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-1-naphthylaniline;

N-phenylnaphthalen-1-amine

EC Number:	**201-983-0**
CAS Number:	**90-30-2**
Index Number:	**-**

Contact details for dossier submitter:

BAuA
Federal Institute for Occupational Safety and Health
Federal Office for Chemicals
Friedrich-Henkel-Weg 1-25
44149 Dortmund, Germany

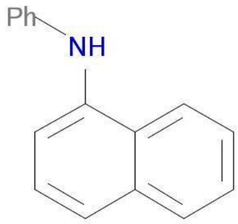
Version number:	**2.0**
Date:	**April 2022**

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-1-naphthylaniline; N-phenylnaphthalen-1-amine
Other names (usual name, trade name, abbreviation)	NPNA, PANA
EC number (if available and appropriate)	201-983-0
EC name (if available and appropriate)	N-1-naphthylaniline
CAS number (if available)	90-30-2
Molecular formula	$C_{16}H_{13}N$
Structural formula	
SMILES notation (if available)	C1=CC=C(C=C1)NC2=CC=CC3=CC=CC=C32
Molecular weight or molecular weight range	219.28 g/mol

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-phenylnaphthalen-1-amine CAS-No.: 90-30-2	100		

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: Proposed harmonised classification and labelling according to the CLP criteria

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	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	No entry										
Dossier submitters proposal	tba	***N*-1-naphthylaniline; *N*-phenylnaphthalen-1-amine**	201-983-0	90-30-2	Acute Tox. 4 Skin Sens. 1	H302 H317	GHS07 Wng	H302 H317		oral: ATE = 1231 mg/kg bw	
Resulting Annex VI entry if agreed by RAC and COM					Acute Tox. 4 Skin Sens. 1	H312 H317	GHS07 Wng	H312 H317		oral: ATE = 1231 mg/kg bw	

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 classes 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	**Acute Tox. 4, H302**	**Yes**
Acute toxicity via dermal route	**Data conclusive but not sufficient for classification**	
Acute toxicity via inhalation route	Data lacking	No
Skin corrosion/irritation	**Data conclusive but not sufficient for classification**	**Yes**
Serious eye damage/eye irritation		
Respiratory sensitisation	Hazard class not assessed in this dossier	No
Skin sensitisation	**Skin Sens. 1, H317**	**Yes**
Germ cell mutagenicity	Hazard class not assessed in this dossier	No
Carcinogenicity		
Reproductive toxicity		
Specific target organ toxicity-single exposure	**Data inconclusive**	**Yes**
Specific target organ toxicity-repeated exposure	**No classification proposed. But data considered as borderline for STOT RE 2, H373 (blood system, liver) classification**	
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

There currently no harmonised classification for *N*-1-naphthylaniline; *N*-phenylnaphthalen-1-amine (NPNA).

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

[B.] Justification that action is needed at Community level is required.

Reason for a need for action at Community level:

Differences in self-classification

Requirement for harmonised classification by other legislation or process.

Further details on the need of action at Community level

NPNA is manufactured and/or imported in the European Economic Area in 100 – 1 000 tonnes per year. Currently there is no harmonised classification for NPNA, however in a preceding and ongoing SEv (CoRAP 2012[1]) performed by the German CA, the need for harmonised classification was identified for acute toxicity, skin sensitisation and specific target organ toxicity following repeated exposure.

NPNA is self-classified by several notifiers. As reported on the ECHA dissemination site, there are in total a number of 1 628 notifiers (42 aggregated notifications) in the C&L inventory (as of 08. July 2021).

Notifications of the 1 628 notifiers for classification and labelling concerning human health are inconsistent and contradictory as seen below.

Acute Tox. 4 = 1 406/1 628
Skin Sens 1 = 1 434/1 628
Skin Sens 1A = 1/1 628
Skin Sens 1B = 114/1628
Skin Irrit. 2 = 8/1 628
Eye Irrit. 2 = 8/1 628
STOT SE 1 = 92/1 628
STOT SE 2 = 1/1 628
STOT SE 3 = 7/1 628
STOT RE 2 = 1145/1 628
Not classified = 69/1 628

Therefore, we consider a proposal for harmonised classification as justified.

5 IDENTIFIED USES

NPNA is used for the manufacture of rubber products. It has widespread uses by professional workers and is used in polymers, lubricants and greases, hydraulic fluids and metal working fluids.

6 DATA SOURCES

Sources: PUBMED, SCOPUS, WEB OF SCIENCE, ECHA dissemination site, IUCLID (registration data), January 2021

[1] https://echa.europa.eu/de/information-on-chemicals/evaluation/community-rolling-action-plan/corap-table/-/dislist/details/0b0236e1807e4181

7 PHYSICOCHEMICAL PROPERTIES

Table 8: Summary of physicochemical properties of NPNA.

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 1013 kPa	purple to brown solid	(Heinisch, 1974)	
Melting/freezing point	62 °C	(Sax and Lewis, 1987)	
Boiling point	335 °C at 558 mm Hg (equivalent to 744 hPa)	(Sax and Lewis, 1987)	
Relative density	1.16 g/cm³ at 20 °C	(Budavari, 2001)	
Vapour pressure	0.0011 Pa at 20 °C	(Budavari, 2001)	
Surface tension	n.a.		
Water solubility	3 mg/L at 20 °C	(Bayer AG, 1989)	
Partition coefficient n-octanol/water	4.28	(National Institute of Technology and Evaluation, 2002)	
Granulometry	D50: 73.49 µm (laser diffractometry) Maximum at 100 100 µm, mostly accompanied by a co-maximum at 1000 µm (agglomeration)	(Rhein Chemie Rheinau GmbH, 2009)	
Stability in organic solvents and identity of relevant degradation products	Good solubility in most organic solvents (e.g. benzene, methylene chloride, acetone and ethanol), soluble in petrol.	(Abele, 1971)	
Dissociation constant	4.93 at 25 °C	(Perrin, 1981)	
Viscosity			The viscosity does not need to be determined, as the substance is a solid. Testing is only appropriate for liquids.

8 EVALUATION OF PHYSICAL HAZARDS

Not assessed in this dossier.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

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

Syracuse (1981) examined absorption, metabolism, distribution and elimination of ^{14}C-NPNA in rats after oral gavage of 160 mg/kg body weight (bw). The test substance was well absorbed, almost completely metabolised, and excreted via faeces and urine. The maximum radioactivity was measured in plasma after 4 hours. After 24 hours, 20 % of the radioactivity was found in the gastrointestinal tract, 2.4 % in fat tissue, 0.4 % in the liver and 0.1 % in the kidneys. More than 90 % of the administered radioactive labelled carbon has been excreted within 48 hours after administration via faeces (60 %) and via urine (32 %). In the ether extract of the urine, using HPLC analysis, five ^{14}C-metabolites were determined while no unchanged NPNA was detected. The elimination half-lives were reported as 1.68 hours for the fast elimination and 33 hours for the slow elimination.

In an *in vit*ro metabolic study conducted with rat liver microsomes Syracuse (1981) detected mono- and di-hydroxylated derivates from NPNA, whereas Xuanxian and Wolff (1992) identified only a mono-hydroxylated metabolite in another *in vitro* study with rat hepatic microsomes. Syracuse (1981) suggested that the metabolism of NPNA is primarily via hydroxylation and subsequently undergoes O-glucoronidation or O-sulfation. In the mono-hydroxy derivate, the hydroxyl group is in the naphthalene moiety at the position para to the amino group and in the di-hydroxy derivate, at least one hydroxyl group is at the available para position in the naphthyl ring. Pre-treatment of male rats with phenobarbital or 3-methylcholanthrene increased the rate of microsomal metabolism of NPNA, indicating that more than one P-450 monooxygenase mediates the reaction (Xuanxian and Wolff, 1992).

Conclusion:

NPNA is well absorbed after oral gavage. Due to its rapid excretion, accumulation in the body is not expected. *In vitro* studies showed that the metabolisms occurs primary via hydroxylation.

10 EVALUATION OF HEALTH HAZARDS

10.1 Acute toxicity – oral

Table 9: 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, reliability
Rat					
LD50-Test, no guideline followed; GLP compliance: not specified	Rat, Sprague-Dawley, male (200 – 300 g), 5/dose	NPNA (CAS 90-30-2 / EC 201-983-0) Purity: no data	500, 1 000, 2 000, 4 000 mg/kg bw, single dose (gavage), 14 d post exposure observation period	1 625 mg/kg bw (male) Calculated by moving average interpolation method of Weil (1952) Mortality: 500 mg/kg: 0/5 animals, 1 000 mg/kg: 0/5 animals, 2 000 mg/kg: 4/5 animals, 4 000 mg/kg: 5/5 animals	AMR (1974) Key study (rel. 2)
Standard acute method, no guideline followed; no GLP compliance	Rat, Wistar, male (90 – 120 g), 5/dose	NPNA (CAS 90-30-2 / EC 201-983-0) Purity: no data	1 000, 2 000, 4 000 mg/kg bw, single dose (gavage), 14 d post exposure observation period	2 380 mg/kg bw (male) Calculated by moving average interpolation method of Weil (1952) Mortality: 1 000 mg/kg: 1/5 animals (9 days post-exposure), 2 000 mg/kg: 1/5 animals (1 day post-exposure), 4 000 mg/kg: 5/5 animals (all died 1 day post-exposure)	Union Carbide (1974) Supporting study (rel. 2)

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Value LD_{50}	Reference, reliability
LD50-Test, no guideline followed; GLP compliance: not specified	Rat, albino, male/female, 3/sex/dose	NPNA (CAS 90-30-2 / EC 201-983-0) Purity: commercial grade	200, 2 000 mg/kg bw, gavage, 14 d post exposure observation period	>2000 mg/kg bw (male) > 200 - < 2 000 mg/kg bw (female) Mortality: 200 mg/kg: 0 animals 2 000 mg/kg: 3/3 females and 1/3 males	Ciba-Geigy (1987b) Supporting study (rel. 2)
LD50-Test, no guideline followed; , no GLP compliance	Rat, Wistar, sex not specified (160 -180 g), 10/sex/dose	NPNA (CAS 90-30-2 / EC 201-983-0) Purity: no data	5 000 mg/kg bw, gavage, 28 d post exposure observation period	> 5 000 mg/kg bw No mortality observed.	Bayer (1978b) Disregarded due to major methodological deficiencies (rel. 4)
LD50-Test, no guideline followed; no GLP compliance	Rat, Wistar, female (160 -180 g), 10/ dose	NPNA (CAS 90-30-2 / EC 201-983-0) Purity: no data	5 000 mg/kg bw, gavage, 28 d post exposure observation period	> 5 000 mg/kg bw (female) Mortality: 5 000 mg/kg: 2/10 animals	Bayer (1978a) Disregarded due to major methodological deficiencies (rel. 4)
Mouse					
LD50-Test, no guideline followed; GLP compliance: not specified	Mouse, CF-1, male (20 – 30 g), 5/dose	NPNA (CAS 90-30-2 / EC 201-983-0) Purity: no data	500, 1 000, 2 000, 4 000 mg/kg bw, single dose (gavage), 14 d post exposure observation period	1 231 mg/kg bw (male) Calculated by moving average interpolation method of Weil (1952) Mortality: 500 mg/kg: 0/5 animals 1 000 mg/kg: 1/5 animals 2 000 mg/kg: 5/5 animals 4 000 mg/kg: 5/5 animals	AMR (1974) Supporting study (rel. 2)

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

None of the tests on acute oral toxicity were carried out in accordance with EU Regulation (EC) No 440/2008 or current OECD test guidelines (TG) for the acute oral testing of chemicals. However, by means of a weight of evidence approach the information is sufficient to conclude on acute oral toxicity of NPNA. Taking the lowest values estimated, an oral LD50 of 1 231 mg/kg bw was determined for mice and an LD50 of 1 625 mg/kg bw was established for rats (AMR, 1974).

10.1.2 Comparison with the CLP criteria

Acute oral toxicity relates to adverse effects occurring after single exposure to a substance. Acute toxicity classification is generally assigned on the basis of evident lethality and typically obtained from animal.

Substances can be allocated to one of four toxicity categories based on the criteria shown in the Table 3.1.1 of Annex I, Part 3, Table 3.1.1 of CLP for acute toxicity by the oral route. For the allocation in the fourth category, following criteria apply:

'Acute oral toxicity - *Category 4: 300 < ATE ≤ 2 000 mg/kg bw.'*

Based on the lowest oral LD50-values in animals (LD50 of 1 231 mg/kg bw in mice; LD50 of 1 625 mg/kg bw in rats) NPNA fulfils the criteria for classification for acute oral toxicity, Category 4.

10.1.3 Conclusion on classification and labelling for acute oral toxicity

The registrants concluded the substance is acute toxic (Cat. 4) and, based on the available information, the dossier submitter (DS) can support this conclusion. With an LD50 oral of 1 231 mg/kg bw NPNA has to be classified as **Acute Tox. 4, H302** (Harmful if swallowed), according to Annex VI of Regulation (EC) 1272/2008.

Regarding the derivation of the Acute Toxicity Estimate (ATE), the lowest LD50 of 1 231 mg/kg bw derived for mice could be used, although neither this study nor any of the other available studies were performed according to any validated guideline or conform with GLP. Alternatively, and taking the limited reliability of the available data into account, the converted acute toxicity point estimate (cATpE) of 500 mg/kg bw as indicated in CLP Regulation, Table 3.1.2, could be used. As this approach is considered rather conservative, and mortality was neither observed in rats nor in mice at ≤ 1 000 mg/kg bw in any of the available studies, **an ATE of 1 231 mg/kg bw is proposed** to be used in weight of evidence based on the lowest LD50 value of all available oral toxicity studies.

It has been noted that the studies with lowest LD50 were conducted in male animals only. The Ciba-Geigy (1987b) study indicated that female rats may be more sensitive than male rats, however, this finding is not supported by Bayer (1978a).

10.2 Acute toxicity - dermal route

Table 10: 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, reliability
LD50-Test, no guideline followed; no GLP compliance	Rabbit, albino, male, 2 animals at 2 000 mg/kg bw, 5 animals at 8 000 mg/kg bw.	NPNA (CAS 90-30-2 / EC 201-983-0) Purity: no data Vehicle: carbowax/ PEG400	2 000 and 8 000 mg/kg bw, epicutaneous, 24 h exposure, at least 8 days of post exposure observation (no further details reported)	> 8 000 mg/kg bw (male, 24 h exposure) Mortality: 2 000 mg/kg bw: 0/2 8 000 mg/kg bw: 1/5 animals (8 days post-exposure).	(Union Carbide, 1974) Key study (rel. 2)

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

The available acute dermal toxicity test (Table 10) does not comply with the current EU or OECD TGs for the testing of chemicals. Based on the applied doses, the test might be considered as a limit test. Compared to the method B.3 of EU Regulation (EC) No 440/2008, there are two main shortcomings i) the number of animals (rabbits) to provide sufficient statistical power was not reached (5 and 2 vs. 5 and 5 for each dose group) and ii) NPNA was only tested in male animals, while males and females are recommended for a limit test. The results obtained in this study indicated an LD_{50} of > 8 000 mg/kg bw (Union Carbide, 1974).

10.2.2 Comparison with the CLP criteria

Acute dermal toxicity relates to adverse effects occurring after a single or relative brief exposure to a substance or mixture. Acute toxicity classification is generally assigned on the basis of evident lethality and typically obtained from animal testing.

Regarding acute toxicity by the dermal route, substances can be allocated to one of four toxicity categories based on the criteria shown in the Table 3.1.1 of Annex I, Part 3, Table 3.1.1 of CLP.

The following applies in comparison to the classification criteria for:

'Acute dermal toxicity - Category 4: 1 000 < ATE ≤ 2000 mg/kg bw'.

In a non-guideline study the application of NPNA to rabbit skin resulted in an LD_{50} of > 8 000 mg/kg bw (24 h exposure) which does not fulfil the criteria for classification for acute dermal toxicity.

10.2.3 Conclusion on classification and labelling for acute dermal toxicity

Based on the dermal LD_{50} of > 5 000 mg/kg a classification of NPNA for acute dermal toxicity is not indicated.

10.3 Acute toxicity - inhalation route

Data lacking, no conclusion possible.

10.4 Skin corrosion/irritation

Table 11: Summary table of animal studies on skin corrosion/irritation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results -Observations and time point of onset -Mean scores/animal -Reversibility	Reference, reliability
OECD TG 404 (Acute Dermal Irritation/Corrosion); no GLP compliance	Rabbit, New Zealand White, male (2 680 – 3 100 g), 3 animals	NPNA (CAS 90-30-2 / EC 201-983-0) Purity: unknown Vehicle: patches moistened with distilled water containing 0.5 % carboxymethyl-cellulose and 0.1 % polysorbate 80	0.5 g (occlusive, shaved flank) Control: untreated other flank Exposure duration: 4 h Observations: 1, 24, 48 and 72 h after removing patches	Slight erythema and oedema (score 1) one hour after removing patches in one animal Other readings (24, 48 and 72 h) without effects Mean erythema score: 0 Mean oedema score: 0	(Ciba-Geigy, 1987a) Key study (rel. 1)
Draize test (1944), "Guide for the Care and Use of Laboratory Animals", DHEW 78-23; GLP-Compliance not	Rabbit, New Zealand White, sex not specified (2.0 -	NPNA (CAS 90-30-2 / EC 201-983-0) Purity: no data Vehicle not specified	Dose level and exposure duration not specified Observations: 24 and 72 h	no irritation effects	AMR (1974) Supporting study (rel. 2)

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results -Observations and time point of onset -Mean scores/animal -Reversibility	Reference, reliability
specified	3.0 kg) 6 animals				
OECD TG 406 (Skin Sensitisation, Guinea pig maximisation test; preliminary test) GLP compliance	guinea pig, Dunkin-Hartley, sex not specified 2 animals	NPNA (CAS 90-30-2 / EC 201-983-0) Purity: unknown Vehicle: paraffin oil	12.5 %, 25 %, 50 %, 100 %, occlusive Observation period & exposure duration: 24 h	Slight erythema at 100 % NPNA in one animal	Phycher (2003) Supporting study (rel. 2)
Skin irritation study (patch-test), no guideline followed; no GLP compliance	Rabbit, New Zealand White, sex not specified 6 animals	NPNA (CAS 90-30-2 / EC 201-983-0) Purity: no data No vehicle	0.5 g (occlusive, 2.5 cm² x 2.5 cm² at backs of the animals) Exposure duration: 24 h Observations: 24 and 72 h after removing patches	Erythema score (after 24 h): 1 (2/6 animals), 2 (1/6 animals) Oedema score (after 24 h): 2 (1/6 animals) Scaliness (after 72 h): 1 (1/6 animals)	(Centraal Instituut voor Voedingsonderzoek, 1977) Supporting study (rel. 2)
Skin irritation study, no guideline followed; no GLP compliance	Rabbit, sex not specified 5 animals	NPNA (CAS 90-30-2 / EC 201-983-0) Purity: no data Vehicle: Carbowax PEG400	0.01 mL in progressive dilutions of 10, 1, 0.1 and 0.01 % in solvent (open, shaved) Exposure period: test material not removed Observation duration: 24 h	moderate capillary injection on 2 rabbits, marked injection on 3 rabbits slightly irritating	(Union Carbide, 1974) Supporting study (rel. 2)

10.4.1 Short summary and overall relevance of the provided information on skin corrosion/irritation

The skin irritation potential of NPNA was investigated in one key study according to OECD TG 404 and four non-guideline studies. In the key study, three New Zealand White rabbits were exposed to 0.5 g of the test substance under occlusive conditions (shaved flank). Animals were exposed for 4 h and observed for 3 days (24, 48 and 72 h). Mean erythema score was 0 and mean oedema score (24, 48, 72 h) was 0 as well.

The four supporting studies likewise found no irritating effects except for one study, in which NPNA was found to be slightly irritating. However, the reliability of this study is questionable, especially since the observation period was only 24 h, so reversibility could not be determined.

10.4.2 Comparison with the CLP criteria

According to the CLP Regulation (Section 3.2.1.1), skin corrosion is the induction of irreversible damage to the skin, following the application of a test substance for up to 4 hours.

On the basis of the results of animal testing a substance is classified as skin irritant (Category 2) (Table 3.2.2, CLP Regulation), if

(1) Mean score of ≥ 2.3 and ≤ 4.0 for erythema/eschar or for oedema in at least 2 of 3 tested animals from gradings at 24, 48 and 72 hours after patch removal or, if reactions are delayed, from grades on 3 consecutive days after the onset of skin reactions; or

(2) Inflammation that persist to the end of the observation period normally 14 days in at least 2 animals, particularly taking into account alopecia (limited area), hyperkeratosis, hyperplasia, and scaling reactions; or

(3) In some cases where there is pronounced variability of response among animals, with very definite positive effects related to chemical exposure in a single animal but less than the criteria above.'

No signs of irritation were observed in a guideline-conform study on rabbits following a 4-hour exposure period at 24, 48 and 72 h after patch removal. Three other supporting non-guideline studies confirm this result or showed slight irritancy without reaching the mean scores of (1).

10.4.3 Conclusion on classification and labelling for skin corrosion/irritation

The registrants concluded that NPNA is not irritating to the skin. Based on the available information the DS considers that classification is not warranted.

10.5 Serious eye damage/eye irritation

Table 12: Summary table of animal studies on serious eye damage/eye irritation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results - Observations and time point of onset - Mean scores/animal - Reversibility	Reference, reliability
OECD TG 405 (Acute Eye Irritation/Corrosion); no GLP compliance	Rabbit, New Zealand White, female (2 510-2 520 g), 3 animals	NPNA (CAS 90-30-2 / EC 201-983-0) Purity: commercial grade No vehicle	0.1 mL (36 mg) Control: untreated right eyes of the test animals Exposure duration: test material not	Cornea opacity: mean score (24, 48, 72 h) = 0; (max. score = 4) Iris score: mean score (24, 48, 72 h) = 0; (max. score = 2) Conjunctivae score: mean score (24, 48, 72 h) = 0.4;	GU 2 Toxicology (1987) Key study (rel. 1)

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results - Observations and time point of onset - Mean scores/animal - Reversibility	Reference, reliability
		used	removed/washed Observations: 1, 24, 48 and 72 h after instillation of test material Further observation period of 10 days.	(max. score = 3) Chemosis score: mean score (24, 48, 72 h) = 0; (max. score = 4) All effects were fully reversible within 10 days.	
Eye irritation study, no guideline followed; no GLP compliance	Rabbit, New Zealand White, 6 animals	NPNA (CAS 90-30-2 / EC 201-983-0) Purity: no data No vehicle used	100 g Control: untreated right eyes of the test animals Exposure duration: test material not removed Observations: 24, 48 and 72 h and 7 days after instillation of test material	Cornea opacity: mean score (24, 48, 72 h) = 0; (max. score = 4) Iris score: mean score (24, 48, 72 h) = 0; (max. score = 2) Conjunctivae score: mean score (24, 48, 72 h) = 0.5; (max. score = 3) Chemosis score: mean score (24, 48, 72 h) = 0.06; (max. score = 4). All effects were fully reversible within 7 days.	Centraal Instituut voor Voedingsonderzoek (1977) Supporting study (rel. 2)
Eye irritation study, no guideline followed; no GLP compliance	Rabbit, 5 animals	NPNA (CAS 90-30-2 / EC 201-983-0) Purity: no data	0.5 mL undiluted, 0.5 mL 50 % dilution Control: not specified Exposure duration: 24 h Observations: 24 h after instillation of test material	No effects	Union Carbide (1974) Supporting study, but disregarded as documentation is considered insufficient for assessment (rel. 4)

10.5.1 Short summary and overall relevance of the provided information on serious eye damage/eye irritation

NPNA was investigated for its eye irritation potential in an OECD TG 405 study (key study). 0.1 ml (36 mg) NPNA was instilled into the conjunctival sac of three New Zealand White rabbits. The untreated right eyes of the test animals served as control. After application, animals were observed for 10 days. After 1 hour, slight chemosis was observed in 2 animals, but was fully reversible within the first 24 hours post-exposure (i.e., before the first observation time point). Slight conjunctival redness was observed in 2/3 animals at the three observation time points (24, 48, 72 h), yielding an overall mean conjunctivae score of 0.4. Effects, however, were fully reversible within 10 days post-exposure. Thus, under the experimental condition, the test material was found to show slight or no irritating effects to eyes. In two supporting non-guideline *in vivo* studies with limited reliability, no or little and reversible eye irritating effects were found.

10.5.2 Comparison with the CLP criteria

Substances that have the potential to seriously damage the eyes are classified in Category 1 (irreversible effects on the eye). Substances that have the potential to induce reversible eye irritation are classified in Category 2 (irritating to eyes).

According to Table 3.3.2 of the CLP Regulation classification criteria for reversible eye effects are as follows:

A substance is considered to cause reversible effects on the eye (Category 2) if, when applied to the eye of an animal, it produces:

- at least in 2 of 3 tested animals, a positive response of: corneal opacity ≥ 1, and/or iritis ≥ 1, and/or conjunctival redness ≥ 2, and/or conjunctival oedema (chemosis) ≥ 2 (calculated as the mean scores following grading at 24, 48 and 72 hours after installation of the test material), and which fully reverses within an observation period of 21 days

No signs of irritation that fulfil these conditions were observed in a guideline-conform study on rabbits and the supporting non-guideline studies confirm this result.

10.5.3 Conclusion on classification and labelling for serious eye damage/eye irritation

According to the available studies, NPNA is not irritating to the eye and based on the available data, the DS concludes that classification is not warranted.

10.6 Respiratory sensitisation

Not evaluated in this CLH proposal.

10.7 Skin sensitisation

Table 13: Summary table of animal studies on skin sensitisation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results	Reference, reliability
OECD TG 406 (Skin Sensitisation, Guinea pig maximisation test) No GLP compliance	Guinea pig, Pirbright White Male/female 10 animals/sex/dose	NPNA (CAS 90-30-2 / EC 201-983-0) Purity: commercial grade Vehicle: Vaseline Control: 'Adjuvant and vehicle' for induction Positive control: Sensitivity of strain is checked every six months with paraphenylene-	Induction: 0.4 g of 10 % test material in vaseline, 24 h intradermal and epicutaneous Challenge: 0.2 g paste of 3 % test material in vaseline, 24 h epicutaneous, occlusive Observations: 24 and 48 h after treatment	90 % of the animals showed skin reactions (erythema and oedema) 24 and 48 h after treatment ➢ Skin Sens. Cat 1, since ≤ 1 % induction dose was not tested, no sub-categorisation appropriate	Ciba-Geigy (1987c) Key study (rel. 1)

		diamine or potassium-dichromate.			
OECD TG 406 (Skin Sensitisation, Guinea pig maximisation test) No GLP compliance	Guinea pig, strain and sex not specified 20/dose 19 control animals	NPNA (CAS 90-30-2 / EC 201-983-0) Purity: no data Vehicle: olive oil for intradermal induction, petrolatum for epicutaneous induction Control: vehicle only Positive control: no information	Induction: Intradermal: 10 % test material in olive oil Topical application: 25 % test substance in vaseline (w/w), with sodium lauryl sulphate pre-treatment Challenge: 0.5, 2.5, 5 % test substance in vaseline, 24 h epicutaneous, occlusive Observations: 24 and 48 h after treatment	~ 73 % of the animals showed skin reactions 24 and 48 h after treatment at highest tested challenge dose (5 %) ~ 63 % of the animals showed skin reactions 24 and 48 h after challenge with 2.5 % ~ 45 % of the animals showed skin reactions 24 and 48 h after challenge with 0.5 % ➢ Skin Sens. Cat 1, since ≤ 1 % induction dose was not tested, no sub-categorisation appropriate.	Boman et al. (1980) Supporting study (rel. 2), but insufficient documentation of methods
OECD TG 406 (Skin Sensitisation, Guinea pig maximisation test) GLP compliance Repeatedly positive findings in the control group, positive findings not discussed or explained, no definition on evaluation criteria	Guinea pig, Dunkin-Haertley Male (main study) 10 animals/dose 5 control animals	NPNA (CAS 90-30-2 / EC 201-983-0) Purity: unknown	Induction: Intradermal: 15 % test material in olive oil Topical application: 100 % test substance in paraffin oil Challenge: First: 12.5 and 25 % test substance in paraffin oil, 24 h epicutaneous, occlusive Second: 6.25 and 12.5 % test substance in paraffin oil, 24 h epicutaneous, occlusive Observations: 24 and 48 h after treatment	Sensitisation rate 10 % (6.25 % challenge dose) to 40 % (12.5 and 25 % challenge dose) Percentage of positive responses in control group (20 %) were subtracted from percentage of positive responses in respective dose groups	Phycher (2003) Supporting data but not used for classification due to significant methodological deficiencies (rel. 3)
Modified Landsteiner Guinea Pig Sensitisation	Guinea pig, albino Male	NPNA (CAS 90-30-2 / EC 201-983-0) Purity: no data	Induction: intradermal, no data on vehicle, concentration and	Non-sensitising Documentation insufficient for assessment	AMR (1974) Study not assignable (rel.

Test (1967) No GLP compliance	18 animals/dose		exposure duration Challenge: intradermal, no data on vehicle, concentration and exposure duration Observations: 24 and 48 h after treatment		4)

Table 14: Summary table of human data on skin sensitisation

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Case report	< 0.01 % NPNA (CAS 90-30-2 / EC 201-983-0) in grease	50-year-old male hydraulic assembler in a plant producing explosives; had atopic dermatitis since childhood and hand dermatitis since the 1980s	In patch tests (standard, epoxy, plastics and glues, oils, and metalworking fluids, coco fatty acid derivatives, methacrylates, formaldehyde resins, and own products), the patient reacted to cocamide diethanolamide (cocamide DEA; ++), NPNA **(+++),** and from the workplace materials to gunpowder containing ethylene glycol dinitrate (10 %; ++), and a grease (?+)	Aalto-Korte et al. (2008)
Case report	1 % NPNA (CAS 90-30-2 / EC 201-983-0) in grease	Worker in aircraft plant had recurrent dermatitis	In patch test, the worker reacted to the ingredient NPNA and a dilution of the grease corresponding to 0.001 % NPNA.	Boman et al. (1980)
Case report	1 % NPNA (CAS 90-30-2 / EC 201-983-0) in grease	Two cases of dermatitis at a manufacturer that uses a fire-resistant grease (FR Grease) and “Alvania grease RA” as lubricant, both containing NPNA	Patch-testing to both greases and its ingredients (incl. NPNA) showed sensitisation by NPNA	Carmichael and Foulds (1990)
Case report	1 % NPNA (CAS 90-30-2 / EC 201-983-0) in grease	Woman worked with a grease for 1 year and developed hand dermatitis after 6 month that spread to her face	Patient was patch-tested to a standard series, the respective grease and NPNA. The Patient reacted to the grease and NPNA, which was the actual allergen therein.	Kalimo et al. (1989)
Case report	NPNA- (CAS 90-30-2 / EC 201-983-0) in grease	Previously healthy man working in an industry where he had contact to grease developed a rash in the face, on the neck, volar aspects of the arms and dorsum of the hands.	Patient was patch-tested with a standard series, a metal-working fluid standard series and materials from work. He tested positively to the grease and an ingredient of the grease that was identified as NPNA.	Svedman et al. (2004)

10.7.1 Short summary and overall relevance of the provided information on skin sensitisation

NPNA was tested in three Guinea Pig Maximisation Tests (OECD TG 406). Two of the tests were conducted pre-GLP, however, the key study (Ciba-Geigy, 1987c) has no methodological and documentary deficiencies and is thus considered reliable without restrictions.

This key study was performed in 10 male and 10 female Pirbright White (Tif:DHP) Guinea pigs per dose with concentrations of 10 % for intradermal and epicutaneous induction and 3 % for challenge and 10 negative control animals. 24 and 48 hours post-challenge, 90 % of the animals showed skin reactions (erythema and oedema).

The supporting study (Boman et al., 1980) was conducted in 20 guinea pigs per dose with concentrations of 10 % for intradermal and 25 % for dermal induction and 0.5, 2.5 and 5 % for challenge and 19 negative control animals. Even at the lowest dose tested, 45 % of the animals showed skin reactions at 24 and 48 hours post-challenge and the percentage increased in a dose related manner. Some methodological information is missing, however, the study is considered acceptable as supporting information for classification and labelling.

The third GPMT (Phycher, 2003) had positive results as well (10 – 40 %); however, the reliability is questionable since there were repeatedly positive findings in the control group (20 %) that were not discussed or explained but simply subtracted from the percentage of positive responses in the respective dose groups. Therefore, although registrants included this study in the registration dossier as “supporting study” and sensitising effects were seen, it cannot be considered for classification and labelling purposes due to major methodological deficiencies.

Case reports also indicate that NPNA may cause allergic skin reactions in humans, as verified by patch tests with patients suffering from contact dermatitis (Aalto-Korte et al., 2008; Boman et al., 1980; Carmichael and Foulds, 1990; Kalimo et al., 1989; Svedman et al., 2004).

10.7.2 Comparison with the CLP criteria

According to the CLP Regulation (Section 3.4.1.4.) a skin sensitiser is a substance that will lead to an allergic response following skin contact. Sensitisation includes two phases: the first phase is induction of specialised immunological memory in an individual by exposure to an allergen. The second phase is elicitation, i.e. production of a cell-mediated or antibody-mediated allergic response by exposure of a sensitised individual to an allergen.

According to Sections 3.4.2.2.3.1 and 3.4.2.2.3.3 and Tables 3.4.2 and 3.4.4 of the CLP Regulation classification criteria for skin sensitising effects are as follows:

<table>
<tr><th colspan="2">Category 1</th></tr>
<tr><td colspan="2">Substances shall be classified as skin sensitisers (Category 1) where data are not sufficient for sub-categorisation in accordance with the following criteria:
(a) if there is evidence in humans that the substance can lead to sensitisation by skin contact in a substantial number of persons; or
(b) if there are positive results from an appropriate animal test (see specific criteria in paragraph 3.4.2.2.4.1).</td></tr>
<tr><td>Criteria for Category 1 A</td><td>Criteria for category 1 B</td></tr>
</table>

Substances showing a high frequency of occurrence in humans and/or a high potency in animals can be presumed to have the potential to produce significant sensitisation in humans. Severity of reaction may also be considered. **Guinea pig maximisation test:** ≥ 30 % responding at ≤ 0.1 % intradermal induction dose or ≥ 60 % responding at > 0.1 % to ≤ 1 % intradermal induction dose	Substances showing a low to moderate frequency of occurrence in humans and/or a low to moderate potency in animals can be presumed to have the potential to produce sensitisation in humans. Severity of reaction may also be considered. **Guinea pig maximisation test:** ≥ 30 % to < 60 % responding at > 0.1 % to ≤ 1 % intradermal induction dose or ≥ 30 % responding at > 1 % intradermal induction dose

Case reports are in general in support of a sensitisation potential of NPNA; however, human data are too limited to conclude on the subcategory for classification.

Both Guinea pig maximisation tests that were considered suitable for classification showed sensitising potential of NPNA in ≥ 30 % animals responding at > 1 % intradermal induction dose, supporting a Skin Sens. 1B classification of NPNA. In the first GPMT (Ciba-Geigy (1987)), however, levels of ≤ 1 % induction dose were not tested and, thus, this data is considered insufficient for sub-categorisation (i.e. 1A or 1B). Although the incidence of animals with positive reactions was very high (90 %) and data is indicative of a Skin Sens. 1B classification, it does not allow for preclusion of a Category 1A classification. In the second GMPT (Boman, A. et al. 1980) a concentration of 10 % intradermal induction dose was tested and 45 % of the Guinea pigs responded. However, again concentrations ≤ 1 % intradermal induction dose were not tested.
Thus, the criteria for classification of NPNA as Skin Sens. 1 are clearly fulfilled, but sub-categorisation (i.e. 1A or 1B) is not possible.

10.7.3 Conclusion on classification and labelling for skin sensitisation

Based on the data presented in Table 13 and in accordance with Annex VI of Regulation (EC) 1272/2008, the DS proposes to classify NPNA as **Skin Sens. 1 (H317: May cause an allergic skin reaction)** without sub-categorisation. A GLC of 1 % (w/v) would apply by default.

10.8 Germ cell mutagenicity

Not evaluated in this CLH proposal.

10.9 Carcinogenicity

Not evaluated in this CLH proposal.

10.10 Reproductive toxicity

Not evaluated in this CLH proposal.

10.11 Specific target organ toxicity-single exposure (STOT SE)

Table 15: Summary table of other studies relevant for STOT SE

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference, reliability
Acute dermal toxicity study	NPNA (CAS 90-30-2 / EC 201-	2 000, 8 000 mg/kg bw, epicutaneous,	Livers congested and mottled; spleens dark; kidneys khaki brown	Union Carbide

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference, reliability
LD50-Test, no guideline followed; no GLP compliance	983-0) Purity: no data	24 h exposure Rabbit, albino, male, 2 animals (2 000 mg) and 5 animals (8 000 mg) LD50: > 8 000 mg/kg bw (male)	in colour No information about number and sex of affected animals and dose level Details on mortality see 10.2	(1974) Key study (rel. 2)
Acute oral toxicity study LD50-Test, no guideline followed; GLP compliance: not specified	NPNA (CAS 90-30-2 / EC 201-983-0) Purity: commercial grade	200, 2 000 mg/kg bw, gavage, 14 d post exposure observation period Rat, albino, male/female, 3/sex/dose LD50: > 2 000 mg/kg bw (male) > 200 - < 2 000 mg/kg bw (female)	At 2 000 mg/kg bw: Dyspnea, exophthalmos, ruffled fur, and abnormal body position, reduced spontaneous activity. No information about number and sex of affected animals. Details on mortality see 10.1	Ciba-Geigy (1987b) Supporting study (rel. 2)
Acute oral toxicity study no guideline followed; no GLP compliance	NPNA (CAS 90-30-2 / EC 201-983-0) Purity: no data	1 000, 2 000, 4 000 mg/kg bw, single dose (gavage), 14 d post exposure observation period Rat, Wistar, male, 5/dose LD50: 2 380 mg/kg bw (calculated)	livers mottled; stomachs transparent, free blood; kidneys and adrenals congested; intestines injected and distended, free blood No information on number of affected animals and dose level At 4 000 mg/kg bw: sluggish, unsteady gait for 1 hour, prostrate for 4 hours (no information about number of affected animals). Details on mortality see 10.1	Union Carbide (1974) Supporting study (rel. 2)

10.11.1 Short summary and overall relevance of the provided information on STOT SE

Two of the supporting oral acute studies report clinical signs or effects on gross pathology. One study (Ciba-Geigy, 1987c) reported that dyspnoea, exophthalmos, ruffled fur, and abnormal body position were seen. These were considered as common symptoms related to moribund status in advance of expected mortalities at lethal or near lethal doses in acute toxicity testing. Additionally, reduced spontaneous activity was observed in the animals of the 2 000 mg/kg bw dose group, in which one female and two males died within 2 days after administration.

The second study in male rats (Union Carbide, 1974) reports mottled livers; transparent stomachs with free blood; congested kidneys and adrenals; injected and distended intestines with free blood. However, it is not specified at which dose these signs of toxicity occur. The study tested 1 000 and 2 000 mg/kg bw without seeing clinical signs in the tested rats. At the highest dose (4 000 mg/kg bw) authors report sluggish, unsteady gait for 1 hour and prostration for 4 hours. However, details about number of the affected animals are not given.

In the key study on acute dermal toxicity (Union Carbide, 1974), 2 000 and 8 000 mg/kg bw were tested under occlusive conditions at the trunk of rabbits. Findings of gross pathology were congested and mottled livers; dark spleens and khaki brown kidneys, without specifications on number and sex of animals or dose level. In the 8 000 mg/kg bw group brown urine (same colour as chemical) and erythema were observed.

One oral 7-day range finding study (n = 2/sex/dose; test doses: 0, 250 and 500 mg/kg bw/d) reported gait abnormalities from day 1 of treatment at all dose groups (Table 16, Bayer (2000)) . Moreover, one female of the high dose group died after 2 days of treatment. No information on haematological or other adverse effects were reported. The additional repeated dose toxicity studies provided in the registration dossier do not furnish acute adverse effects relevant for STOT SE.

10.11.2 Comparison with the CLP criteria

Specific target organ toxicity, single exposure (STOT SE) is defined as specific, non-lethal target organ toxicity arising from a single exposure to a substance. These adverse effects produced by a single exposure include consistent and identifiable toxic effects in humans, or, in experimental animals, which have affected the function or morphology of a tissue/organ, or have produced serious changes to the biochemistry or haematology of the organism, and these changes are relevant for human health.

According to the Guidance on the Application of the CLP Criteria, 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 single exposure:

Substances are classified in Category 2 for specific target organ toxicity (single exposure) based on observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally moderate exposure concentrations.

The following guidance dose/concentration values apply for the classification as

STOT-SE Category 2 – oral exposure: 2 000 mg/kg bw ≥ C > 300 mg/kg bw;

STOT-SE Category 2 –dermal exposure: 2 000 mg/kg bw ≥ C > 1 000 mg/kg bw

No toxic effects (beyond mortalities and associated organ lesions, and clinical findings) that may be considered for STOT SE classification were reported in acute studies designed to determine LD50. Accordingly, little or no detailed information is given on gross pathological or clinical findings, NOAELs or LOAELs were not derived. Effects were only seen at or above 2 000 mg/ kg bw (in cases where dose levels were reported) and/or are considered insufficiently detailed in reporting to justify classification as STOT SE.

10.11.3 Conclusion on classification and labelling for STOT SE

Neither available standard acute toxicity studies nor other studies in the registration dossier (e.g. repeated dose toxicity studies) identified acute adverse effects that were beyond lethality and its associated effects, which are already covered by the classification as Acute Tox. Cat. 4, H302. Hence, the DS proposes that classification as STOT SE is not warranted for NPNA.

10.12 Specific target organ toxicity-repeated exposure

Table 16: Summary table of animal studies on STOT RE with focus on adverse effects on the blood system and the liver

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results** (specifically related to criteria on haemolytic anaemia) **dose-related effects are marked with an asterisk ***	**Effects relevant for classification according to CLP criteria** **-** **Classification/ category**	**Reference, reliability**
Repeated dose oral toxicity study (90 days) in rats (neurotoxicity and haematotoxicity study: OECD TG 424 combined with OECD TG 408) Wistar rat 15 animals/sex/dose Methaemoglobin (MetHb) formation was not investigated Limitations: Urinanalysis with dipsticks does not allow robust quantification, only semiquantitative assessment, at high dose only 1 male and 0 female tested)	NPNA (CAS 90-30-2 / EC 201-983-0), purity: 99.9 % oral: gavage 0, 5, 25, 125 mg/kg bw/d Daily for 3 months, animal sacrificed on day 92. No recovery groups tested.	**General:** No mortality; no indication of neurotoxicity (no adverse neurobehavioral (functional observation battery) and neurohistopathological effects); no gross lesions; **<u>At 5 mg/kg bw/day:</u>** **Blood:** red blood cells (RBC) (m) +3.3 % total protein/ albumin levels (f) +4.1/5.2 % total bilirubin* (m) +56.8 % **Urine:** bilirubin* (f) 5/10 grade 1 (minimal), 5/10 grade 2 (slight) vs. 4/10 grade 1 (minimal) in controls; glucose* (f) 4/10 grade 1 (minimal), (m) 8/10 grade 1 (minimal) vs. 0/10 in controls; blood* (f) 4/10 grade 1 (minimal) vs. 1/10 grade 2 (slight) in control females (incidence and severity statistically not statistically significant) **Liver:** Rel. weight* (f) +8.6 % when compared with control mean **Spleen:** Increased haematopoiesis (f) 1/10 grade 1 (minimal), 1/10 grade 2 (slight) vs. 0/10 in controls (not statistically significant), Pigment storage* (f) 5/10 grade 1 (minimal), 1/10 grade 2 (slight) vs. 1/10 grade 2 (slight) in controls **Kidney:** chronic nephropathy (m) 2/10 grade 1 (minimal) vs. 1/10 grade 1 (minimal) in controls (effect not statistically significant) **<u>At 25 mg/kg bw/day:</u>** **Blood:** creatinine levels* (f) -12.4 % total bilirubin levels* (m/f) +228.4 %/ +131.8 % **Urine:** bilirubin (f) 8/10 grade 2 (slight), 2/10 grade 3 (moderate) vs. 4/10 grade 1	*CLP criteria, Cat. 2, study duration 90 days:* *10 < C ≤ 100 mg/kg bw/day* - *According to 3.9.2.5.2 CLP guidance: Adverse effects are haemolytic anaemia with RBC reduction of ca. 10 % in combination with renal cell degeneration and massive liver weight increase (> 120 % when compared to controls) at 125 mg/kg bw/day which is slightly above guidance value for STOT RE 2* ➢ *Borderline for classification as:* STOT RE Cat. 2	BASF (2016b) Key study (rel. 1) 1 **For details (absolute and/or relative values, incidence and severity) see Tables 1 to 4 in the Confidential Annex.**

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results (specifically related to criteria on haemolytic anaemia) **dose-related effects are marked with an asterisk ***	Effects relevant for classification according to CLP criteria - Classification/ category	Reference, reliability
		(minimal) in controls; (m) 5/10 grade 1 (minimal), 5/10 grade 2 (slight) vs. 9/10 grade 1 (minimal) in controls. glucose (f) 8/10 grade 1 (minimal), 1/10 grade 2 (slight) vs. 10/10 grade 0 (not observed) in controls; (m) 10/10 grade 1 (minimal) vs. 0/10 in controls urobilinogen in urine (m) 6/10 grade 1 (minimal), 4/10 grade 2 (slight) vs. 8/10 grade 0 (not observed), 2/10 grade 1 (minimal) in controls; blood (f) 6/10 grade 1 (minimal), 4/10 grade 2 (slight) vs. 9/10 grade 0 (not observed) and 1/10 grade 2 (slight) in controls; **Liver:** Abs./rel. weight when compared with controls (m) +17.2 %* / +11.1 %* centrilobular hypertrophy* (m;) 5/10 grade 1 (minimal) vs. 0/10 in controls **Spleen:** Increased haematopoiesis (f) 1/10 grade 1 (minimal), vs. 0/10 in controls (not statistically significant), Pigment storage (f) 8/10 grade 1 (minimal), 1/10 grade 2 (slight)* vs. 1/10 grade 2 (slight) in controls **Kidney:** chronic nephropathy* (m) 6/10 grade 1 (minimal) vs. 1/10 in controls degeneration/ regeneration of proximal tubules * (m) 3/10 grade 1 (minimal) vs. 10/10 grade 0 (not observed) in controls (effect not statistically significant) <u>**At 125 mg/kg bw/day:**</u> **Blood:** RBC (m/f) -8.5 %* (mean in both sexes) (in 6/10 females and 4/10 males RBC reduction ≥ 10 %; Fig. 1E and F) haemoglobin (Hb) (m/f) -4-1 %* / -5.9 % haematocrit (HCT) (m/f) -3.9 %* / -5.9 %* mean corpuscular volume (MCV)(m) +4.9 %* mean corpuscular haemoglobin (MCH) (m) + 3.8 % reticulocytes (RET) (m/f) +64.3 % /+58.8 %* urea (m) +8.2 %		

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results (specifically related to criteria on haemolytic anaemia) dose-related effects are marked with an asterisk *	Effects relevant for classification according to CLP criteria - Classification/ category	Reference, reliability
		cholesterol (m) -12.7 % total bilirubin* (m/f) +1 715 % / +1 282 % creatinine* (m/f) -16.6 % / -27.9 % total protein/albumin (f) +5.3 % / +7.1 % **Urine (<u>only 1 male & no females tested; dipstick analysis</u>):** Discoloured urine in all males urobilinogen (m) 1/1 grade 3 (moderate) vs. 8/10 grade 0 (not observed), 2/10 grade 1 (minimal) in controls bilirubin (m) 1/1 grade 3 (moderate) vs. 9/10 grade 1 (minimal) in controls glucose (m) 1/1 grade 2 (slight) vs. 0/10 in controls, **Liver:** Abs./rel. weight when compared with controls (m) +28.4 %* / +28.6 %*, (f) +31 % / +31.9 %, centrilobular hypertrophy* (m) 9/10 grade 2 (slight) vs. 0/10 in controls; (f) 10/10 grade 3 (moderate) vs. 0/10 in controls; **Spleen:** Increased haematopoiesis (f) 2/10 grade 2 (slight), 1/10 grade 3 (moderate) vs. 0/10 in controls (not statistically significant), Pigment storage* (f) 5/10 grade 2 (slight) vs. 1/10 in controls; **Kidney:** Abs./rel. weight when compared with controls (m) +14 %* / +13.9 %*, (f) +14.9 % / +15.8 %, chronic nephropathy* (m) 1/10 grade 1 (minimal), 4/10 grade 2 (slight) vs. 1/10 grade 1 (minimal) in controls, degeneration/regeneration of proximal tubules* (m) 2/10 grade 1 (minimal), 2/10 grade 2 (slight), 4/10 grade 3 (moderate) vs. 0/10 in controls.		
Repeated dose oral toxicity study (28 days) in rats	NPNA (CAS 90-30-2 / EC 201-983-0), purity 99.7 % Oral: gavage 0; 5; 20;	General: No gross findings recorded. No test substance-related histopathological findings. **<u>5 mg/kg bw per day:</u>** **Blood:**	*CLP criteria, Cat. 2, study duration 28 days: 30 < C ≤ 300 mg/kg bw/day (Haber's rule)* -	Bayer (2002) Supporting study (rel. 2: guideline

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results (specifically related to criteria on haemolytic anaemia) **dose-related effects are marked with an asterisk ***	Effects relevant for classification according to CLP criteria - Classification/ category	Reference, reliability
OECD TG 407 Wistar rat 5 animals/sex/dose MetHb formation was not investigated	80 mg/kg bw/day daily for 28 days Recovery groups: 0 and 80 mg/kg bw/d, 28 d of exposure plus 14 d recovery period	MCHC (m) +1.9 %Na (m) -1.4 % **Urine:** bilirubin (m) 5/5 grade 1 (minimal) vs. 0/5 in controls; (f) 4/5 grade 1 (minimal) vs. 0/5 in controls **<u>20 mg/kg bw per day</u>:** **Blood:** RBC* (f) - 4.7 % (not stat. significant) Hb* (f) -7.0 % HCT (f) - 8.2 % mean corpuscular haemoglobin concentration (MCHC) (m) +2.5 % total bilirubin* (m) +41.7 % Na (m) -1.4 % **Urine:** bilirubin* (m+f) 4/5 grade 2 (moderate), 1/5 grade 3 (severe) vs. 0/5 in controls **<u>80 mg/kg bw per day:</u>** **Blood:** RBC* (f) -6.5 % (not stat. significant) (> -10 % in 2/5 f; Fig. 1D) Hb* (f) (mean) -9.2 % , -11 % (median) (reduction > 10 % in 3/5 f; Fig. 1B) HCT (f) -8.4 % total bilirubin* (m/f) +266.7 % / +76.9 % cholesterol/triglyceride (m) -16.1 % / -29.8 %; albumin (m) +6 % **Urine:** bilirubin* (m+f) 5/5 grade 3 (severe) vs. 0/5 in controls urobilinogen (m) 4/5 grade 1 (minimal) vs. 0/5 in controls; (f) 5/5 grade 1 (minimal) vs. 0/5 in controls **Liver:** abs. weight when compared to controls (m) +11.7 %; (f) +10.6 % (the latter not statistically significant) focal Kupffer cell accumulation (f) 3/5 grade 1 (minimal) vs. 1/5 grade 1 (minimal) in control (not statistically significant) **Kidney:** basophilic tubules (m) 5/5 grade 1 (minimal) vs. 3/5 grade 1 in control In some control animals minimal grade basophilic tubules in kidneys (3/5 males	*According to 3.9.2.5.2 CLP guidance: Adverse effects are haemolytic anaemia with significant Hb reduction of ca. 10 % in female rats (> 10 % in 60 % of females) in combination with liver weight increase at 80 mg/kg bw/d (compared to controls: 111.7 % in high dose males, and 114.6 % in females at the end of recovery period). It is noted that the associated adverse effects (here in the liver) are moderate; however, the selected dose for the high dose group is far below the upper limit of the guidance value for STOT RE 2 (300 mg/kg bw/d).* *--> Borderline for classification as:* STOT RE Cat. 2 The interpretation of the findings has some limitations since effects in kidney (basophilic tubules) and liver (Kupffer cell accumulation) were also seen in some control animals.	study, but limited validity due to occurrence of effects linked to haemolytic anaemia in some control animals). **For details (absolute and/or relative values, incidence and severity) see tables 5 to 8 in the Confidential Annex.**

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results** (specifically related to criteria on haemolytic anaemia) **dose-related effects are marked with an asterisk ***	**Effects relevant for classification according to CLP criteria** **-** **Classification/ category**	**Reference, reliability**
		and 1/5 females) and Kupffer cell accumulation in liver (3/5 males and 1/5 females) were also observed, making it impossible to conclude whether the observed cases in treated animals can be considered treatment related. **<u>80 mg/kg bw per day (incl. 14 days of recovery):</u>** **Blood:** MCV (f) +3.6 % Organ weights compared to controls **Liver:** abs. (f) +14.6 % **Spleen:** abs./ rel. (m) +22.4 % / +14.1 % abs. (f) +27.3 % **Kidney:** abs. (f) +7.7 %		
Repeated dose oral toxicity study (28 days) in rats OECD TG 407 Sprague Dawley rats Female and male 5 animals/sex/dose MetHb formation was not investigated	NPNA (CAS 90-30-2 / EC 201-983-0), Purity: 99.4 % Oral: gavage Vehicle: olive oil 0, 4, 20, 100, 500 mg/kg bw/day daily for 28 days Recovery groups: 0 and 500 mg/kg bw/d, 28 d of exposure plus 14 d recovery period	**General:** No indication of neurotoxicity and neurohistopathological effects, Salivation during first 30 min after application in males at ≥ 100 mg/kg bw/d and in females at 500 mg/kg bw/d, non-significant trend towards lower food consumption in males at 500 mg/kg bw/d, no significant effect on bw; significant increase in urine volume in both sexes at 500 mg/kg bw/d. **<u>At 4 mg/kg bw/day:</u>** **Blood:** triglyceride (f) -41.9 % **Liver:** focal necrosis (m) 1/5 grade 1 (slight) vs 0/5 in control (not statistically significant) **<u>At 20 mg/kg bw/day</u>:** **Blood:** triglyceride (f) -48.4 % **Liver:** focal necrosis (m) 2/5 grade 1 (slight) vs 0/5 in control (not statistically significant) **Kidney:** abs. weight when compared to controls (f) +17.3 % (statistically significant, but no clear dose-response) **<u>At 100 mg/kg bw/day:</u>**	*CLP criteria, Cat. 2, study duration 28 days: 30 < C ≤ 300 mg/kg bw/day (Haber's rule)* - *According to CLP guidance: Adverse effects are liver weight increase (around/above 120 % when compared to controls) at 100 mg/kg bw/d. It is noted that the adverse effects (here in the liver) are observed at doses far below the upper limit of the guidance value for STOT RE 2 (300 mg/kg bw/d). Early signs of haematolytic anaemia was seen at 100 mg/kg bw/d (bilirubin, chromaturia), whereas (relevant) Hb reduction was only seen at 500 mg/kg bw/d (-15.2 % (m/f)).* *A large dose space is noted; no dose group was tested at the guidance value for STOT RE 2 (300 mg/kg bw/d).* *→ Based on massive liver weight increase classification as:* STOT RE Cat. 2	Tanabe et al. (2017) Supporting study (rel. 2) **For details (absolute and/or relative values, incidence and severity) see tables 11 to 14 in the Confidential Annex.**

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results (specifically related to criteria on haemolytic anaemia) dose-related effects are marked with an asterisk *	Effects relevant for classification according to CLP criteria - Classification/ category	Reference, reliability
		Blood: total bilirubin (m/f) +52.9 %* / +65.4 %* albumin level (m) +13.8 %* A/G ratio (m) +20 %* triglyceride (f) – 48.4 % **Urine:** purple discolouration of urine (chromaturia) (m) 5/5; (f) 3/5 **Liver:** rel. weight when compared to controls (m/f) +24.2 %* (not statistically significant)/ +16.2 %* (statistically significant) abs. weight when compared to controls (m/f) +21.7 %* / +19.7 %* (both not statistically significant); centrilobular hypertrophy (f) 1/5 grade 1 (slight) vs 0/5 controls (not significant); focal necrosis (m) 1/5 grade 2 (moderate) vs 0/5 controls (not significant) **Spleen:** extramedullary haematopoiesis (m) 3/5 grade 2 (moderate) and 2/5 grade 1 (slight) vs. 5/5 grade 1 (slight) in controls, extramedullary haematopoiesis (f) 1/5 grade 3 (severe) and 4/5 grade 2 (moderate) vs. 5/5 grade 2 (moderate) in controls (not statistically significant); **Kidney:** abs. weight when compared to controls (f) +13.3 % (not statistically significant and no clear dose-response) **<u>500 mg/kg bw/day:</u>** General: 1 male died one day before necropsy (day 28; findings: soiling of fur over lower abdomen by faeces and urine, chromodacryorrhea, large sized kidney, slight tubular dilatation in kidney, moderate basophilic tubules, slight papillary necrosis, slight hyaline casts, slight hypertrophy of hepatocytes, slight thymus atrophy, slight thymus haemorrhage, minimal extramedullary haematopoieisis in spleen, minimal pigment storage in spleen, slight splenic congestion).		

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results (specifically related to criteria on haemolytic anaemia) **dose-related effects are marked with an asterisk ***	Effects relevant for classification according to CLP criteria - Classification/ category	Reference, reliability
		Blood: RBC (f) -15.3 %* (decreasing trend in m) Hb (m/f) -15.2 % / -15.2 % HCT(m/f) -11.8 % / -9.6 % MCHC (m/f) -4 % / -5.6 % RET (m/f) +132.5* / +267.4 total bilirubin (m/f) +202.9 %* / +361.5 %* albumin (m/f) +29.9 %* / +30.7 %* A/G ratio (m/f) +51.1 %* / +40.2 % blood-urea-nitrogen (m) +21.3 %* Na (m) +1.4 % total protein (f) 12.1 % **Urine:** purple discolouration of urine (chromaturia; (all m+f), also day 1 of recovery **Liver:** increased liver sizes (m+f) abs. weight when compared to controls (m/f) +40.5 %* / +71.1 % * Rel. weight when compared to controls (m/f) +70.1 %* / +75.2 % *; centrilobular hypertrophy (m) 5/5 grade 1 (slight) vs 0/5 controls (f) 5/5 grade 2 (moderate) vs 0/5 controls focal necrosis (m/f) 1/5 grade 1 (slight) vs 0/5 controls (not statistically significant) **Spleen:** abs./rel. spleen weights when compared to controls (f) +40.0 % / +45.0 % pigment storage (f) 5/5 grade 3 (severe) vs 0/5 controls extramedullary haematopoiesis (m) 3/5 grade 1 (slight) vs. 5/5 grade 1 (slight) in controls, (f) 3/5 grade 3 (severe) vs. 5/5 grade 2 (moderate) in controls **Kidney:** abs. weight when compared to controls (f) +14.7 % dilatation of distal and collecting tubules (m) 3/5 grade 1 (slight) and 1/5 grade 2 (moderate); (f) 3/5 grade 1 (slight) vs. 0/5 controls (not statistically significant) papillary necrosis (m) 2/5 grade 2 (moderate) (f) 1/5 grade 1 (slight) and 2/5 grade 3 (severe) vs. 0/5 in controls (not statistically significant)		

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results (specifically related to criteria on haemolytic anaemia) **dose-related effects are marked with an asterisk ***	Effects relevant for classification according to CLP criteria - Classification/ category	Reference, reliability
		basophilic tubules (m) 4/5 grade 2 (moderate) and 1/5 grade 3 (severe) vs 2/5 grade 1 (slight) in controls basophilic tubules (f) 4/5 grade 1 (slight) vs 2/5 grade 1 (slight) in controls (not statistically significant) **<u>At 500 mg/kg bw/d (incl. recovery period):</u>** General: Decreased bw **Blood:** RBC (m/f) -13.5 % / -10.9 % Hb (m/f) -7.1 % / -8.1 % HCT (f) -6.2 %/ MCHC (m) -3.3 % RET (m) +99.1 % RET (f) +43.6 % (not statist. significant) MCH (m) +7.1 % MCV (m/f) +10.9 % / +5.4 % Total bilirubin (m) +20.6 % K (m) +12.5 % **Liver:** rel. weight when compared to controls (f) +11.2 % hypertrophy of centrilobular hepatocytes (m/f) 1/5 grade 1 (slight) vs. 0/5 controls (not statistically significant) focal necrosis (m) 1/5 grade 1 (slight) and 1/5 grade 2 (moderate) vs 1/5 controls grade 1 (slight) (not statistically significant) **Spleen:** abs./rel. weight when compared to controls (m) +28.8 % / +47.1 % extramedullary haematopoiesis (m) 3/5 grade 1 (slight) and 2/5 grade 2 (moderate) vs. 5/5 controls grade 1 (not statistically significant) pigment storage (m) 3/5 grade 1 (slight) and 2/5 grade 2 (moderate) vs 5/5 controls grade 1 (slight) (not statistically significant) pigment storage (f) 5/5 grade 3 (severe) vs 5/5 controls grade 2 (moderate) (statistically significant) **Kidney:** papillary necrosis (f) 3/5 grade 1 - 3 (slight to severe) vs 0/5 controls (not statistically significant)		

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results** (specifically related to criteria on haemolytic anaemia) **dose-related effects are marked with an asterisk ***	**Effects relevant for classification according to CLP criteria** **-** **Classification/ category**	**Reference, reliability**
		basophilic tubule (m) 3/5 grade 1 (slight) and 2/5 grade 2 (moderate) vs 1/5 grade 1 (slight) in controls (not statistically significant) post-necrotic mineralisation of papilla (m) 1/5, grade 1 (slight) (not statistically significant).		
Prenatal Developmental Toxicity Study OECD TG 414 Wistar rats females 25/sex/dose MetHb formation was not investigated No organs were weighed and no histopathology was performed.	NPNA (CAS 90-30-2 / EC 201-983-0), purity: 99.9 % Oral: gavage 0, 15, 50 and 150 mg/kg bw/day daily for 14 days (gestation days (GD) 6 through 19); animal sacrificed on GD 20	General: Dams: No mortality; salivation at ≥ 50 mg/kg bw/d, dose-response); no test-substance-related clinical or behavioural changes; no test substance-related findings at necropsy; organ weights not measured (except uterus); gross pathology but no histopathology performed; no MetHb measurements or urinalysis performed. Foetuses: few variations at the top and mid dose (altered rib cages (wavy ribs), incomplete ossification). **<u>At 15 mg/kg bw/day</u>:** **Blood:** bilirubin +30.8 %* urea +10.2 %* **<u>At 50 mg/kg bw/day:</u>** **Blood:** bilirubin +98.5 %* urea +18.1 %* **<u>At 150 mg/kg bw/day</u>:** **General:** water consumption (+24 % on GD 6 - 19; +19 % on GD 0 - 19, start at GD 10) food consumption (-16 % on GD 6 - 13, recovered afterwards; -9 % GD 6 - 19); bw gain (uncorrected): 41 % below controls on GD 6 - 8 (+4.1 g vs. +6.9 g); 9 % below controls on GD 6 - 19 (+82.4 g vs. +91.4 g); corrected (net) terminal bw gain (terminal body weight on GD 20 minus weight of the unopened uterus minus body weight on GD 6): 26 % below controls (+25.7 g vs. +34.6 g);	*CLP criteria, Cat. 2, study duration 14 days: 60 < C ≤ 600 mg/kg bw/day (Haber's rule)* - *According to 3.9.2.5.2 CLP guidance: Adverse effects are haemolytic anaemia with Hb reduction of ≥ -10 % in 8/25 f if compared to the median control value (group median at 150 mg/kg bw: -11 %) and RBC reduction ≥ -10 % in 6/25 f at 150 mg/kg bw/d. No data on organ weight and histopathology available. Increased ALT and urea are indicative of dysfunctions of the liver and kidney.* *It is noted that the selected dose for the high dose group is far below the upper limit of the guidance value for STOT RE 2 (600 mg/kg bw/d).* → *Supporting classification as:* STOT RE Cat. 2	(BASF, 2016a) Supporting study (rel. 1) **For details (absolute and/or relative values, incidence and severity) see tables 9 and 10 in the Confidential Annex.**

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results** (specifically related to criteria on haemolytic anaemia) **dose-related effects are marked with an asterisk ***	**Effects relevant for classification according to CLP criteria - Classification/ category**	**Reference, reliability**
		carcass weight (terminal bw minus uterine weight): 5 % below controls (225.5 g vs. 236.3 g); **Blood:** RBC -5.8 % (mean) (in 6/25 f RBC reduction ≥ -10 %; Fig. 1C) Hb -5.9 % (mean) (> -9 % in 8/25 (based on mean control value, thereof ≥ - 10 % in 4/25), and ≥ -10 % in in 8/25 f (based on median control value); Fig. 1A) HCT -4.8 % MCHC –1.2 % platelets +7.9 % RET +88.9 % relative eosinophils -27.3 % total bilirubin +474.6 %* urea +43.5 %* ALT +16.9 % cholesterol -19.2 % total protein/albumin -6.5 % / -3.8 % no Heinz bodies detected Foetuses: No relevant effects reported.		
7-d range finding study No data on GLP compliance Wistar rats Males and females 2/sex/dose MetHb formation was not investigated. No information on organ weights and histopathology reported.	NPNA (CAS 90-30-2 / EC 201-983-0), purity: no data Oral: gavage 0, 250 and 500 mg/kg bw per day Daily for 7 days	No haematological effects reported gait abnormalities in all dose groups from day 1 of treatment **<u>250 mg/kg bw/day:</u>** **General:** reduced motility, decreased reactivity, uncoordinated gait, laboured breathing, discoloured urine, piloerection, increased water consumption **Adrenals**: enlarged adrenals (f); **Kidneys**: kidneys with rough and discoloured surface (f) **<u>500 mg/kg bw per day:</u>** **General:** death of 1 female after 2 days of treatment reduced motility, decreased reactivity, uncoordinated gait, laboured breathing, discoloured urine, piloerection, increased water consumption **Kidney:** enlarged kidneys (m); kidneys with rough and discoloured surface (f)	*Kidneys were consistently identified as target organs (incl. chromaturia).* *Due to 7-day treatment and low no. of animals, study is not considered for drawing a conclusion on STOT RE classification.*	Bayer (2000) Supporting information (rel. 2)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results (specifically related to criteria on haemolytic anaemia) dose-related effects are marked with an asterisk *	Effects relevant for classification according to CLP criteria - Classification/ category	Reference, reliability
		Adrenals: enlarged adrenals (f);		
Study on sulfhaemoglobin and MetHb formation No GLP compliance Mouse, ddY, 10-15 males Statistics not reported	NPNA (CAS 90-30-2 / EC 201-983-0), purity not reported Intraperitoneal 219 mg/kg Single administration, observation points at 10, 30, 90, 150 min and 24, 48, 72 and 96 h. No positive or negative controls reported.	No sulfhaemoglobin formation at any time point. MetHb levels (mean ± standard error (SE); *significant at p = 0.05; **significant at p = 0.01): 10 min: 4.1 % ± 0.6 %** 30 min: 5.7 % ± 0.6 %** 90 min: 7.4 % ± 0.8 %** 150 min: 7.4 % ± 1.0 %** 24 h: 2.4 % ± 0.4 %** 48 h: 0.6 % ± 0.3 % 72 h: 0.4 % ± 0.1 % 96 h: 1.2 % ± 0.2 %** It is noted that the SE ($SE = SD/\sqrt{n}$) is used instead of SD and the SE is rather high, which usually implicates a large standard deviation and, hence, non-parametrically distributed data.	**Significant MetHb formation** during the first 24 h after single IP injection, and again 96 h post-administration. Peak MetHb levels at 90 and 150 min after IP injection.	Nomura (1977) Supporting information (rel. 2)
Study on sulfhaemoglobin and MetHb formation No GLP compliance Mouse, ddY, 10-15 males Statistics not reported.	NPNA (CAS 90-30-2 / EC 201-983-0), purity not reported Intraperitoneal 0, 219 mg/kg Daily injection for 3 days, 48 h observation. No positive or negative controls reported.	Statistically significant increase in MetHb levels but no sulfhaemoglobin formation 48 h after last administration compared to control. MetHb levels (mean ± standard error (SE); **significant at p = 0.01): Control: 0.07 g/dl ± 0.01 g/dl corresponding to 0.4 % ± 0.1 % NPNA: 0.24 g/dl ± 0.02 g/dl** Corresponding to 1.6 % ± 0.2 %** It is noted that the SE ($SE = SD/\sqrt{n}$) is used instead of SD and the SE is rather high, which usually implicates a large standard deviation and, hence, non-parametrically distributed data.	**Significant increase in MetHb** 48 h after 3 consecutive IP injections (1/day). Confirms MetHb production. It is noted that only one (late) MetHb measurement has been done. The absolute level of MetHb may be underestimated, as the peak time point has not been estimated and considered.	Nomura (1977) Supporting information (rel. 2)

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

For specific target organ toxicity – repeated exposure (STOT RE), several *in vivo* repeated dose toxicity studies (RDT) are available, including a sub-acute guideline study (Bayer, 2002) and a sub-chronic guideline study (BASF, 2016b) in rats performed according to OECD TG 407 and 408, respectively, and in compliance with GLP. Another subacute study in rats was found in the scientific literature (Tanabe et al., 2017). The authors indicated that the study was performed in accordance with GLP and OECD TG 407; however, reporting did not include individual data, but short summaries of effects (e.g. on body weight and food/water consumption) and only mean values (± SD) for blood parameters, and organ weights, as well as results of some relevant histopathological findings are reported. The results of a prenatal developmental toxicity (PNDT) study in rats (according to OECD TG 414, GLP compliant) delivered additional information on this hazard class (BASF, 2016a).

These studies provide data regarding CLP classification of NPNA for STOT RE as they indicate that NPNA (purity > 99 %) causes significant haemolytic anaemia in rats and further affects liver weight of exposed rats. In all studies, significant effects on blood parameters were reported that are typical indicators of haemolytic anaemia. Furthermore, the studies described effects secondary to haematotoxicity on spleen, kidney and liver. In addition to the haematotoxicity, massive liver weight increases were seen in several of the available repeated dose toxicity studies.

Detailed tabulated information on the observed effects on blood and histopathology in the four different studies with NPNA can be found in the Confidential Annex, Tables 1 to 14.

<u>*General health and clinical signs*</u>

No mortality was observed in most of the studies, except for one rat which died one day before necropsy at exposure day 28 in the subacute literature study by (Tanabe et al., 2017) and one female receiving 500 mg/kg, which died after 2 days of treatment in the respective 7-day range-finding study (Bayer, 2000). Further effects in the range finding study were reduced motility, decreased reactivity, uncoordinated gait, laboured breathing, discoloured urine, piloerection and increased water consumption. In the PNDT study as well as after 28 days (Bayer, 2002) and 90 days of exposure (BASF, 2016b) the only test-substance-related clinical or behavioural changes were discoloured urine (for detail see below) and salivation after test material administration. After 28 days of exposure (Bayer, 2002), gait abnormalities were noted in some females, this effect however, could not be confirmed in a more recent 90-day study at similar concentration levels (BASF, 2016b). No statistically significant test substance-related effects on body weight development, i.e. mean body weight and body weight change values, were observed in any of the studies, except for body weight reductions in male rats after 28 days of exposure to NPNA at a high dose of 500 mg/kg bw/d (Tanabe et al., 2017).

<u>*Haematotoxicity*</u>

General Overview

Chronic haemolytic anaemia is known to be caused by (prolonged) exposure to numerous chemicals via several different mechanisms. The most prominent primary effects of substance-induced haemolytic anaemia include the decreased survival of mature erythrocytes (erythrotoxicity/-lysis) due to their increased destruction outside the bone marrow yielding a reduction in red blood cell (RBC) counts, haemoglobin (Hb) concentration and haematocrit (HCT) (Muller et al., 2006). Although these effects are considered reversible (as long as the effect size is not becoming fatal or the primary erythrocyte production in the bone marrow is not hampered), they can vary in degree and can in particular after repeated/chronic exposures be accompanied by severe secondary effects, including organ dysfunction and organ damage outside the blood system. A result of decreased Hb and RBC counts (due to diminished oxygen supply) may be decreased physical activity, including reduced motility and decreased reactivity, as well as other symptoms like pallor, dyspnoea, or tachycardia. These "overt clinical signs of hypoxia represent a clear undesirable impact on health" (Muller et al., 2006). Hb decreases of > 20 % are considered adverse per se, while decreases around 10 % usually are only considered for classification in combination with haemoglobinuria or relevant histopathological findings (ECHA, 2017).

Hb from destroyed RBCs can form complexes with haptoglobin and is subsequently metabolised. Hence, free Hb in plasma is observed, when the haptoglobin binding capacity is exceeded. Eventually, oxidisation of Hb to methaemoglobin (MetHb) can occur, and methaemoglobinaemia of a certain extent can lead to life-threatening conditions due to its high oxygen binding affinity, which interferes with normal Hb functions (i.e. oxygen delivery). ECHA (2017) states: “If methaemoglobinaemia does not result in lethality but exposure to methaemoglobin generating agents results in signs of damage to the erythrocytes and haemolysis, anaemia or hypoxemia, the formation of methaemoglobin shall be classified accordingly either in STOT-SE or STOT-RE”.

(Met)Hb is filtered in the kidneys where the “iron is extracted and incorporated into haemosiderin” (Muller et al., 2006). Thus, if the renal resorption capacity is exceeded, Hb and in severe cases also haemosiderin can be detected in urine (haemoglobinuria and haemosiderinuria, respectively). The ECHA Guidance (ECHA, 2017) states that “haemoglobinuria that is not limited to the first three days of treatment in the repeated dose study in combination with other changes indicating significant haemolytic anaemia (e.g. a reduction in Hb at ≥10 %)” are effects relevant for classification on STOT RE. In excessive cases of haemolytic anaemia, deposition of insoluble haemosiderin aggregates can also be observed in the spleen, kidney, and liver or in the bone marrow and other organs (haemosiderosis), being an indicator of persistent erythrophagocytosis in tissues without clearance mechanisms (Muller et al., 2006). This pigment deposition is considered not fully reversible and can potentially lead to (multi-)organ iron overload and subsequent cell and organ damage, including fibrosis and necrosis. In the spleen, haemosiderosis commonly results in splenomegaly, and after chronic exposure fibrosis may be observed in the red pulp area or in the (sub-)capsular regions (Muller et al., 2006), which is usually only visible after prolonged exposure (e.g. three months). In the liver, deposition of haemosiderin (e.g. in phagocytic Kupffer cells) has to be considered as adverse, as it may result in iron overload inducing fibrosis and more advanced lesions, such as (single-cell to advanced) necrosis (Muller et al., 2006). In kidney, haemosiderosis can yield degeneration (e.g. desquamated cells and/or necrosis/apoptosis) and subsequent regeneration (replacement of degenerated cells) of the proximal tubules, an effect that may be fully or partially reversible depending on the severity of effects. In this regard, ECHA (2017) notes that “marked increase of haemosiderosis in the spleen, liver or kidney in combination with other changes indicating significant haemolytic anaemia (e.g. a reduction in Hb at ≥10 %) in a 28 day study” has to be considered relevant for classification.

The haeme moiety of Hb from destroyed erythrocytes is generally degraded via biliverdin to bilirubin, independent of the site of destruction, leading to increased levels of bilirubin in blood (hyperbilirubinaemia) and urine (hyperbilirubinuria) (Muller et al., 2006). In the gastro-intestinal tract, bilirubin can also be converted to urobilinogen by bacterial reduction. Hence, in extreme cases the latter can be detected in urine as well.

As adaptive response to the accelerated RBC loss, erythropoiesis – stimulated by increases in the production of erythropoietin (Epo) in kidney – is elevated. Specifically, reticulocytosis is enhanced in the bone marrow, resulting in increased counts of reticulocytes (RET), the precursors of erythrocytes, which are generally larger than mature erythrocytes, contributing to an increase in mean corpuscular volume (MCV). Hence, in case of chronic haemolytic anaemia, the (young) erythrocytes are usually larger (↑MCV) and contain more Hb per single RBC (↑mean corpuscular haemoglobin (MCH)), while the MCH concentration (MCHC, average concentration of Hb in packed RBC) is reduced and cell morphology may be abnormal. The intensely elevated blood cell formation is regularly accompanied by bone marrow hyperplasia (due to medullary haematopoiesis) and extramedullary haematopoiesis, the latter mainly occurring in spleen but also in liver and other tissues. As accelerated erythrocyte production as such is usually reversible, it can only be considered adverse (per se) in case the compensatory regenerative capacity is exceeded by the haemolytic toxicity resulting in clinical anaemia (Muller et al., 2006).

Generally ECHA (2017) notes that “in the case where multiple less severe effects with regenerative capacity were observed, the classification should apply as “Assessment shall take into consideration not only significant changes in a single organ or biological system but also generalised changes of a less severe nature involving several organs. (CLP Annex I, 3.9.1.4)”.

Findings in studies with NPNA

Reductions in Hb were seen in several of the available repeated dose studies. In the PNDT-study, **reductions in Hb were around or above 10 %** (= guidance level relevant for classification) **in numerous dams after being exposed to NPNA for 14 days through gestation days (GDs) 6 - 19 at 150 mg/kg bw/d (Fig. 1A). More precisely, at that dose reductions in Hb were above 9 % in 8/25 dams (32 %) and > 10 % in 4/25 dams (16 %)**, although the mean value for this parameter (on all animals) was -6 % (statistically significant effect; Fig. 1A). There was no obvious correlation of the reductions in Hb with either body weight, uterine weight or number of offspring. **After subacute exposure to 20 and 80 mg/kg bw/d (28 days; Bayer (2002)), mean reductions in Hb were slightly below 10 % in females (mean of -7 % and -9.2 %, respectively). However, at 80 mg/kg bw/d, a dose well below the guidance thresholds for STOT RE Cat. 2 classification, the median reduction in Hb was –11 %, meaning that ≥ 50 % of the females showed a reduction in Hb of ≥ 11 % (Fig. 1B). In fact, at that dose 3/5 (60 %) females showed Hb reductions above 11 %** (Fig. 1B)**.** Effects in male animals were only slight, suggesting that females might be more susceptible to NPNA effects than males. Changes in Hb levels were not observed in high dose recovery animals (80 mg/kg bw/d) when compared to recovery controls (Bayer, 2002). In the subchronic study (90 days; (BASF, 2016b), Hb reductions were below 10 % (guidance value according to (Muller et al., 2006)) in males and females at the high dose of 125 mg/kg bw/d (-4 % and -6 %, respectively). In the supporting subacute literature study (28 days; Tanabe et al. (2017), the effects on blood parameters were not significant at 100 mg/kg bw/d (Hb: males: -4.0 %, females: -1.4 %). Reductions in Hb, however, exceeded 15 % at 500 mg/kg bw/d in both sexes, supporting the observed dose-response-relationship of this parameter. No information is available on a dose level close to the guidance level for classification, which is 300 mg/kg bw/d for a 28-day study. The lack of significant Hb effects at 100 mg/kg bw/d compared to the above mentioned guideline studies might indicate that the SD rats used by Tanabe et al. (2017) may be less susceptible to the haemolytic effects of NPNA compared to the Wistar rats used in the other studies. Strain and sex differences in haematological parameters in general were also reported in a recent literature study (de Kort et al., 2020).
It is noteworthy that effects on Hb levels in SD rats of the subacute literature study were still significant after a recovery period of 14 days (500 mg/kg bw/d: Hb -7 % and -8 % in males and females, respectively (Tanabe et al., 2017)). No reductions in Hb could be observed in the recovery animals of the 28-day guideline study when compared to the respective recovery controls, but much lower dose levels (up to 80 mg/kg bw/d) were tested in this study (Bayer, 2002). The other two studies did not employ recovery groups. Thus, data suggest that sufficiently high doses of NPNA generate non-compensated anaemia.

RET counts were highly increased in high dose animals of the subchronic (+59 % and +64 % in females and males, respectively, at 125 mg/kg bw/d and marked but non-significant increases at 25 mg/kg bw/d; BASF (2016b)) and PNDT (+89 % at 150 mg/kg bw/d and marked but non-significant increases at 50 mg/kg bw/d; BASF (2016a)) study. After subacute exposure, RET counts were significantly increased at a high dose of 500 mg/kg bw/d in both sexes, while a non-significant increase (+41 %) occurred in males at 100 mg/kg bw/d (Tanabe et al., 2017); no RET effects were recorded after a 28-day exposure to ≤ 80 mg/kg bw/d (Bayer, 2002). RBC counts were reported to be significantly reduced in all studies at the high doses (i.e., ≥ 80 mg/kg bw/d). Both effects, RET and RBC counts, suggest a significant decrease in oxygen transporting capacity of the blood due to substantial haemolytic anaemia, which demands compensatory erythrocyte production, i.e. increased erythropoiesis. **Reductions in RBC counts in the PNDT study were > 10 % in 6/25 dams (24 %) after being exposed through GDs 6 - 19 at 150 mg/kg bw/d (Fig. 1C)**, although the mean reduction was -6 % (statistically significant effect; BASF (2016a)). **After 28 days of exposure, reductions in RBC counts were reported to be > 10 % in 2/5 females (40 %) at 80 mg/kg bw/d** (mean of -6.5 %; effect not statistically significant; Bayer (2002); **Fig. 1D**). No effects on RBC and RET counts were noted in male animals treated with NPNA at this dose. **Reductions in RBC counts were significant in both sexes at 125 g/kg bw/d after subchronic exposure** (mean of -8.5 % in both **sexes, median of approximately -10 % (males: -9.2 %, females: -10.6 %; Fig. 1E and 1F).** At this dose, which is marginally above guidance threshold for STOT RE classification according to (ECHA, 2017)), **reductions in RBC exceeded 10 % in 6/10 females (60 %) and 4/10 males (40 %)** (BASF (2016b); Fig. 1E and F). In the subacute literature study (Tanabe et al., 2017), RBC counts were still highly affected after recovery of subacute NPNA exposure at 500 mg/kg bw/d (reduction > 10 % in both sexes). Except for a significant increase in MCV, no additional haematological parameters were affected in recovery animals of the subacute guideline study (Bayer, 2002).

Effects on HCT, MCV, MCHC, and MCH in the main and recovery animals of all studies were usually < 10 %.

In the available studies, **marked and significant dose-dependent increases in bilirubin serum levels, a hallmark of erythrolysis,** were detected in animals of almost all treatment groups. Starting at low doses of 4 mg/kg bw/d (Tanabe et al. (2017)) not yet statistically significant, albeit dose-related increases of serum bilirubin indicate that increased erythrolysis already occurs at doses below those associated with clinical anaemia. In the PNDT study, **dams exposed to NPNA doses of 15 mg/kg bw/d and higher during gestation showed significant increases in bilirubin levels of up to +475 %** (BASF, 2016a). **After subacute exposure, significantly increased bilirubin levels were detected in male serum at ≥ 20 mg/kg bw/d (up to +267 %; Bayer (2002)). In females, the increases were visible at 20 mg/kg bw/d as well; and statistical significance was reached at the high dose of 80 mg/kg bw/d (+77 %). In the 90-day study** (BASF, 2016b), **significant increases in bilirubin levels were reported for males and females at doses at and above 5 mg/kg bw/d and 25 mg/kg bw/d, respectively. Similarly, significant bilirubin plasma level increases were detected at ≥ 100 mg/kg bw/d in the subacute literature study** by Tanabe et al. (2017). It is noted that the study authors of both RDT guideline studies (BASF, 2016b; Bayer, 2002) postulated these increases in serum bilirubin levels as an interference of the test substance with the test kits. In the subchronic study, the interference tests are said to result in an interference of NPNA at least down to a concentration of 1 mg/L. The study authors stated: "According to Sikka et al. (1981) one oral gavage of 160 mg/kg bw of the compound led to peak levels of about 3 mg/L serum after two hours with minimal residues of parent compound left after 24 hours." (BASF, 2016b). Thus, they concluded that at least at a dose of 125 mg/kg bw/d the increases in bilirubin may have been due to NPNA interference. Nevertheless, lower test concentrations (i.e. 5 and 20 mg/kg bw/d NPNA) also resulted in elevation of bilirubin in serum. This effect was also observed in some of the control animals at low severity. Thus, it is not entirely clear whether the increase in serum bilirubin was in fact due to NPNA interferences or whether it has to be considered an actual treatment-related effect. As **increased bilirubin levels were consistently seen in several studies** and, furthermore, **no interference of NPNA with bilirubin detection was reported in the PNDT study, although the same detection method was used (DPD method), this interpretation is considered as of limited plausibility. In the PNDT study, significant treatment-related increases of bilirubin were reported by the respective study authors, questioning the assumptions on the interference made by the study authors of the other two studies.**

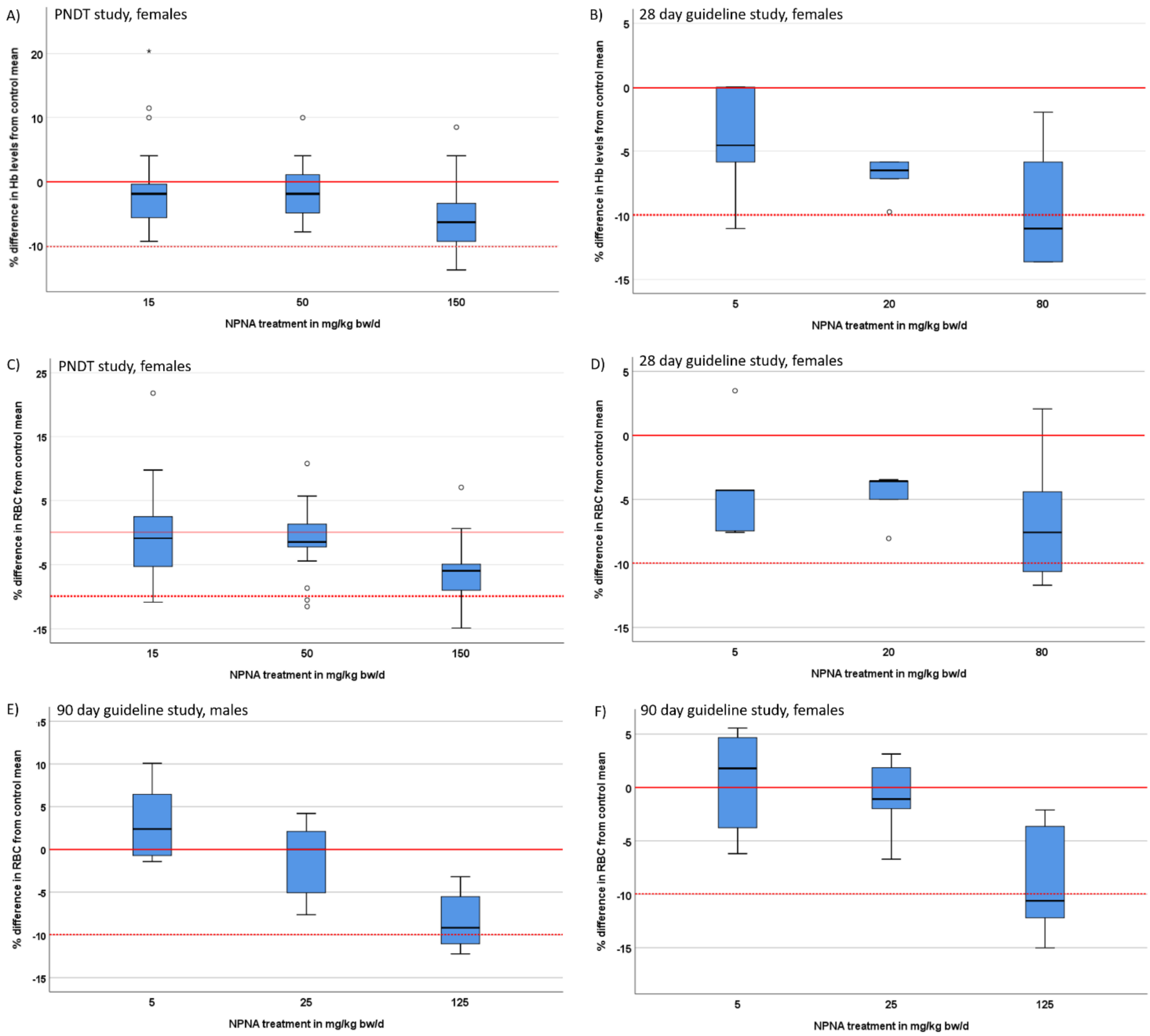


Figure 1: % Difference in haemoglobin (Hb) blood levels compared to the control mean in:

A) the PNDT study (BASF, 2016a) and B) females of the 28 day guideline study by (Bayer, 2002).

% Difference in RBC compared to the respective control mean in C) the PNDT study (BASF, 2016a), D) females of the 28 day guideline study by (Bayer, 2002), E) males and F) females of the 90 day study by (BASF, 2016b). The solid red line depicts the mean control values (0 % difference to control mean), while the dotted red line depicts the guidance value of > 10 % Hb and RBC reduction, respectively, according to Muller, 2006.

Moreover, bilirubin was detected in urine of males and/or females after subchronic exposure to NPNA (males: ≥ 25 mg/kg bw/d; females: ≥ 5 mg/kg bw/d; (BASF, 2016b)). Similarly, bilirubin was found in urine of both sexes after subacute exposure to NPNA at ≥ 5 mg/kg bw/d (Bayer, 2002). Again, the study authors of both of the studies claimed that the increases in bilirubin urine levels may have be due to an interference of NPNA with the test kits. In the subchronic study, the interference test was reported to result in an interference of NPNA at least down to a compound concentration of 100 mg/L. Thus, the study authors questioned the results for bilirubin in urine, again hampering a firm assessment. **However, no interferences of NPNA with urobilinogen testing were reported and urobilinogen values were significantly elevated in both, the 28-day study (both sexes: 80 mg/kg bw/d; (Bayer, 2002)) and the 90-day guideline study (males:**

≥ 25 mg/kg bw/d; (BASF, 2016b)). Hence, if urobilinogen levels are increased due to elevated bilirubin conjugation, analogous increases in bilirubin serum (and urine) levels could be expected as well. Overall, there is a rather high uncertainty whether NPNA interference was in fact the reason for the observed increases in bilirubin urine/serum levels or whether they were due to the interferences reported for the detection methods used. No urinalysis results were presented for the animals of the PNDT study (BASF, 2016a) and the 28-day literature study by Tanabe et al. (2017).

Urine of NPNA treated animals was regularly described as purple/reddish coloured (subchronic study (m, 125 mg/kg bw/d), subacute literature study (m+f, >100 mg/kg bw/day)), and blood cells (indicative of haemoglobinuria or haematuria) were found in the urine of females at ≥ 5 mg/kg bw/d after 90 days of NPNA exposure with increasing incidence and severity the higher the dose; statistical significance was reached at ≥ 25 mg/kg bw/d (BASF, 2016b). It is reported that it **was not tested for blood in urine at the high dose of 125 mg/kg bw/d due to expected/assumed interferences** of the test substance as mentioned above. As for bilirubin testing, the study authors claimed that testing for haemoglobinuria was affected by compound excretion as well, as they found an interference at least in one urine sample (at 100 mg/L). Therefore, the study authors considered the detected blood in urine of no pathophysiological relevance. A statistically significant dose-response relationship of NPNA dose and the correlating grade of interference with Hb-measurement, as stated by the authors, could however only be established for females. On the contrary, blood was also regularly found in male urine samples of all groups, including the controls (dip-stick analysis for occurrence of blood cells) with no clear dose-response relationship regarding incidence and severity (and not significant when compared to controls), contradicting the assumption of the study authors (BASF, 2016b). Thus, and taking into account the additional haemolytic effects found after NPNA exposure, a pathophysiological relevance of the detected dose-related increase in incidence and severity of blood in the urine of NPNA-treated female rats at doses relevant for STOT RE classification can in fact not be excluded**.**

Enlarged kidneys with rough and discoloured surfaces were found in the 7-day range finding study at 250 and 500 mg/kg bw/day (Bayer, 2000). **Dose-dependent kidney degeneration (increase of cellular eosinophilia, desquamated cells or necrosis/apoptosis) and regeneration (increase in basophilia and large vacuolar nuclei of tubular epithelial cells) of the proximal tubules were reported in males at 125 mg/kg bw/d in the subchronic study** (BASF, 2016b). Although this tubular cell loss can be partially or fully reversed via cell regeneration, haemosiderin-related tubular cell death is considered an adverse effect, which in severe cases can yield secondary nephrotoxic effects, such as haemosiderinuria, tubular necrosis, interstitial inflammation and fibrosis (Muller et al., 2006). Unfortunately, haemosiderinuria was not investigated in any of the studies. However, **significant increases in urea levels (males at 125 mg/kg bw/d) in NPNA-treated animals of the subchronic study, in addition to significant increases in absolute and relative kidney weights at the high dose of 125 mg/g bw/d (both sexes, approx. +15 %;** BASF (2016b)), **are as well indicative of deterioration of renal function**. **Increases in urea blood levels (indicative of kidney dysfunction) were observed in dams of the PNDT study (at ≥ 15 mg/kg bw/d),** but histopathological analyses of kidneys were not performed in this study (BASF, 2016a). **A slight increase in incidence of basophilic tubules were observed in kidneys of males in the subacute guideline study (5/5 males, grade 1 at 80 mg/kg bw/d)**, although the significance of these cases is hard to interpret, as high baseline incidences of this effects were observed as well (3/5 males, grade 1 in controls) (Bayer, 2002). Basophilic tubule, slight to moderate signs of renal papillary necrosis and post-necrotic mineralisation of papilla were in fact reported (predominantly) in males at higher doses which are not considered relevant for classification (i.e. 500 mg/kg bw/d) in the subacute literature study by (Tanabe et al., 2017).

In addition, **chronic progressive nephropathy (CPN) was observed frequently in males in the subchronic study starting at a dose of 5 mg/kg bw/d with higher incidence at ≥ 25 mg/kg bw/d and a clear dose-response-relationship regarding severity (BASF, 2016b). The severity of this effect (but not incidence) was statistically significant at ≥ 25 mg/kg bw/d.** No such findings were reported in any of the other studies. The study authors considered this lesion treatment-related although they indicated that CPN, a frequently observed effect in the male aging rat, is (only) exacerbated by the chemical treatment and thus may not be of relevance for humans. As no specific histopathological findings with respect to CPN were reported in the study report, final assessment of the relevance of this finding is hampered. Due to the lack of this specific information**, it is not possible to fully exclude a potential impact of the observed haematotoxicity on the**

progression of the reported CPN in the treated rats, as additional kidney effects elicited by haemolysis were observed as well.

Pigment storage (haemosiderin deposition, as iron was confirmed by specific staining) was observed in the spleen of rats in the subchronic study (BASF, 2016b), supporting the above summarised findings regarding haematotoxic effects after NPNA exposure. **Haemosiderosis in spleen was significantly evident in females at ≥ 5 mg/kg bw/d with significantly increasing incidence and severity (dose-response). In males, pigment storage was observed at the high dose of 125 mg/kg bw/d in 6/10 animals (grade 2 = slight),** whereas 2/10 males with grade 2 pigment storage were also seen in controls. Severe pigment storage in the spleen of female rats was similarly found in the subacute literature study, however, only at the high dose of 500 mg/kg bw/day, and after recovery, whereas the control (recovery) females showed moderate pigment storage as well (Tanabe et al., 2017). The spleen is the main site of erythrolysis. The cascade of secondary effects, such as phagocytosis and deposition of cellular remnants, inflammation, fibrosis and extramedullary haematopoiesis (depending on severity and duration) may explain the significantly **increased spleen weights found** in animals of the recovery groups in the subacute guideline study at 80 mg/kg bw/d (Bayer, 2002) and in recovery animals at 500 mg/kg bw/d in the subacute literature study (Tanabe et al., 2017).

A dose-dependent increase in incidence and severity of extramedullary haematopoiesis was observed in the spleen of females after subchronic exposure to ≥ 5 mg/kg bw/d, although incidence and severity of this effect did not reach statistical significance (BASF, 2016b). Males did not exhibit this effect in the available studies, again suggesting a higher susceptibility of females compared to males with regard to NPNA-elicited haematotoxicity. Slight to severe extramedullary haematopoiesis in the spleen was also observed in the subacute literature study at ≥ 100 mg/kg bw/d in both sexes, however, in this study also control animals showed slight (m) to moderate (f) extramedullary haematopoiesis (Tanabe et al., 2017).

Increased incidence in focal Kupffer cell accumulation in the liver of females, an indicator for severe chronic haemolysis, was reported after subacute exposure to NPNA at 80 mg/kg bw/d (effect not statistically significant and minimal Kupffer cells were also reported in 1/5 control females; (Bayer, 2002)). It is noted that at this dose, Kupffer cell accumulation was only observed in females showing a reduction in Hb > 10 %. **This effect was accompanied by increases in absolute liver weight (> +10 %**), being statistically significant in recovery females (+14.6 %). Magnitude of female liver weight increases, however, did neither correlate with incidence of Kupffer cell accumulation nor severity of Hb reduction. In males, 3/5 animals at the high dose and in the respective controls showed minimal Kupffer cell accumulation in liver as well. Rather massive liver weight increases were also regularly observed in the other repeated dose studies (for details see "liver toxicity" below). Single incidences of (slight to moderate) hypertrophy of centrilobular hepatocytes were observed in the subacute literature study by Tanabe et al. (2017) at 100 mg/kg bw/d in females (not significant) and at 500 mg/kg bw/d in all treated animals (significant in both sexes; effect without clear signs of regression). Moreover, almost all NPNA-treated animals showed this effect (slight to moderate severity) after 90 days of exposure to a dose of 125 mg/kg bw/d (BASF, 2016b). In the subacute literature study (Tanabe et al., 2017), also single incidences of focal necrosis – slight to moderate in severity – were observed in males of all treatment-groups but not in the respective controls. Thus, it cannot be excluded that the observed slight necrosis was caused by the NPNA treatment. Overall, these effects on liver together with the increased haematopoiesis and pigment storage in spleen are indicative of iron overload due to haemolysis caused by NPNA treatment. As the distribution of Kupffer cell accumulation, centrilobular liver cell hypertrophy and necrosis is focal (assumed to be multifocal as only single liver sections were assessed) and of slight to moderate severity (for hypertrophy) and not diffuse, these effects cannot fully explain the massive weight increase at doses of 100 mg/kg and above.

MetHb levels are rarely reported in RDT studies and were also not measured in the above-mentioned repeated dose toxicity studies. However, significant MetHb formation was reported in two supporting *in vivo* short-term experiments in mice after IP injection(s), suggesting slight to moderate methaemoglobinaemia (Nomura, 1977). As mice are less sensitive to Hb oxidation than humans (healthy humans: ≤ 1 % MetHb; healthy mice: 0 – 2 % MetHb), an increase of MetHb > 2 % in mice can generally be considered as biologically relevant (Muller et al., 2006). However, due to the physiologically non-relevant exposure route used in this study, the validity of the quantitative information on this finding is questionable and can only be regarded as supporting information, confirming that NPNA is a MetHb generating substance and this mode of action is plausible as to the observed haematotoxicity.

It is noted that the haemolytic effects of NPNA are comparable with haematotoxicity observed after repeated exposure to other aromatic amines (e.g. classification of aniline as STOT RE 1, H372 (blood and haematopoietic system)), although the magnitude of haemolytic NPNA effects is not as marked.

Liver toxicity

Increases of relative and/or absolute liver weights were frequently observed except for the PNDT-study, where organ weights were not monitored.

Liver weight was reported to be increased when compared to the control in both sexes after a 28-day exposure at 80 mg/kg bw/d, even after a recovery period of 14 days (absolute weight: around/above +11 % in both sexes; +8 % (m) and +15 % (f) after recovery; relative weight: +7 % (m) and +13 % (f), and +7 % (f) after recovery; Bayer (2002)). Increases were more marked at ≥ 100 mg/kg bw/d (absolute weight around/above +20 % in both sexes, relative weight: +24 % and +16 % in males and females, respectively; Tanabe et al. (2017)). The relative liver weight increases in the latter study exceeded +70 % when compared to controls at a dose of 500 mg/kg bw/d (both sexes), while the absolute liver weight at that dose was +41 % and +71 % in males and females, respectively. The authors of this study further noted centrilobular hypertrophy (statistically significant at 500 mg/kg bw/d, single incidences at lower doses) and single cell necrosis (not statistically significant, single incidences) in treated rats that cannot explain the high levels of weight increases. **In the subchronic study, NPNA doses of ≥ 25 mg/kg bw/d similarly yielded dose-dependent increases in absolute and relative liver weight, respectively, from +17.2 % and +11.1 % in males (effects significant) and ca. +6 % in females (relative weight; effect not significant) at 25 mg/kg bw/d up to approximately +30 % (absolute and relative weights) in both sexes at a dose marginally above the upper guidance limit for STOT RE 2 classification, i.e. at 125 mg/kg bw/d. The weight increases seen in the subchronic study were accompanied by slight to moderate centrilobular hypertrophy in males (incidence and severity with dose-response) and females (only at the high dose).** This effect was considered adverse at 125 mg/kg bw/d by the study authors, as the severity was moderate in all females (10/10) and mild in 9/10 male animals, and effects showed a dose-response relationship (effects significant regarding incidence and severity at 25 mg/kg bw/d and 125 mg/kg bw/d, respectively, for males and females). The mild to moderate centrilobular hypertrophy in treated animals at the high dose of 125 mg/kg bw/d was accompanied by significant increases in liver weight around (males) or exceeding (females) +30 %. At 25 mg/kg bw/d, however, incidence and severity of centrilobular liver hypertrophy did neither correlate with the observed increases in absolute nor in relative liver weight.

Liver weight increases through centrilobular hypertrophy of hepatocytes are likely and hypertrophy can in certain cases be regarded as adaptive, if increased activity of metabolic enzymes have been demonstrated and other degenerative/adverse liver effects are absent. For NPNA the occurrence of liver cell hypertrophy at doses below or close to the guidance value for STOT RE classification was reported in the subchronic study only, which suggests that the effect may be related to the subchronic exposure duration. In the supporting subacute literature study, significant increases in incidence of hypertrophy were reported only at a high dose of 500 mg/kg bw. Due to the lack of further data on metabolic enzyme action, liver weight increases at this size (> 20 %) is considered as a pathological effect elicited by NPNA. The severe liver weight increases (> 20 %) observed in the 28-day studies (without liver cell hypertrophy) and in the 90-day study cannot be explained as secondary to haemolytic anaemia. The weight increases were not corroborated by other histopathological findings, except in the one study (BASF, 2016b), in which centrilobular hypertrophy was observed in both sexes. This mild to moderate hypertrophy, however, is considered not severe enough to fully explain the massive liver weight increases observed in the study animals. Moreover, the mild to moderate hypertrophy did not correlate with the absolute and relative liver weight increases at lower dose (i.e. 25 mg/kg /bw/d in the subchronic study). The lack of other relevant histopathological findings accompanying these weight increases is noteworthy, as one would assume that microscopic effects are generally observable in cases of increases in organ weights of ≥ 20 % (Hall et al., 2012). Nevertheless, because of the severity, these drastic dose-dependent increases in absolute and relative liver weight are judged as adverse per se, and, as this effect was seen consistently in all available studies, the liver is considered as additional target organ for NPNA.

10.12.2 Comparison with the CLP criteria

Specific target organ toxicity, repeated exposure, comprises specific, target organ toxicity arising from a repeated exposure to a substance or mixture, including all significant health effects that can impair function, both reversible and irreversible, immediate and/or delayed.

According to CLP Regulation, section 3.9.2.2., classification of substances as specific target organ toxicants following repeated exposure is based on the weight of all evidence available, including the use of recommended guidance values that take into account the duration of exposure and the dose/concentration, which produced the effect. The substances are placed in one of two categories, depending upon the nature and severity of the observed effect(s).

The threshold values for category 1 are based on significant toxic effects observed in a 90-day repeated-dose study conducted in experimental animals (study duration 90 days: $C \leq 10$ *mg/kg bw/d*).

As no significant adverse effects were observed at ≤ 10 mg/kg bw/d in any of the available studies, classification of NPNA as STOT RE 1 is considered unjustified.

Substances are classified in category 2 for target organ toxicity (repeated 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. Guidance dose/concentration values are to be used as part of a weight-of-evidence evaluation. In exceptional cases, human evidence can also be used to place a substance in Category 2 (see CLP Regulation, section 3.9.2.2.). The threshold values for category 2 are based on significant toxic effects observed in a 90-day repeated-dose study conducted in experimental animals (study duration 90 days: 10 *mg/kg bw/d* $< C \leq 100$ *mg/kg bw/d*).

Based on the available data and related only to the primary effect of haematotoxicity, it is not entirely clear if an adjustment of threshold values for varying study durations, using dose/exposure time extrapolations according to Haber's rule as proposed in the ECHA Guidance, can be considered appropriate in the present case. Considering the nature of the adverse effects, its potential to compensate the clinical signs of anaemia at least partly and the similarity of observations on other haemolytic substances, the level of effective doses is less clearly linked to the duration of exposure. However, any adverse haemolytic effect observed at a dose close to 100 mg/kg either in a 28-day or 90-day study should be considered as relevant for classification.

Haematotoxicity

According to CLP Regulation, section 3.9.2.5.2., the criteria for haematotoxicity are 'any consistent and significant' adverse changes in haematology. Specifically, e.g. any consistent and significant adverse effect in clinical biochemistry, haematology or urinalysis parameters; significant organ damage noted at necropsy and/or subsequently seen or confirmed at microscopic examination, such as multifocal or diffuse necrosis, fibrosis or granuloma formation in vital organs with regenerative capacity are considered relevant. As an example, haematotoxicity is considered severe if an increase of haemosiderosis in the spleen, liver or kidney in combination with other changes indicating significant haemolytic anaemia is found. In the ECHA 'Guidance on the Application of CLP Criteria' (ECHA, 2017), it is further mentioned that in "**the case where multiple less severe effects with regenerative capacity were observed, the classification should apply as "Assessment shall take into consideration not only significant changes in a single organ or biological system but also generalised changes of a less severe nature involving several organs.**" (CLP Annex I, 3.9.1.4).

No information is available on toxicity after repeated exposure to NPNA in humans. Studies in rats and mice indicate that the haematological system is a relevant target of the toxicity of NPNA. Toxic effects indicative for haematotoxicity were observed in the OECD TG 408 study (BASF, 2016b), in a OECD TG 407 study (Bayer, 2002) and in a prenatal developmental toxicity study (BASF, 2016a). A second subacute study supposedly performed according to OECD TG 407 (Tanabe et al., 2017) showed haematotoxic effects in exposed rats as well.

For NPNA, several aspects of its toxicity on the blood system can be related to criteria set out in the CLP guidance, Section 3.9.2.5.2., supporting classification for STOT RE 2, H373:

Blood

- reductions in Hb above 10 % in 16 % of the dams and Hb reductions above 9 % in 32 % of the dams (PNDT study, at 150 mg/kg bw/d (BASF, 2016a), Fig. 1A), indicating a rather high variability in susceptibility of NPNA effects on Hb levels,
- mean reductions in Hb around 10 % in the 28-day guideline study (females: -7 % and -9.2 % at 20 and 80 mg/kg bw/d, respectively) with a median reduction in Hb in females of -11 % at 80 mg/kg bw/d, meaning that over half of the individual females showed Hb reductions above 11 % (Bayer, 2002) (Fig. 1B), again highlighting a high variability in affected individuals,
- reductions in RBC counts > 10 % in several individuals in the PNDT study at 150 mg/kg bw/d (24 % of the dams (BASF, 2016a), Fig. 1C), in the subacute guideline study at 80 mg/kg bw/d (40 % of the females; (Bayer, 2002), Fig. 1D),
- significant dose-dependent increases in bilirubin plasma levels in all studies (at ≥ 5 mg/kg bw/d; up to +1 715 %),
- significant MetHb formation in mice after IP injection (Nomura, 1977);
- significant increases in plasma urea levels indicating deterioration of renal function in the PNDT study at ≥ 15 mg/kg bw/d; (BASF, 2016a).

Urine

- (abnormal) bilirubin as well as urobilinogen levels in urine at rather low NPNA doses (at ≥ 5 mg/kg bw/d in the two repeated dose toxicity guideline studies (subacute (Bayer, 2002) and subchronic (BASF, 2016b), at ≥ 15 mg/kg bw/d in the PNDT study (BASF, 2016a), at ≥ 100 mg/kg bw/d in the 28-day literature study (Tanabe et al., 2017)),
- haemoglobinuria (dose-dependent) that is not limited to the first three days of treatment in the 90-day study (females at ≥ 25 mg/kg bw/d) (BASF, 2016b).

Organs

- pigment storage (haemosiderosis) in spleen of females in the subchronic study (≥ 5 mg/kg bw/d; dose-related significant increases in incidence and severity; (BASF, 2016b));
- extramedullary haematopoiesis in spleen of females in the subchronic study at ≥ 5 mg/kg bw/d (not observed in controls; (BASF, 2016b)) and in the subacute literature study (Tanabe et al., 2017) at ≥ 100 mg/kg bw/d (and after recovery); in the latter study effects were not statically significant and slight (males) to moderate (females) effects were also observed in controls,
- focal Kupffer cell accumulation (haemosiderosis) in liver of females in the 28-day guideline study at 80 mg/kg bw/d (effect not significant; (Bayer, 2002))
- single cases of focal liver cell necrosis in males of the subacute literature study at ≥ 4 mg/kg bw/d (Tanabe et al., 2017),
- deterioration of renal function in the subchronic study (BASF, 2016b): degeneration/regeneration of renal tubules (in males at ≥ 25 mg/kg bw/d).

Additional adverse effects on the blood system (and secondary effects in spleen and kidney) were observed at doses (slightly) above the guidance value of 100 mg/kg bw/d:

Blood

- reductions in RBC counts > 10 % in several individuals in the subchronic study at 125 mg/kg bw/d (40 % of males, Fig. 1E; 60 % of the females with a median reduction of 11 % (BASF, 2016b), Fig. 1F),
- significant increases in plasma urea levels in the subchronic study (BASF, 2016b) (males: 125 mg/kg bw/d) indicating deterioration of renal function,

Urine

- reddish discoloured urine in all males and females at 125 mg/kg bw/d (from study day 55 - 60 onwards) (BASF, 2016b).

Organs

- pigment storage (haemosiderosis) in spleen in females of the subacute literature study at 500 mg/kg bw/d (and after recovery; (Tanabe et al., 2017)),
- significant increases in absolute and relative kidney weights (approx. +15 %, both sexes) at 125 mg/kg bw/d.

In weight of evidence, data clearly indicate that NPNA causes significant haemolytic anaemia, affecting multiple organs and general health of rats and mice, although data for the latter species is limited. It is noted that the effects on blood parameters and histomorphology have to be seen as borderline with respect to the criteria as laid down in Muller et al. (2006) and the ECHA Guidance on the Application of the CLP Criteria (ECHA, 2017)(e.g. regarding Hb reduction of ≥ 10 %) and further considering that several haematotoxic effects were only observed at a dose slightly above the upper limit value for STOT RE 2 classification (i.e. at 125 mg/kg bw/d). Nevertheless, it is noted that the ECHA Guidance states that in a weight of evidence "**the emphasis should be on the interpretation of the whole biological picture to judge the impact on health**" (Muller et al., 2006). In several studies the limit values of relevant blood parameters were exceeded in numerous individual test animals, while the mean values may be below 10 %, demonstrating high individual variability in the strength of effect that should be considered as relevant for humans. With regard to the guidance threshold for classification, it is further stated in the CLP Regulation that the "**guidance values and ranges mentioned in paragraphs 3.9.2.9.6 and 3.9.2.9.7 are intended only for guidance purposes, i.e., to be used as part of the weight of evidence approach, and to assist with decisions about classification. They are not intended as strict demarcation values**". Overall the adverse effects observed in the various repeated dose toxicity studies are considered borderline for classification for STOT RE 2, particularly with regards to severity of histopathological and blood effects. Thus, classification is currently not proposed.

Liver toxicity

Liver weight increases of around and above 20 % were seen in the subacute literature study at ≥ 100 mg/kg bw/day and in the subchronic study at 125 mg/kg bw/day, the latter is only marginally above the upper threshold guidance value for STOT RE 2 classification. Absolute and relative liver weight increases > 10 % were also observed after subchronic exposure of male rats to 25 mg/kg bw/d NPNA (+17.2 % and +11.1 %, respectively). Also in the subacute guideline study relative liver weights increased at 80 mg/kg bw/day, however, increases at this dose did not exceed 15 %.

Although not accompanied by relevant histological findings, the massive and dose-related increases in absolute and relative liver weights are judged as adverse per se.

Thus, based on the available data **the liver is to be identified as target organ for NPNA toxicity.**

10.12.2.1 Conclusion on classification and labelling for STOT RE

Considering the entirety of adverse effects on the blood and the additional multiple secondary effects in spleen, kidney and liver after repeated exposure of rats to NPNA doses relevant for classification, and further taking into account the clear dose-response relationship for numerous parameters related to haematotoxicity starting at doses as low as 5 mg/kg bw/d, it is concluded that NPNA elicits haemolytic anaemia of borderline severity when compared to the CLP classification criteria. Hence, **classification of NPNA for STOT RE 2, H373 (blood system) is currently not proposed**.

10.13 Aspiration hazard

Not assessed in this dossier.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

Not assessed in this dossier.

12 EVALUATION OF ADDITIONAL HAZARDS

Not assessed in this dossier.

13 ADDITIONAL LABELLING

Not assessed in this dossier.

14 REFERENCES

Aalto-Korte K., Suuronen K., Kuuliala O., and Jolanki R. (2008): Contact allergy to 2,5-dimercapto-1,3,4-thiadiazole and phenyl-a-naphtylamine, allergens in industrial greases and lubricant oils - Contact allergy to water-insoluble greases is uncommon but needs to be considered in some workers. Contact Dermatitis 58 (2), 93-96

Abele M.e.a. (1971): Alterungsschutzmittel. Bayer AG, Handbuch für die Gummi-Industrie, Leverkusen

AMR (1974): Toxic hazards research unit annual technical report: 1974; Acute toxicity studies on four amine compounds. Report no. F33615-73-C-4059, date: 1974-07. University of California, Irvine Overlook Branch. Laboratory A.M.R., unpublished

BASF (2016a): Prenatal developmental toxicity study in Wistar rats, oral administration (gavage) Report no. 30R0068/14R049, date: 2016-06-30. BASF SE, unpublished

BASF (2016b): Repeated dose 90-day combined oral toxicity-/neurotoxicity study in Wistar rats, administration by gavage. Report no. 64C0068/14S024, date: 2016-04-08. BASF SE - Experimental Toxicology and Ecology, 67056 Ludwigshafen, Germany unpublished

Bayer (1978a): Acute oral toxicity (april 1978), date: 1978-04-29. Bayer, Institut für Toxikologie, Wuppertal-Elberfeld AG R.C.G.L.

Bayer (1978b): Acute oral toxicity (january 1978), date: 1978-01-31. Bayer, Institut für Toxikologie, Wuppertal-Elberfeld, Germany AG R.C.G.L.

Bayer (2000): Pilot tolerability study in rats (application by gavage). Report no. T 8063878, date: 2000-05-09. Bayer AG (Toxicology), unpublished

Bayer (2002): Additin 30 (N-phenyl-1-naphthylamine) - Study for subacute oral toxicity in rats (gavage study over about 4 weeks and 2 weeks recovery period). Report no. PH-32318, study no. T 0069825, date: 2002-08-26. Bayer AG (Toxicology). AG R.C.G.L., unpublished

Bayer AG (1989): Bestimmung der Wasserlöslichkeit von Vulkanox PAN. Bayer AG, Leverkusen, Germany

Boman A., Hagelthorn G., Jeansson I., Karlberg A.T., Rystedt I., and Wahlberg J.E. (1980): Phenyl-alpha-naphthylamine - Case report and guinea pig studies. Contact Dermatitis 6 (4), 299-300. DOI: 10.1111/j.1600-0536.1980.tb04948.x

Budavari S. (2001): The Merck Index. An Encyclopedia of Chemicals, Drugs and Biologicals. 13th Edition, 1543-1544

Carmichael A.J. and Foulds I.S. (1990): Isolated naphthylamine allergy to phenyl-alpha-naphthylamine. Contact Dermatitis 22 (5), 298-299. DOI: 10.1111/j.1600-0536.1990.tb01607.x

Centraal Instituut voor Voedingsonderzoek (1977): Primary skin- and eye irritation tests with the compound WTR 10 in albino rabbits. R 5468, date: 1977-09-16. Testing laboratory: Centraal Instituut voor Voedingsonderzoek, Central Institute for nutrition and food research, Utrechtsweg 48, Zeist, Netherland. Rhein Chemie GmbH / Lanxess AG as former member Bayer AG of the working group on the toxicity of rubber auxiliaries W.G.

Ciba-Geigy (1987a): Acute dermal irritation/corrosion study in the rabbit. 874157, date: 1987-07-13. CIBA-GEIGY Ltd., Basle, Switzerland, GU 2 Toxicology

Ciba-Geigy (1987b): Exploratory acute oral toxicity in the rat. . OTS0533598; New Doc. I.D. 86-920000032 date: 1987-09-04. Corporation C.-G.

Ciba-Geigy (1987c): Skin sensitization test in the guinea pig modified maximisation test (final report) with cover letter dated 100291. 874158,

TSCA, OTS0533601; New Doc I.D. 86-920000035, study no. R 1066.434, date: 1987-07-24. Ciba-Geigy Limited, Toxicology GU 2.5, 4002 Basel, Switzerland Corp. C.G.

de Kort M., Weber K., Wimmer B., Wilutzky K., Neuenhahn P., Allingham P., and Leoni A.-L. (2020): Historical control data for hematology parameters obtained from toxicity studies performed on different Wistar rat strains: Acceptable value ranges, definition of severity degrees, and vehicle effects. Toxicology Research and Application 4, 2397847320931484. DOI: 10.1177/2397847320931484

ECHA (2017): Guidance on the Application of the CLP Criteria. European Chemicals Agency, Helsinki, FI. https://echa.europa.eu/documents/10162/23036412/clp_en.pdf/58b5dc6d-ac2a-4910-9702-e9e1f5051cc5 (last accessed 2021-07-14).

GU 2 Toxicology (1987): N-phenyl-1-naphtylamine: acute eye irritation/corrosion study in the rabbit. GU 874156

OTS0533600 / new doc ID 86-920000034 date: 1987-07-20. GU 2 Toxicology, Basle, Switzerland. Ciba-Geigy L., Werk Stein WST 458, CH-4332, Switzerland

Hall A., Elcombe C., Foster J., Harada T., Kaufmann W., Knippel A., Küttler K., Malarkey D., Maronpot R., and Nishikawa A. (2012): Liver hypertrophy: a review of adaptive (adverse and non-adverse) changes—conclusions from the 3rd International ESTP Expert Workshop. Toxicologic pathology 40 (7), 971-994

Heinisch K.F. (1974): Dictionary of rubber. Applied Science Publishers Ltd. L., 350-353

Kalimo K., Jolanki R., Estlander T., and Kanerva L. (1989): Contact allergy to antioxidants in industrial greases. Contact Dermatitis 20 (2), 151-152. <Go to ISI>://A1989T283700017

Muller A., Jacobsen H., Healy E., McMickan S., Istace F., Blaude M.-N., Howden P., Fleig H., and Schulte A. (2006): Hazard classification of chemicals inducing haemolytic anaemia: An EU regulatory perspective. Regulatory Toxicology and Pharmacology 45 (3), 229-241

National Institute of Technology and Evaluation J. (2002): Biodegradation and Bioaccumulation of Existing Chemical Substances under the Chemical Substance Control Law. . National Institute of Technology and Evaluation, Japan MITI List

Nomura A. (1977): Studies of sulfhemoglobin formation by various drugs (3). Folia Pharmacologica Japonica 73 (7), 793-802. DOI: 10.1254/fpj.73.793

Perrin D.D.e.a. (1981): pKa prediction for organic acids and bases. Chapman & Hall, London

Phycher (2003): Assessment of sensitising properties on albino guinea pig-Maximisation test according to Magnusson and Kligman. Report no. SMK-PH-03/0095, date: 2003-05-28. Phycher Bio Development, 18 chmin Luo Tribail, Z1 de Toctoucau, 33611 Cestas Cedex Rhein Chemie Rheinau GmbH, unpublished

Rhein Chemie Rheinau GmbH (2009): Granulometry of PANA measured by laser diffractometry. PA-0907-0450

Sax N.I. and Lewis R.J. (1987). Hawley's Condensed Chemical Dictionary. 11th edition. Von Nostrand Reinhold Company N.

Svedman C., Isaksson M., Zimerson E., and Bruze M. (2004): Occupational contact dermatitis from a grease. Dermatitis 15 (1), 41-44

Syracuse (1981): Environmental fate and effects of n-phenyl-1-naphthylamine and its disposition and metabolism in the rat. Report no. F49620-77-C-0027, study no. AFOSR-TR-81-0703, date: 1981/12/08. Syracuse Research Corporation. U. S. Air Force Office of Scientific Research, unpublished

Tanabe S., Ohara M., Ito M., Noda A., Kobayashi K., Matsumoto M., and Hirose A. (2017): Toxicity in repeated 28-day oral administration of N-phenyl-1-naphthylamine in rats. Fundamental Toxicological Sciences 4 (5), 207-218

Union Carbide (1974): Phenyl alpha naphthylamine; PANA range finding toxicity studies. Report no. 37-35/NTIS/OTS 0535072, New Doc ID 86-920000742, date: 1974-06-05. Chemical Hygiene Fellowship "Division of Sponsored Research", Carnegie-Mellon University. Union Carbide Corporation Chemicals and Plastics Operation Division, unpublished

Xuanxian X. and Wolff T. (1992): Metabolism of N-phenyl-2-naphtylamine and N-phenyl-1-naphtylamine by rat hepatic microsomes and hepatocytes. Journal of Environmental Science 4 (1), 74-83

15 ANNEXES

Confidential Annex with detailed tabulated data for the assessment of the hazard class STOT RE (see extra file).