MICHIGAN DEPARTMENT OF ENVIRONMENTAL QUALITY

INTEROFFICE COMMUNICATION

TO: Files for: Dibasic Esters (DBE)(CAS# 95481-62-2)
(Mix. of 10-25% DMA, 55-65% DMG and 15-25% DMS)
Dimethyl Succinate (DMS, butanedioic acid, dimethyl ester)(106-65-0)
Dimethyl Glutarate (DMG, pentanedioic acid, dimethyl ester)(1119-40-0)
Dimethyl Adipate (DMA, hexanedioic acid, dimethyl ester)(627-93-0)

FROM: Michael Depa, Air Quality Division, Toxics Unit

DATE: August 1, 2012

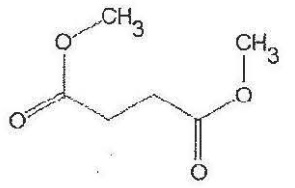
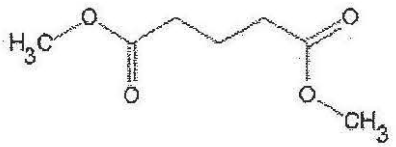
SUBJECT: Screening Level Change

This is an update to the Initial Threshold Screening Level (ITSL) for the mixture commonly known as dibasic ester (DBE) established in 2004, which was in turn an update of the 1993 screening level. The now-superseded ITSL for DBE was 0.6 µg/m³ with an annual averaging time. All screening levels for DBE, including the new ITSL of 1 µg/m³ (annual) are based on a 90-day inhalation study Keenan *et al.*(1990), which identified a lowest-observed-adverse-effect-level (LOAEL) of 20 mg/m³ for degeneration of nasal epithelium in female rats. Up to this time, the individual dimethyl esters each had ITSLs of 0.1 µg/m³ (annual). The ITSLs for the individual compounds are being rescinded and replaced with a new footnote that states:

> The combined ambient impact of dimethyl adipate, dimethyl glutarate and dimethyl succinate (collectively known as dibasic ester) cannot exceed the ITSL of 1 µg/m³ with annual averaging time.

DBE is a mixture of three dimethyl esters of four, five and six carbon dicarboxylic acids: succinate, glutarate and adipate, respectively (see Figure 1).

Figure 1. Compounds that Comprise the Dibasic Esters Mixture

		
Dimethyl Succinate (DMS)	Dimethyl Glutarate (DMG)	Dimethyl Adipate (DMA)
Metabolized to 4-Carbon dicarboxcylic Acid*	Metabolized to 5-Carbon dicarboxcylic Acid*	Metabolized to 6-Carbon dicarboxcylic Acid*

* Keenan *et al.*, 1990; Bogdanffy *et al.*, 1991; Trela *et al.*, 1991a; Morris *et al.*, 1991; Trela *et al.*, 1991b; Trela *et al.*; 1992

Toxicological data on the individual constituents of DBE, namely DMS, DMG and DMA, indicate that the effects are the same as the mixture. Given that the consistency of the physiochemical and toxicological relationships among these chemicals is strong, and since DMA, DMG and DMS

are never emitted individually from an air pollution source in Michigan, it was deemed appropriate to derive a screening level solely on the mixture: DBE.

Toxicological Database

A literature search was performed to find toxicological data for an inhalation risk assessment on DBE. All relevant data found was published before 2008. Data pertinent for derivation of a screening level is discussed below, however, additional data, including developmental toxicity data can be found in Appendix A. There are two 90-day inhalation studies on the mixture DBE (Keenan *et al.*, 1990; Dupont, 1986). Additionally, in a study sponsored by DuPont, Bamberger (2000) exposed rats to the individual constituents of DBE for 90 days via inhalation, 6 hours per day, 5 days per week (see Table 1 for protocol).

Table 1. Protocol for Bamberger (2000) 90-day Inhalation Study

DBE Constituent	Dose levels $\mu g/m^3$	Toxicological Parameters Measured
DMS	0, 400	Food, body/organ weight, ophthalmological eval., hematology, serum chemistry, urinalysis, histopathology, functional observational battery (fore- and hindlimb grip strength, hindlimb foot splay, open field defecation, arousal (motor activity), cell proliferation (CP): liver, lung, nose levels II and III
DMG	0, 10, 50, 400	
DMA	0, 400	

In 2008, the US Environmental Protection Agency (EPA) evaluated all of the dose-response studies available on DBE and individual components and published their findings in: *Screening-Level Hazard Characterization of High Production Volume Chemicals. Chemical Category Name: Dibasic Esters (DBE)* (see Table 2 and text below).

Subchronic Inhalation Study: Dimethyl adipate (CAS No. 627-93-0)(from EPA, 2008)
Sprague-Dawley rats were exposed to DMA via inhalation at 0 or 400 mg/m^3 (measured as 390 mg/m^3), 6 hours/day, 5 days/week for 90 days (Bamberger, 2000). A 1-month recovery group was included in the study. Neurobehavioral test battery; evaluation of male reproductive organs including sperm count, motility, and morphology; cell proliferation (CP—hepatic, lung, and nasal tissues); female estrous cycle determination; and hormonal analysis (serum LH, FSH and testosterone in males and serum estradiol and progesterone concentrations in females) were included in the test. Test-substance-related effects seen in the noses of male and female rats at 400 mg/m^3 DMA included degeneration/atrophy and focal respiratory metaplasia of the olfactory mucosa with minimum to mild severity. Degeneration/atrophy of the olfactory mucosa was evident in recovery animals in the same locations as observed in the animals examined after 90 days of exposure. Male rats exposed to 400 mg/m^3 showed marked increase in CP in the liver and had greater CP in the nose level II relative to controls. Female rats exposed to 400 mg/m^3 had greater CP in the lungs relative to controls. In male rats, although not statistically significant, an increase in epididymal sperm counts was noted.

LOAEL = 400 mg/m^3 based on effects on nasal tissues, increase in CP in liver, lungs and nose
NOAEL = Not established

Subchronic Inhalation Study: Dimethyl glutarate (CAS No. 1119-40-0)(from EPA, 2008)
Sprague-Dawley rats were exposed to DMG via inhalation at 0, 10, 50 or 400 mg/m^3, 6 hours/day, 5 days/week for 90 days (Bamberger, 2000). A 1-month recovery group was included in the study. Neurobehavioral test battery; evaluation of male reproductive organs including sperm count, motility and morphology; cell proliferation (CP—hepatic, lung, and nasal tissues); female estrous cycle determination; and hormonal analysis (serum LH, FSH and testosterone in males and serum estradiol and progesterone concentrations in females) were included in the test. Male rats exposed to 400 mg/m^3 showed lower mean body weight and body weight gains during the

study and male and female rats had lower food consumption. Test substance-related effects seen in the noses of male and female rats at 400 mg/m³ DMG included degeneration/atrophy and focal respiratory metaplasia of the olfactory mucosa with minimum to mild severity. Degeneration/atrophy of the olfactory mucosa was evident in recovery animals in the same locations as observed in the animals examined after 90 days of exposure. Male and female rats exposed to 400 mg/m³ DMG showed marked increase in CP in the nose level III. Male rats showed a statistically significant decrease in serum testosterone levels at 50 and 400 mg/m³ (59 and 50% of control, respectively). Serum LH concentration was decreased in a dose-dependent manner and was statistically significant at 400 mg/m³ (71% of control). In addition, a significant increase in epididymal sperm count was seen in the animals exposed to 50 and 400 mg/m³ (124 and 131% of control, respectively).

LOAEL = 50 mg/m³ based on decrease in serum LH concentration in a dose-dependent manner, decrease in testosterone concentration, effects on nasal tissues, increased epididymal sperm counts at 50 mg/m³ and above in males
NOAEL = 10 mg/m³

Subchronic Inhalation Study: Dimethyl Succinate (CAS No. 106-65-0) (from EPA, 2008)
Sprague-Dawley rats were exposed to DMS via inhalation at 0 or 400 mg/m³, 6 hours/day, 5 days/week for 90 days (Bamberger, 2000). A 1-month recovery group was included in the study. Neurobehavioral test battery; evaluation of male reproductive organs including sperm count, motility and morphology; cell proliferation (CP—hepatic, lung, and nasal tissues); female estrous cycle determination; and hormonal analysis (serum luteinizing hormone (LH), follicle stimulating hormone (FSH) and testosterone in males and serum estradiol and progesterone concentrations in females) were included in the test. Test-substance-related effects seen in the noses of male and female rats at 400 mg/m³ DMS included degeneration/atrophy and focal respiratory metaplasia of the olfactory mucosa with minimum to mild severity. Degeneration/atrophy of the olfactory mucosa was evident in recovery animals in the same locations as observed in the animals examined after 90 days of exposure. Male rats exposed to 400 mg/m³ showed marked increase in CP in the liver and the females had greater CP in the nose level III relative to controls. Females showed a statistically significant decrease (43% of control) in serum estradiol concentrations. In male rats, epididymal sperm counts were significantly increased (141 – 153% of control)(biological significance not established).

LOAEL = 400 mg/m³ based on effects on nasal tissues, decrease in estradiol concentration in females and increased epididymal sperm counts in males
NOAEL = Not established

Subchronic Inhalation Study: DBE—Mixture (CAS No. 95481-62-2) (from EPA, 2008)
In a 90-day inhalation toxicity study, rats were exposed to DBE aerosol-vapor mixture at 160, 400 or 1000 mg/m³, 6 hours/day, 5 days/week for approximately 14 weeks (Dupont,1986). Histopathological examination of nasal tissues showed degeneration of the olfactory epithelium in all DBE-exposed groups. Effects were of minimal severity in the 160 mg/m³ group and mild to moderate at the mid- and high-concentrations. A dose-dependent decrease in liver to body weight ratio was seen in male and female rats from the 400 and 1000 mg/m³ groups and a slight increase in lung/body weight ratio and decreased body weights in animals from the 1000 mg/m³ group.

LOAEL = 160 mg/m³ based on degeneration of olfactory epithelium
NOAEL = Not established

In another 90-day inhalation toxicity study, male and female rats were exposed to DBE at 20, 76 or 390 mg/m³ for 13 weeks (Keenan *et al.*, 1990). A 6-week recovery group was also included in the study. The results indicated degeneration of olfactory epithelium in male rats exposed to 76 or 390 mg/m³ and in female rats exposed to all test concentrations. At the end of the 6-week recovery period, these effects were still visible in affected animals. In female rats exposed to 390 mg/m³, depressed body weight gain and liver weights were evident compared to controls. A slight decrease in sodium levels was evident at 76 and 390 mg/m³ in male and female rats. After 6

weeks of recovery, the sodium level was still low in animals exposed to 390 mg/m³. A NOAEL was not demonstrated in female rats.

NOAEL = 20 mg/m³ based on degeneration of olfactory epithelium in males
LOAEL = 20 mg/m³ based on degeneration of olfactory epithelium at all concentrations in females

Table 2. Toxicity Data: Dibasic Esters Category (US EPA, 2008)

	DMA	DMG	DMS	DBE
LD50 (mg/kg-bw)	> 5000	> 5000	> 500; < 5000	> 500; < 5000
Acute Dermal Toxicity LD50 (mg/kg-bw)	> 5000	> 5000	> 5000	> 5000
Acute Inhalation LC50 (mg/m³)	No Data	No Data	No Data	> 11000
Repeated-Dose Oral (mg/kg-bw/day)	No Data	No Data	No Data	NOAEL = 1000
90-day Repeated-Dose Inhalation (mg/m³) (see description below)	NOAEL = Not Established LOAEL = 400	NOAEL = 10 LOAEL = 50	NOAEL = Not Established LOAEL = 400	NOAEL = Not Established LOAEL = 20
Reproductive Toxicity Inhalation (mg/m³)	Evaluation of reproductive parameters from 90-day study—Increased epididymal sperm counts, and decreased testosterone and LH levels in males	Evaluation of reproductive parameters from 90-day study—Increased epididymal sperm counts, and decreased testosterone and LH levels in males	Evaluation of reproductive parameters from 90-day study—Increased epididymal sperm counts and increased estradiol in females	NOAEL = 400 LOAEL = 1000
Maternal Toxicity - Maternal (mg/m³)	NOAEL = 100 LOAEL = 500	NOAEL = 100 LOAEL = 400	NOAEL = 100 LOAEL = 300	NOAEL = 160 LOAEL = 400
Developmental Toxicity - fetus (mg/m³)	NOAEL = 300 LOAEL = 1200	NOAEL = 300 LOAEL = 1100	NOAEL = 300 LOAEL = 1000	NOAEL = 1000 LOAEL = Not Established
Gene Mutation In vitro	Negative	Negative	Negative	Negative
Genetic Toxicity Chromosomal	Negative	Negative	Negative	Negative
Skin Irritation	Not a skin irritant	Not a skin irritant	Not a skin irritant	Not a skin irritant
Eye Irritation	Mild to moderate	Mild to moderate	Mild to moderate	Mild to moderate

Discussion

As noted in Table 1, the 90-day inhalation studies performed on DMA and DMS had one dose level other than control rats, at 400 mg/m³ (Bamberger, 2000). A more thorough study was performed on DMG using three dose levels where rats were exposed to 0, 10, 50 and 400 mg/m³. The DMG study is the only study on a constituent of DBE or the DBE mixture as a whole that identified a NOAEL. As noted above, DMG produced a statistically significant decrease in serum testosterone levels at 50 and 400 mg/m³ (the effect was more pronounced at high dose of 400 mg/m³) and significant increase in epididymal sperm count at the same dose levels. The biological significance of decreased testosterone is unclear since it apparently had no effect on sperm motility, morphology or decrease in number of sperm, in fact an increase in number of epididymal sperm was observed. Degeneration/atrophy of olfactory mucosa was not observed at

50 mg/m³ DMG, but it was increased at the 400 mg/m³ dose level, as it was with exposures to DMA and DMS at the 400 mg/m³ dose level. This olfactory degeneration dose-response threshold for DMA, DMS and DMG differs from that of the mixture DBE, which showed degeneration/atrophy of olfactory mucosa at 20 mg/m³. Concerning systemic effects (i.e., effects outside the respiratory tract), in a 90-day study (Keenan *et al.* 1990) on DBE found a slight decrease in sodium levels at 76 and 390 mg/m³ in male and female rats, which may indicate a threshold for systemic effects near the 50 mg/m³ DMG dose level (Bamberger 2000) where decreased testosterone level was observed. Even though there is clearly a systemic effect at the low dose of 50 mg/m³ DMG, EPA (2008) did not consider the decreased testosterone toxicologically relevant in identifying 50 mg/m³ DMG an effect level:

> The potential health hazard of chemicals in the dibasic esters category is considered low because the effects observed were: (a) local effects to the nasal epithelium that are likely the result of irritation; (b) changes in hormone levels that do not appear to have toxicological significance; or (c) developmental toxicity at high doses.

A screening level for DMG can be developed using the mean testosterone levels and benchmark dose software (BMDS; Version 2.2), rather than the NOAEL/LOAEL methodology. Using EPA's BMDS, a screening level for DMG would be 0.5 µg/m³, or almost the same as the current ITSL of 0.6 µg/m³ based on the LOAEL of 20 µg/m³ for a DBE mixture (see appendix B for calculations).

The repeated dose 90-day inhalation studies on DMA, DMG, DMS and DME indicate that the nasal epithelia represent the target organ and that nasal effects are the most toxicologically relevant effect of this class of compounds. This is the same conclusion made during previous reviews of the toxicological database (Gary Butterfield's Memo to the File for Dibasic Esters dated April 22, 2004).

Consideration of Screening Level for DBE

Using EPA's BMDS (dichotomous option) and the data from Keenan *et al.* (1990) a new screening level was developed. The incidence of olfactory epithelium degeneration (at any level) is shown in Table 3.

Table 3. Olfactory Epithelia Degeneration (from Keenan, *et al.* 1990)

DBE Dose (mg/m³)	Number of Rats (female)	Degeneration Incidence
0	20	1
20	20	5
76	19	7
390	20	19

These models were used to fit the data points: Logistic, LogLogistic, LogProbit, Multistage, Probit, Quantal-Linear. Gamma and Weibull were designated for use in the initial run session, however, error messages were produced:

> Error Log Entry: 10:31:41 AM Tuesday, July 17, 2012
>
> Row 1: Gamma plotter encountered some problem...
> 002 file input for plotter 10GAMMHIT.exe does not exist...
> please check your data or option selection..
>
> Row 7: Weibull executable failed to process the data
> C:\Documents and Settings\depam\My Documents\BMDS220\Data\DBE_Nasl_degeneration.dax.

The BMDS output automatically deleted the output of the Gamma and Weibull models (see Table 4). After checking for any obvious problems with the input, session or options files, it was decided to see if dropping the high dose group from the input data and re-running the software produced a result for Weibull and Gamma models. The Weibel model did produce an output file, but the Gamma model still failed to produce a BMDL for DBE, which is described by "error" in Table 4. It was decided not to use the data produced by dropping the high dose group since it diminishes the degrees of freedom and, when doing so, the results were not any better than keeping all three exposure groups[1].

Using all four dose groups (i.e., 0, 20, 76 and 390 mg/m^3 DBE), the BMDS generated graphs for 6 of the 8 models available for dichotomous data (see graphs in Appendix B). The six graphs indicated adequate fits at low dose levels, however, the Multistage model showed two peaks (i.e., "multiple local maxima") and the graph actually dipped below zero response into a negative response region, therefore, it was rejected. The LogProbit model produced a goodness of fit p-value equal to 0.1 (i.e., not "greater than"), which is the cutoff for minimum "goodness of fit", and had the highest Akaike's Information Criterion (AIC), therefore, it was viewed as poor quality and excluded from further BMDL consideration. At this point four models were still being considered for the BMDL: Quantal-Linear, Logistic, Probit, and LogLogistic. However, two of the models, Logistic and Probit produced BMDLs of 30 mg/m^3. Remember that the low dose of 20 mg/m^3 produced a statistically increased incidence of olfactory degeneration in 5 out of 20 female rats (i.e., 25%) and the control rats had an incidence of 1 out of 20 (5%). The BMDL is designed to generate the lower bound (at 95% confidence level) of a BMDL that approximates a 10% incidence rate of extra risk. It is biologically implausible that the modeled dose of 10% response would be greater than that of a experimental (measured) incidence of 25%, therefore, the validity of the Logistic and Probit models was rejected.

Table 4. Results of BMDS Modeling of Olfactory Degeneration of DBE (Sorted by AIC)

Model Name	AIC (sorted ↑)	P-value	BMDL	Scaled Residual of Interest	Comments	Result of Dropping High Dose
Quantal-Linear	68.512	0.560	**10.24***	0.76	Best AIC	BMDL = 10
Logistic	70.218	0.297	29.24	0.742		BMDL = 22
Probit	70.249	0.291	31.74	0.746		BMDL = 20
Multistage	71.383	NA	3.20	0	Bad Fit (two maxima): Rejected	Failed p-value
LogLogistic	72.018	0.103	**5.32***	1.112	P-value approaching cutoff of 0.1	Failed p-value
LogProbit	72.096	0.100	5.60	0.964	P-value = cutoff of 0.1, Highest AIC	Failed p-value
Wiebull	error	error	error	error	error	BMDL = 10
Gamma	error	error	error	error	error	error

* Can be used to obtain average BMDL value of 7.78 mg/m^3.

[1] BMDS guidance suggests that the high dose can be dropped, although it is not necessary if good fitting models are obtained using all the data, as was found in the initial run. Nonetheless, after dropping the high dose in order to obtain a better fit, the Multistage model still produced a bad fit as assessed by failing the goodness of fit criteria (i.e., reject if p-values are less than 0.1).

The LogLogistic Model produced the lowest BMDL of 5 mg/m³, although the goodness of fit p-value of 0.103 was only slightly above the 0.1 cutoff, and had a higher AIC (i.e., 72.018) than Quantal-Linear (i.e., 68.512) BMDL. According to BMDS guidance, "all thing being equal, lower AIC values are preferred".

The BMDL of 10.24 mg/m³ was adjusted to continuous duration because the experimental exposure conditions were 6 hours per day 5 days per week. The BMDL of 10.24 mg/m³ was multiplied by 6/24 and 5/7, resulting in BMDL(Adj) of 1.8 mg/m³. The human equivalent concentration (HEC) was derived by multiplying BMDL(Adj) by the RGDR of 0.156 (Butterfield, 2004) resulting in a BMDL(HEC) of 0.29 mg/m³. The screening level was then calculated using uncertainty factors of 10, 10, and 3, for subchronic to chronic, sensitive individuals and animal to human, respectively, resulting in a screening level of 0.00095 mg/m³ or, rounding to 1 significant figure and converting to micrograms yields 1 µg/m³.

In 2009, EPA published a detailed review of advances in the state-of-the-science for inhalation gas dosimetry related to the upper respiratory tract (URT) or nasal tract region (EPA, 2009). EPA's principal conclusion is that internal dose equivalency for rats and humans in achieved through similar external exposure concentrations. Hence, the human equivalent concentration (HEC), under certain circumstances, is the same as the duration adjusted exposure concentration in animals. Typically a dosimetric adjustment factor (DAF) is applied to convert from animal exposure concentration to HEC, but when the target tissue is the nasal tract region (i.e., URT) and the gas is reactive or is metabolized into a reactive species within the nasal mucosa, as is DBE via carboxyesterase (Bogdanffy *et al.*, 1991; Keenan *et al.*, 1990; Morris *et al.*, 1991; Trela *et al.*, 1991a; Trela *et al.*, 1991b; Trela *et al.*, 1992), then the DAF would equal 1. However, EPA is not known to have superseded EPA (1994) RfC methodology for determining dose equivalency between animals and humans and has not used the DAF of 1 in a draft or finalized risk assessment where it would be applicable. Therefore, even though DBE's physical-chemical and toxicological properties indicate that a DAF of 1 could be applied, it will not be used until a peer-reviewed and well documented risk assessment using this method is published or other compelling reasoning can be discerned.

The derivation of a health based screening level for DBE using the LOAEL/NOAEL methodology (US EPA, 1994) and the Benchmark Dose methodology (US EPA, 2011) yielded very similar results: 0.6 µg/m³ and 1 µg/m³, respectively. The methods are compared below in Table 5.

Table 5. Compare Uncertainty Factors and Screening Levels by Risk Assessment Method

	DBE LOAEL/NOAEL Method	DBE Benchmark Dose Method	DMG Benchmark Dose Method
Toxicological Endpoint	Olfactory Degeneration	Olfactory Degeneration	Decreased Testosterone
Animal to Human	3	3	3
Sensitive Individuals	10	10	10
Subchronic to Chronic	10	10	10
LOAEL to NOAEL	3		
TOTAL Uncertainty	1000	300	300
Screening Level	0.6 µg/m³	1 ug/m³	0.5 µg/m³

The screening level for DMG was not established for reasons mentioned above, however, using the benchmark dose method would produce a screening level of 0.5 µg/m³. Considering that the DBE mixture typically is composed of 55-65% DMG and that the DMG screening level is 50% of that of DBE, it provides a level of assurance that the screening level for DBE would protect for the potential effects of decreased testosterone observed in the DMG study.

Conclusion

Typically the averaging time for a screening level would be based on Air Pollution Control Rule 232(2)(b) that states:

> If the initial threshold screening level is derived as in subrule 1(a) and (b) of this rule, then the averaging time is 24-hours.

However, the screening level for DBE was determined pursuant to Rule 229(2)(b):

> Any alternative methodology to assess noncarcinogenic health effects that can be demonstrated to the department to be more appropriate based on toxicological grounds and that is supported by the scientific data.

Since Rule 229 does not have a subrule similar to Rule 232(2)(b) that specifies an averaging time, it was reasoned that because the screening level is protective of chronic effects, an annual averaging time should be used.

The ITSL for DBE is 1 ug/m^3 based on annual averaging time.

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Appendix A: Summary of Repeated-dose 90-day Inhalation Studies on DMA, DMG, DMS and DBE

Under the High Production Volume Program, the U.S. Environmental Protection Agency published a summary of the toxicological database for DBE. The following is paraphrased from this report (US EPA, 2007).

Introduction

The sponsor, Dibasic Esters Group of Synthetic Organic Chemical Manufactures Association, Inc. (SOCMA), submitted a Test Plan and Robust Summaries to EPA for the Dibasic Ethers. The sponsor provided EPA with revised documents on November 8, 2002 and May 30, 2003, which were posted to the ChemRTK website.

Summary-Conclusion

The dibasic esters are liquids at room temperature with moderate vapor pressures and high water solubilities. They are moderately volatile and will be slowly photolyzed in the atmosphere. They are highly mobile in soil and water systems. They are not persistent and are not bioaccumulative. They are expected to hydrolyze slowly and biodegrade rapidly.

The category members are not skin irritants, but cause mild to moderate eye irritation.

Repeated exposures to these chemicals via inhalation show local effects (likely a result of irritation at the point of contact in the nasal region) as well as some changes in hormone levels that, although consistently observed, are not considered to be toxicologically significant. In all 90-day inhalation studies (one each with DMS, DMA, and DMG and two with the mixture DBE), degeneration/atrophy and focal respiratory metaplasia of the olfactory mucosa with minimum to mild severity was observed in both males and females. Exposed animals also showed marked microscopic alterations in the DMS, DMA, and DMG studies as measured by increases in cell proliferation (CP) in the liver (males), nasal area (males and females) and lung (females). The following effects on reproductive parameters were observed in the 90-day studies with DMS, DMA, and DMG: increase in epididymal sperm counts (2/3 studies), decrease in testosterone levels (1/3 studies), and decrease in leutenizing hormone levels (1/3 studies) - all in males, and decrease in estradiol levels in females (1/3 studies). The significance of these findings is unclear because the decrease in male hormone levels should result in a decrease in sperm counts, yet the opposite effect was observed. The single study showing changes in estradiol was not observed in the other two studies. Other reproductive parameters evaluated in these studies but which were not affected by treatment were: follicle stimulating hormone (FSH) and sperm motility/morphology in males and progesterone level and estrous cyclicity in females. In addition, a reproductive study was conducted with the fourth member of the category (DBE) and there were no effects on the following reproductive parameters: fertility, viability of pups at birth, and the ability of the mothers to lactate.

In a developmental toxicity study in rats, a marked reduction in maternal body weight gain and food consumption was seen during the exposure period at the highest concentration tested. No effects on fetal survival, fetal weight, litter size, implantations, or increased incidences of fetal malformations/variations were seen. In rabbits, reductions in body weights in dams and a marked increased in delayed ossification in fetuses were seen in the high dose group only. The dibasic esters category was not mutagenic in tested strains of Salmonella typhimurium and did not induce statistically significant increase in the mean number of micronucleated polychromatic erythrocytes in the bone marrow of CD-1 mice.

The potential health hazard of chemicals in the dibasic esters category is considered low because the effects observed were: (a) local effects to the nasal epithelium that are likely the result of irritation; (b) changes in hormone levels that do not appear to have toxicological significance; or (c) developmental toxicity at high doses. No data gaps have been identified under the HPV Challenge Program.

Appendix B: Derivation of Screening Levels for Dimethyl Glutamate (DMG) and Dibasic Esters (DBE) Using EPA Benchmark Dose Software (BMDS)

First, the US EPA Benchmark Dose Software (BMDS) (EPA, 2011) was used in order to develop a benchmark concentration for Dimethyl Glutamate (DMG) (CAS No. 1119-40-0). Dose-response input data is shown in Table B-1. Model output data is shown in Table B-2. Because the testosterone levels in blood were measured as concentrations the "continuous" option was chosen in the BMDS.

Table B-1. Benchmark Dose Input data for DMG for Testosterone Repsonse

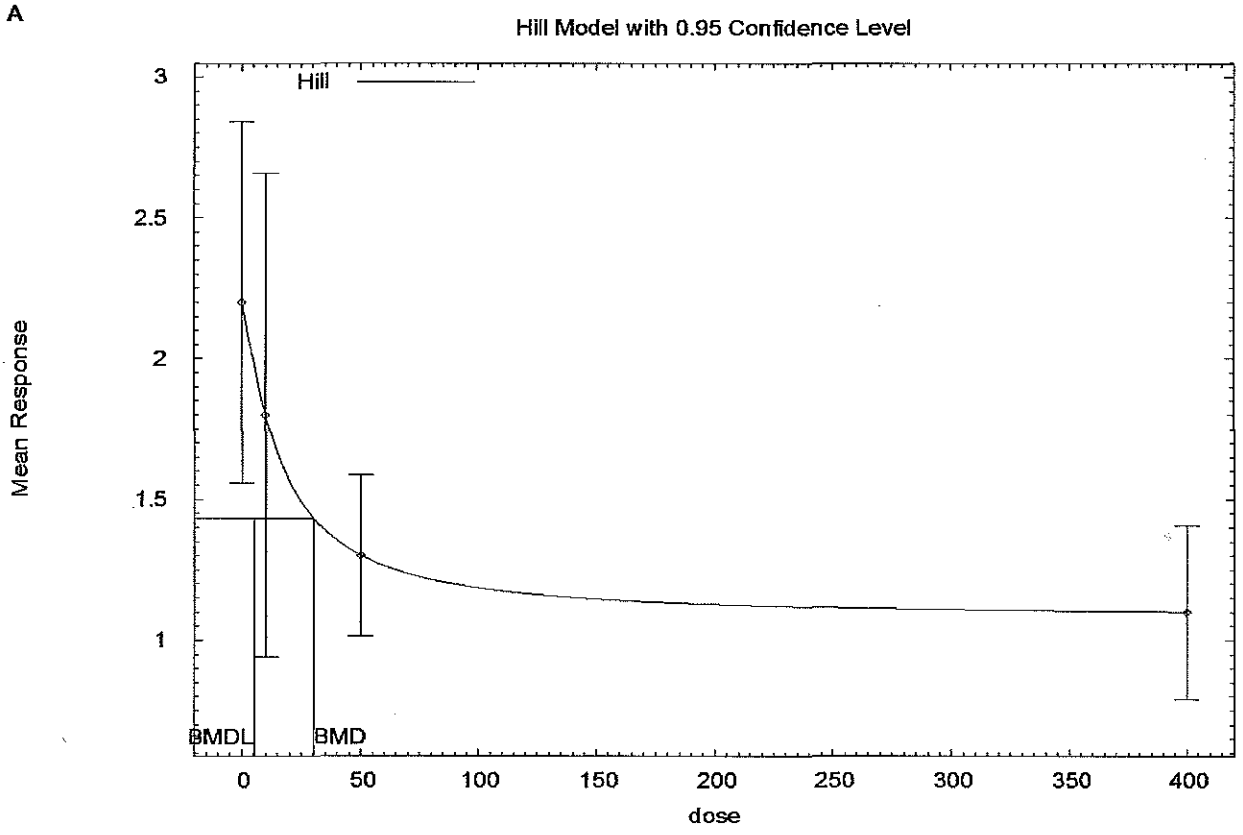
Dose Group (mg/m^3)	N (number of animals per group)	Observed Mean Testosterone (ng/mL)	Observed Stardard Deviation (ng/mL)
0	10	2.2	0.9
10	10	1.8	1.2
50	10	1.3	0.4
400	9	1.1	0.4

Table B-2. Benchmark Dose Output Data for DMG for Testosterone Response

Model Name	Expo* 2	Expo* 3	Expo* 4	Expo* 5	Hill	Linear	Poly-nomial	Power
BMDL	Bad Completion	Bad Completion	Bad Completion	0	5.15	245.08	26.09	245.08

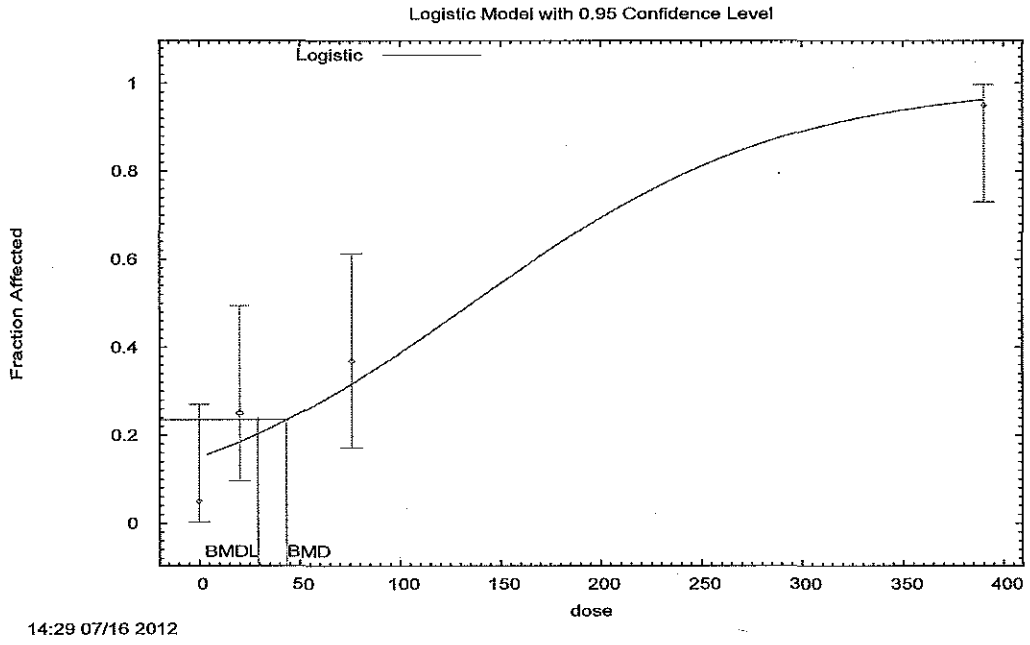
*Exponential

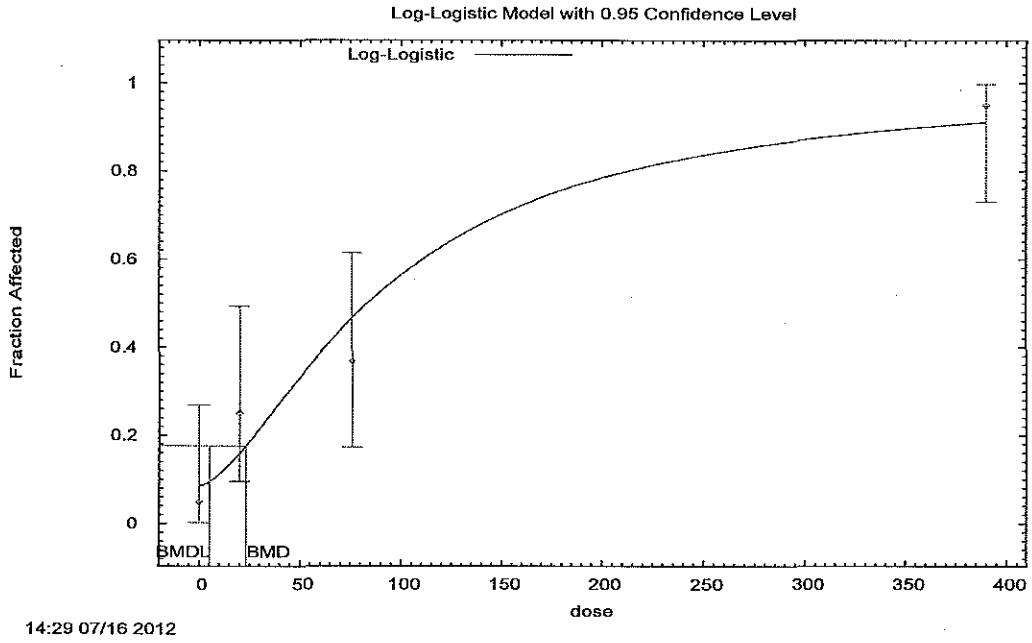
A

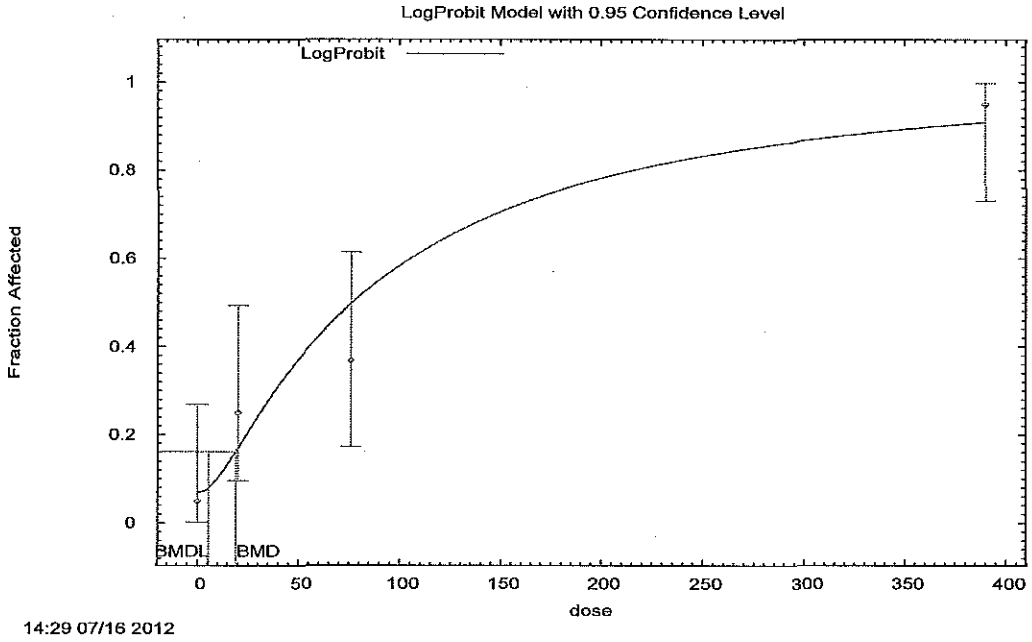


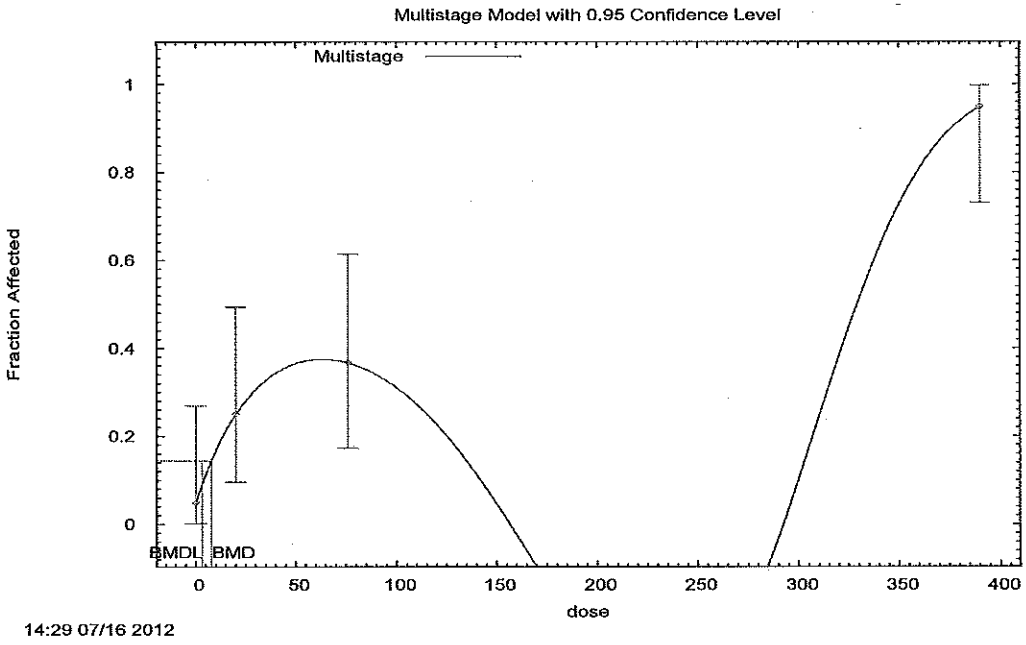
The Hill Continuous Model produced a perfect fit to the data and was deemed the most appropriate to use to derive the point of departure (POD)(i.e., BMDL). The BMDL of 5.15 mg/m³ DMG represents the 95% lower confidence limit on the dose at 1 standard deviation below the mean testosterone level. The DMG BMDL was duration adjusted, then converted to human equivalent concentration (HEC) using a regional gas dose ratio (RGDR) to adjust the dose in amimals (in this case rats) to that of humans, as described by the EPA (1994) RfC Methodology and Butterfield (2004). Finally the screening level was derived using standard uncertainty factors (UFs) recommended by EPA (1994). Adjusted BMDL = 6/24 x 5/7 = 0.9196 mg/m³. RDGR for extrathoracic region (ET) was calculated as 0.156 (Butterfield, 2004). The HEC is the adjusted dose multiplied by RGDR of 0.156 to yield BMDL(HEC) of 0.143 mg/m³. The total UF of 300 was composed of 3 for animal to human (interspecies), 10 for subchronic to chronic, and 10 for sensitive individuals (intraspecies). The screening level for DMG becomes 0.143 mg/m³ ÷ 300 = 0.478 mg/m³, or rounding to 1 significant figure yields a screening level of 0.5 µg/m³ for DMG.

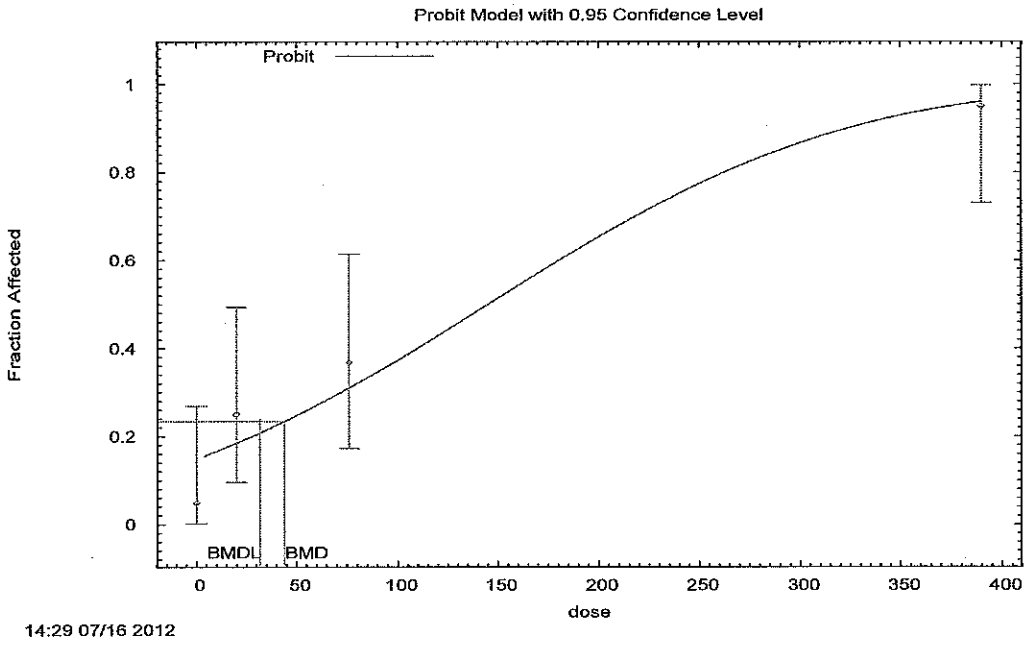
The following graphs represent the model-fitting exercise for DBE using the dichotomous data from olfactory degeneration as the input (Keenan *et al.*, 2001). The quantal-linear model produced the best fit as measured by AIC.

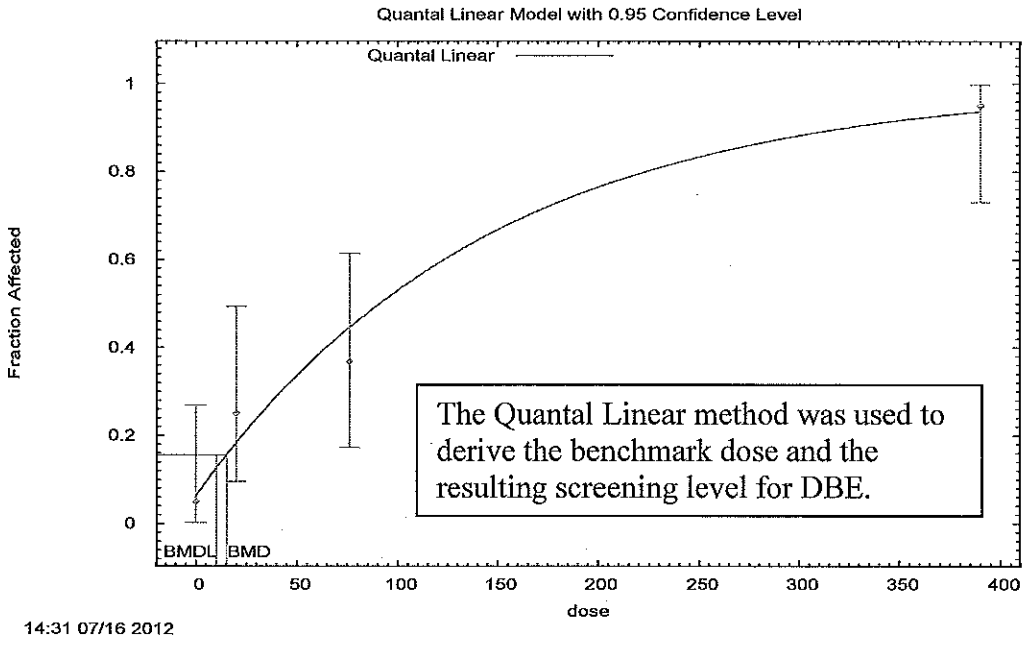



Log-Logistic Model with 0.95 Confidence Level
Log-Logistic
Fraction Affected
1
0.8
0.6
0.4
0.2
0
BMDL
BMD
0
50
100
150
200
250
300
350
400
dose
14:29 07/16 2012


LogProbit Model with 0.95 Confidence Level
LogProbit
Fraction Affected
1
0.8
0.6
0.4
0.2
0
BMDL
BMD
0
50
100
150
200
250
300
350
400
dose
14:29 07/16 2012


Multistage Model with 0.95 Confidence Level
Multistage
Fraction Affected
1
0.8
0.6
0.4
0.2
0
BMDL
BMD
0
50
100
150
200
250
300
350
400
dose
14:29 07/16 2012


Probit Model with 0.95 Confidence Level
Probit
Fraction Affected
1
0.8
0.6
0.4
0.2
0
BMDL
BMD
0
50
100
150
200
250
300
350
400
dose
14:29 07/16 2012


Quantal Linear Model with 0.95 Confidence Level
Quantal Linear
Fraction Affected
1
0.8
0.6
0.4
0.2
0
BMDL
BMD
0
50
100
150
200
250
300
350
400
dose
14:31 07/16 2012
The Quantal Linear method was used to derive the benchmark dose and the resulting screening level for DBE.