MICHIGAN DEPARTMENT OF ENVIRONMENT, GREAT LAKES, AND ENERGY

INTEROFFICE COMMUNICATION

TO: File for diethyl methyl benzenediamine (CAS# 68479-98-1)

FROM: Keisha Williams, Air Quality Division

DATE: November 8, 2023

SUBJECT: Screening level derivation for diethyl methyl benzenediamine

The initial risk screening level (IRSL) for diethyl methyl benzenediamine is 0.2 µg/m^3 (annual averaging time) and the secondary risk screening level is 2.0 µg/m^3 (annual averaging time) based on the Michigan Department of Environment, Great Lakes, and Energy (EGLE), Air Quality Division (AQD) Rule 336.1231 (3). The acute initial threshold screening level is 80 µg/m^3 (24-hour averaging time) based on Rule 336.1233.

The following references or databases were searched to identify data to determine the screening level: United States Environmental Protection Agency's (EPA's) Integrated Risk Information System (IRIS), EPA's CompTox Chemicals Dashboard; ChemView: the EPA's database on chemical health and safety data for chemicals subject to the Toxic Substances Control Act (TSCA), the TSCA National Technical Reports Library; the American Conference of Governmental Industrial Hygienists (ACGIH) Threshold Limit Values (TLV), the national Library of Medicine PubChem and PubMed websites; Agency for Toxic Substances and Disease Registry (ATSDR) Minimal Risk Levels, International Agency for Research on Cancer (IARC) Monographs, the American Chemical Society's SciFinder database (searched in August 2022), National Toxicology Program (NTP) Study Database, EPA Superfund Provisional Peer Reviewed Toxicity Values, EPA Acute Exposure Guideline Levels (AEGLs) for Airborne Chemicals, United States Department of Labor Occupational Safety and Health Administration (OSHA) Permissible Exposure Limits (PELs), the Texas Commission on Environmental Quality (TCEQ) Texas Air Monitoring Information System; the Canadian Centre for Occupational Health and Safety's Registry of Toxic Effects of Chemical Substances (RTECS), California Office of Environmental Health Hazard Assessments Reference Exposure Levels, OECD Existing Chemicals Database; German occupational exposure limits (abbreviated MAK values); and European Chemicals Agency Registered Substances Dossiers.

Background Information

Diethyl methyl benzenediamine, also called diethyl toluenediamine (DETDA), has been used as a reactant in paint manufacturing, an adhesive and sealant chemical, as an intermediate in paint and coating manufacturing, and in an engine filament winding process (2015 CDR Data and Chemical Test Rule Data from Chemview database; ECHA, 2020). The chemical structure is shown in Figure 1. At room temperature, diethyl methyl benzenediamine is a colorless or yellow liquid (ECHA, 2020). Chemical properties are listed in Table 1.

Figure 1. Chemical structure for diethyl methyl benzenediamine (CompTox Chemicals Dashboard)

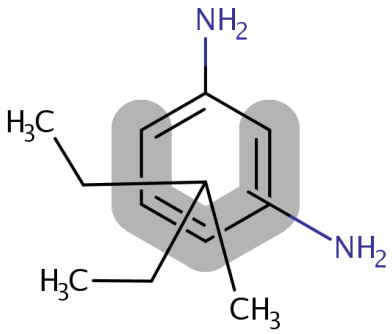

Table 1. Chemical properties of DETDA (Referenced from ECHA, 2020 unless otherwise noted)

Molecular weight: 178.274 grams/mole
Boiling point: 308.3°C
Melting point: -21.6 +/- 0.2°C (Chemview)
Vapor pressure: 32.4 Pa (or less) at 20°C

The only other health benchmarks found were developed by TCEQ at 90 µg/m^3 short-term ESL and 9 µg/m^3 long-term ESL based on occupational exposure limits for o-toluidine (CAS# 95-53-4) (TAMIS database).

Controlled human studies and epidemiological studies have not been found.

Only one inhalation study was identified as summarized in ECHA (2020). In this acute mortality study, no male or female Sprague-Dawley rats died after a 1-hour exposure to 2.45 mg/L (2450 mg/m^3).

As compared to inhalation exposure, there has been more research on the toxicity of DETDA via the oral route of exposure. Pancreatic effects that may lead to hyperglycemia and decreased body weight are the adverse effects most often observed as critical effects with both acute and chronic oral exposure (ECHA, 2020). Effects in the liver have only been shown after chronic dosing in rat studies (ECHA, 2020; Ethyl Corp, 1992). Based on summaries from ECHA, dermal and eye irritation experiments have shown that DETDA is either mildly irritating or non-irritating to skin and irritating to the eyes of rabbits, and DETDA was not found to be sensitizing in two different guinea pig studies (ECHA, 2020). Given the systemic effects expected to occur after DETDA exposure, route to route extrapolation was deemed appropriate and oral studies will be considered for screening level development. However, with the potential for portal of entry effects given the evidence of eye irritation from this TAC, potential portal of entry critical effects should be considered as inhalation route of exposure studies become available.

Given the limited toxicity database for DETDA, the structurally similar chemical classes of simple aromatic amines and toluene diamines will be discussed here as well. Similar to DETDA, human studies have not been identified. Furthermore, repeat dose inhalation animal studies have not been identified. Toluene diamine mixtures have been observed to be mild/slight irritants (OECD, 2007). In short-term, repeated dose oral studies for toluene-2,4-diamine (CAS# 95-80-7), critical effects included decreased body weight as well as increased liver to

body weight ratios. With chronic administration of 2,4-TDA, liver and kidney toxicity as well as liver tumors were observed. At relatively higher oral doses, reproductive and developmental adverse effects were observed. Given the toxicological similarities in decreased weight and liver toxicity, the toxicity database for the class of toluene diamines will be considered to supplement the limited database for DETDA.

As noted above, only one agency was found to currently have screening levels for DETDA, and those screening levels are based on occupational exposure limits for o-toluidine (CAS# 95-53-4) (TAMIS). The ACGIH documentation has noted that the TLV is to protect against methemoglobinemia (ACGIH, 2001). This TLV is expected to also then be protective of irritation. Similar to aromatic amines like toluidines, TDAs tend to also have methemoglobinemia as a major critical effect. This effect has not been described following DETDA exposure. So, while the weight changes and liver effects as well as the structural similarities suggest that it is appropriate to use the overall toxicity database for toluene diamines in this screening level development, there may be significant differences in toxicity and structure between DETDA and simple aromatic amines.

Review of relevant studies for acute, noncarcinogen effects
Only one acute inhalation study was identified. The original study publication was not obtained, but the summary has been presented by two different agencies (ECHA, 2020; Chemview Database). Briefly, zero mortality was seen in male and female rats (N=5 for each sex) after 1 hour of exposure to up to 2.45 mg/l (2450 mg/m^3). However, clinical signs noted included, “excessive lacrimation and salivation, mucoid nasal discharge, labored breathing, and inactivity” (Chemview). At the time of necropsy, lung discoloration was also noted.

Summaries of eye and dermal irritation studies suggest that none to mild irritation is expected with DETDA exposure (ECHA, 2020 and Dow, 1992).

Three different summaries of the same reproductive and developmental study were found (Chemview; ECHA, 2020; Jacobi, 2014). The original publication was not obtained. In this study, 0, 50, 150, and 500 mg/kg per day were given to pregnant female rats (N=24 per dose group) in gestation days 0-20. This was estimated to be actual dietary levels of 0, 2.63, 7.83, and 20.45 mg/kg per day.

For sample collection:
Daily clinical observations were made. Body weights were taken on gestation days 0, 3, 7, 10, 14, 17, 20, and 21. Food consumption for select females were taken periodically over the study. During the necropsy, gross examination of organs was done, reproductive organ weights were taken, number of corpora lutea were taken, histopathological examination of the pancreas were performed, and blood was collected for hematology and clinical chemistry. The number of implantation sites, early and late resorptions, live and dead fetuses, fetus weight, placenta weight, fetus sex and external abnormalities were recorded. The pre-implantation loss and post-implantation loss were also calculated.

The critical effect was maternal toxicity-related with dose-dependent decreased body weight and histopathological changes in the pancreas. The No-observable effect level (NOEL) was observed to be 2.63 mg/kg per day. As summarized by one program, “In the high dose group maternal toxicity was observed as represented by a body weight loss during the first three days of gestation, followed by a decreased mean body weight gain and decreased food consumption during gestation, reduced ovary weight and induction of acinar cell apoptosis and mononuclear cell inflammation in the pancreas in most of the animals of the high dose group (13 animals with

minimal to moderate apoptosis of the pancreatic acinar cells, 15 animals with minimal to mild mononuclear cell inflammation). In the mid dose group minimal induction of acinar cell apoptosis was observed in two animals and minimal mononuclear cell inflammation in one animal. Although this finding was of low incidence and severity in this group, it could be regarded as a first indication of an effect on the target organ. Consequently, the low dose level was considered a No Observed Effect Level (NOEL) for maternal toxicity…the effects on embryo-fetal development that were observed at the high dose level were considered to be secondary to maternal toxicity" (Jacobi, 2014).

An acute ITSL could be derived as shown in Equation 1 based on a modification of AQD Rule 233.

$$Equation\ 1.\ ITSL = \frac{POD_{HED}}{UF_H\ x\ UF_A\ x\ UF_L}\ x \frac{hours\ exposed}{AT}\ x \frac{70\ kg}{20 m^3}$$

Where,

$$POD_{HED} = 2.63 \frac{mg}{kg} per\ day\ x\ DAF$$

$$DAF = \left(\frac{Weight_{Animal}}{Weight_{Human}}\right)^{1/4}$$

UFs=3 for interspecies extrapolation, 10 for intraspecies extrapolation, and 1 for LOAEL to NOAEL extrapolation

Hours exposed does not apply, but a 24-hour averaging time will be used since the critical effects were observed within the first three days of dosing.

Inhalation rate for an adult female is 20 m^3

Rat body weight = 0.319 kg

Human body weight = 70 kg

$$POD_{HED} = 2.63 \frac{mg}{kg}\ per\ day\ x\ \left(\frac{0.319\ kg}{70\ kg}\right)^{0.25} = 0.683 \frac{mg}{kg}$$

$$acute\ ITSL = \frac{0.683\ \frac{mg}{kg}}{3\ x\ 10}\ x \frac{10^3 \mu g}{mg}\ x \frac{70\ kg}{20\ m^3} = \ 79.683 \approx 80\ \frac{\mu g}{m^3}, 24\ hour\ AT$$

As more inhalation study information becomes available, this determination should be reconsidered.

Evaluation of Cancer Risk and IRSL Derivation

There is an unpublished carcinogenicity bioassay for DETDA that was submitted to TSCA (Ethyl Corp, 1992). In this study, 0, 10, 35, or 70 ppm DETDA was given to male and female rats (N=50 for each gender) via their diet for 24 months. For dosed animals, this was estimated to be 0.4, 1.4, and 3.2 mg/kg for male rats and 0.5, 1.8, and 3.8 mg/kg for female rats, respectively (ECHA, 2020).

As noted in Table 2, statistically significant increases in hepatocellular carcinomas and follicular cell adenomas were observed in male rats fed the highest dose. There seemed to be a trend for a dose-response as two low dose and three mid dose male rats had hepatocellular carcinomas as compared to one male rat in the control group. Statistically significant increases in hepatocellular adenomas were observed in female rats fed the highest dose. However, there did not seem to be a trend toward a dose-response at the lower doses compared to the control group.

As noted in Table 3, statistically significant increases in fibroadenomas were observed in female rats at the mid-level and high dose groups. No significant increases in follicular cell carcinoma were noted for any of the dosed groups, although two low dose, male rats showed follicular cell carcinomas. A statistically significant increase in breast tissue adenomas was not observed. However, a trend for increased adenomas may have occurred as 1 control female rat presented with adenomas compared to 0, 2, and 3 females in the low dose, mid dose, and high dose female groups, respectively. Interestingly, there was an inverse trend for adenocarcinomas, where 10 control group female rats had adenocarcinomas compared to 4, 7, and 4 female rats from the low dose, mid dose, and high dose groups, respectively.

Table 2. Summary of proliferative lesions identified.

Dose (ppm)	0	0	10	10	35	35	70	70
Gender (N=50)	Male	Female	Male	Female	Male	Female	Male	Female
Hepatocellular carcinoma	1	0	2	0	3	2	9[a]	1
Hepatocellular Adenoma	0	2	1	0	3	1	1	8[b]
Follicular Cell Carcinoma	0	0	2	0	0	0	0	0
Follicular Cell Adenoma	0	0	3	0	4	0	5[b]	2

a: significant increase over same gender controls; p=0.01
b: significant increase over same gender controls; p=0.05

Table 3. Summary of female rats presenting with breast tumors

Dose (ppm)	0	10	35	70
Number of Female rats	47	50	50	50
Adenocarcinoma	10	4	7	4
Adenoma	1	0	2	3
Fibroadenoma	12	16	23[a]	25[a]

a: significant increase over same gender controls; p=0.05

Notably, the authors of the study stated, “Since the incidence of malignant mammary gland tumors (adenocarcinomas) was high in the control females of this study, the relevance of the incidence of the fibroadenomas in the mammary gland of the high dose females is unknown.” The NTP’s review of control data indicate that this tumor type can have a 67% incidence in female Harlan Sprague-Dawley rats (Dinse et al., 2010), which provides further evidence that the statistically significant increase in this tumor type at the mid and high doses is not chemical-specific.

Besides the tumors themselves, other notable adverse effects included decreased body weight and pancreatic atrophy in the high dose males as compared to the controls. While these effects might typically indicate exceedance of the maximum tolerated dose, these effects have been consistently observed with DETDA administration in other studies and are likely to indicate a DETDA-specific adverse effect. As noted by the authors, "The results of this study should be evaluated in conjunction with subchronic DETDA studies conducted in this laboratory. The 90-day subchronic and 28-day progression/reversibility studies identified the pancreas as the target organ in the rat. Male rats were more severely affected and at an earlier time and lower dose than females…pathology in other organs occurred after or in conjunction with islet cell involvement…effects on the liver and thyroid, not seen in the subchronic studies, were detected in the two-year study…liver and thyroid effect[s] appear related to chronic, long-term continuous exposure." As a result of these chemical-specific effects, all of the dose groups were evaluated for increased tumor development.

Summaries of another carcinogenicity study, where male and female rats were administered DETDA via diet or gavage was available (ECHA, 2020). Via the diet, male and female rats were dosed with 0, 6, or 12 mg/kg per day DETDA for 24 months. Via gavage, male and female rats were initially dosed with 0, 4, or 12 mg/kg/day DETDA for 5 days per week. After significant reduction in body weight in DETDA-gavaged animals that suggested the dosing was above the maximum tolerated level, doses were lowered two times: first to 2 or 6 mg/kg per day for 5 days per week, then to 1 or 3 mg/kg per day for 5 days per week. At the end of the 24-month dose administration via either diet or gavage, no treatment-related increase in tumors was observed. Also, notable given the chemical-specific critical effects noted in other studies, no treatment-related effects in the pancreas were observed either. Taken together, given the dosing concerns and changes in the gavage study and the lack of pancreatic effects observed, there is less confidence in this study, and it will not be used to determine the carcinogenicity of DETDA.

The relatively more robust toxicity database for 2, 4-TDA and 2, 5-TDA have been evaluated for the weight of evidence related to carcinogenicity. As shown in Table 4, the results from oral studies for other TDAs have shown carcinogenic potential (EPA, 2021). The increased tumor types have varied for different TDAs, but this carcinogenicity indicates that the chemical-specific data for the unpublished study is supported. As a result, the DETDA-specific data will be used here.

Table 4. Comparison of Oral Carcinogenicity Data for TDAs

Chemical	2,4-TDA (CAS# 95-80-7)	2,5-TDA (CAS# 95-70-5)	2,6-TDA (CAS# 823-40-5)
EPA Weight of Evidence Characterization	N/A	"Suggestive Evidence of Carcinogenic Potential"	"Inadequate Information to Assess Carcinogenic Potential"
Oral slope factor $(mg/kg\text{-}day)^{-1}$	4×10^{0}	Screening p-OSF: 1×10^{-1} (as sulfate); screening p-OSF: 1.8×10^{-1} (as free base)	ND
Data set used for slope factor derivation	Mammary gland tumors in female rats (NTP, 1978)	Interstitial-cell tumors of the testis in male rats (NTP, 1978)	Studies were considered insufficient to assess carcinogenic potential; results were not considered treatment related but doses were too low, and a maximum tolerated dose was not achieved.
Other tumors observed in animal bioassays	Liver tumors in rats and mice; subcutaneous fibroma in male rats; lymphoma in female mice	Lung tumors in female mice	N/A
Study doses (mg/kg-day)	0, 3.2, 7.0 (M); 0, 3.95, 8.55 (F)	Adjusted daily dose: 0, 47, 158 (M); 0, 55, 183 (F)	N/A
Administration Duration	103 wk	78 wk	2 yr
POD type	$BMDL_{10}$	$BMDL_{10}$ (HED)	N/A
Source	(CALEPA, 2011)	(EPA, 2013)	(EPA, 2005)

Note: Table is modified from Table C-3 in (EPA, 2021)

To determine whether DETDA is genotoxic or not, summaries of *in vitro* and *in vivo* genotoxicity testing were obtained (ECHA, 2021; EPA, 2022). As shown in Table 5 and in Appendix 1, the summaries show that DETDA mostly tested positive for mutagenicity upon activation in both bacterial and somatic cell *in vitro* studies. At the same time, DETDA tested negative for mutagenicity in bacteria without activation, and negative/inconclusive for genotoxicity testing (aside from mutagenicity) in mammalian cells. DETDA never tested positive for mutagenicity in any of the *in vivo* studies, nor in the *in vitro* studies without activation. However, the *in vivo* testing was limited to micronucleus testing and a dominant lethal assay. The comparable testing in 2,4-TDA produced similar results (ECHA, 2022; EPA, 2021): positive results with activation and negative results with micronucleus testing and a dominant lethal assay. However, the additional *in vivo* testing conducted with 2,4-TDA shows positive results for both DNA strand breaks and DNA adducts *in* vivo. Taken together, DETDA is assumed to be genotoxic based on both chemical-specific *in vitro data* and results from the similar chemical 2,4-TDA. If more data becomes available, this determination should be re-evaluated.

Table 5. Summary of *in vitro* and *in vivo* genotoxicity testing

	DETDA	2,4-TDA
Mutagenicity testing in bacteria	Negative without activation; positive with activation.	Positive with activation
Mutagenicity testing in mammalian cells	In some studies, negative without activation; positive with and without activation in some studies.	Positive with activation
Genotoxicity testing aside from mutagenicity in mammalian cells	Inconclusive with one study; negative for other studies.	Positive in one study
Mutagenicity testing in animals	Negative in one rodent dominant lethal assay.	Negative in one rodent dominant lethal assay; Negative with sperm morphology test; Positive for testicular DNA synthesis.
Genotoxicity testing aside from mutagenicity in animals	Negative in two studies micronucleus tests.	Positive for DNA strand breaks in mice and rats; Positive for DNA adducts in rats; negative or questionable effects in micronucleus test.

As a result of the outcomes from the Ethyl Corp study as well as the possibility for DETDA to be genotoxic, Benchmark Dose (BMD) modeling was performed across the full range of dose groups when statistically significant increases in specific tumor types was observed, except for the fibroadenomas in female rats where both the study controls and historical controls have a relatively high incidence rate. BMD modeling software (EPA, Version 3.2) was used to determine the benchmark dose lower bound confidence limit (BMDL) given a benchmark dose response (BMR) at 10%, the typical response level used when considering extra risk in BMD modeling. Subsequently, the cancer slope factor (CSF) was derived as shown in Table 6. Further details from the model runs are provided in Appendix 2.

Table 6. BMD results based on tumor types identified from Ethyl Corp, 1992 study

Tumor type (Gender)	Selected Model	BMD (mg/kg per day)	BMDL (mg/kg per day)	CSF=BMR/BMDL (risk per mg/kg per day)
Follicular Cell Adenoma (Male rats)	Multistage Degree 1	2.77035865783691	1.41251112281207	0.070796
Hepatocellular Carcinoma (Male rats)	Multistage Degree 1	**2.13821601867675**	**1.28835978537782**	**0.077618**
Hepatocellular Adenoma (Female rats)	Multistage Degree 3	2.83595294952393	2.19986792988514	0.045457274

Based on Rule 231(1) and (3)(f)(i), an IRSL is derived as shown in Equation 2.

Equation 2. $IRSL = (1x10^{-6})/(unit\ risk)$,

Where:

$$unit\ risk_{inhalation} = unit\ risk_{oral} x \left(\frac{20\ m^3}{70\ kg}\right) x \frac{1\ mg}{10^3\ \mu g}$$

$$CSF_{HED} = CSF_{animal}\ x\ DAF$$

HED stands for Human Equivalent Dose and DAF stands for dosimetric adjustment factor

CSF_{animal}= 0.077618 (mg/kg) per day^{-1}

$$DAF = \left(\frac{W_A}{W_H}\right)^{0.25}$$

W_A = weight of animal = 0.47 kg

W_H = weight of human = 70 kg

$$CSF_{HED} = 0.077618\ \left(\frac{\text{mg}}{\text{kg}}\right)\text{per day}^{-1}\ x\ \left(\frac{0.47\ kg}{70\ kg}\right)^{0.25} = 0.0222184\ \left(\frac{mg}{kg}\right) per\ day^{-1}$$

$$unit\ risk_{inhalation} = 0.0222184\ \left(\frac{\text{mg}}{\text{kg}}\right)\text{per day}^{-1}\ x \left(\frac{20\ m^3}{70\ kg}\right)\ x\ \left(\frac{1\ mg}{10^3 \mu g}\right) = 6.34811\text{E-}06\ \left(\frac{\mu g}{m^3}\right)^{-1}$$

$$IRSL = \frac{(1x10^{-6})}{\left(6.34811\text{E}-06\ \left(\frac{\mu g}{m^3}\right)^{-1}\right)} = 0.15752709 \approx 0.2\ \frac{\mu g}{m^3}, \text{annual averaging time}$$

Review of relevant studies for chronic, noncarcinogen effects:
The same 2-year study on which the carcinogenic effects were observed showed increased proliferative and degenerative changes in the liver, where the LOAEL was 0.4 mg/kg in male rats (Ethyl Corp, 1992). An oral reference dose (RfD)-derived ITSL could be derived as shown in Equation 3.

$$Equation\ 3. ITSL = Oral\ RfD\ x \frac{70\ kg}{20\ m^3}$$

Where,

$$Oral\ RfD = \frac{LOAEL_{HED}}{UFs}$$

UFs = 3 for interspecies extrapolation, 10 for intraspecies extrapolation, and 10 for LOAEL to NOAEL extrapolation.

$$LOAEL_{HED} = LOAEL_{animal}\ x\ DAF$$

$LOAEL_{animal}$ = 0.4 mg/kg per day

$$DAF = \left(\frac{animal\ body\ weight}{human\ body\ weight}\right)^{0.25}$$

Rat body weight = 0.47 kg

Human body weight = 70 kg

$$LOAEL_{HED} = 0.4\frac{mg}{kg}\ per\ day\ x\ \left(\frac{0.47\ kg}{70\ kg}\right)^{0.25} = 0.11\frac{mg}{kg}$$

$$Oral\ RfD = \frac{0.11\frac{mg}{kg}per\ day}{3\ x\ 10\ x\ 10}x\frac{10^3\ \mu g}{mg} = 0.3\frac{\mu g}{kg}\ per\ day$$

$$ITSL = 0.11\ \frac{\mu g}{kg}\ x\frac{70\ kg}{20\ m^3} \approx\ 0.4\ \frac{\mu g}{m^3}, annual\ AT$$

Since BMD modeling was done on this same data in this same organ to derive a cancer slope factor, the IRSL will be used to protect against noncancer effects as well and the potential chronic ITSL will not be adopted at this time.

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KW:lh

Appendix 1: Detailed Genotoxicity Information

Table A. Summary of *in vitro* findings for DETDA

Table A1. Mutagen tests in vitro for DETDA

Negative	Inconclusive	Positive
Bacterial gene mutations without S-9 mix		Bacterial gene mutations with S-9 mix
Mammalian cell mutagenicity (tk locus) without S-9 mix		Mammalian cell mutagenicity (tk locus) with S-9 mix
Mammalian cell mutagenicity (BALB/3T3 Clone A31) with and without S-9 mix		
	Chromosome aberration in lymphocytes	

Table A2. Genotoxicity tests other than mutagenicity in vitro for DETDA

Negative Effects	Inconclusive Effects	Positive Effects
Unscheduled DNA synthesis with primary hepatocytes		

Table B. Summary of *in vivo* findings for DETDA

Table B1. Mutagen tests in vivo for DETDA

Negative	Inconclusive	Positive
Dominant lethal in rats		
Micronucleus testing in mice		

Table B2. Genotoxicity tests other than mutagenicity in vivo for DETDA

Negative Effects	Inconclusive Effects	Positive Effects
Erythrocyte micronucleus in mice		

Table C. Summary of *in vitro* findings for 2,4-TDA

Table C1. Mutagen tests *in vitro* for 2,4-TDA

Negative	Inconclusive	Positive
		Bacterial gene mutations with S-9 mix
Mammalian cell mutagenicity (hprt locus) with and without S-9 mix		
Mammalian cell mutagenicity (tk locus) with S-9 mix	Mammalian cell mutagenicity (tk locus) without S-9 mix	
		Chromosomal aberrations in CHO cells with and without S-9 mix

Table C2. Genotoxicity tests other than mutagenicity *in vitro* for 2,4 TDA

Negative Effects	Inconclusive Effects	Positive Effects
		Sister chromatid exchange in mammalian cells with and without S-9 mix
		Unscheduled DNA synthesis with primary hepatocytes
		DNA strand breaks in mammalian cells with and without S-9 mix
		DNA adducts in mammalian cells with and without S-9 mix

Table D. Summary of *in vivo* findings for 2,4-TDA

Table D1. Mutagen tests *in vivo* for 2,4-TDA

Negative	Inconclusive	Positive
Micronuclei in mice and rats (bone marrow; peripheral blood)	Micronuclei in rats at a highly toxic dose (bone marrow)	Gene mutations in transgenic mice (liver)
Dominant lethals in mice		

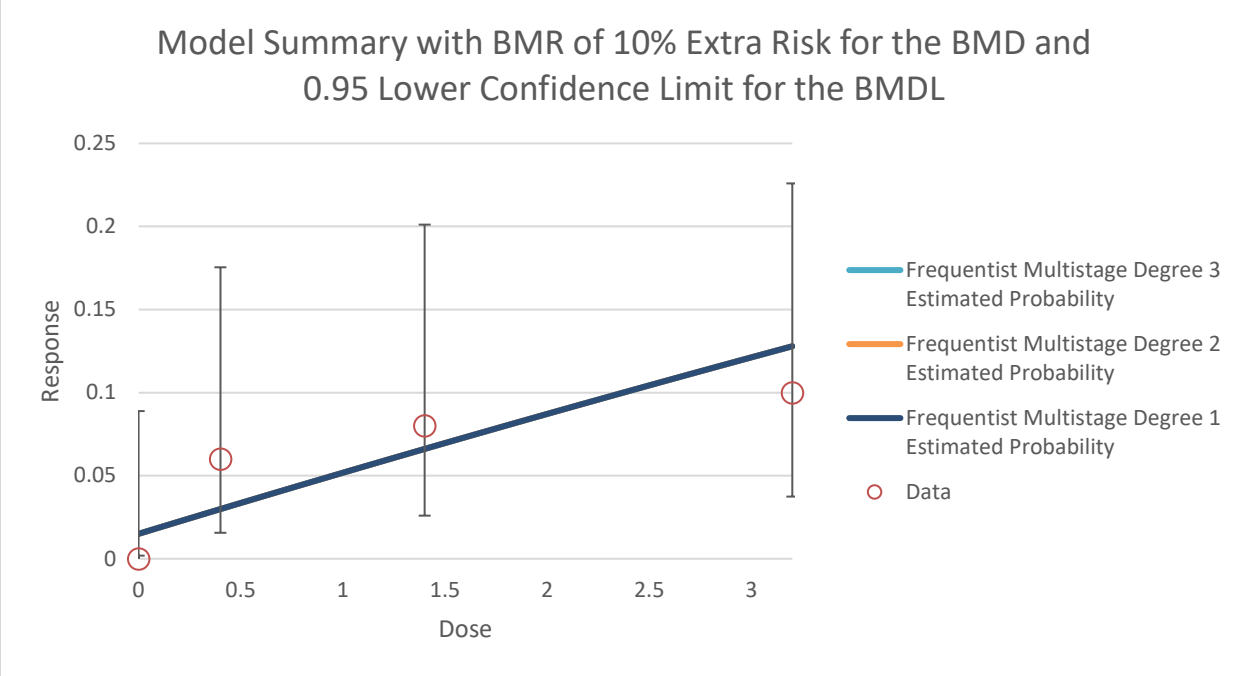
Table D2. Genotoxicity tests other than mutagenicity *in vivo* for2,4-TDA

Negative Effects	Inconclusive Effects	Positive Effects
Sperm morphology in mice		Sister chromatid exchange in mice (bone marrow)
		Unscheduled DNA synthesis in rats (liver)
		DNA strand-breaks in mice and rats (liver, kidney, lung, stomach)
		DNA adducts in rats (liver, mammary gland, kidney, lung)
		Reduction of testicular DNA-synthesis in mice

Appendix 2: Goodness of Fit of model predictions for each tumor type exhibiting a dose-response relationship

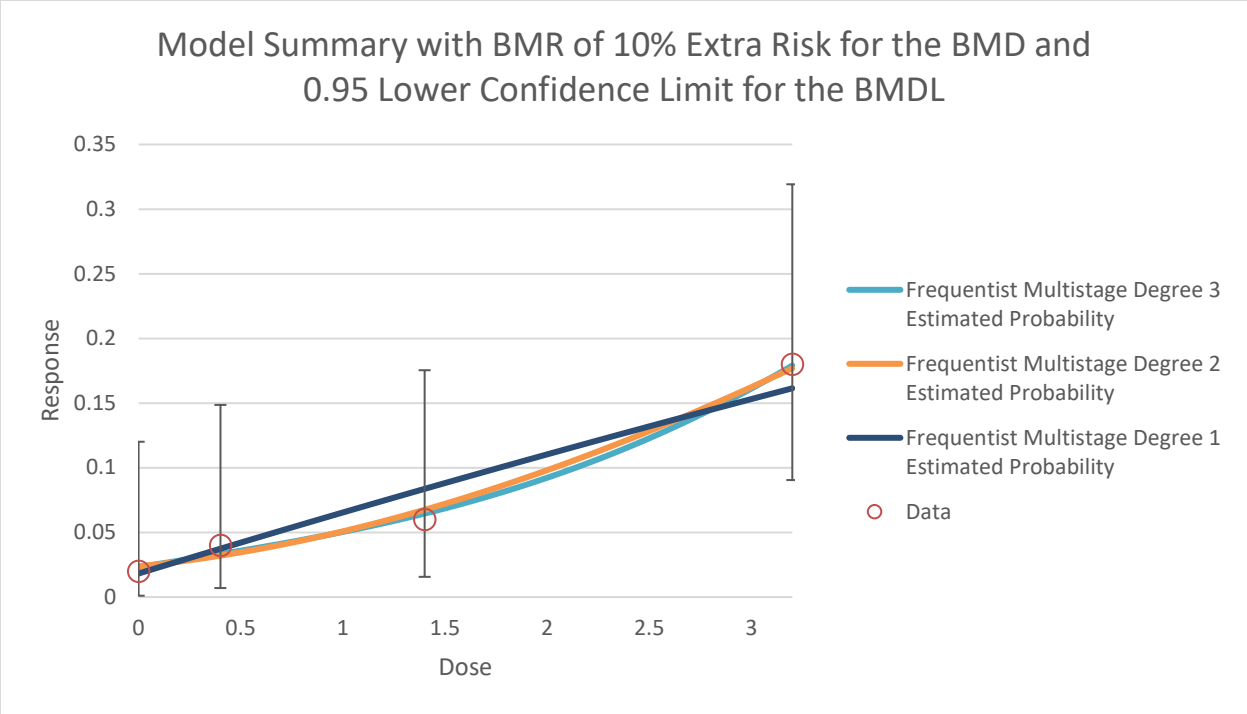
Follicular Cell Adenoma (Male rats)

Model	P Value	AIC	Scaled Residual for Dose Group near BMD
Multistage Degree 3	0.242768	90.33289012	-0.590363826
Multistage Degree 2	0.242768	90.33289012	-0.590363726
Multistage Degree 1	0.2427681	90.33289012	-0.590363809



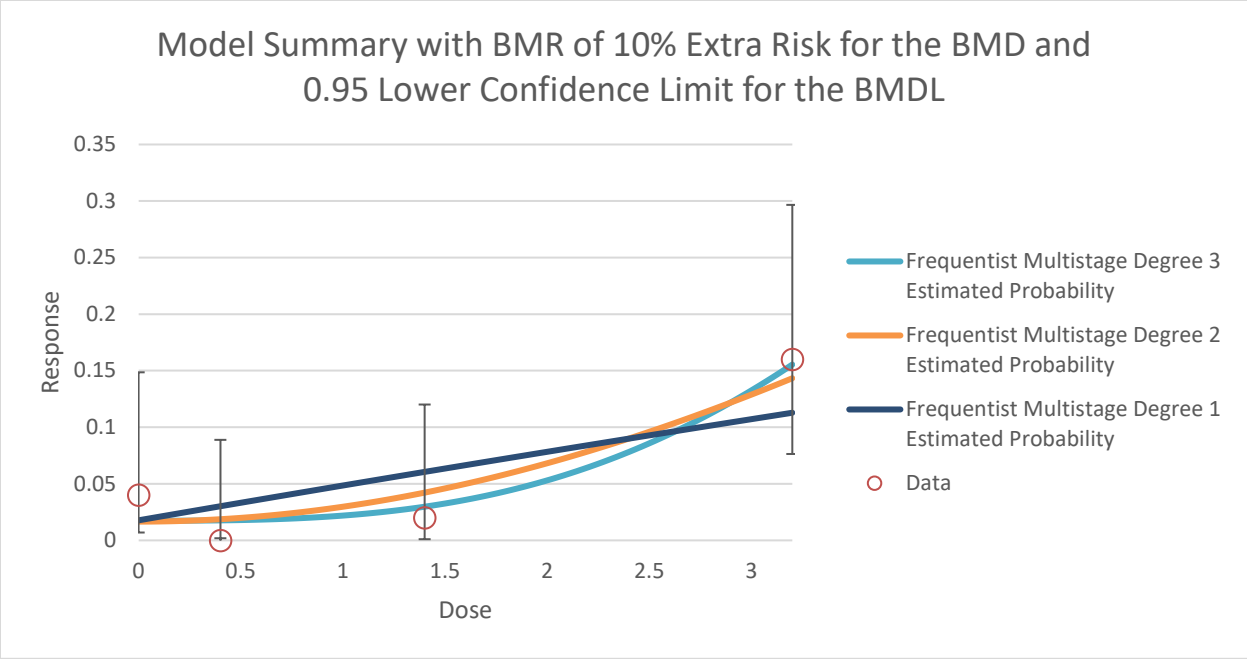
Hepatocellular Carcinoma (Male rats)

Model	P Value	AIC	Scaled Residual for Dose Group near BMD
Multistage Degree 3	0.7386266	102.5430233	0.018243699
Multistage Degree 2	0.6708255	102.6109033	0.056880446
Multistage Degree 1	0.7745791	100.9777943	-0.60432406



Hepatocellular Adenoma (Female rats)

Model	P Value	AIC	Scaled Residual for Dose Group near BMD
Multistage Degree 3	0.2769872	77.64051659	0.089321195
Multistage Degree 2	0.1868204	78.52423274	0.336584315
Multistage Degree 1	0.0629927	81.60374261	1.054122868


Model Summary with BMR of 10% Extra Risk for the BMD and 0.95 Lower Confidence Limit for the BMDL
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
Response
0
0.5
1
1.5
2
2.5
3
Dose
Frequentist Multistage Degree 3 Estimated Probability
Frequentist Multistage Degree 2 Estimated Probability
Frequentist Multistage Degree 1 Estimated Probability
Data