Committee for Risk Assessment

RAC

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

Adopted

10 June 2021

10 June 2021

CLH-O-0000007005-83-01/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name: ***N*,*N*-dimethyl-*p*-toluidine**

EC Number: **202-805-4**

CAS Number: **99-97-8**

The proposal was submitted by **Germany** and received by RAC on **26 May 2020.**

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

Germany has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **22 June 2020**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **21 August 2020**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Gerlienke Schuur**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on **10 September 2020** by **consensus**.

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	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	612-056-00-9	*N,N-d*imethyl-*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	GHS08 GHS06 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	**Add** Carc. 2 **Modify** Acute Tox. 4 Acute Tox. 3 STOT RE 2 **Remove** Acute Tox. 3	**Add** H351 **Modify** H332 H301 H373 (blood*; nasal cavity) **Remove** H311	**Retain** GHS08 GHS06 Dgr	**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
RAC opinion	612-RST-VW-Y	*N*,*N*-dimethyl-*p*-toluidine	202-805-4	99-97-8	**Add** Carc. 1B **Modify** Acute Tox. 4 Acute Tox. 3 STOT RE 2 **Remove** Acute Tox. 3	**Add** H350 **Modify** H332 H301 H373 (blood system; respiratory tract) **Remove** H311	**Retain** GHS08 GHS06 Dgr	**Add** H350 **Modify** H332 H301 H373 (blood system; respiratory tract) **Remove** H311		**Add** oral: ATE = 140 mg/kg bw inhalation: ATE = 1,4 mg/L (mists) **Remove** *	**Remove** C
Resulting Annex VI entry if agreed by COM	612-RST-VW-Y	*N*,*N*-dimethyl-*p*-toluidine	202-805-4	99-97-8	Carc. 1B Acute Tox. 4 Acute Tox. 3 STOT RE 2 Aquatic Chronic 3	H332 H301 H373 (blood system; respiratory tract) H350 H412	GHS08 GHS06 Dgr	H350 H332 H301 H373 (blood system; respiratory tract) H412		oral: ATE = 140 mg/kg bw inhalation: ATE = 1,4 mg/L (mists)	

*) In the text of the CLH report, the designated organ was noted as blood system.

GROUNDS FOR ADOPTION OF THE OPINION

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 and 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.

HUMAN HEALTH HAZARD EVALUATION

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 ($50< LD_{50} \leq 300$ 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 the existing classification should be removed**.

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)**.

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, n=50, 2-year study Equiv. OECD TG 451	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) - 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)

Study	dose (mg/kg bw/day)	Results
		- 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 **125**	0 0 0 0 9**	 (2.3)	0 0 0 **5*** 8**	 **(1.8)** **(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 $\geqslant$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**.

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)

Method, guideline, deviations if any	Information	Observations	Reference
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			

Method, guideline, deviations if any	Information	Observations	Reference
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**.

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****						

F344/N rats	Male					Female				
Dose (mg/kg bw/day)	0	6	20	60	HCD	0	6	20	60	HCD
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)

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)
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.

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).