics Inc. (1992). Primary Eye Irritation Study in Rabbits (DME, Dimethyl Ester). Submitted to Monsanto Company Reference Numbers: 92-6319, BD-92-242, October 22, 1992.

Remarks: This study was designed as one in a series of four acute studies conducted with each of four different dibasic ester test materials: DBE (mixture), DMA, DMG, and DMS.

Additional References for Acute Eye Irritation:

Instillation of 0.1 ml of DBE into the eyes of rabbits produced minimal conjunctival irritation. Prompt washing reduced the degree of irritation. All treated eyes had returned to normal 24 hours after treatment.

Dupont Co. (1974a). Unpublished Data, Haskell Laboratory Report No. 108-74.

Instillation of 0.1 ml of a 1% aqueous DBE solution into a rabbit eye produced a localized area of slight corneal opacity and transient conjunctival irritation. The treated eyes were normal three days after.

Dupont Co. (1974b). Unpublished Data, Haskell Laboratory Report No. 483-74.

Instillation of 0.1 ml of DBE into the eyes of rabbits produced mild to slight clouding of the cornea, which became slight in eight days. Immediate washing decreased the clouding.

Dupont Co. (1980). Unpublished Data, Haskell Laboratory Report No. 858-80.

A crude sample of DBE containing 21% methanol produced severe corneal opacity, moderate iritis, and severe conjunctival irritation in rabbits. The injury did not appear to be reversible and washing with tap water or a 25% propylene glycol solution did not significantly reduce the injury. This material is not typical of the DBE used as an industrial solvent.

Dupont Co. (1978). Unpublished Data, Haskell Laboratory Report No. 120-78.

DBE (10 ml) was instilled into the rabbit eye. Mild corneal opacity was observed in 2/4 of the rabbits. This opacity cleared in one rabbit by two days after treatment. No changes in the anterior chamber depth were found.

Dupont Co. (1992). Unpublished Data, Haskell Laboratory Report No. 362-92.

Imsol R (an ICI equivalent of DBE) was instilled into the eyes of groups of three rabbits at 0.01, 0.03, and 0.1-ml. Moderate initial pain was noted in all the rabbits administered 0.01 ml and evidence of corneal anesthesia was seen in two rabbits, which persisted for up to 1 hour after treatment. All the rabbits showed conjunctival effects, ranging from slight redness to moderate redness and ocular discharge, persisting up to eight days after treatment. Corneal or iridal effects were not seen in 2/3 rabbits, but the third showed slight to moderate corneal opacity between days 3 and 8, reaching a maximum on days 4-5. Significant corneal swelling (44%) was observed in this rabbit with the maximum response on day one. Corneal swelling and corneal surface irregularity persisted in this rabbit up to day eight. Moderate initial pain and corneal anesthesia was again evident in the rabbits administered 0.03 ml, which was of a similar duration as seen in the 0.01 ml group. Conjunctival effects of slight to moderate redness, slight chemosis, and slight to severe discharge were observed, with the highest response on day three and full regression by day seven. Evidence of corneal opacity was apparent until day seven, with slight iritis from day two to day six. Significant corneal swelling (25%) was observed on day two, again accompanying signs of corneal surface irregularity. In the group administered 0.1 ml, a similar pattern of pain reaction, and corneal anesthesia was noted. Conjunctival effects (slight to moderate redness, slight chemosis, and slight to moderate discharge) persisted up to day 10. Slight to moderate corneal opacity covering up to the entire area of the cornea occurred up to day six, with the maximum response on days 3-4. Significant corneal swelling (53%) was seen on day two. All indications of ocular irritancy had regressed by day 10.

ICI, Ltd. (1991). Central Toxicology Laboratory, Report CTL/T/2750 (J-8948).

5.2. Repeated Dose Toxicity

Type:	Oral Gavage
Species/Strain	Sprague-Daley (CD) rats
Sex/Number	10/sex/dose

Exposure
Period: One month
Frequency
of Treatment: Daily, seven days per week.
Levels: 0, 100, 300, and 1,000 mg/kg/day
Method: Groups of 10 animals/sex/dose were dosed daily for one month via oral gavage at 0, 100, 300, and 1,000 mg/kg/day. The test material DBE (DME) (mixture of 12% DMA, 62% DMG, and 26% DMS; 99.5% total esters) was administered in corn oil with corn oil control. Concentrations were verified using gas-liquid chromatography. Water and food were available *ad libitum*. In-life observations included: mortality and other signs of toxicity, body weight, and food consumption. Clinical observations included: body weights, food consumption, and clinopathological measurements. All animals were necropsied at test termination and tissues were retained.

GLP: Yes
Test Substance: DBE (DME) (99.5% total dibasic esters) (mixture of 12% DMA, 62% DMG, and 26% DMS)

Results: Analytical confirmation of dose solutions showed from 0.0 to 8.0% below target concentrations. Relative to control animals there were no significant differences observed in treated group mean body weights or cumulative body weight gains, no unscheduled mortalities, no treatment related results from ophthalmoscopic examination, no treatment-related changes in food consumption, clinical signs, or gross or microscopic pathology. The one exception is a small decrease in urine pH in males (non significant) and female at 1,000 mg/kg/day dose level. The authors stated this could have been due to excretion of test material, but there was no evidence on any direct or indirect effect on the urinary system. The 1,000 mg/kg/day dose level was considered an NOAEL.

Reliability: High because scientifically defensible or guideline, and GLP
Reference: Monsanto. (1992). One-Month Gavage Study of DME (Dimethyl Esters) in Sprague Dawley Rats. Report # MSL-12509

Type: **90-d Inhalation Study**

Species: Rats/Crl:CD®(CD)BR
 Value: No No-Effect Level
 Method: Groups of 10 male and 10 female rats were exposed six hours a day, five days a week, for approximately 14 weeks to DBE vapor concentrations of 160 or 400 mg/m³ or to 1000 mg/m³ of a DBE aerosol-vapor mixture.

GLP: Yes
 Test Substance: 99.5% total esters (DBE) (16.5% DMA, 66.6% DMG, 16.9%).

Results: Histopathological evaluation of nasal tissues showed degeneration of the olfactory epithelium in all DBE-exposed groups. The effect was minimal in the 160 mg/m³ group and of mild to moderate severity in the 400 and 1000 mg/m³ groups. Other than the nasal lesions, histopathological examination showed no deleterious effects in DBE-exposed rats at any tested concentration. Other effects of DBE exposure included dose-dependent decreased liver/body weight ratios in male and female rats in the 400 and 1000 mg/m³ groups, and slightly increased lung/body weight ratios and slightly depressed body weights in male and female rats in the 1000 mg/m³ group. A no-effect exposure concentration was not demonstrated.

Reliability: High (Scientifically defensible or guideline method, GLP)

References: Dupont Co. (1986). Unpublished Data, Haskell Laboratory Report No. 194-86.
 Kelly, D.P. et al. (1986). The Toxicologist, 6(1):136 (Abstract 551).

Type: **90-day Inhalation**
 Species/Strain: Rats/ Crl:CD®(SD)IGS BR
 Sex/Number: 40 male and 40 female/treatment level
 Route of Administration: inhalation
 Exposure Period: 13 wk
 Frequency Of treatment: 6 hours/day, 5 days/wk
 Exposure levels: 20,76, or 390 mg/m³ of DBE.

Type: **90-d Inhalation Study**
 Species: Rats/Crl:CD®(CD)BR
 Value: NOEC = 20 mg/m³ in males rats relative to changes in the olfactory mucosa.

Method: Ten rats per group were sacrificed after approximately seven weeks of exposure, and 20 rats per group were sacrificed after approximately 13 weeks of exposure. The remaining 10 rats in each group were sacrificed after a recovery period of six weeks. Gross necropsy and nasal histopathological examinations were conducted on all control and DBE-exposed rats.

GLP: Yes

Test Substance: 99.5% total esters (DBE) (16.5% DMA, 66.6% DMG, 16.9% DMS)

Results: Degeneration of the olfactory epithelium was observed in male and female rats that were exposed to 76 or 390 mg/m³ of DBE for seven weeks. After 13 weeks of exposure, there was degeneration of the olfactory epithelium in female rats in all exposure groups and in male rats exposed to 76 or 390 mg/m³. At the end of the 6-week recovery period, DBE exposure-related effects were still visible in affected groups, and histological changes were compatible with repair of the olfactory mucosa. During the exposure period, clinical signs in DBE-exposed rats were similar to those of the controls. However, female rats exposed to 390 mg/m³ of DBE had slightly depressed rates of weight gain compared to controls during the exposure period. During the recovery period the weight-gain rate of these rats was similar to those of the controls. At the end of the exposure period, absolute liver weights in female rats exposed to 390 mg/m³ were decreased compared to those of the controls. This effect was not present in rats at the end of the recovery period. In the absence of corroborative histopathological findings, the biological significance of this effect is not known. A slight decrease in serum sodium concentration was observed in male and female rats exposed to 76 or 390 mg/m³ of DBE for 13 weeks. After the six-week recovery period, sodium concentrations in the 390 mg/m³ group remained slightly low, but the sodium concentrations in the other affected groups were no longer different from those of the control group. The slightly decreased sodium concentrations were considered to

be of minimal biological significance. A no-effect exposure concentration of 20 mg/m³ was demonstrated in male rats for the olfactory epithelial degeneration observed. A no-effect concentration was not demonstrated in female rats.

Reliability: High (Scientifically defensible or Guideline, GLP)
References: Dupont Co. (1987). Unpublished Data, Haskell Laboratory Report No. 312-87.
Keenan, C.M. et al. (1988). The Toxicologist, 9(1):284 (Abstract 23).
Keenan, C.M. et al. (1990). Fundam. Appl. Toxicol., 15(2):381-393.

Additional References for Repeated Dose Inhalation:

Groups of 10 rats were exposed six hours a day, five days a week, for two weeks to 0.1, 0.3, or 0.9 mg/L of DBE. The exposed rats were indistinguishable from controls in terms of growth and histopathological examination.

Dupont Co. (1981). Unpublished Data, Haskell Laboratory Report No. 244-81.

Dupont Co. (1981). Unpublished Data, Haskell Laboratory Report No. 815-81.

Morris, J.B. et al. (1991) Toxicol. Appl. Pharmacol., 108(3):538-546.

5.3. Developmental Toxicity

Type:	Teratogenicity
Species/Strain:	Rats/Crl:CD [®] (SD)BR
Sex/Number:	24 female (pregnant)
Route of Administration:	Inhalation
Exposure Period:	Days 7-16 of gestation
Frequency of Treatment:	Six hours/day.
Value:	NOECs for the dam and fetus are 160 and 1000 mg/m ³ , respectively.
Method:	Groups of 24 pregnant rats were exposed six hours a day, on days 7 -16 of gestation to 160, 400, or 1000 mg/m ³ of DBE.
GLP:	Yes
Test Substance:	99.5% total esters (DBE) (16.8% DMA, 65.1% DMG, 17.8% DMS)

Results: Maternal body weight gains were significantly reduced during the period of administration (days 7 -16) of DBE at 400 and 1000 mg/m³; the reduction on days 9-11 was also significant. Maternal feed consumption was significantly reduced at the same exposure levels during the first six days of DBE exposure (days 7-9, 9-11, and 11-13). Clinical observations related to treatment (increased staining of the periocular, perinasal, and head fur, and wet fur) were significant only at 1000 mg/m³ and were not severe. No other evidence of maternal toxicity was seen. No effects on fetal survival, fetal weight, litter size, or nidations were seen. The incidence of fetal malformations and variations showed no exposure-related changes. Since maternal toxicity was demonstrated at 400 mg/m³ and fetal toxicity was absent at the highest exposure level, 1000 mg/m³, the NOEL for the dam and fetus are 160 and 1000 mg/m³, respectively (Dupont Co, 1987e; Kelly et al., 1986).

Reliability: High (Scientifically defensible or guideline, GLP)

References: Dupont Co. (1987). Unpublished Data, Haskell Laboratory Report No. 562-87. Kelly, D.P. et al. (1986). The Toxicologist, 6(1):136 (Abstract 551).

5.4. Reproductive Toxicity

Type: Repeated Inhalation (14 week)

Species/Strain: Rats/Crl:CD[®](SD)BR

Sex/Number: 20 pairs (male and female)

Exposure Period: 14 weeks

Frequency of Treatment: Daily, five days per week.

Method: Groups of 20 male and 20 female rats were mated after exposure for six hours a day, five days a week, for about 14 weeks to DBE vapor concentrations of 160 or 400 mg/m³, or to 1000 mg/m³ of a DBE aerosol/vapor mixture. DBE exposure continued during breeding (15 days), gestation (21 days), and lactation (21 days) periods. DBE exposures were discontinued for the dams after the 19th gestation day and begun again on day four postpartum.

Offspring were not subjected to DBE exposure.

GLP: Yes

Test Substance: 99.5% total esters (DBE) (16.8% DMA, 65.1% DMG, 17.8% DMS).

Results: The only DBE exposure-related effect was depressed pup weights in the 1000 mg/m³ exposure group from days 1-21 postpartum. No exposure-related differences were observed between control and test groups in the following reproduction parameters: male and female fertility indices, born-alive index, viability index, gestation index, and lactation index. A gross pathological examination of 21-day old rats whose parents had been exposed to DBE showed no exposure-related effects.

Reliability: High (Scientifically defensible or guideline, GLP)

References: Dupont Co. (1987). Unpublished Data, Haskell Laboratory Report No. 76-87. Kelly, D.P. et al. (1986). The Toxicologist, 6(1):136 (Abstract 551).

Additional References for Repeated Dose Inhalation: Not found

5.5. Genetic Toxicity

Type: *In vitro* Bacterial Reverse Mutation Test

Tester Strains: *Salmonella typhimurium* strains TA98 and TA100, and TM677

Exogenous Metabolic Activation: Rat liver (S-9) fraction/female rat olfactory mucosa activation

Exposure Concentrations: Maximum of 8.53 mg DBE/treatment (1.7 mg/ml) in Reverse Suspension Assay// maximum of 100 µg DBE/treatment (0.9 mg/ml) Microforward Suspension Assay

Methods: DBE was tested for mutagenic activity in *Salmonella typhimurium* strains TA98 and TA100 in the presence and absence of a rat-liver activation system using a suspension assay, and in a microforward mutation assay in strain TM677 in the presence of female rat olfactory mucosa activation.

GLP: Yes

Test Substance: 99.5% total esters (DBE) (15.9% DMA, 68.9% DMG, 14.9% DMS).
Results: Negative.
Reliability: High (Scientifically defensible or guideline, GLP)
Reference: Dupont Co. (1987). Unpublished Data, Haskell Laboratory Report No. 584-87.
Vlachos, D.A. et al. (1988). Environ. Mol. Mutagen., 11 (Suppl. 11):109.

Additional References for *in vitro* Bacterial Reverse Mutation Test:

DBE was not mutagenic in the Ames *Salmonella* test, either in the presence or absence of an S-9 activation system.

Dupont Co. (1977). Unpublished Data, Haskell Laboratory Report No. 396-77.

Dupont Co. (1980). Unpublished Data, Haskell Laboratory Report No. 462-80.

Type: *In vitro* Chromosome Aberration Study
Cell Type: Human lymphocytes
Exposure
Concentrations: $\geq 0.3\%$ v/v (3.3 mg/ml, based on approximate average molecular weight of components).
Methods: DBE was evaluated for *in vitro* clastogenic (chromosome damaging) activity in human lymphocytes with and without metabolic S-9 activation. Additional work was undertaken to elucidate the accuracy of these findings. Based on these results, it was suspected that the positive *in vitro* findings with DBE resulted from an acidic culture environment produced by this chemical under activated conditions. Therefore, experiments were undertaken to more thoroughly evaluate the pH changes induced by DBE under activated conditions and the apparent sex- specific response.
GLP: Yes
Test material: 99.5% total esters (DBE)
Results: As demonstrated by cell-cycle delay and/or reduced mitotic index, cytotoxicity was observed at DBE concentrations, $\geq 0.3\%$ v/v (3.3 mg/ml, based on approximate average molecular weight of components). Chromosome aberration analyses following non-activated treatments showed no statistically significant increases in the

percent abnormal cells in cultures treated with DBE. Under activated conditions, however, statistically significant increases in structural chromosome aberrations were reproducibly observed at DBE concentrations, $\geq 0.3\%$ v/v. These aberrations were predominant in cells from female (but not male) donors. Under the conditions of this assay, DBE is clastogenic.

In the above study, measurements of pH were made with culture medium pooled from male and female cultures following the completion of the three-hour treatment period. In the presence of S9, acidic conditions were observed at concentrations of 3.3 and 4.4 mg/ml. In contrast, the pH of the culture medium from non-activated 4.4 and 6.6 mg/ml treatments were similar to control.

Reliability: High (Scientifically defensible or guideline method, GLP).

References: Dupont Co. (1987). Unpublished Data, Haskell Laboratory Report No. 531-87. Vlachos, D.A. et al. (1988). Environ. Mol. Mutagen., 11 (Suppl. 11):109.

Remarks: Under activated conditions only, DBE causes reductions in the pH of culture medium. This was demonstrated in two independent assessments, where concentrations of DBE similar to those inducing positive results were evaluated. The drop in pH occurs quickly following the addition of DBE, and the resulting pH is in the range that has been reported to induce clastogenic effects with activation.

The induction of chromosome aberrations by DBE was quite variable based on the number of positive and negative results that have been produced with activation. The variability is believed to be attributable, in part, to the severe toxicity that this chemical induces.

Additional References for *in vitro* Chromosome Aberration Study:

None found

Type: *In vivo* Rat Erythrocyte Micronucleus Test

Guideline:	US EPA (1998). Health Effects Test Guidelines. OPPTS 870.5395
Species/strain:	Mice/Crl:CD [®] (ICR)BR
Sex/Number:	4 male and 4 female mice per treatment level
Route of Administration:	Inhalation (nose only)
Exposure Concentrations:	5.5, 11, or 19 mg DBE/L
Exposure Duration:	Six hours/one exposure
Method:	DBE was tested for its ability to induce micronuclei in the bone-marrow polychromatic erythrocytes of male and female mice. The mice were exposed nose-only to atmospheres of 5.5, 11, or 19 mg/L of DBE aerosol in air for 6 hours. Bone-marrow smears were prepared approximately 24, 48, and 72 hours after the beginning of exposure, and 1000 polychromatic erythrocytes per mouse were scored for the presence of micronuclei.
GLP:	Yes
Test Substance:	99.5% total esters (DBE)
Results:	Significant depression in the ratio of young, polychromatic erythrocytes to mature, normochromatic erythrocytes was detected in the 11 and 19 mg/L treated females at the 24-hour sampling as compared to their concurrent negative controls. No statistically significant increases in the frequency of micronucleated cells were seen in the DBE-treated mice at any sampling time. Under the conditions of this assay, DBE does not induce micronuclei.
Reliability:	High (Scientifically defensible or guideline, GLP)
References:	Dupont Co. (1987). Unpublished Data, Haskell Laboratory Report No. 498-87. Vlachos, D.A. et al. (1988). Environ. Mol. Mutagen., 11 (Suppl. 11):109.

6.0 Other Information

6.1 Biochemical/Metabolism Studies

Hepatic mitochondria were used as a model for nasal tissue. DBEs were found to inhibit mitochondrial ATP synthesis 11 to 27% at 100 μ M. The order of potency was DMA > DMG > DMS and paralleled the V_{max}/K_m values for the hydrolysis of the DBEs to their monomethyl esters. Pre-treatment of the rats with 100 mg/kg of bis-nitrophenyl phosphate for three days decreased the rate of hydrolysis of the DBEs approximately 50% and

protected the mitochondria from DBE-induced inhibition of ATP synthesis. These results support the hypothesis that DBE-induced cytotoxicity results from esterase-mediated hydrolysis to acid metabolites and interference with intermediary metabolism (Bogdanffy and Londergan, 1989).

In the study cited above, DBE cytotoxicity was shown to be due to esterase-mediated activation. In this present study, the putative toxic monomethyl and diacid metabolites were evaluated in an *in vitro* nasal explant system. Monomethyl adipate (MMA), glutarate (MMG), and succinate (MMS) induced increases in nasal explant acid phosphatase release, a biochemical index of their cytotoxicity. Metabolism of MMA and MMG to their diacids paralleled cytotoxicity. MMS metabolism was not quantifiable. Pretreatment of rats with a carboxylesterase inhibitor reduced cytotoxicity and metabolism of MMA and MMG, but not cytotoxicity of MMS. It is concluded that both monomethyl ester and diacid metabolites of DBE are cytotoxic. The contribution of each to cytotoxicity *in vivo* may depend on their rate of formation during exposure (Bogdanffy et al., 1991a; Trela and Bogdanffy, 1991b).

The kinetic parameters V_{max} , K_m , K_{si} , and V/K were measured for the hydrolysis of the dibasic esters in the target nasal tissue, olfactory mucosa, and non-target tissue, respiratory mucosa. It was determined under the conditions of these experiments, diacid metabolites were not formed. Esterase activity was inhibited by pretreatment with bis-nitrophenyl phosphate. V_{max} values for the three dibasic esters were 5- to 13-fold greater in olfactory mucosa than respiratory mucosa for male and female rats. V/K values were 4- to 11-fold greater in olfactory mucosa than respiratory mucosa for male and female rats. V/K was similar between male and female olfactory mucosa when DMG was used as the substrate. With DMS or DMA as the substrate, V/K for female olfactory tissue was 0.5- or 2-fold that of males, respectively. Differences in V/K were mainly due to decreases in K_m associated with increasing carbon chain length. Substrate inhibition was observed at DBE concentrations greater than approximately 25 mM, which are unlikely to be achieved *in vivo*. These results lend further support to the hypothesis that organic acid accumulation in the target tissue, olfactory mucosa, plays a significant role in the pathogenesis of DBE-induced nasal lesions (Bogdanffy et al., 1991b).

Since female rats appear to be more sensitive to DBE-induced olfactory toxicity than males, it was of interest to measure the rate of hydrolysis of DBEs in male and female nasal mucosa homogenates and compare these values to those derived from human nasal tissue obtained at autopsy. For both male and female rats, V_{max}/K_m values followed the order $DMA > DMG > DMS$ paralleling carbon chain length. The V_{max}/K_m values for female olfactory mucosa using DMA or DMS as substrates were two times or one-half the values for male olfactory mucosa, respectively. Hydrolysis of DBEs was detectable in only three of six human samples. Activity values that were measurable were two or three orders of magnitude lower than that of rat respiratory or olfactory mucosa, respectively. These data suggest the rate of conversion of DBEs to acid

metabolites in nasal tissue is less significant in humans than in rats, and that the rat may be more sensitive than man to the effects of DBEs on nasal mucosa (Kee et al., 1989).

The enzymatic esterase activity of carboxylesterases is integral to the nasal toxicity of many esters, including DMG, DMS, and DMA. Inhalation of these esters specifically damages the olfactory mucosa of rodents. In this study, the localization differential distribution of a 59 KD carboxylesterase was demonstrated in the nasal tissues of the rat by immunohistochemistry. Rabbit antiserum against the 59 KD rat liver microsomal carboxylesterase bound most prominently to the olfactory mucosa when applied to decalcified, paraffin-embedded sections of rat nasal turbinates. Within the olfactory mucosa, anti-carboxylesterase did not bind to sensory neurons, the target cell for ester-initiated toxicity; these cells apparently lack carboxylesterase. Instead, the antibody was preferentially bound by cells of Bowman's glands and sustentacular epithelial cells that are immediately adjacent to the olfactory nerve cells. In contrast, non-olfactory tissues (respiratory mucosa and squamous epithelium) which are more resistant to the toxicity of esters, had less carboxylesterase content (Olson et al., 1993).

An *in vitro* system was utilized to determine if DBE toxicity is dependent on metabolic activation by carboxylesterase. Explants from the olfactory and respiratory regions of the rat nasal cavity were incubated in a medium containing 10-100 mM of the dimethyl esters of adipic-, glutaric-, and succinic acids. DBE caused a dose-related increase in nasal explant acid phosphatase release, a biochemical index of cytotoxicity. A parallel increase in carboxylesterase-mediated monomethyl ester (MME) formation was seen. In addition, MME concentrations and acid phosphatase release were generally higher in olfactory than respiratory tissues. DME-induced cytotoxicity and MME formation were markedly reduced in nasal tissue excised from rats treated with a carboxylesterase inhibitor, bisnitrophenyl phosphate (Trela and Bogdanffy, 1990; 1991a).

The kinetic constants were determined for carboxylesterase-mediated hydrolysis of DBEs and correlated with lesion formation. No diacid metabolites were found. V_{max} values for the formation of MMS, MMG, and MMA were approximately 8- to 10-times larger in olfactory mucosa than in respiratory mucosa. V/K values for the formation of MMG and MMA were approximately 9- and 10-times larger in olfactory mucosa than respiratory mucosa. For the formation of MMS, V/K was approximately 2 times larger in respiratory mucosa than olfactory mucosa (Patterson et al., 1988).

To determine the biochemical mechanism for the toxic effect of DBE on rat nasal olfactory mucosa, an *in vitro* study was conducted with rat and human nasal tissue. This study demonstrated that the nasal tissue toxicity of DBE is related to enzymatic hydrolysis of DBE within the nasal cavity to form the corresponding monomethyl ester. Additionally, it was found that human nasal tissue hydrolyzes DBE at 1/100 to 1/1000 the rate of rat nasal tissue. For this reason, the nasal tissue of humans is likely to be at

greatly reduced risk of DBE toxicity compared to rats (Bogdanffy and Frame, 1994).

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