Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

2,2-dibromo-2-cyanoacetamide; [DBNPA]

EC Number: 233-539-7
CAS Number: 10222-01-2

CLH-O-0000001412-86-289/F

Adopted

13 June 2019

13 June 2019

CLH-O-0000001412-86-289/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: **2,2-dibromo-2-cyanoacetamide; [DBNPA]**

EC Number: **233-539-7**

CAS Number: **10222-01-2**

The proposal was submitted by **Denmark** and received by RAC on **23 May 2018.**

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

PROCESS FOR ADOPTION OF THE OPINION

Denmark 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 **18 July 2018**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **18 September 2018**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Michal Martínek**

Co-Rapporteur, appointed by RAC: **Riitta Leinonen**

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 **13 June 2019** by **consensus**.

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

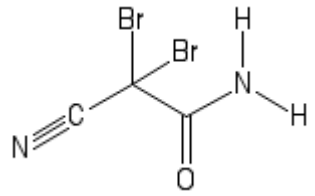
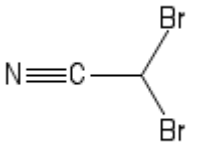
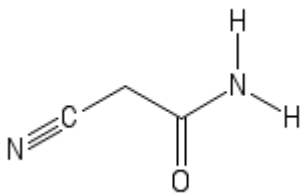
	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	TBD	2,2-dibromo-2-cyanoacetamide; [DBNPA]	233-539-7	10222-01-2	Acute Tox. 2 Acute Tox. 3 STOT RE 1 Skin Irrit. 2 Eye Dam. 1 Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H330 H301 H372 (respiratory tract, thyroid) H315 H318 H317 H400 H410	GHS06 GHS08 GHS05 GHS09 Dgr	H330 H301 H372 (respiratory tract, thyroid) H315 H318 H317 H410		inhalation: ATE = 0,275 mg/L (dust/mist) oral: ATE = 167 mg/kg bw M=1 M=1	
RAC opinion	TBD	2,2-dibromo-2-cyanoacetamide; [DBNPA]	233-539-7	10222-01-2	Acute Tox. 2 Acute Tox. 3 STOT RE 1 Skin Irrit. 2 Eye Dam. 1 Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H330 H301 H372 (respiratory tract) (inhalation) H315 H318 H317 H400 H410	GHS06 GHS08 GHS05 GHS09 Dgr	H330 H301 H372 (respiratory tract) (inhalation) H315 H318 H317 H410		inhalation: ATE = 0,24 mg/L (dust/mist) oral: ATE = 118 mg/kg bw M=1 M=1	
Resulting Annex VI entry if agreed by COM	TBD	2,2-dibromo-2-cyanoacetamide; [DBNPA]	233-539-7	10222-01-2	Acute Tox. 2 Acute Tox. 3 STOT RE 1 Skin Irrit. 2 Eye Dam. 1 Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H330 H301 H372 (respiratory tract) (inhalation) H315 H318 H317 H400 H410	GHS06 GHS08 GHS05 GHS09 Dgr	H330 H301 H372 (respiratory tract) (inhalation) H315 H318 H317 H410		inhalation: ATE = 0,24 mg/L (dust/mist) oral: ATE = 118 mg/kg bw M=1 M=1	

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

2,2-dibromo-2-cyanoacetamide, hereafter referred to as DBNPA is an active substance in biocidal products. It is used as a preservative (e.g., to prevent growth of slime-forming bacteria in paper mills and cooling towers) and as a disinfectant. The bactericidal mode of action appears to involve an attack on electron-rich groups of biomolecules.

The substance is a solid with a water solubility of 15 g/L (20 °C). It is relatively stable in water under acidic conditions but hydrolyses quickly at higher pH values. The main product of abiotic hydrolysis is dibromoacetonitrile. In contact with organic matter, DBNPA is transformed to cyanoacetamide, which is also the main metabolite in the rat. The structures of DBNPA and its two main degradation products are shown below.

		
DBNPA	**Dibromoacetonitrile**	**Cyanoacetamide**

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

No classification was proposed by the Dossier Submitter (DS) for physical hazards based on an evaluation of the hazard classes described below.

There are no chemical groups present in the DBNPA molecule that are associated with explosive or self-reactive properties as specified in Tables A6.1 and 6.2 of the UN Recommendations on Transport of Dangerous Goods, Manual of Tests and Criteria (ST/SG/AC.10/11/Rev.5).

The DS concluded that based on the absence of explosive groups and lack of an effect that would imply explosive properties in a GLP EPA OPP 63-16 guideline test, classification of DBNPA as explosive was not required.

DBNPA did not propagate combustion in a test performed according to EU test method A.10. Therefore, it did not require classification as a flammable solid.

In addition to the absence of chemical groups associated with explosive or self-reactive properties, DBNPA did not ignite before melting in a test conducted using EU test method A.16. Consequently, it did not require classification as a self-reactive substance.

Experience in manufacturing and handling showed that the DBNPA did not ignite spontaneously on coming into contact with air at normal temperatures. Thus, DBNPA did not require classification as a pyrophoric solid.

Considering the hazard class self-heating substances DBNPA was not flammable, explosive or oxidising. ECHA Guidance on the Application of the CLP criteria (v.5.0, hereafter CLP guidance) states the substance with a melting point below 160°C should not be considered for classification as self-heating. Thus, as DBNPA melting point is 124.5°C, no classification as a self-heating solid was needed.

The chemical structure of DBNPA does not contain metals or metalloids and experience in production or handling showed that reaction with water did not take place. In addition, DBNPA is known to form stable aqueous solutions. Therefore, according to the CLP criteria no classification as substance that will emit flammable gases when in contact with water was needed.

DBNPA fulfils the criteria in the CLP Regulation, Annex I 2.14.4.1(b). Therefore, classification as oxidising solid substance was not needed.

DBNPA was not an organic peroxide as the organic peroxide bond grouping is not present in the chemical structure.

Considering hazard class corrosive to metals, dissolution of DBNPA in water to perform UN Transport Test C.1 was inappropriate because DBNPA is solid that is supplied as dry and, as a result, will not cause a corrosion rate exceeding 6.25 mm per year when steel and/or aluminium are tested at a temperature of 55°C. No incidences of damage to metals had occurred during manufacture and use. DBNPA is a stable organic molecule with no functional groups that infer strongly acidic or basic properties. Classification was therefore not required.

Comments received during public consultation

No comments were received during the public consultation.

Assessment and comparison with the classification criteria

RAC supports the DS' view that DBNPA does not meet the criteria and therefore **should not be classified for physical hazards**.

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

Acute oral toxicity

The acute oral toxicity of DBNPA has been investigated in 6 studies, two of which (A6.1.1/01 and A6.1.1/02) were selected by the DS as the key studies due to their reliability and detailed reporting. The DS proposed classification as Acute Tox. 3; H301 and an ATE value (oral) of 167 mg/kg bw for DBNPA based on the lowest LD_{50} of 167 mg/kg bw (in female rats) (A6.1.1/02).

Acute dermal toxicity

The acute dermal toxicity of DBNPA was investigated

Acute inhalation toxicity

The acute inhalation toxicity of DBNPA was investigated in 2 studies, one of which (A6.1.3/01) was selected as the key study. The DS proposed classification as Acute Tox. 2; H330 based on the combined LC_{50} of 0.275 mg/L from this study and an ATE (inhalation) value of 0.275 mg/L (dust and mist).

Comments received during public consultation

A MSCA supported the DS' proposal of Acute Tox. 3 for the oral route and Acute Tox. 2 for the inhalation route. For acute oral toxicity they supported the proposed ATE value of 167 mg/kg bw based on the rat female LD_{50} value in the study A6.1.1/02.

A manufacturer supported Acute Tox. 2 for the inhalation route but disagreed with Acute Tox. 3 for the oral route, proposing Category 4 instead. They considered A6.1.1/01 as the most reliable acute oral toxicity study and argued that since there was no obvious sex difference in the sensitivity, the combined LD_{50} value of 308 mg/kg bw was the most appropriate value for classification. In their reply the DS pointed out that there were two key studies following the OECD Test Guideline (TG) 401 and in those studies 3 out of 4 LD_{50} values were consistent with Category 3. Therefore, the DS considered that Acute Tox. 3 for the oral route was justified.

Assessment and comparison with the classification criteria

Acute oral toxicity

The available acute oral toxicity studies with DBNPA are summarized in the following table.

Acute oral toxicity studies			
Species; Reference; Year	**Method**	**LD_{50}**	**Other information**
Rat A6.1.1/01 1983	OECD TG 401 5/sex/dose Doses: 133, 265, 529, 1056 mg/kg bw Vehicle: corn oil	m: 375 mg/kg bw f: 284 mg/kg bw m+f: 308 mg/kg bw	Clinical signs: decreased activity, ataxia, tremor, hunching, muzzle staining Pathology: gastro-intestinal contents mucoid and haemorrhagic with erosions, gastric perforation, oedematous and haemorrhagic lungs associated with presence of frothy fluid in the trachea
Rat A6.1.1/02 1995	OECD TG 401 GLP 5/sex/dose Doses: 50, 100, 500 mg/kg bw Vehicle: 0.5% Methocel in water	m: 224 mg/kg bw f: 167 mg/kg bw	Clinical signs: decreased activity, lacrimation Pathology: blood in the digestive tract
Rat A6.1.1/03 1968	Non-guideline 2 females/dose Doses: 63, 126, 126, 252, 500, 1000, 2000 mg/kg bw Vehicle: corn oil Post-exposure period: 7 or 16 days	f: between 126 and 252 mg/kg bw	All animals at ≤ 126 mg/kg survived, all animals at ≥ 252 mg/kg died Limited reporting
Rat, guinea pig, rabbit A6.1.1/04 1970	Non-guideline Rat: 5/sex/dose; Doses 126, 252, 500 mg/kg bw Guinea pig: 5 females/dose; doses	Rat: m: 235 mg/kg bw f: 178 mg/kg bw Guinea pig: f: 118 mg/kg bw	Mortality in guinea pigs: 31 mg/kg bw 0/5; 63 mg/kg bw 0/5; 126 mg/kg bw (initial trial) 3/6; 126 mg/kg bw (subsequent trial) 2/5;

	31, 63, 126, 126, 252, 500 mg/kg bw Rabbit: 5 animals/dose (males and females mixed); Doses 63, 126, 252, 500 mg/kg bw Vehicle: corn oil Post-exposure period: 14 days	Rabbit: m+f: 118 mg/kg bw	252 mg/kg bw 5/5; 500 mg/kg bw 5/5 Mortality in rabbits: 63 mg/kg bw 0/5; 126 mg/kg bw 2/5; 252 mg/kg bw 5/5; 500 mg/kg bw 5/5 Limited reporting Clinical signs not reported Necropsy not performed/reported
Rat A6.1.1/05 1972	Non-guideline 3 females/dose Doses: 63, 126, 252, 500, 1000 mg/kg bw Vehicle: corn oil Post-exposure period: 14 days	f: between 126 and 252 mg/kg bw	All animals at ≤ 126 mg/kg bw survived, all animals at ≥ 252 mg/kg bw died Limited reporting
Rat A6.1.1/06 1970	Non-guideline 4 females/dose Doses: 126, 252 mg/kg bw Vehicle: water, pH adjusted to 4 Post-exposure period: 14 days	f: between 126 and 252 mg/kg bw	All animals at 126 mg/kg bw survived, all animals at 252 mg/kg bw died

The results of the two guideline studies (A6.1.1/01 and /02) as well as of the other non-guideline studies support a classification in Category 3 (50 < ATE ≤ 300 mg/kg bw). Although in the study A6.1.1/01 the combined LD_{50} of 308 mg/kg bw is slightly above the cut-off value of 300 mg/kg bw, the female LD_{50} from this study is below 300 mg/kg bw and none of the remaining studies points towards Category 4. Considering all available data, RAC agrees with the DS' proposal that classification as **Acute Tox. 3; H301** is warranted but considers an **ATE** value of **118 mg/kg bw** as the most appropriate (LD_{50} from the most sensitive species, rabbit and guinea pig; A6.1.1/04).

Acute dermal toxicity

The available acute dermal toxicity studies with DBNPA are summarized in the following table.

Acute dermal toxicity studies			
Species; Reference; Year	**Method**	**LD_{50}**	**Other observations**
Rabbit A6.1.2/01 1995	OECD TG 402 GLP 5/sex/dose Dose: 2000 mg/kg bw 10% suspension in vehicle; Vehicle: 0.5% methocel in water	> 2000 mg/kg bw	No mortality Severe skin reactions including burns persisting untill day 15
Rabbit A6.1.2/02 1984	OECD TG 402 GLP 5/sex/dose Dose: 2000 mg/kg bw Major deviation: substance was applied dry, not moistened with water or other vehicle	> 2000 mg/kg bw	No mortality Exfoliations in 3 animals

The study A6.1.2/01 is considered valid by RAC while the other study, A6.1.2/02, is not because the substance was not moistened to ensure good contact with skin as required by the OECD TG.

RAC agrees with the DS that as the LD_{50} value in the A6.1.2/01 study was above 2000 mg/kg bw, **no classification** for acute dermal toxicity is warranted.

Acute inhalation toxicity

The available acute inhalation toxicity studies with DBNPA are summarized in the following table.

Acute inhalation toxicity studies			
Species; Reference; Year	**Method**	**LC_{50}**	**Other observations**
Rat A6.1.3/01 1995	OECD TG 403 GLP 5/sex/concentration Analytical concentrations: 0.10, 0.37, 0.68 mg/L MMAD from 1.1 to 1.5 µm; GSD from 2.6 to 2.9 µm Nose-only exposure	m: 0.31 mg/L f: 0.24 mg/L m+f: 0.275 mg/L	Clinical signs: mouth breathing, laboured breathing, noisy respiration of nasal origin, clear nasal discharge Pathology: visceral congestion, pulmonary congestion, clear fluid in the trachea, congestion/hyperaemia of the nasal tissues Mortality: 0.10 mg/L 0/10 0.37 mg/L 7/10 0.68 mg/L 10/10
Rat A6.1.3/02 1988	OECD TG 403 GLP 5/sex/concentration Analytical concentrations: 0.041, 0.088, 0.42 mg/L MMAD from 4.9 to 6.5 µm (MMAD at 0.041 mg/L not available) Whole-body exposure Major deviations from OECD TG 403 (2009): exposure for 1 hour instead of 4 hours; observation for 48 hours instead of 14 days; MMAD above 4 µm	m: 0.13 mg/L f: 0.13 mg/L	Clinical signs: gasping, laboured breathing, lacrimation Pathology: discoloured lungs and nasal turbinates in spontaneously dying animals Mortality: 0.041 mg/L 2/10 0.088 mg/L 3/10 0.42 mg/L 9/10

Study A6.1.3/01 was conducted in accordance with the current version of the OECD TG 403. The MMAD was within the required range of 1 to 4 µm (OECD TG 403, 2009; CLP, Annex I, 3.1.2.3.2) and also below 2 µm as recommended by the latest version of OECD GD 39. Exposure duration was standard (4 hours). The inhalation mode was nose-only, which is the preferred mode according to the OECD guidelines. The female LC_{50} of 0.24 mg/L (i.e. the lowest LC_{50}) is considered to be the most appropriate ATE (dust and mist) from this study. This ATE corresponds to Category 2 (0.05 mg/L < ATE ≤ 0.5 mg/L).

Study A6.1.3/02 deviated from the current OECD guideline in several aspects. Although the intended exposure duration was 4 hours, the actual duration was only 1 hour. The particles were larger than recommended, which generally leads to lower deposition in the lower respiratory tract and higher loading of the upper airways. The sampling duration of 2 x 1 min at the top concentration is considered too short to sufficiently cover concentration fluctuations. The LC_{50} from this study has to be corrected for classification purposes to a 4-hour exposure by dividing by a factor of 4 (CLP, Annex 1, Note (c) below Table 3.1.1), yielding an ATE of 0.033 mg/L. This ATE corresponds to Category 1 (ATE ≤ 0.05 mg/L). RAC notes that the extrapolation creates additional uncertainty.

The ATE from study A6.1.3/02 would lead to a more stringent classification. However, given the deficiencies of this study and also taking into account absence of mortality after three 6-hour exposures to 0.05 mg/L in a reliable 2-week inhalation study in the rat (summarized in the STOT RE section), preference is given to the ATE from the guideline-compliant study A6.1.3/01.

Thus, RAC agrees with the DS' classification proposal of **Acute Tox. 2; H330** based on the study A6.1.3/01. As to the ATE, RAC prefers the female LC_{50} of **0.24 mg/L** (dust and mist) (the lowest LC_{50} value from the study).

In addition to classification for inhalation toxicity, if data are available that indicate that the mechanism of toxicity is corrosivity, the substance has to be labelled as EUH071: 'corrosive to the respiratory tract'. DBNPA is a skin irritant and the clinical signs and/or pathology findings in the acute inhalation toxicity studies are indicative of respiratory tract irritation. However, the available data do not unequivocally demonstrate that corrosivity is the leading mechanism behind the observed mortality. Therefore, RAC does not consider EUH071 warranted for DBNPA.

In conclusion, and in line with the DS, RAC proposes to **classify DBNPA as Acute Tox. 2; H330 with an ATE of 0.24 mg/L (dust and mist).**

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

Summary of the Dossier Submitter's proposal

The DS proposed no classification for STOT SE. They considered the effects below the LD_{50} in the acute oral toxicity studies to indicate a generalized toxic event rather than specific target organ toxicity, and the effects below LC_{50} following single inhalation exposure as not severe enough to warrant classification for STOT SE 3 (respiratory tract irritation). They were of the view that all the acute effects were sufficiently covered by the proposed classification of the substance as Acute Tox. 3 (oral), Acute Tox. 2 (inhalation), Eye Dam. 1 and Skin Irrit. 2.

Comments received during public consultation

A manufacturer submitted a 2-week inhalation study in the rat (summarized in the section for RAC evaluation of STOT RE). They proposed to consider an appropriate classification to communicate the respiratory irritation potential of the substance identified in this study, without giving a specific classification proposal. The DS reviewed the study and considered the local effects in the respiratory tract relevant for STOT RE rather than for STOT SE.

Assessment and comparison with the classification criteria

Specific, non-lethal target organ toxicity arising from a single or a few exposures and warranting classification for STOT SE 1 or 2 has not been identified in the available studies. Neither were there indications of narcotic effects at non-lethal doses that would warrant classification as STOT SE 3; H336.

Signs of respiratory irritation were seen in the acute inhalation study A6.1.3/01 (at 0.10 mg/L) and in the 2-week inhalation study (after 3 exposures to 0.05 mg/L).

In the acute inhalation toxicity study A6.1.3/01, laboured breathing was observed in all males (but not in females) during the exposure to 0.10 mg/L of DBNPA, and noisy respiration, mouth breathing and sneezing were observed after exposure. There were no mortalities and no abnormalities on necropsy at this concentration (however, it is noted that the necropsy took place 14 days after exposure). Although the observed clinical signs are indicative of respiratory irritation, classification as Acute Tox. 2; H330 with an ATE of 0.24 mg/L, which is relative close

to 0.10 mg/L, is already proposed based on this study; it is also noted that Acute Tox. 2 covers a concentration range (0.05 to 0.5 mg/L) that includes 0.10 mg/L. Therefore, the respiratory effects in males at 0.10 mg/L are considered to be already covered by the acute toxicity classification.

Slow, laboured and noisy breathing was observed after 3 consecutive 6-hour exposures to 0.05 mg/L in the 2-week inhalation study, which is at the border of the range for Acute Tox. 2. Due to excessive toxicity, the concentration was reduced to 0.025 mg/L after 3 days of exposure and the clinical signs gradually receded. Inflammatory changes in the respiratory tract were observed not only at 0.05 and 0.025 mg/L, but also at 0.0054 and 0.00051 mg/L. As the necropsy took place after 9-10 exposures, RAC considers this information relevant for STOT RE rather than for STOT SE.

In view of the above considerations, RAC agrees with the DS that **no classification for STOT SE is warranted**.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

The skin irritation potential of DBNPA has been investigated in 5 *in vivo* studies in the rabbit. The DS proposed classification in Category 2 based on the OECD TG and GLP compliant study A6.1.4/02.

Comments received during public consultation

One manufacturer and one MSCA supported the DS' proposal of Skin Irrit. 2.

Another MSCA, although supporting Skin Irrit. 2, mentioned evidence potentially pointing towards classification as Skin Corr. 1: occurrence of skin necrosis, scabs, exfoliation and scars in three non-guideline studies (A6.1.4/04b, A6.1.4/05b and A6.1.4/06b) and severe skin lesions in accidentally exposed workers (Senoh *et al.*, 2009, summarized under A6.12.2). The DS replied that they had given most weight to the key OECD TG 404 study (A6.1.4/02). They also pointed out that the full composition of the mixture to which the workers were exposed was not known, and therefore the potential impact of co-formulants could not be excluded.

Assessment and comparison with the classification criteria

There are two *in vivo* studies according to OECD TG 404. They are summarized in the following table.

Guideline skin irritation studies		
Type of study; Reference; Year	**Method**	**Observations**
In vivo, rabbit A6.1.4/02 1995	OECD TG 404 GLP 3 males and 3 females 4-hour exposure 15-day observation period	Average score for each animal (mean of 24, 48, 72h observations): Erythema: 4, 4, 4, 3, 1, 4 Oedema: 2.3, 2.7, 2.7, 2, 0, 3 In 5 animals blisters at the application site, in 4 animals blisters persisted till day 7 On day 15, 1 animal had scabs at the application site, no skin lesions in the rest of the animals

In vivo, rabbit A6.1.4/03 1983	OECD TG 404 GLP 3 males and 3 females 4-hour exposure 5-day observation period	Average score for each animal (mean of 24, 48, 67h observations): Erythema: 2, 2, 2, 2, 2, 2 Oedema: 0, 0, 0, 0, 0, 0 On day 5, exfoliation observed on all animals

The results of the study A6.1.4/02 clearly meet the criteria for classification in Category 2 (mean score for erythema or oedema ≥ 2.3 and ≤ 4.0 in at least two thirds of the animals). Although the occurrence of scabs on day 15 in 1 out of 6 animals raises doubts about reversibility of the effect, RAC does not consider this finding sufficient for classification in Category 1. The second OECD TG 404 study, A6.1.4/03, showed skin irritation of a severity slightly below the threshold for classification.

The DS also presented three older non-guideline *in vivo* studies in the rabbit (A6.1.4/04b, A6.1.4/05b, A6.1.4/06b) employing both single and repeated application regimen. Although their results do not contradict those of the OECD TG 404 studies, their methods are non-standardised to such an extent (e.g., exposure duration not 4 hours; low number of animals, probably only 1 in the single dose experiments) that no meaningful comparison with the CLP criteria can be made. The studies are described in detail in Annex II to the CLH report.

The DS also assessed a human case report (Senoh *et al.*, 2009) where 2 workers were accidentally exposed to a liquid containing 20% DBNPA. Immediately after the accidents, both patients had slight pain due to primary irritation. The irritation subsided quickly but generalized erythemic plaques and blisters occurred 17 and 10 days after exposure on the skin of patient 1 and 2, respectively, including non-exposed sites. Treatment with ciclosporin was successful. RAC considers that this case report supports classification for skin sensitisation rather than for Skin Corr. 1.

RAC notes that a 10% suspension of DBNPA caused skin corrosion in an acute dermal toxicity study (A6.1.2/01). However, the exposure time was 24 hours as required by the OECD TG 402, which is a longer exposure than the 4-hour exposure under the skin irritation/corrosion protocol of OECD TG 404.

In summary, the result of the OECD TG 404 and GLP compliant *in vivo* study in rabbits A6.1.4/02 meets the criteria for classification as a skin irritant and therefore RAC agrees with the DS that **classification of DBNPA as Skin Irrit. 2; H315 is warranted**.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

The eye irritation potential of DBNPA has been investigated in 4 *in vivo* studies in the rabbit. The DS proposed classification in Category 1 based on the OECD TG 405 and GLP compliant study A6.1.4/01.

Comments received during public consultation

One MSCA and one manufacturer supported classification with Eye Dam. 1.

Assessment and comparison with the classification criteria

There is one *in vivo* study conducted in accordance with OECD TG 405. It is summarized in the following table.

Guideline eye irritation study		
Type of study; Reference; Year	**Method**	**Observations**
In vivo, rabbit A6.1.4/01 1983	OECD TG 405 3 males and 3 females Exposure period 1 hour	1 hour after exposure: Corneal opacity, discharge and chemosis of the highest grades of severity and conjunctival ulceration. Due to these findings, the animals were killed.

This guideline study, as well as several supporting non-guideline *in vivo* studies in the rabbit (A6.1.4/04a, A6.1.4/05a, A6.1.4/06a), reported severe eye lesions. In the non-guideline studies, where the observation period was 1 week, the damage progressed with time and included severe corneal injury. RAC agrees with the DS that **classification with Eye Dam. 1; H318 is warranted**

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

The skin sensitisation potential of DBNPA has been investigated in three animal studies (two Buehler tests and one non-guideline study) and several human repeat insult patch tests (HRIPT). One case report involving two subjects is also available.

The DS proposed classification as Skin Sens. 1 based on the positive Buehler test A6.1.5/01. The other Buehler test was negative. As to subcategorisation, the DS pointed out that the animal data were conflicting and the positive human studies lacked information on the purity and dose per surface area. In addition, they noted that only a single clinical case of skin sensitisation had been reported during over 45 years of use of the substance. The DS concluded that the available data did not provide sufficient evidence for subcategorisation.

Comments received during public consultation

One MSCA and one manufacturer supported the DS' proposal of Skin Sens. 1 without a subcategorisation.

Assessment and comparison with the classification criteria

Animal studies

There are two Buehler tests and one non-guideline study in guinea pigs employing repeated topical application. One of the Buehler tests was positive (A6.1.5/01) and the other one was negative (A6.1.5/03). The reason for this discrepancy is not clear. The study A6.1.5/02 was considered positive by the study authors but the induction regimen was non-standard (6 inductions instead of 3), no preliminary irritation screen was conducted and no data on negative controls have been reported. Especially the lack of (information on) negative controls is critical in view of the irritant response to 5% DBNPA in negative controls of another study (A6.1.5/03). Therefore, the result of the study A6.1.5/02 is not considered reliable. The three animal studies are summarized in a table below.

Skin sensitization studies in animals		
Type of study; Reference; Year	**Method**	**Observations**
Buehler test A6.1.5/03 1995	OECD TG 406 GLP 1st experiment: No. of animals: 10 treated (male), 5 controls (male) Induction: 25% w/v in 0.5% Methocel Challenge: 5% w/v in 0.5% Methocel 2nd experiment: No. of animals: 10 treated (male), 10 controls (male) Induction: 25% w/v in 0.5% Methocel Challenge: 0.5% w/v in 0.5% Methocel Positive control: neat DER 331 epoxy resin (CAS no. 1675-54-3); 10 animals Choice of concentrations was based on a preliminary screen at 0.1%, 0.5%, 1%, 5%, 7.5%, 10%, 25%, 50%, 75% and pure (moistened)	**Negative** 1st experiment: Erythema in 10/10 treated and 5/5 control males → attributed to irritation 2nd experiment: No positive reaction in any of the treated or negative control animals Positive control: slight to moderate erythema on 5/10 animals
Buehler test A6.1.5/01 1984	Comparable to OECD TG 406 No. of animals: 20 treated (10 m + 10 f), 10 controls (5 m + 5 f) Induction: 2% w/v in acetone Challenge: 0.5% w/v in acetone	**Positive** Patchy or slight erythema after 24h and/or 48h in 11/20 treated animals and 1/10 control animal
Non-guideline in guinea pigs A6.1.5/02 1972	Non-guideline No. of animals: 10 treated (male), 10 controls (male); not clear whether these controls are negative controls or positive controls Induction: topical twice a week for 3 weeks; 5% w/v in 9:1 mixture of Dowanol DPM:Tween 80 Challenge: after two weeks; 5% w/v in 9:1 mixture of Dowanol DPM:Tween 80 Positive control: 15% w/v DER 331 epoxy resin in 9:1 mixture of Dowanol DPM:Tween 80 Unknown purity of the test material Limited reporting No preliminary irritation screen	**Positive** DBNPA: 7/10 sensitised Positive control: 8/10 sensitised No data on negative control

Human data

There are three HRIPTs, each involving ca. 26 subjects. Two of them were negative and one was positive. The dose per area was below 500 µg/cm^2 in all three tests (the patch area in A6.12.6/02, Study III and Study V, is not reported but based on the applied volume of 0.2 mL it is unlikely to be smaller than 1 cm^2). As the number of subjects in the individual studies is relatively low and as the induction concentrations in the negative studies (125 and 250 ppm) were lower than in the positive study (500 ppm), the two negative studies (A6.12.6/01; A6.12.6/02, Study V) do not necessarily contradict the positive result of A6.12.6/02, Study III.

The skin sensitisation potential of DBNPA in humans is further supported by A6.12.6/02, Study I. However, the study design did not follow a HRIPT or HMT protocol and the dose per skin area cannot be estimated. Therefore, this study cannot be used for sub-categorisation.

The case report (Senoh *et al.*, 2009) describes a contact reaction after an accidental exposure of two paper-mill workers to liquid-formulated 20% DBNPA. A relatively severe reaction including erythema and blisters on non-exposed sites (back, chest) occurred approx. 2 weeks after

exposure. The reaction proved to be immune system-mediated and sensitisation to DBNPA was confirmed via patch testing in one of the patients.

The human data on skin sensitisation are summarized in the following table.

Human data on skin sensitisation		
Type of study; Reference; Year	**Method**	**Observations**
HRIPT A6.12.6/01 1982	26 subjects Test substance: a formulation containing 20% DBNPA, 29% water and 48% Polyglycol E-200; pH 3.8 Induction: 10 applications (3 per week); 0.0125% (125 ppm) DBNPA, patch volume approx. 0.2 mL; dose ca. 6 µg/cm^2 Challenge: two weeks after induction; 0.0125% DBNPA	**Negative** 3 subjects reacted during the induction phase None of the subjects reacted to challenge
Cumulative irritancy with delayed challenge A6.12.6/02, Study I and II 2002	<u>Study I</u> 27 subjects (24 for challenge) Test substance: a formulation containing 20% DBNPA (in water and polyethylene glycol) Induction: 15 applications (5 per week) to the same site; concentrations 0, 500, 750, 1000, 1250, 1500, 1750 and 2000 ppm DBNPA; probably simultaneous application of patches at the aforementioned doses Challenge: 10 days after induction, to a previously untreated site; 500 ppm <u>Study II</u> 6 subjects positive in Study I reassembled Single exposure 45 days after Study I Concentrations 0, 1, 2, 4, 8, 16, 31, 63, 125, 250, 500 ppm DBNPA	<u>Study I</u> **Positive** 13 subjects were probably sensitized (according to the DS) Irritancy was observed at ≤ 1250 ppm <u>Study II</u> The lowest level for elicitation was 4 ppm in subject 1; 16 ppm in subjects 2, 3, 4 and 5; subject 6 had an equivocal response at even the highest concentration
HRIPT A6.12.6/02, Study III and IV 2002	<u>Study III</u> 26 subjects Test substance: a formulation containing 20% DBNPA (in water and polyethylene glycol); vehicle used for dilution: distilled water Control: vehicle (the tested formulation without DBNPA) Induction: 10 applications (3 per week) to the same site; 500 ppm (0.05%) DBNPA; patch volume approx. 0.2 mL Challenge: two weeks after induction, to a previously unpatched site; 500 ppm <u>Study IV</u> 5 subjects positive in Study III reassembled 11 months after Study III *Retest:* 500 ppm, patch volume approx. 0.2 mL *Rechallenge:* 1, 5, 50, 125, 250 and 500 ppm, patch volume approx. 0.2 mL	<u>Study III</u> **Positive** 7 subjects were deemed to have developed allergic contact dermatitis to the test sample; 2 out of these 7 subjects had a response to the vehicle believed to represent the excited skin syndrome <u>Study IV</u> *Retest:* all 5 subjects positive *Rechallenge:* 50 ppm (0.005%) or less did not elicit a skin response in any of the subjects; at least one subject reacted to 125 ppm
HRIPT A6.12.6/02, Study V 2002	26 subjects (25 completed the study) Test substance: a formulation containing 20% DBNPA (in water and polyethylene glycol); vehicle used for dilution: distilled water	**Negative** 1 mild reaction during the induction phase None of the subjects reacted to challenge (including the subject with a reaction during induction)

	Induction: 10 applications (3 per week) to the same site; 250 ppm (0.025%) DBNPA; applied amount approx. 0.2 g Challenge: two weeks after induction, to a previously unpatched site; 250 ppm	
Case report Senoh *et al.* 2009, A6.12.2	Two industrial workers from the same paper mill were accidentally exposed to a formulation containing 20% DBNPA Subject 1: exposed on fingers and legs; immediately after the incident he washed the exposed areas with running water Subject 2: probably exposed on hands and arms	Subject 1: Slight pain after exposure. Superficial skin necrosis occurred 3 days later, eruptions subsided after treatment with a topical steroid. 17 days after the incident, blisters developed on the previous lesions on the thigh without further exposure to DBNPA and then extended to other sites (back, chest), associated with fever. Bacterial culture from the bulla did not grow any pathogens. Most of the infiltrating cells in the epidermis were CD8+ T-lymphocytes. Ciclosporin successfully controlled the disease within several days. Subject 2: Transient pain after exposure. After approx. 10 days redness and increased pain in the exposed areas. After additional 10 days widespread, erythematous plaques with blisters on the trunk and conjunctivitis. Infiltrating cells mainly CD8+ T-lymphocytes. Many necrotic and apoptotic keratinocytes. Disease controlled by a combination of methylprednisolone and ciclosporin. In a patch test (0.1% in petrolatum) the patient reacted with oedematous erythema.

Conclusion on classification

RAC finds in the available studies, particularly the Buehler assay A6.1.5/01 and the A6.12.6/02, Study I (human data on cumulative irritancy with delayed challenge) and III (HRIPT), sufficient evidence for classification of DBNPA as a skin sensitiser.

According to the CLP guidance, classification into subcategories is required when data are sufficient.

The reliable animal data do not indicate a high potency. One reliable Buehler assay was negative (A6.1.5/03), while the other one (A6.1.5/01) gave a positive response consistent with subcategory 1B (approximately half of the animals positive at an induction concentration of 2%), but a subcategory 1A could not be excluded as ≤ 0.2% induction concentration was not tested.

As to human data, one HRIPT (A6.12.6/02, Study III) showed positive responses at an induction dose below 500 µg/cm^2, which is consistent with subcategory 1A. There is a single case report involving two subjects with a relatively severe response to a mixture containing 20% of DBNPA. The number of published cases is very low but this may reflect the predominantly industrial use of the chemical where risk management measures are likely to be in place.

Overall, there is one study consistent with subcategory 1A (A6.12.6/02, Study III) and several studies either not allowing sub-categorisation (A6.1.5/01; A6.12.6/02, Study I; case report) or indicating low potency (A6.1.5/03). Taking a weight of evidence approach, RAC agrees with the DS that **classification as Skin Sens. 1; H317 without sub-categorisation** is warranted.

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

Summary of the Dossier Submitter's proposal

The repeat dose toxicity of DBNPA via the oral route has been investigated in the rat, mouse and dog. Two dermal studies in the rat are also available. In the CLH report the DS discussed effects on the thyroid, kidneys, liver, adrenals, and on haematopoiesis. They also addressed dyspnoea observed in rat gavage studies. The original DS' proposal was no classification. Based on the comments and new data received during the public consultation (see below) the DS changed they proposal to STOT RE 1 with the respiratory tract and the thyroid as the target organs.

Comments received during public consultation

A manufacturer submitted a 2-week inhalation study in the rat (summarized in the Background Document under 'additional key elements'). They proposed considering an appropriate classification to communicate the respiratory irritation potential of the substance identified in this study, without giving a specific classification proposal. The DS proposed to address the local effects in the upper and lower respiratory tract observed in this study with a classification as STOT RE 1.

One MSCA pointed out thyroid follicular cell hypertrophy seen in the 90-day dog study and hyperplasia seen in the 2-year rat study, requesting a due consideration of the endocrine disrupting properties of the substance. The DS responded that, according to the ECHA ED Expert Group in October 2018, the hypertrophy in the dogs at 10.7 mg/kg bw/d DBNPA was an adverse effect. Therefore, the DS was of the view that the thyroid follicular cell hypertrophy observed in dogs was sufficient to trigger classification in Category 2 and the thyroid should be stated as a target organ in the hazard statement. The thyroid effects in the rat were considered only as supportive evidence as they occurred above the guidance value range for classification.

Assessment and comparison with the classification criteria

RAC has identified the following effects as potentially relevant for the STOT RE classification in the available studies with DBNPA:

- Effects on the respiratory tract in the 2-week rat inhalation study
- Thyroid findings in the 90-day dog study (A6.4.1/01) and in the 2-year rat study (A6.7/01)
- Histopathological findings in the kidney in a 90-day rat study (A6.4.1/05)
- Effects on haematopoiesis in a 90-day rat study (A6.4.1/02)
- Dyspnoea associated with mortality in several rat gavage studies

These effects are discussed below. Additionally, RAC agrees with the DS that the observed small adrenal (A6.1.4/04) and liver (A6.4.1/05) weight changes without associated histopathological findings are not sufficient for classification.

Respiratory tract

The 2-week inhalation study was performed with concentrations of 0.51, 5.4 and 50/25 mg/m^3 (31 mg/m^3). The default guidance value for STOT RE 1 for a 90-day study is 20 mg/m^3 (dust/mist/fume). For other study durations the guidance values are normally adjusted using Haber's rule. However, according to the CLP guidance, care should be taken when using Haber's rule to assess inhalation data on substances which are locally active. DPNPA is a locally active

substance and the effects observed in the respiratory tract in this study are site-of-contact effects. Therefore, the use of Haber's rule is might not be appropriate in this case.

Nevertheless, already at the mid-dose of 5.4 mg/m^3, which is clearly below the guidance value for STOT RE 1, multifocal necrosis and fibrosis was observed in the larynx and multifocal necrosis in the lung of almost all animals. Such effects normally trigger a STOT RE classification (cf. CLP Regulation, Annex I, 3.9.2.7.3e). Although the severity was very slight to slight, it would most likely increase after longer exposures. Thus, classification with STOT RE 1 for effects on the respiratory tract is considered appropriate.

Thyroid

In the 90-day dietary study in dogs (A6.4.1/01), very slight thyroid follicular cell hypertrophy and increased thyroid weight (slightly above HCD) were observed at doses within the guidance value range for Category 2. The findings are presented in the table below. As DBNPA is unstable in contact with organic matter, a correction factor had to be applied to the nominal dose in all dietary studies to account for the loss of the parent substance. As the thyroid effects are likely to be caused by bromide (see below), which remains in the food after DBNPA degradation, the nominal, uncorrected dose is considered more representative when considering the effects on the thyroid.

Thyroid findings in the 90-day dog dietary study					
Target dose (mg/kg bw/d)	**0**	**15**	**30**	**50**	**HCD**
Dose corrected for degradation of DBNPA (mg/kg bw/d) m/f	0	5.9/6.1	12/11	18	
Males					
No. of animals examined	4	4	4	4	
Thyroid weight absolute (g)	0.68	0.71	0.75*	0.90*	0.68-0.81
Dilatation of the thyroid follicle, diffuse, very slight[b]	0	0	0	2	
Females					
No. of animals examined	4	4	4	4	
Thyroid weight absolute (g)	0.58	0.66	0.88*	0.75*	0.62-0.75
Dilatation of the thyroid follicle, diffuse, very slight[b]	1	1	3	3	

* Statistically significantly different from controls, $p \leq 0.05$

[a] Historical controls group mean range from four 13-week dog studies from 1999-2003 (the study report of the present study is dated 2004)

[b] Statistical analysis not conducted

None of the 90-day studies in the rat (A6.4.1/02, A6.4.1/04, A6.4.1/05) reported any histopathological changes in the thyroid (in the dietary study A6.4.1/02 up to 300/600 mg/kg bw/d nominal m/f). Increased incidence of diffuse thyroid follicular hyperplasia (very slight to slight) was observed after 1 and 2 years at 150 mg/kg bw/d (nominal) in both sexes and in males also at 20 mg/kg bw/d after 2 years only. Thus, the effects in the rat are considered to occur above the guidance values for classification.

The DS mentioned that the observed thyroid effects may have been caused by the bromine component of DBNPA (CLH report, p. 86). Although no specific mechanistic studies on the role of bromide in the development of the weak thyroid effects in the DBNPA-treated rats and dogs are available, RAC finds this explanation plausible. High doses of bromide are known to inhibit thyroid

function in rats, probably by replacing iodine in the thyroid and by accelerating iodide elimination (Pavelka *et al.*, 2001; Loeber *et al.*, 1983). Bromide is readily released from DBNPA upon contact with organic matter and has been identified as a major metabolic product in the rat (A6.8.1/03) with a longer elimination time than that of the organic moiety (A6.2/01). Bromine content in DBNPA is 66% by mass.

The weak thyroid effects in the dog within the guidance value range for classification in Category 2 are likely to be related to treatment with DBNPA. However, RAC considers that they are not of sufficient toxicological significance to meet the STOT RE criteria for classification.

Kidney

In the 90-day rat study (A6.4.1/05), minimal cytoplasmic swelling and vacuolisation of renal tubular epithelial cells was observed in females at 63 mg/kg bw/d (incidence 9/10 vs 0/9 in controls), accompanied by a slight increase in kidney weight (by 5%). DBNPA was administered in drinking water at two pH values, 4 and 8; the kidney effects were present only in the pH 8 group. As the substance degrades rapidly under alkaline conditions (degradation was not measured in this study, but a rapid change in colour and pH of the solution was noted by the study authors), the effects are likely to have been caused by products of abiotic degradation rather than by the parent substance. In addition, the observed effect does not always indicate degeneration and can be spontaneous as evidenced by 2 cases in the pH 4 female controls. Therefore, RAC does not find the kidney effects sufficient for classification.

Haematopoiesis

Increased incidence of haematopoiesis in the spleen was observed within the guidance value range for Category 2 in the 90-day rat dietary study (A6.4.1/02). The incidences in females were 0/10, 1/10, 2/10 and 4/10 at 0, 1.2, 4.3 and 44 mg/kg bw/d respectively, and the severity was "very slight" in all cases. No increase was observed in males at these dose levels. Although the effect is likely to be treatment-related (in view of the further increases in incidence at higher doses above the guidance value), the toxicological significance or severity of the findings within the guidance value range are considered not to be sufficient to warrant classification.

Mortality

Dyspnoea starting during the first weeks of treatment was observed in several rat gavage studies (A6.3.1/01; A6.4.1/04; A6.8.2/02; A6.8.1/03). This effect was associated with mortality (spontaneous or sacrifice *in extremis*) from approximately 30 mg/kg bw/d. On pathological examination the affected animals showed distended stomach and/or intestines and often also congestion of the lungs. The observed dyspnoea is likely to be a consequence of reflux of the irritant solution to the respiratory tract (the pathology of gavage-related reflux has been reviewed by Damsch *et al.*, 2011). As this effect occurred only after administration via gavage and not via diet or drinking water (A6.4.1/02; A 6.4.2/05), i.e. routes more relevant for human health hazard assessment, the dyspnoea and related mortality is not considered relevant for classification.

Mortalities were also observed at 60 mg/kg bw/d in a rabbit PNDT study (A6.8.1/01) where the substance was also administered via gavage. The affected animals had ulcerative gastritis occasionally associated with pulmonary oedema. This is also considered to be an effect related to gavage administration of a strong irritant, not relevant for classification.

Conclusion on classification

RAC agrees with the DS on classification with STOT RE 1 for effects on the respiratory tract. This classification is based on multifocal fibrosis and/or necrosis in the larynx and the lung at 5.4 mg/m^3 in the 2-week rat inhalation study. RAC notes the absence of effects on the respiratory

tract in the rat oral and dermal studies (apart from gavage-related reflux, which is not considered relevant for human health hazard assessment). As to the thyroid, RAC considers that the very slight thyroid hypertrophy seen in the dog is not of sufficient toxicological significance to include the thyroid as the target organ for STOT RE classification.

In conclusion, RAC concludes that **classification with STOT RE 1; H372 (respiratory tract) (inhalation) is warranted**.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

The DS proposed no classification for germ cell mutagenicity based on negative results of the following assays:

- Ames test (A6.6.1/01)
- Chromosomal aberration assay *in vitro* (A6.6.2/01)
- HPGRT assay (A6.6.3/01)
- Sister chromatid exchange assay *in vitro* (A6.6.2/02)
- Unscheduled DNA synthesis *in vitro* (A6.6.3/02)
- Micronucleus test *in vivo* (A6.6.4/01, A6.6.4/02)

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

The genotoxic potential of DBNPA was investigated both *in vitro* and *in vivo*. The *in vitro* assays were all negative and included an OECD TG and GLP compliant Ames test (A6.6.1/03), an OECD TG and GLP compliant HPGRT assay (A6.6.3/01) and a GLP compliant *in vitro* chromosomal aberration assay (A6.6.2/01) showing several deviations from the current version of the OECD guideline.

There are also two *in vivo* micronucleus tests in mice, both negative. The top dose in A6.6.4/01 was chosen on the basis of mortality in a dose range-finding experiment. There was no direct evidence of bone marrow exposure in either study. The toxicokinetic studies in the rat did not investigate tissue distribution but systemic exposure is likely as the substance was excreted mainly via urine (A6.2/01, A6.2/02). Slight effects on the bone marrow were observed in one repeated dose toxicity study in the rat (A6.4.1/02).

The available key studies for classification are summarised in the following table. Some *in vitro* mutagenicity studies have been omitted because of low reliability (A6.6.1/02; A6.6.2/03). *In vitro* assays investigating DNA damage that can potentially be repaired (sister chromatid exchange and unscheduled DNA synthesis) are not included in the table because they are less informative than studies having mutations as the endpoint.

Key genotoxicity studies		
In vitro mutagenicity studies		
Type of study; Reference; Year	**Method**	**Observations**
Ames test A6.6.1/03 2002	OECD TG 471 GLP *S. typhimurium* TA 1538, TA 98, TA 100, TA 1535, TA 1537; *E. coli* WP2uvrA Up to 100 µg/plate	Negative ±S9 Sufficient cytotoxicity achieved Positive controls responded appropriately
Ames test A6.6.1/01 1992	OECD TG 471 GLP *S. typhimurium* TA 1535, TA 1537, TA 98, TA 100 Deviation: *S. typhimurium* TA102 or *E. coli* WP2 not tested Up to 25 µg/plate	Negative ±S9 Sufficient cytotoxicity –S9 Positive controls responded appropriately
Chromosomal aberration assay *in vitro* A6.6.2/01 1989	OECD TG 473 GLP Human lymphocytes Up to 50 µg/mL Harvest after 24h and 48h Deviations from OECD TG 473 (2016): long exposure (1.5 cell cycle length) missing; exposure for 2h instead of 3-6h	Negative ±S9 (a non-significant increase +S9 but only at a cytotoxic concentration) Sufficient cytotoxicity +S9; –S9 mitotic index reduced by 32% (24h) and 18% (48h) at the top concentrations (50 and 37.5 µg/mL respectively), reduction above 50% would have been reached at 75 and 50 µg/mL respectively Positive controls responded appropriately
HPGRT assay A6.6.3/01 1985	OECD TG 476 GLP Chinese hamster ovary cells Up to 400 µM	Negative ±S9 Sufficient cytotoxicity achieved Positive controls responded appropriately
In vivo studies		
Micronucleus test (bone marrow) A6.6.4/01 1994	OECD TG 474 GLP Mouse 5 per dose, sampling time and sex (plus 5 extra animals in the top dose to serve as possible replacements for dead animals) Single oral (gavage) dose; 30, 60, 120 mg/kg bw Sampling time 24h, 48h and 72h (positive control 24h) Top dose chosen based on mortalities in a dose range-finding experiment	Negative Mortality at 120 mg/kg bw (4/30) and 60 mg/kg bw (1/30) No clinical signs No reduction in %PCE Positive control responded appropriately
Micronucleus test (bone marrow) A6.6.4/02 1985	OECD TG 474 GLP Mouse 5 animals (males and females mixed) per dose and sampling time Single oral (gavage) dose; 9, 30, 90 mg/kg bw Sampling time 24h and 48h (positive control 24h)	Negative No reduction in %PCE Positive control responded appropriately

RAC is of the view that the genotoxic potential of DBNPA has been sufficiently investigated. As the available data do not meet the criteria for classification, RAC agrees with the DS' proposal that **no classification is warranted for germ cell mutagenicity.**

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

The DS proposed no classification based on a negative 2-year carcinogenicity study in the rat. A carcinogenicity study in the second species is not available.

Comments received during public consultation

One MSCA requested details about thyroid follicular cell hyperplasia in the rat carcinogenicity study but did not express a preference regarding classification.

Assessment and comparison with the classification criteria

No neoplastic findings were observed in a GLP compliant 2-year rat carcinogenicity/chronic toxicity study conducted according to OECD TG 453 (A6.4.2/01).

The top dose of ca. 70 mg/kg bw/d caused only limited general toxicity (no effect on survival, no significant clinical signs, reduced terminal body weight by 11%/8%). On the other hand, rats exposed for 90 days to ca. 250 mg/kg bw/d DBNPA via diet had to be prematurely sacrificed (A6.4.1/02) and the oral LD_{50} in the rat (via gavage) is around 200 mg/kg bw, which reduces the concern about the choice of the top dose in this study.

As the results of the available carcinogenicity study do not meet the criteria for classification, RAC agrees with the DS that no classification for carcinogenicity is warranted

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

The DS proposed no classification for effects on fertility and sexual function, development or for effects on or via lactation based on the results of a 2-generation study in the rat and a prenatal developmental toxicity study (PNDT) in the rabbit (A6.8.1/01). The slight increases in some skeletal variations in the rabbit were not considered related to the treatment. Following a comment from the public consultation the DS also evaluated a PNDT study in the rat (A6.8.1/03), which was also considered negative and therefore did not change the classification proposal.

Comments received during public consultation

A manufacturer pointed out that a PNDT study in the rat was part of the biocidal dossier but it was not included in the CLH report (although it was present in its annex). The DS acknowledged this inconsistency. As the DS considered the study to be negative, the classification proposal remained unchanged. A summary of this PNDT study in the rat (A6.8.1/03) that was omitted in the CLH report is provided in the BD under Additional key elements.

Assessment and comparison with the classification criteria

Adverse effects on sexual function and fertility

The 2-generation study in the rat (6.8.2/02) was conducted according to the OECD TG 416 (1983) and in compliance with GLP. Some parameters introduced into the OECD TG in 2001, such as

sexual maturation, were not investigated (they were not mandatory at the time the study was performed).

DBNPA was administered via gavage. The top dose of 40 mg/kg bw/d had to be reduced to 30 mg/kg bw/d shortly after the beginning of the study due to dyspnoea and mortality. However, even the reduced top dose lead to additional mortalities in the P and F1 generations (total mortality in P: 4 m, 7 f; F1: 2 m, 7 f). Several cases of dyspnoea occurred also at the mid-dose of 15 mg/kg bw/d.

As no effects on fertility or sexual function were observed in this study, RAC agrees with the DS that **no classification** for adverse effects on sexual function and fertility is warranted.

Adverse effects on development

PNDT studies

In the OECD TG and GLP compliant rat PNDT study (6.8.1/03) no developmental effects occurred up to the top dose of 30 mg/kg bw/d associated with maternal toxicity including mortality.

The OECD TG and GLP compliant PNDT study in the rabbit (6.8.1/01) employed doses up to 60 mg/kg bw/d. The top dose of 60 mg/kg bw/d caused excessive maternal toxicity; 6 out of 14 dams died or were killed *in extremis*. The only developmental effect at the top dose was increased incidence of small foetuses. Increased incidence of some skeletal variations was seen at the lower doses (see the table below). These minor skeletal variations are not considered sufficient for classification.

Skeletal variations in the rabbit PNDT study (%foetuses/no. of litters)						
Dose (mg/kg bw/d)	**0**	**2**	**10**	**30**	**60**	**HCD**
No. of foetuses/litters examined	108/13	138/13	137/14	107/12	58/7	1316/149
Bilaterally lumbar rib	44%/12	44%/13	71%***/14	58%*/12	48%/6	43–66%
Incomplete ossification of sternebra 5 and/or xiphisternum	19%/6	21%/10	26%/11	40%***/12	35%/5	0–37%
Ileum articulating with 1st or 1st+2nd sacral vertebra bilaterally	53%/12	51%/12	37%*/10	36%*/12	40%/7	12–58%
Incomplete ossification of long bone epiphyses	20%/7	23%/10	12%/7	35%*/9	31%/7	11–36%

Statistically significantly different from control (based on foetal inc.): *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$

HCD from the same laboratory, within 5 years, including the present study

2-generation study

An increase in total litter loss was observed in the mid- and high-dose groups in P dams but only at the high dose in F1 dams. As the total litter loss was not affected in the second generation at 15 mg/kg bw/d, the effect in the first generation is not considered to be sufficient evidence to warrant classification for reproductive toxicity. A slight reduction in the litter size was also observed at the top dose in the first generation and was not due to a reduced number of implantation sites. The data are shown in the table below. Due to the low magnitude of the effects and the excessive toxicity at the top dose, the effects are not considered sufficient for classification.

Selected findings in the 2-generation study					
Dose (mg/kg bw/d)		**0**	**5**	**15**	**40/35/30**
Number of dams per group	P	28	28	28	28
	F1	26	26	26	26
Maternal mortality	P	0	0	0	7 (25%)
	F1	0	1	0	7 (27%)
Mean number of implantation sites	P/F1	16.9	16.2	16.3	16.5
	F1/F2	16.9	17.2	14.5	14.6
Litter size[a]	P/F1	15.8	15.0	15.0	14.0
	F1/F2	15.4	15.1	14.5	14.6
Total litter loss	P/F1	1	1	4	2
	F1/F2	0	0	0	2

[a] Excludes litters where all pups died

In the absence of significant developmental findings in well-conducted PNDT studies and in a 2-generation study, RAC agrees with the DS that **no classification** for adverse effect on development is warranted.

Adverse effects on or via lactation

In the absence of adverse findings potentially related to lactation in the 2-generation study, RAC agrees with the DS that **no classification** for adverse effects on or via lactation is warranted.

In summary, RAC agrees with the DS that **DBNPA does not warrant classification reproductive toxicity**.

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Summary

The DS proposed to classify the substance as Aquatic Acute 1; H400 based on the EC_{50} for invertebrates of 0.72 mg/L. M-factor of 1 was warranted because the lowest toxicity value was in range 0.1 mg/L < L(E)C_{50} ≤ 1 mg/L. Based on the lowest NOEC of 0.06 mg/L for invertebrates for a rapidly degradable substance, the DS proposal for chronic classification was Aquatic Chronic 2; H411. During the public consultation, the DS confirmed that they consider the substance as not rapidly degradable and consequently the proposed classification should be Aquatic Acute 1; H400, M=1 and Aquatic Chronic 1; H410, M=1.

Degradation

There were two valid studies on ready biodegradability available. In the OECD TG 301B study following GLP, the concentration of test material ([^{14}C] DBNPA) was reduced to 0.06 and 0.6 mg/L to avoid potential inhibitory effects of DBNPA.[1] After 28 days, mineralisation of 0.06 mg/L

[1] OECD TG 209, GLP, Respiration inhibition test, 3-hour EC50 4.6 mg/L (nominal).

reached 78%. Within the 10-day window, mineralisation of approximately 58% was reached. At concentration 0.6 mg/L the mineralisation reached only 11% after 28 days. In the toxicity control, the solution containing 0.6 mg/L DBNPA reduced DOC removal (83%) suggesting that the higher concentration was at least partly inhibitory to the inoculum.

In the second GLP OECD TG 301B study, the concentration of [^{14}C] DBNPA was reduced to 0.05 mg/L to avoid potential inhibitory effects of DBNPA. After 28 days, mineralisation reached only 35%.

The reason for the different results of the two valid studies was not known but was likely due to variations in the microbial inoculum. Based on these studies the DS concluded that DBNPA is not readily biodegradable.

In the one hydrolysis study with a reliability indicator of 1 (three other studies had a reliability indicator of 3) following OECD TG 111, hydrolysis rates for DBNPA were determined in 0.05M buffered reaction mixtures for a range of different temperatures/pH combinations including; 50, 60, and 70°C at pH 4; 25, 37.5, and 50°C at pH 7; and 13, 25, and 35°C at pH 9. Hydrolysis of DPNBA occurred in all reaction mixtures with the rate increasing with an increase in either the solution pH or temperature. Overall, the mass balance of DBNPA and hydrolysis product concentrations ranged from 83 to 108%. Hydrolysis products included dibromoacetonitrile (DBAN), dibromoacetamide (DBAM), dibromoacetic acid (DBAA), dibromomalonamide (DBMAL) and oxalic acid. The range of maximum yields of the hydrolysis products at pH 4, 7, and 9 were: dibromoacetonitrile – 54 to 79%; dibromoacetamide – from not detectable to 35%; dibromoacetic acid – 6 to 11%; and dibromomalonamide – 6 to 11%. The 12^{o}C half-lives for DBNPA were determined to be 12079 hours, 183 hours and 5.6 hours at pH 4, pH 7 and pH 9, respectively. The DS concluded that the substance was not hydrolytically stable at environmentally relevant pH and temperature.

DBNPA is not degradable by direct photodegradation in water. Indirect photolytic mechanisms appear to be responsible for the observed accelerated degradation under sunlight-irradiated conditions. Several transformation products (oxalic acid, bromoacetamide, dibromoacetic acid) were identified in a study conducted according to a GLP FIFRA Guideline 161-2 investigating photodegradation in water. In another GLP FIFRA Guideline 161-2 test, total sunlight exposure half-lives of 14.8, 6.9 and 0.4 hours at pH 5, 7 and 9, respectively, were determined for DBNPA. The observed degradation in the exposed samples was due to both hydrolysis, oxidation and indirect photolysis. Several degradation products were detected namely dibromoacetic acid, cyanoacetic acid, 2-bromo-2-nitropropionamide, dibromoacetamide, dibromoacetonitrile.

In addition, there was a non-guideline study available on the hydrolytic degradation of DBNPA and the degradation of DBNPA in the presence of organic material occurring in natural waters. The study does not follow GLP. The main purpose of this study was to investigate the influence of organic material present in natural waters on the degradation rates of DBNPA. The results showed rapid primary degradation (DT_{50} of 2 hours) and formation of degradation products including DBAN.

Based on the available information, the DS concluded that DBNPA is not rapidly degradable.

Bioaccumulation

The measured dissociation constant pKa was 8.3 for DBNPA. The measured log K_{OW} for DBNPA was 0.80, 0.80 and 0.82 for pH 5, pH 7 and pH 9, respectively at 20-21°C. A fish bioconcentration study was performed. The study did not follow GLP or the test principles described in OECD TG 305. The depuration phase was started, with some of the fish, before the plateau concentration was reached. Information on the control group is missing. The calculated BCF at steady state in

whole fish was 13. This information can only be used as supportive evidence. Overall, DBNPA is considered to have a low potential for bioaccumulation.

Aquatic Toxicity

***Table**: Reliable aquatic toxicity data on DBNPA*

Method	Species	Test material	Results	Remarks	Reference
Acute Aquatic Toxicity					
EPA-FIFRA 72-3; GLP	Sheepshead minnow (*Cyprinodon variegatus)*	DBNPA	96h LC_{50} = 3.4 mg/L (mm)	Flow-through Saline water nom. 0.78–10 mg/L(1 mm 42-83% of nom.	A7.4.1.1/01
US EPA-FIFRA 72-3; GLP	Mysid shrimp *(Mysidopsis bahia)*	DBNPA	96h LC_{50} = 0.72 mg/L (mm)	Flow-through Saline water 0.23–3 mg/L (1 mm 0 – 2.4 mg/L	A7.4.1.2/05
OECD TG 201 GLP	*Scenedesmus subspicatus*	DBNPA	72h E_bC_{50} = 0.9mg/L (time-weighted mm) 72h E_rC_{50} = 2.3 mg/L (time-weighted mm)	Static 0 hour: 0.9 – 13.7 mg/L (1 72 hours: <LOQ – 3.59 mg/L)	A7.4.1.3/04
Chronic Aquatic Toxicity					
EPA FIFRA 72-4 GLP	Rainbow trout (*Oncorhynchus mykiss*)	DBNPA	NOEC = 0.47 mg/L (mm) LOEC = 0.98 mg/L (mm)	Flow-through, 85 days 0.096 – 4.1 mg/L (1 mm 80 – 103% of nom Embryo viability, survival of embryos at hatch and survival and growth (weight and length) of larvae after 60 days post-hatch exposure	A7.4.3.2/01
OECD TG 211 and USEPA OPPTS 850.1300 GLP	*Daphnia magna*	DBNPA	21d NOEC = 0.060 mg/L (mm); survival, reproduction and growth (total body length, dry weight) 21d LOEC = 0.170 mg/L (mm), survival 21d LOEC > 0.06 mg/L (2 (mm); reproduction, growth (total body length, dry weight)	Flow-through 0 - 0.50 mg/L(1 (mm): <LOQ, 0.0055, 0.020, 0.060 and 0.17 mg/L	A7.4.3.4/02

EPA FIFRA 72-4 GLP	*Daphnia magna*	DBNPA	21-d NOEC 0.07 mg/L reproduction 21-d NOEC 0.54 mg/L growth 21d LOEC = 0.12 mg/L reproduction	Flow-through 0.07 - 0.9 mg/L [(1]	A7.4.3.4/03
OECD TG 201 GLP	*Scenedesmus subspicatus*	DBNPA	72h NOEC = 0.36 mg/L (time-weighted mm)	Static 0 hour: 0.9 – 13.7 mg/L [(1] 72 hours: <LOQ – 3.59 mg/L)	A7.4.1.3/04

nom=nominal concentrations; mm=mean measured concentrations
[(1] Initial concentrations of test substance
[(2] Due to the percent survival in the 0.50 mg/L treatment level (0.17 mg/L mean measured concentration), this treatment level was excluded from statistical analysis for the mean cumulative offspring per female, total body length and dry weight.

Acute aquatic toxicity

There were reliable test data available for fish, invertebrates and algae. The lowest toxicity value was a mean measured 96-hour LC_{50} of 0.72 mg/L for *Mysidopsis bahia* from a US EPA-FIFRA 72-3 test following GLP.

Chronic aquatic toxicity

There were reliable test data available for fish, invertebrates and algae. The lowest toxicity values were from the two *Daphnia magna* tests. In the OECD TG 211 test following GLP, the mean measured 21-day NOEC for survival and growth was 0.06 mg/L for *Daphnia magna*. In the other *Daphnia* test following EPA FIFRA 72-4 and GLP, the 21-day NOEC for reproduction was 0.07 mg/L.

Comments received during public consultation

Four MSCA agreed with the aquatic acute classification proposal. They paid attention to the fact that the CLH Report contained conflicting conclusions on the rapid degradability of DBNPA. The DS confirmed that the substance is not rapidly degradable and agreed that the chronic classification should be Aquatic Chronic 1 (M=1) instead of the initially proposed Aquatic Chronic 2.

Assessment and comparison with the classification criteria

Degradation

The hydrolysis half-lives for DBNPA at 12°C were determined to be 12079 hours, 183 hours and 5.6 hours at pH 4, pH 7 and pH 9, respectively. Hydrolysis rates increased with an increase in either the solution pH or temperature. Several hydrolysis products were detected. Of those, DBAN (Aquatic Acute 1) and DBMAL (Aquatic Chronic 3) were self-classified for environmental hazards in the ECHA C&L Inventory. The range of maximum yields of these two hydrolysis products were, for DBAN from 54 to 79% and for DBMAL from 6 to 11%. RAC agrees with the DS conclusion that the substance is not hydrolytically stable at environmentally relevant pH and temperature.

DBNPA was not degradable by direct photodegradation in water. Indirect photolytic mechanisms appeared to be responsible for the observed accelerated degradation under sunlight-irradiated conditions. The observed degradation in the two studies investigating photodegradation in water was due to hydrolysis, oxidation and indirect photolysis. Several degradation products were

detected. One of the degradation products, 2-bromo-2-nitropropionamide, was self-classified in the ECHA C&L Inventory as Aquatic Acute 1 and Aquatic Chronic 1.

In the OECD TG 301B study, DBNPA mineralized 78% in 28 days at concentration of 0.06 mg/L. Within the 10-day window, mineralisation of approximately 58% was reached which means the 10-day window criteria was not filled. At a concentration of 0.6 mg/L, the mineralisation reached only 11% after 28 days. In the toxicity control solution containing 0.6 mg/L, DBNPA reduced DOC removal (83%) suggests that the higher concentration is at least partly inhibitory to the inoculum. In the second GLP OECD TG 301B study, the concentration of DBNPA was reduced to 0.05 mg/L to avoid potential inhibitory effects of DBNPA. After 28 days, mineralisation reached only 35%. RAC agrees with the DS and considers DBNPA not readily biodegradable.

DBNPA does not fulfil the criteria mentioned in the CLP guidance to consider the substance rapidly degradable:

- the substance is not readily biodegradable,
- the substance showed rapid primary degradation in an aerobic surface water test. At least one of the degradation products fulfil the criteria for classification as hazardous to the aquatic environment.
- the substance is hydrolysed rapidly in the aquatic environment with a half-life < 16 days (at pH 7 and 9, 12°C) but at least some of the degradation products fulfil the criteria for classification as hazardous to the aquatic environment. At pH 4 the half-life for hydrolysis is much longer (around 500 days, 12°C).

Consequently, RAC considers DBNPA not rapidly degradable for classification.

Bioaccumulation

The measured log K_{OW} for DBNPA was 0.80. There was no reliable fish BCF study available. The log K_{OW} of 0.8 is below the bioaccumulation cut-off value of 4 and RAC agrees with the DS that DBNPA has a low potential for bioaccumulation.

Aquatic toxicity

There are reliable acute and chronic toxicity test data available for fish, invertebrates and algae. The lowest acute value is the mean measured 96-hour LC_{50} of 0.72 mg/L for *Mysidopsis bahia*. The value is in the range of 0.1 mg/L < $L(E)C_{50}$ ≤ 1 mg/L, resulting in classification as Aquatic Acute 1 with an M-factor of 1.

The lowest chronic value is the mean measured 21-day NOEC for survival and growth of 0.06 mg/L for *Daphnia magna* supported by the 21-day NOEC for reproduction of 0.07 mg/L in another Daphnia test. The value is in the range 0.01 mg/L < NOEC ≤ 0.1 mg/L, resulting in classification as Aquatic Chronic 1 with an M-factor of 1, for a not rapidly degradable substance.

Overall, RAC agrees with the DS that DBNPA, i.e. 2,2-dibromo-2-cyanoacetamide, warrants classification as **Aquatic Acute 1; H400 (M=1) and Aquatic Chronic 1; H410 (M=1)**.

RAC evaluation of hazards to the ozone layer

Summary of the Dossier Submitter's proposal

DBNPA did not fulfil the criteria for classification for effects to the ozone layer.

No hazard classification for hazards to the ozone layer was proposed by the DS. Exposure of the atmospheric compartment to DBNPA was considered to be of no concern, as DBNPA had a very low vapour pressure (1.19 x 10-3 Pa at 19.2°C). The Henry's law constant of DBNPA, calculated based on the vapour pressure and the water solubility, was 2.04 x 10^{-5} Pa m^3 mol^{-1} at pH 7 and 20°C. Hence volatilisation of dissolved DBNPA from surface waters was expected to be negligible.

DBNPA is not listed on the Annex I of Regulation (EC) 2037/2000 on substances that deplete the ozone layer. DBNPA is not a member of any of the chemical classes expected to be ozone depleting substances in the stratosphere.

Comments received during public consultation

There were no comments received in the public consultation.

Assessment and comparison with the classification criteria

RAC agrees with the DS view that DBNPA does not fulfil the criteria for classification for effects to the ozone layer. DBNPA is not listed on the Annex I of Regulation (EC) 2037/2000 on substances that deplete the ozone layer. DBNPA itself is not a member of any of the chemical classes expected to be an ozone depleting substances in the stratosphere.

However, DBNPA contains bromine, which is known to destroy ozone molecules on contact in the stratosphere. Despite this, none of the information in the CLH dossier indicates that bromine or bromide would be released to the water column and in such an event these would form HBr and/or HOBr, which react extremely quickly with organic material and are unlikely to volatilise. Consequently, bromine is highly unlikely to reach the stratosphere as a result of release from DBNPA.

In conclusion, RAC agrees with the DS that **no classification for hazardous to the ozone layer is warranted**.

Additional references

Damsch *et al.* (2011) Gavage-related reflux in rats: identification, pathogenesis, and toxicological implications (review). Toxicology Pathology 39:348-360

Loeber *et al.* (1983) Effect of sodium bromide on endocrine parameters in the rat as studied by immunocytochemistry and radioimmunoassay. Food and Chemical Toxicology 21(4):391-404

Pavelka *et al.* (2001) Effect of high bromide levels in the organism on the biological half-life of iodine in the rat. Biological Trace Element Research 82:125-132

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).