Committee for Risk Assessment

RAC

Opinion
proposing harmonised classification and labelling
at EU level of

N-1-naphthylaniline;
N-phenylnaphthalen-1-amine

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

CLH-O-0000007248-69-01/F

Adopted

16 March 2023

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16 March 2023

CLH-O-0000007248-69-01/F

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

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

Chemical name: ***N*-1-naphthylaniline; *N*-phenylnaphthalen-1-amine**

EC Number: **201-983-0**

CAS Number: **90-30-2**

The proposal was submitted by **Germany** and received by RAC on **14 April 2022.**

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

PROCESS FOR ADOPTION OF THE OPINION

Germany has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **16 May 2022**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **15 July 2022**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Bogusław Barański**

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

The RAC opinion on the proposed harmonised classification and labelling was adopted on **16 March 2023** by **consensus**.

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

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	TBD	*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	
RAC opinion	TBD	*N*-1-naphthylaniline; *N*-phenylnaphthalen-1-amine	201-983-0	90-30-2	Acute Tox. 4 STOT RE 2 Skin Sens. 1	H302 H373 (blood system, liver) H317	GHS07 GHS08 Wng	H302 H373 (blood system, liver) H317		oral: ATE = 1200 mg/kg bw	
Resulting Annex VI entry if agreed by COM	TBD	*N*-1-naphthylaniline; *N*-phenylnaphthalen-1-amine	201-983-0	90-30-2	Acute Tox. 4 STOT RE 2 Skin Sens. 1	H302 H373 (blood system, liver) H317	GHS07 GHS08 Wng	H302 H373 (blood system, liver) H317		oral: ATE = 1200 mg/kg bw	

GROUNDS FOR ADOPTION OF THE OPINION

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

Oral route

A summary of relevant acute oral toxicity studies provided in the CLH report for *N*-1-naphthylaniline (NPNA) is provided in the table below.

***Table**: Summary table of animal studies on acute oral toxicity (modified from Table 1 of the CLH report).*

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Value LD_{50}	Reference, reliability
Rat					
LD_{50}-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)
LD_{50}-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)

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Value LD_{50}	Reference, reliability
LD_{50}-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)
LD_{50}-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					
LD_{50}-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)

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 the acute oral toxicity of NPNA. Taking the lowest values estimated, an oral LD_{50} of 1 231 mg/kg bw was determined for mice (AMR, 1974) and an LD_{50} of 1 625 mg/kg bw was established for rats (AMR, 1974). Based on these data the DS proposed to classify NPNA as Acute Tox. 4, H302 (Harmful if swallowed), according to Annex VI of Regulation (EC) 1272/2008. The ATE of 1 231 mg/kg bw has been proposed to be used based on the lowest LD_{50} value of all available oral toxicity studies

Dermal route

Summary of relevant acute dermal toxicity studies provided in CLH report for NPNA.

***Table**: Summary table of animal studies on acute dermal toxicity (Table 10 of the CLH report)*

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Value LD_{50}	Reference, reliability
LD_{50}-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: 8 000 mg/kg bw: 1/5 animals 2 000 mg/kg bw: 0/2 animals (8 days post-exposure).	(Union Carbide, 1974) Key study (rel. 2)

Based on the dermal LD_{50} of > 8 000 mg/kg for rabbits, the DS concluded that classification of NPNA for acute dermal toxicity is not required.

Comments received during consultation

One MSCA supported the proposed by the DS a classification for acute oral toxicity as Acute Tox. 4, H302 and the ATE of 1 231 mg/kg bw.

One MSCA agreed that the substance does not warrant a classification for acute dermal toxicity

Assessment and comparison with the classification criteria

Taking into account the oral LD_{50} for mice of 1 231 mg/kg bw (AMR, 1974) and oral LD_{50} for rats of 1 625 mg/kg bw (AMR, 1974) RAC concludes that NPNA meets classification criteria for Acute Tox. 4, H302 with ATE, after rounding down, of 1 200 mg/kg bw as proposed by the DS.

Since the dermal LD_{50} in rabbits is well above 2 000 mg/kg (Union Carbide, 1974) NPNA **does not meet classification criteria and no classification for acute dermal toxicity is warranted**.

RAC evaluation of specific target organ toxicity – single exposure (STOT SE)

Summary of the Dossier Submitter's proposal

Table: *Summary of studies with NPNA considered for STOT SE (modified from Table 15 of the CLH report)*

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference, reliability
Acute dermal toxicity study no guideline followed; no GLP compliance	NPNA Purity: no data	Rabbit, albino, male, 2 animals (2 000 mg) and 5 animals (8 000 mg) mg/kg bw, epicutaneous, 24 h exposure LD_{50}: > 8 000 mg/kg bw (male)	Livers congested and mottled; spleens dark; kidneys khaki brown in colour No information about number and sex of affected animals and dose level	Union Carbide (1974) Key study (rel. 2)
Acute oral toxicity study LD_{50}-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 LD_{50}: > 2 000 mg/kg bw (male) > 200 -< 2000 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. Mortality: 200 mg/kg: 0 animals 2 000 mg/kg: 3/3 females and	Ciba-Geigy (1987b) Supporting study (rel. 2)

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference, reliability
			1/3 males	
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 LD_{50}: 2 380 mg/kg bw (calculated)	livers mottled; stomachs transparent, kidneys and adrenals congested; intestines injected and distended, 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).	Union Carbide (1974) Supporting study (rel. 2)

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 the LD_{50}, therefore the DS concluded that classification as STOT SE is not warranted for NPNA.

Comments received during consultation

One MSCA commented that available data do not allow proposing a classification for STOT SE.

Assessment and comparison with the classification criteria

RAC agrees with the DS that 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. 4, H302. Hence, the **classification as STOT SE is not warranted for NPNA**.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

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.

A summary of the studies relevant for skin irritation/corrosion as provided in CLH report for NPNA is presented below.

***Table**: Summary table of animal studies on skin corrosion/irritation (cf. Table 11 of the CLH report)*

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 specified	Rabbit, New Zealand White, sex not specified (2.0-3.0 kg) 6 animals	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)
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 cm^2 × 2.5 cm^2 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)

Based on the available information the DS concluded that classification is not warranted.

Comments received during consultation

One MSCA agreed that based on available data the substance does not warrant a classification for skin irritation.

Assessment and comparison with the classification criteria

Taking into account that in the reliable key study on rabbits following a 4-hour exposure period no signs of skin irritation were observed at 24, 48 and 72 h after patch removal. In the other three supporting, non-guideline studies, either no sign of skin irritation were observed or a slight irritancy were observed but below the skin irritancy CLP criteria, for which RAC is of the opinion that NPNA **does not warrant classification for skin irritancy**

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

The DS presented results of three studies. In the key study NPNA was investigated for its eye irritation potential in an OECD TG 405 study. NPNA (0.1 mL; 36 mg) 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.

Summary of studies relevant for skin irritation/corrosion provided in CLH report for NPNA is presented below:

Table: *Summary table of animal studies on serious eye damage/eye irritation (cf. Table 11 of the CLH report).*

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 used	0.1 mL (36 mg) Control: untreated right eyes of the test animals Exposure duration: test material not removed/washed Observations: 1,	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	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
			24, 48 and 72 h after instillation of test material Further observation period of 10 days.	score: mean score (24, 48, 72 h) = 0.4; (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 mg 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) DS considered as supporting study, but propose to disregard it as documentation was considered insufficient for assessment (rel. 4)

Noting that NPNA was not irritating to the eye in the available studies the DS concluded that classification is not warranted.

Comments received during consultation

One MSCA has agreed that based on available data the substance does not warrant a classification for eye irritation

Assessment and comparison with the classification criteria

Noting that in the reliable key study on rabbits no conjunctival oedema, no effects in cornea and in iris were observed and only slight and reversible conjunctival redness was found with score below classification criteria and that in other supporting study no or little and reversible eye irritating effects were found RAC is of the opinion that that NPNA **does not warrant classification for eye irritancy**.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

The DS presented three Guinea Pig Maximisation Tests (GPMTs) (OECD TG 406) with NPNA and five case reports on NPNA skin sensitisation in humans. The key GPMT (Ciba-Geigy, 1987c) has no methodological and documentary deficiencies and is thus considered reliable without restrictions. Two other tests were conducted in pre-GLP period; one of them with limited reliability due to insufficient documentation of methods and the other one has not been used for classification due to significant methodological deficiencies.

This key study was performed in 10 negative control animals and 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. 24 and 48 hours post-challenge, 90 % of the treated 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 was regarded by the DS as 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.

***Table**: Summary table of animal studies on skin sensitisation (cf. Table 13 of the CLH report).*

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, GPMT) 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-diamine or potassium-dichromate.	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)
OECD TG 406 (Skin Sensitisation, GPMT) 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 Sensitisatio, (GPMT) GLP compliance Repeatedly positive findings in the	Guinea pig, Dunkin-Haertley Male (main study) 10 animals/dose 5 control	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	Sensitisation rate 10 % (6.25 % challenge dose) to 40 % (12.5 and 25 % challenge dose) Percentage of	Phycher (2003) Supporting data but not used for classification due to significant methodological deficiencies

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results	Reference, reliability
control group, positive findings not discussed or explained, no definition on evaluation criteria	animals		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	positive responses in control group (20 %) were subtracted from percentage of positive responses in respective dose groups	(rel. 3)
Modified Landsteiner Guinea Pig Sensitisation Test (1967) No GLP compliance	Guinea pig, albino Male 18 animals/dose	NPNA (CAS 90-30-2 / EC 201-983-0) Purity: no data	Induction: intradermal, no data on vehicle, concentration and exposure duration Challenge: intradermal, no data on vehicle, concentration and exposure duration Observations: 24 and 48 h after treatment	Non-sensitising Documentation insufficient for assessment	AMR (1974) Study not assignable (rel. 4)

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).

Table: *Summary table of human data on skin sensitisation (cf. Table 14 of the CLH report).*

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	Aalto-Korte et al. (2008)

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
			workplace materials to gunpowder containing ethylene glycol dinitrate (10 %; ++), and a grease (?+)	
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)

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.

Based on the data presented above 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 General Concentration Limit (GCL) of 1 % (w/v) would apply by default.

Comments received during consultation

One MSCA noted that a classification for subcategory 1A cannot be excluded based on experimental data and thus the substance should be classified as Skin Sens. 1. Since only few human case reports are available, this does not contribute to propose a category 1A.

Nevertheless, it would have been useful to provide a comparison to criteria according to CLP guidance.

In response DS pointed out that there are only 5 case reports available, in which NPNA was tested using patch tests in a non-standardised way on single human patients. There are neither data from Human Repeated Insult Patch Tests (HRIPT), Human Maximization Tests (HMT) and Diagnostic patch tests nor from epidemiological studies available, which however are necessary to conclude on the appropriate sub-categorisation according to CLP Annex I, 3.4.2.2.2.1 and 3.4.2.2.2.2.

Due to these reasons, the DS considered that it is inappropriate to draw any conclusions from these few individual reports on the general frequency of occurrence of skin sensitisation in humans and the likelihood of exposure (as foreseen in the CLP Guidance, section 3.4.2.2.2.: "When considering human evidence, it is necessary to take into account the size of the population exposed and the extent of exposure and frequency, and thus the consideration is on a case by case basis.").

Assessment and comparison with the classification criteria

As indicated in the CLH report, both GPMTs 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 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.

Positive patch test with NPNA in several human case reports indicate skin sensitisation property of this substance but, as pointed out by the DS, they do not allow subcategorization.

Taking the available animal and human data RAC is of the opinion that criteria for classification of NPNA as Skin Sens. 1 are clearly fulfilled, and sub-categorisation (i.e. 1A or 1B) is not possible, therefore NPNA **should be classified as Skin Sens. 1; H317** with default GCL of 1 % (w/v).

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

Summary of the Dossier Submitter's proposal

For evaluation of repeated dose toxicity the DS presented four studies: the 90-day repeated oral dose toxicity study in rats (BASF, 2016b), 28-day repeated oral dose toxicity study in rats (Bayer, 2002), 28-day repeated oral dose toxicity study in rats (Tanabe et al., 2017) and prenatal developmental toxicity (PNDT) study in rats (BASF, 2016a).

The results of the animal studies relevant for assessment of STOT RE have been presented in table 16 of the CLH report and in its Confidential Annex and the information is summarised below (dose-related effects are marked with an asterisk *).

In a **90-d repeated dose oral toxicity study (OECD TG 424 combined with OECD TG 408)**, **(BASF, 2016b)**, Wistar rats (15 animals/sex/dose)) were given daily by gavage NPNA at doses of 0, 5, 25, 125 mg/kg bw/d.

No mortality; no indication of neurotoxicity (no adverse neurobehavioral (functional observation battery)) and no neurohistopathological effects were observed. MetHb formation was not investigated.

The following effects were found in male (m) and female (f) rats:

At 5 mg/kg bw/d

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).

At 25 mg/kg bw/d

Blood: creatinine levels* (f) -12.4 %; total bilirubin levels* (m/f) +228.4 %/+131.8 %.

Urine: bilirubin increased (f) 8/10 grade 2 (slight), 2/10 grade 3 (moderate) vs 4/10 grade 1 (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 increased 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).

At 125 mg/kg bw/d

Blood: RBC (m/f) -8.5 %* (mean in both sexes) (in 6/10 females and 4/10 males RBC reduction ≥ 10 %; 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 %; 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.

Considering as adverse such effects at 125 mg/kg bw/d (which is slightly above guidance value for STOT RE 2) as haemolytic anaemia with RBC reduction of ca. 10 % in combination with renal cell degeneration and massive liver weight increase (> 120 % when compared to controls). The DS was of the opinion that data are borderline for classification as STOT RE 2.

In a **28-d repeated dose oral toxicity study (OECD 407) (Bayer, 2002)** Wistar rats (5 animals/sex/dose) were given daily by gavage NPNA at doses of 0; 5; 20; 80 mg/kg bw/d. Recovery groups: 0 and 80 mg/kg bw/d, 28 d of exposure plus 14 d recovery period.

The following effects were found in male (m) and female (f) rats:

General: No gross findings recorded. No test substance-related histopathological findings. MetHb formation was not investigated

5 mg/kg bw per day

Blood: 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.

20 mg/kg bw per day

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.

80 mg/kg bw per day

Blood: RBC* (f) -6.5 % (not stat. significant), ≥10 % in 2/5 f; Hb* (f) (mean) -9.2 %, -11 % (median) (reduction > 10 % in 3/5 f); 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 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.

The DS noted the following adverse effects at 80 mg/kg bw/d: A 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 was admitted that the associated adverse effects 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).

In a **28-d repeated dose oral toxicity study (OECD 407), (Tanabe et al. (2017)** Sprague Dawley rats (5 animals/sex/dose)) were given daily by gavage NPNA at doses of 0, 4, 20, 100, 500 mg/kg bw/d. Recovery groups: 0 and 500 mg/kg bw/d, 28 d of exposure plus 14 d recovery period. MetHb formation was not investigated. The following effects were found in male (m) and female (f) rats:

General: No indication of neurotoxicity and neurohistopathological effects, 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.

At 4 mg/kg bw/d

Blood: triglyceride (f) -41.9 %.

Liver: focal necrosis (m) 1/5 grade 1 (slight) vs 0/5 in control (not statistically significant).

At 20 mg/kg bw/d

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 increase when compared to controls, (f) +17.3 % (statistically significant, but no clear dose-response).

At 100 mg/kg bw/d

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).

500 mg/kg bw/d

General: 1 male died one day before necropsy (day 28; findings: soiling of fur over lower abdomen by faeces and urine, chromodacryorrhea.

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), 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).

The DS noted that the 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 were 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 2.

In the **Prenatal Developmental Toxicity Study (OECD 414), (BASF, 2016a)** Wistar rats (25/sex/dose) were given by gavage NPNA at doses of 0, 15, 50 and 150 mg/kg bw/d for 14 days

(gestation days 6 through 19). The following effects were found in male (m) and female (f) rats:

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.

At 15 mg/kg bw/d

Blood: bilirubin +30.8 %*, urea +10.2 %*

At 50 mg/kg bw/d

Blood: bilirubin +98.5 %* urea +18.1 %*

At 150 mg/kg bw/d

Blood: RBC -5.8 % (mean) (in 6/25 f RBC reduction ≥ -10 %; 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); 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.

The DS noted that according to 3.9.2.5.2 CLP guidance: Adverse effects are haemolytic anaemia with Hb reduction of ≥ -10 % in 8/25 females if compared to the median control value (group median at 150 mg/kg bw: -11 %) and RBC reduction ≥ -10 % in 6/25 females 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 2.

In the short-term experiments after single or several intraperitoneal injections of NPNA in mice considerable formation of methaemoglobin in blood was noted (Nomura, 1977).

Based on the above data, the dossier submitter finally concluded that NPNA causes borderline haemolytic anemia compared to the CLP classification criteria and therefore did not propose a classification of NPNA for STOT RE 2, H373 (blood system).

Comments received during consultation

One MSCA noted that according to CLP guidance: "The guidance developed for classification of substances inducing haemolytic anaemia according to 67/548/EEC (Muller et al., 2006) cannot directly be used under CLP (CLP Annex I, 3.9.2.7.3 c and 3.9.2.8.b, d) because of the changes in the criteria. The major criterion for haemolytic anaemia changed from 'Any consistent changes in haematology which indicate severe organ dysfunction' to 'Any consistent and significant adverse changes in haematology' This indicates that less adverse effects are considered for classification according to CLP".

The overall data clearly show that the substance induces haemolytic anaemia with the main impact being on the liver and kidney.

Regarding haematology, a significant decrease of haemoglobin is reported in the 90-day study at 125 mg/kg bw/d. Even if the dose is slightly above the cut-off for STOT RE 2 (100 mg/kg

bw/d), it should be noted that the lower tested dose is very low (25 mg/kg bw/d). This is not in accordance with OECD guidance that recommends a 2 to 4 interval between tested doses. The large interval creates uncertainties on results that can be expected at a dose close to the CLP cut-off. At 125 mg/kg bw/d, reticulocytes are significantly increased, and total bilirubin is increased at all tested doses. This supports the relevance and significance of haemolytic anaemia. Significant decrease of haemoglobin is also reported in a 28-day study at 80 mg/kg bw/d (CLP cut-off for STOT RE 2: 30 < C ≤ 300 mg/kg bw/d) and in a prenatal developmental toxicity study at 150 mg/kg bw/d (CLP cut-off for STOT RE 2: 60 < C ≤ 600 mg/kg bw/d). Even if the threshold of 10 % for a decrease of haemoglobin set by Muller et al. 2006 is not reached, the decreases observed at these doses are statistically significant and can correspond to the more flexible criteria set in the CLP guidance. The effects can fulfil the CLP criteria: "any consistent and significant adverse change in clinical biochemistry, haematology, or urinalysis parameters".

Concerning liver: increased weight associated with centrilobular hypertrophy is reported in the 90-day study. These effects cannot be considered as an adaptive reaction especially at the dose of 125 mg/kg bw/d: the increase of liver weight is clearly higher than 10 % and almost all animals present a hypertrophy of slight to moderate severity. Even if the dose is slightly above the cut-off for STOT RE 2, similar results can be expected at a dose close to or below 100 mg/kg bw/d. Indeed, increased liver weight and hypertrophy are already observed at 25 mg/kg bw/d, reaching statistically significance, even if these findings are of lower significance.

Concerning kidney: Chronic nephropathy is observed from 25 mg/kg bw/d in males only in the 90-day study. Study authors considered that this lesion may not be of relevance for humans as CNP is a frequently observed effect in the male aging rat, (only) exacerbated by the chemical treatment. However, the relevance of this tumour to humans cannot be neither completely excluded since (1) these effects occurred in animals that cannot be considered as aged at the end of the study, (2) a clear-dose response is observed for severity and (3) haemolytic anaemia can lead to secondary effects on the kidney. In addition, degeneration/regeneration of tubules is reported in males and mostly at the dose of 125 mg/kg bw/d. Even if the dose is slightly above the cut-off for STOT RE 2, similar results can be expected at a dose close to or below 100 mg/kg bw/d. These effects can fulfil to the CLP criteria: "significant organ damage noted at necropsy and/or subsequently seen or confirmed at microscopic examination."

Finally, it has to be noted that these results are consistent with those reported with an analogous substance, diphenylamine classified as STOT RE 2 (RAR, 2008). Data from this substance can support the need to classify NPNA, accordingly.

In their response, the DS agreed 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 observed at a dose only slightly above the upper limit value for STOT RE 2 classification (i.e. at 125 mg/kg bw/d).

In addition, and as noted in the dossier, regarding the chronic progressive nephropathy (CPN) frequently observed 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 the clear dose-response relationship regarding severity (BASF, 2016b), it is not possible to fully exclude a relevance for humans, although the study authors suggested this. However, 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 and it is neither possible to fully exclude nor to verify 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.

Summing up, the DS noted that discussion in RAC, whether classification of NPNA as STOT RE 2, H373 (blood system) is warranted, is welcomed. The DS further agreed that based on the available data the liver is to be identified as target organ for NPNA toxicity and, thus, classification as STOT RE 2, H373 (liver) may be warranted as well.

Assessment and comparison with the classification 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 ≤ 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 STOT RE 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 ≤ 100 mg/kg bw/d*).

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 bw/d 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 hemosiderosis in the spleen, liver or kidney in combination with other changes indicating significant haemolytic anaemia is found. In the '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 blood 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 toxic effects in the blood system can be considered as meeting criteria set out in the CLP guidance, Section 3.9.2.5.2., supporting classification for STOT RE 2, H373:

- mean reductions of Hb in females, but not in males, of 7.0 % and 9.2 % **at 20 and 80 mg/kg bw/d**, respectively) with a median reduction of 11 % in Hb in females **at 80 mg/kg bw/d**, meaning that over half of the individual females showed Hb reductions above 11 % (repeated dose oral toxicity study (28 days) in rats (OECD TG 407) (Bayer, 2002)
- **at 125 mg/kg bw/d** increased haematopoiesis in spleen in 3 females out of 10 tested vs 0/10 in control females and significant increase in pigment storage in females 5/10 grade 2 (slight) vs 1/10 in controls was observed, a haemoglobin level (Hb) reduced by 4.1 % and 5.9 % (m/f) (repeated dose oral toxicity study (90 days) in rats (BASF (2016b))
- **at 500 mg/kg bw/d** haemoglobin level (Hb) reduced by 15.2 %, both in males and females; reduction of RBC by 15.3 % in females and by 9.6 % in males, (all males and females),pigment storage (haemosiderosis) in spleen in females; 5/5 grade 3 (severe) vs 0/5 controls, (Repeated dose oral toxicity study (28 days) in rats (Tanabe et al. (2017))
- **at 150 mg/kg bw/d** reduction in Hb above 10 % in 16 % of the dams and Hb reductions above 9 % in 32 % of the dams (PNDT study, (BASF, 2016a),
- significant dose-dependent increases in bilirubin plasma levels in all studies (in repeated dose oral toxicity study (90 days) (**BASF, 2016b)** at ≥ 5 mg/kg bw/d; up to +1 715 % at 125 mg/kg bw/d),
- urine of NPNA treated animals was regularly described as purple/reddish coloured (in repeated dose oral toxicity study (90 days) in rats (**BASF, 2016b) at 125 mg/kg bw/d**), in repeated dose oral toxicity study (28 days) in rats, (Tanabe et al.,2017) at 100 and 500 mg/kg bw/d),
- 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)
- 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))

The whole degree of haematotoxicity in the repeated dose toxicity studies with NPNA could not be uncovered since methaemoglobin (MetHb) formation was not investigated. NPNA induces transformation of Hb to MetHb. Significant MetHb formation was reported in two in vivo short-term experiments in mice after IP injection of NPNA, with peak MetHb levels of 7.2 % at 90 and 150 min after IP injection (Nomura, 1977). The formation of methaemoglobin could also be induced in the repeated oral toxicity studies however this effect was not measured in these studies.

In conclusion, noting that NPNA at doses within or very close to guidance values for STOT RE 2 is causing haemolytic anaemia with consistent and significant adverse changes in haematology, affecting several organs RAC is of the opinion that the substance warrant classification as STOT RE 2; H373 (blood system).

Hepatotoxicity

NPNA has induced the following toxic effects in liver:

- **in the repeated oral dose toxicity study (90 days) in rats (BASF (2016b)) at 125 mg/kg bw/d** significantly increased an absolute and relative weight when compared with controls in males +28.4 %/+28.6 %; in females +31 %/ 31.9 % combined with centrilobular hypertrophy in males 9/10 grade 2 (slight) vs 0/10 in controls; in females: 10/10 grade 3 (moderate) vs 0/10 in controls
- **in repeated dose oral toxicity study (28 days) (Tanabe et al. (2017)) at dose 100 mg/kg bw/d** increased in relative/absolute weight of liver: in males by 24.2 %/ 21.7 % and female rats +16.2 %/19.7 %; centrilobular hypertrophy in females: 1/5 grade 1 (slight) vs 0/5 controls (not significant); and focal necrosis (males) 1/5 grade 2 (moderate) vs 0/5 controls (not significant); and at dose of 500 mg/kg bw/d increased absolute/relative liver weight when compared to controls by 40.5 %/ 70.1 % in males and 71.1 %/ 75.2 % in females combined with centrilobular hypertrophy in males: 5/5 grade 1 (slight) vs 0/5 controls, in females: 5/5 grade 2 (moderate) vs 0/5 controls; focal necrosis (m/f) 1/5 grade 1 (slight) vs 0/5 controls

The centrilobular hypertrophy which was observed in rats of both sexes could be potentially linked to induction of microsomal enzymes by NPNA, however since no measurement of liver microsomal enzyme activity was made, such a link cannot be considered as demonstrated. Therefore, observed centrilobular hepatocyte hypertrophy cannot be explained by metabolic adaptation to exposure to toxic substance, and in combination with substantial increase of absolute and relative liver weight (> 25 %) is considered as an adverse effect.

Considering that NPNA at doses within or very close to guidance values for STOT RE 2 is causing large, dose-dependent increase in liver weight combined with centrilobular hepatic hypertrophy, RAC is of the opinion that the substance warrant classification as STOT RE 2, H373 (liver).

Nephrotoxicity

The increase of incidence of minimal or slight chronic nephropathy was observed in male rats, but not in female rats, in the 90-day repeated oral dose toxicity study (BASF (2016b)). This increase of chronic nephropathy incidence at doses of 25 and 125 mg/kg bw/d was not dose-dependent, although a dose response was observed for severity.

No treatment related significant, adverse effects were observed in kidneys of male and female rats given orally by gavage NPNA at daily doses of 5, 20 and 80 mg/kg bw/d for 28 days (Bayer, 2002) or in kidneys of male and female rats given orally by gavage NPNA at daily doses of 4, 20 and 100 mg/kg mg/kg bw/d for 28 days (Tanabe et al., 2017). In the latter study (Tanabe et al., 2017) some toxic effects in kidneys were only seen at dose of 500 mg/kg bw/d, well above a guidance value of 300 mg/kg bw/d.

The increase in incidence of chronic nephropathy only in male rats may not be of relevance for humans as such nephropathy is a frequently observed effect in the male aging rats as noted by the authors of the study (BASF, 2016b). Chronic progressive nephropathy (CPN) is a single renal disease of unknown aetiology, occurring in high incidence in laboratory rats, that can confound subchronic and carcinogenicity bioassay interpretation and it does not corresponds with pathogenesis and pathomorphology of human nephropathy (Hard et al. 2009).

Based on these data RAC is of the opinion that NPNA, at doses within or very close to guidance values for STOT RE 2, does not induce in rat kidneys effects meeting classification criteria for STOT RE 2.

Summing up RAC is of the opinion that NPNA warrant classification **STOT RE 2, H373: May cause damage to organs (blood system, liver)** through prolonged or repeated exposure

Additional references

Hard GC, Johnson KJ, Cohen SM. A comparison of rat chronic progressive nephropathy with human renal disease—implications for human risk assessment; Critical Reviews in Toxicology, Volume 39, 2009 - Issue 4, 332-46.

ANNEXES:

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

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