

# CLH report

## Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation),  
Annex VI, Part 2

### International Chemical Identification:

*N,N*-dimethyl-*p*-toluidine

**EC Number:** 202-805-4  
**CAS Number:** 99-97-8  
**Index Number:** 612-056-00-9 (Group Entry)

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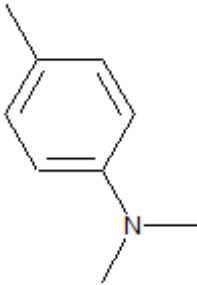
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## 1 IDENTITY OF THE SUBSTANCE

### 1.1 Name and other identifiers of the substance

Table 1: Substance identity and information related to molecular and structural formula of the substance

<b>Name(s) in the IUPAC nomenclature or other international chemical name(s)</b>	<i>N,N</i> -Dimethyl- <i>p</i> -toluidine
<b>Other names (usual name, trade name, abbreviation)</b>	Benzenamine, <i>N,N</i> ,4-trimethyl- <i>N,N</i> ,4-trimethylaniline DMPT 4, <i>N,N</i> -Trimethylaniline 4-Dimethylaminotoluene
<b>EC number (if available and appropriate)</b>	202-805-4
<b>EC name (if available and appropriate)</b>	<i>N,N</i> -Dimethyl- <i>p</i> -toluidine
<b>CAS number (if available)</b>	99-97-8
<b>Molecular formula</b>	C <sub>9</sub> H <sub>13</sub> N
<b>Structural formula</b>	
<b>SMILES notation (if available)</b>	<chem>N(C)(C)c1ccc(C)cc1</chem>
<b>Molecular weight or molecular weight range</b>	135.206 g/mol

There is an entry in Annex VI (Index number 612-056-00-9) where *N,N*-Dimethyl-*o*-toluidine, *N,N*-Dimethyl-*m*-toluidine and *N,N*-Dimethyl-*p*-toluidine are grouped together.

The intention is to generate a new entry in annex VI for *N,N*-Dimethyl-*p*-toluidine and to delete it from the existing entry.

Further explanation is given in chapter 3.

## 1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi- constituent substances)	Current CLH in Annex VI Table 3.1 (CLP)	Current self- classification and labelling (CLP)
<i>N,N</i> -Dimethyl-p-toluidine CAS-No.: 99-97-8	100 %	<ul style="list-style-type: none"> <li>• Acute Tox. 3*; H301</li> <li>• Acute Tox. 3*; H311</li> <li>• Acute Tox. 3*; H331</li> <li>• STOT RE 2*; H373**</li> <li>• Aquatic Chronic 3; H412</li> </ul>	<ul style="list-style-type: none"> <li>• Acute Tox. 2 (inhalation)</li> <li>• STOT RE 2 (e.g. oral and inhalation; reproductive, mouth, pharynx)</li> <li>• Carc. 1B</li> <li>• STOT SE 1 (blood)</li> <li>• Skin Irrit. 2</li> <li>• Eye Irrit. 2</li> <li>• Aquatic Chronic 1</li> </ul>

Table 3: Impurities (non-confidential information) if relevant for the classification of the substance

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self- classification and labelling (CLP)	The impurity contributes to the classification and labelling
-				

Table 4: Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self- classification and labelling (CLP)	The additive contributes to the classification and labelling
-					

Table 5: Test substances (non-confidential information) (this table is optional)

Identification of test substance	Purity	Impurities and additives (identity, %, classification if available)	Other information	The study(ies) in which the test substance is used
-				

## 2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

### 2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 6: 1.1 Proposed harmonised classification and labelling according to the CLP criteria

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATEs	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry (group entry)	612-056-00-9	<i>N,N</i> -dimethyl- <i>p</i> -toluidine [1] <i>N,N</i> -dimethyl- <i>m</i> -toluidine [2] <i>N,N</i> -dimethyl- <i>o</i> -toluidine [3]	202-805-4 [1] 204-495-6 [2] 210-199-8 [3]	99-97-8 [1] 121-72-2 [2] 609-72-3 [3]	Acute Tox. 3 * Acute Tox. 3 * Acute Tox. 3 * STOT RE 2 * Aquatic Chronic 3	H331 H311 H301 H373 ** H412	GHS06 GHS08 Dgr	H331 H311 H301 H373 ** H412		*	C
Dossier submitters proposal	612-RST-VW-Y	<i>N,N</i> -dimethyl- <i>p</i> -toluidine	202-805-4	99-97-8	<b>Retain</b> Aquatic Chronic 3 <b>Add</b> Carc. 2 <b>Modify</b> Acute Tox. 4 Acute Tox. 3 STOT RE 2 <b>Remove</b> Acute Tox. 3	<b>Retain</b> H412 <b>Add</b> H351 <b>Modify</b> H332 H301 H373 (blood; nasal cavity) <b>Remove</b> H311	<b>Retain</b> GHS06 GHS08 Dgr	<b>Retain</b> H412 <b>Add</b> H351 <b>Modify</b> H332 H301 H373 (blood; nasal cavity) <b>Remove</b> H311		<b>Add</b> Oral: ATE = 139 mg/kg bw  Inhalation: ATE = 1,4 mg/L (mists) <b>Remove</b> *	<b>Remove</b> C
Resulting entry in Annex VI if adopted by RAC and agreed by Commission									Carc. 2 Acute Tox. 4 Acute Tox. 3 STOT RE 2 Aquatic Chronic 3	H351 H332 H301 H373 (blood; nasal cavity) H412	GHS06 GHS08 Dgr

Please note that, as a result of this CLH proposal, the current group entry (# 612-056-00-9) shall be modified also.

Table 7: Reason for not proposing harmonised classification and status under public consultation

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	hazard class not assessed in this dossier	No
Flammable gases (including chemically unstable gases)		
Oxidising gases		
Gases under pressure		
Flammable liquids		
Flammable solids		
Self-reactive substances		
Pyrophoric liquids		
Pyrophoric solids		
Self-heating substances		
Substances which in contact with water emit flammable gases		
Oxidising liquids		
Oxidising solids		
Organic peroxides		
Corrosive to metals		
Acute toxicity via oral route	harmonised classification proposed	Yes
Acute toxicity via dermal route	data conclusive but not sufficient for classification	
Acute toxicity via inhalation route	harmonised classification proposed	
Skin corrosion/irritation	hazard class not assessed in this dossier	No
Serious eye damage/eye irritation		
Respiratory sensitisation		
Skin sensitisation		
Germ cell mutagenicity	data conclusive but not sufficient for classification	Yes
Carcinogenicity	harmonised classification proposed	
Reproductive toxicity	hazard class not assessed in this dossier	No
Specific target organ toxicity-single exposure		
Specific target organ toxicity-repeated exposure	harmonised classification proposed	Yes
Aspiration hazard	hazard class not assessed in this dossier	No
Hazardous to the aquatic environment		
Hazardous to the ozone layer		

### 3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

*N,N*-Dimethyl-p-toluidine covered by the group entry (Index No. 612-056-00-9) in Annex VI of the CLP Regulation (EC) No 1272/2008 and is classified in following hazard classes (hazard statement codes):

- Acute Tox 3\* oral (H301)
- Acute Tox 3\* dermal (H311)
- Acute Tox 3\* inhalation route (H331)
- STOT RE 2\* (H373\*\*)
- Aquatic Chronic 3

Hazard classes marked with asterisk (\*) have been adapted referring to the translation table in Annex VII of CLP Regulation from an Annex I entry of the Dangerous Substances Directive (DSD), 67/548/EEC, and should be considered as minimum classifications. The STOT RE 2 classification stems from DSD risk phrase R33 (“Danger of cumulative effects”), which has been translated to STOT RE 2 without specifying the target organ or the route of exposure. The double asterisk (\*\*) indicates that the hazard statement is without specifying the route of exposure as the necessary information was not available.

A documentation of the previous classification process from March 1991 is not available. In the previous classification process, *N,N*-dimethyl-p-toluidine (CAS-No. 99-97-8) and its position isomers *N,N*-dimethyl-o-toluidine (CAS-No. 609-72-3) and *N,N*-dimethyl-m-toluidine (CAS-No. 121-72-2) have been classified as substance group with the same hazards classes.

Because a relevant carcinogenicity study is only available for *N,N*-dimethyl-p-toluidine, a harmonized classification for this human health endpoint can only be made for the *para* isomer, the *ortho*- or *meta*-isomers are not subject of this dossier. The translated endpoints present in Annex VI of the CLP Regulation are reviewed and newly classified according to Regulation (EC) No 1272/2008 and the ECHA Guidance on the Application of CLP Criteria (in short, CLP Guidance) (ECHA, 2017).

### 4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

There is no requirement for justification that action is needed at Community level for CMR properties.

#### Further detail on need of action at Community level

The existing entry in Annex VI to CLP contains minimum classifications for Acute Toxicity and STOT RE and it is concluded that a refinement of the classification based on new available data is justified. Additionally for STOT RE, new data is available that allows updating the existing entry.

### 5 IDENTIFIED USES

According to the REACH registration dossier, *N,N*-dimethyl-p-toluidine is used as formulation in polyacrylic bone cements, as intermediate in the manufacture of other substance(s), in textile dyes, finishing and impregnating products; including bleaches and other processing aids, pH-regulators and manufacture of textiles, leather, fur.

ECHA notes widespread uses by professional workers. *N,N*-dimethyl-p-toluidine is used as an accelerator in polymer chemistry, e.g. in the polymerization of polymethyl methacrylate (PMMA) based bone cement. *N,N*-dimethyl-p-toluidine-cured PMMA is widely used in orthopaedics to anchor artificial joints or in dental applications. It used in glues and in artificial fingernail solutions.

The substance is used in the following products: pH regulators and water treatment products, adhesives and sealants, leather treatment products and laboratory chemicals. This substance is used in the following



areas: health services and scientific research and development. This substance is used for the manufacture of: textile, leather or fur.

Release to the environment of this substance is likely to occur from: indoor use (e.g. machine wash liquids/detergents, automotive care products, paints and coating or adhesives, fragrances and air fresheners).

## 6 DATA SOURCES

In addition to information that is available on the website of ECHA and in the REACH registration dossier, an extensive literature research was conducted in several relevant online resources (e.g. PubMed, SciFinder, SCOPUS, Web of Science, Embase, Wiley) during September and October 2017.

## 7 PHYSICOCHEMICAL PROPERTIES

Table 8: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
<b>Physical state at 20 °C and 101.3 kPa</b>	brown coloured organic liquid having unpleasant odour	REACH registration dossier	Physical observation
<b>Melting/freezing point</b>	- 15 °C	GESTIS - Substance Database	
<b>Boiling point</b>	211.2 °C at 965 hPa	REACH registration dossier	measured, distillation method
<b>Relative density</b>	0.88 g/cm <sup>3</sup> at 35 °C	REACH registration dossier	measured, mass by volume method
<b>Vapour pressure</b>	0.07501 mmHg at 20 °C	GESTIS - Substance Database	
<b>Surface tension</b>	33.97 mN/m	Chemspider - ACD/PhysChem Suite	Estimated
<b>Water solubility</b>	650 mg/L at 37 °C	GESTIS - Substance Database	
<b>Partition coefficient n-octanol/water</b>	1.729 at 35 °C, pH = 5.6	REACH registration dossier	measured, shake flask method
<b>Granulometry</b>			<i>N,N</i> -dimethyl- <i>p</i> -toluidine is a liquid
<b>Stability in organic solvents and identity of relevant degradation products</b>	<i>N,N</i> -dimethyl- <i>p</i> -toluidine was found to be stable in organic solvent dichloro methane and no degradation products were formed after 24 hours as evident from the GC-MS chromatogram obtained at 0 hours and that obtained after 24 hours.	REACH registration dossier	
<b>Dissociation constant</b>	0.05497 (average pKa value) at 35 °C	REACH registration dossier	measured
<b>Viscosity</b>	14.4 mPa s (dynamic) at 35 °C	REACH registration dossier	measured, Redwood/ Ostwald Viscometer

## 8 EVALUATION OF PHYSICAL HAZARDS

Not assessed for this dossier.

## 9 TOXICOKINETICS (ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION)

This section summarizes the toxicokinetic studies on *N,N*-dimethyl-*p*-toluidine (DMPT, CAS 99-97-8).

**Table 9: Summary table of toxicokinetic studies.**

Method	Results	Remarks	Reference
<p><b>Disposition study with radioactive labelled DMPT</b></p> <p><i>In vivo</i></p> <p>Distribution of radioactivity in urine, faeces, VOCs and tissues determined 24 hours after dosing.</p> <p><b>Fischer 344 rats</b></p> <p>Single i.v. (2.5 mg/kg) or oral gavage with 2.5, 25, or 250 mg/kg dose in 10 % aqueous PEG-30 castor oil or 250 mg/kg bw in corn oil</p> <p>4 male animals/dose</p> <p>Additional 4 female animals at 25 mg/kg bw oral</p> <p><b>B6C3F1 mice</b></p> <p>Single i.v. (2.5 mg/kg) or oral gavage with 2.5, 25, or 250 mg/kg dose in 10 % aqueous PEG-30 castor oil</p> <p>4 male animals/dose</p> <p>Additional 4 female animals at 25 mg/kg bw oral</p>	<p>[<sup>14</sup>C]DMPT-derived radioactivity was rapidly absorbed and excreted at oral doses up to 25 mg/kg: Excretion in urine accounted for approximately 75–90 % of the dose in mice and approximately 88–94 % in rats in the 2.5 and 25 mg/kg dose groups. The remaining radioactivity of the administered dose was recovered in faeces and tissues, and minor amounts were excreted as exhaled VOCs. For 2.5 mg/kg dose, recovery of radioactivity in the various matrices, including faeces and tissues, was similar regardless of route of administration. The 250 mg/kg oral dose was acutely toxic to male mice. 1/4 mice died before 24 h, 3/4 were moribund. Clinical and histopathological findings are consistent with acute renal failure. The concentrations of radioactivity in kidneys, liver, and urinary bladder at this dose were relatively high compared to other tissues.</p> <p>Male F344 rats at 250 mg/kg bw exhibited clinical signs of toxicity approximately 12 h after dosing but were clinically normal by 24 h.</p> <p>At 250 mg/kg bw radioactivity in the urine was reduced at 250 mg/kg to about 24 % (male mice) and 73 % (male rats), a higher proportion of the administered dose remained in tissues.</p>	<p>Considered reliable with restrictions.</p> <p>Not performed according to GLP or test guideline.</p> <p>[<sup>14</sup>C] <i>N,N</i>-Dimethyl-<i>p</i>-toluidine</p> <p>(CAS-No: 99-97-8)</p> <p>(Purity: 97.4 %)</p> <p>(Specific activity: 25.3 mCi/mol)</p> <p>The study was partly performed in the presence of an impurity or breakdown product of DMPT (i.e. <i>N</i>-methyl-<i>p</i>-toluidine). Results from “purified” and “nonpurified” i.v. studies at 2.5 mg/kg in male rats performed as control experiments did not differ statistically.</p>	<p>(Dix et al., 2007)</p>
<p><b>Identification of urinary metabolites</b></p> <p>Analytical reversed-phase high performance liquid chromatography (HPLC), spectrometric and spectroscopic methods</p>	<p>Four radiolabelled peaks were observed, isolated, and purified by solid-phase extraction (SPE) and preparative HPLC. The peaks were identified as <i>p</i>-(<i>N</i>-acetylhydroxyamino)-hippuric acid</p>	<p>Considered reliable with restrictions</p> <p>Not performed according to</p>	<p>(Kim et al., 2007a)</p>

Method	Results	Remarks	Reference
<b>Fischer 344 rats</b> <ul style="list-style-type: none"> <li>oral gavage of [<sup>14</sup>C]DMPT (250 mg/kg) in 10 % aqueous PEG-30 castor oil, 4 male rats per dose</li> <li>collection-interval composite yields (from 6, 12, 24, 48, and 72 h) of 4 male rats (10 % by weight of the total urinary output).</li> </ul>	(M1), DMPT N-oxide (M2), N-methyl-p-toluidine (M3), and parent DMPT.  DMPT metabolism is similar to that reported for <i>N,N</i> -dimethylaniline, i.e. phenylhydroxylamine formed from DMA is structurally related to p-methylphenylhydroxylamine, from which the identified major DMPT metabolite p-(N-acetylhydroxyamino)hippuric acid is a putative derivate.	GLP or test guideline  <b>Test material:</b> [ <sup>14</sup> C] <i>N,N</i> -Dimethyl-p-toluidine (CAS-No: 99-97-8) (Purity: 97.4 %) (Specific activity: 25.3 mCi/mol)	

## 9.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

### Absorption

No data on absorption kinetics are available, but absorption of radioactive labelled [<sup>14</sup>C]DMPT is high after single oral administration as indicated by the analysis of radioactivity in tissues, urine and faeces of mice and rat (Dix et al., 2007).

### Distribution

In oral gavage studies with radioactive labelled [<sup>14</sup>C] DMPT (Dix et al., 2007), the highest concentration of radioactivity at 2.5 mg/kg bw are found in the urinary bladder, kidney and liver (male rats) or in the liver (male mice). At 25mg/kg bw, radioactivity is distributed to most organs, with highest concentrations similar to the low dose, but for mice additionally in lung and urinary bladder. 250 mg/kg bw was acutely toxic to male mice (and to a lesser degree also to male rats), and the highest levels of radioactivity were found in urinary bladder, adipose tissue, liver and kidney (rats). In mice, additionally lung, spleen and testis had relatively high concentrations of radioactivity.

### Metabolism

An analysis of DMPT metabolites in the urine of rats after administering a single oral dose of [<sup>14</sup>C]DMPT identified three radioactive labelled metabolites in addition to the parent DMPT: p-(N-acetylhydroxyamino)hippuric acid, DMPT N-oxide and N-methyl-p-toluidine (Kim et al., 2007a). A quantitative analysis of DMPT and its metabolites in the urine is not available, in the HPLC radiochromatogram, peak intensities of DMPT (lowest intensity) and its major metabolites are on the same order of magnitude.

N-demethylation and N-oxidation are known cytochrome P450-mediated metabolic pathways described for DMPT and structurally similar substances like *N,N*-dimethylaniline (DMA) (Seto and Guengerich, 1993) or aniline (Harrison and Jollow, 1987). Based on the metabolism of DMA to phenylhydroxylamine, which produces methaemoglobinaemia, the metabolite putatively responsible for DMPT-induced methaemoglobinaemia is p-methylphenylhydroxylamine. In (Kim et al., 2007a), p-(N-acetylhydroxyamino)hippuric acid was identified as the major metabolite in the urine of DMPT dosed rats, which is the glycine conjugated and N-acetylated derivate of p-methylphenylhydroxylamine (Figure 1). Although the latter has not been identified directly, it can be concluded that p-methylphenylhydroxylamine can be formed from DMPT in vivo. Overall, the DMPT metabolism is consistent with the metabolism of DMA and aniline. Regarding aniline toxicity, it is generally known that phenylhydroxylamine can reduce haemoglobin to MetHb under production of reactive oxygen species (ROS) in a redox-cycle. Thereby, phenylhydroxylamine is oxidized to

nitrosobenzene, which can be reduced back to phenylhydroxylamine, and in turn generates more MetHb, ROS and other protein- or DNA reactive intermediates (Kiese, 1974).

### Excretion

At low and medium dose (2.5 or 25 mg/kg bw), about 90 % of orally administered DMPT (or radioactive labelled metabolites, (Dix et al., 2007)) is excreted via the urine from rats and male mice by 24 h, in female mice at 25 mg/kg bw about 77 % were recovered in the urine (Table 10 and Table 11). About 5 % of the radioactivity at these doses is recovered in faeces and in tissues. At high dose (250 mg/kg bw), [<sup>14</sup>C] recovery in the urine was reduced to about 70 % (rats) and 24 % (mice). Correspondingly, a higher percentage of radioactivity was present in the tissues.

**Table 10: Percent dose recovered 24 h after a single i.v. or oral dose of [<sup>14</sup>C]DMPT to male and female Rats (from (Dix et al., 2007)).**

Dose group <sup>b</sup>	Gender	n	Percent administered dose <sup>a</sup>					Total
			Urine	Feces	VOCs	Tissues <sup>c</sup>	GI tract	
IV, 2.5 mg/kg	Male	4	96.9 (3.4)	3.9 (0.7)	0.4 (0.2)	4.4 (2.3)	0.9 (1.0)	106 (5)
IV (purified), 2.5 mg/kg	Male	4	95.6 (5.9)	3.5 (0.9)	0.5 (0.2)	8.4 (0.9)	1.9 (0.3)	108 (6)
Oral, 2.5 mg/kg	Male	4	90.8 (2.5)	3.4 (1.3)	<0.1	4.2 (0.3)	1.7 (0.3)	98.5 (1.9)
Oral, 25 mg/kg	Male	4	87.7 (1.6)	9.3 (4.6)	<0.1	4.2 (0.2)	1.4 (0.3)	101 (4)
Oral, 25 mg/kg	Female	4	93.6 (5.5)	4.2 (1.1)	0.2 (0.1)	3.9 (0.5)	1.3 (0.3)	102 (6)
Oral, 250 mg/kg	Male	4	69.6 (2.3)	1.8 (0.7)	0.8 (0.5)	18.3 (3.2)	12.3 (3.0)	90.7 (0.3)
Oral (corn oil), 250 mg/kg	Male	4	72.9 (7.1)	1.5 (1.2)	0.5 (0.1)	15.4 (2.0)	10.5 (2.2)	90.3 (5.5)

<sup>a</sup>Mean (SD).

<sup>b</sup>Target dose; actual doses are provided in Table 1.

<sup>c</sup>Includes GI tract.

**Table 11: Recovery of radioactivity 24 h after a single i.v. or oral dose of [<sup>14</sup>C]DMPT to male and female mice (from (Dix et al., 2007)).**

Dose group <sup>b</sup>	Gender	n	Percent administered dose <sup>a</sup>					Total
			Urine	Feces	VOCs	Tissues <sup>c</sup>	GI tract	
IV, 2.5 mg/kg	Male	4	75.7 (15.8)	5.3 (0.8)	1.6 (0.1)	3.7 (1.1)	0.3 (0.2)	86.5 (16.1)
Oral, 2.5 mg/kg	Male	4	89.3 (2.5)	4.4 (0.8)	0.6 (0.3)	2.5 (0.2)	0.3 (<0.1)	97.2 (1.5)
Oral, 25 mg/kg	Male	4	92.0 (1.5)	4.8 (1.2)	0.8 (0.3)	5.4 (1.3)	0.2 (<0.1)	103 (2)
Oral, 25 mg/kg	Female	4	76.9 (4.5)	2.9 (1.6)	0.8 (0.5)	4.2 (2.0)	1.9 (1.7)	84.9 (4.3)
Oral, 250 mg/kg	Male	3	23.8 (11.4)	8.0 (8.4)	1.1 (0.2)	31.8 (5.0)	21.9 (5.2)	64.8 (9.3)

<sup>a</sup>Mean (SD).

<sup>b</sup>Target dose; actual doses are provided in Table 1.

<sup>c</sup>Includes GI tract.

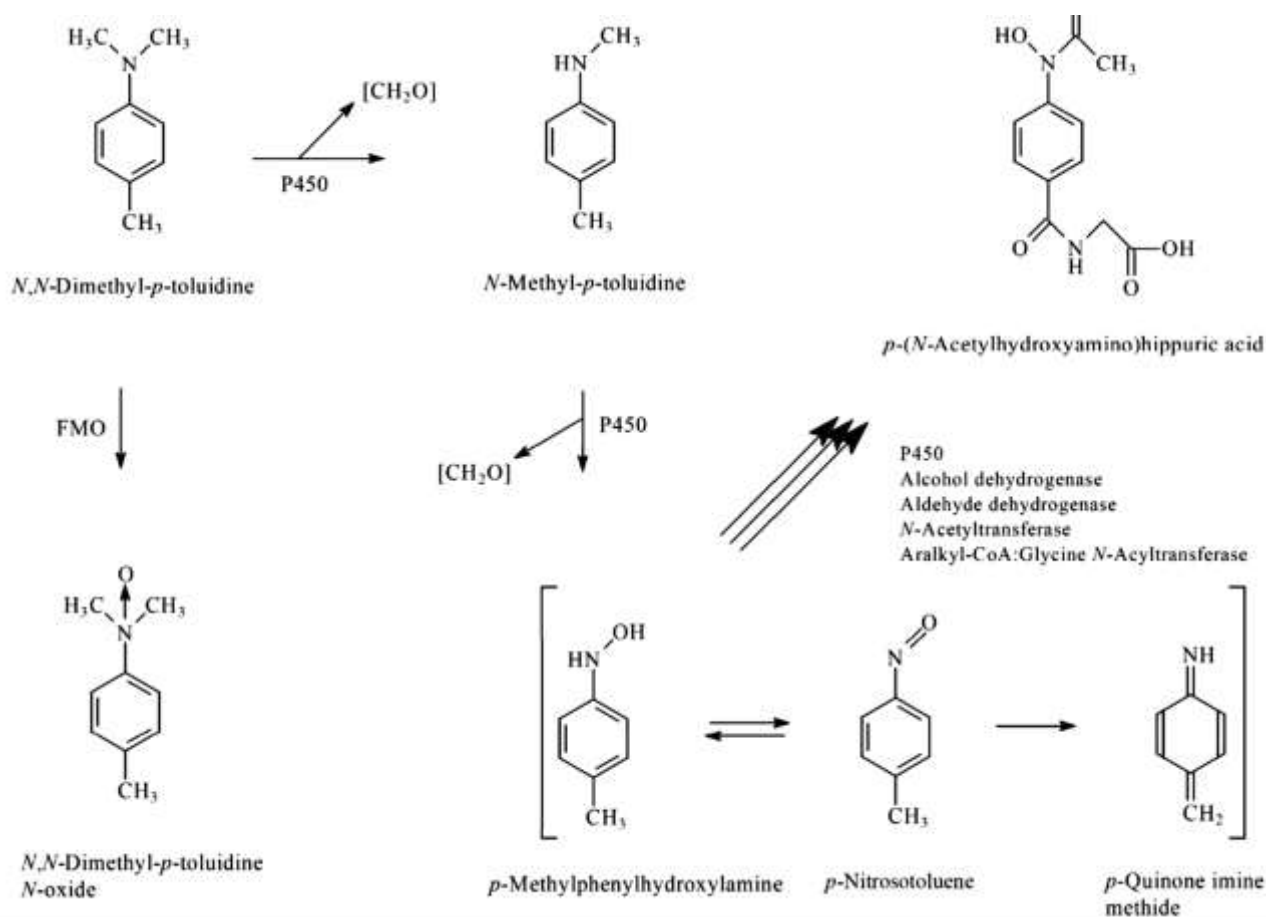


Figure 1 Observed DMPT metabolites including some proposed reactive intermediates of DMPT (*N,N*-Dimethyl-*p*-toluidine, CAS No. 99-98-7), (Kim et al., 2007b) and (Dunnick et al., 2014). FMO: Flavin-containing monooxygenase; P450: cytochrome P450. From (IARC, 2016)

In (Dunnick et al., 2017), metabolism of DMPT and toxicity of its metabolites is summarized as: “*N*-hydroxylated arylamines are capable of covalently binding to hemoglobin and/or DNA (Marques et al. 1997; Pathak et al. 2016). DNA adduct formation may result in mutations, leading to a carcinogenic response. Further, formation of a reactive imine methide via *N*-hydroxylation has been postulated (Dunnick et al. 2014). Imine methides may react with glutathione, other proteins or nucleic acids (Grillo et al. 2008).”

## 9.2 Summary of the toxicokinetic studies for the classification proposal

After oral administration, absorption of DMPT is about 90 % when administered at doses below acute toxicity. DMPT and its metabolites are mainly excreted via the urine, but are also dose dependently concentrated in tissues, e.g. in the liver, urinary bladder and kidney. At higher doses, urinary excretion is limiting and the concentration in tissues is increased, e.g. in rat liver to about 600 nmol (about 80 ug DMPT) per g liver weight at 250 mg/kg bw. (Dix et al., 2007). A major metabolite - putatively *p*-methylphenylhydroxylamine, which is related to the aniline metabolite phenylhydroxylamine - could reduce haemoglobin to MetHb under production of reactive oxygen species (ROS) in a redox-cycle, generating other protein- or DNA reactive intermediates (Kim et al., 2007b).

**10 EVALUATION OF HEALTH HAZARDS****Acute toxicity****10.1 Acute toxicity - oral route****Table 12: Summary table of animal studies on acute oral toxicity**

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Value LD <sub>50</sub>	Reference
<b>LD50-Test</b> Database entry, study details not available According to OECD 401 <b>Reliability not assignable</b>	<b>Rat</b> (Sprague-Dawley) Males and females No information on animal number	<i>N,N</i> -dimethyl-p-toluidine (CAS: 99-97-8) (purity = 99 %)	not available	1650 mg/kg bw	ChemFirst Study No. 3888-91-0105-TX-001, 1987, accessed from (ACToR, 2015)
<b>LD50-Test</b> Database entry, study details not available <b>Reliability not assignable</b>	<b>Mouse</b> No information on sex, strain and animal number	<i>N,N</i> -dimethyl-p-toluidine (CAS: 99-97-8) (purity not available)	not available	139 mg/kg bw	Toksikologicheskii Vestnik, (2),44,2006 and (4),30,2007, accessed from (RTECS, 2012)
<b>LD50-Test</b> Database entry, study details not available <b>Reliability not assignable</b>	<b>Rat</b> No information on sex, strain and animal number	<i>N,N</i> -dimethyl-p-toluidine (CAS: 99-97-8) (purity not available)	not available	980 mg/kg bw	Toksikologicheskii Vestnik, (2),44,2006 and (4),30,2007, accessed from (RTECS, 2012)
<b>Disposition study with radioactive labelled DMPT</b> (see Table 9) No guideline study, single oral gavage <b>Reliable with restrictions</b>	<b>Mouse</b> Male B6C3F1 mice 4 animals per dose	<i>N,N</i> -dimethyl-p-toluidine ([ <sup>14</sup> C]-DMPT, CAS: 99-97-8) (purity: 97.4 %)	2.5, 25, and 250 mg [ <sup>14</sup> C]-DMPT / kg bw administered in a dose volume of 10 ml/kg, 24 h study.	24 h after dosing with 250 mg/kg bw: 1 dead, 3 moribund. No overt signs of toxicity 24 h after dosing at 2.5 or 25 mg/kg bw	(Dix et al., 2007)

CLH REPORT FOR *N,N*-DIMETHYL-P-TOLUIDINE

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Value LD <sub>50</sub>	Reference
<p><b>3 month oral gavage study</b></p> <p>No guideline (NTP internal standards)</p> <p><b>Reliable with restrictions</b></p>	<p><b>Mouse</b></p> <p>B6C3F1/N mice, male and female, 10 mice per sex and dose</p>	<p><i>N,N</i>-dimethyl-p-toluidine (CAS: 99-97-8)</p> <p>(purity &gt;99 %)</p>	<p>0, 15, 30, 60, 125, and 250 mg/kg bw/day</p>	<p>250 mg/kg bw/day: 10/10 males and 9/10 females died within 10 days of dosing.</p> <p>125 mg/kg bw/day: 2/10 males and 1/10 females died within the first 2 weeks of dosing.</p> <p>No data on mortality is available covering the first 72 hours.</p>	(NTP, 2012)
<p><b>3 month oral gavage study</b></p> <p>No guideline (NTP internal standards)</p> <p><b>Reliable with restrictions</b></p>	<p><b>Rat</b></p> <p>F344 rats, male and female, 10 rats per sex and dose</p>	<p><i>N,N</i>-dimethyl-p-toluidine (CAS: 99-97-8)</p> <p>(purity &gt;99 %)</p>	<p>0, 62.5, 125, 250, 500, 1000 mg/kg bw/day</p>	<p>no survival at 1,000 mg/kg bw/day by study day 3 and centrilobular hepatocellular necrosis; fatty change of liver; ulceration of forestomach; renal tubule dilatation; red pulp atrophy of the spleen; necrosis and haemorrhage of thymus.</p> <p>500 mg/kg bw/day: 1/10 male rats dead by study day 3.</p>	(NTP, 2012)

Table 13: Summary table of human data on acute oral toxicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
<b>Single case report</b> <b>Reliability not assignable</b>	Fingernail solution containing <i>N,N</i> -dimethyl-p-toluidine	Accidental oral administration, single case report	Methaemoglobinaemia in 5-month old boy from drinking 30 mL of artificial fingernail solution	(Kao et al., 1997)
<b>Single case report</b> <b>Reliability not assignable</b>	Fingernail solution containing <i>N,N</i> -dimethyl-p-toluidine	Accidental oral administration, single case report	An acute cyanotic episode due to methaemoglobinaemia occurred in a 16-month old girl following the ingestion of <i>N,N</i> -dimethyl-p-toluidine, a commercially available component used in the production of artificial fingernails. The amount of the parent compound ingested was about 6 mg/kg bw. Administration of methylene blue was effective in the reversal of the methaemoglobinaemia (metHb was 43% vs. normal value of < 2%). In vitro studies suggest that the activity of the compound was probably due to its biochemical transformation to the toxic metabolite p-methylphenylhydroxylamine.	(Potter et al., 1988)

Table 14: Summary table of other studies relevant for acute oral toxicity

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
<b>i.v. injection</b> No guideline study <b>Not reliable</b>	<i>N,N</i> -dimethyl-p-toluidine (CAS: 99-97-8) (purity not available)	<b>Mice</b> (SPF-NMRI) i.v. injection, n=10 per dose, 5 doses between about 50 to 100 mg/kg bw	LD <sub>50</sub> : 75,8 mg/kg bw	(Liso et al., 1997)
<b>i.p. injection</b> <b>Reliability not assignable</b>	<i>N,N</i> -dimethyl-p-toluidine (CAS: 99-97-8) (purity not available)	<b>Mice</b> i.p. injection, no study details available	LD <sub>50</sub> : 212 mg/kg bw	(Citroni, 1951) cited in (Taningher et al., 1993)



### 10.1.1 Short summary and overall relevance of the provided information on acute oral toxicity

Detailed study reports to assess acute toxicity of DMPT are not available. Instead, LD<sub>50</sub> values for acute oral toxicity of DMPT can be obtained from chemical / toxicological databases at US EPA (ACToR, 2015) and (RTECS, 2012). For rats, a LD<sub>50</sub> of 1650 mg/kg bw from an OECD test guideline 401 conform study is listed ((ACToR, 2015)). Another entry reports an LD<sub>50</sub> of 980 mg/kg bw for rats and 139 mg/kg bw for mice ((RTECS, 2012)).

Although these database entries could not be verified, they are comparable with acute toxicity data from (Dix et al., 2007), in which mice were administered orally 250 mg/kg bw DMPT. After 24 h, 1 of 4 mice was dead and 3 of 4 animals were moribund. At the 25 mg/kg or 2.5 mg/kg oral doses, there were no signs of overt toxicity. Rats administered with 250 mg/kg bw DMPT showed only reversible signs of toxicity within 24 hours. This study is not conform to OECD test guidelines for acute toxicity, as the aim of this study was to identify the distribution of the radioactively labelled test substance in animals, but the study design is grossly comparable to OECD TG 423. Deviations from the guideline do not diminish the estimated acute toxicity (between 25 and) below 250 mg/kg bw. Main deviations were the number of animals used (4 per dose instead of 3 per step); male mice instead of preferred female rats; all dose levels tested instead of stepwise procedure; dose levels were 2.5, 25 and 250 mg/kg bw instead of 5, 50, 300 and 2000. One limitation might be, that the substance purity of the radioactively labelled DMPT was not in all experiments identical, and a breakdown product or impurity of DMPT (i.e. N-methyl-p-toluidine) was present in some of the experiments, but which is also an *in vivo* metabolite of DMPT (Kim et al., 2007a) and a precursor of the metabolite putatively responsible for DMPT-induced methaemoglobinaemia. A control experiment using *i.v.* administration of DMPT containing the breakdown product / impurity and purified DMPT did not show differences in the distribution of the radioactivity in mice.

In a series of three-month oral gavage studies (NTP, 2012), mice administered with 250 mg/kg bw/day DMPT died within 10 days of dosing (10/10 male and 9/10 female dead). Survival was much higher when dosing 125 mg/kg bw/day (2/10 males and 1/10 females died within 2 weeks of study). For rats, all 1,000 mg/kg males and females and one 500 mg/kg male died by study day 3. Mice and rats treated daily with lower doses of DMPT showed no increased mortality. These repeated dose studies allow conclusions on the acute toxicity of DMPT, as most mice treated with the highest dose (250 mg/kg bw/day) died within the first 10 days of treatment, whereas at 125 mg/kg bw/day the mortality was lower. Conclusively, an LD<sub>50</sub> for mice would be >125 mg/kg bw for the oral uptake route. For rats, the LD<sub>50</sub> would be expected between 500 and 1000 mg/kg bw, at and below 500 mg/kg bw a single dose would be expected to result in <50 % dead animals.

In mice, non-oral LD<sub>50</sub> values from *i.v.* or *i.p.* injections of DMPT are available: 75.8 mg/kg bw (*i.v.*, (Liso et al., 1997)) or 212 mg/kg bw (*i.p.*, (Taningher et al., 1993)).

### 10.1.2 Comparison with the CLP criteria

Acute oral toxicity means those adverse effects occurring following oral administration of a single dose of a substance or a mixture, or multiple doses given within 24 hours. According to the CLP Guidance (ECHA, 2017), mortalities during the first 72 hours after first treatment (in a repeated dose study) may also be considered for the assessment of acute toxicity.

Classification into one of four hazard categories for acute oral toxicity according to CLP is based on LD<sub>50</sub> values or acute toxicity estimates (ATE). The ranges are listed in Table 3.1.1, Annex I of CLP Regulation.

Exposure Route	Category 1	Category 2	Category 3	Category 4
Oral (mg/kg bodyweight)	ATE ≤ 5	5 < ATE ≤ 50	50 < ATE ≤ 300	300 < ATE ≤ 2000

In general, classification is based on the lowest LD<sub>50</sub> or ATE value available, i.e. the lowest LD<sub>50</sub> or ATE in the most sensitive appropriate species tested. Based on these criteria, LD<sub>50</sub> or ATE values derived from mice are used for classification, as these are lower than those obtained from rat experiments and no reasons have been identified why the most sensitive species (mouse) should not be considered.

Mouse data from (Dix et al., 2007) suggest an ATE below 250 mg/kg bw, where 4/4 animal died within 24 h. In the 3-month (NTP, 2012) study, 1/10 and 2/10 mice dosed daily with 125 mg/kg bw died within the first two weeks of study. As this is a repeated dose study, a single dose with 125 mg/kg bw should be considered as being below the ATE value. The ATE is therefore estimated to be between 125 and 250 mg/kg bw. This value includes the database listed LD<sub>50</sub> value of 139 mg/kg bw for mice from (RTECS, 2012), which is conclusively chosen as the ATE value for further derivations. This is supported by LD<sub>50</sub> values derived from i.v. (74.8 mg/kg bw, (Liso et al., 1997)) and i.p. (212 mg/kg bw, (Taningher et al., 1993)) administration in mice, which would indicate a Category 3 classification as well.

In conclusion, DMPT is to be classified into Acute Oral Toxicity Category 3 with an ATE of 139 mg/kg bw. This corresponds to the minimum classification from translation of entries in Annex I of the Dangerous Substances Directive (67/548/EEC).

### 10.1.3 Conclusion on classification and labelling for acute oral toxicity

Derived LD<sub>50</sub>/ATE values of the most sensitive species (mouse) fall in the range between 50 and 300 mg/kg bw, resulting in a classification of *N,N*-Dimethyl-p-toluidine as Acute Toxicity (oral) Category 3. An ATE value of 139 mg/kg bw is proposed.

## 10.2 Acute toxicity - dermal route

Table 15: Summary table of animal studies on acute dermal toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Value LD <sub>50</sub>	Reference
<b>LD<sub>50</sub>-Test</b> <b>According to OECD 402</b> Database entry, study details not available <b>Reliability not assignable</b>	<b>Rabbit</b> (New Zealand White Males and females No information on animal numbers	<i>N,N</i> -dimethyl-p-toluidine (CAS: 99-97-8) (purity >99 %)	unknown	>2000 mg/kg bw	ChemFirst Study No. 3888-91-0106-TX-001, 1987 (ACToR, 2015)

### 10.2.1 Short summary and overall relevance of the provided information on acute dermal toxicity

A single study summary for acute dermal toxicity in rabbits is available from the US EPA database (ACToR, 2015), which lists a dermal LD<sub>50</sub> value of >2000 mg/kg bw. The study is reported as in conformity with OECD TG 402, but study details are not available, therefore the reliability cannot be assigned.

### 10.2.2 Comparison with the CLP criteria

Classification into one of four hazard categories for acute dermal toxicity according to CLP is based on LD<sub>50</sub> or ATE values. The ranges are listed in Table 3.1.1, Annex I of CLP Regulation.

Exposure Route	Category 1	Category 2	Category 3	Category 4
Dermal (mg/kg bodyweight)	ATE ≤ 50	50 < ATE ≤ 200	200 < ATE ≤ 1000	1000 < ATE ≤ 2000

The only available study for acute dermal toxicity of *N,N*-dimethyl-p-toluidine concludes on an LD<sub>50</sub> value >2000mg/kg bw (ChemFirst Study No. 3888-91-0106-TX-001, 1987 (ACToR, 2015)). According to CLP classification criteria this would not result in a classification according to CLP.

### 10.2.3 Conclusion on classification and labelling for acute dermal toxicity

There are no reliable studies on Acute Toxicity (dermal) available. The only study results available would not result in a classification according to CLP criteria. Conclusively, the existing classification for Acute Toxicity, Category 3; H311 should be deleted without replacement.

### 10.3 Acute toxicity - inhalation route

Table 16: Summary table of animal studies on acute inhalation toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, , form and particle size (MMAD)	Dose levels, duration of exposure	Value LC <sub>50</sub>	Reference
Database entry, study details not available GLP conform (TSCA 40CFR 798.1150) <b>Reliability not assignable</b>	<b>Rat</b> (Sprague-Dawley) Males and females, n=10	<i>N,N</i> -dimethyl-p-toluidine (CAS: 99-97-8) (purity: 99 %)	Dose levels not available Exposure: 4 hours	1.4 mg/l	ChemFirst Study No. L08413, 1991 accessed from (ACToR, 2015)
Database entry, study details not available No guideline <b>Reliability not assignable</b>	<b>Mouse</b> No information on sex, strain and animal number	<i>N,N</i> -dimethyl-p-toluidine (CAS: 99-97-8) (purity unknown)	unknown	LC <sub>50</sub> not available (LOAEL: 3.192 mg/l) eye lacrimation, somnolence (general depressed activity), structural or functional change in trachea or bronchi	Toksikologicheskii Vestnik, (4),30,2007 accessed from (RTECS, 2012)
<b>LC<sub>50</sub> study</b> Database entry, study details not available No guideline <b>Reliability not assignable</b>	<b>Mouse</b> No information on sex, strain and animal number	<i>N,N</i> -dimethyl-p-toluidine (CAS: 99-97-8) (purity unknown)	unknown	LC <sub>50</sub> not available (LOAEL: 0.800 mg/l) structural or functional change in trachea or bronchi, dyspnoea	Toksikologicheskii Vestnik, (2),44,2006 (RTECS, 2012)

#### 10.3.1 Short summary and overall relevance of the provided information on acute inhalation toxicity

Detailed study reports for acute toxicity by inhalation are not available. Database records obtained from (ACToR, 2015) or (RTECS, 2012) report an LC<sub>50</sub> value for rats of 1.4 mg/l or an LOAEL value for mice of 0.8 mg/l., respectively The rat study is listed as a 4 hour exposure study with conformity to GLP and TSCA 40CFR 798.1150 (US). The reliability of these studies cannot be assigned because of not available study details.

### 10.3.2 Comparison with the CLP criteria

Classification into one of the four hazard categories for acute inhalation toxicity according to CLP is based on available LC<sub>50</sub> or ATE values. The ranges are listed in Table 3.1.1, Annex I of CLP Regulation. The CLP Guidance (ECHA, 2017) states criteria for differentiation between “vapours” and “dusts and mists” on the basis of the saturated vapour concentration (SVC) for a volatile substance. An LC<sub>50</sub> well below the SVC is considered for classification according to the criteria for vapours; whereas an LC<sub>50</sub> close to or above the SVC is considered for classification according to the criteria for dusts or mists. The SVC can be estimated as follows:

$$\text{SVC [mg/l]} = 0.0412 \times \text{MW} \times \text{vapour pressure (vapour pressure in hPa at 20 °C)}.$$

According to the registration dossier (key study), the vapour pressure of the registered substance is 0.075 mmHg, which equals 0.1 hPa. The estimated SVC is 0.557 mg/l.

Exposure Route	Category 1	Category 2	Category 3	Category 4
Vapours (mg/l)	ATE ≤ 0.5	0.5 < ATE ≤ 2.0	2.0 < ATE ≤ 10.0	10.0 < ATE ≤ 20.0
Dusts and mists (mg/l)	ATE ≤ 0.05	0.05 < ATE ≤ 0.5	0.5 < ATE ≤ 1.0	1.0 < ATE ≤ 5.0

Database entries for acute toxicity by inhalation are a LC<sub>50</sub> of 1.4 mg/l in rats and a LOAEL of 0.8 mg/l in mice, both values are above the estimated SVC of 0.557 mg/l. Therefore the classification criteria for dusts and mists should be applied.

Although details for the study in rats are not available, the ATE derived from the LC<sub>50</sub> value of 1.4 mg/l would indicate a classification into Category 4 of Acute Toxicity (inhalation) for dusts and mists. For mice, no ATE could be obtained, the LOAEL is based on adverse effects, but not on mortality, therefore an ATE value greater than 0.8 mg/l can be assumed. An ATE between 0.8 and 1.0 mg/l would result in classification into Category 3, above 1.0 mg/l into Category 4.

### 10.3.3 Conclusion on classification and labelling for acute inhalation toxicity

Study summaries were obtained from publicly available database entries. Because of the lack of study details in the database entries, reliability of the studies on Acute Toxicity (inhalation) is not assignable. However, based on the study in rats, which is reported as being conform to GLP- and TSCA-guidelines, it is conclusive that the hazard for Acute Toxicity by inhalation is lower than currently considered. A classification of *N,N*-dimethyl-p-toluidine into hazard class Acute Toxicity (inhalation) Category 4 is therefore suggested. An ATE value for dusts and mists of 1.4 mg/l should be noted based on the LC<sub>50</sub> in rats from the only available study report which claims GLP conformity.

The existing classification as Acute Toxicity, Category 3; H331 (inhalation) should be changed to Category 4; H332. The asterisk (\*) indicating transference from the classification under Dangerous Substances Directive (67/548/EEC) should be removed.

### 10.4 Skin corrosion/irritation

Not assessed for this dossier.

### 10.5 Serious eye damage/eye irritation

Not assessed for this dossier.

### 10.6 Respiratory sensitisation

Not assessed for this dossier.

### 10.7 Skin sensitisation

Not assessed for this dossier.

### 10.8 Germ cell mutagenicity

Table 17: Summary table of mutagenicity/genotoxicity tests in vitro

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
<b>Bacteria cell culture</b>				
<p><b>Reverse mutation / Ames Test</b></p> <p>Similar to OECD TG 471</p> <p>GLP: no information</p> <p>Deviations:</p> <ul style="list-style-type: none"> <li>• <i>S. typhimurium</i> TA 1535 not tested</li> <li>• <i>E. coli</i> WP2 uvrA, or <i>E. coli</i> WP2 uvrA (pKM101), or <i>S. typhimurium</i> TA102 not tested</li> <li>• no detailed data on cytotoxicity</li> </ul>	<p><b><i>N,N</i>-dimethyl-p-toluidine</b></p> <p>CAS: 99-97-8</p> <p>Purity: 99 %</p>	<p><b>Supporting study (Reliable with restrictions)</b></p> <p>Tester strains: <i>S. typhimurium</i> TA97, TA98 and TA100</p> <p>Dosing: 0, 1, 2.5, 5, 10, 40, 70, 100 µg/plate (with and without S9 mix):</p> <p>Controls: Negative control: valid Positive control: valid</p>	<p><b>Negative</b></p> <p>Negative in all tested strains (up to 70µg/plate) without and with metabolic activation</p> <p>Cytotoxicity: highest dose (100 µg/plate) was cytotoxic in all strains and conditions tested</p>	(Taningher et al., 1993)
<p><b>Reverse mutation / Ames test</b></p> <p>Similar to OECD TG 471 (NTP internal guideline)</p> <p>GLP: no information</p> <p>Deviations:</p> <ul style="list-style-type: none"> <li>• 5<sup>th</sup> strain missing</li> <li>• No data on cytotoxicity</li> </ul>	<p><b><i>N,N</i>-dimethyl-p-toluidine</b></p> <p>CAS: 99-97-8</p> <p>Purity: &gt;99 %</p>	<p><b>Supporting study (Reliable with restrictions)</b></p> <p>Tester strains: <i>S. typhimurium</i> TA97, TA98, TA100, and TA1535</p> <p>Dosing (with and without S9 mix): 0, (0.33), 1, (3.3), 10, 33, 100, 333, 500, 1 000 µg/plate</p> <p>Controls: Negative control: valid Positive control: valid</p>	<p><b>Negative</b></p> <p>No data on cytotoxicity (“The high dose was limited by cytotoxicity.”)</p>	(NTP, 2012)

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
<p><b>Reverse mutation / Ames Test</b></p> <p>Similar to OECD TG 471 (NTP internal guideline)</p> <p>Deviations:</p> <ul style="list-style-type: none"> <li>• Strains <i>S. typhimurium</i> TA1535, TA1537, TA97 (or TA97a) not tested.</li> <li>• No data on cytotoxicity</li> </ul>	<p><b><i>N,N</i>-dimethyl-p-toluidine</b></p> <p>CAS: 99-97-8</p> <p>Purity: &gt;99 %</p>	<p><b>Supporting study (Reliable with restrictions)</b></p> <p><i>E. coli</i> strain WP2 uvrA/pKM101, <i>S. typhimurium</i> strains TA98 and TA100. 10 % rat liver S9 for exogenous metabolic activation.</p> <p>Dosing (with and without S9 mix): 0, 50, 100, 250, 500, 750, 1 000, 1 500 µg/plate</p> <p>Controls: Negative control: valid Positive control: valid</p>	<p><b>Negative</b></p> <p>No data on cytotoxicity (“The high dose was limited by cytotoxicity.”)</p>	(NTP, 2012)
<p><b>Reverse mutation / Spot Test</b></p> <p>Not conform to OECD TG 471</p> <p>GLP: no information</p> <p>Major deviations:</p> <ul style="list-style-type: none"> <li>• single dose applied as spot</li> <li>• <i>S. typhimurium</i> TA104 instead of TA102</li> <li>• <i>S. typhimurium</i> TA1535 not tested</li> <li>• S9 activation method not described</li> <li>• no data on cytotoxicity</li> <li>• no colony counts available</li> <li>• no information on replicates</li> <li>• no information on relevance of negative controls</li> </ul>	<p><b><i>N,N</i>-dimethyl-p-toluidine</b></p> <p>CAS: 99-97-8</p> <p>Purity: not reported</p>	<p><b>Disregarded study (Not reliable)</b></p> <p>Tester strains: <i>S. typhimurium</i> TA97, TA98, TA100 and TA104</p> <p>Dosing: 3 µl/spot (3 mg pure test substance, single dose)</p> <p>Metabolic activation with rat liver S9 mix</p> <p>Controls: Negative control: no information on colony counts Positive control: valid</p>	<p><b>Negative</b></p> <p>Cytotoxicity: no information</p>	(Miller et al., 1986)

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
<p><b>Reverse mutation / Ames Test (plate incorporation)</b></p> <p>Similar to OECD TG 471 (US NCI standard procedure)</p> <p>GLP: no information</p> <p>Deviations:</p> <ul style="list-style-type: none"> <li>• <i>E. coli</i> WP2 uvrA, or <i>E. coli</i> WP2 uvrA (pKM101), or <i>S. typhimurium</i> TA102 not tested</li> <li>• No information on positive control substances available</li> <li>• Only general information on cytotoxicity available</li> </ul>	<p><b><i>N,N</i>-dimethyl-p-toluidine</b></p> <p>CAS: 99-97-8</p> <p>Purity: information not available</p>	<p><b>Disregarded study (Not reliable)</b></p> <p>Tester strains: <i>S. typhimurium</i> TA98, TA100, TA1535, TA1537, TA1538</p> <p>Dosing: 3, 10, 33, 100, 333 µg/plate (without and with S9 mix from hamster and rat)</p> <p>Controls: Negative control: valid Positive controls: Data present, but no information on positive control substances reported</p>	<p><b>Negative</b></p> <p>Negative in all tested strains, with and without metabolic activation</p> <p>Cytotoxicity: Dose range finding study in TA100 with and without metabolic activation as justification for dosing, but data not reported.</p>	<p>Summary report of the US National Cancer Institute (NCI): (Seifried et al., 2006)</p>
<p><b>Reverse mutation / Ames test</b></p> <p>OECD TG 471 conform</p> <p>GLP: yes</p> <p>Deviations:</p> <ul style="list-style-type: none"> <li>• 5<sup>th</sup> stain missing</li> <li>• No method details available</li> <li>• No study data available (colony counts, controls)</li> </ul>	<p><b><i>N,N</i>-dimethyl-p-toluidine</b></p> <p>CAS: 99-97-8</p> <p>Purity: 99 %</p>	<p><b>Disregarded study (Reliability not assignable)</b></p> <p><i>S. typhimurium</i> strains TA98, TA100, TA1537, TA1538</p> <p>Dosing: 100 – 5 000 µg/plate</p> <p>Controls: Negative control: no data Positive control: no data</p>	<p><b>Negative</b></p> <p>TA98, TA100, TA1537: conclusion/genotoxic effect: <b>negative/negative</b></p> <p>TA1538 conclusion/genotoxic effect: <b>negative/equivocal</b></p> <p>Cytotoxic without metabolic activation: 1000µg/plate</p> <p>Study data not available</p>	<p>ChemFirst Study No. 14506-0-401 (1983) from database entry (ACToR, 2015)</p>

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
<i>mammalian cell culture</i>				
<p><b>L5178Y TK+/- Mouse Lymphoma Mutagenicity Assay</b></p> <p>Equivalent to OECD 476 (1997), similar to OECD 490</p> <p>GLP: no information</p> <p>Deviations:</p> <ul style="list-style-type: none"> <li>No study details reported</li> </ul>	<p><i>N,N</i>-dimethyl-p-toluidine</p> <p>CAS: 99-97-8</p> <p>Purity: not reported</p>	<p><b>Key study (Reliable with restrictions)</b></p> <p>Cells: L5178Y TK<sup>+/-</sup> 3.7.C mouse lymphoma cells</p> <p>Dosing:  <u>Without S9 mix:</u>                      0.05, 0.11, 0.18, 0.24, 0.31 µl/mL</p> <p><u>With S9 mix:</u>                      0.005, 0.011, 0.018, 0.024, 0.031, 0.037, 0.044 µl/mL</p> <p>Treatment time: 4 h</p> <p>Sampling time: 10-12 days incubation time</p> <p>Colonies larger 0.2 mm were counted</p> <p>Controls:                      Negative control: valid                      Positive control: valid</p>	<p><b>Equivocal</b> with/without S9 mix</p> <p>Cytotoxicity: Only doses with total growth rates of 10% or more were used in analysis of induced mutant frequency (MF) or global evaluation factor (GEF).</p> <p><u>Without S9 mix:</u>                      In one of two parallel cultures at 0.24 µl/mL weakly positive rel. MF (2.0-fold) and GEF (90 mutants per 10<sup>6</sup> viable cells over solvent control), overt cytotoxicity in the other parallel culture.</p> <p><u>With S9 mix:</u>                      Weakly positive rel. MF (3.1- and 2.2-fold) and equivocal GEF (106 and 59 mutants per 10<sup>6</sup> viable cells over solvent control) at 0.031 µl/mL.</p>	<p>Summary report of the US National Cancer Institute (NCI): (Seifried et al., 2006)</p>



Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
<p><b>In vitro mammalian micronucleus test</b></p> <p>Equivalent to OECD TG 487</p> <p>GLP: no information</p> <p>Deviations:</p> <ul style="list-style-type: none"> <li>Extended treatment (48h, approx. 3 cell cycles)</li> <li>Metabolic activation: no data</li> </ul>	<p><i>N,N</i>-dimethyl-p-toluidine</p> <p>CAS: 99-97-8</p> <p>Purity: 99 %</p>	<p><b>Supporting study (Reliable with restrictions)</b></p> <p>Cells: V79 cells.</p> <p>Dosing: 0, 0.3, 0.9, 1.2 mM</p> <p>Treatment time: 48 h</p> <p>Sampling time: end of treatment</p> <p>Controls:                      Negative control: valid                      Positive control: valid</p>	<p><b>Positive</b></p> <p>Significant aneugenic activity: CREST positive micronuclei up to about 5.5-fold induced compared to control (<math>p &lt; 0.01</math>, <math>X^2</math> test or Fisher Exact test)</p> <p>Significant clastogenic activity: CREST negative micronuclei up to about 3.6-fold induced compared to control (<math>p &lt; 0.01</math>, <math>X^2</math> test or Fisher-Exact test)</p> <p>Dose dependency: significant for CREST positive and negative micronuclei (<math>p &lt; 0.001</math>, Cochran-Armitage trend test)</p> <p>Cytotoxicity: Survival &gt;10 % (colony formation, data not presented)                      Mitotic index (at 24 and 48 h of treatment) partly increased, no dose dependency.</p>	<p>(Taningher et al., 1993)</p>

Table 18: Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells in vivo

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
<p>Mouse peripheral blood micronucleus, flow cytometric assay</p> <p>Equivalent to OECD TG 474</p> <p>GLP: no information</p> <p>Deviations:</p> <ul style="list-style-type: none"> <li>No information on toxicity; dosing based on 3-month study</li> <li>Clinical observations not available</li> </ul>	<p><i>N,N</i>-dimethyl-p-toluidine</p> <p>CAS: 99-97-8</p> <p>Purity: &gt;99 %</p>	<p><b>Supporting study (Reliable with restrictions)</b></p> <p>Species: male B6C3F1/N mice; n=5 per dose</p> <p>Dosing: 0, 30, 60, or 75 mg/kg bw/day in corn oil daily for 4 days by gavage</p> <p>Sampling time: 4 hours after the fourth dose</p> <p>Controls: Positive control: valid Negative control: valid</p> <p>Toxicity: The highest dose was based on the toxicity information obtained in a 3-month mouse study (NTP, 2012), see Table 42 and Table 46.</p>	<p><b>Negative</b></p> <p>No significant increases in frequencies of micronucleated erythrocytes.</p> <p>Toxicity: No significant alterations in percentage of circulating reticulocytes.</p> <p>Clinical signs: information not available.</p>	(NTP, 2012)

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
<p><b>Mouse peripheral blood micronucleus, slide-based assay</b></p> <p>Equivalent to OECD TG 474</p> <p>GLP: no information</p> <p>Deviations:</p> <ul style="list-style-type: none"> <li>• No positive control</li> <li>• Sampling time not reported</li> </ul>	<p><i>N,N</i>-dimethyl-p-toluidine</p> <p>CAS: 99-97-8</p> <p>Purity: &gt;99 %</p>	<p><b>Supporting study (Reliable with restrictions)</b></p> <p>Species: B6C3F1/N mice; n=5 per dose and sex</p> <p>Dosing: 0, 15, 30, 60 and 125 mg/kg bw/day in corn oil by gavage for 3-months</p> <p>Controls: Negative control: valid Positive control: none</p> <p>Toxicity: Dosing for 3-month study (NTP, 2012) was based on available LD50 values.</p>	<p><b>Negative</b></p> <p>No significant increases in the frequencies of micronucleated erythrocytes (MNE).</p> <p>In male mice, MNE frequencies were slightly increased with dose, but without significant trend.</p> <p>Toxicity: No significant alterations in the percentage of circulating reticulocytes.</p> <p>Other clinical/toxicological observations: Blood was taken from animals of a 3-month study (NTP, 2012) (see Table 42 and Table 46 for details). At 250 mg/kg bw/day, 10/10 (male) and 9/10 (female) animals died within 10 days; at 125 mg/kg bw/day: 2/10 males and 1/10 females died within the first 2 weeks of dosing.</p>	(NTP, 2012)

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
<p><b>Comet assay in mouse blood and liver cells</b></p> <p>Equivalent to OECD TG 489</p> <p>GLP: no information</p> <p>Deviations:</p> <ul style="list-style-type: none"> <li>No information on toxicity; dosing based on 3-month study</li> <li>Clinical observations not available</li> </ul>	<p><i>N,N</i>-dimethyl-p-toluidine</p> <p>CAS: 99-97-8</p> <p>Purity: &gt;99 %</p>	<p><b>Supporting study (Reliable with restrictions)</b></p> <p>Species: male B6C3F1/N mice; n=5 per dose</p> <p>Dosing: 0, 30, 60, or 75 mg/kg bw/day in corn oil daily for 4 days by gavage.</p> <p>Sampling time: 4 hours after the fourth dose</p> <p>Controls: Positive control: valid Negative control: valid</p> <p>Toxicity: The highest dose was based on the toxicity information obtained in a 3-month mouse study (NTP, 2012), see Table 42 and Table 46.</p>	<p><b>Negative</b></p> <p>No increased DNA damage in liver cells or blood leukocytes.</p> <p>Clinical signs: information not available.</p>	(NTP, 2012)

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
<p><b>Comet assay in rat liver cells</b></p> <p>Equivalent to OECD TG 489</p> <p>GLP: no information</p> <p>Deviations:</p> <ul style="list-style-type: none"> <li>• Only single dose tested</li> <li>• No information on toxicity; dosing based on 2-year study</li> <li>• Clinical observations not available</li> </ul>	<p><i>N,N</i>-dimethyl-p-toluidine</p> <p>CAS: 99-97-8</p> <p>purity: &gt;99 %</p>	<p><b>Disregarded study (Not reliable)</b></p> <p>Species: Male F344/N rats; n=5 per dose</p> <p>Dosing: Single dose of 60 mg/kg bw/day in a 1 % acetone/corn oil vehicle by gavage.</p> <p>Sampling time: 4 hours after the fourth dose</p> <p>Toxicity: Same dose as the highest dose in 2-year study (NTP, 2012).</p> <p>Controls: Positive control: valid Negative control: valid</p>	<p><b>Equivocal</b></p> <p>Statistically significant, but weak increase (1.4-fold, p&lt;0.05) compared to vehicle control in percent tail DNA.</p> <p>No information on cytotoxic effect/no information on clinical signs.</p>	(NTP, 2012)
<p><b>Alkaline DNA elution test</b></p> <p>No test guideline followed</p> <p>GLP: no information</p> <p>Only summary data available.</p> <p>No positive control.</p>	<p><i>N,N</i>-dimethyl-p-toluidine</p> <p>CAS: 99-97-8</p> <p>Purity: 99 %</p>	<p><b>Disregarded study (Not reliable)</b></p> <p>Species: Sprague Dawley rats (male); n=2 (neg. control) or 4 (dosing)</p> <p>Organ: liver</p> <p>Dosing (sampling time) by oral gavage: 8 mmol/kg bw (6 h after treatment) 4 mmol/kg bw (24 h after treatment)</p> <p>Controls: Negative control: valid Positive control: none</p>	<p><b>Negative</b></p> <p>DNA elution rate (considered as a marker of genotoxicity) increased after 6h treatment by a factor of 2.4, but not statistically significant. No increase after 24h.</p>	(Taningher et al., 1993)

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
<p><b>Alkaline DNA elution test</b></p> <p>No test guideline followed</p> <p>GLP: no information</p> <p>Only summary data available.</p>	<p><i>N,N</i>-dimethyl-p-toluidine</p> <p>CAS: 99-97-8</p> <p>Purity: 99 %</p>	<p><b>Disregarded study (Not reliable)</b></p> <p>Species: Sprague Dawley rats (male); n=2 to 6</p> <p>i.p. injection, male Sprague Dawley rats, liver n=2 to 6</p> <p>Dosing (sampling time) by i.p injection: 4 mmol/kg bw (2 h and 24 h after treatment) 8 mmol/kg bw (only 2 h after treatment)</p> <p>Toxicity: Doses based on reported LD<sub>50</sub> values.</p> <p>Controls: Positive control: valid Negative control: valid</p>	<p><b>Weakly positive/equivocal</b></p> <p>“... weakly positive results that in some cases were statistically significant”. DNA elution not increased 24h after treatment. After 2 hours, statistically significant response with 4 and 8 mmol/kg (increase of elution rate by factor of 1.9 and 2.0 over control dose).</p>	<p>(Taningher et al., 1993)</p>
<p><b>Alkaline DNA elution test</b></p> <p>No test guideline followed</p> <p>GLP: no information</p> <p>Only summary data available.</p>	<p><i>N,N</i>-dimethyl-p-toluidine</p> <p>CAS: 99-97-8</p> <p>Purity: 99 %</p>	<p><b>Disregarded study (Not reliable)</b></p> <p>Species: BALB/c mice (male); n=4 (neg. control) or 6 (dosing)</p> <p>Organ: liver</p> <p>Dosing (sampling time) by i.p injection: 1 or 2 mmol/kg bw (2 h after treatment) 1 mmol/kg bw (24 h after treatment)</p> <p>Toxicity: Doses based on reported LD<sub>50</sub> values.</p> <p>Controls: Positive control: valid Negative control: valid</p>	<p><b>Weakly positive/equivocal</b></p> <p>“... weakly positive results that in some cases were statistically significant”. DNA elution not increased 2h after treatment (all doses). After 24 hours, marginal but statistically significant response with 1 mmol/kg (increase of elution rate by factor of 1.7 over control dose).</p>	<p>(Taningher et al., 1993)</p>

### 10.8.1 Short summary and overall relevance of the provided information on germ cell mutagenicity

In accordance with the CLP Regulation and the CLP Guidance (ECHA, 2017) only fully reliable (positive) results of well-conducted and scientific validated tests are relevant for justification of toxicological classification of a substance.

Therefore, only those *in vitro* (see Table 17) and *in vivo* studies (see Table 18) are considered for the discussion on mutagenicity of *N,N*-dimethyl-p-toluidine, which are characterized as 'key study' or 'supporting study'. Definition for the study categories are:

**Key studies:** Studies that have been performed according to relevant OECD guidelines or are at least equivalent without major restrictions to the guideline requirements, and where a comprehensible documentation is available, i.e. at least a robust study.

**Supporting studies:** Studies which are in general reliable, but with some deficiencies in either documentation or test guideline conformity.

**Disregarded studies:** Studies with significant shortcomings, such as lack of controls (positive and/or negative control) or lack of detailed information. The results of these studies are therefore considered as not reliable and not relevant for a classification discussion.

#### **In vitro data**

##### Bacterial Mutagenicity Assays

Results from 3 Bacterial Mutagenicity Assays are available. The results from these Ames tests are negative with or without metabolic activation in all tested strains.

Conclusively, there is no evidence for bacterial mutagenicity with or without metabolic activation.

##### Mouse Lymphoma Mutagenicity Assay (MLA) (Seifried et al., 2006)

The results of the MLA are weakly positive for single doses in single parallel cultures close to the border of evaluation criteria and acceptable cytotoxicity; overall the study results are rated as equivocal.

The study data are available as summary publication of the US National Cancer Institute (NCI). The evaluation either

- followed internal standards, equivalent to OECD TG 476 (original evaluation), i.e. among others at least a doubling of the mutation frequency (MF) in relation to the negative control

or

- was similar to OECD TG 490 (re-evaluation), where in the test guideline a global evaluation factor (GEF) of  $90 \times 10^{-6}$  predefines the induced MF based on negative control MF for the soft agar version of the MLA. In (Seifried et al., 2006) a GEF of  $100 \times 10^{-6}$  was used for evaluation according to NCI internal standards.

Severe toxicity was an exclusion criterion for evaluation, i.e. when relative growth rate (RTG) was below 10 %. In the studies, relevant positive and negative controls were performed and the data was available. Experiments were performed in parallel cultures.

Without S9 mix (see Table 19), *N,N*-dimethyl-p-toluidine induced a weakly positive response at or around the evaluation criteria only at the highest dose below overt cytotoxicity response (0.24 µl/ml; GEF:  $90 \times 10^{-6}$ ; rel. MF: 2.0; RTG: 13 %). A parallel culture had a slightly higher rel. MF and GEF, but which is not relevant due to higher cytotoxicity (RTG: 9 %).

With S9 mix (see Table 20), the frequency of mutations in the solvent control was comparably low. With the lowest dose, 0.005 µl/ml, a weak positive response for relative MF of 2.0 was measured (only in one parallel

culture), but which was below the evaluation criterion in the parallel culture. Only at the highest relevant dose tested below overt cytotoxicity (0.031 µl/ml, RTG 12 and 15 %) the rel. MF was reproducibly above 2-fold (3.1 and 2.2), but GEF was only positive in one of the parallel cultures (106 and 59).

For both metabolic activation conditions, MF and cytotoxicity by DMPT are increasing with dose (not statistically tested). Relevant positive rel. MF and GEF are only present at doses with high cytotoxicity and general high variability between the parallel cultures. Although there are positive responses inside the OECD TG 490 RTG limit values (between 10 % and 20 %), these are either only present in one of two parallel cultures, or are dependent on the evaluation criterion, rel. MF or GEF. Accordingly, the MLA response on DMPT is considered as equivocal.

**Table 19: Results from mouse lymphoma assay, non-activated cultures, adapted from (Seifried et al., 2006). Average TFT: mutant cell counts; Average VC: viable cell counts; RTG: relative total growth; MF: mutation frequency. Bold: positive according to OECD TG 476 or OECD TG 490 criteria, grey background: cytotoxic concentration.**

Non-Activated Cultures						
Dose	Average TFT	Average VC	RTG	MF	GEF	rel. MF
µl/mL	counts per 1x10 <sup>6</sup> cells	counts per 200 cells	%	mutations per 10 <sup>6</sup> cells	MF with solvent control subtracted	MF fold-change to solvent control
0.05	82	198	90	83	-4	0.9
	78	159	64	98	11	1.1
0.11	81	180	59	90	3	1.0
	111	205	63	108	21	1.2
0.18	113	150	28	151	63	1.7
	104	144	31	144	57	1.7
0.24	115	130	13	177	90	<b>2.0</b>
	124	128	9	194	107	2.2
Solvent	82	188		87		
Positive	432	105	35	823	736	9.4

**Table 20: Results from mouse lymphoma assay, S9-activated cultures, adapted from (Seifried et al., 2006). Average TFT: mutant cell counts; Average VC: viable cell counts; RTG: relative total growth; MF: mutation frequency. Bold: positive according to OECD TG 476 or OECD TG 490 criteria, grey background: cytotoxic concentration.**

S9-Activated Cultures						
Dose	Average TFT	Average VC	RTG	MF	GEF	rel. MF
µl/mL	counts per 1x10 <sup>6</sup> cells	counts per 200 cells	%	mutations per 10 <sup>6</sup> cells	MF with solvent control subtracted	MF fold-change to solvent control
0.005	36	81	41	89	39	1.8
	68	132	65	103	53	<b>2.0</b>
0.011	47	161	72	58	8	1.2
	66	204	87	65	14	1.3
0.018	60	179	35	67	17	1.3
	84	144	55	117	66	<b>2.3</b>
0.024	76	157	26	97	47	1.9
	85	177	32	96	46	1.9
0.031	113	145	12	156	<b>106</b>	<b>3.1</b>
	86	158	15	109	59	<b>2.2</b>
0.037	121	144	8	168	118	3.3
	104	158	9	132	81	2.6
0.044	118	83	3	284	234	5.7
Solvent	46	183		50		
Positive	181	87	48	416	366	8.3



*In vitro* mammalian micronucleus test (MNT) (Taningher et al., 1993)

The MNT test showed induction of clastogenic effects and aneuploidy (statistically significant increased CREST positive and negative micronuclei). In principle, the study is in conformity with OECD TG 487, although the treatment period was longer (48h, approx. 3 cell cycles) than recommended in the guideline (1.2 to 2 cell cycles). Detailed information on the cytotoxicity was not reported, it was only stated that the survival rate was above 10% for all doses tested. There was no dose-dependency of the mitotic index after 24 and 48 h treatment time, the mitotic index was above 10 % for all doses.

Chemical	Dose (mM)	Mitotic Index		Micronuclei/1,000 interphasic nuclei <sup>a</sup>		
		24 hr	48 hr	CREST+	CREST-	TOTAL
<i>N,N</i> -Dimethyl-p-toluidine	0	16.54	11.74	3.33	2.67	6.00
	0.3	20.25	10.89	4.67	4.00	8.67
	0.9	11.29	30.23	10.67 <sup>b</sup>	6.67	17.34 <sup>c</sup>
	1.2	14.32	10.89	18.26 <sup>c</sup>	9.62 <sup>c</sup>	27.88 <sup>c</sup>
Methylnitrosourea	0			2.70	2.29	4.99
	0.5			28.47 <sup>c</sup>	114.39 <sup>c</sup>	142.86 <sup>c</sup>
Colchicine	0			2.62	2.35	4.97
	0.000025			35.20 <sup>c</sup>	3.33	38.53 <sup>c</sup>

Figure 2: Micronuclei *in vitro* induction by *N,N*-dimethyl-p-toluidine, as evaluated by CREST-antibody immunofluorescent staining in V79 Cells (Taningher et al., 1993). <sup>a</sup>Observed 48 hr after treatment began. The duration of the treatments was 48 hr for all chemicals tested, except for methylnitrosourea whose treatment duration was 30 min. Each reported value is the mean of results obtained in at least two independent experiments in which at least 3,000 cells were scored. <sup>b,c</sup>Significantly different from concurrent controls with a p value less than 0.05 or 0.01, respectively, according to the  $\chi^2$  test or the Fisher Exact Test. The dose-dependency of CREST+ and CREST- micronuclei induction with DMPT is statistically significant with  $p < 0.001$  in both cases, according to the Cochran-Armitage trend test.

**In vivo data – somatic cells**

In (NTP, 2012), mouse-peripheral blood micronucleus assays and comet assays in blood and liver are available that fulfil the criteria for supporting studies.

Mouse-peripheral blood micronucleus assays

Both *in vivo* micronucleus assays (NTP, 2012) did not show increased frequencies of micronucleated erythrocytes from peripheral blood, the results are considered negative.

The tests were performed with peripheral blood samples of mice, either as a

- slide-based assays at the end of a 3-month gavage study with DMPT (see Table 22),
- or as a
- flow cytometric assays after daily gavage for 4 days (see Table 21).

The NTP studies do not fully comply with OECD TG 474. The MNT after daily gavage for 4 days yielded in a negative result, i.e. no significant alterations in the percentage of micronucleated circulating reticulocytes were observed. However, the dosing of the MNT with a highest dose of 75 mg/kg bw/day was based on the results of a 3-month study (NTP, 2012), see

Table 43 and Table 44. In the 3-month study, mice dosed with 60 mg/kg bw/day did not show relevant treatment dependent effects. The relevance of the dosing for the MNT (4-day oral gavage) is therefore questionable.

A second MNT at the end of a 3-month oral gavage study also did not show increased frequencies of micronucleated erythrocytes. Here, the doses were the same as in the 3-month study (see above).

**Table 21: Frequency of micronuclei in peripheral blood erythrocytes of male mice following administration of *N,N*-Dimethyl-*p*-toluidine by gavage for 4 days<sup>a</sup> (NTP, 2012)**

	Dose (mg/kg)	Number of Mice with Erythrocytes Scored	Micronucleated PCEs/1,000 PCEs <sup>b</sup>	P Value <sup>c</sup>	Micronucleated NCEs/1,000 NCEs <sup>b</sup>	P Value <sup>c</sup>	PCEs <sup>b</sup> (%)	P Value <sup>c</sup>
Com oil <sup>d</sup>	0	5	2.59 ± 0.20		1.46 ± 0.02		1.270 ± 0.11	
<i>N,N</i> -Dimethyl- <i>p</i> -toluidine	30	5	2.57 ± 0.19	0.5114	1.49 ± 0.03	0.3095	1.201 ± 0.07	0.748
	60	5	2.66 ± 0.22	0.5200	1.47 ± 0.02	0.3706	1.140 ± 0.15	0.465
	75	5	2.78 ± 0.54	0.4341	1.54 ± 0.04	0.0588	1.103 ± 0.12	0.430
			P=0.327 <sup>e</sup>		P=0.089		P=0.243	
Ethyl methanesulfonate <sup>f</sup>	150	5	12.18 ± 0.34	0.0000	1.69 ± 0.04	0.0004	0.942 ± 0.04	0.015

<sup>a</sup> Study was performed at ILS, Inc. The detailed protocol is presented by Witt *et al.* (2008). NCE=normochromatic erythrocyte; PCE=polychromatic erythrocyte

<sup>b</sup> Mean ± standard error

<sup>c</sup> Pairwise comparison with the vehicle control group; values are significant at P≤0.025 by Williams' test

<sup>d</sup> Vehicle control

<sup>e</sup> Significance tested by a linear regression trend test; significant at P≤0.025

<sup>f</sup> Positive control; pairwise comparison with the vehicle control group; values are significant at P≤0.05 by a one-tailed independent t-test

**Table 22: Frequency of micronuclei in peripheral blood erythrocytes of mice following administration of *N,N*-Dimethyl-*p*-toluidine by gavage for 3 months<sup>a</sup> (NTP, 2012)**

	Dose (mg/kg)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/1,000 NCEs <sup>b</sup>	P Value <sup>c</sup>	PCEs <sup>b</sup> (%)
<b>Male</b>					
Com oil <sup>d</sup>	0	5	2.00 ± 0.32		3.34 ± 0.24
<i>N,N</i> -Dimethyl- <i>p</i> -toluidine	15	5	2.10 ± 0.29	0.4379	2.62 ± 0.05
	30	5	2.40 ± 0.19	0.2730	3.20 ± 0.25
	60	5	2.80 ± 0.90	0.1238	4.16 ± 0.29
	125	5	3.00 ± 0.52	0.0784	3.94 ± 0.11
			P=0.050 <sup>e</sup>		
<b>Female</b>					
Com oil	0	5	1.50 ± 0.16		4.24 ± 0.36
<i>N,N</i> -Dimethyl- <i>p</i> -toluidine	15	5	1.90 ± 0.40	0.2462	3.32 ± 0.29
	30	5	1.70 ± 0.12	0.3617	3.24 ± 0.45
	60	5	1.30 ± 0.41	0.6474	3.58 ± 0.25
	125	5	2.10 ± 0.40	0.1584	5.36 ± 0.60
			P=0.238		

<sup>a</sup> Study was performed at ILS, Inc. The detailed protocol is presented by MacGregor *et al.* (1990). NCE=normochromatic erythrocyte; PCE=polychromatic erythrocyte

<sup>b</sup> Mean ± standard error

<sup>c</sup> Pairwise comparison with the vehicle control group; dosed group values are significant at P≤0.005

<sup>d</sup> Vehicle control

<sup>e</sup> Significance of micronucleated NCEs/1,000 NCEs tested by the one-tailed trend test; significant at P≤0.025

Comet assays in blood and liver (NTP, 2012)

The results from the only acceptable in vivo comet assay indicate no increased DNA damage by DMPT. The assay was conducted to measure induction of DNA damage in liver and blood leukocytes. In the study (equivalent to OECD TG 489), conducted in male B6C3F1/N mice, *N,N*-dimethyl-*p*-toluidine administered by gavage over a range of 30 to 75 mg/kg once daily for 4 days did not produce an increase in DNA migration in liver cells or blood leukocytes (Table 23). Here the same restrictions apply as for the MNT (see above), the dose levels were selected from a 3-month study, and their relevance the assay is questionable.

**Table 23: DNA damage in the blood and liver of Male B6C3F1/N mice following administration of *N,N*-dimethyl-*p*-toluidine by gavage for 4 days<sup>a</sup> (NTP, 2012)**

	Dose (mg/kg)	Number of Animals	Percent Tail DNA <sup>b</sup>	P Value <sup>c</sup>
<b>Blood</b>				
Com oil <sup>d</sup>	0	5	2.0 ± 0.24	
<i>N,N</i> -Dimethyl- <i>p</i> -toluidine	30	5	1.9 ± 0.23	0.549
	60	5	1.5 ± 0.14	0.922
	75	5	2.2 ± 0.30	0.308
			P=0.943 <sup>e</sup>	
Ethyl methanesulfonate <sup>f</sup>	150	5	20.7 ± 1.10	<0.001
<b>Liver</b>				
Com oil	0	5	5.3 ± 0.59	
<i>N,N</i> -Dimethyl- <i>p</i> -toluidine	30	5	5.7 ± 1.70	0.411
	60	5	6.5 ± 0.42	0.067
	75	5	6.3 ± 0.81	0.178
			P=0.364	
Ethyl methanesulfonate	150	5	19.2 ± 1.00	<0.001

<sup>a</sup> Study was performed at ILS, Inc. The detailed protocol is presented by Recio *et al.* (2010).

<sup>b</sup> Mean ± standard error

<sup>c</sup> Pairwise comparison with the vehicle control group; dosed group values are significant at P<0.008 by Student's t-test; positive control values are significant at P<0.05 by a one-tailed independent t-test.

<sup>d</sup> Vehicle control

<sup>e</sup> Significance of percent tail DNA tested by a linear regression trend test: significant at P<0.025

<sup>f</sup> Positive control

**In vivo data – germ cells**

Studies on the mutagenicity/genotoxicity of DMPT in mammalian germ cells are not available.

**Summary**

DMPT did not show gene mutagenicity in bacteria with and without metabolic activation.

In vitro, results from a mouse lymphoma assay were considered equivocal with and without metabolic activation. DMPT induced genotoxicity (positive aneugenic and clastogenic response) in an in vitro micronucleus test.

In vivo, reliable micronucleus and comet assays were negative, no tests for in vivo gene mutagenicity were identified.

### 10.8.2 Comparison with the CLP criteria

Criteria for the classification of germ cell mutagens are listed in Annex I, 3.5.2.2, Table 3.5.1 of the CLP Regulation.

The definition for Category 1 (1A or 1B) is “Substances known to induce heritable mutations or to be regarded as if they induce heritable mutations in the germ cells of humans”. For a classification in Category 1A, either positive evidence from human (epidemiological) studies is needed; or substances are allocated which are to be regarded as if they induce heritable mutations in the germ cells of humans.

→ There are no data available that would support classification into Category 1A.

Classification in Category 1B is based on:

- positive result(s) from *in vivo* heritable germ cell mutagenicity tests in mammals; or
- positive result(s) from *in vivo* somatic cell mutagenicity tests in mammals, in combination with some evidence that the substance has potential to cause mutations to germ cells. It is possible to derive this supporting evidence from mutagenicity/genotoxicity tests in germ cells *in vivo*, or by demonstrating the ability of the substance or its metabolite(s) to interact with the genetic material of germ cells; or
- positive results from tests showing mutagenic effects in the germ cells of humans, without demonstration of transmission to progeny; for example, an increase in the frequency of aneuploidy in sperm cells of exposed people.

→ There is no information available for DMPT that would justify a classification as Category 1 mutagen.

Category 2 comprises “Substances which cause concern for humans owing to the possibility that they may induce heritable mutations in the germ cells of humans”. Classification in Category 2 is based on following experiments:

- Somatic cell mutagenicity tests *in vivo*, in mammals; or
- Other *in vivo* somatic cell genotoxicity tests which are supported by positive results from *in vitro* mutagenicity assays. Note: Substances which are positive in *in vitro* mammalian mutagenicity assays, and which also show chemical structure activity relationship to known germ cell mutagens, shall be considered for classification as Category 2 mutagens.

→ No information on *in vivo* somatic cell mutagenicity studies was available. The reliable *in vivo* tests (micronucleus test, comet assay) taken into account for classification were negative. Therefore, the conditions for classification as Category 2 mutagen are not fulfilled.

### 10.8.3 Conclusion on classification and labelling for germ cell mutagenicity

*N,N*-dimethyl-p-toluidine should not be classified as germ cell mutagen.

## 10.9 Carcinogenicity

Toxicity and carcinogenicity of *N,N*-dimethyl-*p*-toluidine have been investigated by the US National Toxicology Program (NTP). The NTP studies were published 2012 in the Technical Report 579 (NTP, 2012). The report comprises 3-month sub-chronic toxicity studies and 2-year carcinogenesis studies by oral gavage to rats and mice of both sexes. Results from the sub-chronic studies were used as range finding studies for the chronic studies. Additionally, genotoxicity was assayed and reported in the NTP Technical Report.

The NTP carcinogenicity studies in mice and rats resulted in “clear evidence of carcinogenic activity” in both species and in both sexes, which is the highest of the five categories for carcinogenicity defined by NTP. Furthermore, treatment related non-neoplastic lesions were observed in several organs. These can be - at least partly - attributed as secondary effects to the identified methaemoglobinaemia, but also as possible pre-neoplastic stages.

One other long term study was identified (Druckrey et al., 1954), however this study exhibits major deficiencies in terms of study design and reporting. In this study the effects of *N,N*-dimethyl-*p*-toluidine when admixed to the diet was investigated in rats at a single dose levels of 7 mg/day. The study reported an absence of any chronic toxicity, no reduction of body-weight or life expectancy and no carcinogenicity. Instead, both an increased body weight and a longer life span were reported. Based on the average body weight of about 100 g at study start and about 300 g for adult rats, the daily dose of 7 mg/day per rat would resemble a daily average dose of 70 mg/kg bw for animals at study start and 23 mg/kg bw at the end. Because the test substance was mixed into the diet, the effective dosing is unknown, additionally, body weight dependent, individual dosing has not been controlled and only average values were given for the whole study. In addition, three different rat strains were used, but the results were averaged over the tested strains. These major deficiencies of study design and reporting lead to the conclusion, that the study results are not reliable. Therefore, the report not further considered for the assessment of carcinogenicity.

In the following, the NTP carcinogenicity studies are summarized and discussed. The studies follow the standards of the NTP, study design and results are reported transparently. The two 2-year carcinogenicity studies in mice and rats are equivalent to OECD TG 451 (NTP internal guideline) and have been conducted in compliance with Food and Drug Administration (FDA) Good Laboratory Practice Regulations. The dosing regimen (5 days per week instead of 7 days per week as recommended in OECD TG 451) is the only major deviation from the test guideline, the studies are considered as reliable without restrictions.

Statistical significance of lesions has been tested by pairwise comparisons of each dosed group with controls and a test for an overall dose-related trend. Continuity-corrected Poly-3 tests were used in the analysis of lesion incidence, and reported P values are one sided. Poly-3 tests account for mortality in animals that did not reach terminal kill.

Table 24: Summary table of animal studies on carcinogenicity

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
<p><b>2-year study</b></p> <p><b>Reliable without restrictions</b></p> <p>Gavage with corn oil (dosing volume 2.5 ml/kg)</p> <p><b>Rats (F344/N)</b></p> <p>♀ and ♂</p> <p>NTP internal guideline, equivalent to OECD TG 451</p> <p>50 animals per sex and dose</p> <p>Additional clinical pathology groups of 10 male and 10 female rats receiving the same doses for 86 days.</p>	<p><i>N,N</i>-Dimethyl-p-toluidine (CAS No. 99-97-8)</p> <p>purity &gt; 99 %</p> <p>0, 6, 20, or 60 mg/kg in corn oil</p> <p>5 days per week</p> <p>♂: 104 weeks ♀: 105 weeks</p>	<p><u>Clear evidence of carcinogenic activity</u> (summarised in Table 25)</p> <p><b>Liver</b></p> <ul style="list-style-type: none"> <li>hepatocellular carcinoma (♀/♂)</li> <li><b>Nasal cavity</b> nasal cavity neoplasms (♂, primarily nasal cavity transitional epithelium adenoma),</li> <li>nasal cavity transitional epithelium adenoma was considered to be related to treatment (♀)</li> </ul> <p><b>Thyroid gland</b></p> <ul style="list-style-type: none"> <li>thyroid gland follicular cell neoplasms may have been related to treatment (♂)</li> </ul> <p>Additionally increased incidences of <b>non-neoplastic lesions</b> in</p> <ul style="list-style-type: none"> <li>liver (♀/♂), see Table 27;</li> <li>nasal cavity (♀/♂), see Table 28;</li> </ul> <p>kidney (♀/♂), spleen and bone marrow (♀/♂), forestomach (♂), mesenteric lymph node (♂), see</p> <ul style="list-style-type: none"> <li>Table 31.</li> </ul> <p>Hematologic toxicity and increases in methaemoglobin levels (♀/♂, assessed after 86 days), see Table 29, Table 30 and section 10.12 (STOT-RE) for details</p> <p>Body-weight gain ↓ (60 mg/kg; ♀/♂); Survival ↓ (60 mg/kg; ♂)</p>	<p>(NTP, 2012)</p> <p><b>Key study</b></p> <p>(Reliable without restrictions)</p>
<p><b>2-year study</b></p> <p><b>Reliable without restrictions</b></p> <p>Gavage with corn oil (dosing volume 5 ml/kg)</p> <p><b>Mice (B6C3F1/N)</b></p> <p>♀ and ♂</p> <p>NTP internal guideline, equivalent to OECD TG 451</p> <p>50 animals per sex and dose</p>	<p><i>N,N</i>-Dimethyl-p-toluidine (CAS No. 99-97-8)</p> <p>purity &gt; 99 %</p> <p>0, 6, 20, or 60 mg/kg in corn oil</p> <p>5 days per week</p> <p>105 weeks</p>	<p><u>Clear evidence of carcinogenic activity</u> (summarized in Table 32)</p> <p><b>Liver</b></p> <ul style="list-style-type: none"> <li>hepatocellular carcinoma and hepatoblastoma (♀/♂)</li> <li>hepatocellular adenoma (♀/♂, multiple in ♂)</li> </ul> <p><b>Lung</b></p> <ul style="list-style-type: none"> <li>alveolar/ bronchiolar neoplasms (primarily adenoma) (♀)</li> </ul> <p><b>Forestomach</b></p> <ul style="list-style-type: none"> <li>increased incidences of forestomach squamous cell papilloma considered to be related to treatment (♀)</li> </ul> <p>Additionally increased incidences of <b>non-neoplastic lesions</b> in</p> <ul style="list-style-type: none"> <li>liver (♀/♂), see Table 34;</li> <li>lung (♀/♂), see Table 35;</li> <li>forestomach (♀), see Table 36;</li> <li>nasal cavity and olfactory lobe (♀/♂), see Table 37;</li> <li>spleen, bone marrow and mesenteric lymph node (♀), see Table 38.</li> </ul> <p>Body-weight gain ↓ (20 mg/kg; ♂, 60 mg/kg; ♀/♂); Survival ↓ (60 mg/kg; ♂)</p>	<p>(NTP, 2012)</p> <p><b>Key study</b></p> <p>(Reliable without restrictions)</p>

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
<p><b>Lifetime study</b></p> <p><b>Not reliable</b></p> <p>No guideline study, no GLP conformity</p> <p>Detailed study data not available</p> <p>Rats, strains BD I, BD III, W.</p> <p>28 animals per strain (about 100 days old at study start)</p>	<p>“N-Dimethyl-toluidine”, no purity given</p> <p>Test substance mixed into food (leftovers from hospital)</p> <p>Average dose: 7 mg / day</p> <p>Total dose: 5 g/rat</p>	<p>Brief report without traceable study data and gross lacks in study design.</p> <ul style="list-style-type: none"> <li>• Higher than average life-expectancy, higher average body weight compared to controls</li> <li>• No chronic toxicity (although not clear what was investigated)</li> <li>• No carcinogenic effects</li> </ul>	<p>(Druckrey et al., 1954)</p> <p><b>Disregarded study</b> (Not reliable)</p>

**2-year studies in rats (NTP, 2012)**

Groups of 50 male and 50 female F344/N rats were administered 0, 6, 20, or 60 mg *N,N*-dimethyl-p-toluidine/kg body weight in corn oil by gavage, 5 days per week for 104 or 105 weeks. Additional groups of 10 male and 10 female rats (clinical pathology study) received the same doses for 86 days.

Statistically significantly increased incidences for neoplastic lesions were found in liver and the nasal cavity (see Table 25 and more detailed descriptions below). In addition, increased incidences (above historical control, but without reaching statistical significance), of neoplastic lesions were observed in the thyroid glands of males (Follicular Cell Adenoma or Carcinoma) and females (Follicular Cell Adenoma at 20 mg/kg only). Non-neoplastic lesions were identified in several organs, e.g. in spleen, kidneys, forestomach, bone marrow and mesenteric lymph nodes. Neoplastic lesions occurred mainly in the highest dose group of 60 mg/kg bw/day, pre- and non-neoplastic lesions were also observed in lower dose groups, and neoplastic lesions were preceded by precursor stages. For historical control data see Annex A – Historical control values of NTP 2012 study.

**Table 25 Summary of neoplastic incidences in 2-year studies in F344/N rats (NTP, 2012)**

	Male				Female			
	0 mg/kg	6 mg/kg	20 mg/kg	60 mg/kg	0 mg/kg	6 mg/kg	20 mg/kg	60 mg/kg
Number of animals in dosing group	50	50	50	50	50	50	50	50
Surviving animals at termination	37	37	31	21	33	42	33	23
Survival probability (%) <sup>a</sup>	74	76	63	<b>45<sup>ss</sup></b>	66	86	66	<b>47<sup>s</sup></b>
<b>Liver</b>								
Hepatocellular adenoma	0	0	1	1	0	1	1	3
Hepatocellular carcinoma	0 <sup>##</sup>	0	1	<b>6<sup>**</sup></b>	0 <sup>##</sup>	0	0	<b>4<sup>*</sup></b>
H. adenoma or carcinoma	0 <sup>##</sup>	0	2	<b>6<sup>**</sup></b>	0 <sup>##</sup>	1	1	<b>7<sup>**</sup></b>
<b>Nasal cavity</b>								
Glands, olfactory epith., adenoma	0	0	0	1				
Transitional epithelium, adenoma	0 <sup>##</sup>	3	2	<b>11<sup>**</sup></b>	0	1	0	2
Transitional epithelium, carcinoma	0	0	0	2				
Trans. epith. adenoma or carcinoma	0 <sup>##</sup>	3	2	<b>13<sup>**</sup></b>				
<b>Thyroid Gland</b>								
Follicular cell adenoma	1	0	1	3	1	1	2	0
Follicular cell carcinoma	0	2	1	2				
F. cell adenoma or carcinoma	1	2		4				

Data are given as overall incidences (to be compared to the number of animals in dosing group).

\*, \*\* Pairwise comparisons between the vehicle controls and that dosed group, \*: p<0.05; \*\*: p< 0.01. The Poly-3 test accounts for differential mortality.

#, ## Trend test significance levels notated next to vehicle control incidences, #: p<0.01; ##: p< 0.001)

<sup>a</sup> Kaplan-Meier determinations

<sup>s</sup> or <sup>ss</sup> Significance of shorter survival from survival analysis, P<0.05 or P<0.01

### Survival and body weight

Survival of 60 mg/kg male and female animals was significantly lower compared to vehicle controls (see Table 25). Mean body weights of 60 mg/kg males and females were lower compared to vehicle control, with differences of more than 10 % after day 421 in males or day 225 in females, respectively. Body weight gains were reduced in the 60 mg/kg group to about 75 % (males) or 78 % relative to vehicle control (Table 26).

**Table 26 Relative body weights and body weight gains in 2-year studies in rats**

	Male			Female		
	6 mg/kg	20 mg/kg	60 mg/kg	6 mg/kg	20 mg/kg	60 mg/kg
Rel. body weight at end of study (%) <sup>a</sup>	102.5	94.3	80.5	107.3	101.0	84.5
Rel. body weight gain (%) <sup>a, b</sup>	103.3	92.6	74.6	109.9	101.3	77.6

<sup>a</sup> relative to vehicle control

<sup>b</sup> until terminal sacrifice

### Liver

Significantly increased incidences of hepatocellular carcinoma and combined hepatocellular adenoma or hepatocellular carcinoma (Table 25) were observed in rat liver of both sexes at 60 mg/kg. Non-neoplastic liver lesions (Table 27) occurred in dosed males and females primarily in the 20 and 60 mg/kg groups.



**Table 27 Selected non-neoplastic incidences of the liver in F344/N rats. \* or \*\*: Significantly different ( $P \leq 0.05$  or  $P \leq 0.01$ ) from the vehicle control group by the Poly-3 test. Numbers in brackets: Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked. <sup>a</sup>: number of animals examined microscopically.**

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
<b>Male</b>				
Liver <sup>a</sup>	50	50	50	50
Basophilic Focus	28	6**	0**	3**
Eosinophilic Focus	11	21*	21*	29**
Mixed Cell Focus	18	17	17	35**
Bile Duct, Fibrosis	21 (1.0) <sup>b</sup>	27 (1.0)	41** (1.1)	42** (1.5)
Bile Duct, Hyperplasia	40 (1.2)	42 (1.5)	44* (1.6)	44 (1.8)
Degeneration, Cystic	4 (1.3)	10 (1.4)	9 (1.3)	17** (1.3)
Hepatocyte, Hypertrophy	0	0	6* (1.5)	31** (1.5)
<b>Female</b>				
Liver <sup>a</sup>	50	50	50	49
Basophilic Focus	46	45	5**	6**
Clear Cell Focus	7	17*	24**	29**
Eosinophilic Focus	18	24	29*	32**
Mixed Cell Focus	14	20	17	26**
Bile Duct, Fibrosis	6 (1.2)	11 (1.0)	23** (1.0)	27** (1.1)
Bile Duct, Hyperplasia	10 (1.6)	21* (1.0)	27** (1.0)	43** (1.5)
Degeneration, Cystic	0	0	2 (1.0)	10** (1.2)
Hepatocyte, Hypertrophy	0	0	6* (1.3)	22** (1.3)
Hepatocyte, Necrosis	0	0	1 (2.0)	5* (1.8)

Nasal cavity

In the nasal cavity (Table 25), there were significantly increased incidences of transitional epithelium (TE) adenoma and combined TE adenoma or carcinoma in 60 mg/kg males, TE adenoma also occurred in female rats administered 6 or 60 mg/kg.

There were significantly increased incidences of non-neoplastic lesions (see Table 28) in the olfactory epithelia (OE), respiratory epithelia (RE), and transitional epithelia (TE) of dosed rats. These lesions occurred with the greatest incidence and severity in the 60 mg/kg groups. Incidences of inflammation and nerve atrophy (nose, location not further described) were significantly increased in males and females administered 60 mg/kg.

**Table 28 Selected non-neoplastic incidences of the nasal cavity in F344/N rats. \* or \*\*: Significantly different ( $P \leq 0.05$  or  $P \leq 0.01$ ) from the vehicle control group by the Poly-3 test. Numbers in brackets: Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked. <sup>a</sup>: number of animals examined microscopically; OE: Olfactory Epithelium; RE: Respiratory Epithelium; TE: Transitional Epithelium.**

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
<b>Male</b>				
Nose <sup>a</sup>	50	49	50	49
Glands, OE, Dilatation	0	0	3 (1.0)	49** (2.4)
Glands, OE, Hyperplasia	0	2 (1.0)	0	48** (1.9)
Glands, OE, Metaplasia	0	0	0	38** (1.5)
Glands, OE, Necrosis	0	0	0	22** (2.7)
Glands, RE, Dilatation	13 (1.0)	15 (1.0)	19 (1.0)	48** (1.6)
Glands, RE, Hyperplasia	0	8** (1.1)	8** (1.5)	41** (1.7)
Glands, RE, Metaplasia, Respiratory	29 (1.0)	39* (1.0)	39** (1.0)	47** (2.6)
Glands, TE, Dilatation	0	0	5* (1.2)	3 (1.7)
Glands, TE, Hyperplasia	0	1 (1.0)	24** (1.1)	40** (1.6)
Inflammation	35 (1.4)	40 (1.6)	38 (1.2)	48** (1.9)
Nerve, Atrophy	0	0	0	15** (1.3)
OE, Degeneration	0	0	1 (2.0)	47** (2.1)

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
<b>Male</b>				
OE, Hyperplasia, Basal Cell	0	1 (1.0)	2 (1.0)	38** (1.3)
OE, Metaplasia, Respiratory	4 (1.0)	9 (1.4)	9 (1.3)	40** (1.3)
RE, Hyperplasia	15 (1.2)	29** (1.5)	32** (1.3)	49** (1.6)
TE, Hyperplasia	1 (2.0)	1 (1.0)	11** (1.1)	46** (1.7)
<b>Female</b>				
Nose <sup>a</sup>	50	49	50	49
Glands, OE, Dilatation	0	0	0	48** (2.4)
Glands, OE, Hyperplasia	0	0	4 (1.0)	47** (1.9)
Glands, OE, Metaplasia	0	0	0	42** (1.3)
Glands, OE, Necrosis	0	0	0	18** (2.8)
Glands, RE, Dilatation	5 (1.0)	12 (1.0)	27** (1.1)	47** (1.2)
Glands, RE, Hyperplasia	6 (1.2)	9 (1.0)	22** (1.3)	45** (1.6)
Glands, RE, Metaplasia, Respiratory	17 (1.1)	33** (1.1)	44** (1.8)	47** (2.0)
Glands, TE, Dilatation	0	0	0	9** (1.4)
Glands, TE, Hyperplasia	0	4 (1.0)	12** (1.2)	24** (1.4)
Inflammation	23 (1.3)	24 (1.4)	22 (1.1)	45** (1.5)
Nerve, Atrophy	0	0	0	4* (1.8)
OE, Degeneration	0	0	1 (1.0)	46** (2.0)
OE, Hyperplasia, Basal Cell	0	0	0	25** (1.2)
OE, Metaplasia, Respiratory	4 (1.5)	6 (1.5)	1 (2.0)	21** (1.2)
RE, Hyperplasia	10 (1.0)	13 (1.4)	11 (1.1)	41** (1.3)
TE, Hyperplasia	0	1 (1.0)	6* (1.0)	33** (1.1)

#### Thyroid gland

Increased incidence of thyroid tumours in treated groups of male rats, i.e. follicular cell adenoma or carcinoma, was observed. Although statistically not significant, the incidence rate at high dose exceeded the rate of historical controls (by oral route, i.e. corn oil gavage, as well as when data from all administration routes is combined).

**Haematopoietic system**

Haematology parameters were investigated in additional groups of animals at day 86 (see Table 29 and Table 30). Increases in methaemoglobin and Heinz bodies were observed in male and female animals of the 20 and 60 mg/kg groups. Haematocrit values, haemoglobin concentrations, and erythrocyte counts were decreased in the 20 and 60 mg/kg male and female groups. This erythron decrease was accompanied by trends towards erythrocyte macrocytosis and hypochromia evidenced by increases in the mean cell volume and decreases in the mean cell haemoglobin concentration values, respectively. Increases in reticulocyte counts demonstrated increased erythropoiesis. The reduction in functional Hb at 60 mg/kg bw/day of more than 20 % indicates a methaemoglobinaemia according to CLP Guidance (ECHA, 2017) and Muller et al., 2006, which could not be (fully) compensated by the animals. The results at day 86 are comparable to results from 3-months studies in the NTP report (see section 10.12, STOT-RE).

**Table 29 Summarized haematology data at 3 months in 2-year studies in rats (NTP, 2012)**

	Male			Female		
	6 mg/kg	20 mg/kg	60 mg/kg	6 mg/kg	20 mg/kg	60 mg/kg
Haematocrit (%)		↓↓	↓↓		↓↓	↓↓
Haemoglobin (g/dL)	↓	↓↓	↓↓	↓	↓↓	↓↓
Hb change [%] <sup>a</sup>	-2.5	-8.1	-17.5	-4.4	-8.9	-16.5
Funct. Hb change [%] <sup>a,b</sup>	-3.3	-11.0	-28.4	-5.1	-12.1	-27.1
Erythrocytes (10 <sup>6</sup> /μL)		↓↓	↓↓		↓↓	↓↓
Reticulocytes (10 <sup>6</sup> /μL)	↑	↑↑	↑↑		↑↑	↑↑
Mean cell volume (fL)		↑↑	↑↑		↑	↑↑
Mean cell Hb (pg)				↓		
Mean cell Hb concentration (g/dL)		↓↓	↓↓	↓	↓↓	↓↓
Methaemoglobin (g/dL)	↑	↑↑	↑↑		↑↑	↑↑
Methaemoglobin (% Hb)	↑	↑↑	↑↑		↑↑	↑↑
Heinz bodies (% erythrocytes)		↑↑	↑↑	↑	↑↑	↑↑

n=10 for all groups

<sup>a</sup> Calculated from average values without error propagation; percental change compared to vehicle control.

<sup>b</sup> Functional Hb: Haemoglobin concentration minus Methaemoglobin concentration

↓ or ↑ Significantly reduced or elevated (P≤0.05) from the vehicle control group by Dunn's or Shirley's test

↓↓ or ↑↑ Significantly reduced or elevated (P≤0.01) from the vehicle control group by Shirley's test

Table 30 Haematology data at 3 months in 2-year studies in rats (NTP, 2012)

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
n	10	10	10	10
<b>Male</b>				
Hematocrit (%)	48.8 ± 0.5	48.4 ± 0.4	46.5 ± 0.3**	42.6 ± 0.3**
Hemoglobin (g/dL)	16.0 ± 0.2	15.6 ± 0.1*	14.7 ± 0.1**	13.2 ± 0.1**
Erythrocytes (10 <sup>6</sup> /μL)	9.10 ± 0.10	9.02 ± 0.06	8.53 ± 0.04**	7.61 ± 0.06**
Reticulocytes (10 <sup>6</sup> /μL)	0.25 ± 0.01	0.26 ± 0.01*	0.35 ± 0.01**	0.69 ± 0.02**
Mean cell volume (fL)	53.7 ± 0.2	53.6 ± 0.2	54.5 ± 0.2**	56.0 ± 0.1**
Mean cell hemoglobin (pg)	17.5 ± 0.1	17.3 ± 0.1	17.3 ± 0.1	17.3 ± 0.1
Mean cell hemoglobin concentration (g/dL)	32.7 ± 0.2	32.2 ± 0.2	31.6 ± 0.1**	30.9 ± 0.2**
Platelets (10 <sup>3</sup> /μL)	645.4 ± 27.5	682.6 ± 7.8	721.4 ± 18.4**	722.0 ± 26.0*
Leukocytes (10 <sup>3</sup> /μL)	9.44 ± 0.49	9.91 ± 0.45	9.99 ± 0.51	9.31 ± 0.58
Segmented neutrophils (10 <sup>3</sup> /μL)	1.38 ± 0.09	1.42 ± 0.04	1.42 ± 0.09	1.50 ± 0.05
Lymphocytes (10 <sup>3</sup> /μL)	7.70 ± 0.42	8.10 ± 0.41	8.18 ± 0.41	7.46 ± 0.52
Monocytes (10 <sup>3</sup> /μL)	0.23 ± 0.02	0.26 ± 0.02	0.24 ± 0.02	0.20 ± 0.02
Basophils (10 <sup>3</sup> /μL)	0.062 ± 0.007	0.071 ± 0.006	0.079 ± 0.012	0.075 ± 0.009
Eosinophils (10 <sup>3</sup> /μL)	0.08 ± 0.02	0.07 ± 0.01	0.08 ± 0.01	0.06 ± 0.02
Methemoglobin (g/dL)	0.77 ± 0.04	0.88 ± 0.03*	1.14 ± 0.03**	2.30 ± 0.03**
Methemoglobin (% hemoglobin)	4.70 ± 0.26	5.60 ± 0.22*	7.90 ± 0.18**	17.40 ± 0.22**
Heinz bodies (% erythrocytes)	0.0 ± 0.0	0.1 ± 0.1	0.7 ± 0.2**	3.7 ± 0.3**
<b>Female</b>				
Hematocrit (%)	46.9 ± 0.5	45.8 ± 0.6	44.2 ± 0.6**	41.3 ± 0.6**
Hemoglobin (g/dL)	15.8 ± 0.2	15.1 ± 0.2*	14.4 ± 0.2**	13.2 ± 0.1**
Erythrocytes (10 <sup>6</sup> /μL)	8.50 ± 0.09	8.31 ± 0.10	7.88 ± 0.08**	6.95 ± 0.09**
Reticulocytes (10 <sup>6</sup> /μL)	0.24 ± 0.01	0.24 ± 0.01	0.35 ± 0.01**	0.70 ± 0.02**
Mean cell volume (fL)	55.1 ± 0.2	55.1 ± 0.2	56.1 ± 0.3*	59.4 ± 0.2**
Mean cell hemoglobin (pg)	18.6 ± 0.1	18.2 ± 0.1*	18.3 ± 0.1	19.0 ± 0.1
Mean cell hemoglobin concentration (g/dL)	33.8 ± 0.2	33.1 ± 0.2*	32.6 ± 0.2**	32.0 ± 0.2**
Platelets (10 <sup>3</sup> /μL)	597.4 ± 46.6	583.1 ± 46.9	578.8 ± 49.0	719.3 ± 31.9
Leukocytes (10 <sup>3</sup> /μL)	8.04 ± 0.35	8.65 ± 0.22	8.59 ± 0.56	7.46 ± 0.38
Segmented neutrophils (10 <sup>3</sup> /μL)	1.40 ± 0.10	1.51 ± 0.11	1.52 ± 0.15	0.95 ± 0.11
Lymphocytes (10 <sup>3</sup> /μL)	6.29 ± 0.30	6.76 ± 0.26	6.74 ± 0.44	6.24 ± 0.33
Monocytes (10 <sup>3</sup> /μL)	0.21 ± 0.01	0.24 ± 0.02	0.18 ± 0.02	0.15 ± 0.01*
Basophils (10 <sup>3</sup> /μL)	0.060 ± 0.007	0.054 ± 0.003	0.065 ± 0.009	0.052 ± 0.006
Eosinophils (10 <sup>3</sup> /μL)	0.07 ± 0.01	0.09 ± 0.01	0.09 ± 0.02	0.07 ± 0.03
Methemoglobin (g/dL)	0.80 ± 0.03	0.87 ± 0.03	1.21 ± 0.05**	2.26 ± 0.07**
Methemoglobin (% hemoglobin)	5.10 ± 0.23	5.60 ± 0.27	8.40 ± 0.31**	17.10 ± 0.41**
Heinz bodies (% erythrocytes)	0.0 ± 0.0	0.3 ± 0.2*	0.9 ± 0.3**	3.8 ± 0.2**

\* Significantly different ( $P \leq 0.05$ ) from the vehicle control group by Dunn's or Shirley's test

\*\* Significantly different ( $P \leq 0.01$ ) from the vehicle control group by Shirley's test

\* Data are presented as mean ± standard error. Statistical tests were performed on unrounded data.

Non-neoplastic lesions in the 2-year study were observed in organs of the haematopoietic system, e.g. statistically significantly increased hyperplasia of the bone marrow in 20 mg/kg and 60 mg/kg males and 60 mg/kg females (Table 31). Kidney pigmentation (all dosed males groups) or kidney nephropathy (all dosed female groups) had significantly increased incidences. These findings are conclusive with the findings from haematology, i.e. a partly compensated, haemolytic anaemia. The observed haemosiderosis (pigmentation in kidney and spleen) is probably secondary to erythrolysis.

**Table 31 Selected non-neoplastic incidences in F344/N rats. \* or \*\*: Significantly different (P≤0.05 or P≤0.01) from the vehicle control group by the Poly-3 test. Numbers in brackets: Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked. <sup>a</sup>: number of animals examined microscopically.**

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
<b>Male</b>				
Bone Marrow <sup>a</sup>	50	50	50	50
Hyperplasia	17 (2.5)	13 (2.5)	28** (2.1)	50** (2.7)
Forestomach <sup>a</sup>	50	50	50	50
Hyperplasia	0	3 (1.7)	5* (2.2)	11** (2.2)
Inflammation	1 (2.0)	5 (1.6)	5 (2.6)	7* (2.6)
Ulcer	0	2 (2.0)	5* (2.6)	6** (2.0)
Kidney <sup>a</sup>	50	50	50	50
Nephropathy	49 (1.4)	49 (2.0)	48 (2.5)	49 (2.7)
Pigmentation	24 (1.2)	46** (1.0)	37** (1.2)	44** (1.6)
Mesenteric Lymph Node <sup>a</sup>	50	50	50	50
Infiltration Cellular, Histiocyte	21 (1.1)	23 (1.4)	30* (1.3)	34** (1.5)
Spleen <sup>a</sup>	50	50	50	50
Capsule, Fibrosis	1 (2.0)	0	2 (1.5)	46** (1.8)
Capsule, Hypertrophy, Mesothelium	0	1 (1.0)	3 (1.0)	39** (1.1)
Congestion	1 (2.0)	0	0	39** (1.9)
Hematopoietic Cell Proliferation	34 (1.0)	44* (1.1)	42* (1.5)	44** (1.3)
Lymphoid Follicle, Atrophy	0	5* (2.2)	2 (1.5)	19** (2.0)
Pigmentation	36 (1.1)	48** (1.7)	47** (2.1)	48** (2.0)
<b>Female</b>				
Bone Marrow <sup>a</sup>	50	50	50	50
Hyperplasia	18 (2.8)	13 (2.5)	18 (2.7)	49** (2.6)
Kidney <sup>a</sup>	50	50	50	50
Nephropathy	28 (1.1)	38* (1.2)	38* (1.2)	41** (1.8)
Pigmentation	41 (1.0)	45 (1.0)	43 (1.0)	49** (1.4)
Spleen <sup>a</sup>	50	50	50	50
Capsule, Fibrosis	8 (1.1)	0	8 (1.1)	41** (1.3)
Capsule, Hypertrophy, Mesothelium	1 (1.0)	14** (1.0)	10** (1.0)	16** (1.1)
Congestion	0	9** (1.1)	26** (1.3)	28** (1.8)
Hematopoietic Cell Proliferation	32 (1.6)	45** (1.8)	47** (1.9)	42** (1.7)
Lymphoid Follicle, Atrophy	1 (2.0)	2 (3.0)	0	28** (2.4)
Pigmentation	44 (2.0)	47 (2.1)	47 (2.5)	49* (2.2)

**2-year studies in mice (NTP, 2012)**

Groups of 50 male and 50 female B6C3F1/N mice were administered 0, 6, 20, or 60 mg *N,N*-dimethyl-p-toluidine/kg body weight in corn oil by gavage, 5 days per week for 105 weeks.

Neoplastic changes (dose dependent and statistically significant) were observed in the liver (both sexes), lung (females) and in the forestomach (females) at 20 and 60 mg/kg (see below and Table 32). In addition, non-neoplastic effects occurred, more severely at the high dose females, in e.g. liver (hepatocyte hypertrophy, necrosis), nasal cavity (metaplasia, hyperplasia, inflammation, necrosis), lung (hyperplasia, necrosis), forestomach, olfactory lobe (atrophy), bone marrow (hyperplasia), mesenteric lymph node (atrophy), spleen (red pulp atrophy). For historical control data see Annex A – Historical control values of NTP 2012 study.

**Table 32 Summary of neoplastic incidences in 2-year studies in B6C3F1/N mice (NTP, 2012)**

	Male				Female			
	0 mg/kg	6 mg/kg	20 mg/kg	60 mg/kg	0 mg/kg	6 mg/kg	20 mg/kg	60 mg/kg
Number of animals	50	50	50	50	50	50	50	50
Surviving until termination	34	36	31	36	43	40	39	32
Survival probability (%) <sup>a</sup>	71	72	62	72	86	82	80	67 <sup>s</sup>
<b>Liver</b>								
Hepatocellular adenoma	29	34	37	36	17 <sup>##</sup>	19	37 <sup>**</sup>	44 <sup>**</sup>
H. carcinoma	22 <sup>##</sup>	25	30	36 <sup>**</sup>	6 <sup>##</sup>	13 <sup>*</sup>	18 <sup>**</sup>	31 <sup>**</sup>
H. adenoma or carcinoma	38 <sup>##</sup>	44	47 <sup>**</sup>	48 <sup>**</sup>	20 <sup>##</sup>	25	42 <sup>**</sup>	45 <sup>**</sup>
Hepatoblastoma	1	5	10	8 <sup>*</sup>	0 <sup>#</sup>	1	0	4 <sup>*</sup>
H. adenoma, carcinoma, or hepatoblastoma	38 <sup>##</sup>	42	48 <sup>**</sup>	48 <sup>**</sup>	20 <sup>##</sup>	26	42 <sup>**</sup>	45 <sup>**</sup>
<b>Lung</b>								
Alveolar/bronch. adenoma	11	16	18	10	2 <sup>##</sup>	4	8 <sup>*</sup>	12 <sup>**</sup>
Alveolar/bronch. carc.	2	3	0	4	0	1	2	1
Adenoma or carcinoma	13	19	18	12	2 <sup>##</sup>	5	9 <sup>*</sup>	13 <sup>**</sup>
<b>Forestomach</b>								
Squamous cell papilloma	1	1	0	3	1	5	6 <sup>*</sup>	7 <sup>*</sup>
Squamous cell carcinoma					0	1	0	0
Sq. cell papilloma or carc.					1	6	6 <sup>*</sup>	7 <sup>*</sup>

Data are given as overall incidences (to be compared to the number of animals in dosing group).

\*, \*\* Pairwise comparisons between the vehicle controls and that dosed group, \*: p<0.05; \*\*: p< 0.01. The Poly-3 test accounts for differential mortality.

#, ## Trend test significance levels notated next to vehicle control incidences, #: p<0.01; ##: p< 0.005)

<sup>a</sup> Kaplan-Meier determinations

<sup>s</sup> or <sup>ss</sup> Significance of shorter survival from survival analysis, P< 0.05 or P<0.01

**Survival and body weight**

Survival of the 60 mg/kg female group was significantly reduced compared to the vehicle control group; survival of lower dosed females and all dosed groups of males was similar to that of the vehicle control groups (see Table 32 and Table 25).

The mean body weights of 60 mg/kg males and females were reduced by more than 10 % relative to vehicle controls after week 89 (day 617) in males and after week 65 (day 449) in females. Body weight gains were reduced in the 60 mg/kg group to about 69 % (males) and 56 % relative to vehicle control (Table 33).

**Table 33 Relative body weights and body weight gains in 2-year studies in mice.**

	Male			Female		
	6 mg/kg	20 mg/kg	60 mg/kg	6 mg/kg	20 mg/kg	60 mg/kg
Rel. body weight at study end (%) <sup>a</sup>	98.0	92.3	82.3	99.7	101.9	69.9
Rel. body weight gain (%) <sup>a, b</sup>	96.6	86.3	68.6	100.0	103.3	56.3

<sup>a</sup> relative to vehicle control at termination<sup>b</sup> until terminal sacrificeLiver, neoplastic and non-neoplastic lesions

Incidences for hepatocellular carcinoma were statistically significantly increased in 60 mg/kg males and all dosed female groups (Table 32). Hepatocellular adenoma were significantly increased in 20 and 60 mg/kg females. There were also significantly increased incidences of hepatoblastoma in males receiving 20 and 60 mg/kg and in females receiving 60 mg/kg. The historical control incidences for these rare tumours were low with 14/350 (4 %) male and 1/347 (0.3 %) female mice by oral gavage. The incidences of hepatocellular adenoma or carcinoma (combined) and hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined) were significantly increased in males and females receiving 20 and 60 mg/kg compared to vehicle control groups.

In addition to the described neoplasms, significantly increased non-neoplastic effects in the liver (see Table 34) included eosinophilic foci in 20 and 60 mg/kg males and females, mixed cell foci and clear cell foci in 60 mg/kg males. Hepatocellular hypertrophy was significantly increased in all dosed groups of males and females. There were also significantly increased incidences of diffuse fatty change in 60 mg/kg females and necrosis in 6 and 60 mg/kg females. In males, the severity of necrosis was increased in dosed groups although the incidences were not statistically significantly increased.

**Table 34 Selected non-neoplastic incidences of the liver in B6C3F1/N mice. \* or \*\*: Significantly different ( $P \leq 0.05$  or  $P \leq 0.01$ ) from the vehicle control group by the Poly-3 test. Numbers in brackets: Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked. <sup>a</sup>: number of animals examined microscopically.**

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
<b>Male, Liver</b>				
Liver <sup>a</sup>	50	50	50	50
Clear Cell Focus	15	22	15	7*
Eosinophilic Focus	25	30	39**	43**
Mixed Cell Focus	21	25	17	12*
Hepatocyte, Hypertrophy	1 (1.0)	9** (1.2)	11** (1.9)	16** (2.1)
Necrosis	9 (1.6)	8 (2.5)	7 (1.9)	10 (2.0)
<b>Female, Liver</b>				
Liver <sup>a</sup>	50	50	50	50
Clear Cell Focus	0	2	2	3
Eosinophilic Focus	20	18	45**	38**
Mixed Cell Focus	3	9	7	7
Fatty Change	1 (4.0)	0	0	8** (2.5)
Hepatocyte, Hypertrophy	0	11** (1.6)	10** (1.6)	17** (1.9)
Necrosis	1 (2.0)	8* (1.5)	4 (2.0)	10** (1.8)

Lung, neoplastic lesions

Increased incidences for lung neoplasms compared to vehicle controls reached statistical significance in dosed female groups only, although the incidence rate of alveolar/bronchiolar adenoma in the 6 and 20 mg/kg male groups exceeded the historical control ranges for corn oil gavage studies as well as for combined historical controls from all exposure routes. In females, there were statistically significantly increased incidences of alveolar/bronchiolar adenoma and adenoma or carcinoma (combined) in 20 and 60 mg/kg groups (Table 32).

The incidences of alveolar/bronchiolar adenoma in the 20 and 60 mg/kg groups exceeded the historical control ranges for corn oil gavage studies and for all routes of exposure. Non-neoplastic lesions were only observed in single groups of dosed females, e.g. hyperplasia of the alveolar epithelium in the 20 mg/kg group or necrosis in the bronchus in the 60 mg/kg group (see Table 35).

**Table 35 Selected non-neoplastic incidences of the lung in B6C3F1/N mice. \* or \*\*: Significantly different ( $P \leq 0.05$  or  $P \leq 0.01$ ) from the vehicle control group by the Poly-3 test. Numbers in brackets: Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked; <sup>a</sup>: number of animals examined microscopically.**

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
<b>Male, Lung</b>				
Lung <sup>a</sup>	50	50	50	50
Alveolus, Infiltration Cellular, Histiocyte	1 (2.0)	2 (1.5)	2 (2.5)	10** (1.2)
<b>Female, Lung</b>				
Lung <sup>a</sup>	50	50	50	50
Alveolar Epithelium, Hyperplasia	2 (3.0)	3 (2.3)	8* (1.5)	2 (1.0)
Alveolus, Infiltration Cellular, Histiocyte	1 (1.0)	0	0	7* (1.4)
Bronchiole, Epithelium, Regeneration	0	0	0	5* (1.8)
Bronchus, Epithelium, Regeneration	0	0	0	5* (2.0)
Bronchus, Necrosis	0	0	0	5* (1.6)

#### Forestomach, neoplastic lesions

In 20 and 60 mg/kg females, incidences of squamous cell papilloma and squamous cell papilloma or carcinoma (combined) were statistically significantly increased and exceeded historical control ranges (Table 32). In addition, there were significantly increased incidences of epithelial hyperplasia in 20 and 60 mg/kg females and inflammation and ulcer in 60 mg/kg females (Table 36).

**Table 36 Selected non-neoplastic incidences of the forestomach in B6C3F1/N mice. \* or \*\*: Significantly different ( $P \leq 0.05$  or  $P \leq 0.01$ ) from the vehicle control group by the Poly-3 test. Numbers in brackets: Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked. <sup>a</sup>: number of animals necropsied.**

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
<b>Female, Forestomach</b>				
Forestomach <sup>a</sup>	50	50	50	50
Epithelium, Hyperplasia	3 (2.7)	5 (2.8)	12** (2.2)	17** (2.6)
Inflammation	3 (3.0)	4 (2.0)	7 (2.3)	16** (2.3)
Ulcer	2 (2.0)	2 (2.0)	4 (1.3)	7* (1.6)

#### Nasal cavity, non-neoplastic lesions

Non-neoplastic effects in the nasal cavity of mice occurred in dosed males and females with significantly increased incidences in both males and females (Table 37), mainly at 60 mg/kg in many tissues, e.g. dilatation, hyperplasia and metaplasia of the olfactory epithelium (OE) and respiratory epithelium (RE) glands, nerve atrophy (localisation not further described), or necrosis of the OE. Additionally, hyperplasia of OE glands and metaplasia in the OE occurred in females in all dosed groups; hyperplasia and metaplasia of RE glands occurred in females at 20 mg/kg and 60 mg/kg.



**Table 37 Selected non-neoplastic incidences of the nasal cavity in B6C3F1/N mice. \* or \*\*: Significantly different ( $P \leq 0.05$  or  $P \leq 0.01$ ) from the vehicle control group by the Poly-3 test. Numbers in brackets: Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked; \*: number of animals examined microscopically; OE: Olfactory Epithelium; RE: Respiratory Epithelium.**

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
<b>Male, Nose</b>				
Nose <sup>a</sup>	49	50	50	50
Glands, OE, Dilatation	4 (1.0)	11 (1.0)	7 (1.0)	48** (1.8)
Glands, OE, Hyperplasia	4 (1.0)	9 (1.1)	7 (1.3)	49** (2.1)
Glands, OE, Metaplasia, Respiratory	5 (1.0)	5 (1.0)	6 (1.0)	48** (1.7)
Glands, RE, Dilatation	17 (1.0)	19 (1.0)	13 (1.0)	41** (1.8)
Glands, RE, Hyperplasia	4 (1.0)	2 (1.0)	2 (1.0)	11 (1.1)
Glands, RE, Metaplasia, Respiratory	2 (1.5)	2 (1.0)	2 (1.0)	10* (1.1)
Nasolacrimal Duct, Hyperplasia, Regenerative	0	0	0	4 (1.0)
Nerve, Atrophy	2 (1.0)	7 (1.1)	4 (1.3)	42** (2.0)
OE, Metaplasia, Respiratory	10 (1.3)	10 (1.3)	5 (1.2)	49** (2.3)
OE, Necrosis	1 (1.0)	3 (1.3)	3 (1.0)	8* (1.5)
Vomer nasal Organ, Necrosis	0	1 (2.0)	2 (1.0)	3 (1.0)
Olfactory Lobe <sup>a</sup>	38	43	39	34
Atrophy	0	1 (3.0)	0	5* (1.2)
<b>Female, Nose</b>				
Nose <sup>a</sup>	50	49	50	50
Glands, OE, Dilatation	13 (1.0)	14 (1.1)	20 (1.0)	46** (2.3)
Glands, OE, Hyperplasia	2 (1.0)	14** (1.0)	14** (1.1)	50** (2.2)
Glands, OE, Metaplasia, Respiratory	2 (1.0)	5 (1.0)	7 (1.0)	44** (2.3)
Glands, RE, Dilatation	10 (1.0)	17 (1.0)	15 (1.1)	33** (1.4)
Glands, RE, Hyperplasia	0	2 (1.0)	12** (1.2)	13** (1.2)
Glands, RE, Metaplasia, Respiratory	0	0	10** (1.0)	10** (1.4)
Inflammation	3 (1.0)	7 (1.0)	3 (1.0)	32** (1.3)
Nasolacrimal Duct, Hyperplasia, Regenerative	0	0	0	4* (2.5)
Nerve, Atrophy	0	0	0	41** (2.3)
OE, Accumulation, Hyaline Droplet	2 (1.0)	5 (1.0)	8* (1.0)	15** (1.1)
OE, Metaplasia, Respiratory	1 (1.0)	6* (1.0)	14** (1.1)	46** (2.9)
OE, Necrosis	0	0	3 (1.3)	6* (2.3)
RE, Hyperplasia	11 (1.0)	15 (1.0)	11 (1.0)	30** (1.2)
RE, Necrosis	0	0	0	5* (2.0)
Vomer nasal Organ, Necrosis	0	0	0	4* (1.5)
Olfactory Lobe <sup>a</sup>	27	34	24	29
Atrophy	0	0	0	8** (1.6)

Haematopoietic and immune system, non-neoplastic effects

In all dosed female groups, incidence of bone marrow hyperplasia was statistically significantly increased; the incidences of atrophy in the mesenteric lymph nodes were significantly increased in 60 mg/kg females (Table 38).

**Table 38 Selected non-neoplastic incidences of the haematopoietic and immune system in B6C3F1/N mice. \* or \*\*: Significantly different ( $P \leq 0.05$  or  $P \leq 0.01$ ) from the vehicle control group by the Poly-3 test. Numbers in brackets: Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked; <sup>a</sup>: number of animals examined microscopically.**

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
<b>Male, Hematopoietic System</b>				
Spleen <sup>a</sup>	48	50	49	50
Atrophy	4 (2.5)	11 (2.2)	11* (2.4)	6 (1.8)
<b>Female, Hematopoietic System</b>				
Bone Marrow <sup>a</sup>	50	50	50	49
Hyperplasia	5 (2.2)	14* (1.9)	15** (2.1)	14** (2.1)
Lymph Node, Mesenteric <sup>a</sup>	49	49	49	50
Atrophy	1 (2.0)	5 (2.0)	5 (2.2)	12** (2.9)
Hyperplasia, Lymphoid <sup>a</sup>	7 (2.3)	3 (3.7)	1* (2.0)	0*
Spleen <sup>a</sup>	49	49	49	50
Red Pulp, Atrophy	0	0	0	5* (3.2)

### 10.9.1 Short summary and overall relevance of the provided information on carcinogenicity

Treatment with DMPT caused neoplastic lesions in liver and nasal cavity of rats and in lung, liver and forestomach of mice (Dunnick et al., 2014; IARC, 2016; NTP, 2012). The NTP categorized these findings as “Clear Evidence” for carcinogenicity in both species and genders. In addition to neoplastic lesions, non-neoplastic lesions occurred, partly as pre-neoplastic effects at lower doses or in only one species/species/sex. Although most of the neoplastic lesions occurred at the high dose (60 mg/kg bw/d), where survival and body weight gain were reduced in both species (reduction in body weight gain >10 %), the findings are considered relevant for classification of DMPT. Pre-neoplastic lesions, e.g. hyperplasia, inflammation or necrosis were observed in all organs with neoplastic incidences, already at lower doses and/or in sex/species with no significant neoplastic lesions, and it is generally accepted, that there is a continuum between non-neoplastic effects such as chronic inflammation and neoplastic lesions.

The evidence for a genotoxic potential of DMPT is not conclusive. Available in vivo results from micronucleus tests or comet assays are negative although in vitro, a micronucleus test showed a genotoxic potential. In conclusion, DMPT is considered as a non-genotoxic carcinogen. A potential mode of action for carcinogenicity by DMPT is a consequence from chronic oxidative toxicity, resulting in non-neoplastic and pre-neoplastic lesions and further progression to tumour development.

In (Dunnick et al., 2014), a mode of action is discussed where the methaemoglobin formation is a “sentinel response” for oxidative damage induced by DMPT which eventually results in carcinogenic responses in liver, thyroid, lung, or forestomach. The primary effect according to this potential MoA would be the induction of reactive oxygen species by DMPT or its metabolites and/or the ability of DMPT to form free radicals with subsequent cell damage. 2,6-xylidine (or *N,N*-dimethylaniline, DMA, CAS 87-62-7), a substance structurally related to DMPT, is also known to cause methaemoglobinaemia and has a harmonized Classification as Carcinogen, Category 2.

Neoplastic lesions in the nasal cavity are rare findings in NTP carcinogenicity studies, especially by oral gavage. By this route, only dimethylvinylchloride (CAS 513-37-1) gave “Clear Evidence” in male and female

rats for carcinogenesis in the nasal cavity according to NTP criteria. Additionally, also treatment with p-cresidine (CAS 120-71-8), 2,6-xylydine (CAS 87-62-7), pentachlorophenol (CAS 87-86-5) and 1,4-dioxane (CAS 123-91-1) resulted in “Positive” or “Some Evidence” for neoplastic lesions of the nasal cavity in rats when dosed by feeding. A potential mechanism is suggested in (Dunnick et al., 2014): “*The DMPT-induced nasal and pulmonary toxic lesions are not typical of gavage-associated injury or aspiration. The DMPT respiratory epithelial degeneration/necrosis may be due to cytotoxicity as a result of pulmonary/nasal epithelial cytochrome P450 metabolic activation resulting in production of toxic DMPT metabolites.*”

All available studies with DMPT on radical or ROS induction were only performed in the presence of polymerization initiators such as camphorquinone, which generates primary radicals under UV light irradiation. Such studies are of limited usability for the evaluation of carcinogenicity by DMPT. (Dunnick et al., 2014) summarized these studies: “*In a human submandibular gland adenocarcinoma cell line with visible light irradiation, the photosensitizer camphorquinone in the presence of DMPT demonstrated both dose- and time-dependent DMPT induction of reactive oxygen species (Atsumi et al., 2001). This ability of DMPT to form free radicals with subsequent DNA damage may explain the DMPT carcinogenic mechanism (Li et al., 2008; Masuki et al., 2007; Pereira et al., 2008; Winter et al., 2005).*”

For setting a specific concentration limit (SCL), T25 values as measure for the intrinsic carcinogenic potency of *N,N*-dimethyl-p-toluidine were determined according to (EC, 1999). The T25 value estimates the dose in chronic studies, at which particular neoplastic lesions occur in 25 % of the animals of a dose group. For calculations of T25 it was assumed, that the potency is linearly related to the administered doses, which may not necessarily be true. However, the T25 values were calculated for several statistically significant and treatment related incidences of neoplastic lesions from 2-year NTP studies (NTP, 2012) (see Table 39).

The lowest T25 values of 4.9 mg/kg bw/day were obtained for female mice with liver adenoma or carcinoma at medium dose (20 mg/kg bw/day). For hepatocellular carcinoma alone the T25 was 13.1 mg/kg bw/day at the same dose, and 6.7 mg/kg bw/day at low dose (6 mg/kg bw/day). All calculated T25 values were in the medium potency range between 1 and 100 mg/kg bw/day, therefore no SCL is required and the general concentration limit (GCL) should be applied.

Table 39: T25 value calculation for selected neoplastic lesions from NTP 2-year study results (NTP, 2012), data from Table 25 and Table 32, calculated values are printed bold.

Species	mouse			mouse		mouse		rat	
Sex	f			f		f		m	
Organ	liver			liver		lung		liver	nose
Lesion	hepatocellular carcinoma			hepatocellular adenoma / carc.		alveolar/bronch. adenoma / carc.		hep. carc.	trans. epith. ad./carc.
Dose (mg/kg bw/day)	60	20	6	60	20	60	20	60	60
Exposure days/week	5	5	5	5	5	5	5	5	5
Number control	50	50	50	50	50	50	50	50	50
Incidences control	6	6	6	20	20	2	2	0	0
Number dosed	50	50	50	50	50	50	50	50	50
Incidences dosed	31	18	13	45	42	13	9	6	13
<b>Control incidence (%)</b>	<b>12</b>	<b>12</b>	<b>12</b>	<b>40</b>	<b>40</b>	<b>4</b>	<b>4</b>	<b>0</b>	<b>0</b>
<b>Dose incidence (%)</b>	<b>62</b>	<b>36</b>	<b>26</b>	<b>90</b>	<b>84</b>	<b>26</b>	<b>18</b>	<b>12</b>	<b>26</b>
<b>Net incidence (%)<sup>a</sup></b>	<b>56.8</b>	<b>27.3</b>	<b>15.9</b>	<b>83.3</b>	<b>73.3</b>	<b>22.9</b>	<b>14.6</b>	<b>12.0</b>	<b>26.0</b>
<b>Average daily dose (mg/kg bw/day)</b>	<b>42.9</b>	<b>14.3</b>	<b>4.3</b>	<b>42.9</b>	<b>14.3</b>	<b>42.9</b>	<b>14.3</b>	<b>42.9</b>	<b>42.9</b>
<b>T25 (mg/kg bw/day)<sup>b</sup></b>	<b>18.9</b>	<b>13.1</b>	<b>6.7</b>	<b>12.9</b>	<b>4.9</b>	<b>46.8</b>	<b>24.5</b>	<b>89.3</b>	<b>41.2</b>

<sup>a</sup> Net incidence (%) = (Dose incidence (%) - Control incidence (%)) / (100 - Control incidence (%)) \* 100

<sup>b</sup> T25 (mg/kg bw/day) = Average daily dose \* (25 / Net incidence (%))

Exposure duration in the 2-year studies is considered the general life span, therefore no correction is necessary.

**Table 40: Compilation of factors to be taken into consideration in the hazard assessment**

Species and strain	Tumour type and background incidence	Multi-site responses	Progression of lesions to malignancy	Reduced tumour latency	Responses in single or both sexes	Confounding effect by excessive toxicity?	Route of exposure	MoA and relevance to humans
Rats (F344/N) Mice (B6C3F1/N)	<p>Statistically significant, treatment related increased incidences of neoplastic lesions in the <b>liver</b> of both species in both sexes. Background incidences in mice were higher than in rats.</p> <p><b>Nasal cavity and liver</b> tumours in rats have low historical background incidences.</p> <p><b>Hepatoblastoma</b> (in mice) are rare tumours and are found with low incidence in historical controls.</p>	<p>Yes; significant neoplastic lesions in liver and additionally in <b>nasal cavity</b> (rats m), <b>lung</b> (mice f), and <b>forestomach</b> (mice f).</p>	<p>Treatment related non-neoplastic lesions, e.g. hyperplasia, inflammation or necrosis in all organs with neoplastic incidences, already at lower doses and/or in sex/species with no significant neoplastic lesions. In addition, other organs affected:</p> <p><b>liver, nasal cavity and spleen</b> (both species/sexes);</p> <p><b>lung and olfactory lobe</b> (mice m/f);</p> <p><b>kidney</b> (rats m/f)</p> <p><b>forestomach and mesenteric lymph node</b> (rats m, mice f)</p> <p><b>bone marrow</b> (rats m/f, mice f).</p> <p>Most non-neoplastic lesions occurred with mild severity.</p>	<p>Liver tumours (adenoma or carcinoma) in mice have a short latency with first incidences about 100 days or more earlier than in control groups</p>	<p>Liver tumours occurred in both species in both sexes, other neoplastic incidences mainly in one species and in one sex.</p> <p>Pre-neoplastic lesions observed mostly in both sexes.</p>	<p>Treatment related cancer incidences occurred with high dose (60 mg/kg bw/d) in both species (m/f), with mid dose (20 mg/kg bw/d) in mice (m/f), and with low dose (6 mg/kg bw/d) in female mice.</p> <p>Body weight gain at high dose, reduced in both species (m/f), reduction in mice &gt; rats; at mid dose reduced also in male mice. Survival was reduced at high dose in rats (m/f) and female mice compared to control groups.</p> <p>Statistically significant neoplastic incidences at doses below a potential MTD (i.e. body weight gain difference below 10 %) were observed in female mice in liver (e.g. hepatocellular carcinoma), lung (adenoma) and forestomach (papilloma).</p> <p>Pre-neoplastic lesions in these organs occurred already at lower doses.</p>	<p>The studies have been performed by oral gavage, 5 days per week.</p> <p>Other routes of exposure cannot be excluded, and oral, inhalation and skin exposure are relevant exposure routes for humans.</p> <p>Mostly, systemic effects are observed, the occurrence of forestomach tumours in female mice (and non-neoplastic lesions in male rats) could indicate additional local toxicity.</p>	<p>DMPT induces methaemoglobinaemia in rats and mice. A potential metabolite, p-methylphenylhydroxylamine is implicated in the formation of methaemoglobinaemia, and N-hydroxylated arylamines are capable of forming DNA adducts. In addition, formation of a reactive imine methide has been postulated. Without in vivo evidence for genotoxicity of DMPT, the potential MoA is based on indirect effects via oxidative toxicity, e.g. by local ROS production in metabolically active tissues. Methaemoglobinaemia and related chronic toxic effects and/or oxidative DNA damage can propagate cancer development.</p> <p>MoA and target tissues (e.g. liver) are relevant to humans. Oral DMPT exposure in humans leads to acute methaemoglobinemia, MoA in humans and study animals seems comparable, at least for methaemoglobinaemia induced toxic effects.</p>

### 10.9.2 Comparison with the CLP criteria

CLP Regulation, chapter 3.6:

*“Carcinogen means a substance or a mixture of substances which induce cancer or increase its incidence. Substances which have induced benign and malignant tumours in well performed experimental studies on animals are considered also to be presumed or suspected human carcinogens unless there is strong evidence that the mechanism of tumour formation is not relevant for humans. [...]*

*Category 1: Known or presumed human carcinogens.*

*A substance is classified in Category 1 for carcinogenicity on the basis of epidemiological and/or animal data. A substance may be further distinguished as:*

*Category 1A, known to have carcinogenic potential for humans, classification is largely based on human evidence, or*

*Category 1B, presumed to have carcinogenic potential for humans, classification is largely based on animal evidence.”*

Relevant human carcinogenicity studies for DMPT could not be identified, therefore Category 1A cannot be considered.

For Category 1B, *“evidence may be derived from animal experiments for which there is sufficient evidence to demonstrate animal carcinogenicity (presumed human carcinogen)”*. Sufficient evidence of carcinogenicity in animals is defined in the CLP Regulation: *“A causal relationship has been established between the agent and an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in (a) two or more species of animals or (b) two or more independent studies in one species carried out at different times or in different laboratories or under different protocols. An increased incidence of tumours in both sexes of a single species in a well-conducted study, ideally conducted under Good Laboratory Practices, can also provide sufficient evidence. A single study in one species and sex might be considered to provide sufficient evidence of carcinogenicity when malignant neoplasms occur to an unusual degree with regard to incidence, site, type of tumour or age at onset, or when there are strong findings of tumours at multiple sites.”*

Category 1B should be considered, based on incidences for treatment dependent neoplastic lesions at multiple sites in two rodent species and dose-dependent progression from non-neoplastic lesions to neoplasms. It should be noted, that incidence for nasal cavity neoplasms in rats is a rare finding in oral gavage studies. However, in the 2-year studies most neoplastic lesions occurred only at the highest dose in both species, at which survival and body weight gains were reduced. The animals at this dose were possibly above the MTD, average body weight gains were reduced compared to control groups by about 25 % in rats and by more than 30 % in male mice, in female mice by over 40 %. Haematology, measured after 3-months in additional groups of rats showed at the highest dose a reduction in functional haemoglobin by more than 20 % as a result of methaemoglobinaemia. Furthermore, the observed liver tumour incidences in B6C3F1/N mice occur on a high background (vehicle control and historical control data). Except for liver, neoplastic lesions in other organs were limited to one species and one sex. On the other hand it cannot be excluded, that lower body weight gain and lower survival rates in high dose groups were a consequence of the tumour burden. These confounding effects must be acknowledged for classification, therefore rather Category 2 should be considered for classification.

*“Category 2: Suspected human carcinogens*

*The placing of a substance in Category 2 is done on the basis of evidence obtained from human and/or animal studies, but which is not sufficiently convincing to place the substance in Category 1A or 1B, based on strength of evidence together with additional considerations. Such evidence may be derived either from limited evidence of carcinogenicity in human studies or from limited evidence of carcinogenicity in animal studies.”*

No information on human carcinogenicity is available, in experimental animals limited evidence of carcinogenicity is defined in the CLP Regulation: *“The data suggest a carcinogenic effect but are limited for making a definitive evaluation because, e.g. (a) the evidence of carcinogenicity is restricted to a single*

*experiment; (b) there are unresolved questions regarding the adequacy of the design, conduct or interpretation of the studies; (c) the agent increases the incidence only of benign neoplasms or lesions of uncertain neoplastic potential; or (d) the evidence of carcinogenicity is restricted to studies that demonstrate only promoting activity in a narrow range of tissues or organs.”*

Methaemoglobinaemia, severe body weight loss, non-neoplastic lesions in several organs and increased mortality at high dose (60 mg/kg bw/day) indicates a dosing above the maximum tolerable dose. The incidences for neoplastic lesions below MTD, i.e. where the body weight gain reduction compared to vehicle control animals is below 10 %, were exclusively observed in female mice: in liver (hepatocellular adenoma and carcinoma), lung (adenoma) and forestomach (papilloma). Except for the hepatocellular carcinoma, the other neoplastic lesions at mid dose were benign and liver tumours in the mouse strain used (B6C3F1) occur with high spontaneous background incidences.

Although there is in vitro evidence for genotoxicity of DMPT, in vivo data do not support a classification as germ cell mutagen. Therefore, a non-genotoxic mode of action needs to be considered, i.e. metabolic generation of reactive oxygen species, oxidative tissue damage, methaemoglobinaemia and associated progression of non-neoplastic lesions to neoplasms.

The arguments discussed above are conclusive for a classification as carcinogen, a non-classification appears not plausible. The major arguments identified for weighing a classification in either Category 1B or Category 2 are summarized in Table 41. Taken together a classification into Category 2 for carcinogenicity seems more appropriate than Category 1B (see Table 41), considering the confounding factors in the animal experiments. The NTP report (NTP, 2012) concludes on “*clear evidence of carcinogenic activity*” in both species and both sexes, IARC (IARC, 2016) evaluated the available studies as “*possibly carcinogenic to humans (Group 2B)*”.

**Table 41 Identified arguments for a classification of DMPT as a Category 1B or 2 carcinogen.**

Category 1B arguments	Category 2 arguments
<ul style="list-style-type: none"> <li>• liver carcinoma in mice and rats, m/f</li> <li>• dose-dependent progression to neoplasms</li> <li>• pre-neoplastic lesions in all organs with neoplasms</li> <li>• rare/uncommon tumour types                             <ul style="list-style-type: none"> <li>○ historical incidences for transitional epithelium adenomas or carcinomas (nasal cavity) are rare (rats, gavage studies)</li> <li>○ hepatoblastoma are rare tumour types</li> </ul> </li> <li>• Plausible MoA, relevant for humans                             <ul style="list-style-type: none"> <li>○ metabolic generation of ROS and other radicals, methaemoglobinaemia, oxidative tissues damage</li> </ul> </li> </ul>	<ul style="list-style-type: none"> <li>• most neoplastic lesions only at highest dose</li> <li>• excessive toxicity - high dose potentially above MTD                             <ul style="list-style-type: none"> <li>○ methaemoglobinaemia</li> <li>○ reduced body weight / body weight gain</li> <li>○ non-neoplastic lesions in several organs</li> <li>○ increased mortality (not explained by neoplasms)</li> </ul> </li> <li>• single species/single sex neoplasms, i.e.                             <ul style="list-style-type: none"> <li>○ nasal cavity (m rats)</li> <li>○ lung (f mice)</li> <li>○ forestomach (f mice)</li> </ul> </li> <li>• liver (mice): high number of spontaneous incidences</li> <li>• non-genotoxic carcinogen</li> </ul>

### 10.9.3 Conclusion on classification and labelling for carcinogenicity

Based on chronic animal studies in mice and rats, genotoxicity studies and toxicokinetic data, *N,N*-dimethyl-p-toluidine should be classified as Category 2 carcinogen. A specific concentration limit (SCL) is not required, as DMPT is within the medium potency range for carcinogens.

The route of exposure should not be stated, because it cannot be conclusively proven that other route(s) of exposure than oral cannot cause the hazard.

### 10.10 Reproductive toxicity

Not assessed for this dossier.

### **10.11 Specific target organ toxicity-single exposure**

Not assessed for this dossier.

### **10.12 Specific target organ toxicity-repeated exposure**

The uptake of DMPT results in acute methaemoglobinaemia (see chapter 10.1). In chronic studies (chapter 10.9), treatment related neoplastic and non-neoplastic lesions are evidenced in several organs of rats and mice; affected organs are e.g. liver, epithelia in the nasal cavity, spleen, kidney, bone marrow. Whether the lesions observed in the chronic studies are based on direct toxicity to the target organs, incl. by production of reactive oxygen species (ROS) or potential genotoxicity of DMPT and its metabolites, or are effects of the observed methaemoglobinaemia and related haematological changes, is not clear. However, it appears plausible, that at least the effects observed in liver, spleen, kidney (and bone marrow) are at least in part secondary effects of the haematotoxicity. In the following, chronic and sub-chronic studies are evaluated for the assessment of potential STOT-RE hazards with focus on haematotoxicity. The nasal tissue effects are addressed as additional evidence for STOT-RE.

Table 42: Summary table of animal studies on STOT RE

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
<p>2-year study</p> <p>Gavage (vehicle: corn oil; dosing volume 2.5 ml/kg)</p> <p><b>Rats (F344/N)</b></p> <p><b>Reliable with restrictions</b></p> <p>Males and females</p> <p>Equivalent to OECD TG 451</p> <p>50 animals per sex and dose</p>	<p><i>N,N</i>-Dimethyl-p-toluidine (CAS No. 99-97-8)</p> <p>Purity: &gt; 99 %</p> <p>0, 6, 20, or 60 mg/kg</p> <p>5 days per week; 104 (♂) or 105 (♀) weeks</p>	<p><u>Neoplastic lesions</u> (see Table 25)</p> <p><b>Non-neoplastic lesions</b> (see Table 27, Table 28, and Table 31):</p> <ul style="list-style-type: none"> <li>liver: hepatocellular hypertrophy (at 20 and 60 mg/kg, ♀/♂)</li> <li>nasal cavity: hyperplasia of olfactory, respiratory, and transitional epithelia (♀/♂)</li> <li>spleen: pigmentation, congestion, haematopoietic cell proliferation, hypertrophy, fibrosis (♀/♂)</li> <li>kidney: severity of nephropathy ↑ (♀/♂)</li> <li>bone marrow: hyperplasia (♀/♂)</li> <li>forestomach: hyperplasia and ulcer (♂)</li> <li>mesenteric lymph node (♂)</li> </ul>	(NTP, 2012)
<p>86 day study (clinical pathology group from 2-year study)</p> <p>Gavage (vehicle: corn oil; dosing volume 2.5 ml/kg)</p> <p><b>Rats (F344/N)</b></p> <p><b>Reliable with restrictions</b></p> <p>Males and females</p> <p>Equivalent to OECD TG 451</p> <p>10 animals per sex and dose</p>	<p><i>N,N</i>-Dimethyl-p-toluidine (CAS No. 99-97-8)</p> <p>Purity: &gt; 99 %</p> <p>0, 6, 20, or 60 mg/kg</p> <p>5 days per week; 104 (♂) or 105 (♀) weeks</p>	<p>Haematological effects (at 20 and 60 mg/kg ♀/♂, see Table 29 and Table 45)</p> <ul style="list-style-type: none"> <li>methaemoglobin ↑</li> <li>Heinz bodies ↑</li> <li>haematocrit ↓</li> <li>haemoglobin concentrations ↓</li> <li>erythrocyte counts ↓</li> </ul> <p>Functional Hb reduced by more than 20 % compared to vehicle controls in males and females at 60 mg/kg bw/day.</p>	(NTP, 2012)
<p>2-year study</p> <p>Gavage (vehicle: corn oil; dosing volume 5 ml/kg)</p> <p><b>Mice (B6C3F1/N)</b></p> <p><b>Reliable with restrictions</b></p> <p>Males and females</p> <p>NTP internal guideline, equivalent to OECD TG 451</p> <p>50 animals per sex and dose</p>	<p><i>N,N</i>-Dimethyl-p-toluidine (CAS No. 99-97-8)</p> <p>Purity: &gt; 99 %</p> <p>0, 6, 20, or 60 mg/kg</p> <p>5 days per week; 105 weeks</p>	<p><u>Neoplastic lesions</u> (see Table 32)</p> <p><b>Non-neoplastic lesions</b> (see Table 34, Table 35, Table 36, Table 37, and Table 38)</p> <ul style="list-style-type: none"> <li>liver (♀/♂): eosinophilic foci, hep. hypertrophy, necrosis (♀ only)</li> <li>nasal cavity (♀/♂): metaplasia, hyperplasia and necrosis, nerve atrophy</li> <li>olfactory lobe atrophy (♀/♂)</li> <li>bone marrow hyperplasia, mesenteric lymph node and spleen red pulp atrophy (♀)</li> <li>lung: alveolar histiocyte infiltration (♀/♂), necrosis (♀)</li> <li>forestomach hyperplasia, inflammation, ulcer (♀)</li> </ul>	(NTP, 2012)



Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
<p>3-month study</p> <p>Gavage (vehicle: corn oil; dosing volume 2.5 ml/kg)</p> <p><b>Rats (F344/N)</b></p> <p><b>Reliable with restrictions</b></p> <p>Males and females</p> <p>NTP internal guideline, equivalent to OECD TG 408</p> <p>10 animals per sex and dose (core study animals). MetHb and Hb were determined additionally at day 88 from core study animals.</p> <p>Additional clinical pathology groups of 10 male and 10 female rats received the same doses for only 25 days.</p>	<p><i>N,N</i>-Dimethyl-p-toluidine (CAS No. 99-97-8)</p> <p>Purity: &gt; 99 %</p> <p>0, 62.5, 125, 250, 500, and 1,000 mg/kg</p> <p>5 days per week; 14 weeks (98 days)</p>	<ul style="list-style-type: none"> <li>• no survival in the 1,000 mg/kg groups within the first week (♀/♂)</li> <li>• final mean body weights ↓ (&gt; 10 %) with 125, 250, and 500 mg/kg (♂)</li> <li>• treatment-related non-neoplastic lesions in the liver, nasal cavity, spleen, kidney, and bone marrow with increased severity ≥125 mg/kg (see Table 43)</li> <li>• cyanosis, abnormal breathing, and lethargy at ≥ 250 mg/kg</li> <li>• haematology (see Table 44): methaemoglobinaemia and Heinz body formation → macrocytic, hypochromic, responsive anaemia</li> </ul>	<p>(NTP, 2012)</p>
<p>3-month study</p> <p>Gavage (vehicle: corn oil, dosing volume 5 ml/kg)</p> <p><b>Mice (B6C3F1/N)</b></p> <p><b>Reliable with restrictions</b></p> <p>Males and females</p> <p>NTP internal guideline, equivalent to OECD TG 408</p> <p>10 animals per sex and dose</p>	<p><i>N,N</i>-Dimethyl-p-toluidine (CAS No. 99-97-8)</p> <p>Purity: &gt; 99 %</p> <p>0, 15, 30, 60, 125, and 250 mg/kg</p> <p>5 days per week; 14 weeks</p>	<ul style="list-style-type: none"> <li>• increased mortality at 125 and 250 mg/kg bw/day (♀/♂)</li> <li>• reduced body weights at 125 (♂) and 250 mg/kg bw/day (♀/♂)</li> <li>• abnormal breathing, thinness, lethargy, cyanosis, and ruffled fur in 125 and 250 mg/kg males and females</li> <li>• haematology: <ul style="list-style-type: none"> <li>○ f: no erythron changes up to 125 mg/kg</li> <li>○ m: inconsistent and minor decreases in haematocrit values, haemoglobin concentrations, and erythrocyte counts and increased reticulocyte counts (60 mg/kg and greater)</li> </ul> </li> </ul>	<p>(NTP, 2012)</p>
<p><b>Supporting studies</b></p>			
<p>No guideline study</p> <p>Oral gavage</p> <p>Male F344/N rats</p> <p>5 animals/dose</p> <p>Liver examined for lesions and transcriptomic</p>	<p><i>N,N</i>-Dimethyl-p-toluidine (CAS No. 99-97-8)</p> <p>Purity: &gt; 99 %</p> <p>0, 1, 6, 20, 60 or 120 mg/kg/day</p> <p>5 days</p>	<p>Mild hepatic toxicity with individual cell death (20, 60 and 120 mg/kg) and increased mitoses (at 60 and 120 mg/kg) and dose-related transcriptomic alterations in the liver.</p>	<p>(Dunnick et al., 2017)</p>

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference																																			
alterations	<table border="1"> <thead> <tr> <th>Dose</th> <th>0</th> <th>1</th> <th>6</th> <th>20</th> <th>60</th> <th>120 mg/kg</th> </tr> </thead> <tbody> <tr> <td colspan="7">DMPT</td> </tr> <tr> <td>Number examined</td> <td>5</td> <td>5</td> <td>5</td> <td>5</td> <td>5</td> <td>5</td> </tr> <tr> <td>Individual cell death</td> <td>0</td> <td>0</td> <td>0</td> <td>4 [1.0]<sup>a</sup></td> <td>5 [1.4]</td> <td>4 [1.5]</td> </tr> <tr> <td>Increased mitoses</td> <td>0</td> <td>0</td> <td>0</td> <td>0</td> <td>2 [1.0]</td> <td>2 [1.0]</td> </tr> </tbody> </table>	Dose	0	1	6	20	60	120 mg/kg	DMPT							Number examined	5	5	5	5	5	5	Individual cell death	0	0	0	4 [1.0] <sup>a</sup>	5 [1.4]	4 [1.5]	Increased mitoses	0	0	0	0	2 [1.0]	2 [1.0]		
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<p>No guideline study</p> <p>Oral gavage</p> <p>Male F344/N rats</p> <p>5 animals/dose, further 5 for highest dose and control for frozen tissue collection and RNA extraction</p> <p>Exploration of early changes in the nasal cavity after short-term exposure</p>	<p><i>N,N</i>-Dimethyl-p-toluidine (CAS No. 99-97-8)</p> <p>Purity: &gt; 99 %</p> <p>0, 1, 6, 20, 60 or 120 mg/kg/day</p> <p>5 days</p>	<p>Hyperplasia of the nasal cavity transitional epithelium was observed in the 60 and 120 mg/kg dose groups with an increase in cell layers and an increase in the disorganization of the cells.</p> <p>Olfactory epithelial necrosis was characterized by a layer of proteinaceous substance and cell debris overlying a thin, disorganized layer of olfactory epithelium. The remaining olfactory epithelium was hypocellular, disorganized, vacuolated, and contained individual dead cells, which were characterized by shrunken, eosinophilic round bodies. There were focal areas of attenuated epithelium in affected locations, indicative of epithelial cell loss. The dorsal meatus and directly adjacent areas were most commonly and severely affected. Severity of olfactory epithelium necrosis was based upon the extent of the lesion as well as the amount of changes seen in the affected area. Minimal lesions consisted of small areas of dorsal meatus olfactory epithelium characterized by a slight disorganization and vacuolation of the epithelium, which contained decreased numbers of cells within the epithelium. There was a thin layer of proteinaceous and cell debris overlying affected areas. Mild lesions involved a larger area of the dorsal meatus, and the epithelium contained obviously fewer cells. Moderate necrosis involved most of the dorsal meatus and directly adjacent areas. There was a thick layer of debris overlying the epithelium, which was moderately attenuated in areas. The remaining epithelium was disorganized and vacuolated. One occurrence of mild olfactory degeneration was recorded and differed from necrosis in that the epithelium appeared normal in thickness and better organized than that in the animals with necrosis. The epithelium contained numerous vacuoles but lacked evidence of cell death and overlying debris.</p>	<p>(Dunnick et al., 2016)</p>																																			

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference																																			
	<p><b>Table 1. Nasal Cavity Lesions in Male Rats after 5 Days of DMPT Exposure.</b></p> <table border="1"> <thead> <tr> <th>Mg/kg</th> <th>0</th> <th>1</th> <th>6</th> <th>20</th> <th>60</th> <th>120</th> </tr> </thead> <tbody> <tr> <td>Angiectasis</td> <td>1</td> <td>0</td> <td>0</td> <td>1</td> <td>0</td> <td>0</td> </tr> <tr> <td>Transitional epithelium hyperplasia</td> <td>0</td> <td>0</td> <td>0</td> <td>0</td> <td>4 (1.0)<sup>a</sup></td> <td>5 (1.8)</td> </tr> <tr> <td>Olfactory epithelium necrosis</td> <td>0</td> <td>0</td> <td>0</td> <td>0</td> <td>0</td> <td>4 (1.75)</td> </tr> <tr> <td>Olfactory epithelium degeneration</td> <td>0</td> <td>0</td> <td>0</td> <td>0</td> <td>0</td> <td>1 (2.0)</td> </tr> </tbody> </table> <p>Note: Five animals per group. DMPT = N, N-dimethyl-p-toluidine.  <sup>a</sup>Severity of lesion.</p>			Mg/kg	0	1	6	20	60	120	Angiectasis	1	0	0	1	0	0	Transitional epithelium hyperplasia	0	0	0	0	4 (1.0) <sup>a</sup>	5 (1.8)	Olfactory epithelium necrosis	0	0	0	0	0	4 (1.75)	Olfactory epithelium degeneration	0	0	0	0	0	1 (2.0)
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### Blood system

#### 3-month study, rats, (NTP, 2012)

From the NTP study report (NTP, 2012): *“The haematology findings were consistent with methemoglobinemia and Heinz body resulting in a macrocytic, hypochromic, responsive anaemia. In general, these changes were dose-related, occurred at both time points evaluated, and involved all dosed groups of both sexes. The methemoglobinemia was described by a considerable treatment-related increase in methaemoglobin values. The anaemia was characterized by dose-related decreases in the erythron including decreases in haematocrit values, haemoglobin concentrations, and erythrocyte counts. The greatest magnitudes of decrease occurred in the 500 mg/kg groups on day 25; the decrease was greater than 20 % for haematocrit and haemoglobin values and close to 40 % for erythrocyte counts. By week 14, there was some amelioration in the severities of the anaemia. Erythrocyte macrocytosis was characterized by increases in mean cell volume and mean cell haemoglobin values indicating that the circulating erythrocytes were larger than those of the concurrent vehicle controls. Erythrocyte hypochromia was evidenced by decreases in mean cell haemoglobin concentration values, indicating that the circulating erythrocytes did not have the normal intracellular haemoglobin content. An erythropoietic response to the anaemia was characterized by substantially increased reticulocyte and nucleated erythrocyte counts. Decreases in leukocyte counts occurred in 250 and 500 mg/kg male and female rats on day 25. Decreases in lymphocyte counts mimicked the leukocyte count decreases; these changes were consistent with physiologic responses to stress.”*

Table 43: Incidences of Selected Non-neoplastic Lesions in Rats in the 3-Month Gavage Study of *N,N*-Dimethyl-p-toluidine<sup>a</sup> ((NTP, 2012))

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg
<b>Male</b>					
Liver <sup>b</sup>	10	10	10	10	10
Hepatocyte, Hypertrophy <sup>c</sup>	0	2 (1.0) <sup>d</sup>	9** (1.0)	10** (1.2)	10** (1.8)
Pigmentation	0	4* (1.0)	7** (1.0)	9** (1.0)	9** (1.0)
Nose	10	10	10	10	10
Glands, Hyperplasia	0	0	10** (1.8)	10** (2.1)	9** (2.1)
Olfactory Epithelium, Degeneration	0	5* (1.0)	10** (2.5)	10** (3.0)	10** (3.1)
Olfactory Epithelium, Metaplasia	0	0	0	9** (1.9)	9** (2.9)
Respiratory Epithelium, Hyperplasia	1 (1.0)	2 (1.0)	7** (1.4)	10** (1.5)	9** (1.8)
Respiratory Epithelium, Metaplasia, Squamous	0	8** (1.5)	10** (2.5)	10** (2.8)	9** (3.0)
Kidney	10	10	10	10	10
Mineralization	1 (1.0)	4 (1.0)	10** (1.3)	10** (1.8)	8** (2.1)
Pigmentation	0	10** (1.0)	10** (1.0)	10** (1.6)	9** (1.9)
Papilla, Necrosis	0	0	7** (1.3)	7** (1.7)	9** (2.4)
Spleen	10	10	10	10	10
Capsule, Fibrosis	1 (1.0)	5 (1.0)	10** (1.4)	10** (2.7)	9** (2.8)
Congestion	0	10** (1.2)	10** (1.8)	10** (2.4)	9** (3.0)
Hematopoietic Cell Proliferation	9 (1.0)	10 (2.0)	10 (2.0)	10 (1.9)	9 (1.8)
Lymphoid Follicle, Atrophy	0	0	0	8** (1.5)	10** (2.7)
Mesothelium, Hypertrophy	3 (1.3)	5 (1.2)	8* (1.5)	10** (1.5)	9** (1.8)
Pigmentation	10 (1.0)	10 (2.1)	10 (2.2)	10 (2.0)	9 (2.0)
Bone Marrow	10	10	10	10	10
Hyperplasia	0	10** (2.0)	10** (2.9)	10** (3.0)	10** (2.9)
Forestomach	10	10	10	10	10
Inflammation	0	0	1 (1.0)	0	5* (1.4)

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	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg
<b>Female</b>					
Liver	10	10	10	10	10
Hepatocyte, Hypertrophy	0	1 (1.0)	7** (1.0)	9** (1.1)	10** (2.7)
Hepatocyte, Necrosis	1 (1.0)	6* (1.5)	5 (1.4)	7** (1.3)	6* (1.2)
Pigmentation	0	10** (1.0)	10** (1.0)	10** (1.8)	10** (1.9)
Nose	10	10	10	10	10
Glands, Hyperplasia	0	3 (1.0)	9** (1.7)	10** (1.9)	10** (2.0)
Olfactory Epithelium, Degeneration	0	7** (1.3)	10** (2.1)	10** (3.0)	10** (3.0)
Olfactory Epithelium, Metaplasia	0	0	0	7** (1.6)	10** (2.9)
Respiratory Epithelium, Hyperplasia	0	1 (1.0)	7** (1.1)	10** (1.7)	10** (1.7)
Respiratory Epithelium, Metaplasia, Squamous	0	0	6** (1.5)	10** (2.2)	10** (2.6)
Kidney	10	10	10	10	10
Nephropathy	2 (1.0)	2 (1.0)	9** (1.0)	10** (1.0)	10** (1.3)
Pigmentation	0	10** (1.0)	10** (1.0)	10** (1.0)	10** (1.6)
Papilla, Necrosis	0	0	0	6** (1.5)	2 (2.5)
Spleen	10	10	10	10	10
Capsule, Fibrosis	0	3 (1.0)	7** (1.3)	10** (2.2)	10** (2.7)
Congestion	0	2 (1.0)	10** (1.4)	10** (2.4)	10** (3.0)
Hematopoietic Cell Proliferation	10 (1.0)	10 (1.9)	10 (1.9)	10 (2.3)	10 (2.0)
Lymphoid Follicle, Atrophy	0	0	0	0	10** (1.3)
Mesothelium, Hypertrophy	0	1 (1.0)	2 (1.5)	9** (1.1)	9** (1.1)
Pigmentation	10 (1.0)	10 (2.0)	10 (2.0)	10 (1.9)	10 (2.0)
Bone Marrow	10	10	10	10	10
Hyperplasia	0	10** (1.9)	10** (2.7)	10** (3.0)	10** (3.0)
Lymph Node, Mesenteric	10	10	10	10	10
Atrophy	0	0	0	1 (2.0)	6** (2.2)

\* Significantly different ( $P < 0.05$ ) from the vehicle control group by the Fisher exact test

\*\*  $P < 0.01$

\* Data not shown for 1,000 mg/kg groups because all animals died during week 1.

<sup>b</sup> Number of animals with tissue examined microscopically

<sup>c</sup> Number of animals with lesion

<sup>d</sup> Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

Table 44: Selected haematology data for rats in the 3-Month gavage study of *N,N*-Dimethyl-p-toluidine<sup>a</sup> ((NTP, 2012))

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg
<b>Male</b>					
<b>n</b>					
Day 25	10	10	9	10	8
Day 88	10	10	10	10	9
Week 14	10	10	10	10	9
<b>Hematocrit (%)</b>					
Day 25	49.7±0.3	45.0±0.5**	42.8±0.4**	40.2±0.5**	39.2±0.5**
Week 14	46.1±0.4	42.1±0.5**	42.3±0.4**	42.1±0.4**	42.4±0.7**
<b>Hemoglobin (g/dL)</b>					
Day 25	15.3±0.1	13.3±0.1**	12.5±0.1**	11.8±0.1**	11.0±0.1**
Week 14	14.8±0.1	13.0±0.2**	13.0±0.1**	12.9±0.1**	12.7±0.2**
<b>Erythrocytes (10<sup>6</sup>/μL)</b>					
Day 25	8.26±0.05	7.44±0.07**	6.79±0.07**	5.97±0.09**	5.06±0.05**
Week 14	8.62±0.07	7.43±0.08**	6.94±0.05**	6.40±0.07**	6.19±0.07**
<b>Reticulocytes (10<sup>6</sup>/μL)</b>					
Day 25	0.26±0.01	0.50±0.01**	0.64±0.01**	0.94±0.03**	1.08±0.03**
Week 14	0.25±0.01	0.50±0.01**	0.60±0.02**	0.76±0.01**	0.89±0.04**
<b>Nucleated erythrocytes/100 leukocytes</b>					
Day 25	0.2±0.1	1.3±0.4*	1.3±0.5*	4.7±0.7**	21.6±2.1**
Week 14	0.2±0.1	0.9±0.2*	2.0±0.4**	1.7±0.3**	3.6±0.6**
<b>Mean cell volume (fL)</b>					
Day 25	60.2±0.2	60.5±0.2	63.1±0.2**	67.5±0.6**	77.5±0.5**
Week 14	53.5±0.3	56.6±0.3**	61.1±0.3**	65.8±0.3**	68.5±0.6**
<b>Mean cell hemoglobin (pg)</b>					
Day 25	18.5±0.1	17.9±0.1	18.4±0.1	19.7±0.1**	21.8±0.1**
Week 14	17.2±0.1	17.5±0.1*	18.7±0.1**	20.1±0.1**	20.6±0.2**
<b>Mean cell hemoglobin concentration (g/dL)</b>					
Day 25	30.8±0.1	29.7±0.1**	29.2±0.2**	29.2±0.1**	28.2±0.1**
Week 14	32.1±0.1	31.0±0.2**	30.7±0.1**	30.5±0.1**	30.0±0.1**
<b>Methemoglobin (g/dL)</b>					
Day 25	0.35±0.03	0.90±0.04**	1.56±0.04** <sup>b</sup>	1.95±0.05**	1.63±0.06**
Day 88	0.38±0.02	1.37±0.08**	1.95±0.07**	2.29±0.08**	2.03±0.08**
<b>Methemoglobin (% hemoglobin)</b>					
Day 25	2.40±0.22	6.70±0.30**	12.44±0.41**	16.60±0.31**	14.75±0.56**
Day 88	2.44±0.18 <sup>c</sup>	10.10±0.55**	15.50±0.48**	18.20±0.53**	17.67±0.71**
<b>Heinz bodies (% erythrocytes)</b>					
Day 25	0.0±0.0	0.0±0.0	2.0±0.6**	14.5±1.9**	23.5±2.6**
Week 14	0.0±0.0	0.5±0.2**	2.8±0.3**	4.1±0.4**	2.9±0.8**

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg
<b>Female</b>					
<b>n</b>					
Day 25	10	10	10	10	10
Day 88	9	10	10	10	10
Week 14	10	10	9	10	10
<b>Hematocrit (%)</b>					
Day 25	48.8±0.4	44.9±0.3**	43.4±0.6**	40.8±0.5**	37.0±0.5**
Week 14	45.2±0.5	41.3±0.5**	40.0±0.6**	39.0±0.4**	40.7±0.3**
<b>Hemoglobin (g/dL)</b>					
Day 25	15.1±0.1	13.3±0.1**	12.8±0.2**	11.7±0.1**	10.8±0.2**
Week 14	14.8±0.1	12.8±0.1**	12.7±0.1**	12.0±0.2**	12.4±0.1**
<b>Erythrocytes (10<sup>6</sup>/μL)</b>					
Day 25	8.36±0.07	7.42±0.07**	6.90±0.10**	5.93±0.05**	5.15±0.08**
Week 14	8.16±0.07	6.84±0.08**	6.59±0.10**	6.08±0.10**	5.72±0.06**
<b>Reticulocytes (10<sup>6</sup>/μL)</b>					
Day 25	0.18±0.01	0.55±0.02**	0.62±0.03**	0.99±0.05**	1.07±0.04**
Week 14	0.26±0.01	0.50±0.03**	0.54±0.02**	0.90±0.02**	1.11±0.04**
<b>Nucleated erythrocytes/100 leukocytes</b>					
Day 25	0.4±0.2	1.6±0.3**	3.2±0.4**	4.1±0.6**	16.8±1.5**
Week 14	0.7±0.3	1.4±0.3	2.2±0.3**	3.7±0.4**	5.8±0.7**
<b>Mean cell volume (fL)</b>					
Day 25	58.4±0.1	60.5±0.2**	62.9±0.3**	68.7±0.4**	71.9±0.6**
Week 14	55.4±0.2	60.4±0.2**	60.7±0.4**	64.2±0.5**	71.2±0.5**
<b>Mean cell hemoglobin (pg)</b>					
Day 25	18.0±0.1	17.9±0.1	18.5±0.1**	19.8±0.1**	20.9±0.1**
Week 14	18.1±0.0	18.7±0.1**	19.3±0.2**	19.8±0.1**	21.7±0.1**
<b>Mean cell hemoglobin concentration (g/dL)</b>					
Day 25	30.9±0.1	29.5±0.1**	29.4±0.1**	28.8±0.1**	29.0±0.2**
Week 14	32.7±0.1	31.1±0.1**	31.9±0.2**	30.9±0.2**	30.5±0.1**
<b>Methemoglobin (g/dL)</b>					
Day 25	0.37±0.02	0.86±0.07**	1.63±0.05**	1.86±0.05**	1.65±0.03**
Day 88	0.38±0.01	1.49±0.07**	2.20±0.13**	2.49±0.10**	1.75±0.07**
<b>Methemoglobin (% hemoglobin)</b>					
Day 25	2.70±0.15	6.40±0.58**	12.80±0.39**	16.00±0.45**	15.50±0.31**
Day 88	2.88±0.13 <sup>d</sup>	11.20±0.44**	17.22±1.18** <sup>c</sup>	19.70±0.62**	16.00±0.42**
<b>Heinz bodies (% erythrocytes)</b>					
Day 25	0.0±0.0	0.0±0.0	1.5±0.3**	14.4±0.8**	21.2±1.8**
Week 14	0.0±0.0	0.2±0.0**	4.8±0.7**	6.8±0.6**	16.0±1.8**

\* Significantly different (P≤0.05) from the vehicle control group by Dunn's or Shirley's test

\*\* P<0.01

<sup>a</sup> Data are presented as mean ± standard error. Statistical tests were performed on unrounded data. All 1,000 mg/kg rats died before the end of the study; no data are available for these groups.

<sup>b</sup> n=10

<sup>c</sup> n=9

<sup>d</sup> n=8

### **2-years study, rats, haematology group at day 86 (NTP, 2012)**

From ((NTP, 2012), see Table 45): “The haematology findings in this 3-month interim evaluation were consistent with what occurred in the 3-month study. Increases in methaemoglobin and Heinz bodies occurred in the 20 and 60 mg/kg male and female groups. Dose-related decreases occurred in the erythron characterized by decreases in haematocrit values, haemoglobin concentrations, and erythrocyte counts in the 20 and 60 mg/kg male and female groups. The erythron decreases were accompanied by trends toward erythrocyte macrocytosis and hypochromia evidenced by increases in the mean cell volume and decreases in the mean cell haemoglobin concentration values, respectively. Increases in reticulocyte counts demonstrated increased erythropoiesis in response to the decreased erythron. While the magnitudes of the erythron decreases were not sufficient to categorically classify these as anaemias, the patterns of erythron changes were identical to what occurred in the 3-month study. At most, minimally decreased haemoglobin

concentrations (decreased <5 %), increased methaemoglobin values (increased <20 % in males only), and increased Heinz bodies (increased in females only) occurred in the 6 mg/kg groups.”

It should be noted, that for hazard assessment, functional haemoglobin levels were calculated (see Table 48), which show a reduction in functional Hb at 60 mg/kg dosed male and female groups by more than 20 % compared to vehicle control.

Table 45: Haematology data for rats at 3 Months in the 2-year gavage study of *N,N*-Dimethyl-p-toluidine <sup>a</sup>

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
n	10	10	10	10
<b>Male</b>				
Hematocrit (%)	48.8 ± 0.5	48.4 ± 0.4	46.5 ± 0.3**	42.6 ± 0.3**
Hemoglobin (g/dL)	16.0 ± 0.2	15.6 ± 0.1*	14.7 ± 0.1**	13.2 ± 0.1**
Erythrocytes (10 <sup>6</sup> /μL)	9.10 ± 0.10	9.02 ± 0.06	8.53 ± 0.04**	7.61 ± 0.06**
Reticulocytes (10 <sup>6</sup> /μL)	0.25 ± 0.01	0.26 ± 0.01*	0.35 ± 0.01**	0.69 ± 0.02**
Mean cell volume (fL)	53.7 ± 0.2	53.6 ± 0.2	54.5 ± 0.2**	56.0 ± 0.1**
Mean cell hemoglobin (pg)	17.5 ± 0.1	17.3 ± 0.1	17.3 ± 0.1	17.3 ± 0.1
Mean cell hemoglobin concentration (g/dL)	32.7 ± 0.2	32.2 ± 0.2	31.6 ± 0.1**	30.9 ± 0.2**
Platelets (10 <sup>3</sup> /μL)	645.4 ± 27.5	682.6 ± 7.8	721.4 ± 18.4**	722.0 ± 26.0*
Leukocytes (10 <sup>3</sup> /μL)	9.44 ± 0.49	9.91 ± 0.45	9.99 ± 0.51	9.31 ± 0.58
Segmented neutrophils (10 <sup>3</sup> /μL)	1.38 ± 0.09	1.42 ± 0.04	1.42 ± 0.09	1.50 ± 0.05
Lymphocytes (10 <sup>3</sup> /μL)	7.70 ± 0.42	8.10 ± 0.41	8.18 ± 0.41	7.46 ± 0.52
Monocytes (10 <sup>3</sup> /μL)	0.23 ± 0.02	0.26 ± 0.02	0.24 ± 0.02	0.20 ± 0.02
Basophils (10 <sup>3</sup> /μL)	0.062 ± 0.007	0.071 ± 0.006	0.079 ± 0.012	0.075 ± 0.009
Eosinophils (10 <sup>3</sup> /μL)	0.08 ± 0.02	0.07 ± 0.01	0.08 ± 0.01	0.06 ± 0.02
Methemoglobin (g/dL)	0.77 ± 0.04	0.88 ± 0.03*	1.14 ± 0.03**	2.30 ± 0.03**
Methemoglobin (% hemoglobin)	4.70 ± 0.26	5.60 ± 0.22*	7.90 ± 0.18**	17.40 ± 0.22**
Heinz bodies (% erythrocytes)	0.0 ± 0.0	0.1 ± 0.1	0.7 ± 0.2**	3.7 ± 0.3**
<b>Female</b>				
Hematocrit (%)	46.9 ± 0.5	45.8 ± 0.6	44.2 ± 0.6**	41.3 ± 0.6**
Hemoglobin (g/dL)	15.8 ± 0.2	15.1 ± 0.2*	14.4 ± 0.2**	13.2 ± 0.1**
Erythrocytes (10 <sup>6</sup> /μL)	8.50 ± 0.09	8.31 ± 0.10	7.88 ± 0.08**	6.95 ± 0.09**
Reticulocytes (10 <sup>6</sup> /μL)	0.24 ± 0.01	0.24 ± 0.01	0.35 ± 0.01**	0.70 ± 0.02**
Mean cell volume (fL)	55.1 ± 0.2	55.1 ± 0.2	56.1 ± 0.3*	59.4 ± 0.2**
Mean cell hemoglobin (pg)	18.6 ± 0.1	18.2 ± 0.1*	18.3 ± 0.1	19.0 ± 0.1
Mean cell hemoglobin concentration (g/dL)	33.8 ± 0.2	33.1 ± 0.2*	32.6 ± 0.2**	32.0 ± 0.2**
Platelets (10 <sup>3</sup> /μL)	597.4 ± 46.6	583.1 ± 46.9	578.8 ± 49.0	719.3 ± 31.9
Leukocytes (10 <sup>3</sup> /μL)	8.04 ± 0.35	8.65 ± 0.22	8.59 ± 0.56	7.46 ± 0.38
Segmented neutrophils (10 <sup>3</sup> /μL)	1.40 ± 0.10	1.51 ± 0.11	1.52 ± 0.15	0.95 ± 0.11
Lymphocytes (10 <sup>3</sup> /μL)	6.29 ± 0.30	6.76 ± 0.26	6.74 ± 0.44	6.24 ± 0.33
Monocytes (10 <sup>3</sup> /μL)	0.21 ± 0.01	0.24 ± 0.02	0.18 ± 0.02	0.15 ± 0.01*
Basophils (10 <sup>3</sup> /μL)	0.060 ± 0.007	0.054 ± 0.003	0.065 ± 0.009	0.052 ± 0.006
Eosinophils (10 <sup>3</sup> /μL)	0.07 ± 0.01	0.09 ± 0.01	0.09 ± 0.02	0.07 ± 0.03
Methemoglobin (g/dL)	0.80 ± 0.03	0.87 ± 0.03	1.21 ± 0.05**	2.26 ± 0.07**
Methemoglobin (% hemoglobin)	5.10 ± 0.23	5.60 ± 0.27	8.40 ± 0.31**	17.10 ± 0.41**
Heinz bodies (% erythrocytes)	0.0 ± 0.0	0.3 ± 0.2*	0.9 ± 0.3**	3.8 ± 0.2**

\* Significantly different (P<0.05) from the vehicle control group by Dunn's or Shirley's test

\*\* Significantly different (P<0.01) from the vehicle control group by Shirley's test

<sup>a</sup> Data are presented as mean ± standard error. Statistical tests were performed on unrounded data.



**3-month study, mice (NTP, 2012)****Table 46: Incidences of Selected Non-neoplastic Lesions in Mice in the 3-Month Gavage Study ((NTP, 2012))**

	Vehicle Control	15 mg/kg	30 mg/kg	60 mg/kg	125 mg/kg
<b>Male</b>					
Liver <sup>b</sup>	10	10	10	10	10
Hepatocyte,					
Vacuolization Cytoplasmic <sup>c</sup>	9 (2.0) <sup>d</sup>	10 (3.0)	9 (2.6)	10 (2.6)	7 (2.6)
Lung	10	10	10	10	10
Bronchiole, Epithelium, Degeneration	0	0	0	1 (2.0)	10** (2.8)
Bronchiole, Epithelium, Regeneration	0	0	0	1 (2.0)	9** (2.7)
Peribronchiolar, Inflammation, Chronic Active	0	0	0	0	9** (2.2)
Nose	10	10	10	10	10
Glands, Hyperplasia	0	0	0	0	7** (2.0)
Olfactory Epithelium, Degeneration	0	0	0	0	9** (2.9)
Olfactory Epithelium, Metaplasia	0	0	0	0	6** (2.3)
Thymus	10	10	10	10	10
Thymocyte, Necrosis	0	0	0	0	8** (2.0)
<b>Female</b>					
Liver	10	10	10	10	10
Hepatocyte,					
Vacuolization Cytoplasmic	10 (1.0)	10 (2.2)	9 (2.1)	9 (2.3)	8 (2.6)
Lung	10	10	10	10	10
Alveolus, Infiltration Cellular, Histiocyte	0	0	0	0	7** (2.0)
Bronchiole, Epithelium, Degeneration	0	0	0	0	6** (2.5)
Bronchiole, Epithelium, Regeneration	0	0	1 (2.0)	1 (1.0)	7** (3.1)
Peribronchiolar, Inflammation, Chronic Active	0	1 (2.0)	1 (2.0)	1 (2.0)	10** (2.3)
Nose	10	10	10	10	10
Glands, Hyperplasia	0	0	0	0	7** (2.1)
Olfactory Epithelium, Degeneration	0	0	0	5* (1.8)	8** (2.5)
Olfactory Epithelium, Metaplasia	0	0	0	0	4* (2.5)
Thymus	10	10	10	10	10
Thymocyte, Necrosis	0	0	1 (1.0)	0	10** (2.0)

\* Significantly different ( $P \leq 0.05$ ) from the vehicle control group by the Fisher exact test

\*\*  $P \leq 0.01$

<sup>a</sup> Data not shown for 250 mg/kg groups because of mortality during week 1 and week 2.

<sup>b</sup> Number of animals with tissue examined microscopically

<sup>c</sup> Number of animals with lesion

<sup>d</sup> Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

From (NTP, 2012): “*Methaemoglobin values were minimally increased in 30 mg/kg or greater males and females. Heinz bodies demonstrated small increases in 60 mg/kg females, 125 mg/kg males and females, and the lone surviving 250 mg/kg male. In fact, for female mice no erythron changes were detected up to the highest remaining dose (125 mg/kg) and for males, inconsistent and minor decreases in haematocrit values, haemoglobin concentrations, and erythrocyte counts and increased reticulocyte counts occurred in the 60 mg/kg and greater groups (including the lone surviving 250 mg/kg male).*”

The functional haemoglobin levels (see Table 48) were decreased in males and highest dose females, but the reduction in functional Hb in all dosing groups did not exceed 20 % compared to vehicle control.

**Table 47: Selected haematology data for mice in the 3-month gavage study of *N,N*-dimethyl-p-toluidine<sup>a</sup> (NTP, 2012)**

	Vehicle Control	15 mg/kg	30 mg/kg	60 mg/kg	125 mg/kg
<b>Male</b>					
n	10	10	10	10	7
Hematocrit (%)	46.6 ± 0.6	43.7 ± 0.5*	45.4 ± 0.6	43.5 ± 0.5**	44.7 ± 0.5
Hemoglobin (g/dL)	16.4 ± 0.3	15.5 ± 0.2	16.0 ± 0.3	15.0 ± 0.1**	15.3 ± 0.1**
Erythrocytes (10 <sup>6</sup> /μL)	10.82 ± 0.18	10.18 ± 0.14*	10.63 ± 0.15	10.14 ± 0.12*	10.27 ± 0.10
Reticulocytes (10 <sup>6</sup> /μL)	0.25 ± 0.01	0.24 ± 0.01	0.26 ± 0.01	0.27 ± 0.01	0.28 ± 0.01*
Mean cell volume (fL)	43.1 ± 0.2	42.9 ± 0.2	42.8 ± 0.1	42.9 ± 0.2	43.5 ± 0.4
Mean cell hemoglobin (pg)	15.2 ± 0.1	15.2 ± 0.1	15.0 ± 0.2	14.8 ± 0.1*	15.0 ± 0.1
Mean cell hemoglobin concentration (g/dL)	35.3 ± 0.3	35.4 ± 0.3	35.1 ± 0.4	34.5 ± 0.2	34.4 ± 0.3
Methemoglobin (g/dL)	0.35 ± 0.02	0.36 ± 0.02	0.42 ± 0.02*	0.47 ± 0.02**	0.61 ± 0.03**
Methemoglobin (% hemoglobin)	2.10 ± 0.10	2.50 ± 0.17	2.80 ± 0.13**	3.10 ± 0.10**	4.00 ± 0.22**
Heinz bodies (% erythrocytes)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.5 ± 0.1**
<b>Female</b>					
n	10	9	10	10	8
Hematocrit (%)	44.9 ± 0.4	43.8 ± 0.6	45.5 ± 0.6	44.9 ± 0.4	46.4 ± 0.7
Hemoglobin (g/dL)	15.8 ± 0.3	15.5 ± 0.2	16.1 ± 0.2	15.7 ± 0.1	16.1 ± 0.2
Erythrocytes (10 <sup>6</sup> /μL)	10.42 ± 0.11	10.13 ± 0.15	10.57 ± 0.14	10.41 ± 0.07	10.64 ± 0.12
Reticulocytes (10 <sup>6</sup> /μL)	0.26 ± 0.02	0.26 ± 0.02	0.24 ± 0.02	0.24 ± 0.02	0.31 ± 0.02
Mean cell volume (fL)	43.1 ± 0.1	43.2 ± 0.1	43.0 ± 0.1	43.1 ± 0.1	43.6 ± 0.2
Mean cell hemoglobin (pg)	15.1 ± 0.2	15.3 ± 0.1	15.2 ± 0.1	15.1 ± 0.0	15.2 ± 0.0
Mean cell hemoglobin concentration (g/dL)	35.1 ± 0.4	35.4 ± 0.2	35.3 ± 0.2	35.1 ± 0.1	34.8 ± 0.2*
Methemoglobin (g/dL)	0.32 ± 0.01	0.34 ± 0.02	0.43 ± 0.02**	0.53 ± 0.02**	0.58 ± 0.03**
Methemoglobin (% hemoglobin)	2.10 ± 0.10	2.22 ± 0.15	2.60 ± 0.16*	3.40 ± 0.16**	3.88 ± 0.13**
Heinz bodies (% erythrocytes)	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.2 ± 0.1**	0.5 ± 0.1**

\* Significantly different ( $P \leq 0.05$ ) from the vehicle control group by Dunn's or Shirley's test

\*\*  $P \leq 0.01$

<sup>a</sup> Data are presented as mean ± standard error. Statistical tests were performed on unrounded data. No data are presented for the 250 mg/kg groups due to high mortality.

### **Summary: Haematology**

Human repeated dose toxicity data are not available for DMPT.

For rodents, the NTP study report lists 3-month and 2-year studies in rats and mice. Haematology data were obtained after about 4 weeks and 3-months repeated administration on 5 days per week by oral gavage. In particular, methaemoglobin levels were determined at day 25 (3-month study, rats, separate group of animals), day 86 (2-year study, rats) or day 88 (3-month studies, rats and mice). MetHb levels were significantly increased by DMPT in both species, although methaemoglobinaemia associated changes in blood parameters were stronger in rats when compared to mice. In rats at doses relevant for classification, Hb levels were reduced by up to 28 % compared to vehicle controls. The MetHb proportion of total Hb in blood was increased by up to a factor of about 7.4-fold compared to control. The MetHb increase in combination with the decrease of total Hb led to a reduction of functional Hb by up to 33 % (see Table 48). In addition, also the haematocrit and the number of erythrocytes were reduced, whereas Heinz bodies, number of reticulocytes and mean cell volume were increased, which are consistent with methaemoglobinaemia and Heinz body formation, resulting in a macrocytic, hypochromic, responsive anaemia. Similar haematological effects were observed in mice, although the magnitude of changes was lower.

**Table 48: Summary of haemoglobin and methaemoglobin parameters from sub-chronic and chronic NTP studies (NTP, 2012). Values highlighted in bold red are relevant for STOT-RE classification according to the Guidance on the Application of CLP Criteria (European Chemicals Agency, 2017), e.g. reduction in Hb at  $\geq 20\%$  or reduction in functional Hb at  $\geq 20\%$  due to a combination of Hb reduction and MetHb increase at dose levels below 100 mg/kg bw/day in 90-day studies or equivalent. Rows marked in grey are outside relevant doses for STOT RE classification. For easier reading, only mean values without their standard deviations are given in the table.**

species sex study type day	n	dose (mg/kg bw/d)	eq. dose 90-days (mg/kg bw/d) <sup>a</sup>	Hb meas. (g/dL)	Hb (% of control)	MetHb (g/dL)	MetHb (% Hb)	MetHb (% of MetHb fraction in control)	Hb calc. (g/dL) <sup>b</sup>	funct. Hb (g/dL) <sup>c</sup>	funct. Hb (% of control)
rat	10	0.0	0.0	15.3	<b>100.0</b>	0.35	2.40	100.0	14.6	14.2	<b>100.0</b>
male	10	62.5	12.4	13.3**	<b>86.9</b>	0.90**	6.70**	279.2	13.4	12.5	<b>88.1</b>
3-month	10	125.0	24.8	12.5**	<b>81.7</b>	1.56**	12.44**	518.3	12.5	11.0	<b>77.1</b>
day 25	10	250.0	49.6	11.8**	<b>77.1</b>	1.95**	16.60**	691.7	11.7	9.8	<b>68.8</b>
	8	500.0	99.2	11.0**	<b>71.9</b>	1.63**	14.75**	614.6	11.1	9.4	<b>66.2</b>
rat	10	0.0	0.0	14.8 <sup>d</sup>	<b>100.0</b>	0.38	2.44	100.0	15.6	15.2	<b>100.0</b>
male	10	62.5	43.7	13.0 <sup>d</sup> **	<b>87.8</b>	1.37**	10.10**	413.9	13.6	12.2	<b>80.3</b>
3-month	10	125.0	87.3	13.0 <sup>d</sup> **	<b>87.8</b>	1.95**	15.50**	635.2	12.6	10.6	<b>70.0</b>
day 88	10	250.0	174.6	12.9 <sup>d</sup> **	<b>87.2</b>	2.29**	18.20**	745.9	12.6	10.3	<b>67.7</b>
	9	500.0	349.2	12.7 <sup>d</sup> **	<b>85.8</b>	2.03**	17.67**	724.2	11.5	9.5	<b>62.3</b>
rat	10	0.0	0.0	15.1	<b>100.0</b>	0.37	2.70	100.0	13.7	13.3	<b>100.0</b>
female	10	62.5	12.4	13.3**	<b>88.1</b>	0.86**	6.40**	237.0	13.4	12.6	<b>94.3</b>
3-month	10	125.0	24.8	12.8**	<b>84.8</b>	1.63**	12.80**	474.1	12.7	11.1	<b>83.3</b>
day 25	10	250.0	49.6	11.7**	<b>77.5</b>	1.86**	16.00**	592.6	11.6	9.8	<b>73.2</b>
	10	500.0	99.2	10.8**	<b>71.5</b>	1.65**	15.50**	574.1	10.6	9.0	<b>67.5</b>
rat	9	0.0	0.0	14.8 <sup>d</sup>	<b>100.0</b>	0.38	2.88	100.0	13.2	12.8	<b>100.0</b>
female	10	62.5	43.7	12.8 <sup>d</sup> **	<b>86.5</b>	1.49**	11.20**	388.9	13.3	11.8	<b>92.2</b>
3-month	10	125.0	87.3	12.7 <sup>d</sup> **	<b>85.8</b>	2.20**	17.22**	597.9	12.8	10.6	<b>82.5</b>
day 88	10	250.0	174.6	12.0 <sup>d</sup> **	<b>81.1</b>	2.49**	19.70**	684.0	12.6	10.1	<b>79.2</b>
	10	500.0	349.2	12.4 <sup>d</sup> **	<b>83.8</b>	1.75**	16.00**	555.6	10.9	9.2	<b>71.7</b>
rat	10	0.0	0.0	16.0	<b>100.0</b>	0.77	4.70	100.0	16.4	15.6	<b>100.0</b>
male	10	6.0	4.1	15.6*	<b>97.5</b>	0.88*	5.60*	119.1	15.7	14.8	<b>95.0</b>
2-year	10	20.0	13.7	14.7**	<b>91.9</b>	1.14**	7.90**	168.1	14.4	13.3	<b>85.1</b>
day 86	10	60.0	41.0	13.2**	<b>82.5</b>	2.30**	17.40**	370.2	13.2	10.9	<b>69.9</b>
rat	10	0.0	0.0	15.8	<b>100.0</b>	0.80	5.10	100.0	15.7	14.9	<b>100.0</b>
female	10	6.0	4.1	15.1*	<b>95.6</b>	0.87	5.60	109.8	15.5	14.7	<b>98.5</b>
2-year	10	20.0	13.7	14.4**	<b>91.1</b>	1.21**	8.40**	164.7	14.4	13.2	<b>88.6</b>
day 86	10	60.0	41.0	13.2**	<b>83.5</b>	2.26**	17.10**	335.3	13.2	11.0	<b>73.6</b>
mouse	10	0.0	0.0	16.4	<b>100.0</b>	0.35	2.10	100.0	16.7	16.3	<b>100.0</b>
male	10	15.0	10.5	15.5	<b>94.5</b>	0.36	2.50	119.0	14.4	14.0	<b>86.0</b>
3-month	10	30.0	21.0	16.0	<b>97.6</b>	0.42*	2.80**	133.3	15.0	14.6	<b>89.4</b>
day 88	10	60.0	41.9	15.0**	<b>91.5</b>	0.47**	3.10**	147.6	15.2	14.7	<b>90.0</b>
	7	125.0	87.3	15.3**	<b>93.3</b>	0.61**	4.00**	190.5	15.3	14.6	<b>89.7</b>
	1	250.0	174.6	15.7	<b>95.7</b>	0.90	6.00	285.7	15.0	14.1	<b>86.4</b>
mouse	10	0.0	0.0	15.8	<b>100.0</b>	0.32	2.1	100.0	15.2	14.9	<b>100.0</b>
female	9	15.0	10.5	15.5	<b>98.1</b>	0.34	2.2	105.7	15.3	15.0	<b>100.4</b>
3-month	10	30.0	21.0	16.1	<b>101.9</b>	0.43**	2.6*	123.8	16.5	16.1	<b>108.0</b>
day 88	10	60.0	41.9	15.7	<b>99.4</b>	0.53**	3.4**	161.9	15.6	15.1	<b>100.9</b>
	8	125.0	87.3	16.1	<b>101.9</b>	0.58**	3.9**	184.8	14.9	14.4	<b>96.3</b>

\* Significantly different (P<0.05) from the vehicle control group by Dunn's or Shirley's test

\*\* P<0.01

<sup>a</sup> Dosing in studies was 5 days per week, equivalent dose is corrected for 90-day study duration according to Haber's rule using following equation:  $eq. dose = dose * (5 / 7) * (sampling day/90)$

<sup>b</sup>  $Hb calc. = MetHb (g/dl) * 100 / MetHb (\% of Hb)$

<sup>c</sup>  $Functional Hb = Hb (g/dl) - MetHb (g/dl)$

<sup>d</sup> At 14-weeks (~98 days)

**Nasal cavity**

Oral gavage of DMPT in sub-chronic and chronic mouse and rat studies (NTP, 2012) induced dose dependent effects on nasal tissues, e.g. dilatation, hyperplasia, metaplasia, nerve atrophy and necrosis in respiratory epithelia (RE) and olfactory epithelia (OE).

In the 2 year studies, the non-neoplastic effects occurred mainly at high dose (60 mg/kg bw/d), although statistically significant effects are present at 6 mg/kg bw/d and higher (RE hyperplasia / RE glands hyperplasia in male rats and RE glands metaplasia in male and female rats, see Table 28; OE metaplasia in female mice, see Table 37). Chronic exposure at 60 mg/kg bw results additionally in neoplastic lesions of transitional epithelium in the nasal cavity of male rats (without any sign of degeneration/necrosis) (Table 25).

In the 3 month studies, OE degeneration and other effects (e.g. OE/RE metaplasia or hyperplasia) occurred in rats (Table 43) and mice (Table 46) at 125 mg/kg bw/d, statistically significant OE degeneration was observed in female rats at 60 mg/kg bw/d and mice from 62.5 mg/kg bw/d and higher.

The treatment related effects in the nasal tissues are dose dependent, and - notably - are observed after oral gavage. Additional evidence for substance induced alterations of nasal tissue is available from a short-term study (5-days, oral gavage) in male rats (Dunnick et al., 2016).

Conclusively, the nasal cavity appears to be a target organ of DMPT and repeated exposure to DMPT induces effects on nasal tissues such as hyperplasia, metaplasia, and with regard to STOT RE most importantly degeneration, which is considered an adverse effect.

**Table 49 Summary of repeated dose study results (OE degeneration) relevant for classification as STOT-RE (nasal cavity). Values highlighted in bold red are relevant for STOT-RE classification according to (ECHA, 2017), i.e. at dose levels below 100 mg/kg bw/day in 90-day studies or equivalent. Rows marked in grey are above the doses relevant for a STOT RE classification.**

species sex study type sample day	n	dose (mg/kg bw/d)	eq. dose 90- days (mg/kg bw/d) <sup>a</sup>	OE degeneration <sup>b</sup>	(Severity)
rat	10	0.0	0	0	
male	10	<b>62.5</b>	<b>44</b>	<b>5*</b>	<b>(1.0)</b>
3-month	10	<b>125.0</b>	<b>87</b>	<b>10**</b>	<b>(2.5)</b>
day 88	10	250.0	175	10**	(3.0)
	10	500.0	349	10**	(3.1)
rat	10	0.0	0	0	
female	10	<b>62.5</b>	<b>44</b>	<b>7**</b>	<b>(1.3)</b>
3-month	10	<b>125.0</b>	<b>87</b>	<b>10**</b>	<b>(2.1)</b>
day 88	10	250.0	175	10**	(3.0)
	10	500.0	349	10**	(3.0)
mouse	10	0.0	0	0	
male	10	15.0	10	0	
3-month	10	30.0	21	0	
day 88	10	60.0	42	0	
	10	<b>125.0</b>	<b>87</b>	<b>9**</b>	<b>(2.3)</b>
mouse	10	0.0	0	0	
female	10	15.0	10	0	
3-month	10	30.0	21	0	
day 88	10	<b>60.0</b>	<b>42</b>	<b>5*</b>	<b>(1.8)</b>
	10	<b>125.0</b>	<b>87</b>	<b>8**</b>	<b>(2.5)</b>

\* Significantly different (P<0.05) from the vehicle control group by Dunn’s or Shirley’s test

\*\* P<0.01

<sup>a</sup> Dosing in 3-month studies (total 88 days) was 5 days per week, the equivalent dose is corrected for 90-day study duration according to Haber’s rule using following equation:  $eq. \text{ dose} = \text{dose} * (5 / 7) * (88 / 90)$

<sup>b</sup> Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

**Other organs**

Most non-neoplastic lesions in other organs, e.g. inflammation, hyperplasia or necrosis in kidney, liver, thymus and bone marrow, are mild to moderate and potentially secondary to methaemoglobinaemia and/or can be seen as pre-neoplastic lesions already evaluated in chapter 10.9: Carcinogenicity. Therefore those effects on other organs are not considered for a STOT RE classification.

**10.12.1 Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure**

In NTP studies in rats, DMPT induced methaemoglobinaemia with a reduction of total Hb or functional Hb by more than 20 % compared to vehicle controls. The data stem from either 3-month studies at study day 25 or 88 or from 2-year studies at day 86, all studies have been performed by oral gavage using a 5 days per week regimen. The results are summarized in Table 48, and values relevant for classification as STOT RE2 are summarized in Table 50.

Degeneration of the olfactory epithelium occurred in 90-day repeated dose studies in rats and mice statistically significant at doses equivalent to about 40 mg/kg bw/d (rats and female mice) and about 90 mg/kg bw/d (male mice), for a summary of results see Table 49. The number of incidences and/or the severity of the lesions are dose dependent.

**Table 50: Extrapolation of equivalent effective dose for toxicity studies of greater or lesser duration than 90 days.**  
<sup>a</sup>: The length of exposure corresponds to the time point, when blood samples were obtained from rats either at day 25 or at terminal sacrifice in the 3-month study, or at day 86 from a separate clinical control group in the 2-year study. <sup>b</sup>: The extrapolated effective dose was calculated taking into account the length of exposure and the dosing schedule (5 days per week), and has been linearly extrapolated to a 90-day study (see Table 48 for details).

Study reference	Effective dose (mg/kg/d)	Length of exposure (days) <sup>a</sup>	Effective dose when extrapolated to 90-day exposure (mg/kg/d) <sup>b</sup>	Relevant parameter (reduction >20 % compared to control)	Classification supported by the study
(NTP, 2012), rat, male, 3-month study	125	25	24.8	funct. Hb	STOT RE 2
	250	25	49.6	funct. Hb, Hb	STOT RE 2
	500	25	99.2	funct. Hb, Hb	STOT RE 2
(NTP, 2012), rat, male, 3-month study	125	88	87.3	funct. Hb	STOT RE 2
(NTP, 2012), rat, female, 3-month study	250	25	49.6	funct. Hb, Hb	STOT RE 2
	500	25	99.2	funct. Hb, Hb	STOT RE 2
(NTP, 2012), rat, male, 2-year study	60	86	82.5	funct. Hb	STOT RE 2
(NTP, 2012), rat, female, 2-year study	60	86	82.5	funct. Hb	STOT RE 2

**10.12.2 Comparison with the CLP criteria**

The CLP Regulation, Annex I: 3.9.2.1 defines Category 1: “Substances that have produced **significant toxicity** in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following repeated exposure. Substances are classified in Category 1 for target organ toxicity (repeat exposure) on the basis of: reliable and good quality evidence from human cases or epidemiological studies; or observations from appropriate studies in experimental animals in which **significant and/or severe toxic effects**, of relevance to human health, were produced **at generally low exposure concentrations**.”

Category 2: “Substances that, on the basis of evidence from studies in experimental animals can be presumed to have the potential to be harmful to human health following repeated exposure. Substances are classified in category 2 for target organ toxicity (repeat exposure) on the basis of observations from appropriate studies in experimental animals in which **significant toxic effects**, of relevance to human health, were produced at **generally moderate exposure concentrations**.”

No information from human studies is available, which could justify STOT RE Category 1 classification, therefore Category 1 could only be considered, if significant and/or severe toxic effects are observed at generally low exposure in animal studies.

Repeated-dose studies in animals have been performed by the oral (gavage) route in a 5 days per week regimen. Guidance values for a classification as STOT RE, oral, in rats, 90-day are  $\leq 10$  mg/kg bw/d for Category 1 and  $\leq 100$  mg/kg bw/d for Category 2. Equivalent guidance levels can be calculated by linear extrapolation (Haber’s rule).

As indicated in the CLP Regulation, Annex I: 3.9.2.7.3 criteria (c), “any consistent and significant adverse effect in clinical biochemistry, haematology or urinalysis parameters” is sufficient for classification. For haemolytic anaemia, a reduction in Hb at  $\geq 20$  % or a reduction in functional Hb at  $\geq 20$  % due to a combination of Hb reduction and MetHb increase are considered adverse in this respect according to CLP Guidance (ECHA, 2017) and (Muller et al., 2006). Animal studies, which show significant adverse effects below 100 mg/kg bw/d (equivalent to 90 day study) in rats by oral gavage justify classification as STOT RE, Category 2 for blood toxicity. DMPT induces a methaemoglobinaemia with a reduction in haemoglobin and/or functional haemoglobin by equally or more than 20 % compared to controls. Findings in other organs are consistent and presumably secondary to the haemolytic anaemia, e.g. hyperplasia of bone marrow, lesions in kidney, liver and spleen.

In addition, degeneration of olfactory epithelium is a significant adverse effect, which occurred in the 90-day studies summarized above in rats and mice at (equivalent) doses below 100 mg/kg bw/d, but above 40 mg/kg bw/d. Therefore, a classification of DMPT as STOT-RE, Category 2 for the organ “nasal cavity” is justified.

Setting a specific concentration limit (SCL) for DMPT is not indicated, as the SCL is only required for substances with high potency, inducing specific target organ toxicity at dose levels or concentrations clearly below the guidance values according to CLP Annex I, Table 3.9.2, i.e. below 1 mg/kg bw/day adjusted to a 90-day exposure.

### 10.12.3 Conclusion on classification and labelling for STOT RE

Based on

- the reduction in total Hb and/or functional Hb by more than 20 % compared to control animals due to formation of MetHb at equivalent (to 90-day study) effective doses at or below 100 mg/kg bw/d in oral gavage rat studies, and
- the degeneration of the olfactory epithelium at equivalent (to 90-day study) effective doses at or below 100 mg/kg bw/d in oral gavage rat and mouse studies,

a classification as STOT RE, Category 2 (blood system; nasal cavity) is warranted.

No SCL is set, the GCL applies.

The route of exposure should not be stated, because it cannot be conclusively proven that other routes of exposure than oral cannot cause the hazard.

**10.13 Aspiration hazard**

Not assessed for this dossier.

## 11 EVALUATION OF ENVIRONMENTAL HAZARDS

Not assessed for this dossier.

## 12 ADDITIONAL LABELLING

Not assessed for this dossier.

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## 14 ANNEXES

## 14.1 Annex A – Historical control values of NTP 2012 study

## 14.1.1 Historical incidences in control male F344/N rats (NTP, 2012)

Table 51

**Historical Incidence of Hepatocellular Neoplasms in Control Male F344/N Rats<sup>a</sup>**

Study (Study Start)	Adenoma	Carcinoma	Adenoma or Carcinoma
<b>Historical Incidence: Corn Oil Gavage Studies</b>			
<i>N,N</i> -Dimethyl- <i>p</i> -toluidine (October 2004)	0/50	0/50	0/50
<i>Ginkgo biloba</i> extract (March 2005)	0/50	0/50	0/50
Isoeugenol (April 2002)	1/50	0/50	1/50
Kava kava extract (August 2004)	1/49	0/49	1/49
$\beta$ -Myrcene (March 2002)	0/50	0/50	0/50
Pulegone (April 2003)	1/50	0/50	1/50
Total (%)	3/299 (1.0%)	0/299	3/299 (1.0%)
Mean $\pm$ standard deviation	1.0% $\pm$ 1.1%		1.0% $\pm$ 1.1%
Range	0%-2%		0%-2%
<b>Overall Historical Incidence: All Routes</b>			
Total (%)	18/1,249 (1.4%)	5/1,249 (0.4%)	23/1,249 (1.8%)
Mean $\pm$ standard deviation	1.4% $\pm$ 1.9%	0.4% $\pm$ 1.0%	1.8% $\pm$ 1.9%
Range	0%-6%	0%-4%	0%-6%

<sup>a</sup> Data as of May 2011

Table 52

**Historical Incidence of Adenoma of the Nose in Control Male F344/N Rats<sup>a</sup>**

Study (Study Start)	Incidence in Controls
<b>Historical Incidence: Corn Oil Gavage Studies</b>	
<i>N,N</i> -Dimethyl- <i>p</i> -toluidine (October 2004)	0/50
<i>Ginkgo biloba</i> extract (March 2005)	0/50
Isoeugenol (April 2002)	0/50
Kava kava extract (August 2004)	0/49
$\beta$ -Myrcene (March 2002)	0/50
Pulegone (April 2003)	0/50
Total	0/299
<b>Overall Historical Incidence: All Routes</b>	
Total	0/1,248

<sup>a</sup> Data as of May 2011

Table 53

**Historical Incidence of Follicular Cell Neoplasms of the Thyroid Gland in Control Male F344/N Rats<sup>a</sup>**

Study (Study Start)	Adenoma	Carcinoma	Adenoma or Carcinoma
<b>Historical Incidence: Corn Oil Gavage Studies</b>			
<i>N,N</i> -Dimethyl- <i>p</i> -toluidine (October 2004)	1/50	0/50	1/50
<i>Ginkgo biloba</i> extract (March 2005)	2/50	0/50	2/50
Isoeugenol (April 2002)	1/50	1/50	2/50
Kava kava extract (August 2004)	1/49	0/49	1/49
$\beta$ -Myrcene (March 2002)	1/50	2/50	3/50
Pulegone (April 2003)	0/50	0/50	0/50
Total (%)	6/299 (2.0%)	3/299 (1.0%)	9/299 (3.0%)
Mean $\pm$ standard deviation	2.0% $\pm$ 1.3%	1.0% $\pm$ 1.7%	3.0% $\pm$ 2.1%
Range	0%-4%	0%-4%	0%-6%
<b>Overall Historical Incidence: All Routes</b>			
Total (%)	13/1,239 (1.1%)	10/1,239 (0.8%)	23/1,239 (1.9%)
Mean $\pm$ standard deviation	1.0% $\pm$ 1.7%	0.8% $\pm$ 1.5%	1.9% $\pm$ 2.2%
Range	0%-6%	0%-4%	0%-6%

<sup>a</sup> Data as of May 2011

## 14.1.2 Historical incidences in control female F344/N rats (NTP, 2012)

Table 54

**Historical Incidence of Hepatocellular Neoplasms in Control Female F344/N Rats<sup>a</sup>**

Study (Study Start)	Adenoma	Carcinoma	Adenoma or Carcinoma
<b>Historical Incidence: Corn Oil Gavage Studies</b>			
<i>N,N</i> -Dimethyl- <i>p</i> -toluidine (October 2004)	0/50	0/50	0/50
<i>Ginkgo biloba</i> extract (March 2005)	0/50	0/50	0/50
Isoeugenol (April 2002)	0/50	0/50	0/50
Kava kava extract (August 2004)	0/50	0/50	0/50
$\beta$ -Myrcene (March 2002)	0/50	0/50	0/50
Pulegone (April 2003)	1/50	0/50	1/50
Total (%)	1/300 (0.3%)	0/300	1/300 (0.3%)
Mean $\pm$ standard deviation	0.3% $\pm$ 0.8%		0.3% $\pm$ 0.8%
Range	0%-2%		0%-2%
<b>Overall Historical Incidence: All Routes</b>			
Total (%)	11/1,200 (0.9%)	1/1,200 (0.1%)	12/1,200 (1.0%)
Mean $\pm$ standard deviation	0.9% $\pm$ 1.6%	0.1% $\pm$ 0.4%	1.0% $\pm$ 1.6%
Range	0%-4%	0%-2%	0%-4%

<sup>a</sup> Data as of May 2011

Table 55

**Historical Incidence of Adenoma of the Nose in Control Female F344/N Rats<sup>a</sup>**

<b>Study (Study Start)</b>	<b>Incidence in Controls</b>
<b>Historical Incidence: Corn Oil Gavage Studies</b>	
<i>N,N</i> -Dimethyl- <i>p</i> -toluidine (October 2004)	0/50
<i>Ginkgo biloba</i> extract (March 2005)	0/49
Isoeugenol (April 2002)	0/50
Kava kava extract (August 2004)	0/50
$\beta$ -Myrcene (March 2002)	0/50
Pulegone (April 2003)	0/50
Total	0/299
<b>Overall Historical Incidence: All Routes</b>	
Total (%)	1/1,196 (0.1%)
Mean $\pm$ standard deviation	0.1% $\pm$ 0.4%
Range	0%-2%

<sup>a</sup> Data as of May 2011

## 14.1.3 Historical incidences in control male B6C3F1/N mice (NTP, 2012)

Table 56

**Historical Incidence of Liver Neoplasms in Control Male B6C3F1/N Mice<sup>a</sup>**

Study (Study Start)	Hepatocellular Adenoma	Hepatocellular Carcinoma	Hepatocellular Adenoma or Hepatocellular Carcinoma
<b>Historical Incidence: Corn Oil Gavage Studies</b>			
<i>N,N</i> -Dimethyl- <i>p</i> -toluidine (October 2004)	29/50	22/50	38/50
<i>Ginkgo biloba</i> extract (March 2005)	31/50	22/50	39/50
Isoeugenol (May 2002)	24/50	8/50	28/50
Kava kava extract (August 2004)	27/50	20/50	38/50
$\beta$ -Myrcene (April 2002)	26/50	14/50	33/50
Pulegone (April 2003)	22/50	13/50	29/50
3,3',4,4'-Tetrachloroazobenzene (February 2003)	22/50	17/50	34/50
Total (%)	181/350 (51.7%)	116/350 (33.1%)	239/350 (68.3%)
Mean $\pm$ standard deviation	51.7% $\pm$ 6.9%	33.1% $\pm$ 10.5%	68.3% $\pm$ 8.9%
Range	44%-62%	16%-44%	56%-78%
<b>Overall Historical Incidence: All Routes</b>			
Total (%)	658/1,149 (57.3%)	399/1,149 (34.7%)	844/1,149 (73.5%)
Mean $\pm$ standard deviation	57.3% $\pm$ 12.6%	34.7% $\pm$ 10.8%	73.5% $\pm$ 11.3%
Range	24%-78%	16%-56%	52%-90%
	<b>Hepatoblastoma</b>	<b>Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma</b>	
<b>Historical Incidence: Corn Oil Gavage Studies</b>			
<i>N,N</i> -Dimethyl- <i>p</i> -toluidine (October 2004)	1/50	38/50	
<i>Ginkgo biloba</i> extract (March 2005)	3/50	39/50	
Isoeugenol (May 2002)	3/50	30/50	
Kava kava extract (August 2004)	0/50	38/50	
$\beta$ -Myrcene (April 2002)	4/50	34/50	
Pulegone (April 2003)	1/50	29/50	
3,3',4,4'-Tetrachloroazobenzene (February 2003)	2/50	34/50	
Total (%)	14/350 (4.0%)	242/350 (69.1%)	
Mean $\pm$ standard deviation	4.0% $\pm$ 2.8%	69.1% $\pm$ 8.0%	
Range	0%-8%	58%-78%	
<b>Overall Historical Incidence: All Routes</b>			
Total (%)	61/1,149 (5.3%)	852/1,149 (74.2%)	
Mean $\pm$ standard deviation	5.3% $\pm$ 7.1%	74.2% $\pm$ 11.5%	
Range	0%-34%	52%-92%	

<sup>a</sup> Data as of May 2011

Table 57

**Historical Incidence of Alveolar/bronchiolar Neoplasms in Control Male B6C3F1/N Mice<sup>a</sup>**

Study (Study Start)	Adenoma	Carcinoma	Adenoma or Carcinoma
<b>Historical Incidence: Corn Oil Gavage Studies</b>			
<i>N,N</i> -Dimethyl- <i>p</i> -toluidine (October 2004)	11/50	2/50	13/50
<i>Ginkgo biloba</i> extract (March 2005)	8/50	11/50	17/50
Isoeugenol (May 2002)	6/50	2/50	7/50
Kava kava extract (August 2004)	9/50	2/50	11/50
$\beta$ -Myrcene (April 2002)	8/50	5/50	13/50
Pulegone (April 2003)	6/50	3/50	9/50
3,3',4,4'-Tetrachloroazobenzene (February 2003)	5/50	3/50	7/50
Total (%)	53/350 (15.1%)	28/350 (8.0%)	77/350 (22.0%)
Mean $\pm$ standard deviation	15.1% $\pm$ 4.1%	8.0% $\pm$ 6.5%	22.0% $\pm$ 7.3%
Range	10%-22%	4%-22%	14%-34%
<b>Overall Historical Incidence: All Routes</b>			
Total (%)	172/1,150 (15.0%)	144/1,150 (12.5%)	301/1,150 (26.2%)
Mean $\pm$ standard deviation	15.0% $\pm$ 6.9%	12.5% $\pm$ 7.1%	26.2% $\pm$ 6.3%
Range	2%-30%	4%-24%	14%-40%

<sup>a</sup> Data as of May 2011

## 14.1.4 Historical incidences in control female B6C3F1/N mice (NTP, 2012)

Table 58

**Historical Incidence of Liver Neoplasms in Control Female B6C3F1/N Mice<sup>a</sup>**

Study (Study Start)	Hepatocellular Adenoma	Hepatocellular Carcinoma	Hepatocellular Adenoma or Hepatocellular Carcinoma
<b>Historical Incidence: Corn Oil Gavage Studies</b>			
<i>N,N</i> -Dimethyl- <i>p</i> -toluidine (October 2004)	17/50	6/50	20/50
<i>Ginkgo biloba</i> extract (March 2005)	17/50	9/50	20/50
Isoeugenol (May 2002)	11/49	3/49	13/49
Kava kava extract (August 2004)	8/50	3/50	10/50
$\beta$ -Myrcene (April 2002)	6/50	1/50	7/50
Pulegone (April 2003)	13/49	5/49	17/49
3,3',4,4'-Tetrachloroazobenzene (February 2003)	3/49	2/49	4/49
Total (%)	75/347 (21.6%)	29/347 (8.4%)	91/347 (26.2%)
Mean $\pm$ standard deviation	21.6% $\pm$ 10.8%	8.3% $\pm$ 5.5%	26.2% $\pm$ 12.7%
Range	6%-34%	2%-18%	8%-40%
<b>Overall Historical Incidence: All Routes</b>			
Total (%)	380/1,195 (31.8%)	144/1,195 (12.1%)	444/1,195 (37.2%)
Mean $\pm$ standard deviation	31.8% $\pm$ 21.4%	12.1% $\pm$ 10.8%	37.2% $\pm$ 22.9%
Range	2%-78%	0%-46%	6%-82%
	<b>Hepatoblastoma</b>	<b>Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma</b>	
<b>Historical Incidence: Corn Oil Gavage Studies</b>			
<i>N,N</i> -Dimethyl- <i>p</i> -toluidine (October 2004)	0/50	20/50	
<i>Ginkgo biloba</i> extract (March 2005)	1/50	20/50	
Isoeugenol (May 2002)	0/49	13/49	
Kava kava extract (August 2004)	0/50	10/50	
$\beta$ -Myrcene (April 2002)	0/50	7/50	
Pulegone (April 2003)	0/49	17/49	
3,3',4,4'-Tetrachloroazobenzene (February 2003)	0/49	4/49	
Total (%)	1/347 (0.3%)	91/347 (26.2%)	
Mean $\pm$ standard deviation	0.3% $\pm$ 0.8%	26.2% $\pm$ 12.7%	
Range	0%-2%	8%-40%	
<b>Overall Historical Incidence: All Routes</b>			
Total (%)	4/1,195 (0.3%)	444/1,195 (37.2%)	
Mean $\pm$ standard deviation	0.3% $\pm$ 0.8%	37.2% $\pm$ 22.9%	
Range	0%-2%	6%-82%	

<sup>a</sup> Data as of May 2011

Table 59

**Historical Incidence of Alveolar/bronchiolar Neoplasms in Control Female B6C3F1/N Mice<sup>a</sup>**

Study (Study Start)	Adenoma	Carcinoma	Adenoma or Carcinoma
<b>Historical Incidence: Corn Oil Gavage Studies</b>			
<i>N,N</i> -Dimethyl- <i>p</i> -toluidine (October 2004)	2/50	0/50	2/50
<i>Ginkgo biloba</i> extract (March 2005)	0/50	1/50	1/50
Isoeugenol (May 2002)	4/48	0/48	4/48
Kava kava extract (August 2004)	2/50	2/50	4/50
$\beta$ -Myrcene (April 2002)	4/50	2/50	6/50
Pulegone (April 2003)	1/49	2/49	3/49
3,3',4,4'-Tetrachloroazobenzene (February 2003)	3/49	0/49	3/49
Total (%)	16/346 (4.6%)	7/346 (2.0%)	23/346 (6.7%)
Mean $\pm$ standard deviation	4.6% $\pm$ 3.1%	2.0% $\pm$ 2.0%	6.7% $\pm$ 3.2%
Range	0%-8%	0%-4%	2%-12%
<b>Overall Historical Incidence: All Routes</b>			
Total (%)	60/1,196 (5.0%)	44/1,196 (3.7%)	100/1,196 (8.4%)
Mean $\pm$ standard deviation	5.0% $\pm$ 3.6%	3.7% $\pm$ 3.3%	8.4% $\pm$ 4.3%
Range	0%-12%	0%-14%	2%-22%

<sup>a</sup> Data as of May 2011

Table 60

**Historical Incidence of Squamous Cell Neoplasms of the Forestomach in Control Female B6C3F1/N Mice<sup>a</sup>**

Study (Study Start)	Papilloma	Carcinoma	Papilloma or Carcinoma
<b>Historical Incidence: Corn Oil Gavage Studies</b>			
<i>N,N</i> -Dimethyl- <i>p</i> -toluidine (October 2004)	1/50	0/50	1/50
<i>Ginkgo biloba</i> extract (March 2005)	2/50	0/50	2/50
Isoeugenol (May 2002)	1/49	0/49	1/49
Kava kava extract (August 2004)	3/50	0/50	3/50
$\beta$ -Myrcene (April 2002)	1/50	0/50	1/50
Pulegone (April 2003)	2/49	0/49	2/49
3,3',4,4'-Tetrachloroazobenzene (February 2003)	2/50	0/50	2/50
Total (%)	12/348 (3.5%)	0/348	12/348 (3.5%)
Mean $\pm$ standard deviation	3.5% $\pm$ 1.5%		3.5% $\pm$ 1.5%
Range	2%-6%		2%-6%
<b>Overall Historical Incidence: All Routes</b>			
Total (%)	22/1,198 (1.8%)	1/1,198 (0.1%)	23/1,198 (1.9%)
Mean $\pm$ standard deviation	1.8% $\pm$ 1.7%	0.1% $\pm$ 0.4%	1.9% $\pm$ 1.6%
Range	0%-6%	0%-2%	0%-6%

<sup>a</sup> Data as of May 2011



## 15 ABBREVIATIONS

DMA	dimethyl aniline
DMPT	<i>N,N</i> -dimethyl-p-toluidine
GEF	global evaluation factor
MF	mutant frequency
GLP	Good Laboratory Praxis
OECD	Organization for Economic Cooperation and Development
TG	test guideline