Committee for Risk Assessment

RAC

Annex 1
Background document
to the Opinion proposing harmonised classification and labelling at EU level of

N,N-dimethyl-*p*-toluidine

EC Number: 202-805-4
CAS Number: 99-97-8

CLH-O-0000007005-83-01/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

10 June 2021

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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Version number: **3.0**

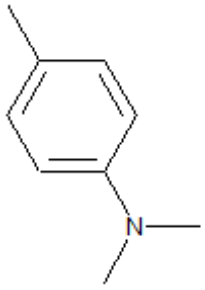
Date: **May 2020**

CONTENTS

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

Name(s) in the IUPAC nomenclature or other international chemical name(s)	*N,N-Dimethyl-p-toluidine*
Other names (usual name, trade name, abbreviation)	Benzenamine, N,N,4-trimethyl- N,N,4-trimethylaniline DMPT 4,*N*,*N*-Trimethylaniline 4-Dimethylaminotoluene
EC number (if available and appropriate)	202-805-4
EC name (if available and appropriate)	*N,N*-Dimethyl-p-toluidine
CAS number (if available)	99-97-8
Molecular formula	C9H13N
Structural formula	
SMILES notation (if available)	N(C)(C)c1ccc(C)cc1
Molecular weight or molecular weight range	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)
N,N-Dimethyl-p-toluidine CAS-No.: 99-97-8	100 %	• Acute Tox. 3*; H301 • Acute Tox. 3*; H311 • Acute Tox. 3*; H331 • STOT RE 2*; H373** • Aquatic Chronic 3; H412	• Acute Tox. 2 (inhalation) • STOT RE 2 (e.g. oral and inhalation; reproductive, mouth, pharynx) • Carc. 1B • STOT SE 1 (blood) • Skin Irrit. 2 • Eye Irrit. 2 • Aquatic Chronic 1

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	*N,N*-dimethyl-*p*-toluidine [1] *N,N*-dimethyl-*m*-toluidine [2] *N,N*-dimethyl-*o*-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	*N,N*-dimethyl-*p*-toluidine	202-805-4	99-97-8	**Retain** Aquatic Chronic 3 **Add** Carc. 2 **Modify** Acute Tox. 4 Acute Tox. 3 STOT RE 2 **Remove** Acute Tox. 3	**Retain** H412 **Add** H351 **Modify** H332 H301 H373 (blood; nasal cavity) **Remove** H311	**Retain** GHS06 GHS08 Dgr	**Retain** H412 **Add** H351 **Modify** H332 H301 H373 (blood; nasal cavity) **Remove** H311		**Add** Oral: ATE = 139 mg/kg bw Inhalation: ATE = 1,4 mg/L (mists) **Remove** *	**Remove** 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	H351 H332 H301 H373 (blood; nasal cavity) H412		Oral: ATE = 139 mg/kg bw Inhalation: ATE = 1,4 mg/L (mists)	

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*-isoforms 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).

RAC general comment

N,N-dimethyl-*p*-toluidine (DMPT) is used as a polymerization catalyst in the production of polyesters, polyacrylates and epoxy resins. It can also be used as a hardener in dental fillings and adhesives. Furthermore, the substance is used as a transition agent in photographic chemicals, dyes and medicines.

The existing Annex VI entry (Index No. 612-056-00-9) is for three substances together: *N,N*-dimethyl-*p*-toluidine [1] 99-97-8 [1], *N,N*-dimethyl-*m*-toluidine [2] 121-72-2 [2], and *N,N*-dimethyl-*o*-toluidine [3] 609-72-3 [3].

The current proposal is for *N,N*-dimethyl-*p*-toluidine (CAS nr 99-97-8) on its own. Data, especially the NTP study data, are only available for the para isomer of the substance. Ortho-, meta- and para-substituted substances can have quite different toxicological properties and/or potency. Therefore, a simple read-across to meta- and ortho-isomers is not possible. The dossier submitter (DS) is not aware of data that would support a read-across to the other isomers.

As a result of this CLH proposal, the current group entry (Index No.612-056-00-9) can be modified and a new entry for the sole DMPT created.

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)
Physical state at 20 °C and 101.3 kPa	brown coloured organic liquid having unpleasant odour	REACH registration dossier	Physical observation
Melting/freezing point	- 15 °C	GESTIS - Substance Database	
Boiling point	211.2 °C at 965 hPa	REACH registration dossier	measured, distillation method
Relative density	0.88 g/cm³ at 35 °C	REACH registration dossier	measured, mass by volume method
Vapour pressure	0.07501 mmHg at 20 °C	GESTIS - Substance Database	

Property	Value	Reference	Comment (e.g. measured or estimated)
Surface tension	33.97 mN/m	Chemspider - ACD/PhysChem Suite	Estimated
Water solubility	650 mg/L at 37 °C	GESTIS - Substance Database	
Partition coefficient n-octanol/water	1.729 at 35 °C, pH = 5.6	REACH registration dossier	measured, shake flask method
Granulometry			*N,N*-dimethyl-p-toluidine is a liquid
Stability in organic solvents and identity of relevant degradation products	*N,N*-dimethyl-p-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	
Dissociation constant	0.05497 (average pKa value) at 35 °C	REACH registration dossier	measured
Viscosity	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
Disposition study with radioactive labelled DMPT *In vivo* Distribution of radioactivity in urine, faeces, VOCs and tissues determined 24 hours after dosing. **Fischer 344 rats** 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 4 male animals/dose	[^{14}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.	Considered reliable with restrictions. Not performed according to GLP or test guideline. [^{14}C] *N,N*-Dimethyl-p-toluidine (CAS-No: 99-97-8) (Purity: 97.4 %)	(Dix et al., 2007)

Method	Results	Remarks	Reference
Additional 4 female animals at 25 mg/kg bw oral **B6C3F1 mice** 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 4 male animals/dose Additional 4 female animals at 25 mg/kg bw oral	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. 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. 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.	(Specific activity: 25.3 mCi/mol) The study was partly performed in the presence of an impurity or breakdown product of DMPT (i.e. N-methyl-p-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.	
Identification of urinary metabolites Analytical reversed-phase high performance liquid chromatography (HPLC), spectrometric and spectroscopic methods **Fischer 344 rats** • oral gavage of [^{14}C]DMPT (250 mg/kg) in 10 % aqueous PEG-30 castor oil, 4 male rats per dose • collection-interval composite yields (from 6, 12, 24, 48, and 72 h) of 4 male rats (10 % by weight of the total urinary output).	Four radiolabelled peaks were observed, isolated, and purified by solid-phase extraction (SPE) and preparative HPLC. The peaks were identified as p-(N-acetylhydroxyamino)-hippuric acid (M1), DMPT N-oxide (M2), N-methyl-p-toluidine (M3), and parent DMPT. DMPT metabolism is similar to that reported for *N,N*-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.	Considered reliable with restrictions Not performed according to GLP or test guideline **Test material:** [^{14}C] *N,N*-Dimethyl-p-toluidine (CAS-No: 99-97-8) (Purity: 97.4 %) (Specific activity: 25.3 mCi/mol)	(Kim et al., 2007a)

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 [^{14}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 [^{14}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 [^{14}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 methaemo-globinaemia is p-methylphenylhydroxylamine. In (Kim et al., 2007a), p-(N-acetylhydroxylamino)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), [^{14}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 [^{14}C]DMPT to male and female Rats (from (Dix et al., 2007)).

			Percent administered dose[a]					
Dose group[b]	Gender	*n*	Urine	Feces	VOCs	Tissues[c]	GI tract	Total
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)

[a]Mean (SD).
[b]Target dose; actual doses are provided in Table 1.
[c]Includes GI tract.

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

			Percent administered dose[a]					
Dose group[b]	Gender	*n*	Urine	Feces	VOCs	Tissues[c]	GI tract	Total
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)

[a]Mean (SD).
[b]Target dose; actual doses are provided in Table 1.
[c]Includes GI tract.

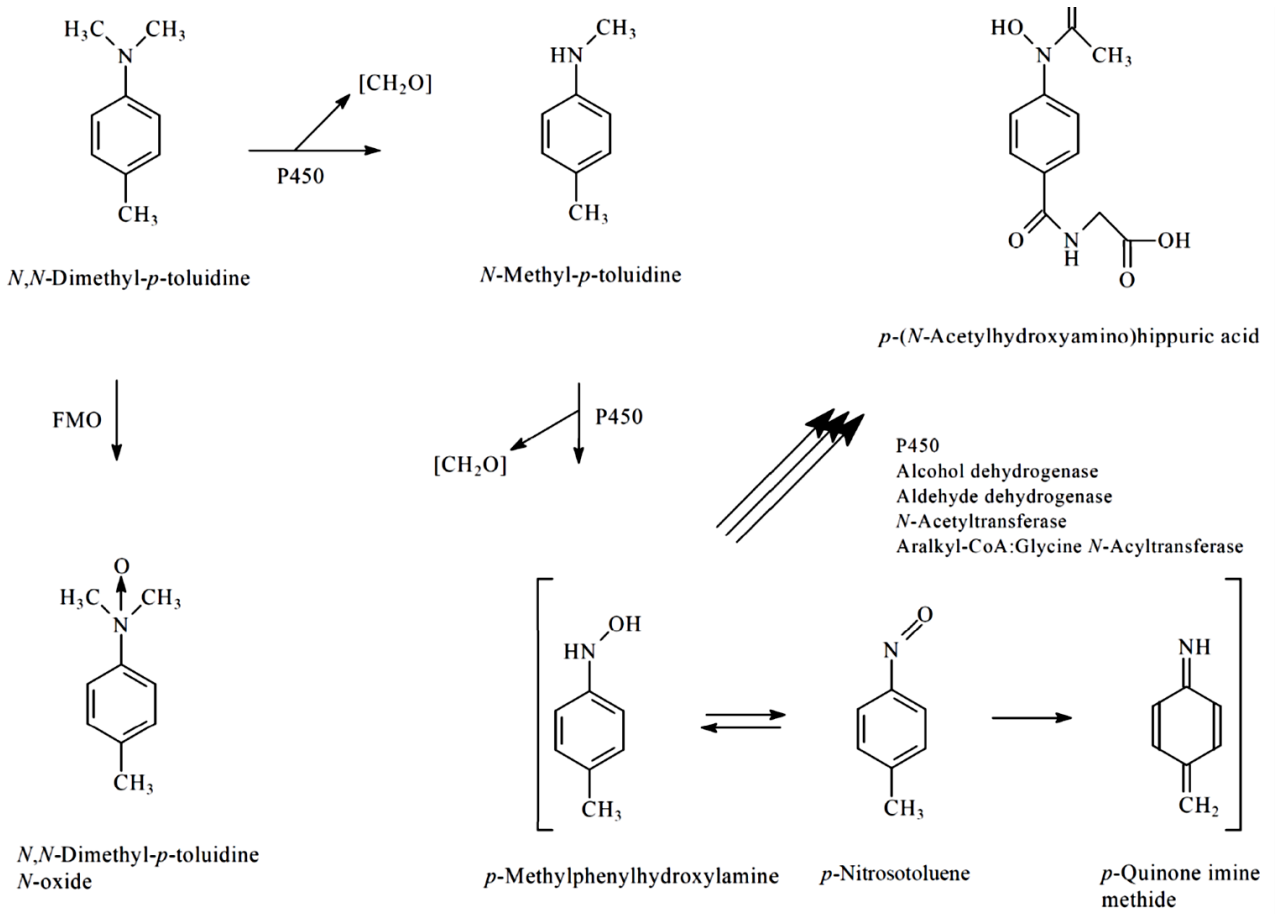


Figure 1 Observed DMPT metabolites including some proposed reactive intermediates of DMPT (*N,N*-Dimethyl-p-toluidine, CAS No. 99-98-7), (Kim et al., 2007b) and (Dunnick et al., 2014). FMO: Flavin-containing monooxygenase; P450: cytochrome P450. From (IARC, 2016)

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_{50}	Reference
LD50-Test Database entry, study details not available According to OECD 401 **Reliability not assignable**	**Rat** (Sprague-Dawley) Males and females No information on animal number	*N,N*-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)
LD50-Test Database entry, study details not available **Reliability not assignable**	**Mouse** No information on sex, strain and animal number	*N,N*-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)
LD50-Test Database entry, study details not available **Reliability not assignable**	**Rat** No information on sex, strain and animal number	*N,N*-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)
Disposition study with radioactive labelled DMPT (see Table 9) No guideline study, single oral gavage **Reliable with restrictions**	**Mouse** Male B6C3F1 mice 4 animals per dose	*N,N*-dimethyl-p-toluidine ([14C]-DMPT, CAS: 99-97-8) (purity: 97.4 %)	2.5, 25, and 250 mg [14C]-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)

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Value LD_{50}	Reference
3 month oral gavage study No guideline (NTP internal standards) **Reliable with restrictions**	**Mouse** B6C3F1/N mice, male and female, 10 mice per sex and dose	*N,N*-dimethyl-p-toluidine (CAS: 99-97-8) (purity >99 %)	0, 15, 30, 60, 125, and 250 mg/kg bw/day	250 mg/kg bw/day: 10/10 males and 9/10 females died within 10 days of dosing. 125 mg/kg bw/day: 2/10 males and 1/10 females died within the first 2 weeks of dosing. No data on mortality is available covering the first 72 hours.	(NTP, 2012)
3 month oral gavage study No guideline (NTP internal standards) **Reliable with restrictions**	**Rat** F344 rats, male and female, 10 rats per sex and dose	*N,N*-dimethyl-p-toluidine (CAS: 99-97-8) (purity >99 %)	0, 62.5, 125, 250, 500, 1000 mg/kg bw/day	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. 500 mg/kg bw/day: 1/10 male rats dead by study day 3.	(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
Single case report **Reliability not assignable**	Fingernail solution containing *N,N*-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)
Single case report **Reliability not assignable**	Fingernail solution containing *N,N*-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 *N,N*-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
i.v. injection No guideline study **Not reliable**	*N,N*-dimethyl-p-toluidine (CAS: 99-97-8) (purity not available)	**Mice** (SPF-NMRl) i.v. injection, n=10 per dose, 5 doses between about 50 to 100 mg/kg bw	LD_{50}: 75,8 mg/kg bw	(Liso et al., 1997)
i.p. injection **Reliability not assignable**	*N,N*-dimethyl-p-toluidine (CAS: 99-97-8) (purity not available)	**Mice** i.p. injection, no study details available	LD_{50}: 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_{50} 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_{50} of 1650 mg/kg bw from an OECD test guideline 401 conform study is listed ((ACToR, 2015)). Another entry reports an LD_{50} 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_{50} for mice would be >125 mg/kg bw for the oral uptake route. For rats, the LD_{50} 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_{50} 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_{50} 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_{50} or ATE value available, i.e. the lowest LD_{50} or ATE in the most sensitive appropriate species tested. Based on these criteria, LD_{50} 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_{50} 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_{50} 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_{50}/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_{50}	Reference
LD_{50}-Test **According to OECD 402** Database entry, study details not available **Reliability not assignable**	**Rabbit** (New Zealand White Males and females No information on animal numbers	*N,N*-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_{50} 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_{50} 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_{50} 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_{50}	Reference
Database entry, study details not available GLP conform (TSCA 40CFR 798.1150) **Reliability not assignable**	**Rat** (Sprague-Dawley) Males and females, n=10	*N,N*-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 **Reliability not assignable**	**Mouse** No information on sex, strain and animal number	*N,N*-dimethyl-p-toluidine (CAS: 99-97-8) (purity unknown)	unknown	LC_{50} 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)
LC_{50} study Database entry, study details not available No guideline **Reliability not assignable**	**Mouse** No information on sex, strain and animal number	*N,N*-dimethyl-p-toluidine (CAS: 99-97-8) (purity unknown)	unknown	LC_{50} 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_{50} 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_{50} 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_{50} well below the SVC is considered for classification according to the criteria for vapours; whereas an LC_{50} 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:

SVC [mg/l] = 0.0412 x MW x 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_{50} 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_{50} 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_{50} 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.

RAC evaluation of acute toxicity

ACUTE ORAL TOXICITY

Summary of the Dossier Submitter's proposal

Three oral acute toxicity studies (without detailed information) with DMPT are available, two with rats, one with mice. They provided LD_{50} values of 1650, 980, and 139 mg/kg bw, respectively.

Supporting information is provided by a toxicokinetic study with mice, which were dosed with 2.5, 25 or 250 mg/kg bw. No overt signs of toxicity were reported for the two lower doses; at the higher dose 1 mouse out of 4 was found dead and the other 3 were moribund. Further information is provided by two 3-month oral gavage studies (NTP, 2012) with rats and mice. At the highest dose of 250 mg/kg bw/day 10/10 male and 9/10 female mice died within 10 days of dosing; in the 125 mg/kg bw/day group 2/10 males and 1/10 females died. The DS concluded that an LD_{50} for mice would be >125 mg/kg bw and for rats, the LD_{50} would be expected between 500 and 1000 mg/kg.

The DS also reported on two human cases of accidental oral administration to DMPT in a fingernail solution. The first case report is on a 5-month old boy drinking 30 mL of artificial fingernail solution resulting in methaemoglobinaemia. The other case report is about a 16-month old girl ingesting about 6 mg/kg bw, resulting in an acute cyanotic episode due to methaemoglobinaemia. MetHb was 43% compared to the normal value of <2%.

The lowest LD_{50} of 139 mg/kg bw compared to the criteria (Category 3: $50<LD_{50}\leq300$) leads to a proposal for classification in Category 3 with an ATE of 139 mg/kg bw, supported by other studies.

Comments received during consultation

One Member state competent authority (MSCA) commented during the consultation. The MSCA agreed with the proposed classification as Acute. Tox. 3. However, considering that no detailed information is available on the studies, the ATE of 139 mg/kg bw was questioned, and a generic ATE of 100 mg/kg bw was proposed.

The DS agreed that indeed the reliability of the LD_{50} data is not assignable. However, the LD_{50} of 139 mg/kg bw listed in RTECS and used to set the ATE falls into the range of estimated toxicity values from a 3-month study in mice (NTP, 2012). Therefore, 139 mg/kg bw is considered reasonable.

Assessment and comparison with the classification criteria

Three acute toxicity studies available reported LD_{50} values of 1650 and 980 mg/kg bw in rats, and 139 mg/kg bw in mice. The species difference is also seen in the mortalities in the 3-month studies (NTP, 2012) in rats and mice.

The LD_{50} of 139 mg/kg bw from the mouse study would result in a classification as Acute Tox. 3 ($50< LD_{50} \leq300$ mg/kg bw). Although no study details are available, the LD_{50} value is supported by information from 1 mouse 3-month study (at 125 mg/kg bw – 2/10 males and 2/10 females died, and at 250 mg/kg bw 9/10 and 10/10 females died within 2 weeks of study).

The lowest LD_{50} value of 139 mg/kg bw results in a (rounded off) ATE of 140 mg/kg bw.

RAC concludes that DMPT meets the criteria ($50<ATE\leq300$ mg/kg bw) and should be classified as **Acute Tox. 3; H301 with an ATE of 140 mg/kg bw**.

ACUTE DERMAL TOXICITY

Summary of the Dossier Submitter's proposal

A single study summary for acute dermal toxicity in rabbits is available from the US EPA database, which lists a dermal LD_{50} 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.

The LD_{50} value of >2000 mg/kg bw leads according to the CLP classification criteria to no classification.

Comments received during consultation

One MSCA commented during the consultation. This MSCA agreed on no classification, based on the presented study. The MSCA asked if the rationale of the existing harmonised classification as Acute Tox 3* - H311 for the grouping entry is known.

The DS reacted by stating that the basis for the existing classification is not known.

Assessment and comparison with the classification criteria

Only one LD_{50} study with rabbits is available, without detailed information, with an LD_{50} value of >2000 mg/kg bw. This leads to no classification.

RAC concludes that **no classification for DMPT for dermal acute toxicity is warranted and <u>the existing classification should be removed</u>**.

ACUTE INHALATION TOXICITY

Summary of the Dossier Submitter's proposal

Detailed study reports for acute toxicity by inhalation are not available. Database (ACToR, 2015; RTECS, 2012) records report an LC_{50} value for rats of 1.4 mg/L and LOAEC values for mice of 0.8 mg/L and 3.192 mg/L based on adverse effects in the respiratory system. The rat study is listed as a 4-hour exposure study with conformity to GLP. The reliability of these studies cannot be assigned because the study details are not available.

With regard to the differentiation between "vapours" and "dusts and mists" on the basis of the saturated vapour concentration (SVC) for a volatile substance, the DS estimated an SVC as follows: 0.0412 x MW (135.206) x vapour pressure (0.1 hPa at 20 °C) = 0.557 mg/L. The LC_{50} is above the SVC, thus classification is considered according to the criteria for dusts and mists. The LC_{50} of 1.4 mg/L compared to the criteria ($1.0<ATE\leq5.0$ mg/L for dusts and mists) then leads to Category 4.

The DS concluded on a classification of DMPT as Category 4. An ATE value for dusts and mists of 1.4 mg/L was selected based on the LC_{50} in rats from the only available study report. Therefore, 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.

Comments received during consultation

One MSCA commented during the consultation. The MSCA agreed with the proposed classification as Acute. Tox. 4. In addition, considering that no detailed information is available on the studies, the relevance of the proposed ATE of 1.4 mg/L was questioned, but nonetheless agreed as the value is very close to the generic ATE (1.5 mg/kg bw).

The DS acknowledged the comment.

Assessment and comparison with the classification criteria

Three database records on acute inhalation toxicity are available, two with mice, one with rats, without detailed information on the underlying studies. The rat study provided an LC_{50} of 1.4 mg/L (n=10 males and females; 4 hr exposure; GLP conform). Neither of the mouse studies provide LC_{50} values, but resulted in LOAECs of 3.192 mg/L and 0.800 mg/L based on adverse effects.

As demonstrated by the DS, the LC_{50} (1.4 mg/L) is above the SVC of DMPT (0.557 mg/L); therefore, classification according to the criteria for mists will be considered. The only LC_{50} of 1.4 mg/L compared to the criteria (Category 4: $1.0<LC_{50}\leq5.0$ mg/L for dusts and mists) leads to a classification in Category 4 and an ATE of 1.4 mg/L.

RAC concludes that DMPT meets the criteria ($1.0<LC_{50}\leq5.0$ mg/L) and should be **classified as Acute Tox. 4; H332 with an ATE of 1.4 mg/L (mist)**.

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
Bacteria cell culture				
Reverse mutation / Ames Test Similar to OECD TG 471 GLP: no information Deviations: • *S. typhimurium* TA 1535 not tested • *E. coli* WP2 uvrA, or *E. coli* WP2 uvrA (pKM101), or *S. typhimurium* TA102 not tested • no detailed data on cytotoxicity	***N,N*-dimethyl-p-toluidine** CAS: 99-97-8 Purity: 99 %	**Supporting study (Reliable with restrictions)** Tester strains: *S. typhimurium* TA97, TA98 and TA100 Dosing: 0, 1, 2.5, 5, 10, 40, 70, 100 µg/plate (with and without S9 mix): Controls: Negative control: valid Positive control: valid	**Negative** Negative in all tested strains (up to 70µg/plate) without and with metabolic activation Cytotoxicity: highest dose (100 µg/plate) was cytotoxic in all strains and conditions tested	(Taningher et al., 1993)
Reverse mutation / Ames test Similar to OECD TG 471 (NTP internal guideline) GLP: no information Deviations: • 5th strain missing • No data on cytotoxicity	***N,N*-dimethyl-p-toluidine** CAS: 99-97-8 Purity: >99 %	**Supporting study (Reliable with restrictions)** Tester strains: *S. typhimurium* TA97, TA98, TA100, and TA1535 Dosing (with and without S9 mix): 0, (0.33), 1, (3.3), 10, 33, 100, 333, 500, 1 000 µg/plate Controls: Negative control: valid Positive control: valid	**Negative** No data on cytotoxicity ("The high dose was limited by cytotoxicity.")	(NTP, 2012)

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
Reverse mutation / Ames Test Similar to OECD TG 471 (NTP internal guideline) Deviations: • Strains *S. typhimurium* TA1535, TA1537, TA97 (or TA97a) not tested. • No data on cytotoxicity	***N,N*-dimethyl-p-toluidine** CAS: 99-97-8 Purity: >99 %	**Supporting study (Reliable with restrictions)** *E. coli* strain WP2 uvrA/pKM101, *S. typhimurium* strains TA98 and TA100. 10 % rat liver S9 for exogenous metabolic activation. Dosing (with and without S9 mix): 0, 50, 100, 250, 500, 750, 1 000, 1 500 µg/plate Controls: Negative control: valid Positive control: valid	**Negative** No data on cytotoxicity ("The high dose was limited by cytotoxicity.")	(NTP, 2012)
Reverse mutation / Spot Test Not conform to OECD TG 471 GLP: no information Major deviations: • single dose applied as spot • *S. typhimurium* TA104 instead of TA102 • *S. typhimurium* TA1535 not tested • S9 activation method not described • no data on cytotoxicity • no colony counts available • no information on replicates • no information on relevance of negative controls	***N,N*-dimethyl-p-toluidine** CAS: 99-97-8 Purity: not reported	**Disregarded study (Not reliable)** Tester strains: *S. typhimurium* TA97, TA98, TA100 and TA104 Dosing: 3 µl/spot (3 mg pure test substance, single dose) Metabolic activation with rat liver S9 mix Controls: Negative control: no information on colony counts Positive control: valid	**Negative** Cytotoxicity: no information	(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
Reverse mutation / Ames Test (plate incorporation) Similar to OECD TG 471 (US NCI standard procedure) GLP: no information Deviations: • *E. coli* WP2 uvrA, or *E. coli* WP2 uvrA (pKM101), or *S. typhimurium* TA102 not tested • No information on positive control substances available • Only general information on cytotoxicity available	***N,N*-dimethyl-p-toluidine** CAS: 99-97-8 Purity: information not available	**Disregarded study (Not reliable)** Tester strains: *S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538 Dosing: 3, 10, 33, 100, 333 µg/plate (without and with S9 mix from hamster and rat) Controls: Negative control: valid Positive controls: Data present, but no information on positive control substances reported	**Negative** Negative in all tested strains, with and without metabolic activation Cytotoxicity: Dose range finding study in TA100 with and without metabolic activation as justification for dosing, but data not reported.	Summary report of the US National Cancer Institute (NCI): (Seifried et al., 2006)
Reverse mutation / Ames test OECD TG 471 conform GLP: yes Deviations: • 5th stain missing • No method details available • No study data available (colony counts, controls)	***N,N*-dimethyl-p-toluidine** CAS: 99-97-8 Purity: 99 %	**Disregarded study (Reliability not assignable)** *S. typhimurium* strains TA98, TA100, TA1537, TA1538 Dosing: 100 – 5 000 µg/plate Controls: Negative control: no data Positive control: no data	**Negative** TA98, TA100, TA1537: conclusion/genotoxic effect: **negative/negative** TA1538 conclusion/genotoxic effect: **negative/equivocal** Cytotoxic without metabolic activation: 1000µg/plate Study data not available	ChemFirst Study No. 14506-0-401 (1983) from database entry (ACToR, 2015)

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
mammalian cell culture				
L5178Y TK+/- Mouse Lymphoma Mutagenicity Assay Equivalent to OECD 476 (1997), similar to OECD 490 GLP: no information Deviations: • No study details reported	***N,N*-dimethyl-p-toluidine** CAS: 99-97-8 Purity: not reported	**Key study (Reliable with restrictions)** Cells: L5178Y TK$^{+/-}$ 3.7.C mouse lymphoma cells Dosing: <u>Without S9 mix:</u> 0.05, 0.11, 0.18, 0.24, 0.31 µl/mL <u>With S9 mix:</u> 0.005, 0.011, 0.018, 0.024, 0.031, 0.037, 0.044 µl/mL Treatment time: 4 h Sampling time: 10-12 days incubation time Colonies larger 0.2 mm were counted Controls: Negative control: valid Positive control: valid	**Equivocal** with/without S9 mix 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). <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 10^6 viable cells over solvent control), overt cytotoxicity in the other parallel culture. <u>With S9 mix:</u> Weakly positive rel. MF (3.1- and 2.2-fold) and equivocal GEF (106 and 59 mutants per 10^6 viable cells over solvent control) at 0.031 µl/mL.	Summary report of the US National Cancer Institute (NCI): (Seifried et al., 2006)

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
In vitro mammalian micronucleus test Equivalent to OECD TG 487 GLP: no information Deviations: • Extended treatment (48h, approx. 3 cell cycles) • Metabolic activation: no data	***N,N*-dimethyl-p-toluidine** CAS: 99-97-8 Purity: 99 %	**Supporting study (Reliable with restrictions)** Cells: V79 cells. Dosing: 0, 0.3, 0.9, 1.2 mM Treatment time: 48 h Sampling time: end of treatment Controls: Negative control: valid Positive control: valid	**Positive** Significant aneugenic activity: CREST positive micronuclei up to about 5.5-fold induced compared to control ($p<0.01$, X^2 test or Fisher Exact test) Significant clastogenic activity: CREST negative micronuclei up to about 3.6-fold induced compared to control ($p<0.01$, X^2 test or Fisher-Exact test) Dose dependency: significant for CREST positive and negative micronuclei ($p<0.001$, Cochrane-Armitage trend test) Cytotoxicity: Survival >10 % (colony formation, data not presented) Mitotic index (at 24 and 48 h of treatment) partly increased, no dose dependency.	(Taningher et al., 1993)

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
Mouse peripheral blood micronucleus, flow cytometric assay Equivalent to OECD TG 474 GLP: no information Deviations: • No information on toxicity; dosing based on 3-month study • Clinical observations not available	***N,N*-dimethyl-p-toluidine** CAS: 99-97-8 Purity: >99 %	**Supporting study (Reliable with restrictions)** Species: male B6C3F1/N mice; n=5 per dose Dosing: 0, 30, 60, or 75 mg/kg bw/day in corn oil daily for 4 days by gavage Sampling time: 4 hours after the fourth dose Controls: Positive control: valid Negative control: valid 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.	**Negative** No significant increases in frequencies of micronucleated erythrocytes. Toxicity: No significant alterations in percentage of circulating reticulocytes. Clinical signs: information not available.	(NTP, 2012)

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Mouse peripheral blood micronucleus, slide-based assay Equivalent to OECD TG 474 GLP: no information Deviations: • No positive control • Sampling time not reported	***N,N*-dimethyl-p-toluidine** CAS: 99-97-8 Purity: >99 %	**Supporting study (Reliable with restrictions)** Species: B6C3F1/N mice; n=5 per dose and sex Dosing: 0, 15, 30, 60 and 125 mg/kg bw/day in corn oil by gavage for 3-months Controls: Negative control: valid Positive control: none Toxicity: Dosing for 3-month study (NTP, 2012) was based on available LD50 values.	**Negative** No significant increases in the frequencies of micronucleated erythrocytes (MNE). In male mice, MNE frequencies were slightly increased with dose, but without significant trend. Toxicity: No significant alterations in the percentage of circulating reticulocytes. 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.	(NTP, 2012)

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Comet assay in mouse blood and liver cells Equivalent to OECD TG 489 GLP: no information Deviations: • No information on toxicity; dosing based on 3-month study • Clinical observations not available	***N,N*-dimethyl-p-toluidine** CAS: 99-97-8 Purity: >99 %	**Supporting study (Reliable with restrictions)** Species: male B6C3F1/N mice; n=5 per dose Dosing: 0, 30, 60, or 75 mg/kg bw/day in corn oil daily for 4 days by gavage. Sampling time: 4 hours after the fourth dose Controls: Positive control: valid Negative control: valid 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.	**Negative** No increased DNA damage in liver cells or blood leukocytes. Clinical signs: information not available.	(NTP, 2012)

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

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Alkaline DNA elution test No test guideline followed GLP: no information Only summary data available.	***N,N*-dimethyl-p-toluidine** CAS: 99-97-8 Purity: 99 %	**Disregarded study (Not reliable)** Species: Sprague Dawley rats (male); n=2 to 6 i.p. injection, male Sprague Dawley rats, liver n=2 to 6 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) Toxicity: Doses based on reported LD_{50} values. Controls: Positive control: valid Negative control: valid	**Weakly positive/equivocal** "… 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).	(Taningher et al., 1993)
Alkaline DNA elution test No test guideline followed GLP: no information Only summary data available.	***N,N*-dimethyl-p-toluidine** CAS: 99-97-8 Purity: 99 %	**Disregarded study (Not reliable)** Species: BALB/c mice (male); n=4 (neg. control) or 6 (dosing) Organ: liver 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) Toxicity: Doses based on reported LD_{50} values. Controls: Positive control: valid Negative control: valid	**Weakly positive/equivocal** "… 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).	(Taningher et al., 1993)

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 $90x10^{-6}$ 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 $100x10^{-6}$ 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: $90x10^{-6}$; 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 $1x10^6$ cells	counts per 200 cells	%	mutations per 10^6 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	**2.0**
	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 $1x10^6$ cells	counts per 200 cells	%	mutations per 10^6 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	**2.0**
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	**2.3**
0.024	76	157	26	97	47	1.9
	85	177	32	96	46	1.9
0.031	113	145	12	156	**106**	**3.1**
	86	158	15	109	59	**2.2**
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[a]		
		24 hr	48 hr	CREST+	CREST−	TOTAL
N,N-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[b]	6.67	17.34[c]
	1.2	14.32	10.89	18.26[c]	9.62[c]	27.88[c]
Methylnitrosourea	0			2.70	2.29	4.99
	0.5			28.47[c]	114.39[c]	142.86[c]
Colchicine	0			2.62	2.35	4.97
	0.000025			35.20[c]	3.33	38.53[c]

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). [a]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. [b,c]Significantly different from concurrent controls with a p value less than 0.05 or 0.01, respectively, according to the χ^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[a] (NTP, 2012)

	Dose (mg/kg)	Number of Mice with Erythrocytes Scored	Micronucleated PCEs/ 1,000 PCEs[b]	P Value[c]	Micronucleated NCEs/ 1,000 NCEs[b]	P Value[c]	PCEs[b] (%)	P Value[c]
Corn oil[d]	0	5	2.59 ± 0.20		1.46 ± 0.02		1.270 ± 0.11	
N,N-Dimethyl-*p*-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[e]		P=0.089		P=0.243	
Ethyl methanesulfonate[f]	150	5	12.18 ± 0.34	0.0000	1.69 ± 0.04	0.0004	0.942 ± 0.04	0.015

[a] Study was performed at ILS, Inc. The detailed protocol is presented by Witt *et al.* (2008). NCE=normochromatic erythrocyte; PCE=polychromatic erythrocyte
[b] Mean ± standard error
[c] Pairwise comparison with the vehicle control group; values are significant at P≤0.025 by Williams' test
[d] Vehicle control
[e] Significance tested by a linear regression trend test; significant at P≤0.025
[f] 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[a] (NTP, 2012)

	Dose (mg/kg)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/1,000 NCEs[b]	P Value[c]	PCEs[b] (%)
Male					
Corn oil[d]	0	5	2.00 ± 0.32		3.34 ± 0.24
N,N-Dimethyl-*p*-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[e]		
Female					
Corn oil	0	5	1.50 ± 0.16		4.24 ± 0.36
N,N-Dimethyl-*p*-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		

[a] Study was performed at ILS, Inc. The detailed protocol is presented by MacGregor *et al.* (1990). NCE=normochromatic erythrocyte; PCE=polychromatic erythrocyte
[b] Mean ± standard error
[c] Pairwise comparison with the vehicle control group; dosed group values are significant at P≤0.006
[d] Vehicle control
[e] 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[a] (NTP, 2012)

	Dose (mg/kg)	Number of Animals	Percent Tail DNA[b]	P Value[c]
Blood				
Corn oil[d]	0	5	2.0 ± 0.24	
N,N-Dimethyl-*p*-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[e]	
Ethyl methanesulfonate[f]	150	5	20.7 ± 1.10	<0.001
Liver				
Corn oil	0	5	5.3 ± 0.59	
N,N-Dimethyl-*p*-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

[a] Study was performed at ILS, Inc. The detailed protocol is presented by Recio *et al.* (2010).
[b] Mean ± standard error
[c] 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.
[d] Vehicle control
[e] Significance of percent tail DNA tested by a linear regression trend test; significant at P≤0.025
[f] 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.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

Several *in vitro* and *in vivo* mutagenicity studies with DMPT are available. Only one study was performed under GLP; most studies had some deviations from the OECD test guidelines. Data in mammalian germ cells are not available.

In vitro tests

Results from three 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.

The results of a Mouse Lymphoma Mutagenicity Assay are weakly positive for single doses in single parallel cultures. Mutation frequencies are just above doubled at the highest concentrations, in both non-activated and S9-activated cultures. However, at the highest concentrations, the relative growth rate was about or below 10%. Overall, the study results are rated as equivocal.

The *in vitro* Mammalian Micronucleus Test showed induction of clastogenic effects and aneuploidy (demonstrated by increased staining of 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 instead of the recommended 1.2 to 2 cell cycles) and detailed information on cytotoxicity was not reported. 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.

***Table**: Overview of in vitro and in vivo mutagenicity studies with DMPT, in short (based on Table 18 in CLH report).*

Method, guideline, deviations if any	Information	Observations	Reference
IN VITRO			
Reverse mutation / Ames Test Similar to OECD TG 471 With deviations: • *S. typhimurium* TA 1535, E. coli WP2 uvrA, or E. coli WP2 uvrA (pKM101), or *S. typhimurium* TA102 not tested • no detailed data on cytotoxicity	Test strains: *S. typhimurium* TA97, TA98 and TA100 Controls: Neg. control: valid Pos. control: valid	**Negative** Negative in all tested strains (up to 70 µg/plate) without and with metabolic activation Cytotoxicity: highest dose (100 µg/plate) was cytotoxic in all strains and conditions tested	1993 Supporting study (Reliable with restrictions)
Reverse mutation / Ames test Similar to OECD TG 471 (NTP internal guideline) With deviations: • 5th strain missing • No data on cytotoxicity	Test strains: *S. typhimurium* TA97, TA98, TA100, TA1535 Controls: Neg. control: valid Pos. control: valid	**Negative** No data on cytotoxicity ("The high dose was limited by cytotoxicity.")	NTP, 2012 Supporting study (Reliable with restrictions)
Reverse mutation / Ames Test Similar to OECD TG 471 (NTP internal guideline) With deviations: • Strains *S. typhimurium* TA1535, TA1537, TA97 (or TA97a) not tested. • No data on cytotoxicity	Test strains: *E. coli* WP2 vrA/pKM101, *S. typhimurium* TA98, TA100. 10% rat liver S9. Controls: Neg. control: valid Pos. control: valid	**Negative** No data on cytotoxicity ("The high dose was limited by cytotoxicity.")	NTP, 2012 Supporting study (Reliable with restrictions)
Reverse mutation / Spot Test Not OECD TG 471 conform Major deviations: • single dose applied as spot • *S. typhimurium* TA104 instead of TA102 • *S. typhimurium* TA1535 not tested • S9 activation method not described • no data on cytotoxicity, replicates, relevance of neg. controls • no colony counts available	Test strains: *S. typhimurium* TA97, TA98, TA100, TA104 Controls: Neg control: no information on colony counts Pos control: valid	**Negative** Cytotoxicity: no information	1986 Disregarded study (Not reliable)

Reverse mutation / Ames Test (plate incorporation) Similar to OECD TG 471 (US NCI standard procedure) Deviations: • *E. coli* WP2 uvrA, or *E. coli* WP2 uvrA (pKM101), or *S. typhimurium* TA102 not tested • Only general information on cytotoxicity available	Test strains: *S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538 Controls: Neg. control: valid Pos. controls: data, but no information on pos. control substances reported.	**Negative** Negative in all tested strains, with and without metabolic activation Cytotoxicity: Dose range finding study in TA100 with and without metabolic activation as justification for dosing, but data not reported.	2006 Disregarded study (Not reliable)
Reverse mutation / Ames test OECD TG 471 conform GLP: yes Deviations: • 5th stain missing • No method details • No study data available (colony counts, controls)	Test strains: *S. typhimurium* strains TA98, TA100, TA1537, TA1538 Controls: Neg. control: no data Pos. control: no data	**Negative** TA98, TA100, TA1537: conclusion/genotoxic effect: **negative/negative** TA1538 conclusion/genotoxic effect: **negative/equivocal** Cytotoxic without metabolic activation: 1000µg/plate	1983 Disregarded study (Reliability not assignable)
L5178Y TK+/- Mouse Lymphoma Mutagenicity Assay Equivalent to OECD TG 476 (1997), similar to OECD TG 490 Deviations: No study details reported	Cells: L5178Y $TK^{+/-}$ 3.7.C mouse lymphoma cells Controls: Neg. control: valid Pos. control: valid	**Equivocal** with/without S9 mix 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). <u>Without S9 mix:</u> In one of two parallel cultures at 0.24 µl/mL weakly positive MF fold-change to solvent control (rel. MF) (2.0-fold) and GEF (90 mutants per 10^6 viable cells over solvent control), overt cytotoxicity in the other parallel culture. <u>With S9 mix:</u> Weakly positive rel. MF (3.1- and 2.2-fold) and equivocal GEF (106 and 59 mutants per 10^6 viable cells over solvent control) at 0.031 µl/mL.	2006 Key study (Reliable with restrictions)

In vitro mammalian micronucleus test Equivalent to OECD TG 487 Deviations: • Extended treatment (48h, approx. 3 cell cycles) • Metabolic activation: no data	Cells: V79 cells Controls: Neg. control: valid Pos. control: valid	**Positive** Significant aneugenic activity: CREST positive micronuclei up to about 5.5-fold induced compared to control ($p<0.01$, X^2 test or Fisher Exact test) Significant clastogenic activity: CREST negative micronuclei up to about 3.6-fold induced compared to control ($p<0.01$, X^2 test or Fisher-Exact test) Dose dependency: significant for CREST positive and negative micronuclei ($p<0.001$, Cochrane-Armitage trend test) Cytotoxicity: Survival >10 % (colony formation, data not presented) Mitotic index (at 24 and 48 h of treatment) partly increased, no dose dependency.	1993 Supporting study (Reliable with restrictions)
IN VIVO			
Mouse peripheral blood micronucleus, flow cytometric assay Equivalent to OECD TG 474 Deviations: • No info on toxicity; dosing based on a 3-month study • No clinical observations	Species: male B6C3F1/N mice; n=5 per dose Dosing: 0, 30, 60, 75 mg/kg bw/day in corn oil daily for 4 days by gavage. Sampling time: 4 hours after 4th dose Toxicity: The highest dose was based on the toxicity information obtained in a 3-month mouse study Controls: Pos. control: valid Neg. control: valid	**Negative** No significant increases in the frequencies of micronucleated erythrocytes (MNE). Toxicity: No significant alterations in percentage of circulating reticulocytes. Clinical signs: information not available.	NTP, 2012 Supporting study (Reliable with restrictions)
Mouse peripheral blood micronucleus, slide-based assay Equivalent to OECD TG 474 Deviations: No positive control Sampling time not reported	Species: B6C3F1/N mice; n=5 per dose and sex Dosing: 0, 15, 30, 60 and 125 mg/kg bw/day in corn oil by gavage for 3-months Toxicity: Dosing for 3-month study was based on available LD_{50} values. Controls: Pos. control: none Neg. control: valid	**Negative** No significant increases in the frequencies of micronucleated erythrocytes (MNE). In male mice, MNE frequencies were slightly increased with dose, but without significant trend. Toxicity: No significant alterations in the percentage of circulating reticulocytes.	NTP, 2012 Supporting study (Reliable with restrictions)

Comet assay in mouse blood and liver cells Equivalent to OECD TG 489 Deviations: No info on toxicity; dosing based on a 3-month study No clinical observations	Species: male B6C3F1/N mice; n=5 per dose Dosing: 0, 30, 60, 75 mg/kg bw/ day in corn oil daily for 4 days by gavage. Sampling time: 4 hours after 4th dose Toxicity: The highest dose was based on the toxicity information obtained in a 3-month mouse study Controls: Pos. control: valid Neg. control: valid	**Negative** No increased DNA damage in liver cells or blood leukocytes. Clinical signs: information not available.	NTP, 2012 Supporting study (Reliable with restrictions)
Comet assay in rat liver cells Equivalent to OECD TG 489 Deviations: • single dose tested • No info on toxicity; dosing based on 2-year study • No clinical observations	Species: Male F344/N rats; n=5 per dose Dosing: Single dose of 60 mg/kg bw/day in 1% acetone/corn oil vehicle by gavage. Sampling time: 4 hours after 4th dose Toxicity: Same dose as the highest dose in 2-year study Controls: Pos. control: valid Neg. control: valid	**Equivocal** Statistically significant, but weak increase (1.4-fold, $p<0.05$) compared to vehicle control in percent tail DNA. No information on cytotoxic effect/no information on clinical signs.	NTP, 2012 Disregarded study (Not reliable)

In vivo tests

Two *in vivo* Micronucleus tests with DMPT in male B6C3F1/N mice are available, a slide-based assay and a flow cytometric assay, both not fully compliant with OECD TG 474. None of the studies showed increased frequencies of micronucleated erythrocytes. The first study used 75 mg/kg bw/day as highest dose used, the second study 125 mg/kg bw/day. A 3-month study in the same mouse strain did not show any effects at 60 mg/kg bw/day, but showed adverse effects at 125 mg/kg bw/day (increase mortality, reduced body weight and effects on haematology).

Two *in vivo* Comet assays are available with DMPT, in mice and in rat. In the study with male B6C3F1/N mice, no increase in DNA migration in liver cells or blood leukocytes was found. Mice were dosed from 30-75 mg/kg bw/day for four days. Similarly to the MNT assay, this dose did not show effects in the 3-month study. The study with Sprague-Dawley rats using a single dose of 60 mg/kg bw/day resulted in a weak increase in percent tail DNA, but was assessed as not reliable by the DS.

Summarising, 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 specific for *in vivo* gene mutagenicity were identified.

The DS concluded that no classification as germ cell mutagen is warranted.

Comments received during consultation

One MSCA commented. The MSCA agreed with no classification, based on mostly negative (or equivocal) results in *in vitro* studies and mostly negative results in *in vivo* studies. The MSCA noted that there is no study in full accordance with OECD test guidelines. In addition, in most *in vivo* studies, there is no data on general toxicity and thus it cannot be confirmed that the exposure was sufficient to identify mutagenicity.

The DS acknowledged the comment.

Assessment and comparison with the classification criteria

Several *in vitro* and *in vivo* studies with DMPT investigating mutagenicity are available. It should be noted that these tests are similar to OECD test guidelines, but all have some deviations or flaws (and one is "not conform"). There were no germ cell mutagenicity studies available.

DMPT tested *in vitro* negative in six Ames tests and equivocal in a mouse lymphoma assay. Results from an *in vitro* micronucleus test were positive.

However, two *in vivo* micronucleus tests (NTP, 2012) were negative. No significant increases in the frequencies of micronucleated erythrocytes were observed in peripheral blood of male or female B6C3F1/N mice DMPT (0, 30, 60, 125 mg/kg bw/day) dosed for 3 months. No significant alterations in the percentage of circulating polychromatic erythrocytes (reticulocytes) were observed, suggesting that DMTP did not induce bone marrow toxicity over the dose range tested. Results of a second micronucleus test in male B6C3F1/N mice DMPT (0, 30, 60, 75 mg/kg bw/day) once daily for 4 days were also negative and again, no significant alterations in the percentage of circulating reticulocytes were observed.

Two comet assays are available. A comet assay in blood and liver cells with B6C4F3F1/N male mice (dosing 0, 30, 60, 75 mg/kg bw/day for 4 days; NTP, 2012) was negative. The other comet assay (1993) in liver cells from male F344/N rats (single dose of 60 mg/kg bw/day) was equivocal, with a weak increase (1.4-fold) in percent tail DNA.

For the evaluation of toxicity, all studies need to be compared with a 3-month (NTP) study (0, 15, 30, 60, 125 or 250 mg/kg bw/day). All ten 250 mg/kg bw/day male and female mice (except for one male mouse) died, whereas three males and two females administered 125 mg/kg died before the end of the study. Other adverse effects at 125 mg/kg bw/day in male mice consisted of lower body weight (12%), affected haematology (lower Hb, increased MetHb, small increases in Heinz bodies), as well as effects in the lungs, nasal cavity, thymus and liver. At 60 mg/kg bw/day, no effect was found on body weight, lungs, nasal cavity, thymus and liver, only significant effects on haematology (lower haematocrit %, lower Hb, higher MetHb).

Further, no specific *in vivo* gene mutagenicity tests are available.

It should be noted that DMPT might have some genotoxic potential based on the positive *in vitro* clastogenicity test, equivocal comet assay, oxidative damage to erythrocytes and multisite carcinogenicity.

As there is no human data, nor data on germ cell mutagenicity, classification in Category 1A/1B is not warranted. No clear positive results were observed in the *in vivo* micronucleus and comet assays to warrant classification in Category 2.

RAC considered that **the criteria for classification for germ cell mutagenicity are not fulfilled**.

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
2-year study **Reliable without restrictions** Gavage with corn oil (dosing volume 2.5 ml/kg) **Rats (F344/N)** ♀ and ♂ NTP internal guideline, equivalent to OECD TG 451 50 animals per sex and dose Additional clinical pathology groups of 10 male and 10 female rats receiving the same doses for 86 days.	*N,N*-Dimethyl-p-toluidine (CAS No. 99-97-8) purity > 99 % 0, 6, 20, or 60 mg/kg in corn oil 5 days per week ♂: 104 weeks ♀: 105 weeks	Clear evidence of **carcinogenic activity** (summarised in Table 25) **Liver** • hepatocellular carcinoma (♀/♂) • **Nasal cavity** nasal cavity neoplasms (♂, primarily nasal cavity transitional epithelium adenoma), • nasal cavity transitional epithelium adenoma was considered to be related to treatment (♀) Thyroid gland • thyroid gland follicular cell neoplasms may have been related to treatment (♂) Additionally increased incidences of **non-neoplastic lesions** in • liver (♀/♂),see Table 27; • nasal cavity (♀/♂), see Table 28; kidney (♀/♂), spleen and bone marrow (♀/♂), forestomach (♂), mesenteric lymph node (♂), see • Table 31. Hematologic toxicity and increases in methaemoglobin levels (♀/♂, assessed after 86 days), see Table 29, Table 30 and section 10.12 (STOT-RE) for details Body-weight gain↓ (60 mg/kg; ♀/♂); Survival ↓ (60 mg/kg; ♂)	(NTP, 2012) **Key study** (Reliable without restrictions)
2-year study **Reliable without restrictions** Gavage with corn oil (dosing volume 5 ml/kg) **Mice (B6C3F1/N)** ♀ and ♂ NTP internal guideline, equivalent to OECD TG 451 50 animals per sex and dose	*N,N*-Dimethyl-p-toluidine (CAS No. 99-97-8) purity > 99 % 0, 6, 20, or 60 mg/kg in corn oil 5 days per week 105 weeks	Clear evidence of **carcinogenic activity** (summarized in Table 32) **Liver** • hepatocellular carcinoma and hepatoblastoma (♀/♂) • hepatocellular adenoma (♀/♂, multiple in ♂) **Lung** • alveolar/ bronchiolar neoplasms (primarily adenoma) (♀) **Forestomach** • increased incidences of forestomach squamous cell papilloma considered to be related to treatment (♀) Additionally increased incidences of **non-neoplastic lesions** in • liver (♀/♂), see Table 34; • lung (♀/♂), see Table 35; • forestomach (♀), see Table 36; • nasal cavity and olfactory lobe (♀/♂), see Table 37; • spleen, bone marrow and mesenteric lymph node (♀), see Table 38. Body-weight gain ↓ (20 mg/kg; ♂, 60 mg/kg; ♀/♂,); Survival ↓ (60 mg/kg; ♂)	(NTP, 2012) **Key study** (Reliable without restrictions)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Lifetime study **Not reliable** No guideline study, no GLP conformity Detailed study data not available Rats, strains BD I, BD III, W. 28 animals per strain (about 100 days old at study start)	"N-Dimethyl-toluidine", no purity given Test substance mixed into food (leftovers from hospital) Average dose: 7 mg / day Total dose: 5 g/rat	Brief report without traceable study data and gross lacks in study design. • Higher than average life-expectancy, higher average body weight compared to controls • No chronic toxicity (although not clear what was investigated) • No carcinogenic effects	(Druckrey et al., 1954) **Disregarded study** (Not reliable)

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 (%)[a]	74	76	63	**45** [ss]	66	86	66	**47** [s]
Liver								
Hepatocellular adenoma	0	0	1	1	0	1	1	3
Hepatocellular carcinoma	0[##]	0	1	**6****	0[##]	0	0	**4***
H. adenoma or carcinoma	0[##]	0	2	**6****	0[##]	1	1	**7****
Nasal cavity								
Glands, olfactory epith., adenoma	0	0	0	1				
Transitional epithelium, adenoma	0[##]	3	2	**11****	0	1	0	2
Transitional epithelium, carcinoma	0	0	0	2				
Trans. epith. adenoma or carcinoma	0[##]	3	2	**13****				
Thyroid Gland								
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)

[a] Kaplan-Meier determinations

[s] or [ss] 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 (%) [a]	102.5	94.3	80.5	107.3	101.0	84.5
Rel. body weight gain (%)[a, b]	103.3	92.6	74.6	109.9	101.3	77.6

[a] relative to vehicle control

[b] 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≤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. [a]: number of animals examined microscopically.

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
Male				
Liver [a]	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)b	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)
Female				
Liver [a]	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≤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. [a]: 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
Male				
Nose [a]	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
Male				
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)
Female				
Nose [a]	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 [%][a]	-2.5	-8.1	-17.5	-4.4	-8.9	-16.5
Funct. Hb change [%][a.b]	-3.3	-11.0	-28.4	-5.1	-12.1	-27.1
Erythrocytes (10^6/µL)		↓↓	↓↓		↓↓	↓↓
Reticulocytes (10^6/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

[a] Calculated from average values without error propagation; percental change compared to vehicle control.

[b] 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
Male				
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 (10^6/µL)	9.10 ± 0.10	9.02 ± 0.06	8.53 ± 0.04**	7.61 ± 0.06**
Reticulocytes (10^6/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 (10^3/µL)	645.4 ± 27.5	682.6 ± 7.8	721.4 ± 18.4**	722.0 ± 26.0*
Leukocytes (10^3/µL)	9.44 ± 0.49	9.91 ± 0.45	9.99 ± 0.51	9.31 ± 0.58
Segmented neutrophils (10^3/µL)	1.38 ± 0.09	1.42 ± 0.04	1.42 ± 0.09	1.50 ± 0.05
Lymphocytes (10^3/µL)	7.70 ± 0.42	8.10 ± 0.41	8.18 ± 0.41	7.46 ± 0.52
Monocytes (10^3/µL)	0.23 ± 0.02	0.26 ± 0.02	0.24 ± 0.02	0.20 ± 0.02
Basophils (10^3/µL)	0.062 ± 0.007	0.071 ± 0.006	0.079 ± 0.012	0.075 ± 0.009
Eosinophils (10^3/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**
Female				
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 (10^6/µL)	8.50 ± 0.09	8.31 ± 0.10	7.88 ± 0.08**	6.95 ± 0.09**
Reticulocytes (10^6/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 (10^3/µL)	597.4 ± 46.6	583.1 ± 46.9	578.8 ± 49.0	719.3 ± 31.9
Leukocytes (10^3/µL)	8.04 ± 0.35	8.65 ± 0.22	8.59 ± 0.56	7.46 ± 0.38
Segmented neutrophils (10^3/µL)	1.40 ± 0.10	1.51 ± 0.11	1.52 ± 0.15	0.95 ± 0.11
Lymphocytes (10^3/µL)	6.29 ± 0.30	6.76 ± 0.26	6.74 ± 0.44	6.24 ± 0.33
Monocytes (10^3/µL)	0.21 ± 0.01	0.24 ± 0.02	0.18 ± 0.02	0.15 ± 0.01*
Basophils (10^3/µL)	0.060 ± 0.007	0.054 ± 0.003	0.065 ± 0.009	0.052 ± 0.006
Eosinophils (10^3/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
[a] 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. [a]: number of animals examined microscopically.

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
Male				
Bone Marrow [a]	50	50	50	50
Hyperplasia	17 (2.5)	13 (2.5)	28** (2.1)	50** (2.7)
Forestomach [a]	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 [a]	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 [a]	50	50	50	50
Infiltration Cellular, Histiocyte	21 (1.1)	23 (1.4)	30* (1.3)	34** (1.5)
Spleen [a]	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)
Female				
Bone Marrow [a]	50	50	50	50
Hyperplasia	18 (2.8)	13 (2.5)	18 (2.7)	49** (2.6)
Kidney [a]	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 [a]	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 (%)[a]	71	72	62	72	86	82	80	**67** [s]
Liver								
Hepatocellular adenoma	29	34	37	36	17[##]	19	**37****	**44****
H. carcinoma	22[##]	25	30	**36****	6[##]	**13***	**18****	**31 ****
H. adenoma or carcinoma	38[##]	44	**47****	**48****	20[##]	25	**42****	**45****
Hepatoblastoma	1	5	10	**8***	0[#]	1	0	**4***
H. adenoma, carcinoma, or hepatoblastoma	38[##]	42	**48****	**48****	20[##]	26	**42****	**45****
Lung								
Alveolar/bronch. adenoma	11	16	18	10	2[##]	4	**8***	**12****
Alveolar/bronch. carc.	2	3	0	4	0	1	2	1
Adenoma or carcinoma	13	19	18	12	2[##]	5	**9***	**13****
Forestomach								
Squamous cell papilloma	1	1	0	3	1	5	**6***	**7***
Squamous cell carcinoma					0	1	0	0
Sq. cell papilloma or carc.					1	6	**6***	**7***

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)

[a] Kaplan-Meier determinations

[s] or [ss] 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 (%) [a]	98.0	92.3	82.3	99.7	101.9	69.9
Rel. body weight gain (%)[a, b]	96.6	86.3	68.6	100.0	103.3	56.3

[a] relative to vehicle control at termination
[b] until terminal sacrifice

Liver, 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≤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. [a]: number of animals examined microscopically.

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
Male, Liver				
Liver [a]	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)
Female, Liver				
Liver [a]	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≤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; [a]: number of animals examined microscopically.

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
Male, Lung				
Lung [a]	50	50	50	50
Alveolus, Infiltration Cellular, Histiocyte	1 (2.0)	2 (1.5)	2 (2.5)	10** (1.2)
Female, Lung				
Lung [a]	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≤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. [a]: number of animals necropsied.

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
Female, Forestomach				
Forestomach [a]	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≤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; [a]: number of animals examined microscopically; OE: Olfactory Epithelium; RE: Respiratory Epithelium.

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
Male, Nose				
Nose [a]	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)
Vomeronasal Organ, Necrosis	0	1 (2.0)	2 (1.0)	3 (1.0)
Olfactory Lobe [a]	38	43	39	34
Atrophy	0	1 (3.0)	0	5* (1.2)
Female, Nose				
Nose [a]	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)
Vomeronasal Organ, Necrosis	0	0	0	4* (1.5)
Olfactory Lobe [a]	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≤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; [a]: number of animals examined microscopically.

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
Male, Hematopoietic System				
Spleen [a]	48	50	49	50
Atrophy	4 (2.5)	11 (2.2)	11* (2.4)	6 (1.8)
Female, Hematopoietic System				
Bone Marrow [a]	50	50	50	49
Hyperplasia	5 (2.2)	14* (1.9)	15** (2.1)	14** (2.1)
Lymph Node, Mesenteric [a]	49	49	49	50
Atrophy	1 (2.0)	5 (2.0)	5 (2.2)	12** (2.9)
Hyperplasia, Lymphoid [a]	7 (2.3)	3 (3.7)	1* (2.0)	0*
Spleen [a]	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-xylidine (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
Control incidence (%)	**12**	**12**	**12**	**40**	**40**	**4**	**4**	**0**	**0**
Dose incidence (%)	**62**	**36**	**26**	**90**	**84**	**26**	**18**	**12**	**26**
Net incidence (%) [a]	**56.8**	**27.3**	**15.9**	**83.3**	**73.3**	**22.9**	**14.6**	**12.0**	**26.0**
Average daily dose (mg/kg bw/day)	**42.9**	**14.3**	**4.3**	**42.9**	**14.3**	**42.9**	**14.3**	**42.9**	**42.9**
T25 (mg/kg bw/day) [b]	**18.9**	**13.1**	**6.7**	**12.9**	**4.9**	**46.8**	**24.5**	**89.3**	**41.2**

[a] Net incidence (%) = (Dose incidence (%) - Control incidence (%)) / (100 - Control incidence (%)) * 100

[b] 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)	Statistically significant, treatment related increased incidences of neoplastic lesions in the **liver** of both species in both sexes. Background incidences in mice were higher than in rats. **Nasal cavity and liver** tumours in rats have low historical background incidences. **Hepatoblastoma** (in mice) are rare tumours and are found with low incidence in historical controls.	Yes; significant neoplastic lesions in liver and additionally in **nasal cavity** (rats m), **lung** (mice f), and **forestomach** (mice f).	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: **liver**, **nasal cavity** and **spleen** (both species/sexes); **lung** and **olfactory lobe** (mice m/f); **kidney** (rats m/f) **forestomach** and **mesenteric lymph node** (rats m, mice f) **bone marrow** (rats m/f, mice f). Most non-neoplastic lesions occurred with mild severity.	Liver tumours (adenoma or carcinoma) in mice have a short latency with first incidences about 100 days or more earlier than in control groups	Liver tumours occurred in both species in both sexes, other neoplastic incidences mainly in one species and in one sex. Pre-neoplastic lesions observed mostly in both sexes.	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. Body weight gain at high dose, reduced in both species (m/f), reduction in mice > 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. 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). Pre-neoplastic lesions in these organs occurred already at lower doses.	The studies have been performed by oral gavage, 5 days per week. Other routes of exposure cannot be excluded, and oral, inhalation and skin exposure are relevant exposure routes for humans. 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.	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. MoA and target tissues (e.g. liver) are relevant to humans. Oral DMPT exposure in humans leads to acute methaemoglobinaemia, MoA in humans and study animals seems comparable, at least for methaemoglobinaemia induced toxic effects.

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

• liver carcinoma in mice and rats, m/f
• dose-dependent progression to neoplasms
• pre-neoplastic lesions in all organs with neoplasms
• rare/uncommon tumour types
  ○ historical incidences for transitional epithelium adenomas or carcinomas (nasal cavity) are rare (rats, gavage studies)
  ○ hepatoblastoma are rare tumour types
• Plausible MoA, relevant for humans
  ○ metabolic generation of ROS and other radicals, methaemoglobinaemia, oxidative tissues damage
	• most neoplastic lesions only at highest dose
	• excessive toxicity - high dose potentially above MTD
	  ○ methaemoglobinaemia
	  ○ reduced body weight / body weight gain
	  ○ non-neoplastic lesions in several organs
	  ○ increased mortality (not explained by neoplasms)
	• single species/single sex neoplasms, i.e.
	  ○ nasal cavity (m rats)
	  ○ lung (f mice)
	  ○ forestomach (f mice)
	• liver (mice): high number of spontaneous incidences
	• non-genotoxic carcinogen

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.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

For DMPT, two 2-year carcinogenicity NTP studies in mice and rats are available, equivalent to OECD TG 451 (NTP internal guideline) and under GLP. 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.

Next to the NTP studies, one other long-term study is available (1954), a rat diet study with a single dose level of 7 mg/day. The study has major deficiencies in design and reporting (effective dosing unknown, three rat strains used but average values provided). This study is not further considered.

***Table**: Overview of neoplastic incidences in two carcinogenicity 2-year studies with DMPT (0, 6, 20 or 60 mg/kg bw/day in corn oil per gavage, 5 days/week, n=50 females/males) according NTP internal guideline, equivalent to OECD TG 451.*

F344/N rats	**Male**					**Female**				
Dose (mg/kg bw/day)	0	6	20	60	HCD	0	6	20	60	HCD
Number of animals	50	50	50	50	299	50	50	50	50	300
Surviving animals at termination	37	37	31	21		33	42	33	23	
Survival probability (%)[a]	74	76	63	**45** [ss]		66	86	66	**47** [s]	
Body weight (g) [e]	487	495	475	424		331	341	324	275	
Relative bw at study end (%) [c]		102.5	94.3	80.5			107.3	101.0	84.5	
Relative bw gain (%)[c,d]		103.3	92.6	74.6			109.9	101.3	77.6	
Liver										
Hepatocellular adenoma	0	0	1	1	3	0	1	1	3	1
Hepatocellular carcinoma	0[##]	0	1	**6****	0	0[##]	0	0	**4***	0
H. adenoma or carcinoma	0[##]	0	2	**6****	3	0[##]	1	1	**7****	1
Nasal cavity										
Glands, olf. epith., adenoma	0	0	0	1	0					0
Transitional epith., adenoma	0[##]	3	2	**11****	0	0	1	0	2	
Transitional epith., carcinoma	0	0	0	2						
Trans. epith. adenoma or carcinoma	0[##]	3	2	**13****						
Thyroid Gland										
Follicular cell adenoma	1	0	1	3	6	1	1	2	0	3
Follicular cell carcinoma	0	2	1	2	3					
F. cell adenoma or carcinoma	1	2		4	9					
B6C3F1/N mice	**Male**					**Female**				
Dose (mg/kg bw/day)	0	6	20	60	HCD	0	6	20	60	HCD
Number of animals	50	50	50	50	350	50	50	50	50	347
Surviving until termination	34	36	31	36		43	40	39	32	
Survival probability (%)[a]	71	72	62	72		86	82	80	**67** [s]	
Body weight (g) [e]	55.0	54.7	55.0	52.2		63.4	63.8	65.9	53.0	
Rel. bw at study end (%) [c]		98.0	92.3	82.3			99.7	101.9	69.9	
Rel. bw gain (%)[c,d]		96.6	86.3	68.6			100.0	103.3	56.3	
Liver										
Hepatocellular adenoma	29	34	37	36	181	17[##]	19	**37****	**44****	75

Hepatocellular carcinoma	22##	25	30	**36****	116	6##	**13***	**18****	**31****	29
Hepatocellular adenoma or carcinoma	38##	44	**47****	**48****	239	20##	25	**42****	**45****	91
Hepatoblastoma	1	5	**10****	**8***	14	0#	1	0	**4***	1
Hepatocellular adenoma, carcinoma, or hepatoblastoma	38##	42	**48****	**48****	242	20##	26	**42****	**45****	91
Lung										
Alveolar/bronchiolar adenoma	11	16	18	10	53	2##	4	**8***	**12****	16
Alveolar/bronchiolar carcinoma	2	3	0	4	28	0	1	2	1	7
Adenoma or carcinoma	13	19	18	12	77	2##	5	**9***	**13****	23
Forestomach										
Squamous cell papilloma	1	1	0	3		1	5	**6***	**7***	12
Squamous cell carcinoma						0	1	0	0	0
Squamous cell papilloma or carcinoma						1	6	**6***	**7***	12

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)
a Kaplan-Meier determinations
s or ss Significance of shorter survival from survival analysis, P<0.05 or P<0.01
c relative to vehicle control at termination
d until terminal sacrifice
e mean for weeks 53-101
Historical Control Data (HCD) are from other corn oil gavage F344/N rat NTP studies in the same period (March 2002 – March 2005), study with DMPT was in October 2004.
Historical Control Data (HCD) are from other corn oil gavage B6C3F1/N mice NTP studies in the same period (April 2002 – March 2005), study with DMPT was in October 2004.

Table: *Overview of neoplastic incidences (in percentages) in two carcinogenicity 2-year studies with DMPT (0, 6, 20 or 60 mg/kg bw/day in corn oil per gavage, 5 days/week, n=50 females/males) according NTP internal guideline, equivalent to OECD TG 451.*

F344/N rats	**Male**						**Female**					
Dose (mg/kg bw/day)	0	6	20	60	HCD (corn oil)	HCD (all routes)	0	6	20	60	HCD (corn oil)	HCD (all routes)
Number of animals	50	50	50	50	299		50	50	50	50	300	
Liver												
Hepatocellular adenoma	0	0	2	2	1 (0-2)	1.4 (0-6)	0	2	2	6	0.3 (0-2)	0.9 (0-4)
Hepatocellular carcinoma	0##	0	2	**12****	0	0.4 (0-4)	0##	0	0	**8***	0	0.1 (0-2)
H. adenoma or carcinoma	0##	0	4	**12****	1 (0-2)	1.8 (0-6)	0##	2	2	**14****	0.3 (0-2)	1.0 (0-4)
Nasal cavity												
Glands, olf. epith., adenoma	0	0	0	2	0	0					0	0.1 (0-2)
Transitional epith., adenoma	0##	6	4	**22****	0	0	0	2	0	4	0	0.1 (0-2)
Transitional epith., carcinoma	0	0	0	4	n.a.	n.a.						
Trans. epith. adenoma or carcinoma	0##	6	4	**27****	n.a.	n.a.						
Thyroid Gland												
Follicular cell adenoma	2	0	2	6	2 (0-4)	1 (0-6)	2	2	4	0	1 (0-2)	0.7 (0-2)
Follicular cell carcinoma	0	4	2	4	1 (0-4)	0.8 (0-4)						
F. cell adenoma or carcinoma	2	4	4	8	3 (0-6)	1.9 (0-6)						

B6C3F1/N mice	**Male**						**Female**					
Dose (mg/kg bw/day)	0	6	20	60	HCD		0	6	20	60	HCD	
Number of animals	50	50	50	50	350		50	50	50	50	347	
Liver												
Hepatocellular adenoma	58	68	74	72	51.7 (44-62)	57.3 (24-78)	17##	19	**37****	**44****	32.6 (6-34)	31.8 (2-78)
Hepatocellular carcinoma	44##	50	60	**72****	33.1 (16-44)	34.7 (16-56)	6##	**13***	**18****	**31****	8.3 (2-18)	12.1 (0-46)
Hepatocellular adenoma or carcinoma	76##	88	**94****	**96****	68.3 (56-78)	73.5 (52-90)	20##	25	**42****	**45****	26.2 (8-40)	37.2 (6-82)
Hepatoblastoma	2	10	**20****	**16***	4.0 (0-8)	5.3 (0-34)	0#	1	0	**4***	0.3 (0-2)	0.3 (0-2)
Hepatocellular adenoma, carcinoma, or hepatoblastoma	76##	90	**96****	**96****	69.1 (58-78)	74.2 (52-92)	20##	26	**42****	**45****	26.2 (8-40)	37.2 (6-82)
Lung												
Alveolar/bronchiolar adenoma	22	32	36	20	15.1 (10-22_	15.0 (2-30)	4##	8	**16***	**24****	4.6 (0-8)	5.0 (0-12)
Alveolar/bronchiolar carcinoma	4	6	0	8	8.0 (4-22)	12.5 (4-24)	0	2	4	2	2.0 (0-4)	3.7 (0-14)
Adenoma or carcinoma	26	38	36	24	22.0 (14-34)	26.2 (14-40)	4##	10	**18***	**26****	6.7	
Forestomach												
Squamous cell papilloma	2	2	0	6	n.a.	n.a.	2	10	**12***	**14***	3.5 (2-6)	1.8 (0-6)
Squamous cell carcinoma							0	2	0	0	0	0.1 (0-2)
Squamous cell papilloma or carcinoma							2	12	**12***	**14***	3.5 (2-6)	1.9 (0-6)

n.a. Not available.

The DS summarised that treatment with DMPT caused neoplastic lesions in liver (both sexes) and nasal cavity (males) of rats and in lung (females), liver (both sexes) and forestomach (females) of mice. In addition to neoplastic lesions, non-neoplastic lesions occurred, partly as pre-neoplastic effects at lower doses or in only one species/sex. Although most of the neoplastic lesions occurred at the high dose (60 mg/kg bw/day), 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.

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.

Regarding mode of action (MoA), two MoAs are presented that could contribute to the tumour formations. 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. The MoA and target tissues (e.g. liver) are relevant to humans. Oral DMPT exposure in humans leads to acute methaemoglobinaemia, MoA in humans and study animals seems comparable, at least for methaemoglobinaemia induced toxic effects.

Treatment related cancer incidences occurred with high dose in both species (males/females), with mid dose in mice (males/females), and with low dose in female mice. Body weight gain at high dose was reduced in both species (males/females), with larger reduction in mice; at mid dose reduced also in male mice. Survival was reduced at high dose in rats (males/females) and female mice compared to control groups. Statistically significant neoplastic incidences at doses below a potential MTD (i.e. body weight gain difference below 10%; high dose) were observed in female mice in liver (e.g. hepatocellular carcinoma), lung (adenoma) and forestomach (papilloma). Pre-neoplastic lesions in these organs occurred already at lower doses.

The arguments discussed above are conclusive for classification as carcinogen. The major arguments identified for classification in either Category 1B or Category 2 are summarised in the table below. Taken together the DS concluded that classification in Category 2 for carcinogenicity seems more appropriate than Category 1B, 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, and IARC (IARC, 2016) evaluated the available studies as "possibly carcinogenic to humans (Group 2B)".

***Table**: Identified arguments for a classification of DMPT as a Category 1B or 2 carcinogen (Table 41 from CLH report).*

Category 1B arguments	**Category 2 arguments**

• liver carcinoma in mice and rats, m/f
• dose-dependent progression to neoplasms
• pre-neoplastic lesions in all organs with neoplasms
• rare/uncommon tumour types
  ○ historical incidences for transitional epithelium adenomas or carcinomas (nose) are rare (rats, gavage studies)
  ○ hepatoblastoma are rare tumour types
• Plausible MoA, relevant for humans
  ○ metabolic generation of ROS and other radicals, methaemoglobinaemia, oxidative tissues damage
	• most neoplastic lesions only at highest dose
	• excessive toxicity - high dose potentially above MTD
	  ○ methaemoglobinaemia
	  ○ reduced body weight/body weight gain
	  ○ non-neoplastic lesions in several organs
	  ○ increased mortality (not explained by neoplasms)
	• single species/single sex neoplasms, i.e.
	  ○ nose (male rats)
	  ○ lung (female mice)
	  ○ forestomach (female mice)
	• liver (mice): high number of spontaneous incidences
	• non-genotoxic carcinogen

The DS concluded, based on chronic animal studies in mice and rats, genotoxicity studies and toxicokinetic data, that DMPT should be classified as a Category 2 carcinogen.

For setting an SCL, T25 values as measure for the intrinsic carcinogenic potency of DMPT were determined according to EU guidelines (directive 67/548/EEC). The lowest T25 values of 4.9 mg/kg bw/day were obtained for female mice with liver adenoma or carcinoma at mid 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, i.e. between 1 and 100 mg/kg bw/day; therefore, no SCL is required and the GCL applies.

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.

Comments received during consultation

Two MCSAs commented.

One MSCA summarised the various tumours induced by DMPT in the NTP studies:

- Liver tumours, mainly carcinomas, occurred in both sexes of rats and mice. These tumours occurred in rats at a dose associated with excessive toxicity (survival <50%). B6C3F1 mice are known to be very sensitive to liver tumorigenesis. It should be noted that in mice, the increased incidence of hepatoblastoma is statistically significant. This type of tumour is very rare and may not be combined with adenoma and hepatocarcinoma.
- Nasal cavity tumours occurred only in male rats, principally as adenomas, at a dose associated with excessive toxicity.
- Thyroid tumours occurred only in male rats, at a dose associated with excessive toxicity.
- Lung tumours occurred in mice, principally as adenomas.
- Forestomach tumours – squamous cell papilloma - occurred only in female mice.

The MSCA concluded that overall, the clearest evidence of carcinogenicity is principally the malignant liver tumours in both sexes and both species (all other tumours are rather benign). This can fulfil the criteria for Carc. Category 1B. However, considering the excessive toxicity at the highest tested dose in rats and the high sensitivity of mice, they agree that the proposed classification as Carc. Category 2 seems more appropriate.

Another MSCA considered that a Carc. Category 1B classification may be more appropriate. The MSCA argued that an increased incidence of tumours is observed mainly in animals exposed to the highest dose, with simultaneous general toxicity, including the weight gain clearly reduced. However, preneoplastic lesions are observed at all lower doses, in a dose-dependent manner, where animals show no signs of general toxicity. Furthermore, in female mice, liver tumours were statistically increased at lower doses without simultaneous general toxicity. They considered that the effects seen at the lower doses should be given more weight as the general toxicity appears to "obscure" the carcinogenic effect of this chemical at this high dose level. Some rare tumours were also observed in rats (nasal cavity, liver) and mice (hepatoblastoma), which adds further evidence to the carcinogenic potential of the substance.

The DS acknowledged both comments. Regarding the second MSCA, the DS generally agreed with the comment that the observed effects, low-dose pre-neoplasia and rare/uncommon tumour findings could also be considered for classification as Carc. Category 1B. However, in the CLH dossier, the DS weighted the arguments for Carc. Category 1B or Carc. Category 2 and came to the conclusion that classification as Carc Category 2 would be more appropriate: A number of uncertainties are present, e.g. most neoplastic lesions only appeared at the highest dose, with likely excessive general toxicity. The proposed MoA, i.e. non-genotoxic carcinogen with induction of severe methaemoglobinaemia, and the generally high number of spontaneous incidences of mice

liver tumours are further factors that should be considered, as well as the limitation of neoplasms (nose, lung, forestomach) to single species and sexes.

Assessment and comparison with the classification criteria

There are no data on long-term exposure and carcinogenicity of DMPT in humans. In animal experiments (a 2-year study with F344 rats and B6C3F1 mice), administration of DMPT by gavage resulted in increased incidences of neoplastic lesions in the liver of both species in both sexes. Other neoplastic lesions were found in nasal cavity (male rats), lung (female mice) and forestomach (female mice).

Liver tumours

Liver tumours, mainly carcinomas, occurred in both sexes in the rat at the high dose. At this dose, there was also general toxicity, with a survival lower than 50%. However, as can be seen in the NTP report of the study, both mortality and lower body weight occurred mainly at the later stages of the study period.

In B6C3F1 mice liver carcinomas occurred at the high dose in both sexes, but in females carcinomas also occurred at the low and mid dose. B6C3F1 mice are known to be very sensitive to liver tumorigenesis. However, incidences for hepatocellular carcinoma (72% high dose males, and 26%, 36%, 62% low, mid, high dose females respectively) are higher compared to HCD (33.1% male and 8.4% female mice). It should be noted that in mice, there was also an increased incidence of hepatoblastoma at the mid and high dose in males (10/50 and 8/50vs 1/50 in the control group), and at the high dose in female mice (4/50 vs 0/50); this is 20%, 16% and 8% compared to HCD of 4% and 0.29% for male and female mice respectively.

Nasal cavity tumours

Tumours in the nasal cavity occurred only in male rats, principally as adenomas, at the high dose associated with general toxicity. Low incidence of adenomas also occurs at the low and mid dose (with HCD of 0%).

Thyroid tumours

Follicular cell adenomas and carcinomas occurred in male rats, at the high dose associated with general toxicity, and with low incidence in female rats, but not in the top dose. No dose-response relationship is shown and the increases are not statistically significant.

Lung tumours

Alveolar and bronchiolar adenomas and carcinomas occurred in male and female mice. Especially in female mice, a dose-response relationship is seen in the adenomas.

Forestomach tumours

An increase in the incidence of squamous cell papilloma occurred only in female mice, which was statistically significant at the mid and high dose (above HCD and a dose-response is seen).

In summary, treatment-related cancer incidences occurred at the high dose (60 mg/kg bw/day) in both species in combination with a reduced survival (especially in rats and

female mice). Although general toxicity is present at the highest dose in the form of lower body weight and higher mortality, this occurs only at the end of the study period. This means that these effects are likely to coincide with the induction of tumours, and may be secondary to the carcinogenic effects. For this reason, RAC considers the tumours occurring at the high dose relevant for classification. Further, as noted above, cancer incidences were also increased at the mid dose in mice.

The potential mechanism behind the carcinogenicity is based on indirect effects via oxidative toxicity, e.g. by local ROS production in metabolically active tissues. DMPT also induces methaemoglobinaemia in rats and mice, through a metabolite that may also induce DNA adduct formation. Methaemoglobinaemia and related chronic toxic effects and/or oxidative DNA damage can both cause cancer development.

This MoA and the target tissues are relevant to humans. Oral DMPT exposure in humans leads to acute methaemoglobinaemia.

RAC concludes that, based on the dose-dependent induction of liver carcinomas in two species (mice and rats) in both sexes, dose-dependent progression to neoplasms, pre-neoplastic lesions in all organs with neoplasms, the induction of rare hepatoblastomas in mice and nasal cavity tumours in rats (above HCD) and the presence of a plausible MoA which is relevant to humans, **DMPT fulfils the criteria for Carc. Category 1B**.

Because the calculated T25 values (see above) were all in the medium potency range, between 1 and 100 mg/kg bw/day, **no SCL is required** and the GCL applies.

The route of exposure should not be specified, because there is no information that other route(s) of exposure besides the oral could not cause carcinogenicity.

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
2-year study Gavage (vehicle: corn oil; dosing volume 2.5 ml/kg) **Rats (F344/N)** **Reliable with restrictions** Males and females Equivalent to OECD TG 451 50 animals per sex and dose	*N,N*-Dimethyl-p-toluidine (CAS No. 99-97-8) Purity: > 99 % 0, 6, 20, or 60 mg/kg 5 days per week; 104 (♂) or 105 (♀) weeks	Neoplastic lesions (see Table 25) **Non-neoplastic lesions** (see Table 27, Table 28, and Table 31): • liver: hepatocellular hypertrophy (at 20 and 60 mg/kg, ♀/♂) • nasal cavity: hyperplasia of olfactory, respiratory, and transitional epithelia (♀/♂) • spleen: pigmentation, congestion, haematopoietic cell proliferation, hypertrophy, fibrosis (♀/♂) • kidney: severity of nephropathy ↑ (♀/♂) • bone marrow: hyperplasia (♀/♂) • forestomach: hyperplasia and ulcer (♂) • mesenteric lymph node (♂)	(NTP, 2012)
86 day study (clinical pathology group from 2-year study) Gavage (vehicle: corn oil; dosing volume 2.5 ml/kg) **Rats (F344/N)** **Reliable with restrictions** Males and females Equivalent to OECD TG 451 10 animals per sex and dose	*N,N*-Dimethyl-p-toluidine (CAS No. 99-97-8) Purity: > 99 % 0, 6, 20, or 60 mg/kg 5 days per week; 104 (♂) or 105 (♀) weeks	Haematological effects (at 20 and 60 mg/kg ♀/♂, see Table 29 and Table 45) • methaemoglobin ↑ • Heinz bodies ↑ • haematocrit ↓ • haemoglobin concentrations ↓ • erythrocyte counts ↓ Functional Hb reduced by more than 20 % compared to vehicle controls in males and females at 60 mg/kg bw/day.	(NTP, 2012)
2-year study Gavage (vehicle: corn oil; dosing volume 5 ml/kg) **Mice (B6C3F1/N)** **Reliable with restrictions** Males and females NTP internal guideline, equivalent to OECD TG 451 50 animals per sex and dose	*N,N*-Dimethyl-p-toluidine (CAS No. 99-97-8) Purity: > 99 % 0, 6, 20, or 60 mg/kg 5 days per week;105 weeks	Neoplastic lesions (see Table 32) **Non-neoplastic lesions** (see Table 34, Table 35, Table 36, Table 37, and Table 38) • liver (♀/♂): eosinophilic foci, hep. hypertrophy, necrosis (♀ only) • nasal cavity (♀/♂): metaplasia, hyperplasia and necrosis, nerve atrophy • olfactory lobe atrophy (♀/♂) • bone marrow hyperplasia, mesenteric lymph node and spleen red pulp atrophy (♀) • lung: alveolar histiocyte infiltration (♀/♂), necrosis (♀) • forestomach hyperplasia, inflammation, ulcer (♀)	(NTP, 2012)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
3-month study Gavage (vehicle: corn oil; dosing volume 2.5 ml/kg) **Rats (F344/N)** **Reliable with restrictions** Males and females NTP internal guideline, equivalent to OECD TG 408 10 animals per sex and dose (core study animals). MetHb and Hb were determined additionally at day 88 from core study animals. Additional clinical pathology groups of 10 male and 10 female rats received the same doses for only 25 days.	*N,N*-Dimethyl-p-toluidine (CAS No. 99-97-8) Purity: > 99 % 0, 62.5, 125, 250, 500, and 1,000 mg/kg 5 days per week; 14 weeks (98 days)	• no survival in the 1,000 mg/kg groups within the first week (♀/♂) • final mean body weights ↓ (> 10 %) with 125, 250, and 500 mg/kg (♂) • treatment-related non-neoplastic lesions in the liver, nasal cavity, spleen, kidney, and bone marrow with increased severity ≥125 mg/kg (see Table 43) • cyanosis, abnormal breathing, and lethargy at ≥ 250 mg/kg • haematology (see Table 44): methaemoglobinaemia and Heinz body formation → macrocytic, hypochromic, responsive anaemia	(NTP, 2012)
3-month study Gavage (vehicle: corn oil, dosing volume 5 ml/kg) **Mice (B6C3F1/N)** **Reliable with restrictions** Males and females NTP internal guideline, equivalent to OECD TG 408 10 animals per sex and dose	*N,N*-Dimethyl-p-toluidine (CAS No. 99-97-8) Purity: > 99 % 0, 15, 30, 60, 125, and 250 mg/kg 5 days per week; 14 weeks	• increased mortality at 125 and 250 mg/kg bw/day (♀/♂) • reduced body weights at 125 (♂) and 250 mg/kg bw/day (♀/♂) • abnormal breathing, thinness, lethargy, cyanosis, and ruffled fur in 125 and 250 mg/kg males and females • haematology: ○ f: no erythron changes up to 125 mg/kg ○ m: inconsistent and minor decreases in haematocrit values, haemoglobin concentrations, and erythrocyte counts and increased reticulocyte counts (60 mg/kg and greater)	(NTP, 2012)
Supporting studies			
No guideline study Oral gavage Male F344/N rats 5 animals/dose Liver examined for lesions and transcriptomic	*N,N*-Dimethyl-p-toluidine (CAS No. 99-97-8) Purity: > 99 % *0, 1, 6, 20, 60 or 120 mg/kg/day* *5 days*	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.	(Dunnick et al., 2017)

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, route of exposure, dose levels, duration of exposure</th><th colspan="2">Results</th><th>Reference</th></tr>
<tr><td>alterations</td><td colspan="4"><table>
<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>
<tr><td>DMPT</td><td></td><td></td><td></td><td></td><td></td><td></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][a]</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>
</table></td></tr>
<tr><td>No guideline study
Oral gavage
Male F344/N rats
5 animals/dose, further 5 for highest dose and control for frozen tissue collection and RNA extraction
Exploration of early changes in the nasal cavity after short-term exposure</td><td>*N,N*-Dimethyl-p-toluidine (CAS No. 99-97-8)
Purity: > 99 %
0, 1, 6, 20, 60 or 120 mg/kg/day
5 days</td><td colspan="2">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.
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.</td><td>(Dunnick et al., 2016)</td></tr>
</table>

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference

Table 1. Nasal Cavity Lesions in Male Rats after 5 Days of DMPT Exposure.

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)[a]	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)

Note: Five animals per group. DMPT = *N, N*-dimethyl-p-toluidine.
[a]Severity of lesion.

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[a] ((NTP, 2012))

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg
Male					
Liver[b]	10	10	10	10	10
Hepatocyte, Hypertrophy[c]	0	2 (1.0)[d]	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)

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg
Female					
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
[a] Data not shown for 1,000 mg/kg groups because all animals died during week 1.
[b] Number of animals with tissue examined microscopically
[c] Number of animals with lesion
[d] 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[a] ((NTP, 2012))

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg
Male					
n					
Day 25	10	10	9	10	8
Day 88	10	10	10	10	9
Week 14	10	10	10	10	9
Hematocrit (%)					
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**
Hemoglobin (g/dL)					
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**
Erythrocytes (10^6/µL)					
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**
Reticulocytes (10^6/µL)					
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**
Nucleated erythrocytes/100 leukocytes					
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**
Mean cell volume (fL)					
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**
Mean cell hemoglobin (pg)					
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**
Mean cell hemoglobin concentration (g/dL)					
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**
Methemoglobin (g/dL)					
Day 25	0.35 ± 0.03	0.90 ± 0.04**	1.56 ± 0.04**[b]	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**
Methemoglobin (% hemoglobin)					
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[c]	10.10 ± 0.55**	15.50 ± 0.48**	18.20 ± 0.53**	17.67 ± 0.71**
Heinz bodies (% erythrocytes)					
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
Female					
n					
Day 25	10	10	10	10	10
Day 88	9	10	10	10	10
Week 14	10	10	9	10	10
Hematocrit (%)					
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**
Hemoglobin (g/dL)					
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**
Erythrocytes (10^6/µL)					
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**
Reticulocytes (10^6/µL)					
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**
Nucleated erythrocytes/100 leukocytes					
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**
Mean cell volume (fL)					
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**
Mean cell hemoglobin (pg)					
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**
Mean cell hemoglobin concentration (g/dL)					
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**
Methemoglobin (g/dL)					
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**
Methemoglobin (% hemoglobin)					
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[d]	11.20 ± 0.44**	17.22 ± 1.18**[c]	19.70 ± 0.62**	16.00 ± 0.42**
Heinz bodies (% erythrocytes)					
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
[a] 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.
[b] n=10
[c] n=9
[d] 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 [a]

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
n	10	10	10	10
Male				
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 (10^6/µL)	9.10 ± 0.10	9.02 ± 0.06	8.53 ± 0.04**	7.61 ± 0.06**
Reticulocytes (10^6/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 (10^3/µL)	645.4 ± 27.5	682.6 ± 7.8	721.4 ± 18.4**	722.0 ± 26.0*
Leukocytes (10^3/µL)	9.44 ± 0.49	9.91 ± 0.45	9.99 ± 0.51	9.31 ± 0.58
Segmented neutrophils (10^3/µL)	1.38 ± 0.09	1.42 ± 0.04	1.42 ± 0.09	1.50 ± 0.05
Lymphocytes (10^3/µL)	7.70 ± 0.42	8.10 ± 0.41	8.18 ± 0.41	7.46 ± 0.52
Monocytes (10^3/µL)	0.23 ± 0.02	0.26 ± 0.02	0.24 ± 0.02	0.20 ± 0.02
Basophils (10^3/µL)	0.062 ± 0.007	0.071 ± 0.006	0.079 ± 0.012	0.075 ± 0.009
Eosinophils (10^3/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**
Female				
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 (10^6/µL)	8.50 ± 0.09	8.31 ± 0.10	7.88 ± 0.08**	6.95 ± 0.09**
Reticulocytes (10^6/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 (10^3/µL)	597.4 ± 46.6	583.1 ± 46.9	578.8 ± 49.0	719.3 ± 31.9
Leukocytes (10^3/µL)	8.04 ± 0.35	8.65 ± 0.22	8.59 ± 0.56	7.46 ± 0.38
Segmented neutrophils (10^3/µL)	1.40 ± 0.10	1.51 ± 0.11	1.52 ± 0.15	0.95 ± 0.11
Lymphocytes (10^3/µL)	6.29 ± 0.30	6.76 ± 0.26	6.74 ± 0.44	6.24 ± 0.33
Monocytes (10^3/µL)	0.21 ± 0.01	0.24 ± 0.02	0.18 ± 0.02	0.15 ± 0.01*
Basophils (10^3/µL)	0.060 ± 0.007	0.054 ± 0.003	0.065 ± 0.009	0.052 ± 0.006
Eosinophils (10^3/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
[a] 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
Male					
Liver[b]	10	10	10	10	10
Hepatocyte, Vacuolization Cytoplasmic[c]	9 (2.0)[d]	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)
Female					
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≤0.05) from the vehicle control group by the Fisher exact test
** P≤0.01
[a] Data not shown for 250 mg/kg groups because of mortality during week 1 and week 2.
[b] Number of animals with tissue examined microscopically
[c] Number of animals with lesion
[d] 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[a] (NTP, 2012)

	Vehicle Control	15 mg/kg	30 mg/kg	60 mg/kg	125 mg/kg
Male					
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 (10^6/µL)	10.82 ± 0.18	10.18 ± 0.14*	10.63 ± 0.15	10.14 ± 0.12*	10.27 ± 0.10
Reticulocytes (10^6/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**
Female					
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 (10^6/µL)	10.42 ± 0.11	10.13 ± 0.15	10.57 ± 0.14	10.41 ± 0.07	10.64 ± 0.12
Reticulocytes (10^6/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≤0.05) from the vehicle control group by Dunn's or Shirley's test
** P≤0.01
[a] 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 ≥20 % or reduction in functional Hb at ≥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) [a]	Hb meas. (g/dL)	Hb (% of control)	MetHb (g/dL)	MetHb (% Hb)	MetHb (% of MetHb fraction in control)	Hb calc. (g/dL) [b]	funct. Hb (g/dL) [c]	funct. Hb (% of control)
rat	10	0.0	0.0	15.3	**100.0**	0.35	2.40	100.0	14.6	14.2	**100.0**
male	10	62.5	12.4	13.3**	**86.9**	0.90**	6.70**	279.2	13.4	12.5	**88.1**
3-month	10	125.0	24.8	12.5**	**81.7**	1.56**	12.44**	518.3	12.5	11.0	**77.1**
day 25	10	250.0	49.6	11.8**	**77.1**	1.95**	16.60**	691.7	11.7	9.8	**68.8**
	8	500.0	99.2	11.0**	**71.9**	1.63**	14.75**	614.6	11.1	9.4	**66.2**
rat	10	0.0	0.0	14.8 [d]	**100.0**	0.38	2.44	100.0	15.6	15.2	**100.0**
male	10	62.5	43.7	13.0 [d] **	**87.8**	1.37**	10.10**	413.9	13.6	12.2	**80.3**
3-month	10	125.0	87.3	13.0 [d] **	**87.8**	1.95**	15.50**	635.2	12.6	10.6	**70.0**
day 88	10	250.0	174.6	12.9 [d] **	**87.2**	2.29**	18.20**	745.9	12.6	10.3	**67.7**
	9	500.0	349.2	12.7 [d] **	**85.8**	2.03**	17.67**	724.2	11.5	9.5	**62.3**
rat	10	0.0	0.0	15.1	**100.0**	0.37	2.70	100.0	13.7	13.3	**100.0**
female	10	62.5	12.4	13.3**	**88.1**	0.86**	6.40**	237.0	13.4	12.6	**94.3**
3-month	10	125.0	24.8	12.8**	**84.8**	1.63**	12.80**	474.1	12.7	11.1	**83.3**
day 25	10	250.0	49.6	11.7**	**77.5**	1.86**	16.00**	592.6	11.6	9.8	**73.2**
	10	500.0	99.2	10.8**	**71.5**	1.65**	15.50**	574.1	10.6	9.0	**67.5**
rat	9	0.0	0.0	14.8 [d]	**100.0**	0.38	2.88	100.0	13.2	12.8	**100.0**
female	10	62.5	43.7	12.8 [d] **	**86.5**	1.49**	11.20**	388.9	13.3	11.8	**92.2**
3-month	10	125.0	87.3	12.7 [d] **	**85.8**	2.20**	17.22**	597.9	12.8	10.6	**82.5**
day 88	10	250.0	174.6	12.0 [d] **	**81.1**	2.49**	19.70**	684.0	12.6	10.1	**79.2**
	10	500.0	349.2	12.4 [d] **	**83.8**	1.75**	16.00**	555.6	10.9	9.2	**71.7**
rat	10	0.0	0.0	16.0	**100.0**	0.77	4.70	100.0	16.4	15.6	**100.0**
male	10	6.0	4.1	15.6*	**97.5**	0.88*	5.60*	119.1	15.7	14.8	**95.0**
2-year	10	20.0	13.7	14.7**	**91.9**	1.14**	7.90**	168.1	14.4	13.3	**85.1**
day 86	10	60.0	41.0	13.2**	**82.5**	2.30**	17.40**	370.2	13.2	10.9	**69.9**
rat	10	0.0	0.0	15.8	**100.0**	0.80	5.10	100.0	15.7	14.9	**100.0**
female	10	6.0	4.1	15.1*	**95.6**	0.87	5.60	109.8	15.5	14.7	**98.5**
2-year	10	20.0	13.7	14.4**	**91.1**	1.21**	8.40**	164.7	14.4	13.2	**88.6**
day 86	10	60.0	41.0	13.2**	**83.5**	2.26**	17.10**	335.3	13.2	11.0	**73.6**
mouse	10	0.0	0.0	16.4	**100.0**	0.35	2.10	100.0	16.7	16.3	**100.0**
male	10	15.0	10.5	15.5	**94.5**	0.36	2.50	119.0	14.4	14.0	**86.0**
3-month	10	30.0	21.0	16.0	**97.6**	0.42*	2.80**	133.3	15.0	14.6	**89.4**
day 88	10	60.0	41.9	15.0**	**91.5**	0.47**	3.10**	147.6	15.2	14.7	**90.0**
	7	125.0	87.3	15.3**	**93.3**	0.61**	4.00**	190.5	15.3	14.6	**89.7**
	1	250.0	174.6	15.7	**95.7**	0.90	6.00	285.7	15.0	14.1	**86.4**
mouse	10	0.0	0.0	15.8	**100.0**	0.32	2.1	100.0	15.2	14.9	**100.0**
female	9	15.0	10.5	15.5	**98.1**	0.34	2.2	105.7	15.3	15.0	**100.4**
3-month	10	30.0	21.0	16.1	**101.9**	0.43**	2.6*	123.8	16.5	16.1	**108.0**
day 88	10	60.0	41.9	15.7	**99.4**	0.53**	3.4**	161.9	15.6	15.1	**100.9**
	8	125.0	87.3	16.1	**101.9**	0.58**	3.9**	184.8	14.9	14.4	**96.3**

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

** P≤0.01

[a] 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)*

[b] *Hb calc. = MetHb (g/dl) *100/MetHb (% of Hb)*

[c] *Functional Hb = Hb (g/dl) – MetHb (g/dl)*

[d] 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) [a]	OE degeneration [b]	(Severity)
rat	10	0.0	0	0	
male	10	**62.5**	**44**	**5***	**(1.0)**
3-month	10	**125.0**	**87**	**10****	**(2.5)**
day 88	10	250.0	175	10**	(3.0)
	10	500.0	349	10**	(3.1)
rat	10	0.0	0	0	
female	10	**62.5**	**44**	**7****	**(1.3)**
3-month	10	**125.0**	**87**	**10****	**(2.1)**
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	**125.0**	**87**	**9****	**(2.3)**
mouse	10	0.0	0	0	
female	10	15.0	10	0	
3-month	10	30.0	21	0	
day 88	10	**60.0**	**42**	**5***	**(1.8)**
	10	**125.0**	**87**	**8****	**(2.5)**

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

** P≤0.01

[a] 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. dose = dose * (5 / 7) * (88/90)*

[b] 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. [a]: 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. [b]: 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) [a]	Effective dose when extrapolated to 90-day exposure (mg/kg/d) [b]	Relevant parameter (reduction >20 % compared to control)	Classification supported by the study
(NTP, 2012), rat, male, 3-month study	125 250 500	25 25 25	24.8 49.6 99.2	funct. Hb funct. Hb, Hb funct. Hb, Hb	STOT RE 2 STOT RE 2 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	250 500	25 25	49.6 99.2	funct. Hb, Hb funct. Hb, Hb	STOT RE 2 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 ≤ 10 mg/kg bw/d for Category 1 and ≤ 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: 3.9.2.7.3criteria (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 ≥ 20 % or a reduction in functional Hb at ≥ 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.

RAC evaluation of specific target organ toxicity – repeated exposure (STOT RE)

Summary of the Dossier Submitter's proposal

The uptake of DMPT results in acute methaemoglobinaemia (as demonstrated in two human cases).

In subchronic and chronic studies, treatment related neoplastic and non-neoplastic lesions are evidenced in several organs of rats and mice; affected organs are liver, epithelia in the nasal cavity, spleen, kidney, and bone marrow.

Chronic and sub-chronic studies were evaluated for the assessment of potential STOT RE hazards with focus on haematotoxicity. The nasal tissue effects were addressed as additional target organ for STOT RE.

***Table**: Overview of repeated dose toxicity studies (NTP, 2012). Only non-neoplastic lesions reported. Only key studies included.*

Study	dose (mg/kg bw/day)	Results
rat F344/N, male/female (M/F), n=50, 2-year study Equiv. OECD TG 451	0, 6, 20, 60 gavage 5 days/week	At 6 mg/kg bw: - nasal cavity: metaplasia (F/M)/hyperplasia (M) of respiratory epithelia - spleen: pigmentation, hematopoietic cell proliferation (M) and congestion, hematopoietic cell proliferation fibrosis (F) - kidney: nephropathy (F), pigmentation (M) ▪ at 20 mg/kg bw: - liver: hepatocellular hypertrophy - nasal cavity: metaplasia/hyperplasia of respiratory and transitional epithelia (F/M) - spleen: pigmentation, hematopoietic cell proliferation (M) and congestion, hematopoietic cell proliferation fibrosis (F) - kidney: nephropathy (F), pigmentation (M) - bone marrow: hyperplasia (M) - forestomach: hyperplasia and ulcer (M) - mesenteric lymph node (M) at 60 mg/kg bw: - liver: hepatocellular hypertrophy (F/M) - nasal cavity: metaplasia/hyperplasia of olfactory, respiratory, and transitional epithelia (F/M) - spleen: pigmentation, congestion, hematopoietic cell proliferation, hypertrophy, fibrosis (F/M) - kidney: nephropathy, pigmentation (F/M) - bone marrow: hyperplasia (F/M) - forestomach: hyperplasia and ulcer (M) - mesenteric lymph node: histiocyte cellular infiltration (M)
rat F344/N, male/female, n=10, 86-day study	0, 6, 20, 60 gavage 5 days/week	Haematological effects at 20 and 60 mg/kg bw; both males and females: - methaemoglobin ↑ - Heinz bodies ↑ - haematocrit ↓ - haemoglobin concentrations ↓ - erythrocyte counts ↓ Functional Hb reduced by more than 20 % compared to vehicle controls in males and females at 60 mg/kg bw.
mouse B6C3F1/N, male/female,	0, 6, 20, 60 gavage 5 days/week	At 6 mg/kg bw: - liver: hepatocyte hypertrophy (M/F), necrosis (F) - nasal cavity: metaplasia of olfactory epithelia (F) - kidney: nephropathy (F), pigmentation (M)

n=50, 2-year study Equiv. OECD TG 451		- bone marrow: hyperplasia (F) ▪ at 20 mg/kg bw: - liver: hepatocyte hypertrophy, eosinophilic foci (M/F) - nasal cavity: metaplasia/hyperplasia of olfactory and respiratory epithelia (F) - spleen: red pulp atrophy (M) - kidney: nephropathy (F), pigmentation (M) - bone marrow: hyperplasia (F) - forestomach: hyperplasia (F) ▪ at 60 mg/kg bw: - liver: hepatocyte hypertrophy, eosinophilic foci (M/F), necrosis (F) - nasal cavity: metaplasia, hyperplasia and necrosis, nerve atrophy (M/F) - olfactory lobe atrophy (F/M) - spleen: red pulp atrophy (F) - kidney: nephropathy, pigmentation (F/M) - bone marrow: hyperplasia (F) - forestomach: hyperplasia and ulcer (M) - mesenteric lymph node atrophy (F) - lung: alveolar histiocyte infiltration (F/M), necrosis (F) - forestomach: hyperplasia, inflammation, ulcer (F)
Rat F344/N, male/female, n=10 3-month study Equiv. OECD TG 408 Also N=10 per dose for only 25 days	0, 62.5, 125, 250, 500, 1,000 gavage 5 days/week	- no survival in the 1,000 mg/kg bw groups within first week (M/F) - decreased final mean bw (>10%) at 125, 250, 500 mg/kg bw (M) - cyanosis, abnormal breathing, and lethargy at ≥ 250 mg/kg bw - Liver: pigmentation (all doses M/F), hypertrophy (≥125 mg/kg bw M/F), necrosis (≥250 F) - Nasal cavity: hyperplasia/metaplasia of respiratory epithelium (≥62.5 M; ≥125 F), degeneration (all doses M/F), metaplasia of olfactory epithelium (≥250 M/F), hyperplasia glands (≥125 M/F) - Spleen: congestion (all dose levels M, ≥ 125 F), hypertrophy, fibrosis, atrophy (≥250 M/F) - Kidney: pigmentation (all dose levels M/F), nephropathy (≥125 F), mineralisation and necrosis (⩾ 250 M) - bone marrow: hyperplasia (all dose levels M/F) - haematology: decrease in Hb, methaemoglobinemia, Heinz body formation (all dose levels M/F) (also present at day 25)
mouse B6C3F1/N, male/female, n=10 3-month study Equiv. OECD TG 408	0, 15, 30, 60, 125, 250 gavage 5 days/week	- increased mortality at 125 and 250 mg/kg bw (F/M) - reduced body weights at 125 (F) and 250 mg/kg bw (F/M) - abnormal breathing, thinness, lethargy, cyanosis, and ruffled fur in 125 and 250 mg/kg bw (M/F) - at 125 mg/kg bw (M/F): - lung: bronchiole epithelium degeneration - nose: degeneration/metaplasia of olfactory epithelium, hyperplasia glands - thymus: necrosis - haematology: decrease in Hb (≥ 60 M), methaemoglobin increase (≥ 30 M/F), Heinz bodies increase (≥125 M, ≥60 F)

Haematology

Haematology data were obtained after about 4 weeks and 3 months repeated administration (5 days/week oral gavage; NTP, 2012) in rats and mice. In particular, methaemoglobin levels were determined at day 25 (3-month study, rats, separate group of animals), day 86 (part of 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 compared to mice.

At doses relevant for classification, Hb levels were reduced by up to 28% compared to vehicle controls. For the comparison with guidance values, the DS corrected the dosing in

the CLH report, as dosing in the studies was 5 days per week, and for some 86 or 88 days instead of 90 days. 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%. 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.

Nasal tissue effects

Oral gavage of DMPT in subchronic and chronic mouse and rat studies induced dose-dependent effects on nasal tissues, e.g. dilatation, hyperplasia, metaplasia, nerve atrophy and necrosis in respiratory epithelia and olfactory epithelia.

In the 2-year rat and mice studies, the non-neoplastic effects (dilatation, hyperplasia, metaplasia, necrosis) in olfactory, respiratory and transitional epithelium occurred mainly at the high dose (60 mg/kg bw/day). Additionally, respiratory epithelia hyperplasia was already present at the low and mid (6 and 20 mg/kg bw/day) dose in male rats and respiratory epithelia metaplasia in female rats (Table 28 CLH report). Further, olfactory epithelia hyperplasia was already present at the low and mid (6 and 20 mg/kg bw/day) dose in the female mice (Table 37 CLH report). Additionally, chronic exposure resulted in neoplastic lesions.

In the 3-month studies, olfactory epithelia degeneration (see Table below) and respiratory epithelia and olfactory epithelia metaplasia or hyperplasia occurred in both rats and mice at 125 mg/kg bw/day. Olfactory epithelia degeneration was also observed in female mice at 60 mg/kg bw/day and rats at 62.5 mg/kg bw/day.

In conclusion, treatment related and dose-dependent effects on nasal tissues are observed after oral gavage to DMPT. The nasal cavity is a target organ of DMPT, and repeated exposure induces effects on nasal tissues such as hyperplasia, metaplasia, and (for STOT RE important) degeneration.

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 to be evaluated under carcinogenicity. Therefore, the DS did not consider the effects on other organs for STOT RE classification.

In summary

The DS concluded that in the NTP studies in rats, DMPT induced methaemoglobinaemia with a reduction of total Hb or functional Hb by more than 20% compared to vehicle controls. These data are 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.

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/day (rats and

female mice) and about 90 mg/kg bw/day (male mice). The incidences and/or the severity of the lesions are dose dependent.

The DS concluded that 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/day in oral gavage rat and mouse studies,

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

Setting a specific concentration limit (SCL) for DMPT is not justified, 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. No SCL is set, the generic concentration limit (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.

Comments received during consultation

One MSCA commented. The MSCA agreed with the proposed classification as STOT RE 2. Nevertheless, the need to adjust the effective dose considering the frequency of exposure (from 5 days/week to continuous administration) was questioned, even if there is no impact on overall conclusion.

The DS responded that according to OECD Test Guideline 408, "the animals are dosed with the test chemical daily seven days each week for at least 90 days". Dosing in the NTP protocol is five days per week, on average over the study period, and the animals received a lower dose per week than reported. The DS therefore considered it necessary to calculate the corrected dose and to use these values for classification.

Assessment and comparison with the classification criteria

In animal subchronic and chronic studies, effects on liver, lungs, kidney, thyroid, spleen, forestomach, nasal cavity and bone marrow are noted. Three-month and 2-year studies with B6C3F1/N mice and F344/N rats are available (NTP, 2012), including an 86 days group within the 2-year studies and a 25-day investigation in the 3-month study, both on haematology.

RAC considered that it was not appropriate to correct the dose from the study with regard to 5 days dosing regimen (in all NTP studies) instead of 7 days, and to correct for the shorter duration (86 or 88 days instead of 90 days).

According to CLP criteria, significant adverse effects observed in an 28-day study at dose ranges $3<C\leq300$ mg/kg bw/day, in a 90-day repeated dose study at dose ranges $10<C\leq100$ mg/kg bw/day or in a 2-year study at dose ranges $1.25<C\leq12.5$ mg/kg bw/day, warrant classification for STOT RE in Category 2.

Liver

In the 3-month rat study, pigmentation occurred at all dose levels (starting at 62.5 mg/kg bw/day), and hypertrophy from 125 mg/kg bw/day and higher. In the 2-year studies, hepatocellular hypertrophy occurred at the mid and high dose, in both species and sexes (20 and 60 mg/kg bw/day). Of these effects, only pigmentation occurred below the guidance value for STOT RE 2. However, this effect on its own is usually an adaptive response and not sufficiently severe to warrant classification.

Lungs

In the 3-month rat study, no lung lesions were reported. In male and female mice alveolar histiocyte infiltration as well as necrosis (only female) was found in the 2-year study at the highest dose of 60 mg/kg bw/day, which is above the guidance value for classification.

Kidney

In the 3-month rat study, pigmentation in kidney was demonstrated at all dose levels (males and females), from 125 mg/kg bw/day also nephropathy was reported. No kidney effects were reported in the 3-month mice study. In the 2-year study in rats and mice, nephropathy (female) and pigmentation (male) in kidneys was reported at the low, mid and high doses (starting at 6 mg/kg bw/day). Although the effects at the lowest dose of 6 mg/kg bw/day occurred below the guidance value for classification, the effects at this dose were insufficiently severe (minimal to mild) to warrant classification for kidney toxicity. Furthermore, the observed pigmentation in kidney (haemosiderosis) is probably secondary to erythrolysis.

Nasal cavity

In the 3-month studies, degeneration in olfactory epithelium was observed in male and female rats starting at the low dose (62.5 mg/kg bw/day), showing a dose response in severity of the effect. At higher doses, also metaplasia of the olfactory epithelium as well as hyperplasia and metaplasia of the respiratory epithelium was observed. In mice, metaplasia and degeneration were reported from 60 mg/kg bw/day (female) and 125 mg/kg bw/day (male). Further, metaplasia, hyperplasia and necrosis were observed in olfactory and respiratory epithelia in female mice at all dose levels (starting at 6 mg/kg bw/day) and in male mice at the high dose in the 2-year studies. In the rats, metaplasia in respiratory epithelia were reported in males and females in the low dose, with even more effects at higher levels.

***Table**: Summary of repeated dose study results (OE degeneration) relevant for classification as STOT RE (nasal cavity) (NTP, 2012; adapted from Table 49 CLH report).*

Study	Dose (mg/kg bw/day)	Olfactory epithelia degeneration	(Severity)	Olfactory epithelia degeneration	(Severity)
		male		female	
male rat 3-month study	0 **62.5** 125 250 500	0 **5*** 10** 10** 10**	 **(1.0)** (2.5) (3.0) (3.1)	0 **7**** 10** 10** 10**	 **(1.3)** (2.1) (3.0) (3.0)
male mouse 3-month study	0 15 30 60	0 0 0 0		0 0 0 **5***	 **(1.8)**

	125	9**	(2.3)	8**	**(2.5)**

Values highlighted in **bold blue** are relevant for STOT RE classification according to CLP, 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.
* Significantly different (P≤0.05) from the vehicle control group by Dunn's or Shirley's test
** P≤0.01
b Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

These effects are considered relevant for classification as they already occurred at dose levels relevant for STOT RE (90-day study; 10<dose≤100 mg/kg bw/day), and were present in all studies, in mice and rats and both sexes.

Dunnick *et al*. (2016) investigated molecular changes in the nasal cavity after DMPT exposure in rats after 4 days of exposure. They found that the DMPT nasal transcript expression pattern was similar to that found in the rat nasal cavity after formaldehyde exposure, with over 1,000 transcripts in common. Molecular changes in the nasal cavity after DMPT exposure suggest that oxidative damage is a mechanism of the DMPT toxic and/or carcinogenic effects.

Haematology

Haematology data were obtained after about 4 weeks and 3 months repeated administration 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).

***Table**: Summary of haemoglobin and methaemoglobin parameters from sub-chronic and chronic NTP studies (NTP, 2012).*

species sex study type day	**n**	**dose (mg/kg bw/day)**	**Hb meas. (g/dL)**	**Hb (% of control)**	**MetHb (g/dL)**	**MetHb (% Hb)**	**MetHb (% of MetHb fraction in control)**	**Hb calc. (g/dL) [b]**	**funct. Hb (g/dL) [c]**	**funct. Hb (% of control)**
rat	10	0.0	15.3	**100.0**	0.35	2.40	100.0	14.6	14.2	**100.0**
male	10	62.5	13.3**	**86.9**	0.90**	6.70**	279.2	13.4	12.5	**88.1**
3-month	10	125.0	12.5**	**81.7**	1.56**	12.44**	518.3	12.5	11.0	**77.1**
day 25	10	250.0	11.8**	**77.1**	1.95**	16.60**	691.7	11.7	9.8	**68.8**
	8	500.0	11.0**	**71.9**	1.63**	14.75**	614.6	11.1	9.4	**66.2**
rat	10	0.0	14.8 [d]	**100.0**	0.38	2.44	100.0	15.6	15.2	**100.0**
male	10	62.5	13.0 [d] **	**87.8**	1.37**	10.10**	413.9	13.6	12.2	**80.3**
3-month	10	125.0	13.0 [d] **	**87.8**	1.95**	15.50**	635.2	12.6	10.6	**70.0**
day 88	10	250.0	12.9 [d] **	**87.2**	2.29**	18.20**	745.9	12.6	10.3	**67.7**
	9	500.0	12.7 [d] **	**85.8**	2.03**	17.67**	724.2	11.5	9.5	**62.3**
rat	10	0.0	15.1	**100.0**	0.37	2.70	100.0	13.7	13.3	**100.0**
female	10	62.5	13.3**	**88.1**	0.86**	6.40**	237.0	13.4	12.6	**94.3**
3-month	10	125.0	12.8**	**84.8**	1.63**	12.80**	474.1	12.7	11.1	**83.3**
day 25	10	250.0	11.7**	**77.5**	1.86**	16.00**	592.6	11.6	9.8	**73.2**
	10	500.0	10.8**	**71.5**	1.65**	15.50**	574.1	10.6	9.0	**67.5**
rat	9	0.0	14.8 [d]	**100.0**	0.38	2.88	100.0	13.2	12.8	**100.0**
female	10	62.5	12.8 [d] **	**86.5**	1.49**	11.20**	388.9	13.3	11.8	**92.2**
3-month	10	125.0	12.7 [d] **	**85.8**	2.20**	17.22**	597.9	12.8	10.6	**82.5**
day 88	10	250.0	12.0 [d] **	**81.1**	2.49**	19.70**	684.0	12.6	10.1	**79.2**
	10	500.0	12.4 [d] **	**83.8**	1.75**	16.00**	555.6	10.9	9.2	**71.7**
rat	10	0.0	16.0	**100.0**	0.77	4.70	100.0	16.4	15.6	**100.0**
male	10	6.0	15.6*	**97.5**	0.88*	5.60*	119.1	15.7	14.8	**95.0**
2-year	10	20.0	14.7**	**91.9**	1.14**	7.90**	168.1	14.4	13.3	**85.1**
day 86	10	60.0	13.2**	**82.5**	2.30**	17.40**	370.2	13.2	10.9	**69.9**
rat	10	0.0	15.8	**100.0**	0.80	5.10	100.0	15.7	14.9	**100.0**

female	10	6.0	15.1*	**95.6**	0.87	5.60	109.8	15.5	14.7	**98.5**
2-year	10	20.0	14.4**	**91.1**	1.21**	8.40**	164.7	14.4	13.2	**88.6**
day 86	10	60.0	13.2**	**83.5**	2.26**	17.10**	335.3	13.2	11.0	**73.6**
mouse	10	0.0	16.4	**100.0**	0.35	2.10	100.0	16.7	16.3	**100.0**
male	10	15.0	15.5	**94.5**	0.36	2.50	119.0	14.4	14.0	**86.0**
3-month	10	30.0	16.0	**97.6**	0.42*	2.80**	133.3	15.0	14.6	**89.4**
day 88	10	60.0	15.0**	**91.5**	0.47**	3.10**	147.6	15.2	14.7	**90.0**
	7	125.0	15.3**	**93.3**	0.61**	4.00**	190.5	15.3	14.6	**89.7**
	1	250.0	15.7	**95.7**	0.90	6.00	285.7	15.0	14.1	**86.4**
mouse	10	0.0	15.8	**100.0**	0.32	2.1	100.0	15.2	14.9	**100.0**
female	9	15.0	15.5	**98.1**	0.34	2.2	105.7	15.3	15.0	**100.4**
3-month	10	30.0	16.1	**101.9**	0.43**	2.6*	123.8	16.5	16.1	**108.0**
day 88	10	60.0	15.7	**99.4**	0.53**	3.4**	161.9	15.6	15.1	**100.9**
	8	125.0	16.1	**101.9**	0.58**	3.9**	184.8	14.9	14.4	**96.3**

Values highlighted in bold blue are relevant for STOT RE classification, e.g. reduction in Hb at ⩾20 % or reduction in functional Hb at ⩾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.

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

** P≤0.01

[b] *Hb calc. = MetHb (g/dl) *100/MetHb (% of Hb)*

[c] *Functional Hb = Hb (g/dl) – MetHb (g/dl)*

[d] At 14 weeks (~98 days)

MetHb levels were significantly increased by DMPT in both species starting with the dose of 6 mg/kg bw/day (male rat) and 30 mg/kg bw/day (mouse) at day 86. The methaemoglobinaemia associated changes in blood parameters were stronger in rats compared to mice. In rats, Hb levels were reduced to 23% at a dose of 250 mg/kg bw/day at day 25 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 of 33% at 125 mg/kg bw/day at day 25. 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, leading to haemolytic anaemia. Similar haematological effects were observed in mice, although the magnitude of changes was lower.

The adverse effects (reduced Hb, MetHb proportion of total Hb, and functional Hb reduction) are severe enough (reduction in functional Hb at ⩾20%), although they occur at borderline dose levels for classification and are highest at the earlier time points. Further, they are consistent in both species and sexes.

Regarding the organs affected in the repeated dose toxicity studies, RAC considered the designation as "respiratory tract" more appropriate than "nasal cavity" since effects in other parts of the respiratory tract cannot be excluded. In addition, the "respiratory tract" is a more comprehensible term and is more consistent with previous STOT RE classifications.

Regarding the effects on haematology, the primary effects were observed in the blood, while secondary effects were also seen in the organs involved in blood cell generation or removal (spleen, bone marrow). As the effects may occur in other organs than the blood itself, "blood system", as opposed to "blood" is the preferred designation for the target organ in the present case.

In conclusion, RAC considers that the effects described above with regard to methaemoglobinaemia and degeneration of olfactory epithelium fulfils the criteria for **classification as STOT RE Category 2; H373 for blood system and respiratory tract**.

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[a]

Study (Study Start)	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence: Corn Oil Gavage Studies			
N,N-Dimethyl-*p*-toluidine (October 2004)	0/50	0/50	0/50
Ginkgo biloba 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
β-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 ± standard deviation	1.0% ± 1.1%		1.0% ± 1.1%
Range	0%-2%		0%-2%
Overall Historical Incidence: All Routes			
Total (%)	18/1,249 (1.4%)	5/1,249 (0.4%)	23/1,249 (1.8%)
Mean ± standard deviation	1.4% ± 1.9%	0.4% ± 1.0%	1.8% ± 1.9%
Range	0%-6%	0%-4%	0%-6%

[a] Data as of May 2011

Table 52

Historical Incidence of Adenoma of the Nose in Control Male F344/N Rats[a]

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

[a] Data as of May 2011

Table 53

Historical Incidence of Follicular Cell Neoplasms of the Thyroid Gland in Control Male F344/N Rats[a]

Study (Study Start)	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence: Corn Oil Gavage Studies			
N,N-Dimethyl-*p*-toluidine (October 2004)	1/50	0/50	1/50
Ginkgo biloba 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
β-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 ± standard deviation	2.0% ± 1.3%	1.0% ± 1.7%	3.0% ± 2.1%
Range	0%-4%	0%-4%	0%-6%
Overall Historical Incidence: All Routes			
Total (%)	13/1,239 (1.1%)	10/1,239 (0.8%)	23/1,239 (1.9%)
Mean ± standard deviation	1.0% ± 1.7%	0.8% ± 1.5%	1.9% ± 2.2%
Range	0%-6%	0%-4%	0%-6%

[a] 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[a]

Study (Study Start)	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence: Corn Oil Gavage Studies			
N,N-Dimethyl-*p*-toluidine (October 2004)	0/50	0/50	0/50
Ginkgo biloba 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
β-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 ± standard deviation	0.3% ± 0.8%		0.3% ± 0.8%
Range	0%-2%		0%-2%
Overall Historical Incidence: All Routes			
Total (%)	11/1,200 (0.9%)	1/1,200 (0.1%)	12/1,200 (1.0%)
Mean ± standard deviation	0.9% ± 1.6%	0.1% ± 0.4%	1.0% ± 1.6%
Range	0%-4%	0%-2%	0%-4%

[a] Data as of May 2011

Table 55

Historical Incidence of Adenoma of the Nose in Control Female F344/N Rats[a]

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

[a] 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[a]

Study (Study Start)	Hepatocellular Adenoma	Hepatocellular Carcinoma	Hepatocellular Adenoma or Hepatocellular Carcinoma
Historical Incidence: Corn Oil Gavage Studies			
N,N-Dimethyl-*p*-toluidine (October 2004)	29/50	22/50	38/50
Ginkgo biloba 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
β-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 ± standard deviation	51.7% ± 6.9%	33.1% ± 10.5%	68.3% ± 8.9%
Range	44%-62%	16%-44%	56%-78%
Overall Historical Incidence: All Routes			
Total (%)	658/1,149 (57.3%)	399/1,149 (34.7%)	844/1,149 (73.5%)
Mean ± standard deviation	57.3% ± 12.6%	34.7% ± 10.8%	73.5% ± 11.3%
Range	24%-78%	16%-56%	52%-90%

	Hepatoblastoma	Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma
Historical Incidence: Corn Oil Gavage Studies		
N,N-Dimethyl-*p*-toluidine (October 2004)	1/50	38/50
Ginkgo biloba extract (March 2005)	3/50	39/50
Isoeugenol (May 2002)	3/50	30/50
Kava kava extract (August 2004)	0/50	38/50
β-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 ± standard deviation	4.0% ± 2.8%	69.1% ± 8.0%
Range	0%-8%	58%-78%
Overall Historical Incidence: All Routes		
Total (%)	61/1,149 (5.3%)	852/1,149 (74.2%)
Mean ± standard deviation	5.3% ± 7.1%	74.2% ± 11.5%
Range	0%-34%	52%-92%

[a] Data as of May 2011

Table 57

Historical Incidence of Alveolar/bronchiolar Neoplasms in Control Male B6C3F1/N Mice[a]

Study (Study Start)	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence: Corn Oil Gavage Studies			
N,N-Dimethyl-*p*-toluidine (October 2004)	11/50	2/50	13/50
Ginkgo biloba 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
β-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 ± standard deviation	15.1% ± 4.1%	8.0% ± 6.5%	22.0% ± 7.3%
Range	10%-22%	4%-22%	14%-34%
Overall Historical Incidence: All Routes			
Total (%)	172/1,150 (15.0%)	144/1,150 (12.5%)	301/1,150 (26.2%)
Mean ± standard deviation	15.0% ± 6.9%	12.5% ± 7.1%	26.2% ± 6.3%
Range	2%-30%	4%-24%	14%-40%

[a] 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[a]

Study (Study Start)	Hepatocellular Adenoma	Hepatocellular Carcinoma	Hepatocellular Adenoma or Hepatocellular Carcinoma
Historical Incidence: Corn Oil Gavage Studies			
N,N-Dimethyl-*p*-toluidine (October 2004)	17/50	6/50	20/50
Ginkgo biloba 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
β-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 ± standard deviation	21.6% ± 10.8%	8.3% ± 5.5%	26.2% ± 12.7%
Range	6%-34%	2%-18%	8%-40%
Overall Historical Incidence: All Routes			
Total (%)	380/1,195 (31.8%)	144/1,195 (12.1%)	444/1,195 (37.2%)
Mean ± standard deviation	31.8% ± 21.4%	12.1% ± 10.8%	37.2% ± 22.9%
Range	2%-78%	0%-46%	6%-82%

	Hepatoblastoma	Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma
Historical Incidence: Corn Oil Gavage Studies		
N,N-Dimethyl-*p*-toluidine (October 2004)	0/50	20/50
Ginkgo biloba extract (March 2005)	1/50	20/50
Isoeugenol (May 2002)	0/49	13/49
Kava kava extract (August 2004)	0/50	10/50
β-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 ± standard deviation	0.3% ± 0.8%	26.2% ± 12.7%
Range	0%-2%	8%-40%
Overall Historical Incidence: All Routes		
Total (%)	4/1,195 (0.3%)	444/1,195 (37.2%)
Mean ± standard deviation	0.3% ± 0.8%	37.2% ± 22.9%
Range	0%-2%	6%-82%

[a] Data as of May 2011

Table 59

Historical Incidence of Alveolar/bronchiolar Neoplasms in Control Female B6C3F1/N Mice[a]

Study (Study Start)	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence: Corn Oil Gavage Studies			
N,N-Dimethyl-*p*-toluidine (October 2004)	2/50	0/50	2/50
Ginkgo biloba 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
β-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 ± standard deviation	4.6% ± 3.1%	2.0% ± 2.0%	6.7% ± 3.2%
Range	0%-8%	0%-4%	2%-12%
Overall Historical Incidence: All Routes			
Total (%)	60/1,196 (5.0%)	44/1,196 (3.7%)	100/1,196 (8.4%)
Mean ± standard deviation	5.0% ± 3.6%	3.7% ± 3.3%	8.4% ± 4.3%
Range	0%-12%	0%-14%	2%-22%

[a] Data as of May 2011

Table 60

Historical Incidence of Squamous Cell Neoplasms of the Forestomach in Control Female B6C3F1/N Mice[a]

Study (Study Start)	Papilloma	Carcinoma	Papilloma or Carcinoma
Historical Incidence: Corn Oil Gavage Studies			
N,N-Dimethyl-*p*-toluidine (October 2004)	1/50	0/50	1/50
Ginkgo biloba 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
β-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 ± standard deviation	3.5% ± 1.5%		3.5% ± 1.5%
Range	2%-6%		2%-6%
Overall Historical Incidence: All Routes			
Total (%)	22/1,198 (1.8%)	1/1,198 (0.1%)	23/1,198 (1.9%)
Mean ± standard deviation	1.8% ± 1.7%	0.1% ± 0.4%	1.9% ± 1.6%
Range	0%-6%	0%-2%	0%-6%

[a] Data as of May 2011

15 ABBREVIATIONS

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